Design and synthesis of next generation carbohydrate-mimetic cyclitols: towards deactivators of inverting glycosidases and glycosyl transferases

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Design and synthesis of next generation carbohydrate-mimetic cyclitols

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Towards deactivators of inverting glycosidases and glycosyl transferases

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Chapter 1

Introduction and outline

Carbohydrates and glycoconjugates, collectively termed glycans, comprise a large and structurally widely diverse class of biomolecules. The glycan structural diversity stems from the wide array of monosaccharide building blocks they are assembled from; the nature (alpha or beta) and position by which these building blocks are connected through glycosidic linkages; the wealth of aglycons (peptides, proteins, lipids, nucleotides, and other biomolecules) that are found to bear mono- or oligosaccharides; and the numerous chemical modifications (phosphates, sulfates, esters, amongst others) glycan chains may carry themselves.[1] This structural diversity is reflected in the numerous biological functions mediated by glycans. Bulk glycans such as cellulose and starch provide structure and serve as energy storage for several kingdoms of life.[2,3] Moreover, specific glycans play key roles in many physiological processes by mediating cell-cell interaction and signal transduction processes amongst others.[1,4,5] The structural and functional diversity of glycans is also reflected in the wealth of glycoprocessing enzymes that make (glycosyltransferases) and break (glycosidases) glycosidic linkages.[6,7] These large enzyme families are found in all branches of life[8] and are main factors in nutrient digestion and acquisition, protein post-translational modification and glycan turnover.[9,10]

Inhibitors, often designed to mimic the substrate structure of glycoprocessing enzymes (known as glycomimetics), have been widely used to study glycoprocessing enzymes.[11–23] As is the case for enzyme inhibitors in general, glycoprocessing enzyme inhibitors come in various flavors that broadly, but not exclusively, fall in two categories. Reversible inhibitors compete with substrates for the enzyme active site where they bind in a reversible manner. Such inhibitors, also termed competitive inhibitors, are useful starting points for drug discovery and development, in case the underlying glycoprocessing enzyme is causative of human disease, but are less useful for reporting on enzyme activities in complex biological substrates. The latter is done more effectively using irreversible inhibitors. Such compounds, especially those that react within an enzyme active site to form a covalent and irreversible bond, also termed suicide substrates, form the basis for the design of activity-based probes (ABPs). These compounds are equipped with a reporter entity (fluorophore, affinity tag) which allow detection, isolation, identification and quantification of active enzyme molecules by a variety of biochemical and analytical means.[16,21–24] The design of glycoprocessing enzyme inhibitors and probes requires accessibility through synthetic methodologies, which have become increasingly complex over the years, necessitating the development of versatile chemistries for their synthesis.[12,13,22,25]

In this thesis, the design and synthesis of potential glycosidase and glycosyltransferase inhibitors using novel synthetic methodologies are presented. A crucial element in the design is the use of a pyranose-configured carbasugar isostere, in which the endocyclic oxygen of the parent monosaccharide is replaced for a methylene functionality. This all-carbon six-membered ring motif is prominently featured in the natural mechanism-based retaining β-glucosidase inhibitor cyclophellitol, which has a remarkable structural similarity to β-glucose. Cyclophellitol adopts a half-chair conformation, resembling the transition state conformation that evolves during enzymatic hydrolysis of a β-glucosidic bond. Upon binding to a retaining β-glucosidase active site, the epoxide then reacts with the active site nucleophile to form a covalent, irreversible enzyme-inhibitor adduct. These unique features have inspired the design of numerous cyclophellitol analogues, and also form the basis of all inhibitor designs presented in this thesis. The mode of action of cyclophellitol as a retaining β-glucosidase inhibitor is reviewed in this chapter, which also presents a concise overview of the synthetic methodologies and chemical transformations developed over the years to create the cyclophellitol scaffold. The chapter concludes with an outline of the thesis.

As first proposed by Koshland in 1953, most glycosidases can be divided in two different classes, based on their catalytic mechanism, as retaining or inverting glycosidases.[26,27] Both mechanisms of action lead to hydrolysis of the substrate glycosidic linkage; in both cases similar catalytic site residues are involved during hydrolysis; and both reactions proceed through an oxocarbenium-like transition state.[28] The fundamental difference concerns the mechanistic itinerary displayed by these enzymes.

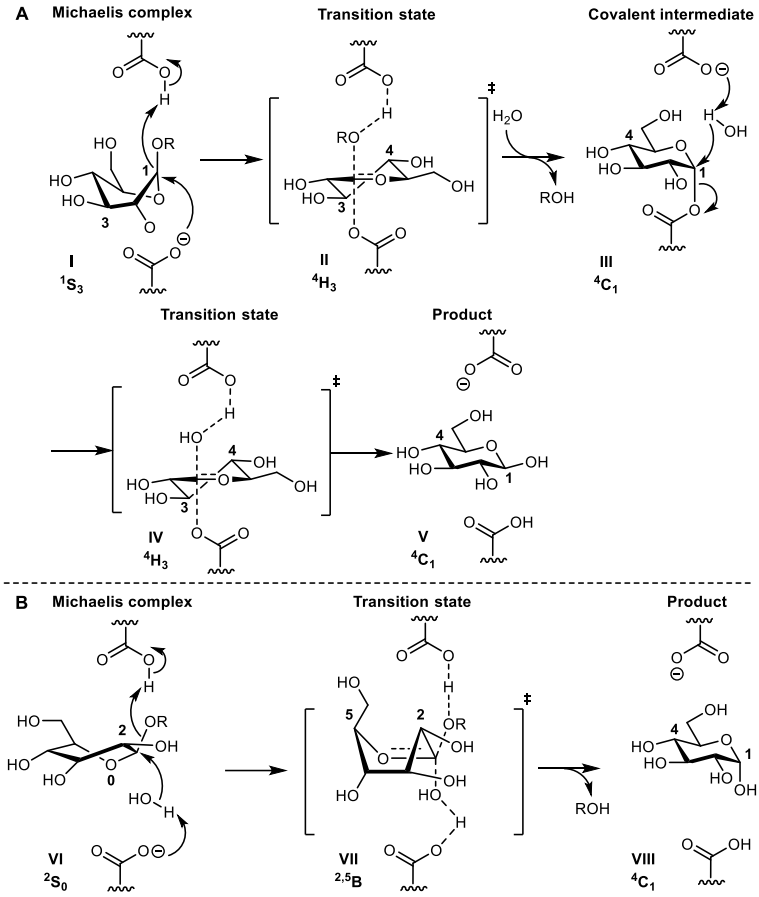

Figure 1. Catalytic itinerary of enzymatic hydrolysis of β-glucosides as mediated by β-glucosidases. **A**) In retaining β-glucosidases the first S_N2 displacement step results in a covalently bound enzyme-substrate complex (**III**), which in the second displacement step is hydrolyzed by an incoming water molecule to give β-glucose as the product. Both steps are mediated by the enzyme's catalytic acid/base residues (Asp/Glu). **B)** In inverting β-glucosidases, the aglycon is directly substituted in a single S_N2 event by a water molecule. This step is mediated by the enzyme's more distal catalytic residues (Asp/Glu). Here, no covalently bound enzyme-substrate complex is formed during catalysis, and α-glucose is produced.

Retaining glycosidases hydrolyze their substrates through a double-displacement mechanism resulting in a net retention of the anomeric configuration of the hydrolyzed substrate (Figure 1A).[29–31] The first displacement step comprises protonation of the aglycon oxygen (**I**) by the catalytic acid/base residue (either a glutamic acid or aspartic acid functionality, depending on the nature of the retaining β-glucosidase at hand) and concomitant S_N2 nucleophilic aglycon displacement by the catalytic nucleophile (a glutamate or aspartate) resulting in covalently bound intermediate **III**. In a reversal of steps, the covalently bound intermediate undergoes anomeric substitution by an incoming water molecule which is deprotonated by the carboxylate site residue resulting in expulsion of β-glucoside as the product (**V**) and regeneration of the enzyme active site.

The relatively large distance (6–12 Å) between the two catalytic side residues in inverting glycosidases allows for the presence of a water molecule upon binding of the substrate in the active side (**VI**, Figure 1B).[32–35] As a result, hydrolysis of the substrate occurs in a single displacement with net inversion of stereochemistry at the anomeric position. This single step process is, as for retaining glycosidases, mediated by a glutamic or aspartic acid residue. In this case, the substrate is directly hydrolyzed by water in a process in which the two active site residues assist the attack of water and the protonation of the aglycon leaving group.[32,36,37]

Each catalytic mechanism performed by glycosidases follows a well-defined conformational itinerary, during which complex substrate distortions take place in order to accommodate optimal orbital overlap between the leaving group and the nucleophile.[28] For retaining glucosidases, the first step in the itinerary is represented by the formation of the Michaelis complex (**I**), in which distortion of the substrate forces the aglycon leaving group in (pseudo)-axial orientation upon initial enzyme binding. This allows the empty σ* orbital of the leaving group to become accessible for the incoming lone pair of the activated nucleophile, approaching the electrophile with an angle of approximately 180° relative to the leaving group. This substitution is assisted by the endocyclic oxygen, of which a lone pair is perfectly positioned to interact with the leaving group σ* (also being positioned under an angle of 180°), weakening the glycosidic bond – thus promoting substitution by the incoming nucleophile *via* an oxocarbenium transition state (**II**).

Investigations on retaining β-glucosidases suggests a $^{1}S_{3} \rightarrow [^{4}H_{3}]^{\ddagger} \rightarrow {}^{4}C_{1}$ conformational itinerary to be most common, as depicted in Figure 1A, while the reversed itinerary $^{4}C_{1} \rightarrow [^{4}H_{3}]^{\ddagger} \rightarrow {}^{1}S_{3}$ is generally followed by retaining α-glucosidases.[30,32,36–38] In contrast, the conformational itinerary of inverting β-glucosidases is suggested to go through a $^{2}S_{0} \rightarrow$

$[^{2,5}B]^{‡} \rightarrow {}^{4}C_1$ conformational itinerary.[28,39] However, many itineraries, especially those employed by inverting glycosidases, are as of yet unknown. Metadynamic simulations have provided crucial insights in glucosidase mechanistical itineraries and the structural distortions of the substrates.[40–42] Such studies are very useful in the design of glycosidase inhibitors, and mimicry of the Michaelis complex or transition state conformations have led to the conception of effective glycosidase inhibitors.[34,43,44]

A prime example of a transition state mimicking glycosidase inhibitor is the natural product (+)-cyclophellitol (**1**, Figure 2A), first isolated in 1989 from a species of the *Phellinus sp.* mushroom.[45,46] Cyclophellitol is a potent irreversible inhibitor of retaining β-*exo*-glucosidases.[47] Upon binding in a transition-state mimicking $^{4}H_3$ conformation to the active site of a retaining β-glucosidase (**IX**, Figure 2B), epoxide protonation and concomitant nucleophilic opening of the epoxide warhead by the active site nucleophile results in the formation of a stable ester-linked enzyme-inhibitor adduct (**X**), effectively incapacitating the enzyme. Stability of the ester linkage arises from the absence of an endocyclic oxygen, which as aforementioned, provides the necessary destabilization of the acetal intermediate during catalysis of the natural substrate *via* the oxocarbenium ion transition state.

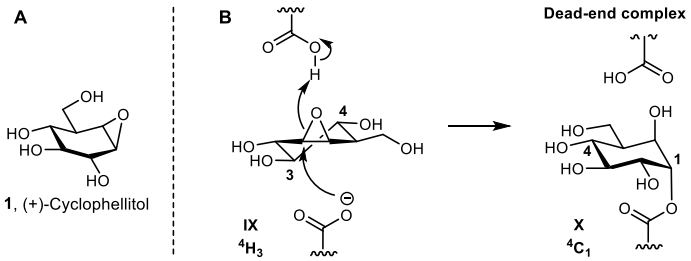

Figure 2. **A**) The potent irreversible inhibitor (+)-cyclophellitol (**1**) exhibits a $^{4}H_3$ conformation. **B**) The mode of action of cyclophellitol upon covalently and irreversibly binding to a retaining β-*exo*-glucosidase.

Cyclophellitol represents a synthetic challenge due to its six chiral centers – all carbons that form the cyclohexane ring are substituted and chiral – as well as the presence of the reactive group that makes the compound a mechanism-based glucosidase inhibitor: the epoxide. The most used strategy to address this stereochemical challenge is by starting from chiral pool building blocks: that is, by starting with an enantiopure reagent resembling the desired molecule as much as possible – thereby limiting the required amount of chemical transformations.[48–50] Ideally, large quantities of the starting material are readily accessible and cheap. With cyclophellitol being a carbasugar, many commercially available monosaccharides have proven to be a sensible starting point to access this scaffold.

The first total synthesis of cyclophellitol was achieved by Tatsuta[51] in 1990 and counted fourteen consecutive steps starting from intermediate **1A** (Figure 3A), which in turn is readily available through known transformations from L-glucose. Key transformations in the synthesis entail a diastereoselective *syn* intramolecular [2+3] cycloaddition of intermediate **2A** resulting in bicycle **3A**, which upon chemical manipulation involving the reduction of the N-O bond using Raney-nickel and subsequent hydrolysis of the imine provided the corresponding ketone at the C-1'-position. A stereoselective reduction of the ketone and subsequent mesylation provided structure **4A**. Reductive deprotection of the benzyl protecting groups sets the stage for a base induced intramolecular substitution, affording the 1,7-anhydro functionality. Upon removal of the remaining silyl protecting groups, cyclophellitol **1** is obtained.

An alternative route has been developed by Fraser-Reid a few years later (Figure 3B).[52] Here, commercially available tri-*O*-acetyl-D-glucal is transformed into both 1,7-epimers of cyclophellitol in a nineteen-step synthesis route. To this end, D-glucal **1B** was subjected to a series of transformations leading to the introduction of an external alkyne at the 6-position (**2B**).

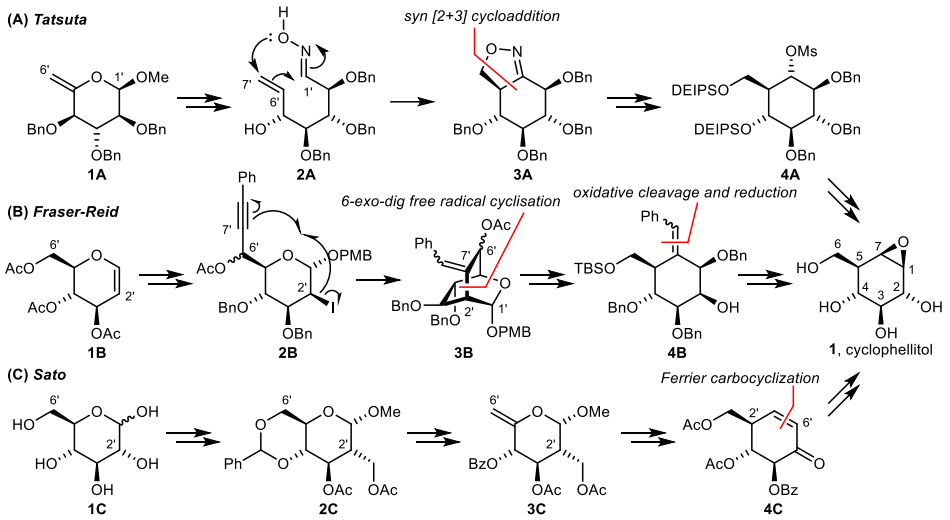

Figure 3. Three different routes towards cyclophellitol **1** starting from commercially available monosaccharides. **A**) Tatsuta *et al*.[51] started from sugar derivative **1A** and exploited an 1,3-dipolar cycloaddition to build the carbacycle backbone. **B**) Fraser-Reid *et al.*[52] started from the commercially available tri-*O*-acetyl-D-glucal (**1B**) and employed a radical cyclization for the construction of the carbacycle backbone. **C**) Sato *et al*.[53] started from D-glucose, and installed the carbocyclic backbone *via* a Ferrier carbocyclization.

A radical induced intramolecular 6-*exo-dig* cyclization under oxidative conditions effectively allowed for C-C bond formation between C2' and C7' to afford [2.2.2]oxabicyclopyranoside **3B**. Oxidative cleavage of the PMB protecting group at C-1' afforded an hemi-acetal which rapidly collapsed into an aldehyde. The aldehyde was successively reduced and protected as a silyl ether. Oxidative cleavage of the exo-alkene at C-7', stereoselective reduction and subsequent transformations set the stage for an intramolecular substitution to form the epoxide ring. Global deprotection then afforded cyclophellitol **1**.

In the following years four different routes were reported,[53–56] all based on a common key synthetic transformation, as first exploited by Sato *et al.*[53] in their route towards cyclophellitol: the Ferrier carbocyclization (Figure 3C).[57] This reaction allows to open the pyranose ring and, through a subsequent intramolecular aldol reaction, forms the carbacycle. This very useful transformation has been exploited with different Lewis acids ($HgCl_2$, $PdCl_2$) to neatly provide the desired carbocycles in high yields.[58] In Sato's approach, many steps involving protection of hydroxyl functionalities and formation of a new C-C bond at the 2'-position (**2C**) are required to convert the starting material (D-glucose, **1C**) into Ferrier precursor **3C**. The cyclic α,β unsaturated ketone **4C**, which is the product of the Ferrier carbocyclization, is subjected to stereoselective reduction of the carbonyl group and direct epoxidation of the double bond. Transesterification conditions then resulted in removal of the acetyl protecting groups and isolation of cyclophellitol **1**. With the Ferrier carbocyclization as key transformation, Letellier and co-workers managed to drastically reduce the number of steps, successfully obtaining cyclophellitol **1** in just eight steps from methyl α-D-glucopyranoside.[54]

In an alternative approach towards highly decorated cyclitol constructs, synthesis routes have been designed starting from a chiral carbocyclic structure present in some easily accessible natural products. These routes were based on the idea that circumventing the need to replace the endocyclic oxygen for a methylene group would increase the synthetic accessibility.

Inositols are naturally occurring compounds with many biological functions,[59] and feature a carbocyclic structure with various stereochemistries. In the context of cyclophellitol synthesis, such structures represent a real advantage, since they already possess a carbocyclic backbone featured with exploitable chiral centers. The synthesis developed by Ozaki[60,61] starts from L-quebrachitol (**1D**, Figure 4D), the structure of which possesses four chiral centres also present in cyclophellitol. Here, asymmetric protection of four of the hydroxyls as cyclohexylidenes allowed for regioselective oxidation of the 5-OH (**2D**). At this point, installation of the hydroxymethyl functionality

was accomplished *via* addition of Me_3SiCH_2MgCl, followed by a hydroboration to afford the primary 6-OH which was consequently benzoylated yielding cyclophellitol backbone **3D**. The protecting groups on the 2-, 3- and 4-position were selectively exchanged for benzyl ethers, after which the *cis* cyclohexylidene moiety spanning the 1,2-diol was removed (TFA, MeOH). Regioselective triflation of the equatorial 1-OH, acetylation of the axial 7-OH, and subsequent substitution of the triflate with iodide afforded cyclohexane **4D**. Deacetylation under alkaline conditions resulted directly in the 1,7-anhydro functionality, *via* an intramolecular substitution. Global deprotection then afforded cyclophellitol **1**.

The natural product (-)-quinic acid **1E** (Figure 4E) has been chosen as starting material by Shing *et al.*[62] in the synthesis of cyclophellitol. A total of eighteen transformations are required for all functionalities and stereocenters to be installed. The first part of the route comprised the formation of the correct stereocenters at the 3-, 4- and 5-carbons *via* inversion of the 3- and 5-position and the introduction of a hydroxyl moiety at the 4-position (**2E**). This is accomplished through oxidation and stereoselective reduction of the 3-position and formation of a C-C double between C-4- and C-5, followed by a stereocontrolled hydroboration. Introduction of a cyclic sulfate spanning the 1,2-diol allowed for a regioselective substitution, resulting in the corresponding iodide or selenide at the C-1 position (**3E**). In turn, these leaving groups could be eliminated regioselectively to form a double bond spanning the 1- and 7-position. All that remained then was inverting the stereochemistry at the C-2 position to transform the mannose configured cyclohexene to the glucose configuration. This was achieved using Mitsunobu conditions. Direct epoxidation of the 1,7-alkene by *m*-CPBA afforded the 1,7-epoxide as a mixture of diastereoisomers which after global deprotection and separation resulted in cyclophellitol **1**.

An elegant approach towards the cyclophellitol scaffold was described by Trost and co-workers in 1999.[63] One of the key transformations entails a palladium catalysed kinetic resolution of racemic conduritol B tetraacetate (**1F**, Figure 4F) using a chiral phosphine ligand as a means to acquire enantiopure intermediate **2F(-)**. The use of a chiral ligand guaranteed that only the (-)-conduritol B **1F(-)** underwent transesterification of the C-1-OAc, yielding a separable mixture of unreacted (+)-conduritol B **1F(+)** and product **2F(-)**. In addition, orthogonal differentiation between the hydroxyl functionalities in **2F(-)** allowed for further functionalization of the 1-OH delivering stannane **3F(-)**. Next, **3F(-)** underwent a [2,3]-Wittig-Still rearrangement resulting in the installation of the hydroxymethyl functionality at the C-5-position with correct regio- and stereochemistry completing the synthesis of cyclophellitol scaffold **4F(-)**. All that remained was

epoxidation (*m*-CPBA) of the alkene and subsequent hydrogenation to afford cyclophellitol **1**.

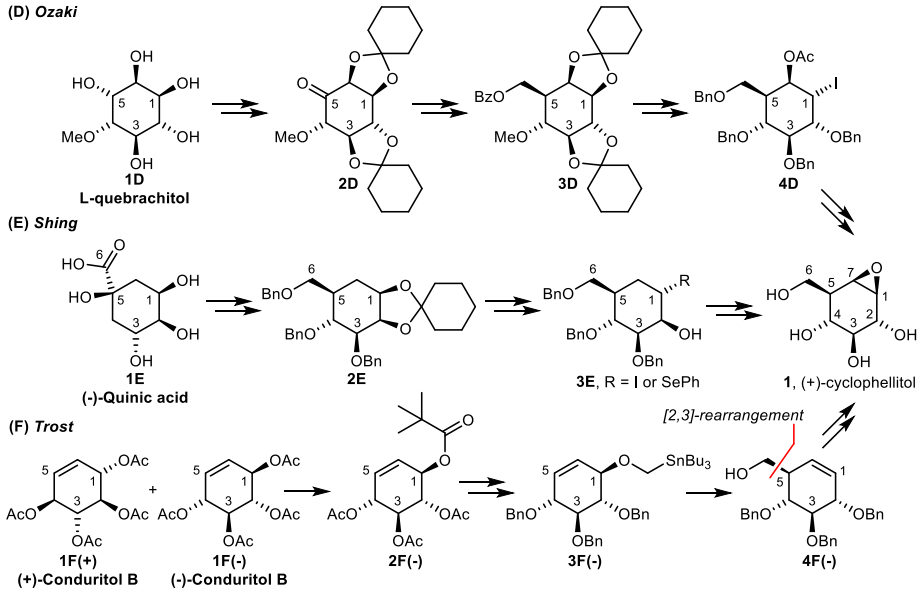

Figure 4. Three different routes towards cyclophellitol **1** starting from inositols. **D**) Ozaki: utilizing L-quebrachitol (**1D**); **E**) Shing: starting from (-)-quinic acid (**1E**); **F**) Trost: using racemic conduritol B (**1F**).

The most recently established strategy for the synthesis of cyclophellitol-based inhibitors is founded on the work by Madsen and co-workers.[64] Minor adjustments and modifications, which on the whole do not distort the scheme, have been made since.[21] This strategy provides the cyclitol backbone in three key transformations, starting from commercially available D-xylose (**1G**, Figure 5). Following standard manipulation, D-xylose is readily converted to iodofuranoside **2G**. A subsequent zinc-mediated Vasella fragmentation is used to promote the formation of aldehyde **3G**. In the transformation towards diene **4G**, an asymmetric allylation is required to give rise to two new chiral centers (the intended 4- and 5-position in cyclophellitol). This diastereoselective step cannot be accomplished by using standard Grignard reagents but requires usage of a Barbier reaction with a commercially available bromocrotonate in combination with expensive materials such as the rare-earth metals lanthanum and indium. A Zimmerman-Traxler transition-state allows for the correct stereocenters to be installed in high overall yield. In addition, a small percentage of the C-5 epimer is formed as well.[65]

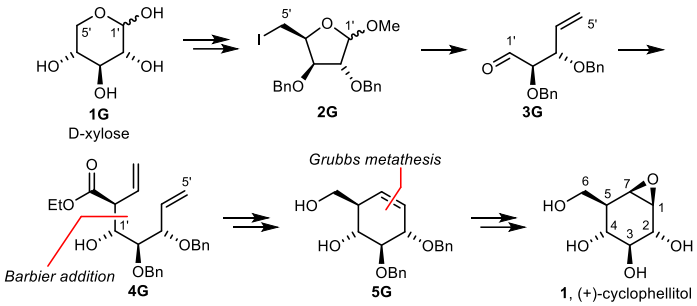

Figure 5. Synthetic scheme towards cyclophellitol **1** as reported by Madsen and co-workers,[64] accomplished in nine steps starting from commercially available D-xylose (**1G**).

The newly formed diene **4G** can undergo a ruthenium-catalyzed ring-closing metathesis to form cyclohexene **5G** using 5 mol% of the second-generation Grubbs catalyst.[66] The use of a smaller amount or the first-generation catalyst[67] led to lower yields and unidentified regioisomers.[21] Eventually, cyclophellitol **1** was obtained through a stereoselective epoxidation aided by the guidance of the homo-allylic alcohol followed by global deprotection.

Over the years, understanding of the conformational itineraries of retaining glycosidases has allowed for the design and synthesis of cyclophellitol based analogues and isosteres tailored towards predetermined glycosidases and for specific purposes.[11,13,15,16,19–21] In this way, inhibitors and probes targeting specific *endo*- or *exo*-glycosidases,[22,68] but also inhibitors to study broader ranges of glycosidases have been constructed and applied successfully.[24,69] In contrast, and hindered by the absence of a covalently-bound substrate-enzyme adduct during catalytic hydrolysis, no covalent and irreversible inhibitor designs for inverting glycosidases exist to date. As a consequence, an absence of activity-based probes, suited to selectively profile the activity of inverting glycosidases *in situ* and *in vivo*, is noted.[70] To this end, novel irreversible inhibitor designs are required, expanding beyond the inhibitory scope of cyclophellitol. This challenge formed the inspiration of part of the work described in this thesis: are mechanism-based inverting glycosidase inhibitor designs feasible and if so, can these be based on expanding on the cyclophellitol theme. Other questions addressed during the research described in this thesis – summarized below per chapter – entailed the design of suitable alternative strategies for the construction and glycosylation of orthogonally protected cyclophellitols, as well as the design of putative competitive glycosyltransferase inhibitors that also feature structural elements characteristic for the cyclophellitol scaffold.

Outline of this thesis

The covalent, irreversible inhibitor of retaining β-glucosidases, (+)-cyclophellitol, in combination with numerous analogues and activity based probes, has fueled the study of retaining glycosidases in recent decades. Although inverting glycosidases are equally widespread and of equal societal importance, a complete lack of covalent, irreversible inhibitors holds back the field. To tackle this hurdle, this thesis describes novel methodologies and inhibitor designs to increase accessibility of carba-glycoside backbones and complex inhibitors targeting inverting glucosidases. Further raising the bar, the design and synthesis of putative competitive glycosyltransferase inhibitors is presented. **Chapter 2** describes a novel methodology towards an orthogonal cyclophellitol building block with the aim of extending the scope of synthetic cyclophellitol-based glycosidase inhibitors and probes. The key orthogonal cyclohexene was obtained in twelve steps starting from acetylated D-glucal and the versatility of the strategy is demonstrated in the construction of a small series of α-cyclophellitols mimicking linear and branched dextran substructures. **Chapter 3** describes a series of twenty configurational and functional cyclophellitol analogues, featuring a systematic array of electrophiles spanning the 1,2- and 1,7-position. Their inhibitory potencies were assessed *in vitro* assays and combined with calculated conformational free energy landscapes to find structural and electronic activity relationships.

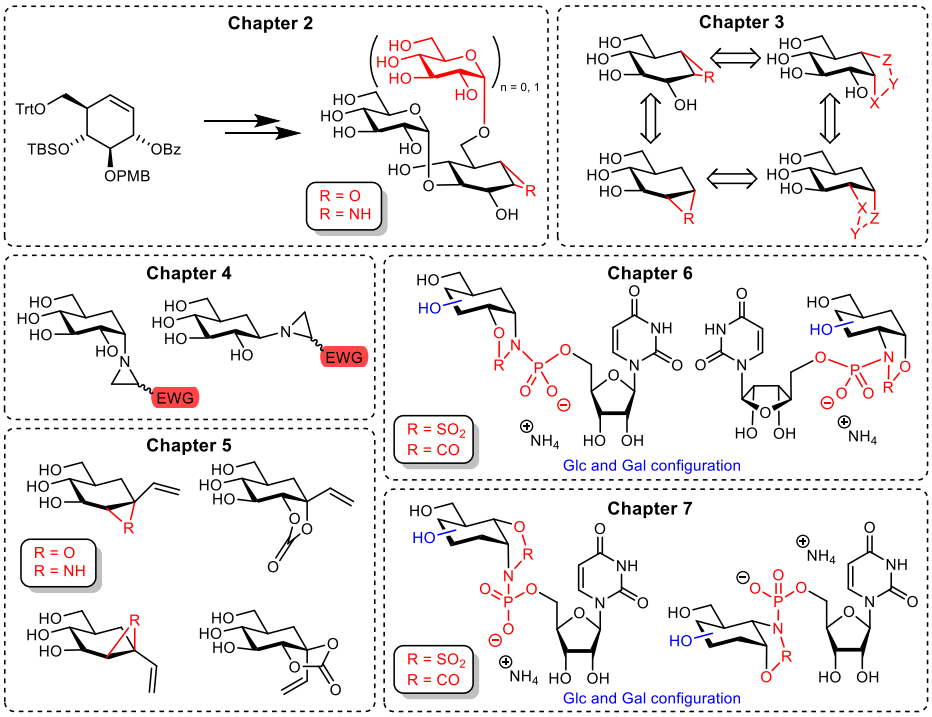

Figure 6. Generic overview of the constructs described in this thesis.

Chapter 4 describes the synthesis of eight exocyclic aziridine cyclitols envisioned as putative irreversible, covalent deactivators of inverting α- and β-glucosidases. Key in the synthesis is a divergent aza-Michael initiated ring closure reaction (aza-MIRC) on unprotected carbasugars. In **Chapter 5**, a series of cyclophellitol-derived constructs, equipped with an anomeric vinyl moiety, is described and synthesized. These inhibitors are envisioned to act *via* an 1,4-Michael addition as putative inhibitors of inverting α- and β-glucosidases. Further expanding the scope, **Chapter 6** describes the design, methodology, and synthesis of eight UDP-glucose and UDP-galactose mimetics as potential inhibitors of glucosyltransferases and galactosyltransferases. Further capitalizing on this strategy, **Chapter 7** presents the corresponding 1,7-regioisomers. **Chapter 8** summarizes the work presented in this thesis and provides some suggestions for future work capitalizing on the here developed inhibitor designs and methodologies.

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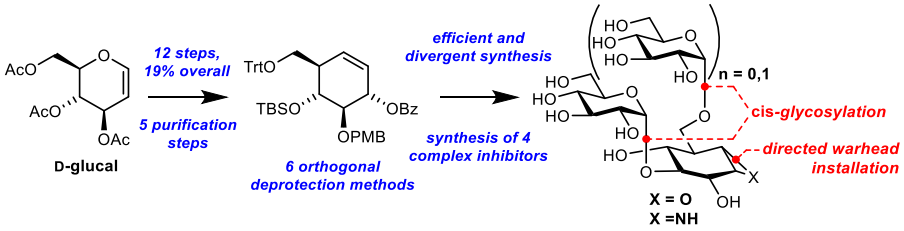
Chapter 2

An orthogonally protected cyclitol for the construction of nigerose- and dextran-mimetic cyclophellitols



ABSTRACT Cyclophellitols are potent inhibitors of *exo*- and *endo*glycosidases. Efficient synthetic methodologies are needed to fully capitalize on this intriguing class of mechanism-based enzyme deactivators. This chapter reports on the synthesis of an orthogonally protected cyclitol from D-glucal (19% yield over 12 steps), and its use in the synthesis of α-(1,3)-linked di- and trisaccharide dextran mimetics. These new glycomimetics may find use as dextranase inhibitors, and the developed chemistries in widening the palette of glycoprocessing enzyme targeting glycomimetics.

PUBLISHED Ofman, T. P.; Küllmer, F.; van der Marel, G. A.; Codée, J. D. C.; Overkleeft, H. S. An orthogonally protected cyclitol for the construction of nigerose- and dextran-mimetic cyclophellitols. *Org. Lett.* **2021**, *23*, 9516–9519.

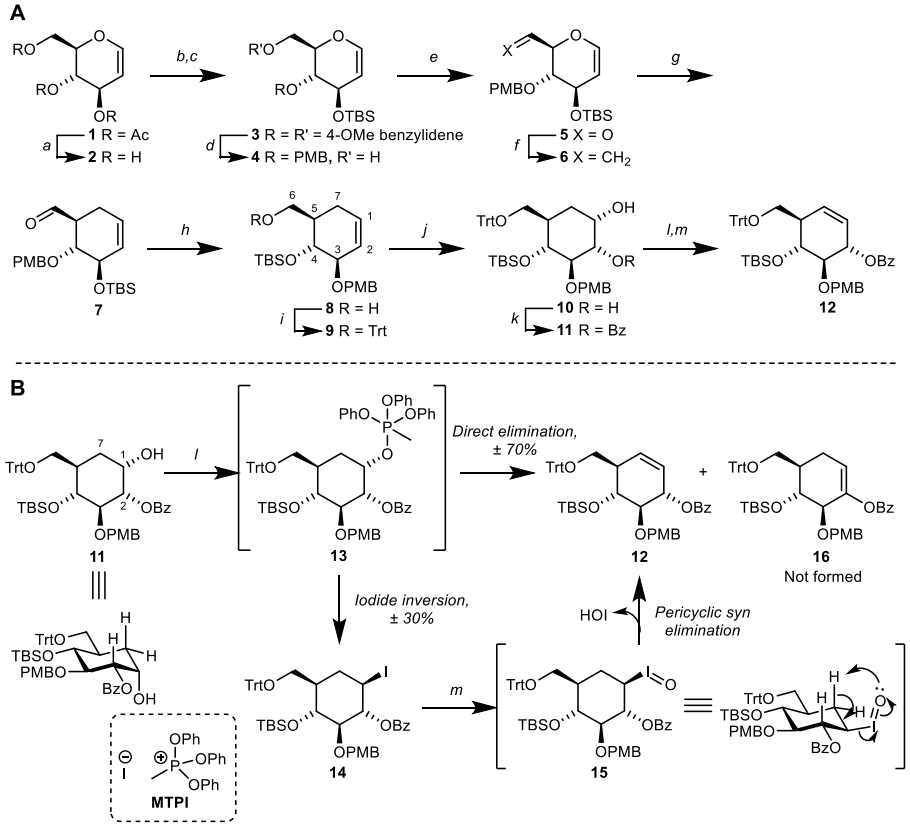
Introduction

Cyclophellitol is a natural product isolated from species of the *Phellinus sp.* mushroom, and is a potent irreversible inhibitor of retaining β-exoglucosidases.[1,2] Since its discovery, a number of syntheses of cyclophellitol have appeared in literature.[3–6] Cyclophellitol is a densely functionalized cyclohexane featuring the β-D-glucopyranose configuration with an epoxide bridging C-1 and C-7.[1,2] The epoxide forces the cyclohexane ring in a $^4H_3$ conformation, which is also the expected conformation of the transition state oxocarbenium ion that emerges during the enzyme-catalyzed hydrolysis of β-glucosidic linkages by retaining β-glucosidase enzymes.[7] Binding in the active site results in a stable ester-linked enzyme-inhibitor adduct, effectively incapacitating the enzyme. This mode of action makes it attractive for use in activity-based protein profiling (ABPP).[7–10] Previous studies revealed that tagging cyclophellitol, and also its nitrogen congener, cyclophellitol aziridine, with a reporter entity (biotin, fluorophore), allows for very sensitive profiling of retaining β-glucosidases.[11] In a follow-up study, it was revealed that the same holds true for 1,7-*epi*-cyclophellitol (or α-cyclophellitol) and the corresponding aziridine in inhibiting and tagging retaining α-glucosidases.[12] ABPP now finds wide use in glycobiology research and a host of configurational and functional cyclophellitol analogues have been reported, each targeting unique retaining exo- and endoglycosidases in the context of drug discovery and bulk polysaccharide processing enzyme discovery.[13–18] However, retaining glycosidase ABPP has not been exploited to the full of its potential yet and this is -at least in part- due to the challenges associated with the synthesis of cyclophellitol-based inhibitors and probes. Challenges that rest in the manipulations of the functionalized cyclohexene/cyclitol epoxide/aziridine cores that are required to create diverse substitution patterns, including glycosidic linkages. With the aim of extending the scope of synthetic cyclophellitol-based glycosidase inhibitors and probes the synthesis strategies towards α-cyclophellitols were revisited, with special focus on the orthogonality of protection group arrays in advanced intermediates. The results of these studies are presented here and entail the synthesis of a fully orthogonal cyclophellitol building block in twelve steps, starting from commercially available 3,4,6-tri-*O*-acetyl-D-glucal, and the demonstration of its versatility in the construction of glycosylated α-cyclophellitols mimicking linear and branched dextran substructures.

Results and discussion

The synthesis of orthogonally protected cyclohexene **12** started from commercially available per-acetylated D-glucal (scheme 1A). Saponification of the acetyl protecting groups (Et_3N, H_2O, MeOH) yielded unprotected D-glucal **2**. Subsequently, a regioselective protection of the 4- and 6-OH followed, using anisaldehyde dimethyl acetal and catalytic amounts of PPTS. Treatment with TBSCl, imidazole and DMAP allowed for the 3-OH to be protected as a TBS ether **3**. Reductive opening of the 4-methoxybenzylidene towards the 4-position was accomplished by treatment with DiBAl-H yielding intermediate **4** in good yield (51% over four steps, >50 gram scale). The primary hydroxyl of compound **4** was oxidized by treatment with Dess-Martin periodinane, resulting into the corresponding aldehyde **5** which was transformed into alkene **6** under Wittig conditions in good yield (80% over two steps, >20 gram scale).[19] The ensuing key thermal [3,3]-sigmatropic Claisen rearrangement by heating of **6** in diphenyl ether to 210 °C yielded intermediate aldehyde **7**, which was directly reduced with $NaBH_4$ to give alcohol **8** (80% over two steps, 20 gram scale) according to literature precedent.[20–22] Tritylation of the primary hydroxyl of compound **8** (TrtCl, Et_3N), followed by dihydroxylation of the alkene using catalytic amounts of OsO_4 and NMO as co-oxidant yielded solely the glucose configured *syn*-diol **10**. Subsequently, the 2-OH could be regioselectively protected as a benzoyl ester under mild conditions (BzCl, pyridine, -15 °C) due to the higher reactivity of the equatorial 2-OH over the axial 1-OH, to yield compound **11** (79% over three steps, >15 gram scale).[23]

Scheme 1. Synthesis of orthogonally protected cyclohexene **12** (A) and the proposed mechanism of the dehydration reaction of compound **11** *via* an iodide intermediate (B).



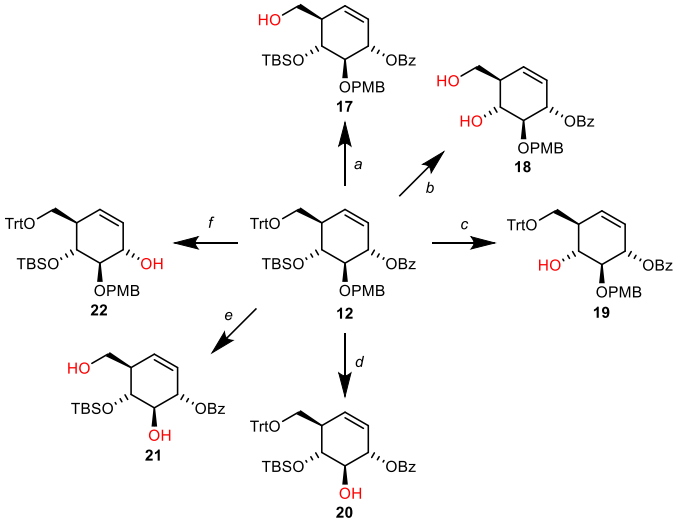
Reagents and conditions: *a*) MeOH, Et_3N, H_2O; 8:1:1, 16 h, rt; *b*) anisaldehyde dimethyl acetal, PPTS, DMF, 1 h, 35 °C; *c*) TBSCl, DMAP, imidazole, DMF, 1 h, 35 °C; *d*) DiBAL-H, DCM, 2 h, -15 °C (51% over four steps); *e*) Dess-Martin periodinane, $NaHCO_3$, DCM, 2 h, rt; *f*) $MePPh_3Br$, *n*-BuLi, THF, 2 h, -78 °C to 0 °C (80% over two steps); *g*) Ph_2O, 2 h, 210 °C; *h*) $NaBH_4$, THF, EtOH, 20 min., 0 °C (80% over two steps); *i*) TrtCl, Et_3N, DMAP, DCM, 16 h, rt; *j*) OsO_4, NMO, acetone, H_2O, 16 h, rt; *k*) BzCl, pyridine, 1.5 h, -15 °C (79% over three steps); *l*) MTPI, 2,6-lutidine, DMF, 1.5 h, 100 °C; *m*) m-CPBA, $NaHCO_3$, 4 h, 0 °C (75%).

With intermediate **11** in hand, attempts were made to regioselectively eliminate the 1-OH to alkene **12**. Compound **11** was subjected to a myriad of dehydration conditions (see Appendix Table S1). The best results were obtained when treating compound **11** with methyl triphenoxyphosphonium iodide (MTPI) and the sterically hindered base 2,6-lutidine, in DMF and at elevated temperature.[24] This procedure gave the desired elimination product **7** together with the corresponding iodide **14**, which formed in a competing substitution reaction, in a ratio of 7:3 of compound **12** to **14** respectively (Scheme 1B). Exposure of this mixture of compounds to *m*-CPBA and $NaHCO_3$ resulted in oxidation and subsequent elimination towards compound **12**. Under these oxidative

conditions, iodide **11** was oxidized to the corresponding iodoso intermediate **15**. This iodoso species quickly undergoes a pericyclic *syn* elimination which, again, solely yields the desired alkene **12**. Due to the high reaction rates, reaction times could be limited to only a few hours and over-oxidation of the relatively electron poor alkene was not observed.[25] Alkene **12** was thus obtained in a total yield of 75%.[26]
It is postulated that the regioselectivity of this reaction sequence results from favoring the Hoffman elimination. This is caused by the use of a mild, sterically hindered base like 2,6-lutidine, but also the steric interactions of the large phosphonate leaving group prevailing the deprotonation of the more acidic H-2 which would have resulted in the more stable Zaitsev product, compound **16**. Instead, the more accessible H-7 is abstracted resulting solely in **12**. Co-elution of the formed methyl diphenoxy phosphonate resulted in troublesome purification of cyclohexene **12**, therefore **12** was directly subjected to the deprotection method of choice.

Attention was then turned to the orthogonal deprotection of **12**, using various conditions (Scheme 2). Treatment of **12** with Lewis acid ($ZnCl_2$) in the presence of a nucleophile (methanol) resulted in the clean removal of the trityl protecting group in high yield (compound **17**, 87%), whereas treatment with a Brønsted acid (*p*-TsOH), under the same conditions resulted in simultaneous removal of the trityl and TBS protecting groups (compound **18**, 84%). Selective, orthogonal removal of the TBS protecting group could be achieved by treatment with TBAF in THF to yield compound **19** (95%). The PMB protecting group was oxidatively removed using DDQ in a biphasic medium consisting of DCM and aqueous phosphate buffer[27], leading to compound **20** (91%). The PMB and trityl protecting groups were removed by subjecting **12** to TFA and TES in anhydrous DCM to yield diol **21** (84%). The TBS protecting group was left untouched due to the absence of a nucleophile. The benzoyl protecting group was removed by saponification with NaOMe in DCM/MeOH, delivering compound **22** (86%). Larger scale deprotections were performed successfully using crude cyclohexene **12**.

Scheme 2. Orthogonal deprotection methods on cyclohexene **12**.

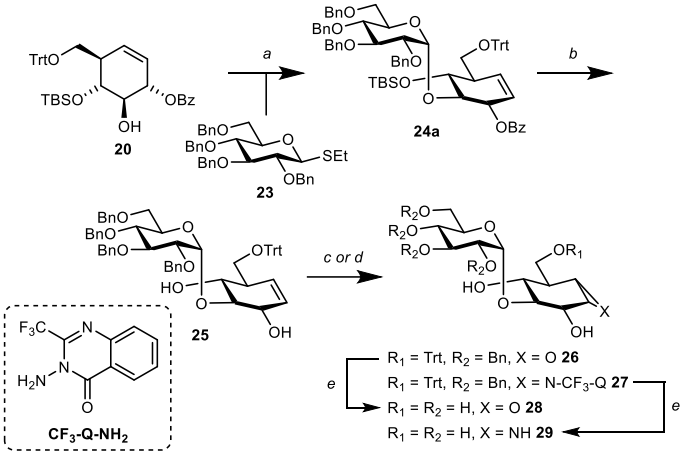


Reagents and conditions: a) $ZnCl_2$, MeOH, DCM, 16 h, rt (87%); *b) p*-TsOH, MeOH, DCM, 16 h, rt (84%); *c)* TBAF, THF, 1 h, rt (95%); *d)* DDQ, DCM, aq. phosphate buffer pH = 7.4[27], 1 h, 0 °C to rt (91%); *e)* TFA, TES, DCM, 1 h, 0 °C to rt (84%); *f)* NaOMe, MeOH, DCM, 16 h, rt (86%).

Having established the full orthogonality of the protective group pattern in cyclohexene **12**, their value was then demonstrated by the synthesis of a set of α(1,3)-linked di- and trisaccharide structures. These structures (**28**/**29** and **36**/**37**; Scheme 3 and 4 respectively) can be regarded as cyclophellitol derivatives of nigerose (α(1,3)-linked glucose) and dextran (α(1,6)-branched α(1,3)-linked glucose), and are thus envisioned as potential inhibitors for the corresponding nigerase and dextranase enzymes.[28,29]

Pre-activation based glycosylation with compound **20** acting as acceptor and glucose donor **23**[30] yielded a separable mixture of stereoisomers (**24a**:**24b**, 4:1, 88%). Removal of both the TBS and benzoyl protecting groups from disaccharide **24a** by standard deprotection procedures gave **25** in 66% yield over two steps. The stereoselective installation of the epoxide and aziridine warheads was achieved making use of the directing effect of the allylic alcohol on the C-2 position. Treatment of **25** with *m*-CPBA and $NaHCO_3$ in anhydrous DCM yielded exclusively α-epoxide **26** in 70% yield. Conversion of precursor **25** to the aziridine was accomplished using 3-amino-2-(trifluoromethyl)quinazolin-4(3*H*)-one (CF_3-Q-NH_2) and BAIB in anhydrous DCM, exclusively yielding α-aziridine **27** in 76% yield.[31] Birch reduction of compounds **26** and **27** resulted in the clean removal of the benzyl, trityl and CF_3-Q protecting groups, yielding compound **28** and **29** in 71% and 86% yield respectively.

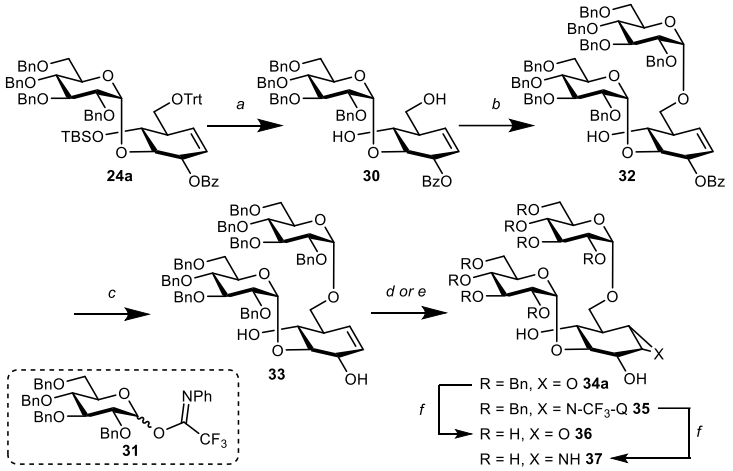
Scheme 3. Assembly of disaccharide target structures **28** and **29**.



Reagents and conditions: a) Tf_2O, TTBP, Ph_2SO, donor **23**[30], 3Å molecular rods, DCM, -78 °C, *then* **20**, 2 h, -78 °C to -10 °C (88%; α:β, 4:1); b) i. TBAF, THF, 1 h, rt; ii. NaOMe, DCM, MeOH, 16 h, rt (66%); *c) m*-CPBA, $NaHCO_3$, DCM, 16 h, rt (70% for **26**); *d)* BAIB, CF_3-Q-NH_2, DCM, 48 h, -40 °C to rt (76% for **27**); *e)* Na, *t*-BuOH, NH_3, 2 h, -60 °C (71% for **28**, 86% for **29**).

The synthesis of dextran-analogues trisaccharidic compounds **36** and **37** (Scheme 4) started by subjecting alkene **24a** to TFA and TES in anhydrous DCM, to selectively remove the trityl protecting group, followed by removal of the TBS protecting group by treatment with TBAF in THF, yielding compound **30** (72% over two steps). Acceptor **30** was treated with an excess of *N*-phenyltrifluoroacetimidate donor **31**[32] in the presence of PPh_3O and TMSI in anhydrous DCM, following literature procedures[33,34], to stereoselectively yield α(1,6)-linked trimer **32** (79%). Saponification of the benzoyl protecting group then liberated the 2-OH to direct the epoxidation/aziridination reactions. Surprisingly, conversion of alkene **33** to the epoxide with *m*-CPBA, $NaHCO_3$ in anhydrous DCM, proceeded with no stereoselectivity to yield a mixture of separable stereo-isomers (**34a** and **34b**), with a combined yield of 69%. The stereochemistry of the two isomers was unequivocally established by NOESY NMR experiments. Conversion of **33** to the aziridine was accomplished by treatment with CF_3-Q-NH_2 and BAIB in anhydrous DCM and this transformation did proceed with complete diastereotopic selection to yield α-aziridine **35** in 76%. Final deprotection of both **34a** and **35** under Birch conditions resulted in the cleavage of all benzyl and CF_3-Q protecting groups to yield trisaccharide **36** and **37** in 91% and 87% respectively.

Scheme 4. Assembly of trisaccharide target structures **36** and **37**.



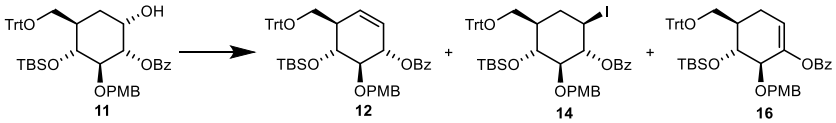
Reagents and conditions: a) i. TES, TFA, DCM, 1 h, 0 °C; *ii.* TBAF, THF, 1 h, rt (72%); *b)* donor **31**, PPh_3O, TMSI, 3Å molecular rods, DCM, 48 h, rt (79%); *c)* NaOMe, MeOH, DCM, 16 h, rt (72%); *d)* *m*-CPBA, $NaHCO_3$, DCM, 48 h, 0 °C (69% for **34a**:**34b**; α:β, 1:1); *e)* BAIB, CF_3-Q-NH_2, DCM, 48 h, -40 °C to rt (62% for **35**); *f)* Na, *t*-BuOH, NH_3, 2 h, -60 °C (91% for **36**, 87% for **37**).

Conclusion

To conclude, this Chapter describes the development of a synthetic route towards a versatile, fully orthogonal cyclophellitol building block, which can be obtained on multi-gram scale with an overall yield of 19% over 12 steps. The synthesis route has been optimized to only require five column purification steps. The key transformation involved the two-step, regioselective elimination of the C1-OH in carbaglucose **11** using MTPI and subsequent treatment with *m*-CPBA, leading to the overall transposition of the initially formed 1,2-alkene to the corresponding 1,7-alkene. Subjection of this building block to several deprotection methods demonstrated the orthogonal nature of the protecting groups. To illustrate its versatility, a number of complex glycomimetics resembling the structures of the natural polysaccharides nigerose and dextran was synthesized. Combined, the methodology as presented here may assist in the generation of complex inhibitors and activity-based probes for use in understanding and modulating carbohydrate-processing enzymes in glycobiology.

Appendix

Table S1. Screened dehydration conditions on compound **11**



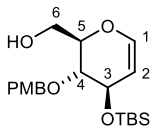
Conditions	Observations
DEAD, PPh_3, Imidazole, THF, reflux, 16 h.	43% (mixture of **12** : **16**, 8:2)
$SOCl_2$, Et_3N, DCM, 0 ˚C, *then* DBU, 16 h.	No conversion, **11** recovered
Martin sulfurane, DCM, 0 ˚C, 16 h.	Degradation of starting material
Burgess reagent, THF, 0 ˚C, 16 h.	Degradation of starting material
NBS, PPh_3, Imidazole, THF, reflux, 16 h.	Small amounts of **12** + Degradation of starting material
TCCA, PPh_3, Imidazole, THF, reflux, 16 h.	Small amounts of **12** + Degradation of starting material
MTPI, 2,6-Lutidine, DMPU, 100 ˚C, 1.5 h.	Full conversion, 68% (mixture of **12** : **14**, 7:3)
MTPI, 2,6-Lutidine, DMF, 100 ˚C, 1.5 h.; *then*: *m*-CPBA, $NaHCO_3$, DCM, 0 ˚C, 3 h.	75%, clean **12**

Experimental

General procedures.

All chemicals were of commercial grade and were used as received unless stated otherwise. Solvents used in synthesis were dried and stored over 4Å molecular sieves. 2,6-Lutidine was stored over KOH pellets. Trifluoromethanesulfonic anhydride (Tf_2O) was distilled over P_2O_5 and stored at 3 °C under a nitrogen atmosphere. Deuterated chloroform was stored over activated 3 Å molecular rods (rods, size 1/16 in., Sigma Aldrich) and potassium carbonate. Flash column chromatography was performed on silica gel 60 Å (0.04 – 0.063 mm, Screening Devices B.V.). TLC analysis was performed on TLC Silica gel 60 (Kieselgel 60 F254, Merck) with UV detection (254 nm) and by spraying with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ (10 g/L) in 10% sulfuric acid in water followed by charring at ± 200 °C. TLC-MS analysis was performed on a Camag TLC-MS Interface coupled with an API165 (SCIEX) mass spectrometer (eluted with *tert*-butylmethylether/EtOAc/MeOH, 5/4/1, v/v/v +0.1% formic acid, flow rate 0.12 mL/min). High-resolution mass spectra (HRMS) were recorded on a Waters Synapt G2-Si (TOF) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV) and an internal lock mass LeuEnk ($M+H^+$ = 556.2771). ^1H and ^{13}C NMR spectra were recorded on a Bruker AV-400 NMR, a Bruker AV-500 NMR or a Bruker AV-850 NMR instrument. All samples were measured in $CDCl_3$, unless stated otherwise. Chemical shifts (δ) are given in ppm relative to tetramethyl silane as internal standard or the residual signal of the deuterated solvent. Coupling constants (*J*) are given in Hz. All given ^{13}C APT spectra are proton decoupled. NMR peak assignment was accomplished using COSY, HSQC. If necessary, additional NOESY, HMBC and HMBC-gated experiments were used to further elucidate structures. Stereochemical product ratios were based on integration of ^1H NMR (crude and purified). Proton and carbon numbering for NMR peak assignment was done as followed: numbering was done similarly to their glucose counterparts and not their respective nomenclature. Numbering starts at the 'anomeric' center and progresses similarly as their glucose counterpart. 'H-7' or 'C-7' is used where the intramolecular oxygen is replaced for the substituted carbon.

3-*O*-*Tert*-butyldimethylsilyl-4-*O*-(4-methoxybenzyl)-D-glucal (4).



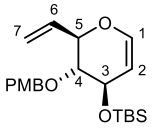
3,4,6-tri-*O*-acetyl-D-glucal (54 g, 0.20 mol) was dissolved in MeOH/Et_3N/H_2O (8:1:1, 1.0 L, 0.2 M) and stirred overnight at room temperature. Upon full conversion (R_f 0.5 (MeOH:DCM, 1:9 v:v)), The reaction mixture was concentrated under reduced pressure and co-evaporated with DMF (50 mL) twice.

The crude was dissolved in anhydrous DMF (0.29 L, 0.70 M). Anisaldehyde dimethyl acetal (44 mL, 0.26 mol, 1.3 eq.) and pyridinium *p*-toluene sulfonate (1.0 g, 4.0 mmol, 0.02 eq.) were added and the mixture was stirred for 1 hour under reduced pressure at 35 °C. Upon full conversion (R_f 0.3 (EtOAc:pentane, 2:8 v:v)), the reaction mixture was quenched by addition of imidazole (27 g, 0.40 mol, 2.0 eq.) and concentrated to 80% of its original volume to secure the removal of any remaining methanol.

The solution was diluted with anhydrous DMF (final concentration: 0.33 L, 0.60 M) followed by the addition of DMAP (2.4 g, 20 mmol, 0.1 eq.) and TBSCl (39 g, 0.26 mol, 1.3 eq.). The mixture was stirred for 1 hour at 35 °C (oil bath) after which full conversion was observed (R_f 0.7 (Toluene)). The reaction was quenched by addition of methanol (10 mL) followed by concentration to a fifth of its original volume. The mixture was diluted with water (1.0 L) and Et_2O (300 mL) and separated in a separation funnel. The aqueous layer was extracted two more times with Et_2O (200 mL), the combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (toluene:pentane, 1:1 v:v) to result in the desired intermediate contaminated with anisaldehyde dimethyl acetal.

This mixture was dissolved in anhydrous DCM (0.33 L, 0.6 M) and cooled to -15 °C. A 1 M solution of DIBAL-H in DCM (0.26 L, 0.26 mol, 1.3 eq.) was slowly added. Subsequently, the reaction mixture was allowed to attain room temperature. Upon stirring for an additional two hours TLC showed full conversion (R_f 0.7 (EtOAc:pentane, 3:7 v:v)). The solution was quenched by the dropwise addition of methanol (10 mL) followed by the addition of a saturated solution of Rochelle's salt (500 mL) and sat. aq. NaOH (100 mL), this was added whilst keeping the solution below 0 °C. Upon full addition, the solution was transferred to a separation funnel followed by separation of the organic layer. The aqueous layer was extracted two more times with EtOAc (200 mL), the combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (10:90 EtOAc:pentane → 20:80 EtOAc:pentane) to obtain the title compound as a colorless oil (39 g, 0.1 mol, 51% over four steps). Spectral data was in accordance with literature precedence.[19] ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.28 (d, *J* = 8.6 Hz, 2H, CH_{arom}), 6.89 (d, *J* = 8.7 Hz, 2H, CH_{arom}), 6.33 (dd, *J* = 6.1, 1.4 Hz, 1H, H-1), 4.77 (d, *J* = 11.1 Hz, 1H, C*H*H PMB), 4.67 (dd, *J* = 6.1, 2.9 Hz, 1H, H-2), 4.63 (d, *J* = 11.1 Hz, 1H, CH*H* PMB), 4.34 (dddd, *J* = 5.9, 2.9, 1.4, 0.6 Hz, 1H, H-3), 3.94 (ddd, *J* = 8.2, 4.3, 4.3 Hz, 1H, H-5), 3.81 (s, 5H, OMe, H-6), 3.62 (dd, *J* = 8.0, 5.8 Hz, 1H, H-4), 2.05 (dd, *J* = 6.8, 6.8 Hz, 1H, 6-OH), 0.92 (s, 9H, $C(CH_3)_3$), 0.12 (s, 3H, $SiCH_3$), 0.12 (s, 3H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 160.1 ($C_{q\text{-}arom}$), 143.6 (C-1), 130.2 ($C_{q\text{-}arom}$), 129.8 (CH_{arom}), 114.1 (CH_{arom}), 103.7 (C-2), 77.2 (C-5), 76.5 (C-4), 74.1 (CH_2 PMB), 68.7 (C-3), 62.1 (C-6), 55.4 (OMe), 26.0 ($C(CH_3)_3$), 25.9 ($C(CH_3)_3$), 18.1 ($C(CH_3)_3$), -4.2 ($SiCH_3$), -4.5 ($SiCH_3$); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{20}H_{32}NaO_5Si$ 403.1911; Found 403.1910.

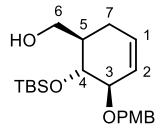
(2*R*,3*R*,4*R*)-3-*O*-(4-Methoxybenzyl)-4-*O*-*tert*-butyldimethylsilyl-2-vinyl-2,3-dihydro-2*H*-pyran (6).



Compound **4** (25 g, 67 mmol) was dissolved in DCM (0.33 L, 0.2 M) followed by the addition of $NaHCO_3$ (56 g, 0.67 mol, 10 eq.) and consecutively cooled on ice. Dess-Martin periodinane (42 g, 0.10 mol, 1.5 eq.) was added to the mixture after which the reaction was stirred for 2 hours while attaining to room temperature. Upon full conversion (R_f 0.1-0.6 (EtOAc:pentane, 3:7 v:v)), the mixture was quenched by the addition of a saturated aqueous $Na_2S_2O_3$ solution (100 mL). The mixture was filtered over celite and rinsed with water (100 mL) and DCM (100 mL) followed by transferring to a separation funnel. Additional 500 mL of water were added and the organic layer was separated. The aqueous layer was extracted two more times with EtOAc after which the combined organic layers were dried over $MgSO_4$, filtered over celite and concentrated under reduced pressure to yield the crude intermediate as a yellow oil. The crude intermediate was co-evaporated twice with toluene and dissolved in THF (70 mL).

Ph_3PCH_3Br (36 g, 0.10 mmol, 1.5 eq.) was dissolved in anhydrous THF (200 mL, 0.5 M) and cooled to -78 °C. *n*-BuLi (2.5 M in hexane, 38 mL, 94 mmol, 1.4 eq.) was added dropwise, stirring continued at 0 °C for 1 hour. Subsequently, the mixture was cooled back to -78 °C and the previously prepared aldehyde solution was added dropwise. This reaction mixture was allowed to attain to 0 °C and stirring continued for another hour. Upon full conversion (R_f 0.5 (toluene:pentane, 1:1 v:v)), the reaction was quenched by addition of sat. aq. $NaHCO_3$ (50 mL) and diluted with 500 mL of water and 100 mL of Et_2O. The organic layer was separated and the aqueous layer was extracted twice with 200 mL of Et_2O. The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (30:70 toluene:pentane → 90:10 toluene:pentane) to obtain the title compound as a colorless oil (20 g, 54 mmol, 80% over two steps). Spectral data was in accordance with literature precedence.[19] ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.27 – 7.25 (m, 2H, CH_{arom}), 6.87 (m, 2H, CH_{arom}), 6.33 (dd, *J* = 6.1, 1.5 Hz, 1H, H-1), 6.01 (ddd, *J* = 17.2, 10.5, 6.7 Hz, 1H, H-6), 5.40 (ddd, *J* = 17.2, 1.5, 1.5 Hz, 1H, H-7), 5.27 (ddd, *J* = 10.5, 1.6, 1.1 Hz, 1H, H-7), 4.70 (d, *J* = 10.7 Hz, 1H, C*H*H PMB), 4.67 (dd, *J* = 6.1, 2.6 Hz, 1H, H-2), 4.59 (d, *J* = 10.7 Hz, 1H, CH*H* PMB), 4.35 (ddd, *J* = 6.2, 2.6, 1.4 Hz, 1H, H-3), 4.28 (dddd, *J* = 9.2, 6.8, 1.2, 1.2 Hz, 1H, H-5), 3.80 (s, 3H, OMe), 3.42 (dd, *J* = 8.6, 6.2 Hz, 1H, H-4), 0.92 (s, 9H, $C(CH_3)_3$), 0.10 (s, 3H, $SiCH_3$), 0.10 (s, 3H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.4 ($C_{q\text{-}arom}$), 142.7 (C-1), 134.8 (C-6), 131.4 ($C_{q\text{-}arom}$), 129.8 (CH_{arom}), 118.2 (C-7), 115.3 (CH_{arom}), 104.1 (C-2), 80.2 (C-4), 78.3 (C-5), 74.0 (CH_2 PMB), 69.3 (C-3), 55.4 (OMe), 26.0 ($C(CH_3)_3$), 17.6 ($C(CH_3)_3$), -4.3 ($SiCH_3$), -4.4 ($SiCH_3$); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{21}H_{32}NaO_4Si$ 399.1968; Found 399.1959.

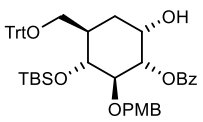
3-*O*-(4-Methoxybenzyl)-4-*O*-*tert*-butyldimethylsilyl-carba-D-glucal (8).



Compound **6** (20 g, 54 mmol) was dissolved in anhydrous diphenyl ether (2.5 M, 21 mL) under a N_2 atmosphere. The solution was heated to 210 °C (oil bath) and stirred for 2 hours. Upon full conversion (EtOAc:pentane, 1:9 v:v), the reaction mixture was transferred to a second flask containing a solution of $NaBH_4$ (3.0 g, 80 mmol, 1.5 eq.) in a 2:1 mixture of THF:EtOH (0.21 L, 0.25 M) and stirred at 0 °C. After stirring for 20 minutes, full conversion was observed (R_f 0.3 (EtOAc:pentane, 2:8 v:v)) and

the reaction was quenched using sat. aq. $NaHCO_3$ (50 mL), diluted with water (600 mL) and Et_2O (200 mL). The organic layer was separated and the aqueous layer extracted twice with Et_2O (200 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (10:90 Et_2O:pentane → 60:40 Et_2O:pentane) to obtain the title compound as a colorless oil (16 g, 43 mmol, 80% over two steps). ^1H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.31 – 7.20 (m, 2H, CH_{arom}), 6.87 (m, 2H, CH_{arom}), 5.78 (dddd, *J* = 10.1, 4.2, 3.1, 1.4 Hz, 1H, H-1), 5.68 (dddd, *J* = 10.1, 2.4, 2.4, 1.6 Hz, 1H, H-2), 4.58 (d, *J* = 11.2 Hz, 1H, C*H*H PMB), 4.49 (d, *J* = 11.2 Hz, 1H, CH*H* PMB), 3.88 – 3.81 (m, 2H, H-3, H-4), 3.80 (s, 3H, OMe), 3.73 (ddd, *J* = 11.0, 6.7, 4.3 Hz, 1H, H-6), 3.61 (ddd, *J* = 11.0, 7.6, 4.8 Hz, 1H, H-6), 2.42 (dd, *J* = 7.6, 4.4 Hz, 1H, 6-OH), 2.23 (ddddd, *J* = 17.8, 5.8, 3.9, 1.6, 1.6 Hz, 1H, H-7), 1.98 (ddddd, *J* = 7.8, 7.8, 6.2, 4.9, 4.9 Hz, 1H, H-5), 1.89 (dddd, *J* = 18.0, 5.4, 5.4, 2.7 Hz, 1H, H-7), 0.89 (s, 9H, $C(CH_3)_3$), 0.09 (s, 3H, $SiCH_3$), 0.07 (s, 3H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.2, 131.7 ($C_{q\text{-}arom}$), 129.4 (CH_{arom}), 128.5 (C-1), 125.1 (C-2), 113.8 (CH_{arom}), 78.9 (C-3), 73.7 (C-4), 70.8 (CH_2 PMB), 64.9 (C-6), 55.8 (OMe), 41.7 (C-5), 27.2 (C-7), 26.0 ($C(CH_3)_3$), 18.3 ($C(CH_3)_3$), -3.5 ($SiCH_3$), -4.7 ($SiCH_3$); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{21}H_{34}NaO_4Si$ 401.2124; Found 401.2116.

2-*O*-Benzoyl-3-*O*-(4-methoxybenzyl)-4-*O*-*tert*-butyldimethylsilyl-6-*O*-trityl-carba-α-D-glucose (11).



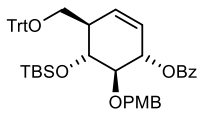
Compound **8** (16 g, 43 mmol) was dissolved in anhydrous DCM (0.22 L, 0.2 M) followed by the addition of Et_3N (30 mL, 0.22 mol, 5.0 eq.), DMAP (0.53 g, 4.3 mmol, 0.1 eq.) and TrtCl (18 g, 65 mmol, 1.5 eq.) and stirred overnight at room temperature. Upon full conversion (R_f 0.7 (Toluene)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ followed by diluting the reaction mixture with 500 mL of water. The organic layer was separated and the aqueous layer was extracted twice with 200 mL of Et_2O. The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated.

The crude was dissolved in an acetone/water mixture (6:1, 0.43 L, 0.1 M) followed by the addition of an aqueous NMO solution (50% w:w, 18 mL, 87 mmol, 2.0 eq.) and an aqueous osmium tetra oxide solution (2.5 % w/w, 18 mL, 1.7 mmol, 0.04 eq.). The reaction mixture was stirred overnight after which full conversion was observed (R_f 0.4 (EtOAc:pentane, 3:7 v:v)). The reaction was quenched by addition of sat. aq. $NaHCO_3$ (50 mL) and sat. aq. $Na_2S_2O_3$ (50 mL), This mixture was transferred to a separation funnel and diluted with water (1.0 L) and EtOAc (300 mL). The organic layer was separated and the aqueous layer was extracted twice with EtOAc (300 mL). The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated.

The crude was dissolved in pyridine (0.22 L, 0.2 M) and cooled to -15 °C. Subsequently, BzCl (7.5 mL, 65 mmol, 1.5 eq.) was added dropwise after which the reaction mixture was stirred for another 1.5 hours at this temperature. Upon full conversion (R_f 0.3 (Et_2O:pentane, 3:7 v:v)), the reaction was quenched by the addition of MeOH (10 mL) and allowed to attain to room temperature followed by diluting the reaction mixture with water (1.0 L) and Et_2O (300 mL). The organic layer was separated and the aqueous layer extracted twice with Et_2O (200 mL). The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (10:90 Et_2O:pentane → 50:50 Et_2O:pentane) to obtain the title compound as a white foam (26 g, 34 mmol, 79% over three steps).

^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 8.00 – 7.91 (m, 2H, CH_{arom}), 7.53 (dd, *J* = 7.8, 7.1 Hz, 1H, CH_{arom}), 7.46 – 7.35 (m, 8H, CH_{arom}), 7.28 – 7.18 (m, 9H, CH_{arom}), 7.04 – 6.97 (m, 2H, CH_{arom}), 6.64 – 6.57 (m, 2H, CH_{arom}), 5.10 (dd, *J* = 9.7, 3.0 Hz, 1H, H-2), 4.64 (d, *J* = 11.1 Hz, 1H, C*H*H PMB), 4.61 (d, *J* = 11.1 Hz, 1H, CH*H* PMB), 4.24 (dd, *J* = 3.3, 3.3 Hz, 1H, H-1), 3.91 (dd, *J* = 9.7, 8.3 Hz, 1H, H-3), 3.68 (s, 3H, OMe), 3.62 (dd, *J* = 8.4, 3.9 Hz, 1H, H-6), 3.32 (dd, *J* = 9.7, 8.3 Hz, 1H, H-4), 2.65 (dd, *J* = 9.8, 8.4 Hz, 1H, H-6), 2.52 (ddd, *J* = 14.2, 3.9 , 3.9 Hz, 1H, H-7), 2.49 – 2.42 (m, 1H, H-5), 2.08 (bs, 1H, 1-OH), 1.27 (ddd, *J* = 14.3, 11.9, 2.4 Hz, 1H, H-7), 0.70 (s, 9H, $C(CH_3)_3$), -0.14 (s, 3H, $SiCH_3$), -0.36 (s, 3H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 165.4 (C=O Bz), 158.2, 143.6 ($C_{q\text{-}arom}$), 133.3 (CH_{arom}), 130.8 ($C_{q\text{-}arom}$), 129.9, 129.8, 128.9, 128.5, 127.9, 127.0, 113.5 (CH_{arom}), 86.2 (CPh_3), 81.6 (C-3), 77.7 (C-2), 75.0 (CH_2 PMB), 75.0 (C-4), 67.7 (C-1), 65.8 (C-6), 55.2 (OMe), 38.8 (C-5), 31.1 (C-7), 26.1 ($C(CH_3)_3$), 18.0 ($C(CH_3)_3$), -3.5 ($SiCH_3$), -4.4 ($SiCH_3$); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{47}H_{54}NaO_7Si$ 781.3537; Found 781.3526.

2-*O*-Benzoyl-3-*O*-(4-methoxybenzyl)-4-*O*-*tert*-butyldimethylsilyl-6-*O*-trityl-cyclophellitol alkene (12).

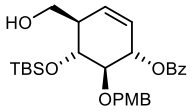


Compound **11** (0.94 g, 1.2 mmol) was dissolved in anhydrous DMF (12 mL, 0.1 M) followed by the addition of 2,6-lutidine (0.71 mL, 6.2 mmol, 5.0 eq.) and subsequently methyl triphenoxy phosphonium iodide (1.1 g, 2.5 mmol, 2.0 eq.). The reaction was kept under N_2 atmosphere while heating to 100 °C (oil bath), after 1.5 hours at this temperature the reaction was cooled to room temperature. Upon full conversion (R_f 0.5 (Et_2O:pentane, 2:8 v:v)), the reaction was quenched by the addition of sat. aq. NH_4Cl (10 mL) and sat. aq. $Na_2S_2O_3$ (5.0 mL) followed by diluting the reaction mixture with water (200 mL) and Et_2O (50 mL). The organic layer was separated and the aqueous layer was extracted twice with Et_2O (50 mL). The combined organic layers were washed with sat. aq. NH_4Cl before subsequent drying over $MgSO_4$, filtration over a glass filter and concentration.

The crude mixture was dissolved in anhydrous DCM (12 mL, 0.1 M), cooled on ice, followed by the addition of $NaHCO_3$ (1.0 g, 12 mmol, 10 eq.) and *m*-CPBA (1.4 g, 8.4 mmol, 7.0 eq.). This reaction mixture was stirred on ice for 4 hours after which full conversion was observed (R_f 0.55 (Et_2O:pentane, 2:8 v:v)). The reaction was quenched by addition of sat. aq. $NaHCO_3$ (5.0 mL) and sat. aq. $Na_2S_2O_3$ (5.0 mL), The mixture was transferred to a separation funnel and diluted with water (100 mL) and Et_2O (50 mL). The organic layer was separated and the aqueous layer was extracted twice with Et_2O (50 mL). The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (40:60 DCM:pentane was used to flush off methyl diphenyl phosphonate *then* 10:90 Et_2O:pentane) to obtain the title compound as a colorless oil (0.68 g, 0.92 mmol, 75% over two steps). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.98 – 7.86 (m, 2H, CH_{arom}), 7.62 – 7.05 (m, 20H, CH_{arom}), 6.73 – 7.55 (m, 2H, CH_{arom}), 6.16 – 6.02 (m, 1H, H-1), 5.67 – 5.62 (m, 2H, H-2, H-7), 4.66 (d, *J* = 11.4 Hz, 1H, C*H*H PMB), 4.61 (d, *J* = 11.4 Hz, 1H, CH*H* PMB), 3.79 (dd, *J* = 9.2, 7.3 Hz, 1H, H-3), 3.69 (s, 3H, OMe), 3.61 (dd, *J* = 9.2, 7.9 Hz, 1H, H-4), 3.57 (d, *J* = 3.6 Hz, 1H, H-6), 2.78 – 2.76 (m, 2H, H-5, H-6), 0.72 (s, 9H, $C(CH_3)_3$), -0.08 (s, 3H, $SiCH_3$), -0.31 (s, 3H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 166.2 (C=O Bz), 158.9, 143.7 ($C_{q\text{-}arom}$), 133.0 (CH_{arom}), 130.8 ($C_{q\text{-}arom}$), 130.6 (C-1), 130.3 ($C_{q\text{-}arom}$), 129.8, 129.4, 128.9, 128.4, 127.9, 127.1 (CH_{arom}), 124.9 (C-7), 114.2 (CH_{arom}), 86.8 (CPh_3), 82.1 (C-

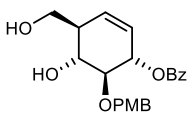
3), 75.6 (C-2), 74.4 (CH_2 PMB), 72.2 (C-4), 64.7 (C-6), 55.2 (OMe), 46.1 (C-5), 26.0 (C(CH_3)$_3$), 18.1 (*C*(CH_3)$_3$), -3.6 ($SiCH_3$), -4.7 ($SiCH_3$); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{47}H_{52}NaO_6Si$ 763.3431; Found 763.3423.

2-*O*-Benzoyl-3-*O*-(4-methoxybenzyl)-4-*O*-*tert*-butyldimethylsilyl-cyclophellitol alkene (17).



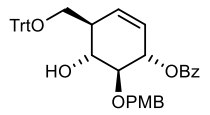
Compound **12** (0.74 g, 1.0 mmol) was dissolved in a 3:1 mixture of DCM and MeOH (5.0 mL, 0.2 M). subsequently, $ZnCl_2$ (1.4 g, 10 mmol, 10.0 eq.) was added and the reaction mixture was stirred overnight at room temperature. Upon full conversion (R_f 0.2 (EtOAc:pentane, 1:9 v:v)), the reaction was diluted with water (50 mL) and Et_2O (25 mL). The organic layer was separated and the aqueous layer was extracted twice with Et_2O (25 mL). The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (20:80 Et_2O:pentane → 30:70 Et_2O:pentane) to obtain the title compound as a colorless oil (0.37 g, 0.74 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 8.00 – 7.90 (m, 2H, CH_{arom}), 7.63 – 7.49 (m, 1H, CH_{arom}), 7.45 – 7.34 (m, 2H, CH_{arom}), 7.19 – 7.12 (m, 2H, CH_{arom}), 6.74 – 6.67 (m, 2H, CH_{arom}), 5.83 – 5.74 (m, 2H, H-1, H-7), 5.70 – 5.63 (m, 1H, H-2), 4.71 (s, 2H, CH_2 PMB), 3.90 (dd, *J* = 8.2, 6.9 Hz, 1H, H-4), 3.84 – 3.75 (m, 3H, H-3, H-6), 3.72 (s, 3H, OMe), 2.48 (dddd, *J* = 6.7, 4.1, 2.4, 2.4 Hz, 1H, H-5), 1.86 (bs, 1H, 6-OH), 0.88 (s, 9H, C(CH_3)$_3$), 0.06 (s, 3H, $SiCH_3$), -0.00 (s, 3H, $SiCH_3$); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 166.2 (C=O Bz), 133.1 (CH_{arom}), 130.9, 130.4 ($C_{q\text{-}arom}$), 130.3 (C-1), 130.1, 129.8, 129.6, 128.4 (CH_{arom}), 126.4 (C-7), 113.7 (CH_{arom}), 80.9 (C-3), 74.1 (CH_2 PMB), 73.9 (C-2), 71.4 (C-4), 62.8 (C-6), 55.3 (OMe), 47.4 (C-5), 26.1 (C(*C*H_3)$_3$), 18.3 (*C*(CH_3)$_3$), -3.7 ($SiCH_3$), -4.9 ($SiCH_3$); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{28}H_{38}NaO_6Si$ 521.2335; Found 521.2328.

2-*O*-Benzoyl-3-*O*-(4-methoxybenzyl)-cyclophellitol alkene (18).



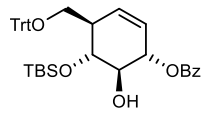
Compound **12** (0.37 g, 0.50 mmol) was dissolved in a 1:1 mixture of DCM and MeOH (10 mL, 0.05 M). subsequently, *p*-TsOH (0.43 g, 2.5 mmol, 5.0 eq.) was added and the reaction mixture was stirred overnight at room temperature. Upon full conversion (R_f 0.3 (EtOAc:pentane, 1:1 v:v)), the reaction was diluted with water (50 mL) and EtOAc (25 mL). The organic layer was separated and the aqueous layer was extracted twice with EtOAc (25 mL). The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (40:60 EtOAc:pentane → 60:40 EtOAc:pentane) to obtain the title compound as a colorless oil (0.12 g, 0.32 mmol, 84%). NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 8.08 – 7.97 (m, 2H, CH_{arom}), 7.64 – 7.52 (m, 1H, CH_{arom}), 7.50 – 7.41 (m, 2H, CH_{arom}), 7.23 – 7.12 (m, 2H, CH_{arom}), 6.85 – 6.66 (m, 2H, CH_{arom}), 5.77 – 5.71 (m, 1H, H-2), 5.67 (ddd, *J* = 10.1, 2.9, 2.2 Hz, 1H, H-7), 5.60 (ddd, *J* = 10.1, 1.9, 1.7 Hz, 1H, H-1), 4.75 (d, *J* = 11.0 Hz, 1H, C*H*H PMB), 4.63 (d, *J* = 11.0 Hz, 1H, CH*H* PMB), 3.90 – 3.69 (m, 7H, H-3, H-4, H-6, OMe), 3.23 (d, *J* = 1.7 Hz, 1H, 4-OH), 2.74 (s, 1H, 6-OH), 2.61 – 2.52 (m, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 166.1 (C=O Bz), 159.5 ($C_{q\text{-}arom}$), 133.3 (CH_{arom}), 130.0 ($C_{q\text{-}arom}$), 129.8 (CH_{arom}), 128.9 (C-1), 128.6 (CH_{arom}), 126.7 (C-7), 114.0 (CH_{arom}), 82.1 (C-3), 75.8 (C-2), 74.5 (CH_2 PMB), 72.7 (C-4), 65.1 (C-6), 55.3 (OMe), 45.3 (C-5); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{22}H_{24}NaO_6$ 407.1471; Found 407.1464.

2-*O*-Benzoyl-3-*O*-(4-methoxybenzyl)-6-*O*-trityl-cyclophellitol alkene (19).



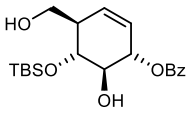
Compound **12** (0.37 g, 0.50 mmol) was dissolved in THF (2.5 mL, 0.2 M) followed by the addition of a 1 M solution of TBAF in THF (4.0 mL, 4.0 mmol, 8.0 eq.). The reaction was stirred for 1 hour at room temperature. Upon full conversion (R_f 0.3 (Et_2O:pentane, 3:7 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ (5.0 mL) followed by diluting the reaction mixture with water (50 mL) and Et_2O (25 mL). The organic layer was separated and the aqueous layer was extracted twice with Et_2O (25 mL). The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (5:95 Et_2O:pentane → 10:90 Et_2O:pentane) to obtain the title compound as a colorless oil (0.20 g, 0.32 mmol, 95%). NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 8.07 – 8.01 (m, 2H, CH_{arom}), 7.59 – 7.52 (m, 1H, CH_{arom}), 7.47 – 7.40 (m, 9H, CH_{arom}), 7.32 – 7.26 (m, 6H, CH_{arom}), 7.26 – 7.15 (m, 4H, CH_{arom}), 6.80 – 6.72 (m, 2H, CH_{arom}), 5.78 (dd, *J* = 7.9, 3.2 Hz, 1H, H-2), 5.69 – 5.60 (m, 2H, H-1, H-7), 4.76 (d, *J* = 11.0 Hz, 1H, C*H*H PMB), 4.67 (d, *J* = 11.0 Hz, 1H, CH*H* PMB), 3.92 (dd, *J* = 9.5, 9.5 Hz, 1H, H-4), 3.84 (dd, *J* = 10.1, 7.8 Hz, 1H, H-3), 3.71 (s, 3H, OMe), 3.34 (dd, *J* = 8.8, 5.2 Hz, 1H, H-6), 3.28 (dd, *J* = 8.8, 5.0 Hz, 1H, H-6), 2.92 (bs, 1H, 4-OH), 2.59 (dddd, *J* = 9.3, 4.5, 4.5, 4.5 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 166.1 (C=O Bz), 159.4, 144.0 ($C_{q\text{-}arom}$), 133.2 (CH_{arom}), 130.3 ($C_{q\text{-}arom}$), 130.2 (C-1), 130.2 ($C_{q\text{-}arom}$), 129.8, 129.8, 128.8, 128.5, 128.0, 127.1 (CH_{arom}), 125.7 (C-7), 113.8 (CH_{arom}), 86.8 (CPh_3), 82.4 (C-3), 75.8 (C-2), 74.6 (CH_2 PMB), 71.3 (C-4), 63.9 (C-6), 55.3 (OMe), 44.2 (C-5); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{41}H_{38}NaO_6$ 649.2566; Found 649.2562.

2-*O*-Benzoyl-4-*O*-*tert*-butyldimethylsilyl-6-*O*-trityl-cyclophellitol alkene (20).



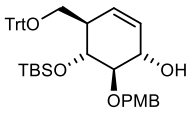
Compound **12** (0.37 g, 0.50 mmol) was dissolved in DCM (10 mL, 0.05 M) followed by the addition of a pH 7.4 aq. phosphate buffer (5.0 mL, 0.1 M). The solution was cooled on ice and subsequently DDQ (0.68 g, 3.0 mmol, 6.0 eq.) was added. The reaction was stirred for 1 hour while attaining to room temperature. Upon full conversion (R_f 0.6 (Et_2O:pentane, 2:8 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ (5.0 mL) and sat. aq. $Na_2S_2O_3$ (5.0 mL) followed by diluting the reaction mixture with water (50 mL) and Et_2O (25 mL). The organic layer was separated and the aqueous layer was extracted twice with Et_2O (25 mL). The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (20:80 Et_2O:pentane → 50:50 Et_2O:pentane) to obtain the title compound as a colorless oil (0.21 g, 0.35 mmol, 91%). NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 8.30 – 8.20 (m, 2H, CH_{arom}), 7.76 – 7.67 (m, 1H, CH_{arom}), 7.66 – 7.56 (m, 9H, CH_{arom}), 7.52 – 7.37 (m, 8H, CH_{arom}), 6.26 (ddd, *J* = 10.2, 2.1, 2.1 Hz, 1H, H-1), 5.86 (ddd, *J* = 10.2, 2.5, 2.5 Hz, 1H, H-7), 5.80 (ddd, *J* = 8.1, 3.5, 2.0 Hz, 1H, H-2), 4.15 – 4.05 (m, 1H, H-3), 3.80 – 3.71 (m, 2H, H-4, H-6), 3.14 – 3.01 (m, 1H, H-6), 2.86 (dddd, *J* = 10.4, 7.5, 3.6, 3.6 Hz, 1H, H-5), 2.74 (d, *J* = 2.8 Hz, 1H, 3-OH), 0.91 (s, 9H, $C(CH_3)_3$), 0.20 (s, 3H, $SiCH_3$), -0.08 (s, 3H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 166.9 (C=O Bz), 144.1 ($C_{q\text{-}arom}$), 133.2 (CH_{arom}), 130.9 (C-1), 130.1 ($C_{q\text{-}arom}$), 129.9, 128.8, 128.5, 128.0, 128.0, 127.9, 127.3, 127.2 (CH_{arom}), 124.8 (C-7), 86.8 (CPh_3), 75.8 (C-2), 75.6 (C-3), 73.1 (C-4), 64.3 (C-6), 45.4 (C-5), 26.0 ($C(CH_3)_3$), 18.2 ($C(CH_3)_3$), -3.7 ($SiCH_3$), -4.7 ($SiCH_3$); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{39}H_{44}NaO_5Si$ 643.2856; Found 643.2847.

2-*O*-Benzoyl-4-*O*-*tert*-butyldimethylsilyl-cyclophellitol alkene (21).



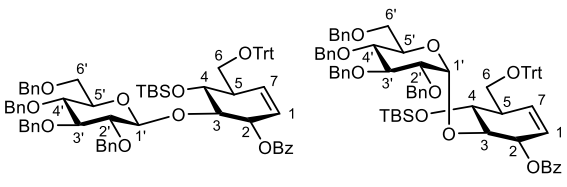
Compound **12** (0.37 g, 0.50 mmol) was dissolved in anhydrous DCM (10 mL, 0.05 M) followed by the addition of TES (0.24 mL, 1.5 mmol, 3.0 eq.). The mixture was cooled on ice after which TFA (0.62 mL, 8.0 mmol, 16 eq.) was added, the solution was stirred for 1 hour while attaining to room temperature. Upon full conversion (R_f 0.5 (Et_2O:pentane, 6:4 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ (5.0 mL) followed by diluting the reaction mixture with water (50 mL) and Et_2O (25 mL). The organic layer was separated and the aqueous layer was extracted twice with Et_2O (25 mL). The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (20:80 Et_2O:pentane → 40:60 Et_2O:pentane) to obtain the title compound as a colorless oil (0.13 g, 0.35 mmol, 84%). NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 8.10 – 8.03 (m, 2H, CH_{arom}), 7.59 – 7.52 (m, 1H, CH_{arom}), 7.46 – 7.40 (m, 2H, CH_{arom}), 5.77 (ddd, *J* = 10.2, 1.5, 1.4 Hz, 1H, H-1/H-7), 5.73 (ddd, *J* = 10.2, 2.0, 1.8 Hz, 1H, H-1/H-7), 5.61 (dddd, *J* = 7.7, 2.9, 1.6, 1.5 Hz, 1H, H-2), 3.93 (dd, *J* = 9.5, 7.7 Hz, 1H, H-3), 3.88 – 3.81 (m, 2H, H-4, H-6), 3.75 (dd, *J* = 10.5, 4.8 Hz, 1H, H-6), 2.76 (s, 1H, 3-OH/6-OH), 2.43 (dddd, *J* = 9.8, 4.9, 2.3, 1.0 Hz, 1H, H-5), 1.86 (s, 1H, 3-OH/6-OH), 0.92 (s, 9H, $C(CH_3)_3$), 0.17 (s, 3H, $SiCH_3$), 0.16 (s, 3H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 166.9 (C=O Bz), 133.3 (CH_{arom}), 130.3 (C-1/C-7), 130.1 ($C_{q\text{-}arom}$), 129.9, 128.5 (CH_{arom}), 126.6 (C-1/C-7), 75.8 (C-2), 75.4 (C-3), 72.1 (C-4), 62.3 (C-6), 46.9 (C-5), 26.1 ($C(CH_3)_3$), 18.4 ($C(CH_3)_3$), -3.7 ($SiCH_3$), -4.6 ($SiCH_3$); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{20}H_{30}NaO_5Si$ 401.1760; Found 401.1752.

3-*O*-(4-Methoxybenzyl)-4-*O*-*tert*-butyldimethylsilyl-6-*O*-trityl-cyclophellitol alkene (22).



Compound **12** (2.2 g, 3.0 mmol) was dissolved in a 1:1 mixture of DCM and MeOH (60 mL, 0.05 M) followed by the addition of NaOMe (0.78 g, 12 mmol, 4.0 eq.). The reaction was stirred overnight at room temperature. Upon full conversion (R_f 0.3 (EtOAc:pentane, 1:9 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ (5.0 mL) followed by diluting the reaction mixture with water (200 mL) and Et_2O (50 mL). The organic layer was separated and the aqueous layer was extracted twice with Et_2O (50 mL). The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (10:90 Et_2O:pentane → 30:70 Et_2O:pentane) to obtain the title compound as a colorless oil (1.2 g, 1.9 mmol, 86%). NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.50 – 7.34 (m, 6H, CH_{arom}), 7.33 – 7.07 (m, 11H, CH_{arom}), 6.86 – 6.79 (m, 2H, CH_{arom}), 5.84 (ddd, *J* = 10.2, 3.3, 1.2 Hz, 1H, H-7), 5.77 (ddd, *J* = 10.2, 3.5, 2.1 Hz, 1H, H-1), 4.50 (d, *J* = 12.0 Hz, 1H, C*H*H PMB), 4.41 (d, *J* = 12.0 Hz, 1H, CH*H* PMB), 4.05 – 3.89 (m, 2H, H-2, H-4), 3.80 (s, 3H, OMe), 3.53 (dd, *J* = 6.5, 4.6 Hz, 1H, H-3), 3.35 (dd, *J* = 8.6, 6.9 Hz, 1H, H-6), 2.99 (dd, *J* = 8.6, 7.7 Hz, 1H, H-6), 2.67 – 2.57 (m, 1H, H-5), 2.46 (d, *J* = 7.9 Hz, 1H, 2-OH), 0.80 (s, 9H, $C(CH_3)_3$), 0.04 (s, 3H, $SiCH_3$), -0.09 (s, 3H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.4, 144.3, 130.7 ($C_{q\text{-}arom}$), 129.4, 128.9, 127.9 (CH_{arom}), 127.8 (C-1), 127.8 (C-7), 127.1, 114.0 (CH_{arom}), 86.8 (CPh_3), 81.1 (C-3), 73.0 (CH_2 PMB), 70.1 (C-4), 68.9 (C-2), 65.0 (C-6), 55.4 (OMe), 45.4 (C-5), 26.0 ($C(CH_3)_3$), 18.1 ($C(CH_3)_3$), -4.3 ($SiCH_3$), -4.7 ($SiCH_3$); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{40}H_{48}NaO_5Si$ 659.3169; Found 659.3157.

3-*O*-(Perbenzyl-α-D-glucopyranosyl)-2-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-6-*O*-trityl-cyclophellitol alkene (24a) and 3-*O*-(perbenzyl-β-D-glucopyranosyl)-2-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-6-*O*-trityl-cyclophellitol alkene (24b).



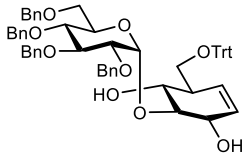
To perbenzylated donor **23** (1.2 g, 2.1 mmol, 2.0 eq.), synthesized according to literature precedence [30], was added TTBP (2.0 g, 8.0 mmol, 7.5 eq.) and Ph_2SO (0.56 g, 2.8 mmol, 2.6 eq.) and co-evaporated twice with toluene. This mixture was subsequently dissolved in anhydrous DCM (20 mL, 0.05 M) followed by the addition of activated 3Å molecular rods and stirred under inert atmosphere. The solution was cooled to -78 °C after which Tf_2O (0.39 mL, 2.3 mmol, 2.2 eq.) was added, the reaction mixture was allowed to warm to -60 °C and stirring continued at this temperature for 15 minutes. Subsequently, the solution was cooled back to -78 °C and compound **20** (0.66 g, 1.1 mmol, 1.0 eq.) dissolved in 2 mL of anhydrous DCM was added dropwise. The reaction was allowed to warm to -10 °C over the course of 2 hours after which full conversion was observed (R_f 0.3 and 0.5 for **24a** and **24b** respectively (Et_2O:pentane, 2:8 v:v)). The reaction was quenched by the addition of sat. aq. $NaHCO_3$ (5.0 mL) followed by diluting the reaction mixture with water (200 mL) and Et_2O (50 mL). The organic layer was separated and the aqueous layer was extracted twice with Et_2O (50 mL). The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (10:90 Et_2O:pentane → 20:80 Et_2O:pentane) to obtain the title compounds as colorless oils (0.85 g, 0.74 mmol for **24a** and 0.21 g, 0.19 mmol for **24b**, α:β ratio; 4:1 with an overall yield of 88%).

Analytical data for **24a**: NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 8.05 – 7.92 (m, 2H, CH_{arom}), 7.55 – 7.08 (m, 38H, CH_{arom}), 6.22 (dd, *J* = 10.1, 3.8 Hz, 1H, H-7), 5.79 (ddd, *J* = 9.9, 3.7, 1.8 Hz, 1H, H-1), 5.49 (ddd, *J* = 3.2, 3.0, 1.6 Hz, 1H, H-2), 4.93 (d, *J* = 3.7 Hz, 1H, H-1'), 4.76 (d, *J* = 10.8 Hz, 1H, C*H*H Bn), 4.72 (d, *J* = 10.9 Hz, 1H, C*H*H Bn), 4.61 (d, *J* = 12.4 Hz, 1H, C*H*H Bn), 4.58 (d, *J* = 11.0 Hz, 1H, C*H*H Bn), 4.56-4.43 (m, 2H, CH*H* Bn, CH*H* Bn), 4.47 (d, *J* = 10.8 Hz, 1H, CH*H* Bn), 4.34 (d, *J* = 12.0 Hz, 1H, CH*H* Bn), 3.92 (dd, *J* = 4.9, 2.9 Hz, 1H, H-3), 3.79 (dd, *J* = 4.9, 3.4 Hz, 1H, H-4), 3.77 – 3.59 (m, 5H, H-3', H-4', H-5', H-6'), 3.48 (dd, *J* = 14.0, 7.0 Hz, 1H, H-6), 3.41 (dd, *J* = 9.7, 3.7 Hz, 1H, H-2'), 3.31 (dd, *J* = 9.3, 9.1 Hz, 1H, H-6), 2.54 (dddd, *J* = 7.4, 6.0, 3.6, 1.4 Hz, 1H, H-5), 0.75 (s, 9H, $C(CH_3)_3$), -0.03 (s, 3H, $SiCH_3$), -0.11 (s, 3H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 166.1 (C=O Bz), 144.5, 139.2, 138.7, 138.4 ($C_{q\text{-}arom}$), 132.9 (CH_{arom}), 132.0 (C-7), 130.6 ($C_{q\text{-}arom}$), 129.9, 128.9, 128.5, 128.5, 128.4, 128.4, 128.3, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.7, 127.6, 127.1 ($C_{q\text{-}arom}$), 122.4 (C-1), 99.0 (C-1'), 87.0 (CPh_3), 82.2 (C-3'/C-4'/C-5'), 80.5 (C-3), 79.7 (C-2'), 77.4 (C-3'/C-4'/C-5'), 75.6, 75.2, 74.0, 73.1 (CH_2 Bn), 71.5 (C-3'/C-4'/C-5'), 70.5 (C-2), 69.2 (C-4), 68.3 (C-6'), 65.7 (C-6), 44.4 (C-5), 26.0 ($C(CH_3)_3$), 18.2 ($C(CH_3)_3$), -3.9 ($SiCH_3$), -4.7 ($SiCH_3$); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{73}H_{78}NaO_{10}Si$ 1165.5262; Found 1164.5253.

Analytical data for **24b**: NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 8.03 (d, *J* = 8.4 Hz, 1H, CH_{arom}), 7.63 – 7.07 (m, 39H, CH_{arom}), 6.16 (ddd, *J* = 10.3, 3.1, 1.4 Hz, 1H, H-7), 5.74 (ddd, *J* = 10.3, 2.8, 2.1 Hz, 1H, H-1), 5.57 (ddd, *J* = 5.4, 3.5, 1.7 Hz, 1H, H-2), 4.84 – 4.76 (m, 2H, H-1', C*H*H Bn), 4.71 (d, *J* = 11.0 Hz, 1H, C*H*H Bn), 4.67 – 4.60 (m, 2H, C*H*H Bn, C*H*H Bn), 4.59 – 4.51 (m, 3H, CH*H* Bn, CH*H* Bn, CH*H* Bn), 4.38 (d, *J* = 11.2, Hz, 1H, CH*H* Bn), 4.22 (ddd, *J* = 7.1, 5.5, 1.4 Hz, 1H, H-3), 3.84 (ddd, *J* =

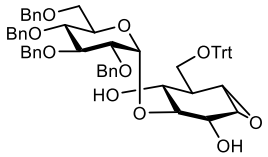
7.3, 5.8, 1.4 Hz, 1H, H-4), 3.78 (ddd, *J* = 10.8, 1.6, 1.6 Hz, 1H, H-6'), 3.68 (ddd, *J* = 10.7, 5.0, 1.2 Hz, 1H, H-6'), 3.58 – 3.40 (m, 4H, H-6, H-3', H-4', H-5'), 3.23 (ddd, *J* = 9.2, 7.9, 1.3 Hz, 1H, H-2'), 2.93 (ddd, *J* = 8.7, 8.7, 1.4 Hz, 1H, H-6), 2.83 – 2.76 (m, 1H, H-5), 0.76 (s, 9H, $C(CH_3)_3$), 0.04 (s, 3H, $SiCH_3$), -0.13 (s, 3H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 166.1 (C=O Bz), 144.2, 138.8, 138.7, 138.5, 138.3 ($C_{q\text{-}arom}$), 133.0 (CH_{arom}), 131.1 (C-7), 130.4 ($C_{q\text{-}arom}$), 129.8, 128.9, 128.5, 128.4, 128.4, 128.2, 128.1, 128.1, 127.9, 127.7, 127.6, 127.5, 127.1 (CH_{arom}), 123.7 (C-1), 102.8 (C-1'), 86.8 (CPh_3), 84.9 (C-3'), 82.2 (C-2'), 78.2 (C-4'), 78.0 (C-3), 75.7, 75.1 (CH_2 Bn), 75.1 (C-5'), 74.8 (CH_2 Bn), 73.6 (C-2), 73.6 (CH_2 Bn), 69.9 (C-4), 69.5 (C-6'), 65.1 (C-6), 45.4 (C-5), 26.1 ($C(CH_3)_3$), 18.2 ($C(CH_3)_3$), -3.3 ($SiCH_3$), -5.2 ($SiCH_3$); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{73}H_{78}NaO_{10}Si$ 1165.5262; Found 1164.5254.

3-*O*-(Perbenzyl-α-D-glucopyranosyl)-6-*O*-trityl-cyclophellitol alkene (25).



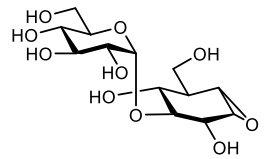
Compound **24a** (0.23 g, 0.20 mmol) was dissolved in THF (4.0 mL, 0.05 M) after which a 1 M TBAF solution in THF (0.60 mL, 0.60 mmol, 3.0 eq.) was added. The reaction mixture was stirred for 1 hour at room temperature. Upon full conversion (R_f 0.2 (Et_2O:pentane, 3:7 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ (5.0 mL) followed by diluting the reaction mixture with water (50 mL) and Et_2O (25 mL). The organic layer was separated and the aqueous layer was extracted twice with Et_2O (25 mL). The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was dissolved in a 1:1 mixture of DCM and MeOH (4.0 mL, 0.05 M) followed by the addition of NaOMe (0.26 g, 4.0 mmol, 20 eq.). The reaction was stirred overnight at room temperature. Upon full conversion (R_f 0.2 (Et_2O:pentane, 4:6 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ (5.0 mL) followed by diluting the reaction mixture with water (50 mL) and Et_2O (25 mL). The organic layer was separated and the aqueous layer was extracted twice with Et_2O (25 mL). The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (30:70 Et_2O:pentane → 40:60 Et_2O:pentane) to obtain the title compound as a colorless oil (0.12 g, 0.13 mmol, 66% over two steps). NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.49 – 7.41 (m, 6H, CH_{arom}), 7.36 – 7.18 (m, 27H, CH_{arom}), 7.18 – 7.09 (m, 2H, CH_{arom}), 5.67 (ddd, *J* = 10.2, 2.0, 2.0 Hz, 1H, H-1), 5.60 (ddd, *J* = 10.2, 2.6, 2.6 Hz, 1H, H-7), 4.93 (d, *J* = 10.9 Hz, 1H, C*H*H Bn), 4.87 – 4.80 (m, 3H, H-1', C*H*H Bn, C*H*H Bn), 4.77 (d, *J* = 11.8 Hz, 1H, C*H*H Bn), 4.68 (d, *J* = 11.8 Hz, 1H, CH*H* Bn), 4.56 (d, *J* = 12.4 Hz, 1H, CH*H* Bn), 4.50 (d, *J* = 12.4 Hz, 1H, CH*H* Bn), 4.46 (d, *J* = 10.9 Hz, 1H, CH*H* Bn), 4.26 – 4.19 (m, 1H, H-2), 4.07 (ddd, *J* = 10.2, 6.5, 1.9 Hz, 1H, H-5'), 4.04 – 3.98 (m, 1H, H-3'), 3.83 (dd, *J* = 9.9, 9.7 Hz, 1H, H-4), 3.75 – 3.71 (m, 2H, 2-OH, 4-OH), 3.64 (dd, *J* = 10.1, 2.0 Hz, 1H, H-6'), 3.58 (dd, *J* = 9.6, 3.8 Hz, 1H, H-2'), 3.50 (dd, *J* = 10.1, 6.6 Hz, 1H, H-6'), 3.44 (dd, *J* = 10.2, 8.9 Hz, 1H, H-4'), 3.40 – 3.34 (m, 2H, H-3, H-6), 3.25 (dd, *J* = 8.7, 6.0 Hz, 1H, H-6), 2.52 (dddd, *J* = 5.7, 5.7, 5.7, 2.6 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 144.3, 138.6, 137.9, 137.5 ($C_{q\text{-}arom}$), 129.0 (CH_{arom}), 128.9 (C-1), 128.7, 128.6, 128.5, 128.3, 128.1, 128.0 (CH_{arom}), 127.9 (C-7), 127.9, 127.0 (CH_{arom}), 100.1 (C-1'), 91.6 (C-3), 86.5 (CPh_3), 82.3 (C-3'), 79.7 (C-2'), 78.1 (C-4'), 75.9, 75.2, 74.1, 73.6 (CH_2 Bn), 71.4 (C-5'), 71.1 (C-2), 69.7 (C-4), 68.7 (C-6'), 63.4 (C-6), 44.2 (C-5); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{60}H_{60}NaO_9$ 947.4135; Found 947.4130.

3-*O*-(Perbenzyl-α-D-glucopyranosyl)-6-*O*-trityl-1,7-*epi*-cyclophellitol (26).



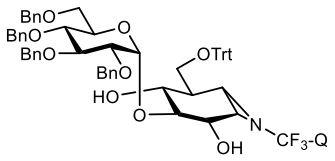
Compound **25** (58 mg, 56 µmol) was dissolved in anhydrous DCM (1.2 mL, 0.05 M). $NaHCO_3$ (24 mg, 0.28 mmol, 5.0 eq.) and *m*-CPBA (29 mg, 0.17 mmol, 3.0 eq.) were subsequently added. The mixture was stirred overnight at room temperature. Upon full conversion (R_f 0.4 (Et_2O:pentane, 6:4 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ (5.0 mL) and sat. aq. $Na_2S_2O_3$ (5.0 mL) followed by diluting the reaction mixture with water (25 mL) and Et_2O (25 mL). The organic layer was separated and the aqueous layer was extracted twice with Et_2O (25 mL). The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (50:50 Et_2O:pentane → 70:30 Et_2O:pentane) to obtain the title compound as a colorless oil (42 mg, 39 µmol, 70%). NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.48 – 7.40 (m, 6H, CH_{arom}), 7.36 – 7.21 (m, 27H, CH_{arom}), 7.16 – 7.10 (m, 2H, CH_{arom}), 4.92 (d, *J* = 10.9 Hz, 1H, C*H*H Bn), 4.85 (d, *J* = 10.9 Hz, 1H, CH*H* Bn), 4.81 (d, *J* = 10.9 Hz, 1H, C*H*H Bn), 4.76 (d, *J* = 11.8 Hz, 1H, C*H*H Bn), 4.76 (d, *J* = 3.9 Hz, 1H, H-1'), 4.66 (d, *J* = 11.8 Hz, 1H, CH*H* Bn), 4.57 (d, *J* = 12.4 Hz, 1H, C*H*H Bn), 4.51 (d, *J* = 12.4 Hz, 1H, CH*H* Bn), 4.47 (d, *J* = 10.9 Hz, 1H, CH*H* Bn), 4.05 (ddd, *J* = 10.3, 6.1, 2.0 Hz, 1H, H-5'), 3.98 (dd, *J* = 9.7, 8.9 Hz, 1H, H-3'), 3.90 (ddd, *J* = 8.1, 3.7, 2.0 Hz, 1H, H-2), 3.81 (s, 1H, 2-OH), 3.70 – 3.60 (m, 3H, H-4, 4-OH, H-6'), 3.56 (dd, *J* = 9.7, 3.8 Hz, 1H, H-6'), 3.52 (dd, *J* = 10.2, 6.1 Hz, 1H, H-2'), 3.46 (dd, *J* = 10.1, 8.9 Hz, 1H, H-4'), 3.42 – 3.34 (m, 4H, H-1, H-3, H-6), 3.10 (d, *J* = 3.9 Hz, 1H, H-7), 2.24 (ddd, *J* = 9.4, 4.3, 4.1 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 144.1, 138.6, 138.0, 137.6, 137.4 ($C_{q\text{-}arom}$), 128.8, 128.8, 128.6, 128.6, 128.6, 128.4, 128.1, 128.0, 128.0, 127.9, 127.2 (CH_{arom}), 100.5 (C-1'), 88.2 (C-3), 86.8 (CPh_3), 82.4 (C-3'), 79.5 (C-2'), 78.0 (C-4'), 75.9, 75.3, 74.3, 73.7 (CH_2 Bn), 71.5 (C-5'), 71.0 (C-2), 69.3 (C-4), 68.6 (C-6'), 62.1 (C-6), 56.7 (C-1), 54.8 (C-7), 42.7 (C-5); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{60}H_{60}NaO_{10}$ 963.4084; Found 963.4079.

3-*O*-(α-D-Glucopyranosyl)-1,7-*epi*-cyclophellitol (28).



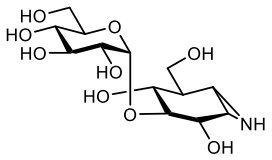
To liquid ammonia (3 mL) at -60 °C, sodium metal (51 mg, 2.2 mmol, 50 eq.) was added. This mixture was stirred for 30 minutes while maintaining a temperature of -60 °C. Subsequently, Compound **26** (42 mg, 44 µmol) was dissolved in THF (1.0 mL) followed by the addition *t*-BuOH (42 µL, 0.44 mmol, 10 eq.). This solution was added dropwise to the flask containing ammonia. The solution was stirred for 1 hour while maintaining a temperature of -60 °C. The reaction was quenched by addition of water (500 µL) and let to attain to room temperature. Upon concentration under reduced pressure, the residue was purified by size exclusion chromatography over HW-40 eluted with water to obtain the title compound as a colorless oil (11 mg, 31 µmol, 71%). NMR (500 MHz, MeOD, HH-COSY, HSQC, HMBC, NOESY): δ 4.99 (d, *J* = 3.9 Hz, 1H, H-1'), 3.93 – 3.79 (m, 4H, H-2, H-6, H-3', H-6'), 3.71 (dd, *J* = 10.9, 6.2 Hz, 1H, H-6), 3.69 – 3.60 (m, 2H, H-4', H-6'), 3.51 – 3.39 (m, 3H, H-3, H-4, H-2'), 3.32 – 3.28 (m, 2H, H-1, H-5'), 3.20 (d, *J* = 4.0 Hz, 1H, H-7), 1.98 (ddd, *J* = 9.6, 6.2, 3.4 Hz, 1H, H-5); ^{13}C NMR (126 MHz, MeOD, HSQC, HMBC): δ 102.7 (C-1'), 86.1 (C-3), 75.3 (C-4'), 74.1 (C-3'), 74.0 (C-2'), 71.8 (C-2), 71.5 (C-5'), 71.2 (C-4), 62.5 (C-6'), 61.8 (C-6), 58.5 (C-1), 55.4 (C-7), 46.0 (C-5); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{13}H_{22}NaO_{10}$ 361.1111; Found 361.1104.

3-*O*-(Perbenzyl-α-D-glucopyranosyl)-6-*O*-trityl-1,7-*epi*-cyclophellitol CF_3-Q-aziridine (27).



BAIB (81 mg, 0.25 mmol, 5.0 eq.) was dissolved in anhydrous DCM (1.5 mL, 0.17 M) and cooled to -80 °C. To this, a solution of 2-trifluoromethyl-3-aminoquinazolin-4-one (57 mg, 0.25 mmol, 5.0 eq.) in anhydrous DCM (3 mL, 0.086 M) was added dropwise over the course of 30 minutes. Afterwards, the solution was allowed to warm to -40 °C followed by the dropwise addition of a solution of compound **25** (47 mg, 50 µmol) in DCM (1.0 mL, 0.05 M) over the course of 15 minutes. The reaction was allowed to attain to room temperature and stirred for another 48 hours. Upon full conversion (R_f 0.6 (Et_2O:pentane, 1:1 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ (5.0 mL) and sat. aq. $Na_2S_2O_3$ (5.0 mL) followed by diluting the reaction mixture with water (50 mL) and Et_2O (25 mL). The organic layer was separated and the aqueous layer was extracted twice with Et_2O (25 mL). The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (30:70 Et_2O:pentane → 40:60 Et_2O:pentane) to obtain the title compound as a colorless oil (44 mg, 38 µmol, 76%). NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC, NOESY): δ 8.26 – 7.14 (m, 1H, CH_{arom}), 7.86 – 7.74 (m, 2H, CH_{arom}), 7.59 (dd, *J* = 8.2, 4.8 Hz, 1H, CH_{arom}), 7.50 – 7.09 (m, 35H, CH_{arom}), 4.95 (d, *J* = 10.9 Hz, 1H, C*H*H Bn), 4.89 (d, *J* = 10.9 Hz, 1H, CH*H* Bn), 4.85 – 4.79 (m, 3H, H-1', C*H*H Bn, C*H*H Bn), 4.68 (d, *J* = 11.7 Hz, 1H, CH*H* Bn), 4.58 (d, *J* = 12.4 Hz, 1H, C*H*H Bn), 4.51 – 4.44 (m, 2H, CH*H* Bn, CH*H* Bn), 4.32 (dd, *J* = 7.4, 3.7 Hz, 1H, H-1), 4.13 (ddd, *J* = 10.2, 4.2, 2.8 Hz, 1H, H-5'), 4.03 (dd, *J* = 9.3, 9.3 Hz, 1H, H-3'), 3.95 – 3.89 (m, 2H, H-2, 2-OH/4-OH), 3.88 – 3.83 (m, 2H, H-7, 2-OH/4-OH), 3.71 (dd, *J* = 10.0, 9.8 Hz, 1H, H-4), 3.66 – 3.62 (m, 2H, H-6'), 3.62 – 3.55 (m, 2H, H-2', H-4'), 3.52 (dd, *J* = 9.2, 4.7 Hz, 1H, H-6), 3.46 (dd, *J* = 10.0, 8.5 Hz, 1H, H-3), 3.36 (dd, *J* = 9.2, 3.3 Hz, 1H, H-6), 2.31 (dd, *J* = 9.4, 4.5 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 161.0 (C=O Bz), 144.1, 138.7, 138.2, 137.8, 137.5 ($C_{q\text{-}arom}$), 135.0, 129.4, 128.9, 128.8, 128.7, 128.6, 128.5, 128.5, 128.4, 128.1, 128.0, 127.9, 127.9, 127.9, 127.8, 127.1, 126.7 (CH_{arom}), 123.2 ($C_{q\text{-}arom}$), 100.7 (C-1'), 87.6 (C-3), 86.8 (CPh_3), 82.4 (C-3'), 79.7 (C-2'), 78.0 (C-4'), 75.8, 75.2, 74.5, 73.6 (CH_2 Bn), 71.3 (C-5'), 70.1 (C-2), 69.9 (C-4), 68.5 (C-6'), 62.6 (C-6), 43.6 (C-1), 42.5 (C-5), 41.9 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{69}H_{64}F_3N_3NaO_{10}$ 1174.4441; Found 1174.4435.

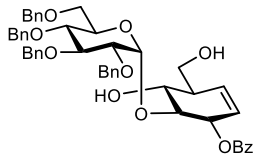
3-*O*-(α-D-Glucopyranosyl)-1,7-*epi*-cyclophellitol aziridine (29).



To liquid ammonia (3 mL) at -60 °C, sodium metal (52 mg, 2.2 mmol, 80 eq.) was added. This mixture was stirred for 30 minutes while maintaining a temperature of -60 °C. Subsequently, Compound **27** (32 mg, 28 µmol) was dissolved in THF (1.0 mL) followed by the addition *t*-BuOH (27 µL, 0.28 mmol, 10 eq.). This solution was added dropwise to the flask containing ammonia. The solution was stirred for 1 hour while maintaining a temperature of -60 °C. The reaction was quenched by addition of water (500 µL) and let to attain to room temperature. Upon concentration under reduced pressure, the residue was purified by size exclusion chromatography over HW-40 eluted with water to obtain the title compound as a colorless oil (8.0 mg, 24 µmol, 86%). NMR (500 MHz, D_2O, HH-COSY, HSQC, HMBC, NOESY): δ 4.99 (d, *J* = 3.9 Hz, 1H, H-1'), 3.88 (dd, *J* = 8.5, 3.7 Hz, 1H, H-2), 3.83 (ddd, *J* = 10.1, 5.0, 2.5 Hz, 1H, H-5'), 3.78 (dd, *J* = 11.1, 3.5 Hz, 1H, H-6), 3.74 (dd, *J* = 12.3, 2.5 Hz, 1H, H-

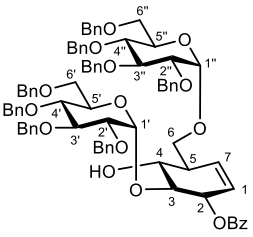
6'), 3.71– 3.62 (m, 2H, H-6, H-6'), 3.58 (dd, *J* = 9.3, 9.1 Hz, 1H, H-3), 3.41 (dd, *J* = 9.6, 3.9 Hz, 1H, H-2'), 3.35 (dd, *J* = 10.0, 9.7 Hz, 1H, H-4), 3.32 – 3.25 (m, 2H, H-3, H-4'), 2.50 (dd, *J* = 6.4, 3.7 Hz, 1H, H-1), 2.26 (dd, *J* = 6.4, 0.8 Hz, 1H, H-7), 1.78 (ddd, *J* = 10.0, 6.4, 3.7 Hz, 1H, H-5); ^{13}C NMR (126 MHz, D_2O, HSQC, HMBC): δ 102.1 (C-1'), 84.6 (C-3), 74.7 (C-3'), 72.9 (C-2'), 72.6 (C-5'), 70.8 (C-4), 70.0 (C-2/C-4), 70.0 (C-2/C-4), 61.9 (C-6), 60.8 (C-6'), 44.7 (C-5), 35.9 (C-1), 31.7 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{13}H_{23}NNaO_9$ 360.1271; Found 360.1263.

3-*O*-(Perbenzyl-α-D-glucopyranosyl)-2-*O*-benzoyl-cyclophellitol alkene (30).



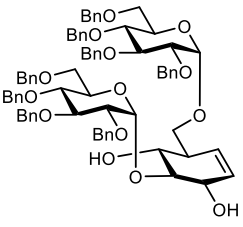
Compound **24a** (0.14 g, 0.12 mmol) was dissolved in anhydrous DCM (2.4 mL, 0.05 M) and cooled on ice. Subsequently, TES (38 µL, 0.24 mmol, 2.0 eq.) and TFA (27 µL, 0.24 mmol, 2.0 eq.) were added respectively. The solution was kept stirring for 1 hour while attaining a temperature of 0 °C. Upon full conversion (R_f 0.3 (Et_2O:pentane, 3:7 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ (5.0 mL) followed by diluting the reaction mixture with water (25 mL) and Et_2O (25 mL). The organic layer was separated and the aqueous layer was extracted twice with Et_2O (25 mL). The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The crude was dissolved in THF (1.2 mL, 0.05 M) after which a 1 M TBAF solution in THF (0.24 mL, 0.24 mmol, 4.0 eq.) was added. The reaction mixture was stirred for 1 hour at room temperature. Upon full conversion (R_f 0.2 (Et_2O:pentane, 1:1 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ (5.0 mL) followed by diluting the reaction mixture with water (25 mL) and Et_2O (25 mL). The organic layer was separated and the aqueous layer was extracted twice with Et_2O (25 mL). The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (60:40 Et_2O:pentane → 80:20 Et_2O:pentane) to obtain the title compound as a colorless oil (68 mg, 86 µmol, 72%). NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 8.15– 8.01 (m, 2H, CH_{arom}), 7.61 – 7.53 (m, 1H, CH_{arom}), 7.48 – 7.41 (m, 2H, CH_{arom}), 7.37 – 7.27 (m, 10H, CH_{arom}), 7.27 – 7.18 (m, 6H, CH_{arom}), 7.14 – 7.06 (m, 2H, CH_{arom}), 7.05 – 6.96 (m, 2H, CH_{arom}), 5.68 – 5.60 (m, 2H, H-2, H-7), 5.56 (ddd, *J* = 10.5, 2.0, 1.7 Hz, 1H, H-1), 5.04 (s, 1H, 4-OH), 4.96 (d, *J* = 3.7 Hz, 1H, H-1), 4.90 – 4.83 (m, 3H, C*H*H Bn, C*H*H Bn, C*H*H Bn), 4.75 – 4.67 (m, 2H, CH*H* Bn, CH*H* Bn), 4.35 (d, *J* = 10.7 Hz, 1H, CH*H* Bn), 4.22 (d, *J* = 12.1 Hz, 1H, C*H*H Bn), 3.97 (dd, *J* = 9.4, 6.6 Hz, 1H, H-3'), 3.91 – 3.71 (m, 5H, CH*H* Bn, H-3, H-4, H-6), 3.68 – 3.63 (m, 2H, H-4', H-5'), 3.58 (dd, *J* = 9.7, 3.7 Hz, 1H, H-2'), 3.34 (dd, *J* = 10.9, 1.5 Hz, 1H, H-6), 2.85 (d, *J* = 8.2 Hz, 1H, 6-OH), 2.68 (dd, *J* = 10.9, 1.4 Hz, 1H, H-6), 2.65 – 2.59 (m, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 166.1 (C=O Bz), 138.6, 138.3, 137.8, 137.0 ($C_{q\text{-}arom}$), 133.3, 130.1, 128.9, 128.8 (C-1), 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 128.1, 127.8, 127.8, 127.8, 127.7 (CH_{arom}), 126.3 (C-7), 101.7 (C-1'), 86.6 (C-3), 82.4 (C-3'), 79.6 (C-2'), 77.5 (C-4'/C-5'), 75.7 (CH_2 Bn), 75.0 (CH_2 Bn), 74.7 (CH_2 Bn), 74.3 (C-4), 74.0 (C-2), 73.4 (CH_2 Bn), 71.3 (C-4'/C-5'), 67.8 (C-6'), 65.6 (C-6), 44.9 (C-5); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{48}H_{50}NaO_{10}$ 809.3302; Found 809.3292.

3,6-Di-*O*-(perbenzyl-α-D-glucopyranosyl)-2-*O*-benzoyl-cyclophellitol alkene (32).



Acceptor **30** (89 mg, 0.11 mmol) and perbenzyl-D-glucopyranosyl N-phenyltrifluoroacetimidate donor **31** (0.20 g, 0.28 mmol, 2.5 eq.), synthesized according to literature precedence[32], were combined and co-evaporated twice with toluene. PPh_3O (0.50 g, 1.8 mmol, 16 eq.), activated 3Å molecular rods and anhydrous DCM (2.3 mL, 0.05 M) were added and kept under N_2 atmosphere. Subsequently, TMSI (40 µL, 0.28 mmol, 2.5 eq.) was added dropwise and the reaction mixture was stirred for 48 hours at room temperature. Upon full conversion (R_f 0.4 (Et_2O:pentane, 1:1 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ (5.0 mL) followed by diluting the reaction mixture with water (50 mL) and Et_2O (25 mL). The organic layer was separated and the aqueous layer was extracted twice with Et_2O (25 mL). The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (40:60 Et_2O:pentane → 60:40 Et_2O:pentane) to obtain the title compound as a colorless oil (0.12 g, 89 µmol, 79%). NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC, HMBC-GATED): δ 8.11 – 8.07 (m, 2H, CH_{arom}), 7.60 – 7.49 (m, 1H, CH_{arom}), 7.46 – 7.40 (m, 2H, CH_{arom}), 7.38 – 6.97 (m, 40H, CH_{arom}), 5.86 (ddd, *J* = 10.2, 2.0, 1.8 Hz, 1H, H-1), 5.67 (dddd, *J* = 7.8, 4.0, 1.6, 1.5 Hz, 1H, H-2), 5.58 (ddd, *J* = 10.2, 2.6, 2.5 Hz, 1H, H-7), 5.00 (d, *J* = 10.9 Hz, 1H, C*H*H Bn), 4.96 (d, *J* = 3.7 Hz, 1H, H-1'), 4.91 – 4.81 (m, 6H, C*H*H Bn, C*H*H Bn, CH*H* Bn, CH*H* Bn, CH*H* Bn, H-1''), 4.78 – 4.60 (m, 5H, C*H*H Bn, C*H*H Bn, C*H*H Bn, CH*H* Bn, CH*H* Bn), 4.52 – 4.44 (m, 2H, C*H*H Bn, CH*H* Bn), 4.34 (d, *J* = 10.8 Hz, 1H, C*H*H Bn), 4.21 (d, *J* = 12.1 Hz, 1H, CH*H* Bn), 3.99 (m, 2H, H-3', H-3''), 3.86 – 3.72 (m, 7H, CH*H* Bn, H-3, H-5', H-5'', H-6'', 4-OH), 3.72 – 3.62 (m, 5H, H-4, H-6, H-4', H-4''), 3.59 (dd, *J* = 9.6, 3.6 Hz, 1H, H-2'/H-2''), 3.55 (dd, *J* = 9.7, 3.6 Hz, 1H, H-2'/H-2''), 3.35 (dd, *J* = 10.8, 1.6 Hz, 1H, H-6'), 2.77 – 2.68 (m, 2H, H-5, H-6'); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC, HMBC-GATED): δ 166.1 (C=O Bz), 139.0, 138.7, 138.4, 138.4, 138.3, 138.0, 137.8, 137.4 ($C_{q\text{-}arom}$), 133.2 (CH_{arom}), 130.3 ($C_{q\text{-}arom}$), 130.2 (C-1), 130.1, 128.7, 128.6, 128.5, 128.5, 128.5, 128.3, 128.3, 128.1, 128.0, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6 (CH_{arom}), 125.2 (C-7), 101.6 (C-1'), 97.4 (C-1''), 86.8 (C-3), 82.4, 82.1 (C-3', C-3''), 80.2, 79.4 (C-2', C-2''), 77.8, 77.4 (C-4', C-4''), 75.8, 75.7, 75.3, 74.9, 74.2 (CH_2 Bn), 74.2 (C-2), 73.6, 73.3, 73.1 (CH_2 Bn), 71.3 (C-4), 70.9, 70.4 (C-5', C-5''), 68.5 (C-6, C-6''), 67.3 (C-6'), 43.5 (C-5); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{82}H_{84}NaO_{15}$ 1331.5708; Found 1331.5702.

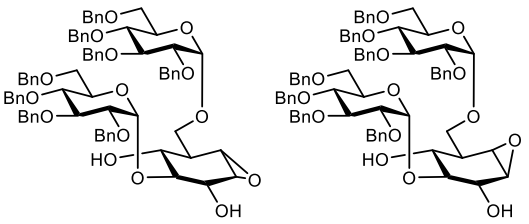
3,6-Di-*O*-(perbenzyl-α-D-glucopyranosyl)-cyclophellitol alkene (33).



Compound **32** (94 mg, 72 µmol) was dissolved in a 1:1 mixture of DCM and MeOH (1.5 mL, 0.05 M) followed by the addition of NaOMe (94 mg, 1.4 mmol, 20 eq.). The reaction was stirred overnight at room temperature. Upon full conversion (R_f 0.2 (Et_2O:pentane, 7:3 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ (5.0 mL) followed by diluting the reaction mixture with water (50 mL) and Et_2O (25 mL). The organic layer was separated and the aqueous layer was extracted twice with Et_2O (25 mL). The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (60:40 Et_2O:pentane → 80:20 Et_2O:pentane) to obtain the title compound as a colorless oil (62 mg, 51

µmol, 72%). NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.41 – 7.19 (m, 36H, CH_{arom}), 7.18 – 7.10 (m, 4H, CH_{arom}), 5.73 (ddd, *J* = 10.2, 2.2, 2.0 Hz, 1H, H-1), 5.58 (ddd, *J* = 10.2, 2.5, 2.2 Hz, 1H, H-7), 4.99 (d, *J* = 10.9 Hz, 1H, C*H*H Bn), 4.93 (d, *J* = 10.9 Hz, 1H, C*H*H Bn), 4.87 – 4.77 (m, 7H, H-1', H-1'', C*H*H Bn, C*H*H Bn, C*H*H Bn, CH*H* Bn, CH*H* Bn), 4.73 (d, *J* = 12.0 Hz, 1H, C*H*H Bn), 4.69 (d, *J* = 11.9 Hz, 1H, CH*H* Bn), 4.67 – 4.60 (m, 2H, C*H*H Bn, CH*H* Bn), 4.55 (d, *J* = 12.4 Hz, 1H, C*H*H Bn), 4.52 – 4.44 (m, 4H, CH*H* Bn, CH*H* Bn, CH*H* Bn, CH*H* Bn), 4.16 (dd, *J* = 6.6, 3.6 Hz, 1H, H-2), 4.05 (ddd, *J* = 10.1, 6.6, 2.0 Hz, 1H, H-5'/H-5''), 4.03 – 3.95 (m, 2H, H-3',H-3''), 3.85 (s, 1H, 4-OH), 3.79 – 3.72 (m, 2H, H-5'/H-5'', H-6/H-6'/H-6''), 3.72 – 3.60 (m, 7H, H-4, H-4'/H-4'', H-6/H-6'/H-6'', H-6/H-6'/H-6'', H-6/H-6'/H-6'', H-6/H-6'/H-6''), 3.60 – 3.56 (m, 2H, H-2', H-2''), 3.48 (dd, *J* = 10.1, 6.7 Hz, 1H, H-6/H-6'/H-6''), 3.42 (dd, *J* = 10.2, 9.0 Hz, 1H, H-4'/H-4''), 3.37 (dd, *J* = 9.9, 7.4 Hz, 1H, H-3), 2.71 – 2.59 (m, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 139.1, 138.6, 138.5, 138.3, 138.1, 138.0, 137.6, 137.6 ($C_{q\text{-}arom}$), 128.7, 128.6, 128.6, 128.5, 128.5, 128.5, 128.4, 128.3, 128.2, 128.1, 128.1, 128.1, 128.0, 128.0, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.7 (CH_{arom}), 100.3, 97.2 (C-1', C-1''), 91.5 (C-3), 82.4, 82.1 (C-3', C-3''), 80.3, 79.6 (C-2', C-2''), 78.1, 77.4 (C-4', C-4''), 75.9, 75.8, 75.4, 75.2, 74.1, 73.6, 73.6, 73.0 (CH_2 Bn), 71.4 (C-5'/C-5''), 71.1 (C-2), 70.4 (C-5'/C-5''), 69.8 (C-4), 68.7, 68.6, 68.5 (C-6, C-6', C-6''), 43.5 (C-5); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{75}H_{80}NaO_{14}$ 1227.5446; Found 1227.5440.

3,6-Di-*O*-(perbenzyl-α-D-glucopyranosyl)-cyclophellitol (34a) and 3,6-di-*O*-(perbenzyl-α-D-glucopyranosyl)-1,7-*epi*-cyclophellitol (34b).

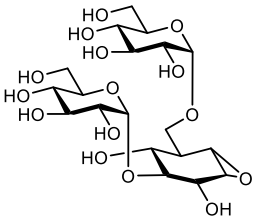


Compound **33** (20 mg, 16 µmol) was dissolved in anhydrous DCM (1.6 mL, 0.01 M) followed by the addition of $NaHCO_3$ (6.7 mg, 80 µmol, 5.0 eq.). The solution was cooled on ice and *m*-CPBA (8.4 mg, 49 µmol, 3.0 eq.) was added. The reaction was stirred for 48 hours at 4 °C. Upon full conversion (R_f 0.2 and 0.3 for **34a** and **34b** respectively (Et_2O:pentane, 7:3 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ (5.0 mL) and sat. aq. $Na_2S_2O_3$ (5.0 mL) followed by diluting the reaction mixture with water (50 mL) and Et_2O (25 mL). The organic layer was separated and the aqueous layer was extracted twice with Et_2O (25 mL). The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (50:50 Et_2O:pentane → 80:20 Et_2O:pentane) to obtain the title compounds as colorless oils (7.0 mg, 5.7 µmol, 35% for **34a** and 6.8 mg, 5.6 µmol, 34% for **34b**). Analytical data for **34a**: NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC, NOESY): δ 7.39 – 7.23 (m, 35H, CH_{arom}), 7.15 – 7.10 (m, 5H, CH_{arom}), 4.98 (d, *J* = 10.8 Hz, 1H, C*H*H Bn), 4.90 (d, *J* = 10.9 Hz, 1H, C*H*H Bn), 4.85 – 4.77 (m, 7H, H-1'/H-1'', C*H*H Bn, C*H*H Bn, C*H*H Bn, CH*H* Bn, CH*H* Bn, CH*H* Bn), 4.72 (d, *J* = 3.9 Hz, 1H, H-1'/H-1''), 4.70 – 4.62 (m, 3H, C*H*H Bn, C*H*H Bn, CH*H* Bn), 4.57 – 4.42 (m, 5H, C*H*H Bn, CH*H* Bn, CH*H* Bn, CH*H* Bn, CH*H* Bn), 4.03 – 3.88 (m, 4H, 2-OH/4-OH, H-3', H-3'', H-5'/H-5''), 3.88 – 3.74 (m, 5H, H-2, H-6, H-5'/H-5'', H-6'/H-6''), 3.72 – 3.58 (m, 5H, 2-OH/4-OH, H-2'/H-2'', H-4'/H-4'', H-6', H-6''), 3.55 (dd, *J* = 9.7, 3.8 Hz, 1H, H-2'/H-2''), 3.49 – 3.42 (m, 2H, H-7, H-6'/H-6''), 3.38 (dd, *J* = 10.2, 8.9 Hz, 1H, H-4'/H-4''), 3.31 (dd, *J* = 9.8, 9.6 Hz, 1H, H-4), 3.15 – 3.10 (m, 2H, H-1, H-3), 2.44 – 2.35 (m, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 139.1, 138.5, 138.4, 138.4,

138.1, 137.8, 137.5, 137.4 ($C_{q\text{-}arom}$), 128.8, 128.6, 128.6, 128.6, 128.5, 128.5, 128.5, 128.4, 128.2, 128.2, 128.1, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.7 (CH_{arom}), 100.5, 97.3 (C-1', C-1''), 91.4 (C-3), 82.3, 82.3 (C-3', C-3''), 80.2, 79.5 (C-2', C-2''), 78.2, 77.8 (C-4', C-4''), 75.9, 75.8, 75.3, 75.3, 74.3, 73.7, 73.6, 73.4 (CH_2 Bn), 71.5, 70.3 (C-5', C-5''), 70.2 (C-2), 68.7, 68.5 (C-6', C-6''), 67.0 (C-6), 66.5 (C-4), 56.1 (C-1), 54.7 (C-7), 41.6 (C-5); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{75}H_{80}NaO_{15}$ 1243.5395; Found 1243.5389.

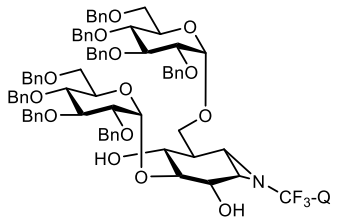
Analytical data for **34b**: NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC, NOESY): δ 7.39 – 7.20 (m, 36H, CH_{arom}), 7.18 – 7.10 (m, 4H, CH_{arom}), 4.99 (d, *J* = 11.0 Hz, 1H, C*H*H Bn), 4.91 (d, *J* = 10.9 Hz, 1H, C*H*H Bn), 4.86 – 4.72 (m, 8H, H-1', H-1'', C*H*H Bn, C*H*H Bn, C*H*H Bn, C*H*H Bn, CH*H* Bn, CH*H* Bn), 4.68 – 4.60 (m, 3H, C*H*H Bn, CH*H* Bn, CH*H* Bn), 4.55 (d, *J* = 12.4 Hz, 1H, CH*H* Bn), 4.51 – 4.44 (m, 4H, C*H*H Bn, CH*H* Bn, CH*H* Bn, CH*H* Bn), 4.05 – 3.88 (m, 5H, 2-OH/4-OH, H-3', H-3'', H-5'/H-5'', H-6), 3.84 (d, *J* = 7.8 Hz, 1H, H-2), 3.78 – 3.72 (m, 2H, H-5'/H-5'', H-6'/H-6''), 3.70 – 3.58 (m, 6H, H-6, H-2'/H-2'', H-4'/H-4'', H-6'/H-6'', H-6'/H-6''), 3.55 (dd, *J* = 9.7, 3.8 Hz, 1H, H-2'/H-2''), 3.52 – 3.41 (m, 3H, H-4, H-4'/H-4'', H-6'/H-6''), 3.34 (dd, *J* = 9.9, 8.0 Hz, 1H, H-3), 3.29 (dd, *J* = 3.9, 2.0 Hz, 1H, H-1), 3.22 (d, *J* = 3.9 Hz, 1H, H-7), 2.31 (ddd, *J* = 9.6, 5.9, 3.3 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 139.0, 138.6, 138.5, 138.2, 138.1, 138.0, 137.6, 137.4 ($C_{q\text{-}arom}$), 128.8, 128.6, 128.6, 128.5, 128.5, 128.4, 128.2, 128.1, 128.1, 128.0, 127.9, 127.9, 127.9, 127.7, 125.7 (CH_{arom}), 100.6, 97.6 (C-1', C-1''), 88.2 (C-3), 82.4, 82.0 (C-3', C-3''), 80.4, 79.4 (C-2', C-2''), 78.0, 77.4 (C-4', C-4''), 76.0, 75.8, 75.5, 75.2, 74.2, 73.7, 73.6, 73.2 (CH_2 Bn), 71.5 (C-5'/C-5''), 70.9 (C-2), 70.6 (C-5'/C-5''), 69.0 (C-4), 68.6, 68.5 (C-6', C-6''), 67.4 (C-6), 56.7 (C-1), 54.4 (C-7), 42.2 (C-5); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{75}H_{80}NaO_{15}$ 1243.5395; Found 1243.5389.

3,6-Di-*O*-(α-D-glucopyranosyl)-1,7-*epi*-cyclophellitol (36).



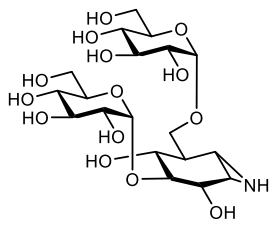
To liquid ammonia (3 mL) at -60 °C, sodium metal (20 mg, 0.88 mmol, 80 eq.) was added. This mixture was stirred for 30 minutes while maintaining a temperature of -60 °C. Subsequently, Compound **34a** (13 mg, 11 µmol) was dissolved in THF (1.0 mL) followed by the addition *t*-BuOH (10 µL, 0.11 mmol, 10 eq.). This solution was added dropwise to the flask containing ammonia. The solution was stirred for 1 hour while maintaining a temperature of -60 °C. The reaction was quenched by addition of water (500 µL) and let to attain to room temperature. Upon concentration under reduced pressure, the residue was purified by size exclusion chromatography over HW-40 eluted with water to obtain the title compound as a colorless oil (5.0 mg, 10 µmol, 91%). NMR (850 MHz, D_2O HH-COSY, HSQC, NOESY): δ 5.15 (d, *J* = 3.9 Hz, 1H, H-1'/H-1''), 4.93 (d, *J* = 3.8 Hz, 1H, H-1'/H-1''), 4.02 (dd, *J* = 8.5, 2.1 Hz, 1H, H-2), 3.97 – 3.93 (m, 2H, H-5'/H-5'', H-6), 3.81 (dd, *J* = 12.4, 2.3 Hz, 1H, H-6''), 3.77 – 3.76 (m, 2H, H-6'), 3.74 – 3.66 (m, 4H, H-6, H-3', H-3'', H-6''), 3.63 – 3.58 (m, 2H, H-5'/H-5'', H-4), 3.54 (m, 2H, H-2', H-2''), 3.47 (dd, *J* = 10.1, 8.5 Hz, 1H, H-3), 3.44 (dd, *J* = 4.1, 2.1 Hz, 1H, H-1), 3.42 (dd, *J* = 9.7, 9.5 Hz, 1H, H-4'/H-4''), 3.40 – 3.36 (m, 2H, H-7, H-4'/H-4''), 2.20 (ddd, *J* = 9.7, 5.9, 3.2 Hz, 1H, H-5); ^{13}C NMR (214 MHz, D_2O, HSQC): δ 100.9, 99.1 (C-1', C-1''), 82.6 (C-3), 73.9 (C-3', C-3''), 72.9, 72.7 (C-5', C-5''), 72.6, 72.2 (C-2', C-2''), 70.9 (C-2), 70.7 (C-4), 70.4, 70.1 (C-4', C-4''), 67.0 (C-6), 61.4, 61.0 (C-6', C-6''), 58.9 (C-1), 56.3 (C-7), 43.0 (C-5); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{19}H_{32}NaO_{15}$ 523.1639; Found 523.1633.

3,6-Di-*O*-(perbenzyl-α-D-glucopyranosyl)-1,7-*epi*-cyclophellitol CF_3-Q-aziridine (35).



BAIB (28 mg, 86 µmol, 5.0 eq.) was dissolved in anhydrous DCM (0.5 mL, 0.17 M) and cooled to -80 °C. To this, a solution of 2-trifluoromethyl-3-aminoquinazolin-4-one (20 mg, 86 µmol, 5.0 eq.) in anhydrous DCM (1 mL, 0.086 M) was added dropwise over the course of 30 minutes. Afterwards, the solution was allowed to warm to -40 °C followed by the addition of a solution of compound **33** (21 mg, 17 µmol) in DCM (0.5 mL, 0.034 M) over the course of 15 minutes. The reaction was allowed to attain to room temperature and stirred for another 48 hours. Upon full conversion (R_f 0.7 (Et_2O:pentane, 7:3 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ (5.0 mL) and sat. aq. $Na_2S_2O_3$ (5.0 mL) followed by diluting the reaction mixture with water (50 mL) and Et_2O (25 mL). The organic layer was separated and the aqueous layer was extracted twice with Et_2O (25 mL). The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (30:70 Et_2O:pentane → 50:50 Et_2O:pentane) to obtain the title compound as a colorless oil (15 mg, 11 µmol, 62%). NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC, HMBC, NOESY): δ 8.24 – 8.09 (m, 1H, CH Q), 7.82 – 7.79 (m, 2H, CH Q), 7.58 – 7.53 (m, 1H, CH Q), 7.36 – 7.19 (m, 32H, CH_{arom}), 7.18 – 7.03 (m, 8H, CH_{arom}), 4.94 (d, *J* = 11.0 Hz, 1H, C*H*H Bn), 4.88 – 4.74 (m, 8H, H-1', H-1'', C*H*H Bn, C*H*H Bn, C*H*H Bn, CH*H* Bn, CH*H* Bn, CH*H* Bn), 4.73 – 4.59 (m, 4H, C*H*H Bn, C*H*H Bn, CH*H* Bn, CH*H* Bn), 4.57 (d, *J* = 12.3 Hz, 1H, C*H*H Bn), 4.50 – 4.43 (m, 4H, C*H*H Bn, CH*H* Bn, CH*H* Bn, CH*H* Bn), 4.13 – 4.07 (m, 2H, H-1, H-5'/H-5''), 4.06 – 4.01 (m, 3H, H-6, H-3'/H-3''), 4.00 (s, 1H, 4-OH), 3.95 – 3.89 (m, 2H, H-7, H-3'/H-3''), 3.89 – 3.82 (m, 2H, H-2, 2-OH), 3.77 (m, 2H, H-5'/H-5'', H-6'/H-6''), 3.71 – 3.38 (m, 9H, H-3, H-4, H-2', H-2'', H-4', H-4'', H-6'/H-6'', H-6'/H-6'', H-6'/H-6''), 2.37 (ddd, *J* = 9.4, 3.8, 3.6 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC, HMBC): δ 160.9 (C=O Q), 144.1, 139.0, 138.8, 138.5, 138.3, 138.3, 138.1, 137.8, 137.5, 135.7, 135.0 ($C_{q\text{-}arom}$), 129.5, 129.4, 128.9, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 128.5, 128.4, 128.4, 128.2, 128.1, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.1, 126.7, 125.7, 123.2 (CH_{arom}), 100.8, 97.9 (C-1', C-1''), 87.7 (C-3), 82.5, 82.0 (C-3', C-3''), 80.3, 79.5 (C-2', C-2''), 78.0, 77.4 (C-4', C-4''), 75.9, 75.7, 75.4, 75.1, 74.4, 73.6, 72.9 (CH_2 Bn), 71.3, 70.5 (C-5', C-5''), 69.9 (C-2), 69.5 (C-4), 68.5 (C-6', C-6''), 67.9 (C-6), 43.9 (C-1), 42.2 (C-5), 42.0 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{84}H_{84}F_3N_3NaO_{15}$ 1454.5752; Found 1454.5747.

3,6-Di-*O*-(α-D-glucopyranosyl)-1,7-*epi*-cyclophellitol aziridine (37).



To liquid ammonia (3 mL) at -60 °C sodium metal (20 mg, 0.85 mmol, 80 eq.) was added. This mixture was stirred for 30 minutes while maintaining a temperature of -60 °C. Subsequently, Compound **35** (15 mg, 11 µmol) was dissolved in THF (1.0 mL) followed by the addition *t*-BuOH (10 µL, 0.11 mmol, 10 eq.). This solution was added dropwise to the flask containing ammonia. The solution was stirred for 1 hour while maintaining a temperature of -60 °C. The reaction was quenched by addition of water (500 µL) and let to attain to room temperature. Upon concentration under reduced pressure, the residue was purified by size exclusion chromatography over HW-40 eluted with water to obtain the title compound as a

colorless oil (4.8 mg, 9.6 µmol, 87%). ^{1}H NMR (500 MHz, D_2O, HH-COSY, HSQC, HMBC, NOESY): δ 5.14 (d, *J* = 4.0 Hz, 1H, H-1'), 4.94 (d, *J* = 3.7 Hz, 1H, H-1''), 3.98 – 3.94 (m, 2H, H-2, H-5'/H-5''), 3.92 – 3.85 (m, 1H, H-6), 3.84 – 3.67 (m, 7H, H-3', H-3'', H-6, H-6', H-6''), 3.66 – 3.62 (m, 1H, H-5'/H-5''), 3.55 (dd, *J* = 3.8, 1.2 Hz, 1H, H-2'/H-2''), 3.54 – 3.48 (m, 2H, H-4, H-2'/H-2''), 3.44 – 3.35 (m, 3H, H-3, H-4', H-4''), 2.57 (dd, *J* = 6.4, 3.7 Hz, 1H, H-1), 2.41 (d, *J* = 6.4 Hz, 1H, H-7), 2.03 (ddd, *J* = 10.2, 6.7, 3.4 Hz, 1H, H-5); ^{13}C NMR (126 MHz, D_2O, HSQC, HMBC): δ 100.0 (C-1'), 98.2 (C-1''), 82.8 (C-3), 73.1, 73.0 (C-3', C-3''), 72.0, 72.0 (C-5', C-5''), 71.8, 71.4 (C-2', C-2''), 70.7 (C-4), 70.2 (C-2), 69.6, 69.3 (C-4', C-4''), 67.6 (C-6), 60.5, 60.3 (C-6', C-6''), 42.5 (C-5), 35.9 (C-1), 31.9 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{19}H_{34}NNaO_{14}$ 500.1979; Found 500.1975.

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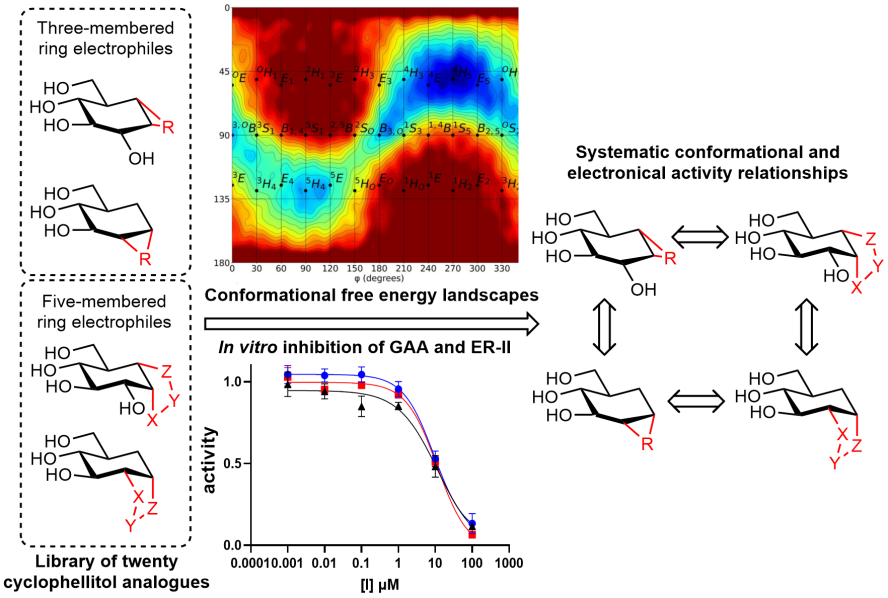
Chapter 3

Conformational and electronic differences of 1,2- and 1,7-cyclophellitols and their impact on α-glucosidase inhibition



ABSTRACT Twenty configurational and functional cyclophellitol analogues, featuring a systematic array of electrophiles were synthesized and evaluated as putative retaining α-glucosidase inhibitors. The inhibitory properties of the focused library of compounds were determined on human α-glucosidases after which the conformational free energy landscapes of the most active compounds were mapped. Our results add to the growing list of competitive and covalent α-glucosidase inhibitors and may help in the design of new therapeutics targeting these enzymes.

Introduction

Carbohydrates are found abundantly in nature and are essential in numerous biological processes.[1–4] The huge structural diversity of carbohydrates, oligosaccharides and glycoconjugates (glycoproteins, glycolipids) is reflected in the large variety of hydrolytic enzymes that have evolved and that are responsible for their processing and degradation. This large family of glycoside hydrolases is categorized in over 170 subfamilies, based on their primary sequence, tertiary structure and function.[5] Understanding their mode of action and reaction itineraries is an important stepping stone in the rational design of compounds that can selectively and efficiently inhibit specific glycoside hydrolases.

Retaining glycoside hydrolases, which comprise a large number of the known glycosidases and that encompass the human retaining α-glucosidases subject of the here-presented studies, employ a Koshland double displacement mechanism (Figure 1A).[6] Two carboxylic acid residues residing in the enzyme active site are positioned in such a way that one residue can act as a nucleophile and the other as a catalytic acid/base. Upon enzyme active site binding of the substrate, a Michaelis complex is formed with the substrate adopting a $^4C_1$-conformation. In this way, the leaving group is positioned in a (pseudo) axial fashion, allowing protonation by the catalytic acid-base and subsequent nucleophilic displacement of the aglycon by the nucleophilic acid residue. This process proceeds through a glucosyl $^4H_3$ oxocarbenium ion-like transition state and results in the formation of a covalent intermediate, with the bound glucose adopting a $^1S_3$-conformation. Next and following expulsion of the aglycon, water enters the active site. Following a reversed conformational itinerary ($^1S_3$-$^4H_3$-$^4C_1$), α-glucose is released and the enzyme returned to its resting phase ready for another catalytic cycle.[7–10]

In the past decades, a vast array of α- and β-retaining glycosidase inhibitors have been identified, many of which are based on the natural product, cyclophellitol (**1**). Cyclophellitol is a potent inhibitor of retaining β-glucosidases found in the *Phellinus sp*. Mushroom.[11] Cyclophellitol is the carbocyclic analogue of the natural retaining β-glucosidase substrates (β-glucopyranosides), bearing an epoxide bridging the C1 and C7 position.[12,13] This epoxide effectively constrains the cyclohexane into a $^4H_3$ conformation thereby mimicking the transition state (TS) during hydrolysis of β-glucopyranoses by retaining β-glucosidases. Not long after the discovery of cyclophellitol and fueled by its unique mode of action, the 1,7-epimer of cyclophellitol was constructed and shown to be a mechanism-based inhibitor of retaining α-glucosidases.[14] As soon as 1,7-*epi*-cyclophellitol (**2**) enters the active site of a retaining α-glucosidase, the nucleophilic carboxylate opens the epoxide in a trans diaxial fashion

forming an irreversible, covalent ester linkage with the inhibitor, thereby incapacitating the enzyme (Figure 1B). This *modus operandi* has been well-appreciated in the design of activity-based protein profiling (ABPP) as tools in chemical glycobiology.[15–19]

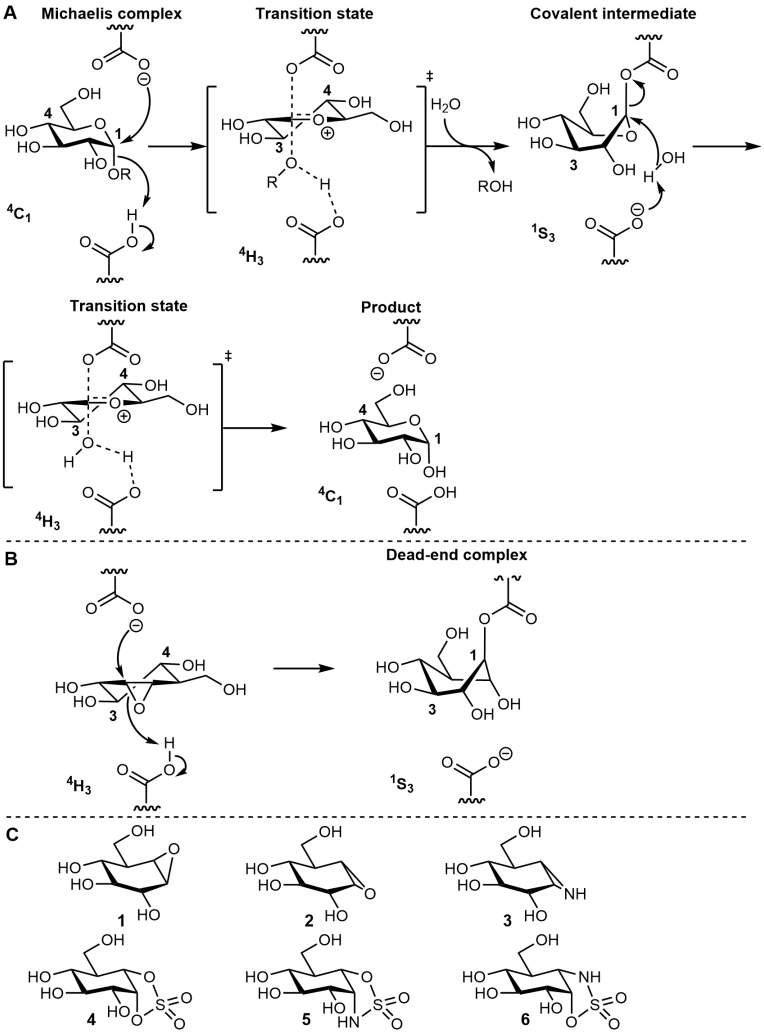


Figure 1. (A) Mechanistic itinerary of retaining α-glucosidases, (B) mechanism of inhibition by 1,7-*epi*-cyclophellitol **2** and (C) a selection of established covalent and non-covalent inhibitors of retaining β-glucosidases (**1**) and α-glucosidases (**2** – **6**).

Previously, the 1,7-epimer of cyclophellitol (**2**) and its nitrogen congener (**3**) were shown to be effective irreversible inhibitors and probes with micromolar to nanomolar affinities for human acid α-glucosidase (GAA) and endoplasmic reticulum glucosidase II (ER-II, Figure 1C).[18,20] As well, it was found that replacing the epoxide by a cyclic sulfate (as in **4**) gave an inhibitor with excellent potency and selectivity for α-glucosidases (1,7-

epi-cyclophellitol has retaining β-glucosidases as off-target).[21] Conformationally, these inhibitors do not exhibit $^4H_3$ character since the ring is not distorted by a strained three-membered ring. Rather, a $^4C_1$ conformation is adopted mimicking the structure of the α-glucosyl substrate in the Michaelis complex instead. As a follow up study, the corresponding cyclic sulfamidates (**5** and **6**) were revealed to be, due to their reduced electrophilicity, competitive retaining α-glucosidase inhibitors.[22]. Due to this reversible binding mode, **6** could be further developed into an enzyme stabilizer and thereby as pharmacological chaperone for the possible treatment of Pompe disease, in which the lysosomal α-glucosidase GAA is genetically impaired.[23–25] These results invite for a more in-depth study of modified cyclophellitol analogues as mechanism-based inhibitors. Here, the synthesis and inhibitory potential of α-1,2-cyclophellitol (**12** – **21**, Figure 2) in comparison with α-1,7-*epi*-cyclophellitols (**2** – **11**) is described. The inhibitory potencies and mode of action of the focused library of cyclophellitols on GAA and ER-II was studied in comparison to their parent α-1,7-*epi*-cyclophellitol. Although no improved inhibitors were found, low micromolar affinities were observed for some.

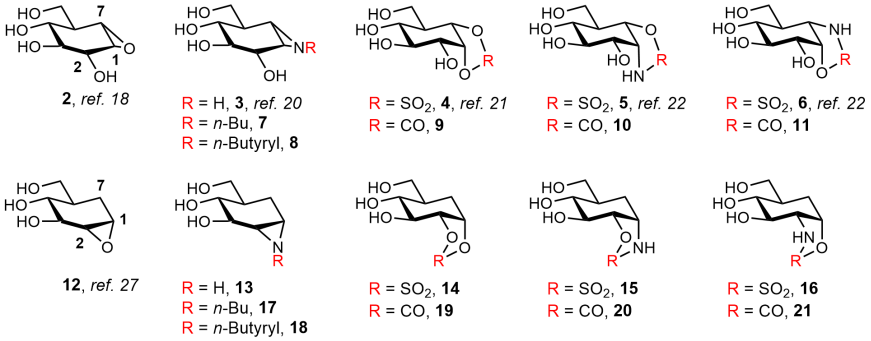


Figure 2. Twenty cyclophellitol analogues studied in this chapter for their inhibitory potencies against human retaining α-glucosidases.

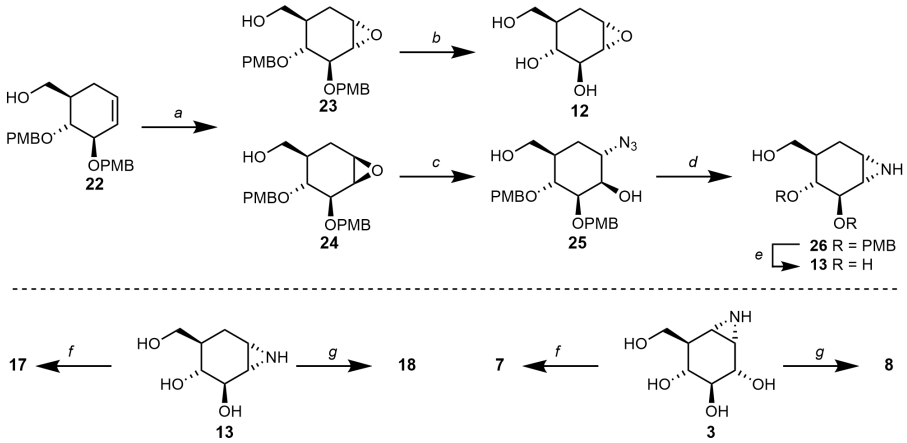
In all, the work presented here comprises expansion of the cyclophellitol scaffold in the design of both covalent and competitive retaining glycosidase inhibitors, including chemistries that can be readily adapted to differently configured carbohydrate mimetics and that would target glycosidases other than the α-glucosidase ones studied here.

Results and discussion

Compound synthesis. Compound **22** (Scheme 1), the key intermediate from which all 1,2-cyclophellitols were derived, was synthesized in eight steps according to procedures of Crotti and co-workers (See experimental, Scheme S1).[26] Epoxidation of the double bond in **22** under the *aegis* of *m*-CPBA yielded a separable mixture of epoxides **23** and **24** in 53% and 26% yield, respectively. Reductive deprotection (Pd/C, H_2) of α-epoxide

23 yielded **12** (83%) as the first target compound, of which all spectroscopic data were in full agreement with those reported in literature.[27] Following established procedures[28], opening of the β-epoxide with sodium azide under elevated temperatures yielded **25** (60%) which was subsequently treated with triphenylphosphine to undergo an intramolecular Staudinger cyclisation to yield α-aziridine **26** as the single regioisomer (43%). Removal of the 4-methoxybenzyl protecting groups in **26** was accomplished using Birch conditions (Na, NH_3) yielding target compound **13** (92%). Treatment of the α-aziridine with either butyl iodide or butyryl chloride yielded the butylated aziridine **17** and butyrylated aziridine **18** in 84% and 50% respectively.

Scheme 1. Synthesis of compounds **7**, **8**, **12**, **13**, **17** and **18**[a]



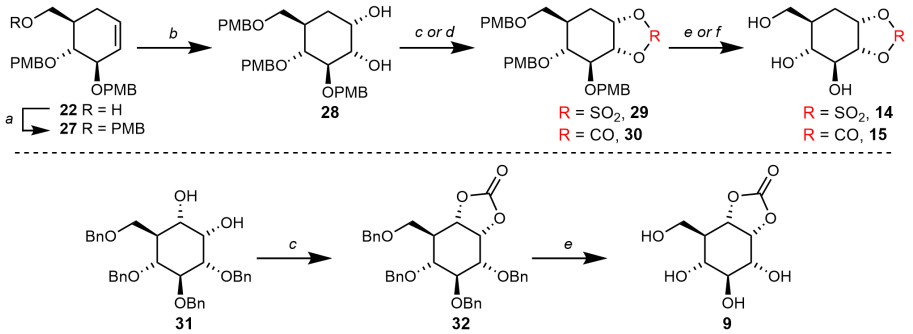
[a]Reagents and conditions: *a) m*-CPBA, DCM, 18 h, rt, 53% (**23**), 26% (**24**); *b)* Pd/C, H_2, MeOH, 1 h, rt (83%); *c)* NaN_3, DMF, 18 h, 130 °C (60%); *d)* TPP, CH_3CN, 18 h, 60 °C (43%); *e)* Na, NH_3, 1 h, -60 °C (92%); *f)* butyl iodide, K_2CO_3, DMF, 18 h, 80 °C, 84% (**17**), 24% (**7**); *g)* butyryl chloride, Et_3N, MeOH, 30 min, 0 °C, 50% (**18**), 64% (**8**).

The butylated and butyrylated 1,7-α-aziridine (**7** and **8**) were obtained *via* identical conditions starting from 1,7-α-aziridine **3**, which was in turn synthesized according to procedures optimized as published previously.[20,29]

Cyclic sulfate **14** and carbonate **15** were constructed starting with protection of the primary hydroxyl in **22** as the 4-methoxybenzyl ether under Williamson etherification conditions (NaH, PMBCl) to yield compound **27** in 85% (Scheme 2). Subsequent dihydroxylation of the alkene ($RuCl_3$, $NaIO_4$) yielded solely α-*cis*-diol **28** (85%) which was either sulfurylated ($SOCl_2$, Et_3N, *then* $RuCl_3$, $NaIO_4$) or carbonylated (triphosgene, Et_3N) to yield cyclic sulfate **29** (46%) and cyclic carbonate **30** (86%) respectively. Global deprotection proceeded smoothly *via* hydrogenation yielding the final compounds **14** and **15** in 83% and 68% respectively. In addition, the 1,7-α-carbonate **9** was obtained *via*

identical conditions starting from 1,7-α-*cis*-diol **31**, which was in turn synthesized according to procedures published previously.[21]

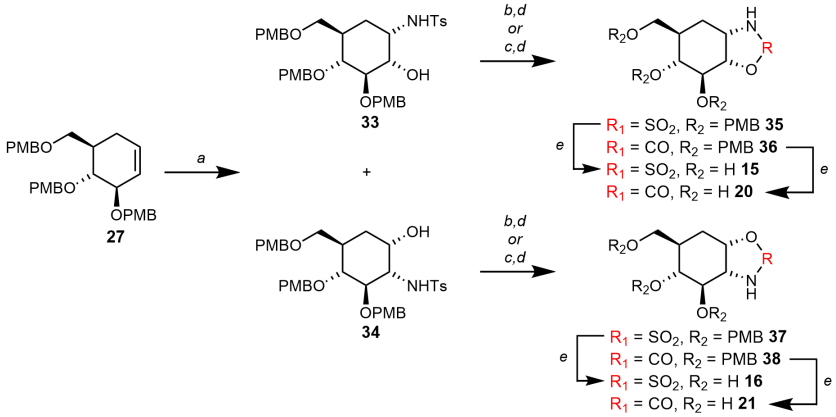
Scheme 2. Synthesis of **9**, **14** and **15**[a]



[a]Reagents and conditions: *a)* PMBCl, NaH, DMF, 16 h, rt (85%); *b)* $RuCl_3$, $NaIO_4$, 1:4:4 H_2O:CH_3CN:EtOAc, 1 h, 0 °C (85%); *c)* triphosgene, pyridine, DCM, 1.5 h, rt, 86% (**29**), 90% (**32**); *d)* (i) $SOCl_2$, Et_3N, DCM, 1 h, rt; (ii) $RuCl_3$, $NaIO_4$, 1:1 H_2O, CH_3CN, 15 min, 0 °C, (46%); *e)* $Pd(OH)_2/C$, H_2, MeOH, 18 h, rt, 68% (**15**), quant. (**9**); *f)* Pd/C, H_2, MeOH, 18 h, rt, 83% (**14**).

Cyclic sulfamidates **15** and **16** and carbamates **20** and **21** were constructed *via* a stereoselective Sharpless aminohydroxylation on alkene **27** ($K_2[OsO_2(OH)_4]$, chloramine-T, TEBACl) to give a separable, regioisomeric mixture of α-*cis*-amino alcohols **33** and **34** in 54% and 31% respectively (Scheme 3). Both α-*cis*-amino alcohols could be transformed into their corresponding cyclic sulfamidates by treatment with sulfuryl chloride and Et_3N at low temperatures (-78 °C) in quantitative yields. Subsequent removal of the *N*-tosyl functionality under reductive conditions (Na, naphthalene) gave rise to cyclic sulfamidates **35** and **38** in 75% and 85% yield, respectively. Alternatively, treatment of the individual amino alcohols **33** and **34** with triphosgene and pyridine followed by subsequent reductive detosylation (Na, naphthalene) yielded cyclic carbamates **36** and **38** (71% and 79% respectively, yield over two steps).

Scheme 3. Synthesis of cyclic sulfamidates **15** and **16** and carbamates **20** and **21**[a]

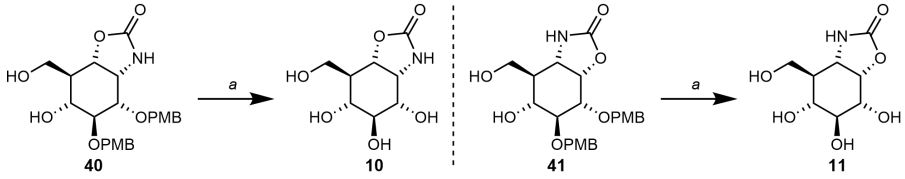


[a]Reagents and conditions: *a)* Chloramine-T, TEBACl, $K_2[OsO_2(OH)_4]$, 1:1 $CHCl_3$:H_2O, 18 h, 60 °C, 54% (**33**), 31% (**34**); *b)* SO_2Cl_2, Et_3N, DCM, 2 h, -78 °C, quant. (**S4**), quant. (**S5**); *c)* triphosgene, pyridine, DCM, 3 h, rt; *d)* naphthalene, Na, THF, 30 min, -78 °C, 75% (**35**), 71% (**36**), 85% (**37**), 79% (**38**); *e)* TFA, TES, DCM, 1 h, 0 °C, 94% (**15**), 67% (**20**), 81% (**16**), 32% (**21**).

Global deprotection with TFA and triethylsilane as cation scavenger afforded target sulfamidates **15** and **16** and carbamates **20** and **21** in 94%, 81%, 67% and 32% respectively.

Cyclic carbamates **10** and **11** were constructed *via* global deprotection of intermediates **40** and **41**. The synthesis of **40** and **41**, prepared *via* modified literature procedures,[29,30] is part of the research described in chapter 7. Deprotection proceeded smoothly by treating compounds **40** and **41** with TFA and triethylsilane (Scheme 4). This afforded the target structures **10** and **11** in 74% and 86% yield respectively.

Scheme 4. Synthesis of cyclic carbamates **10** and **11**[a]



[a]Reagents and conditions: *a)* TFA, TES, DCM, 1 h, 0 °C, 74% (**10**), 86% (**11**).

***In vitro* inhibition of human acid α-glucosidase and ER α-glucosidase II**. With inhibitors **2** – **21** in hand, attention was turned to evaluating their inhibitory potencies as inhibitors against human acid α-glucosidase (GAA) and ER α-glucosidase II (ER-II, Table 1). Apparent IC_{50} values were determined by measuring hydrolysis of the fluorogenic substrate, 4-methylulbelliferyl-α-D-glucose, where release of fluorescent product (4-methylumbelliferonate) is determined in terms of relative absorption (see SI).

Epoxide **12** proved to be a micromolar inhibitor of GAA (IC_{50} = 47 μM) making it slightly less potent than its 1,7-counterpart **2** (IC_{50} = 24 μM). In contrast, superior inhibitory potency was observed for **12** (IC_{50} = 13 μM) when screened against ER-II.[21] Aziridine **13** showed to be inactive on GAA, while inhibiting ER-II in the micromolar range (IC_{50} = 80 μM), whilst its 1,7-counterpart **3** tested to be a sub-micromolar inhibitor of GAA (IC_{50} = 0.37 μM) and ER-II (IC_{50} = 0.98 μM).[21] *n*-Butyl- and *n*-butyryl 1,2-aziridines **17** and **18** proved inactive as inhibitors of GAA and ER-II. Their 1,7-analogues, compounds **7** and **8**, however, yielded micromolar inhibitors. Slightly reduced inhibitory potency was observed for compound **7** and **8** (IC_{50} = 1.7 μM and 1.0 μM respectively) in comparison to unfunctionalized

Table 1. Apparent IC_{50} values for *in vitro* inhibition of GAA and ER-II[a]

Compound	GAA IC_{50} (μM)	ER-II IC_{50} (μM)	Compound	GAA IC_{50} (μM)	ER-II IC_{50} (μM)
2	24[b]	>100[b]	**12**	47	13
3	0.37[b]	0.98[b]	**13**	>100	80
4	0.051[b]	0.035[b]	**14**	2.5	48
5	3.4[c]	>100[c]	**15**	>100	>100
6	40[c]	1.2[c]	**16**	>100	>100
7	1.7	0.27	**17**	>100	>100
8	1.0	4.1	**18**	>100	>100
9	>100	>100	**19**	>100	>100
10	10	>100	**20**	>100	>100
11	>100	>100	**21**	>100	>100

[a]All apparent IC_{50} values are determined from three technical triplicates. [b]Values in accordance to literature data[21]. [c]Values in accordance to literature data[22].

aziridine **3**. Turning to ER-II, compound **7** was shown to be a 3-fold more potent inhibitor (IC_{50} = 0.27 μM) whilst compound **8** showed a 3-fold reduction in inhibitory potency (IC_{50} = 4.1 μM) compared to aziridine **3**.

Cyclic sulfate **14** appeared to be a low-micromolar inhibitor of GAA (IC_{50} = 2.5 μM), in contrast to its somewhat weaker inhibition of ER-II (IC_{50} = 48 μM), giving compound **14** a roughly 20-fold selectivity against GAA over ER-II. Its 1,7-counterpart **4** proved to be a 100-fold more active towards both GAA and ER-II (IC_{50} = 0.051 μM and 0.035 μM respectively).[21] Both the 1,2-cyclic sulfamidates **15** and **16**, lacked the ability to reduce enzyme activities of both GAA as ER-II up to concentrations of 100 μM, demonstrating that migration of the sulfamidate from 1,7- to 1,2-position does not lead to effective

inhibitors. In regards to the cyclic carbamates **10**, **11**, **20** and **21**, only compound **10** appeared to be an active inhibitor of GAA (IC_{50} = 10 μM), which is in line with the structural relationship observed for the cyclic sulfamidates. Again, the 1,2-modified constructs are not accepted by the binding pocket. In addition, superior inhibitory potencies are observed for the 1,7-(*N*,*O*)-regioisomers (**5** and **10**), which are over an order of magnitude more potent inhibitors when compared to the 1,7-(*O*,*N*)-regioisomers (**6** and **11**). Both cyclic carbonates **9** and **19** did not show a reduction of enzyme activities in both GAA as ER-II.

Having identified the inhibitory potencies of compounds **2** – **21**, focus was shifted to determining the kinetic parameters and the mode of binding of some of the most active inhibitors on recombinant human GAA (rhGAA). For this, rhGAA was incubated with a fixed substrate concentration and various inhibitor concentrations. Subsequently, apparent IC_{50} values were measured under varying incubation times. Compounds **2**, **3**, **4**, **6**, **7**, **8**, **12** and **14** showed a gradual decrease in enzyme activity indicating these compounds to be covalent and irreversible binders (Table 2). In contrast, both compound **5** and **10** appeared to be competitive inhibitors of rhGAA, as indicated by the observed lack of time dependency of the enzyme activity. Compounds **12** and **14** display pseudo first order kinetics due to fast inhibition against GAA, limiting measurement of a combined k_{inact}/K_I ratio.

Table 2. Inhibitor kinetic constants for recombinant human α-glucosidase (rhGAA)[a]

Compound	k_{inact}/K_I (min^{-1} mM^{-1})	Mode of binding	Compound	k_{inact}/K_I (min^{-1} mM^{-1})	Mode of binding
2	0.1511[b]	Covalent[b]	**12**	0.1526	Covalent
3	N.D.	Covalent[b]	**13**	N.D.	N.D.
4	62.41[b]	Covalent[b]	**14**	1.389	Covalent
5	0.06169[c]	Non-covalent[c]	**15**	N.D.	N.D.
6	N.D.	Covalent	**16**	N.D.	N.D.
7	N.D.	Covalent	**17**	N.D.	N.D.
8	N.D.	Covalent	**18**	N.D.	N.D.
9	N.D.	N.D.	**19**	N.D.	N.D.
10	N.D.	Non-covalent	**20**	N.D.	N.D.
11	N.D.	N.D.	**21**	N.D.	N.D.

[a]All apparent IC_{50} values are determined from two technical triplicates. [b]Values in accordance to literature data[21]. [c]Values in accordance to literature data[22]. N.D.: not determined due to low inhibitory potency.

Conformational free energy landscapes. The conformational energy landscapes of the most potent 1,2-cyclophellitol inhibitors (**12** – **14**) were mapped next. Free energy landscapes (FELs) were computed by means of metadynamics simulations based on density functional theory (DFT). For compounds **12** and **13**, the lowest energy conformation calculated is centered at $^{4}H_{5}$, with relative energies quickly inclining around this energy minimum (Figure 3D,E, respectively). This suggests compounds **12** and **13** to be relatively rigid and to mainly occupy this $^{4}H_{5}$ conformation. In contrast, their 1,7-counterparts, **2** and **3**, exhibit local energy minima around the $^{3}H_{4}$ conformation, related to a 60° displacement along the φ-axis (Figure 3A, B, respectively). The lack of flexibility exhibited by compounds **12** and **13** prevents adoption of the $^{3}H_{4}$ conformation, required for suitable mimicry of the transition state. This could, at least partly, explain the observed reduced inhibitory potencies of compounds **12** and **13** relative to their parent structures **2** and **3**. The energy minimum of cyclic sulfate **14** is located around $^{4}C_{1}$, with a relatively wide minimum expanding toward the $^{4}H_{3}$–^{4}E–$^{4}H_{5}$ region, with even an additional energy minimum extending toward the $B_{3,0}$–$^{1}S_{3}$ region (Figure 3F).

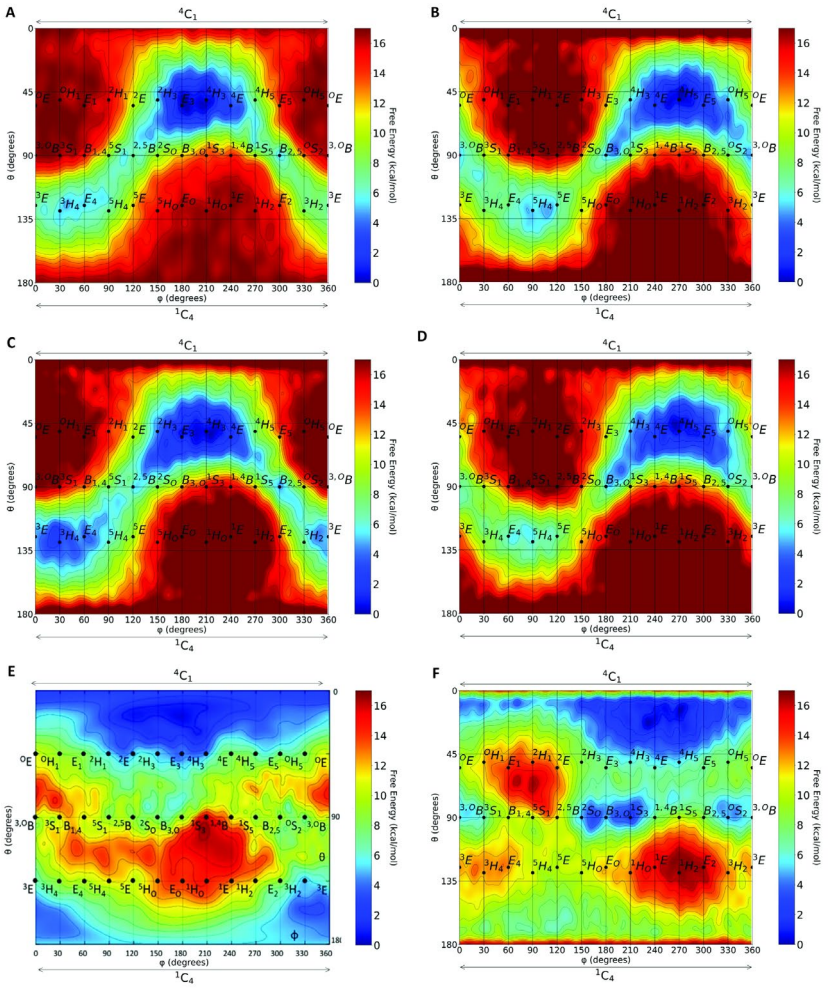


Figure 3. Computational free energy landscapes of 1,7-*epi*-cyclophellitol **2** (A), 1,7-aziridine **3** (B), cyclic 1,7-sulfate **4** (adapted from Artola *et al.*[21]) (C), 1,2-epoxide **12** (D), 1,2-aziridine **13** (E) and cyclic 1,2-sulfate **14** (F). Isolines are 1 kcal/mol with the x and y axis representing the angle (in degrees) in Cremer-Pople puckering coordinates (φ and θ respectively).

Again a 60° shift along the φ-axis is observed when compared to **4** (Figure 3C).[21] Interestingly, here an additional energy minimum is observed around the $B_{3,0}$–$^{1}S_{3}$ region not observed in parent structure **4**. These many low-energy conformations suggest **14** not only to adopt a $^{4}C_{1}$ conformation, but to be flexible enough to reach the $^{4}H_{3}$ transition state conformation. Thus, compound **14** should, as observed during *in vitro* assays, inhibit α-glucosidases with higher potencies in comparison to compounds **12** and **13**.

Conclusion

In this study, cyclophellitol based inhibitors of α-glucosidases were investigated, bearing a warhead over the 1,2-position of the cyclitol backbone. In addition to the preparation of 1,2-analogues (**12** – **16**) of the most prominent 1,7-*epi*-cyclophellitols known to date (**2** – **6**), additional warheads were proposed (**7** – **11**, **17** – **21**) and studied in *in vitro* assays for their inhibitory potencies against human acid α-glucosidase (GAA) and ER α-glucosidase II (ER-II). *N*-alkyl-1,7-aziridine **7** has improved inhibitory potency for ER-II compared to its non-alkylated counterpart **3**, resulting in a nanomolar IC_{50} against ER-II with a 6-fold selectivity over GAA. Additionally, 1,7-(*N*,*O*)-carbamate **10** revealed to be a non-covalent, low-micromolar inhibitor of GAA. Here, a strong structural relationship can be drawn with 1,7-(*N*,*O*)-sulfamidate **5**, suggesting identical enzyme interactions are at play. Therefore, compound **10** may be an interesting candidate for further study as enzyme stabilizer that can be potentially used in treatment of Pompe disease. All 1,2-analogues (**12** – **21**) revealed reduced inhibitory potencies in comparison to their 1,7-counterpart, with only compounds **12** – **14** exhibiting inhibitory potencies below 100 μM. Free energy landscapes revealed the ground state of compounds **12** – **14** to have undergone a shift in lowest energy conformation in comparison to their parent structures (**2** – **4**). As a result of this shift, the conformation does not resemble the conformation of either the Michaelis complex or transition state during hydrolysis. This conformational shift may explain the overall reduction in observed inhibitory potencies of the 1,2-cyclophellitols in contrast to their 1,7-counterparts. Overall, this study into structure-activity relationships of cyclophellitol analogues as human α-glucosidase inhibitors may fuel future design of constructs to effectively act on glycoside hydrolases of various sources and acting on various substrate glycosides.

Acknowledgements

Alba Nin-Hill and Carme Rovira from the University of Barcelona are kindly acknowledged for the metadynamics simulations and valuable discussion. Jurriaan Heming is acknowledged for the *in vitro* IC_{50} and time dependent inhibition experiments and valuable discussion. Roy Steneker and Anne-mei Klein are kindly acknowledged for the synthesis of compounds **10**, **11**, **15**, **16**, **20**, and **21** in the context of their MSc internships.

Biochemical methods

Cell culture/lysates

Fibroblast cell lines were cultured in HAMF12-DMEM medium (manufactor) supplied with 10% (v/v) FCS, 0.1% (w/v) penicillin/streptomycin, and 0.5% (w/v) sodium pyruvate, under 7% CO_2 at 37 °C. Confluent fibroblasts were cultured 1:3 each week. Cell pellets were stored at -80 °C until lysates were prepared. Cell lysates were prepared in potassium phosphate (KPi) lysis buffer (25 mM K_2HPO_4/KH_2PO_4, pH 6.5, supplemented with protease inhibitor cocktail (EDTA-free, Roche, Basel, Switzerland) and 0.1 % (v/v) triton X-100) *via* one Freeze-thaw cycle, followed by sonication on ice. Protein concentration was determined with the BCA Protein Assay Kit (ThermoFisher Pierce™) with 10x lysate dilution in KPi buffer (without protease inhibitor). Lysates were stored in aliquots at -80 °C until use.

IC_{50} determination

Enzymes used for IC_{50} were obtained as follows: recombinant human GAA (Myozyme) were obtained from Genzyme, USA and fibroblast cell lysates were used for ER-II α-glucosidase. Apparent IC_{50} values were determined throughout pre-incubation of 12.5 µL enzyme-mixture with 12.5 µL inhibitor for 30 minutes at 37 °C. GAA activity was measured with 47 nM enzyme (Myozyme) and 100 µL 3 mM 4-MU-α-D-glucopyranoside for 30 minutes at 37 °C. ER-II activity was measured using fibroblast cell lysates containing 10 µg protein (concentration was determined with BCA protein assay kit; Thermo Fisher) and 100 µL, 3 mM 4-MU α-D-glucopyranoside for 1 hour at 37 °C. After incubation with substrate mixture, the enzymatic reactions were quenched with 200 µL 1 M NaOH-Glycine (pH 10.3) and hydrolyzed 4-MU fluorescence is measured with a LS55 fluorescence spectrophotometer (Perkin Elmer: λ_{EX} 366 nm, λ_{EM} 445 nm). Background fluorescence (enzyme-mixture without substrate) is subtracted from the mean value and normalized with maximal activity (without inhibitor). GAA is diluted in 150 mM McIlvain buffer pH 4.0 supplemented with 0.1% bovine serum albumin (BSA, w/v%) and 0.01% NaN_3 as bacteriostatic. ER-II is diluted in 150 mM McIlvain buffer pH 7.0 supplemented with 0.1% bovine serum albumin (BSA, w/v%) and 0.01% NaN_3 as bacteriostatic. Values plotted for concentration inhibitor are those in the final reaction mixture containing enzyme, inhibitor and substrate (125 µL total). The IC_{50} value is the average of two-/triplicates from technical triplicates.

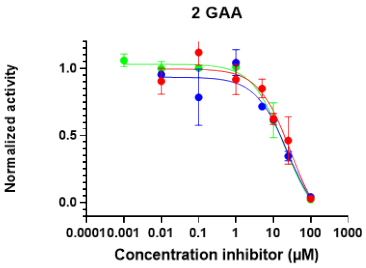
Time dependent inhibition

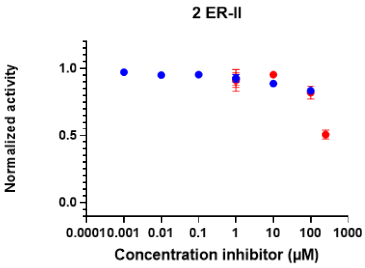
To study the type of inhibition, GAA and fibroblast cell lysates were pre-incubated for 5, 10, 15, 30, and 60 minutes with inhibitor (2x IC_{50} value) at 37 °C. Thereafter, 100 µL of substrate mixture (3 mM 4-MU α-D-glucopyranoside pH 4.0 for GAA, pH 7.0 for ER-II α-glucosidase) is added and incubated for 30 minutes (GAA) or 60 minutes (ER-II α-glucosidase). Finally, stop buffer (1 M glycine-NaOH pH 10.3) was added to stop the reaction and hydrolyzed 4-MU fluorescence was measured. Background fluorescence (enzyme-mixture without substrate) is subtracted from the mean value and normalized with maximal activity (without inhibitor). Time was plotted *vs* residual enzyme activity. either a straight line was observed or decreased activity over time, relating to non-covalent or covalent inhibition, respectively.

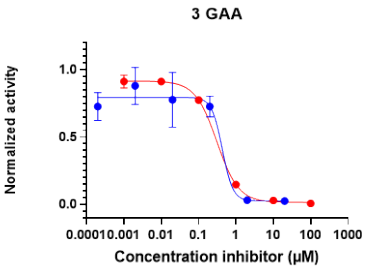
Kinetics

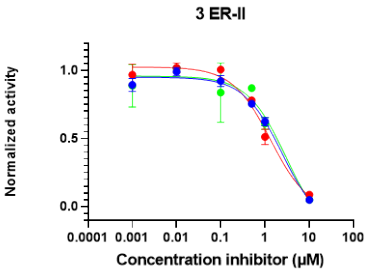
Kinetic parameters for inhibitor of GAA were determined using a continuous fluorescence assay involving simultaneous incubation of the enzyme with substrate and inhibitor. In the assay, the concentration of enzyme (GAA) and substrate (4-MU α-D-glucopyranoside) were 47 nM and 3 mM, respectively. For each inhibitor, sets of eight 2 mL Eppendorf tubes were prepared. Each Eppendorf tube contained 8.13 µL of various inhibitor concentration (diluted in DMSO, 200x assay concentration) or, 8.13 µL DMSO as control, and 154.4 µL McIlvain buffer (150 mM, pH 4.0, 0.1% BSA, 0.01% NaN_3) and 1300 µL substrate mixture. The Eppendorf tubes were pre-warmed on a thermoshaker at 37 °C for 5 minutes, together with a separate Eppendorf tube with 1250 µL enzyme dilution in McIlvain buffer pH 4.0 and enzyme blank (only McIlvain buffer pH 4.0). Prior to the start of the assay, 200 µL stop buffer (1 M glycine-NaOH, pH 10.3) was added to each well of a black 96-well plate. The t = 0 samples were prepared by adding 12.5 µL enzyme solution/blank into the first two columns of the plate and 112.6 µL of inhibitor/substrate mixture per row in duplicates. The reaction was starting by adding 137.6 µL aliquots of the pre-warmed enzyme/blank solution to the pre-warmed tubes containing the inhibitor/substrate mixture, with a time interval of 20 seconds between samples. The reaction tubes were incubated under constant shaking at 800 rpm at 37 °C. At depicted timepoints, 125 µL aliquots from each reaction tube was transferred to the 96-well plate in duplicates, with a 20-second interval between each tube. The 4-MU fluorescence was measured and for each timepoint the blank signal is subtracted and all values are normalized with the maximal activity of the latest timepoint without inhibitor. The observed pseudo-first order rates (k_{obs}) was determined for each concentration inhibitor by fitting the date with the one-phase exponential association function of GraphPad Prism. The obtained k_{obs} values are plotted *vs* the concentration inhibitor and the resulting plots were fitted using a linear function that gives the combined apparent inhibitor parameter k_{inact}/K'_I as the slope.

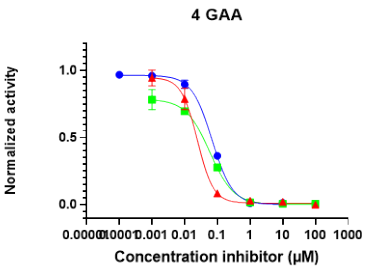
Fluorescent IC_{50} assays on recombinant human GAA (Myozyme) and ER-II for compounds 2 – 21.

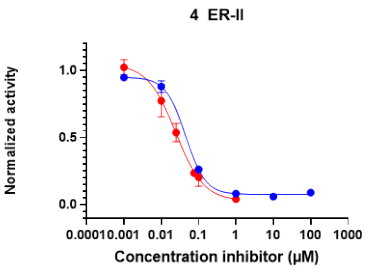


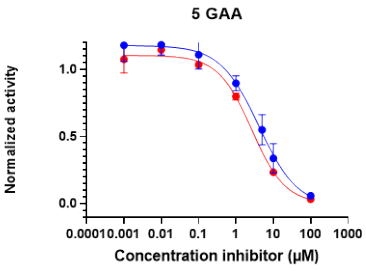


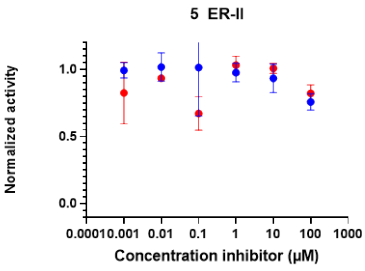


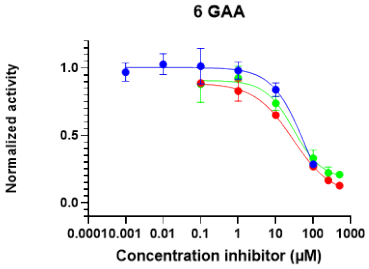








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Normalized activity
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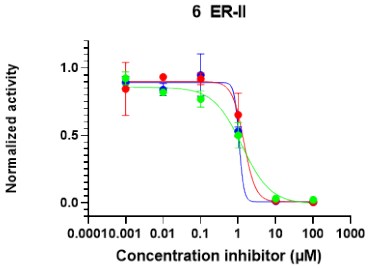
0.5
0.0
0.00010.001 0.01 0.1 1 10 100 1000
Concentration inhibitor (µM)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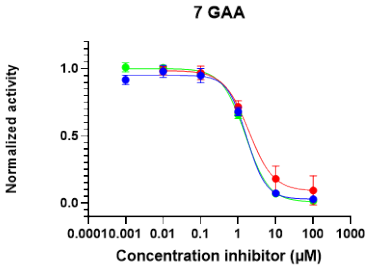
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Normalized activity
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0.5
0.0
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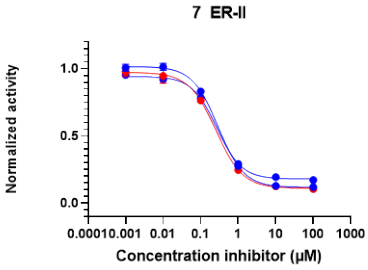
Concentration inhibitor (µM)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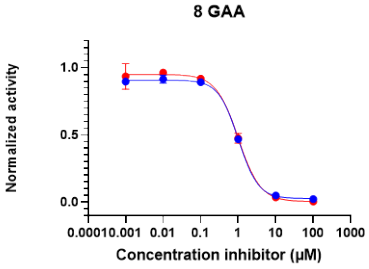
Concentration inhibitor (µM)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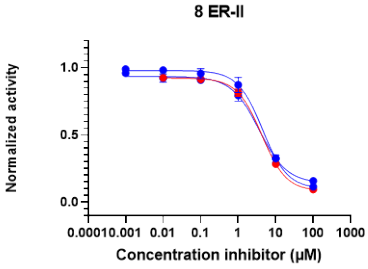
Concentration inhibitor (µM)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Normalized activity
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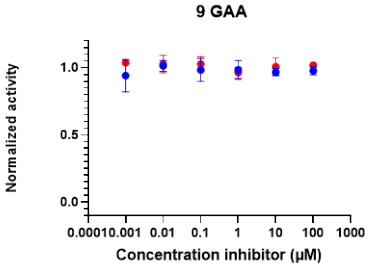
0.5
0.0
0.00010.001 0.01 0.1 1 10 100 1000
Concentration inhibitor (µM)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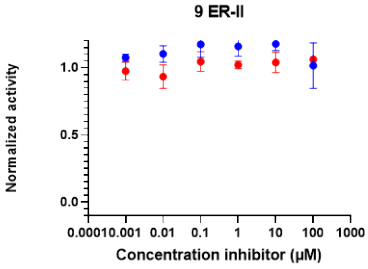
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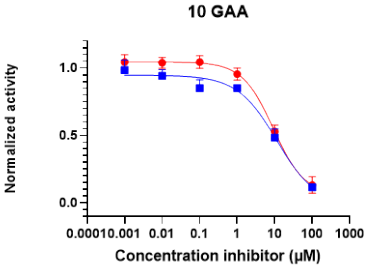
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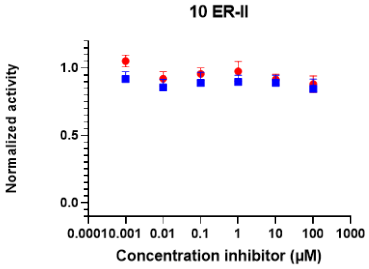
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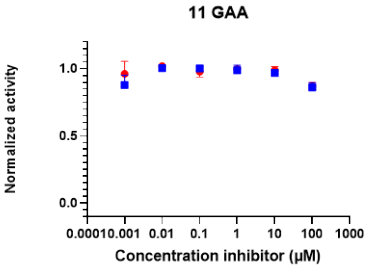
Concentration inhibitor (μM)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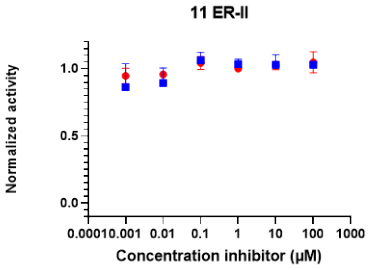
Concentration inhibitor (μM)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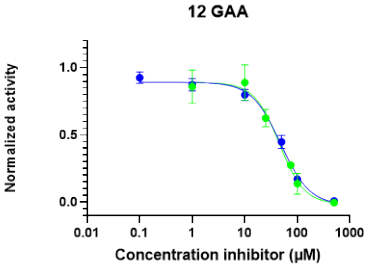
Concentration inhibitor (μM)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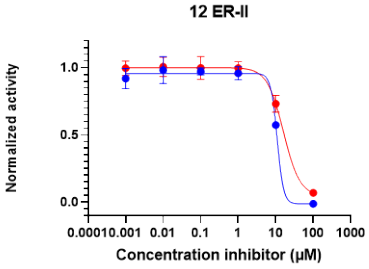
Concentration inhibitor (μM)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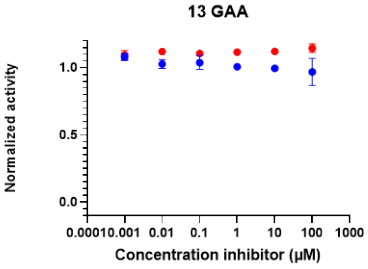
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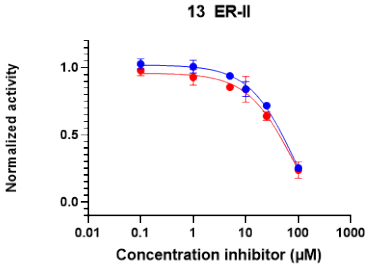
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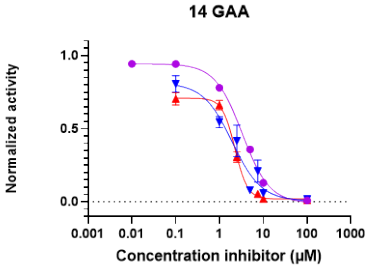
Concentration inhibitor (μM)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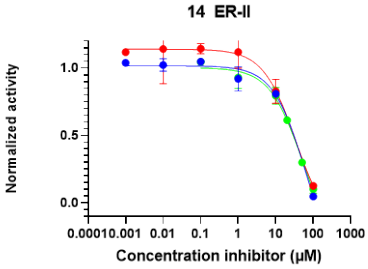
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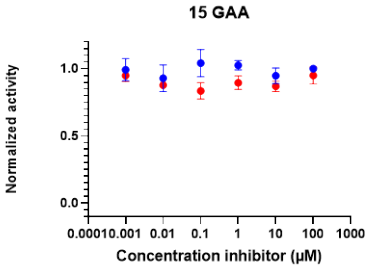
Concentration inhibitor (μM)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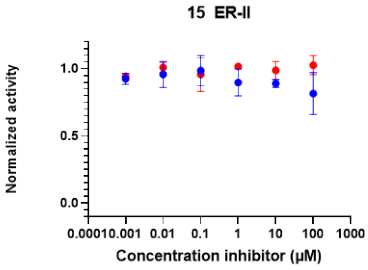
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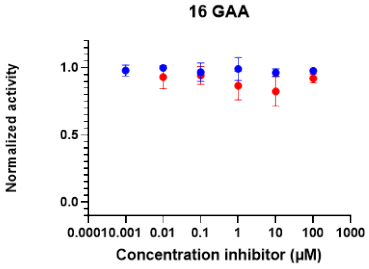
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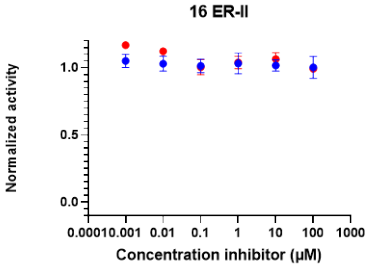
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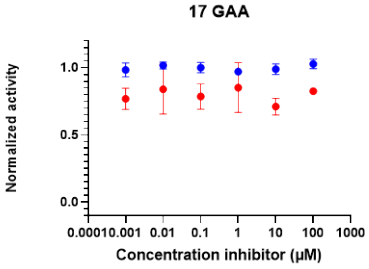
Concentration inhibitor (μM)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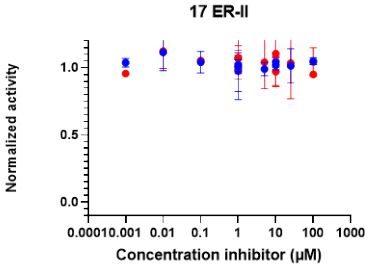
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Normalized activity
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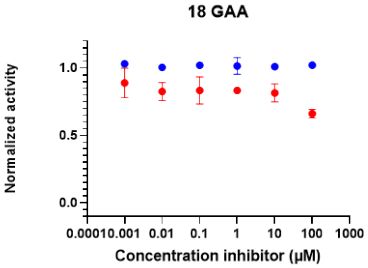
Concentration inhibitor (μM)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Normalized activity
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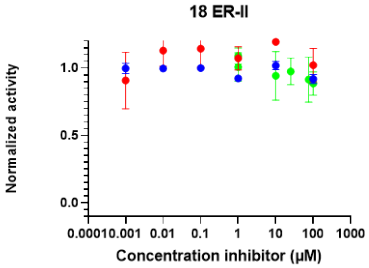
Concentration inhibitor (μM)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0.0
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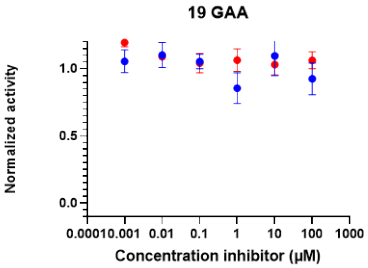
Concentration inhibitor (μM)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Normalized activity
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0.0
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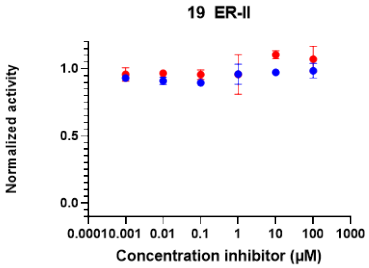
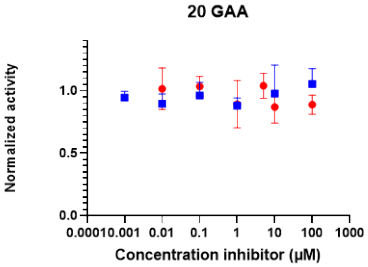
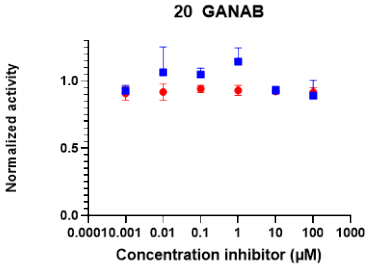
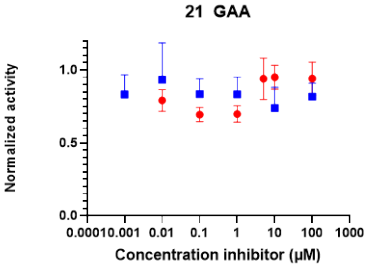
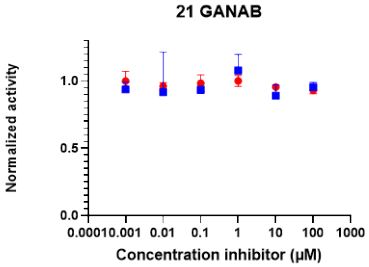
Concentration inhibitor (μM)


19 ER-II
Normalized activity
1.0
0.5
0.0
0.00010.001 0.01 0.1 1 10 100 1000
Concentration inhibitor (μM)

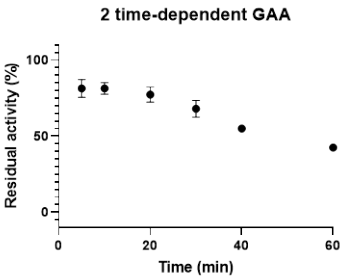

20 GAA
Normalized activity
1.0
0.5
0.0
0.00010.001 0.01 0.1 1 10 100 1000
Concentration inhibitor (μM)

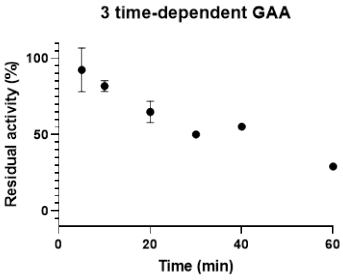

20 GANAB
Normalized activity
1.0
0.5
0.0
0.00010.001 0.01 0.1 1 10 100 1000
Concentration inhibitor (μM)

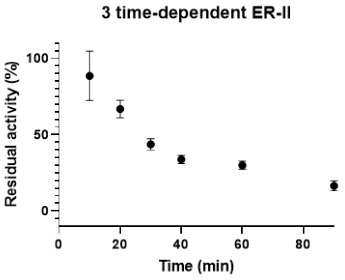

21 GAA
Normalized activity
1.0
0.5
0.0
0.00010.001 0.01 0.1 1 10 100 1000
Concentration inhibitor (μM)

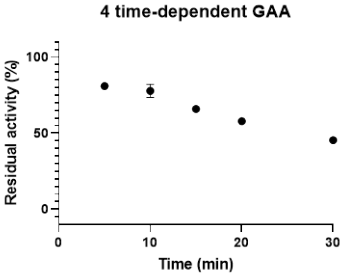

21 GANAB
Normalized activity
1.0
0.5
0.0
0.00010.001 0.01 0.1 1 10 100 1000
Concentration inhibitor (μM)

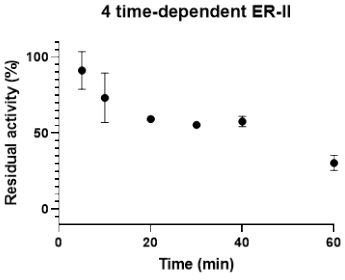
Time dependent inhibition relating to covalent or non-covalent inhibition

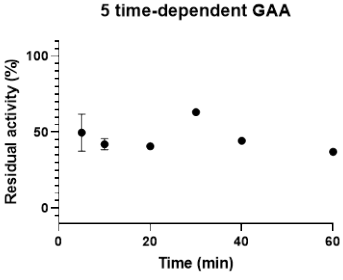


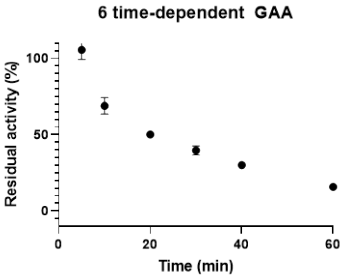


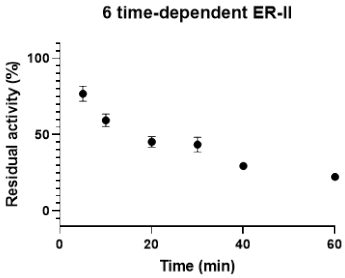


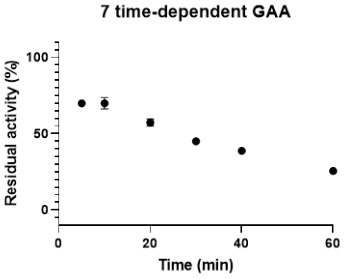


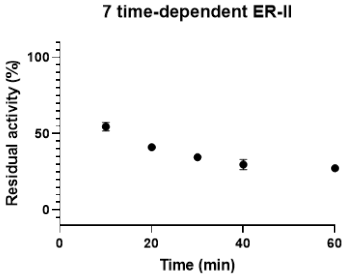


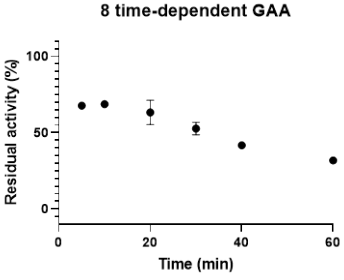


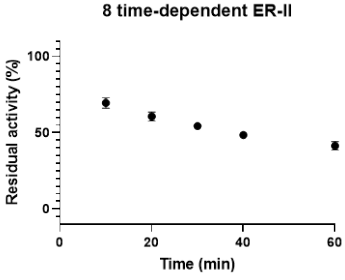

6 time-dependent GAA
Residual activity (%)
Time (min)

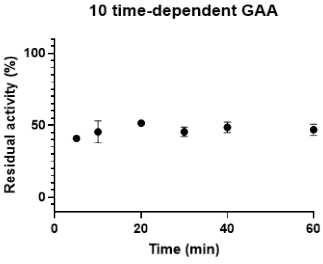

6 time-dependent ER-II
Residual activity (%)
Time (min)

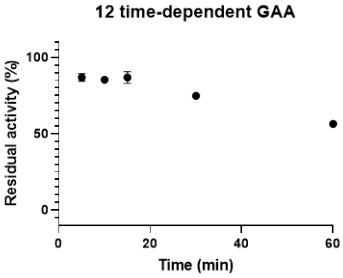

7 time-dependent GAA
Residual activity (%)
Time (min)

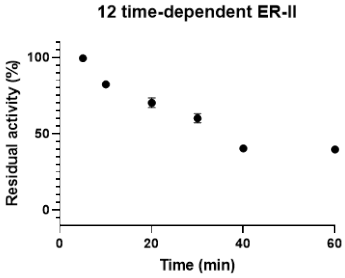

7 time-dependent ER-II
Residual activity (%)
Time (min)

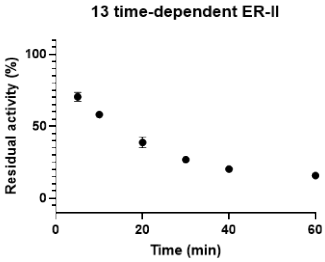

8 time-dependent GAA
Residual activity (%)
Time (min)

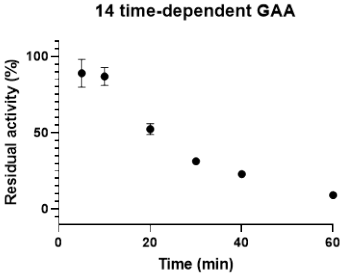

8 time-dependent ER-II
Residual activity (%)
Time (min)

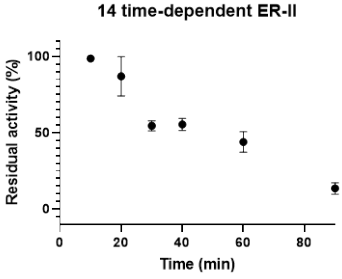

10 time-dependent GAA
Residual activity (%)
Time (min)


12 time-dependent GAA
Residual activity (%)
100
50
0
0
20
40
60
Time (min)


12 time-dependent ER-II
Residual activity (%)
100
50
0
0
20
40
60
Time (min)


13 time-dependent ER-II
Residual activity (%)
100
50
0
0
20
40
60
Time (min)


14 time-dependent GAA
Residual activity (%)
100
50
0
0
20
40
60
Time (min)

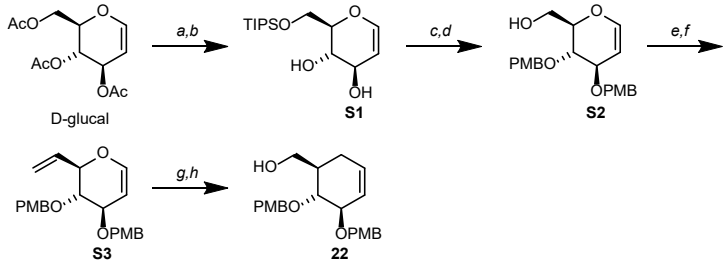

14 time-dependent ER-II
Residual activity (%)
100
50
0
0
20
40
60
80
Time (min)

Molecular modeling

The conformational free energy landscapes of the best cyclophellitol inhibitors (1,2-epoxide **12**, 1,2-aziridine **13** and 1,2-cyclic sulfate **14**) were computed by means of quantum mechanical calculations, with the Car-Parrinello approach,[32] based on the density functional theory (DFT),[33,34] using the CPMD 3.15.1 program.[35] The epoxide, aziridine and cyclic sulfate derivatives were enclosed in orthorhombic boxes of size 12.44Å x 14.57Å x 11.26Å, 13.81Å x 13.12Å x 12.90Å and 12.78Å x 13.53Å x 10.28Å, respectively. The electronic structure was computed using the Perdew, Burke and Ernzerhoff generalized gradient-corrected approximation (PBE)[36] which has been proven to give a good performance in previous works on GHs and glycosyltransferases,[37] besides being proved that it is the cheapest computational method which reaches chemical accuracy. [38] Kohn-Sham orbitals were expanded in a plane wave basis set with a kinetic energy cutoff of 70 Ry. Norm-conserving Trouiller-Martins pseudopotentials[39] were employed. The fictitious electronic mass and time step were set to 700 and 5 a.u. The conformational free energy landscape were calculated *via* the enhanced sampling method called metadynamics[40]; using the CPMD program and the Plumed driver.[41] The collective variables (CVs) used are the Cremer-Pople puckering coordinates[42] θ and ϕ. Initially, the height of these Gaussian terms was set at 0.6 kcal·mol^{-1} and a new Gaussian-like potential was added every 500 molecular dynamics steps. Once the whole free energy space was explored, the height of the Gaussian terms was reduced to 0.35 kcal·mol^{-1}. The width of the Gaussian terms was set to 0.10 Å. The number of deposited gaussians were 12000, 6448 and 12000 corresponding to 720, 387 and 720ps for aziridine **12**, epoxide **13** and cyclic sulfate **14**, respectively. The statistical error calculated with the free energy estimator developed by Tiwary[42] was below 1 kcal·mol^{-1}.

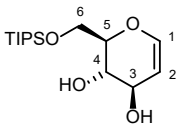
Synthetic procedures.

Scheme S5. Synthesis of key intermediate **22** according to procedures of crotti and co-workers.[26]



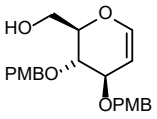
[a]Reagents and conditions: *a)* K_2CO_3, MeOH, 3 h, 40 °C; *b)* TIPSCl, imidazole, DMF, 16 h, rt, (85%); *c)* PMBCl, NaH, DMF, 16 h, rt; *d)* TBAF, THF, 1 h, rt (89%); *e)* Dess-Martin periodinane, $NaHCO_3$, DCM, 3 h, rt; *f)* Ph_3PCH_3Br, *n*-BuLi, THF, 16 h, -78 °C to rt (62%); *g)* diphenyl ether, 3 h, 225 °C; *h)* $NaBH_4$, THF:EtOH (2:1, v:v), 30 min., 0 °C (97%).

6-*O*-Triisopropylsilyl-D-glucal (S1).



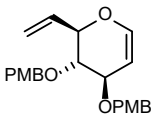
3,4,6-Tri-*O*-acetyl-D-glucal (109 g, 400 mmol) was dissolved in MeOH (400 mL, 1.0 M). K_2CO_3 (16.6 g, 120 mmol, 0.3 eq.) was added and the reaction mixture was stirred for 3 hours at 40 °C. Upon full conversion was observed (R_f 0.4 (MeOH:DCM, 2:8 v:v)), the product was concentrated, co-evaporated twice using DMF and dissolved in DMF (800 mL, 0.5 M). Imidazole (81.6 g, 1.2 mol, 3.0 eq.) was added and the reaction mixture was cooled on ice. Triisoproylsilyl chloride (TIPSCl; 112 mL, 520 mmol, 1.3 eq.) was added and the solution was stirred overnight at room temperature. Upon full conversion (R_f 0.6 (EtOAc:pentane, 1:1 v:v)), the reaction was quenched by addition of MeOH (10 mL) and subsequently concentrated to dryness. The concentrated product was extracted with EtOAc (3x) and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash column chromatography (20:80 EtOAc:pentane → 60:40 EtOAc:pentane) yielded title compound **S1** (103 g, 340 mmol, 85% over 2 steps). Spectral data was in accordance with literature precedence.[26] ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 6.26 (dd, *J* = 6.0, 1.8 Hz, 1H, H-1), 4.68 (dd, *J* = 6.1, 2.2 Hz, 1H, H-2), 4.28 – 4.21 (m, 1H, H-3), 4.05 – 3.95 (m, 3H, 3-OH, H-6), 3.81 – 3.74 (m, 2H, H-4, H-5), 3.56 (d, *J* = 5.3 Hz, 1H, 4-OH), 1.17 – 1.00 (m, 21H, Si(C*H*$(CH_3)_2$), Si(CH(C*H*$_3)_2$)); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 144.1 (C-1), 102.6 (C-2), 76.8 (C-5), 72.4 (C-4), 69.5 (C-3), 64.4 (C-6), 18.0, 18.0 (Si(CH(*C*$H_3)_2$)), 11.9 (Si(*C*H$(CH_3)_2$)); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{15}H_{30}O_4SiNa$ 325.1811, found 325.1806.

3,4-Di-*O*-(4-methoxybenzyl)-D-glucal (S2).



To an ice-cooled solution of **S1** (103 g, 341 mmol) in DMF (560 mL, 0.6 M) was added PMBCl (140 mL, 1.0 mol, 3.1 eq.) followed by the portion-wise addition of NaH (60 wt% dispersion in mineral oil; 47.6 g, 1.2 mol, 3.5 eq.). The reaction mixture was slowly warmed to room temperature and stirred overnight. Upon full conversion was observed (R_f 0.5 (EtOAc:pentane 1:9 v:v)), the reaction was quenched on ice by addition of water (25 mL) and concentrated to a fifth of its original volume. The crude product was extracted with Et_2O (3x), and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude was dissolved in THF (120 mL) and TBAF (1.0 M in THF; 360 mL, 360 mmol, 1.05 eq.) was added. The reaction mixture was stirred at room temperature for 1 hour. Upon full conversion (R_f 0.2 (EtOAc:pentane, 3:7 v:v)), the product was concentrated and extracted with Et_2O (3x), after which the organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Recrystallization from EtOAc/pentane yielded title compound **S2** (86.5 g, 224 mmol) as a pale-yellow solid. Flash column chromatography of the mother liquor (20:80 EtOAc:pentane → 70:30 EtOAc:pentane) yielded additional **S2**, giving and overall yield of 116.5 g, 302 mmol, 89% over 2 steps. Spectral data was in accordance with literature precedence.[26] ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.29 – 7.22 (m, 4H, CH_{arom}), 6.91 – 6.84 (m, 4H, CH_{arom}), 6.39 (dd, *J* = 6.1, 1.4 Hz, 1H, H-1), 4.87 (dd, *J* = 6.2, 2.7 Hz, 1H, H-2), 4.78 (d, *J* = 11.0 Hz, 1H, C*H*H PMB), 4.65 (d, *J* = 11.1 Hz, 1H, CH*H* PMB), 4.60 (d, *J* = 11.3 Hz, 1H, C*H*H PMB), 4.51 (d, *J* = 11.2 Hz, 1H, CH*H* PMB), 4.19 (ddd, *J* = 6.2, 2.8, 1.4 Hz, 1H, H-3), 3.92 (ddd, *J* = 8.5, 4.2, 4.2 Hz, 1H, H-5), 3.83 (d, *J* = 4.2 Hz, 2H, H-6), 3.81 (s, 3H, OMe PMB), 3.80 (s, 3H, OMe, PMB), 3.76 (dd, *J* = 8.6, 6.2 Hz, 1H, H-4), 2.05 (bs, 1H, 6-OH); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.5, 159.4 ($C_{q\text{-}arom}$), 144.6 (C-1), 130.4, 130.2 ($C_{q\text{-}arom}$), 129.8, 129.5 (CH_{arom}), 114.0, 114.0, (CH_{arom}), 100.4 (C-2), 77.4 (C-5), 75.3 (C-3), 74.3 (C-4), 73.5, 70.4 (CH_2 PMB), 62.0 (C-6), 55.4 (OMe PMB); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{22}H_{26}O_6Na$ 409.1627, found 409.1622.

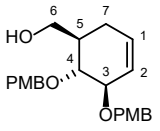
2-Vinyl-3,4-di-*O*-(4-methoxybenzyl)-3,4-dihydro-2*H*-pyran (S3).



Compound **S2** (7.7 g, 20 mmol) was co-evaporated with toluene, dissolved in dry DCM (100 mL, 0.2 M) and kept under a N_2 atmosphere. $NaHCO_3$ (33.6 g, 400 mmol, 20 eq.) was added and the suspension was cooled on ice. Dess-Martin periodinane (12.7 g, 30 mmol, 1.5 eq.) was added, after which the ice bath was removed, and the reaction mixture was stirred at room temperature for 3 hours. Upon full conversion to the aldehyde (R_f 0.5 (EtOAc:pentane, 3:7 v:v)), the reaction was quenched with sat. aq. $NaHCO_3$ and sat. aq. $Na_2S_2O_3$, after which the mixture was filtered over Celite. The product was then extracted with DCM (3x), after which the organic layer was dried over $MgSO_4$, filtered over Celite, and concentrated *in vacuo* to obtain the crude aldehyde as a yellow oil. Wittig reagent Ph_3PCH_3Br (10.7 gr, 30 mmol, 1.5 eq.) was dissolved in dry THF (60 mL) and cooled to –78 °C. *n*-BuLi (11.6 mL, 2.5 M in hexane, 29 mmol, 1.45 eq.) was added over a period of 5 minutes. The reaction mixture was then transferred to an ice bath and stirred for 1 hour. The mixture was cooled back to –78 °C, during which the crude aldehyde was co-evaporated with toluene 3x and dissolved in dry THF (40 mL), followed by the addition to the cooled phosphorus ylide over a period of 10 minutes. The resulting reaction mixture was transferred to an ice bath and stirred overnight while allowing for the reaction to attain to room temperature. Upon full conversion was observed (R_f 0.7 (EtOAc:pentane, 3:7 v:v)), the reaction was quenched with sat. aq. $NaHCO_3$, extracted with

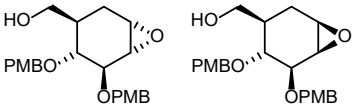
EtOAc (3x) and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Flash column chromatography (dry loading on Celite; 5:95 Et_2O:pentane → 50:50 Et_2O:pentane) yielded title compound **S3** as a yellow oil, which solidified upon standing (4.7 g, 12.3 mmol, 62% over 2 steps). Spectral data was in accordance with literature precedence.[26] ^1H NMR (400 MHz, $CDCl_3$): δ 7.30 – 7.23 (m, 4H, CH_{arom}), 6.90 – 6.85 (m, 4H, CH_{arom}), 6.41 (dd, *J* = 6.1, 1.4 Hz, 1H, H-1), 6.05 (ddd, *J* = 17.1, 10.5, 6.5 Hz, 1H, H-6), 5.43 (ddd, *J* = 17.3, 1.4, 1.4 Hz, 1H, H-7), 5.31 (ddd, *J* = 10.6, 1.4, 1.4 Hz, 1H, H-7), 4.87 (dd, *J* = 6.2, 2.7 Hz, 1H, H-2), 4.71 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 4.62 (d, *J* = 10.9 Hz, 1H, CH*H* PMB), 4.58 (d, *J* = 11.3 Hz, 1H, C*H*H PMB), 4.53 (d, *J* = 11.3 Hz, 1H, CH*H* PMB), 4.31 (dddd, *J* = 8.0, 6.5, 1.3, 1.3 Hz, 1H, H-5), 4.17 (ddd, *J* = 6.2, 2.9, 1.5 Hz, 1H, H-3), 3.81 (s, 3H, OMe PMB), 3.81 (s, 3H, OMe PMB), 3.57 (dd, *J* = 8.6, 6.2 Hz, 1H, H-4); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 159.4, 159.3 ($C_{q\text{-}arom}$), 144.5 (C-1), 134.5 (C-6), 130.6, 130.3 ($C_{q\text{-}arom}$), 129.8, 129.5 (CH_{arom}), 118.4 (C-7), 113.9, 113.9 (CH_{arom}), 100.6 (C-2), 78.2 (C-5), 78.0 (C-4), 75.3 (C-3), 73.6, 70.5 (CH_2 PMB), 55.4 (OMe PMB); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{23}H_{26}O_5Na$ 405.1678, found 405.1673.

3,4-Di-*O*-(4-methoxybenzyl)-carba-D-glucal (22).



Compound **S3** (4.7 g, 12.3 mmol) was co-evaporated with toluene and dissolved in anhydrous diphenyl ether (48 mL, 0.25 M). The resulting solution was transferred to microwave reaction vials which were subsequently purged with N_2 and placed in an aluminum heating block and heated to 225 °C for 3 hours. After full conversion to the aldehyde was observed (R_f 0.5 (EtOAc:pentane, 3:7 v:v)), the yellow solution was directly poured into a stirring suspension of $NaBH_4$ (705 mg, 18.6 mmol, 1.5 eq.) in a mixture of THF:EtOH (2:1 v:v, 120 mL, 0.1 M). The reaction mixture was stirred at room temperature for 30 minutes. Upon full conversion (R_f 0.2 (EtOAc:pentane, 3:7 v:v)), the reaction was quenched with sat. aq. $NaHCO_3$. The mixture was extracted with EtOAc (3x), after which the combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash column chromatography (30:70 EtOAc:pentane → 40:60 EtOAc:pentane) yielded title compound **22** (4.6 g, 11.9 mmol, 97% over 2 steps) as a yellow oil which solidified upon standing. Spectral data was in accordance with literature precedence.[26] ^1H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.32 – 7.20 (m, 4H, CH_{arom}), 6.90 – 6.81 (m, 4H, CH_{arom}), 5.71 (dddd, *J* = 10.2, 4.0, 1.8, 1.8 Hz, 1H, H-1), 5.68 – 5.63 (m, 1H, H-2), 4.88 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 4.66 – 4.61 (m, 2H, CH*H* PMB, C*H*H PMB), 4.56 (d, *J* = 11.1 Hz, 1H, CH*H* PMB), 4.17 (ddd, *J* = 7.4, 3.4, 1.7 Hz, 1H, H-3), 3.76 (s, 3H, OMe PMB), 3.75 (s, 3H, OMe PMB), 3.62 – 3.52 (m, 3H, H-4, H-6), 2.87 (dd, *J* = 8.1, 3.3 Hz, 1H, 6-OH), 2.09 (ddd, *J* = 17.8, 4.8, 4.8 Hz, 1H, H-7), 2.02 – 1.91 (m, 1H, H-5), 1.85 (dddd, *J* = 18.0, 10.3, 2.8, 2.7 Hz, 1H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.3, 159.2, 130.5, 130.5 ($C_{q\text{-}arom}$), 129.8, 129.4 (CH_{arom}), 128.0 (C-1), 126.0 (C-2), 113.9, 113.8 (CH_{arom}), 81.5 (C-4), 80.9 (C-3), 73.8, 70.9 (CH_2 PMB), 65.3 (C-6), 55.2, 55.2 (OMe PMB), 40.5 (C-5), 28.0 (C-7); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{23}H_{28}O_5Na$ 407.1834, found 407.1829.

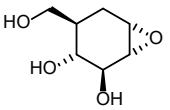
1,2-Anhydro-3,4-di-*O*-(4-methoxybenzyl)-carba-α-D-glucose (23) and 1,2-Anhydro-3,4-di-*O*-(4-methoxybenzyl)-carba-β-D-mannose (24).



Compound **22** (0.52 g, 1.35 mmol) was dissolved in DCM (13.5 mL, 0.1 M) and *m*-CPBA (0.47 g, 2.7 mmol, 2.0 eq.) was added and stirred overnight at room temperature. Upon full conversion (R_f 0.3 and 0.2 for **23** and **24** respectively (EtOAc:Pentane, 1:1 v:v)), the reaction was quenched with sat. aq. $NaHCO_3$ and sat. aq. NaS_2O_3 followed by extraction with EtOAc. The organic layer was then washed with brine and subsequently dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash column chromatography (40:60 EtOAc:pentane → 70:30 EtOAc:pentane) yielded title compounds **23** (284 mg, 0.71 mmol, 53%) and **24** (142 mg, 0.36 mmol, 26%).

Analytical data for **23**: 1H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.38 – 7.15 (m, 4H, CH_{arom}), 6.98 – 6.80 (m, 4H, CH_{arom}), 4.84 (d, *J* = 11.0 Hz, 1H, C*H*H PMB), 4.76 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 4.65 (d, *J* = 11.0 Hz, 1H, CH*H* PMB), 4.61 (d, *J* = 11.0 Hz, 1H, CH*H* PMB), 3.81 (s, 3H, OMe), 3.82 – 3.77 (m, 4H, OMe, H-3), 3.56 (dd, *J* = 11.0, 3.0 Hz, 1H, H-6), 3.45 (dd, *J* = 11.0, 6.2 Hz, 1H, H-6), 3.30 (dd, *J* = 10.7, 8.0 Hz, 1H, H-4), 3.22 (ddd, *J* = 3.5, 1.7, 1.7 Hz, 1H, H-1), 3.13 (dd, *J* = 3.8, 0.7 Hz, 1H, H-2), 2.14 – 2.05 (m, 1H, H-7), 1.77 – 1.57 (m, 2H, H-5, H-7); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 159.6, 159.5, 130.3 ($C_{q\text{-}arom}$), 130.1 (CH_{arom}), 129.9 ($C_{q\text{-}arom}$), 129.4, 114.1 (CH_{arom}), 82.0 (C-4), 80.7 (C-3), 74.0, 72.2 (CH_2 PMB), 65.3 (C-6), 55.4 (OMe), 55.4 (OMe), 54.1 (C-2), 52.9 (C-1), 34.4 (C-5), 27.3 (C-7). HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{23}H_{28}O_6Na$ 423.1784, found 423.1778.
Analytical data for **24**: 1H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.40 – 7.21 (m, 4H, CH_{arom}), 6.97 – 6.80 (m, 4H, CH_{arom}), 4.88 (d, *J* = 10.8 Hz, 1H, C*H*H PMB), 4.79 – 4.73 (m, 2H, C*H*H PMB, CH*H* PMB), 4.59 (d, *J* = 10.7 Hz, 1H, CH*H* PMB), 3.84 (dd, *J* = 8.2, 1.8 Hz, 1H, H-3), 3.81 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.59 (dd, *J* = 10.4, 8.1 Hz, 1H, H-4), 3.49 – 3.43 (m, 2H, H-6), 3.30 (dd, *J* = 4.0, 1.8 Hz, 1H, H-2), 3.25 (dd, *J* = 4.9, 4.0 Hz, 1H, H-1), 2.49 (bs, 1H, 6-OH), 1.97 (ddd, *J* = 14.4, 5.4, 5.4 Hz, 1H, H-7), 1.81 – 1.60 (m, 2H, H-5, H-7); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 159.5, 159.4, 130.4, 130.3 ($C_{q\text{-}arom}$), 130.2, 129.7, 114.1, 114.0 (CH_{arom}), 81.5 (C-3), 79.8 (C-4), 75.0, 72.0 (CH_2 PMB), 64.8 (C-6), 55.4, 55.4 (OMe), 55.3 (C-2), 53.5 (C-1), 41.3 (C-5), 25.8 (C-7); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{23}H_{28}O_6Na$ 423.1784, found 423.1780.

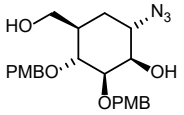
1,2-Anhydro-carba-α-D-glucose (12).



Compound **23** (116 mg, 0.29 mmol) was dissolved in MeOH (14 mL, 0.02 M,) and 5% palladium on carbon (12.3 mg, 60 µmol, 0.2 eq.) was added. The system was flushed with nitrogen and subsequently with hydrogen. The mixture continued to stir for 1 hour at rt whilst kept under a positive hydrogen atmosphere. Upon full conversion was observed (R_f 0.3 (MeOH:DCM, 1:9 v:v)) the mixture was filtered over Celite, and rinsed with MeOH. The filtrate was concentrated under reduced pressure. Flash column chromatography (5:95 MeOH:DCM → 10:90 MeOH:DCM) yielded the title compound **12** (38.6 mg, 0.24 mmol, 83%). Spectral data was in accordance with literature precedence.[27] 1H NMR (500 MHz, D_2O, HH-COSY, HSQC): δ 3.68 (dd, *J* = 8.5, 0.8 Hz, 1H, H-3), 3.65 (m, 2H, H-6), 3.43 (ddd, *J* = 4.0, 2.0, 1.9 Hz, 1H, H-1), 3.25 (dd, *J* = 11.4, 8.4 Hz, 1H, H-4), 3.20 (dd, *J* = 3.9, 0.8 Hz, 1H, H-2), 2.24 (dddd, *J* = 15.4, 4.5, 2.2, 0.8 Hz, 1H, H-7), 1.80 (ddd, *J* = 15.4, 12.1, 1.8 Hz, 1H, H-7), 1.51 (ddddd, *J* = 9.6, 9.5, 4.2, 4.1, 4.0 Hz, 1H, H-5); ^{13}C NMR (126 MHz, D_2O, HSQC):

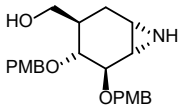
δ 73.0 (C-4), 71.9 (C-3), 61.8 (C-6), 57.0 (C-2), 54.3 (C-1), 34.4 (C-5), 26.5 (C-7); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_7H_{12}O_4Na$ 183.0633, found 183.0628.

1-Deoxy-1-azido-3,4-di-*O*-(4-methoxybenzyl)-carba-α-D-mannose (25).



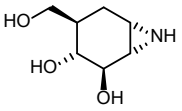
Compound **24** (200 mg, 0.5 mmol) was dissolved in DMF (5.0 mL, 0.1 M) followed by the addition of NaN_3 (488 mg, 7.5 mmol, 15 eq.). The reaction mixture was heated to 130 °C and stirring continued for 16 hours. Upon full conversion was observed (R_f 0.3 (EtOAc:pentane, 3:7 v:v)), the mixture was diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (40:60 → 50:50; EtOAc:pentane) yielded the title compound (133 mg, 0.3 mmol, 60%). 1H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.34 – 7.18 (m, 4H, CH_{arom}), 6.97 – 6.74 (m, 4H, CH_{arom}), 4.79 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 4.65 (d, *J* = 11.1 Hz, 1H, C*H*H PMB), 4.59 (d, *J* = 11.1 Hz, 1H, CH*H* PMB), 4.55 (d, *J* = 10.9 Hz, 1H, CH*H* PMB), 3.94 – 3.87 (m, 2H, H-1, H-2), 3.81 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.72 (dd, *J* = 8.3, 3.0 Hz, 1H, H-3), 3.65 – 3.58 (m, 3H, H-4, H-6), 2.58 (d, *J* = 2.3 Hz, 1H, 2-OH), 2.04 (dd, *J* = 6.9, 4.5 Hz, 1H, 6-OH), 1.96 – 1.88 (m, 1H, H-5), 1.83 (ddd, *J* = 14.4, 11.4, 3.2 Hz, 1H, H-7), 1.67 (dddd, *J* = 14.0, 4.0, 4.0, 1.1 Hz, 1H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.7, 159.5, 130.4 ($C_{q\text{-}arom}$), 129.9 (CH_{arom}), 129.9 ($C_{q\text{-}arom}$), 129.8, 114.2, 114.1 (CH_{arom}), 81.3 (C-3), 78.3 (C-4), 74.3 (CH_2 PMB), 72.7 (CH_2 PMB), 69.7 (C-2), 64.9 (C-6), 60.0 (C-1), 55.4 (OMe), 39.2 (C-5), 26.5 (C-7); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{23}H_{29}N_3O_6Na$ 466.1954, found 466.1949.

1,2-Dideoxy-1,2-azabicyclo[4.1.0]-3,4-di-*O*-(4-methoxybenzyl)-carba-α-D-glucose (26).



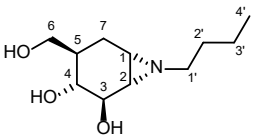
Compound **25** (133 mg, 0.3 mmol) was dissolved in anhydrous acetonitrile (3.0 mL, 0.1 M) followed by the addition of polymer bound triphenyl phosphine (~3 mmol/gram loading, 200 mg, 0.6 mmol, 2.0 eq.) and stirred for 16 hours at 60 °C under protective atmosphere. Upon full conversion was observed (R_f 0.3 (MeOH:DCM, 1:9, v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ solution and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (3:97 → 5:95; MeOH:DCM) yielded the title compound (51 mg, 0.13 mmol, 43%) as a colorless oil. 1H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.40 – 7.29 (m, 2H, CH_{arom}), 7.29 – 7.18 (m, 2H, CH_{arom}), 6.94 – 6.82 (m, 4H, CH_{arom}), 4.85 (d, *J* = 11.0 Hz, 1H, C*H*H PMB), 4.74 (d, *J* = 11.0 Hz, 1H, C*H*H PMB), 4.61 (m, , 2H, CH*H* PMB, CH*H* PMB), 3.81 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.75 (d, *J* = 7.8 Hz, 1H, H-3), 3.52 (dd, *J* = 11.0, 3.2 Hz, 1H, H-6), 3.45 (dd, *J* = 11.0, 6.8 Hz, 1H, H-6), 3.28 (dd, *J* = 10.9, 7.8 Hz, 1H, H-4), 2.64 (s, 1H, 6-OH), 2.33 (d, *J* = 5.7 Hz, 1H, H-1), 2.24 (d, *J* = 5.9 Hz, 1H, H-2), 1.95 (ddd, *J* = 13.7, 4.2, 1.5 Hz, 1H, H-7), 1.69 (dddd, *J* = 14.1, 7.1, 7.1, 3.5 Hz, 1H, H-5), 1.52 (dd, *J* = 13.6, 13.6 Hz, 1H, H-7), 0.59 (s, 1H, NH); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.4, 130.5, 130.3 ($C_{q\text{-}arom}$), 130.0, 129.7, 114.0 (CH_{arom}), 83.2 (C-4), 82.4 (C-3), 73.9 (CH_2 PMB), 71.9 (CH_2 PMB), 66.1 (C-6), 55.4 (OMe), 55.4 (OMe), 34.6 (C-5), 33.2 (C-2), 30.4 (C-1), 27.5 (C-7); HRMS (ESI) m/z: [M+H$^+$] calcd for $C_{23}H_{30}NO_5$ 400.2124, found 400.2118.

1,2-Dideoxy-1,2-azabicyclo[4.1.0]-carba-α-D-glucose (13).



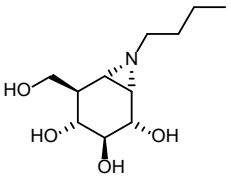
Ammonia (3.0 mL) was condensed at -60 °C followed by the addition of sodium metal (39.0 mg, 1.6 mmol, 20 eq.) and stirring continued for 30 minutes at -60 °C. Compound **26** (32 mg, 80 µmol) was dissolved in THF (1.0 mL) and slowly added to the prepared solution. After stirring for 1 hour at -60 °C, the reaction was quenched by the dropwise addition of water. The mixture was allowed to attain to room temperature followed by direct concentration under reduced pressure. Flash column chromatography (20:80 MeOH:DCM → 30:70 MeOH:DCM) yielded the title compound (11.7 mg, 74 µmol, 92%) as a colorless oil. TLC: R_f 0.3 (MeOH:DCM, 3:7, v:v). ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 3.64 – 3.58 (m, 2H, H-6), 3.53 (d, *J* = 8.2 Hz, 1H, H-3), 3.13 (dd, *J* = 11.1, 8.2 Hz, 1H, H-4), 2.36 (bs, 1H, H-1), 2.17 (d, *J* = 6.1 Hz, 1H, H-2), 2.05 (dd, *J* = 14.7, 4.4 Hz, 1H, H-7), 1.65 (dd, *J* = 13.3, 13.3 Hz, 1H, H-7), 1.47 (ddddd, *J* = 11.7, 11.7, 4.8, 4.8, 4.8 Hz, 1H, H-5); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 76.5 (C-4), 75.2 (C-3), 64.4 (C-6), 36.5 (C-2, C-5), 31.4 (C-1), 28.3 (C-7); HRMS (ESI) m/z: [M+H$^+$] calcd for $C_7H_{14}NO_3$ 160.0974, found 160.0969.

***N*-Butyl-1,2-dideoxy-1,2-azabicyclo[4.1.0]-carba-α-D-glucose (17).**



Compound **13** (8.0 mg, 50 µmol) was dissolved in anhydrous DMF (1.0 mL, 0.05 M) followed by the addition of butyl iodide (12 µL, 0.1 mmol, 2.0 eq.) and K_2CO_3 (21 mg, 0.15 mmol, 3.0 eq.). The reaction was stirred overnight at 80 °C under protective atmosphere. Upon full conversion was observed (R_f 0.6 (MeOH:DCM, 3:7, v:v)), the reaction mixture was concentrated and purified by flash column chromatography (4:96 MeOH:DCM → 12:88 MeOH:DCM) yielded the title compound (9.0 mg, 42 µmol, 84%) as a colorless oil. ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 3.61 (m, 2H, H-6), 3.54 (d, *J* = 8.2 Hz, 1H, H-3), 3.09 (dd, *J* = 11.0, 8.2 Hz, 1H, H-4), 2.37 – 2.24 (m, 2H, H-1'), 2.08 (ddd, *J* = 14.2, 4.5, 1.3 Hz, 1H, H-7), 1.80 (ddd, *J* = 6.4, 3.5, 1.2 Hz, 1H, H-1), 1.66 – 1.47 (m, 5H, H-2, H-7, H-2', H-5), 1.45 – 1.34 (m, 2H, H-3'), 0.95 (t, *J* = 7.4 Hz, 3H, H-4'); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 76.3 (C-4), 74.9 (C-3), 64.5 (C-6), 61.4 (C-1'), 45.7 (C-2), 40.9 (C-1), 37.5 (C-5), 32.8 (C-2'), 28.2 (C-7), 21.6 (C-3'), 14.4 (C-4'); HRMS (ESI) m/z: [M+H$^+$] calcd for $C_{11}H_{22}NO_3$ 216.1600, found 216.1594.

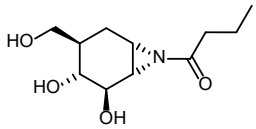
***N*-Butyl-1,7-*epi*-cyclophellitol aziridine (7).**



Compound **3** (8.0 mg, 46 µmol) was dissolved in anhydrous DMF (0.5 mL, 0.1 M) followed by the addition of butyl iodide (16 µL, 137 µmol, 3.0 eq.) and K_2CO_3 (31.5 mg, 228 µmol, 5.0 eq.). The reaction was stirred for 40 hours at 110 °C under protective atmosphere. Upon full conversion was observed (R_f 0.5 (H_2O:MeCN, 4:1, v:v)), the reaction mixture was concentrated and purified by flash column chromatography (10:90 MeOH:DCM → 67:33 MeOH:DCM) followed by reversed phase silica gel purification (95:5 H_2O:MeCN → 1:1 H_2O:MeCN) yielded the title compound (2.5 mg, 11 µmol, 24%) as a colorless solid. ^{1}H NMR (500 MHz, D_2O, HH-COSY, HSQC): δ 3.91 (dd, *J* = 11.2, 3.5 Hz, 1H, H-6), 3.84 (dd, *J* = 8.8, 3.8 Hz, 1H, H-2), 3.73 (dd, *J* = 11.2, 6.6 Hz, 1H, H-6), 3.33 (dd, *J* = 10.2, 8.8 Hz, 1H, H-3), 3.21 (dd, *J* = 10.2, 10.2 Hz, 1H, H-4), 2.31 (dd, *J* = 8.5, 6.7 Hz, 2H, H-1'), 2.07 (dd, *J* = 6.6, 3.9 Hz, 1H, H-1), 1.93 – 1.85 (m, 1H, H-5), 1.83 (dd, *J* = 6.6, 0.9 Hz, 1H, H-7), 1.54 (tq, *J* = 13.1, 8.2

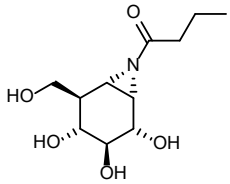
Hz, 2H, H-2'), 1.39 – 1.30 (m, 2H, H-3'), 0.90 (t, *J* = 7.4 Hz, 3H, H-4'); ^{13}C NMR (126 MHz, D_2O, HSQC): δ 74.1 (C-3), 71.5 (C-2), 70.3 (C-4), 61.6 (C-6), 59.8 (C-1'), 44.4 (C-5), 44.3 (C-1), 40.6 (C-7), 30.8 (C-2'), 19.9 (C-3'), 13.3 (C-4'); HRMS (ESI) m/z: [M+H$^+$] calcd for $C_{11}H_{22}NO_4$ 232.15433, found 232.15440.

***N*-Butyryl-1,2-dideoxy-1,2-azabicyclo[4.1.0]-carba-α-D-glucose (18).**



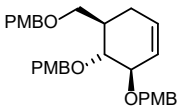
Compound **13** (8.0 mg, 50 µmol) was dissolved in MeOH (1.0 mL, 0.05 M) and cooled on ice. Subsequently, Et_3N (28 µL, 0.2 mmol, 4.0 eq.) and butyryl chloride (11 µL, 0.1 mmol, 2.0 eq.) were added respectively. Stirring continued for 30 minutes while the reaction mixture was kept on 0 °C. Upon full conversion was observed (R_f 0.5 (MeOH:DCM, 2:8, v:v)), the reaction mixture was quenched by adding sat. aq. $NaHCO_3$ (3.0 mL) and then concentrated under reduced pressure to afford the crude product. Flash column chromatography (0:100 MeOH:DCM → 10:90 MeOH:DCM) yielded the title compound (5.7 mg, 25 µmol, 50%) as a colorless oil. ^{1}H NMR (400 MHz, Acetone-d6, HH-COSY, HSQC): δ 4.51 (d, *J* = 4.6 Hz, 1H, 3-OH/6-OH), 4.28 (d, *J* = 3.6 Hz, 1H, 4-OH), 3.71 (d, *J* = 5.4 Hz, 1H, 3-OH/6-OH), 3.62 (m, 3H, H-3, H-6), 3.22 (ddd, *J* = 10.7, 7.9, 2.9 Hz, 1H, H-4), 2.77 (ddd, *J* = 5.9, 2.7, 1.5 Hz, 1H, H-1), 2.60 (d, *J* = 5.8 Hz, 1H, H-2), 2.37 (t, *J* = 7.3 Hz, 2H, 2'), 2.11 (ddd, *J* = 13.8, 3.6, 1.3 Hz, 1H, H-7), 1.71 – 1.49 (m, 4H, H-5, H-7, H-3'), 0.91 (t, *J* = 7.4 Hz, 3H, H-4'); ^{13}C NMR (101 MHz, Acetone-d6, HSQC): δ 185.8 (C-1'), 77.2 (C-4), 74.4 (C-3), 64.8 (C-6), 41.8 (C-2), 38.8 (C-2'), 37.7 (C-1), 36.4 (C-5), 27.9 (C-7), 19.0 (C-3'), 14.0 (C-4'); HRMS (ESI) m/z: [M+H$^+$] calcd for $C_{11}H_{20}NO_4$ 230.1392, found 230.1387.

***N*-Butyryl-1,7-*epi*-cyclophellitol aziridine (8).**



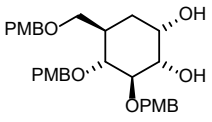
Compound **3** (5.0 mg, 29 µmol) was dissolved in anhydrous MeOH (1.0 mL, 0.03 M) under an inert atmosphere and cooled on ice. Subsequently, Et_3N (59 µL, 0.43 mmol, 15 eq.) and butyryl chloride (8.9 µL, 86 µmol, 3.0 eq.) were added respectively. Stirring continued for 1 hour while the reaction mixture was allowed to attain to room temperature. Upon full conversion was observed (R_f 0.2 (MeOH:DCM, 1:9, v:v)), the reaction mixture was quenched by adding sat. aq. $NaHCO_3$ (3.0 mL) and then concentrated under reduced pressure to afford the crude product. The residue was purified by reversed phase silica gel purification (95:5 H_2O:MeCN → 0:100 H_2O:MeCN) providing aziridine **8** (4.5 mg, 19 µmol, 64%) as colourless solid. ^{1}H NMR (500 MHz, D_2O, HH-COSY, HSQC): δ 3.92 (dd, *J* = 11.3, 3.5 Hz, 1H, H-6), 3.86 (dd, *J* = 8.7, 3.3 Hz, 1H, H-2), 3.75 (dd, *J* = 11.3, 6.4 Hz, 1H, H-6), 3.40 (dd, *J* = 10.2, 8.7 Hz, 1H, H-3), 3.30 (dd, *J* = 10.0, 10.0 Hz, 1H, H-4), 3.10 (dd, *J* = 6.1, 3.3 Hz, 1H, H-1), 2.87 (dd, *J* = 6.1, 0.7 Hz, 1H, H-7), 2.49 (t, *J* = 7.4 Hz, 2H, H-2'), 2.04 (ddd, *J* = 9.8, 6.4, 3.4 Hz, 1H, H-5), 1.61 (sxt, *J* = 7.4 Hz, 2H, H-3'), 0.90 (t, *J* = 7.4 Hz, 3H, H-4'); ^{13}C NMR (126 MHz, D_2O, HSQC): δ 189.8 (C-1'), 73.3 (C-3), 70.4 (C-2), 70.1 (C-4), 61.1 (C-6), 44.3 (C-5), 41.9 (C-1), 38.3 (C-7), 38.0 (C-2'), 18.2 (C-3'), 12.9 (C-4'); HRMS (ESI) m/z: [M+H$^+$] calcd for $C_{11}H_{20}NO_5$ 246.13360, found 246.13349.

3,4,6-Tri-*O*-(4-methoxybenzyl)-carba-D-glucal (27).



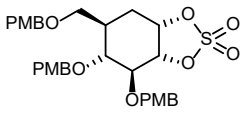
Compound **22** (7.7 g, 20 mmol) was dissolved in DMF (100 mL, 0.2 M) and cooled on ice. Subsequently, PMBCl (4.0 mL, 30 mmol, 1.5 eq.) and NaH (1.6 g, 40 mmol, 2.0 eq.) were added respectively. The mixture was allowed to attain to room temperature and stirred overnight. Upon full conversion was observed (R_f 0.4 (EtOAc:pentane, 3:7, v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (10:90 → 20:80; EtOAc:pentane) yielded the title compound (8.6 g, 17 mmol, 85%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.32 – 7.14 (m, 6H, CH_{arom}), 6.91 – 6.78 (m, 6H, CH_{arom}), 5.79 – 5.69 (m, 1H, H-1), 5.64 (dd, *J* = 10.1, 1.9 Hz, 1H, H-2), 4.79 (d, *J* = 10.6 Hz, 1H, C*H*H PMB), 4.61 (m, 2H, CH_2 PMB), 4.53 (d, *J* = 10.6 Hz, 1H, CH*H* PMB), 4.42 (s, 2H, CH_2 PMB), 4.13 (ddd, *J* = 7.2, 3.6, 1.5 Hz, 1H, H-3), 3.79 (s, 9H, OMe), 3.66 – 3.50 (m, 3H, H-4, H-6), 2.26 – 2.18 (m, 2H, H-7), 2.09 – 1.98 (m, 1H, H-5); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 159.2, 159.2, 131.3, 131.0, 130.8 ($C_{q\text{-}arom}$), 129.7, 129.5, 129.2, 128.5 (C-1), 126.3 (C-2), 113.9, 113.9, 113.8 (CH_{arom}), 80.9 (C-3), 79.3 (C-4), 74.1, 72.8, 71.2 (CH_2 PMB), 70.3 (C-6), 55.4 (OCH_3), 39.4 (C-5), 29.0 (C-7); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{31}H_{36}O_6Na$ 527.2410, found 527.2404.

3,4,6-Tri-*O*-(4-methoxybenzyl)-carba-α-D-glucose (28).



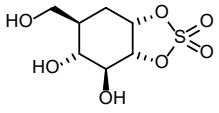
Compound **27** (8.1 g, 16 mmol) was dissolved in a mixture of acetonitrile:EtOAc (1:1, 320 mL, 0.05 M) and cooled on ice. in a second flask, $NaIO_4$ (5.1 gr, 29 mmol, 1.5 eq.) and $RuCl_3$ (232 mg, 1.12 mmol, 0.07 eq.) were dissolved in H_2O (90 mL) and was slowly added to the substrate solution. After 1 hour stirring at 0 ºC, TLC confirmed full conversion (R_f 0.1 (EtOAc:pentane, 1:1, v:v)) and the reaction was quenched by the addition of sat. aq. $Na_2S_2O_3$ and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (50:50 → 70:30; EtOAc:pentane) yielded the title compound (7.8 g, 14.5 mmol, 85%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.33 – 7.11 (m, 6H, CH_{arom}), 6.98 – 6.77 (m, 6H, CH_{arom}), 4.93 (d, *J* = 11.3 Hz, 1H, C*H*H PMB), 4.76 (d, *J* = 10.4 Hz, 1H, C*H*H PMB), 4.59 (d, *J* = 11.3 Hz, 1H, CH*H* PMB), 4.49 (d, *J* = 10.4 Hz, 1H, CH*H* PMB), 4.43 (d, *J* = 11.6 Hz, 1H, C*H*H PMB), 4.38 (d, *J* = 11.6 Hz, 1H, CH*H* PMB), 4.05 (d, *J* = 3.3 Hz, 1H, H-1), 3.81 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.73 (dd, *J* = 9.1, 4.1 Hz, 1H, H-6), 3.67 (dd, *J* = 9.2, 9.2 Hz, 1H, H-3), 3.50 – 3.42 (m, 2H, H-2, H-4), 3.38 (dd, *J* = 9.1, 2.6 Hz, 1H, H-6), 2.36 (d, *J* = 2.2 Hz, 1H, 2-OH), 2.34 (dd, *J* = 1.7, 1.7 Hz, 1H, 1-OH), 2.14 (dddd, *J* = 16.8, 10.3, 3.2, 3.2 Hz, 1H, H-5), 1.88 (ddd, *J* = 14.7, 3.7, 3.7 Hz, 1H, H-7), 1.64 (ddd, *J* = 12.7, 2.3, 2.3 Hz, 1H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.5, 159.3, 159.3, 131.0, 130.9, 130.7 ($C_{q\text{-}arom}$), 129.7, 129.7, 129.4, 114.3, 113.9, 113.9 (CH_{arom}), 83.1 (C-3), 81.0 (C-4), 74.9, 74.6 (CH_2 PMB), 74.4 (C-2), 72.9 (CH_2 PMB), 69.4 (C-6), 68.3 (C-1), 55.4, 55.4 (OMe), 37.5 (C-5), 30.5 (C-7); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{31}H_{38}O_8Na$ 561.2464, found 561.2459.

1,2-*O*-Sulfate-3,4,6-tri-*O*-(4-methoxybenzyl)-carba-α-D-glucose (29).



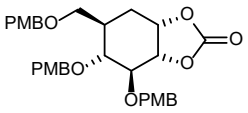
Compound **28** (170 mg, 0.3 mmol) was dissolved in anhydrous DCM (15 mL, 0.02 M) and cooled on ice. Subsequently, Et_3N (166 µL, 1.2 mmol, 4.0 eq.) and $SOCl_2$ (77 µL, 1.05 mmol, 3.5 eq.) were added respectively. The reaction was stirred for 1 hour during which the solution was allowed to attain to room temperature. Upon full conversion was observed (R_f 0.3 (EtOAc:pentane, 3:7, v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ solution and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. The crude product was dissolved in acetonitrile:H_2O (1:1, 3.0 mL, 0.1 M) and cooled on ice. Subsequently, $RuCl_3$ (6.0 mg, 30 µmol, 0.1 eq.) and $NaIO_4$ (77 mg, 0.36 mmol, 1.2 eq.) were dissolved in H_2O (0.5 mL) and slowly added to the substrate solution. After 15 minutes, the reaction was quenched by the addition of sat. aq. $Na_2S_2O_3$ solution and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (20:80 → 30:70; EtOAc:pentane) yielded the title compound (83 mg, 0.14 mmol, 46% over two steps) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.29 – 7.09 (m, 6H, CH_{arom}), 6.89 – 6.80 (m, 6H, CH_{arom}), 5.18 (ddd, *J* = 5.3, 2.9, 2.9 Hz, 1H, H-1), 4.82 – 4.69 (m, 4H, H-2, C*H*H PMB, C*H*H PMB, CH*H* PMB), 4.43 (d, *J* = 10.5 Hz, 1H, CH*H* PMB), 4.40 – 4.33 (m, 2H, C*H*H PMB, CH*H* PMB), 4.10 (dd, *J* = 9.6, 8.1 Hz, 1H, H-3), 3.80 (s, 6H, OMe, OMe), 3.78 (s, 3H, OMe), 3.71 (dd, *J* = 9.2, 3.7 Hz, 1H, H-6), 3.41 (dd, *J* = 9.9, 9.9 Hz, 1H, H-4), 3.37 (dd, *J* = 9.1, 2.4 Hz, 1H, H-6), 2.30 (ddd, *J* = 15.7, 2.9, 2.9 Hz, 1H, H-7), 2.07 – 1.92 (m, 2H, H-5, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.6, 159.5, 159.4, 130.4, 130.1, 130.0 ($C_{q\text{-}arom}$), 129.9, 129.8, 129.6, 129.5, 114.0, 114.0, 113.9, 113.9 (CH_{arom}), 88.4 (C-2), 82.4 (C-3), 82.0 (C-1), 77.8 (C-4), 75.2, 75.0, 73.0 (CH_2 PMB), 68.2 (C-6), 55.4, 55.4 (OMe), 36.5 (C-5), 27.4 (C-7); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{31}H_{36}O_{10}SNa$ 623.1927, found 623.1921.

1,2-*O*-Sulfate-carba-α-D-glucose (14).



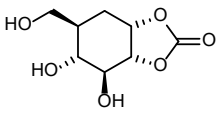
Compound **29** (27.3 mg, 46 µmol) was dissolved in MeOH (5.0 mL, 0.01 M) followed by the addition of $Pd(OH)_2$/C (20% loading, 105 mg, 0.15 mmol, 3.0 eq.). the reaction mixture was kept under 50 atm H_2 atmosphere and stirred for 16 hours. Upon full conversion was observed (R_f 0.2 (MeOH:DCM, 2:8, v:v)), the mixture was filtered over celite and concentrated to yield the crude product. Flash column chromatography (5:95 → 10:90; MeOH:DCM) yielded the title compound (7.5 mg, 31 µmol, 68%) as a colorless oil. ^{1}H NMR (500 MHz, D_2O, HH-COSY, HSQC): δ 5.54 – 5.49 (m, 1H, H-1), 5.02 (dd, *J* = 8.5, 4.8 Hz, 1H, H-2), 4.00 (dd, *J* = 9.9, 8.5 Hz, 1H, H-3), 3.76 (m, 2H, H-6), 3.42 (dd, *J* = 10.1, 10.1 Hz, 1H, H-4), 2.47 (dd, *J* = 12.8, 2.5 Hz, 1H, H-7), 1.95 – 1.83 (m, 2H, H-5, H-7); ^{13}C NMR (126 MHz, D_2O, HSQC): δ 88.9 (C-2), 83.9 (C-1), 74.2 (C-3), 70.3 (C-4), 61.0 (C-6), 37.6 (C-5), 26.3 (C-7); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_7H_{12}O_7SNa$ 263.0201, found 263.0196.

1,2-*O*-Carbonate-3,4,6-tri-*O*-(4-methoxybenzyl)-carba-α-D-glucose (30).



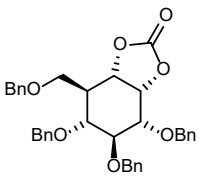
Compound **28** (76.0 mg, 0.14 mmol) was dissolved in DCM (0.7 mL, 0.2 M) and cool on ice. Subsequently, pyridine (50 µL, 0.64 mmol, 4.5 eq.) and triphosgene (27.1 mg, 84 µmol, 0.6 eq.) were added respectively. The reaction was allowed to attain to room temperature and stirred for 1.5 hours. Upon full conversion was observed (R_f 0.8 (EtOAc:pentane, 4:6, v:v)), the mixture was quenched by the addition of sat. aq. $NaHCO_3$ solution and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (30:70 → 40:60; EtOAc:pentane) yielded the title compound (68.1 mg, 0.12 mmol, 86%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.30 – 7.09 (m, 6H, CH_{arom}), 6.91 – 6.81 (m, 6H, CH_{arom}), 4.86 (ddd, *J* = 7.1, 3.5, 3.5 Hz, 1H, H-1), 4.74 – 4.68 (m, 2H, C*H*H PMB, C*H*H PMB), 4.67 – 4.60 (m, 2H, H-2, CH*H* PMB), 4.42 (d, *J* = 10.7 Hz, 1H, CH*H* PMB), 4.40 – 4.32 (m, 2H, C*H*H PMB, CH*H* PMB), 3.80 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.70 (dd, *J* = 8.4, 6.4 Hz, 1H, H-3), 3.62 (dd, *J* = 9.1, 4.1 Hz, 1H, H-6), 3.46 (dd, *J* = 8.7, 8.7 Hz, 1H, H-4), 3.37 (dd, *J* = 9.1, 3.1 Hz, 1H, H-6), 2.21 (ddd, *J* = 15.3, 3.7, 3.7 Hz, 1H, H-7), 1.98 (dddd, *J* = 12.3, 7.4, 3.7, 3.7 Hz, 1H, H-5), 1.89 (ddd, *J* = 15.6, 11.8, 3.9 Hz, 1H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.5, 159.4, 154.6 (C=O), 130.5, 130.3, 129.9 ($C_{q\text{-}arom}$), 129.8, 129.7, 129.4, 114.0, 113.9, 113.9 (CH_{arom}), 82.4 (C-3), 81.1 (C-2), 76.8 (C-4), 76.4 (C-1), 74.2, 73.8, 72.9 (CH_2 PMB), 69.4 (C-6), 55.4, 55.4 (OMe), 36.2 (C-5), 27.3 (C-7); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{32}H_{36}O_9Na$ 587.2257, found 587.2252.

1,2-*O*-Carbonate-carba-α-D-glucose (15).



Compound **30** (113 mg, 0.2 mmol) was dissolved in MeOH (5.0 mL, 0.02 M) followed by the addition of Pd/C (5% loading, 43 mg, 20 µmol, 0.1 eq.). the reaction mixture was kept under 1 atm H_2 atmosphere and stirred for 16 hours. Upon full conversion was observed (R_f 0.2 (MeOH:DCM, 2:8, v:v)), the mixture was filtered over celite and concentrated *in vacuo* to yield the title compound (34 mg, 0.17 mmol, 83%) as a colorless oil. ^{1}H NMR (600 MHz, MeOD, HH-COSY, HSQC): δ 4.87 (ddd, *J* = 6.3, 3.4, 2.4 Hz, 1H, H-1), 4.49 (ddd, *J* = 7.2, 6.5, 0.6 Hz, 1H, H-2), 3.68 (dd, *J* = 10.9, 3.4 Hz, 1H, H-6), 3.61 (dd, *J* = 10.9, 5.4 Hz, 1H, H-6), 3.45 (dd, *J* = 9.5, 7.4 Hz, 1H, H-3), 3.22 (dd, *J* = 9.7, 9.7 Hz, 1H, H-4), 2.24 (dd, *J* = 11.9, 3.1 Hz, 1H, H-7), 1.73 – 1.65 (m, 2H, H-5, H-7); ^{13}C NMR (151 MHz, MeOD, HSQC): δ 157.1 (C=O), 83.5 (C-2), 78.7 (C-1), 77.6 (C-3), 72.2 (C-4), 63.5 (C-6), 39.8 (C-5), 27.9 (C-7); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_8H_{12}O_6Na$ 227.0532, found 227.0526.

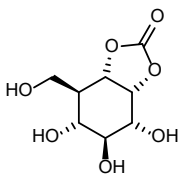
1,7-*Epi*-carbonate-2,3,4,6-tetra-*O*-benzyl-cyclophellitol alkane (32).



Compound **31** (34 mg, 61 µmol) was dissolved in DCM (1.0 mL, 0.06 M) and cooled on ice. Subsequently, pyridine (22 µL, 0.27 mmol, 4.5 eq.) and triphosgene (11 mg, 40 µmol, 0.6 eq.) were added respectively. The reaction was allowed to attain to room temperature and stirred for 1.5 hours. Upon full conversion was observed (R_f 0.8 (EtOAc:pentane, 3:7, v:v)), the mixture was quenched by the addition of sat. aq. $NaHCO_3$ solution and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by

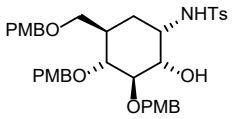
washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (10:90 → 20:80; EtOAc:pentane) yielded the title compound (32 mg, 55 µmol, 90%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.38 – 7.16 (m, 20H, CH_{arom}), 5.00 (dd, *J* = 9.3, 8.1 Hz, 1H, H-7), 4.75 (dd, *J* = 8.1, 3.5 Hz, 1H, H-1), 4.69 (d, *J* = 12.0 Hz, 1H, C*H*H Bn), 4.63 (d, *J* = 9.8 Hz, 1H, C*H*H Bn), 4.61 (d, *J* = 10.7 Hz, 1H, CH*H* Bn), 4.52 – 4.38 (m, 5H, C*H*H Bn, C*H*H Bn, CH*H* Bn, CH*H* Bn, CH*H* Bn), 3.87 (dd, *J* = 3.3, 3.3 Hz, 1H, H-2), 3.86 – 3.81 (m, 2H, H-3, H-6), 3.62 (dd, *J* = 9.4, 2.3 Hz, 1H, H-6), 3.57 (dd, *J* = 12.3, 5.5 Hz, 1H, H-4), 2.55 (dddd, *J* = 11.8, 9.3, 2.3, 2.3 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 154.8 (C=O), 138.2, 138.0, 137.4, 137.2 ($C_{q\text{-}arom}$), 128.7, 128.6, 128.5, 128.3, 128.3, 128.2, 128.1, 127.9, 127.9, 127.8 (CH_{arom}), 81.8 (C-3), 76.4 (C-4), 75.1 (C-2), 74.6 (C-1), 74.3 (C-7), 73.8, 73.6, 73.4, 72.6 (CH_2 Bn), 65.4 (C-6), 42.2 (C-5); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{36}H_{36}O_7Na$ 603.2359, found 603.2353.

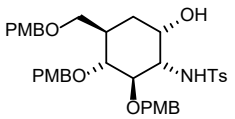
1,7-*Epi*-carbonate-cyclophellitol alkane (9).



Compound **32** (23 mg, 40 µmol) was dissolved in MeOH (1.0 mL, 0.02 M) followed by the addition of $Pd(OH)_2$/C (20% loading, 6.0 mg, 8.0 µmol, 0.2 eq.). the reaction mixture was kept under 1 atm H_2 atmosphere and stirred for 16 hours. Upon full conversion was observed (R_f 0.5 (MeOH:DCM, 3:7, v:v)), the mixture was filtered over celite and concentrated *in vacuo* to yield the title compound (8.4 mg, 40 µmol, quant.) as a colorless oil. ^{1}H NMR (400 MHz, D_2O, HH-COSY, HSQC): δ 5.07 (dd, *J* = 6.2, 4.0 Hz, 1H, H-1), 4.94 (dd, *J* = 9.2, 6.2 Hz, 1H, H-7), 3.88 (dd, *J* = 11.8, 3.9 Hz, 1H, H-6), 3.80 (dd, *J* = 9.6, 4.1 Hz, 1H, H-2), 3.75 (dd, *J* = 11.8, 3.0 Hz, 1H, H-6), 3.63 (dd, *J* = 9.4, 9.4 Hz, 1H, H-3), 3.35 (dd, *J* = 11.5, 9.2 Hz, 1H, H-4), 1.94 – 1.82 (m, 1H, H-5); ^{13}C NMR (101 MHz, D_2O, HSQC): δ 156.4 (C=O), 79.6 (C-1), 76.6 (C-7), 73.3 (C-3), 69.1 (C-2), 67.4 (C-4), 57.7 (C-6), 45.4 (C-5); HRMS (ESI) m/z: [M+H$^+$] calcd for $C_8H_{13}O_7$ 221.0661, found 221.0656.

1-Deoxy-1-(*p*-toluenesulfonamido)-3,4,6-tri-*O*-(4-methoxybenzyl)-carba-α-D-glucose (33) and 2-Deoxy-2-(*p*-toluenesulfonamido)-3,4,6-tri-*O*-(4-methoxybenzyl)-carba-α-D-glucose (34).





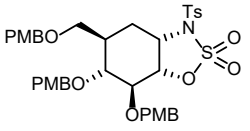
The title compounds were prepared according to a modified literature procedure.[43] Compound **27** (6.9 gr, 13.8 mmol) was dissolved in $CHCl_3$:H_2O (1:1 v/v; 280 mL, 0.05 M), after which were added chloramine-T trihydrate (7.8 gr, 27.6 mmol, 2.0 eq.), benzyltriethylammonium chloride (188 mg, 0.83 mmol, 6 mol%) and potassium osmate (262 mg, 0.71 mmol, 5 mol%). The biphasic reaction mixture was heated to 60 °C and stirred vigorously overnight. Upon full conversion was observed (R_f 0.6 and 0.4 for **33** and **34** respectively (EtOAc:Pentane, 1:1 v:v)), sat. aq. $Na_2S_2O_3$ was added, and the mixture was heated to 60 °C for 0.5-1 hour to effectively quench the unreacted chloramine-T. The organic layer was separated from the aqueous layer, after which the aqueous layer was extracted with EtOAc (3x). The combined organic layers were washed 3x with 1 wt% aq. NaOH, once with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash column chromatography (dry loading on Celite,

30:70 → 50:50; EtOAc:pentane) yielded target compounds **33** (5.1 gr, 7.4 mmol, 54%) as a yellow oil and **34** (2.9 gr, 4.25 mmol, 31%) as an off-white solid, giving an overall yield of 85%.

Analytical data for **33**: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HH-NOESY, HSQC): δ 7.76 – 7.70 (m, 2H, CH_{arom}), 7.29 – 7.12 (m, 8H, CH_{arom}), 6.91 – 6.80 (m, 6H, CH_{arom}), 4.97 (d, *J* = 3.2 Hz, 1H, 1-NH), 4.76 (d, *J* = 11.3 Hz, 1H, C*H*H PMB), 4.63 (d, *J* = 10.7 Hz, 1H, C*H*H PMB), 4.48 (m, 2H, CH*H* PMB, CH*H* PMB), 4.36 (m, 2H, C*H*H PMB, CH*H* PMB), 3.81 (s, 3H, OMe), 3.80 (s, 6H, OMe, OMe), 3.65 (dd, *J* = 9.2, 4.5 Hz, 1H, H-6), 3.55 (dd, *J* = 7.8, 7.8 Hz, 1H, H-3), 3.50 (dd, *J* = 8.1, 8.1 Hz, 1H, H-4), 3.45 (ddd, *J* = 7.8, 3.9, 3.9 Hz, 1H, H-2), 3.42 (dddd, *J* = 5.0, 3.5, 3.2, 3.2 Hz, 1H, H-1), 3.33 (dd, *J* = 9.2, 3.7 Hz, 1H, H-6), 2.69 (d, *J* = 3.3 Hz, 1H, 2-OH), 2.40 (s, 3H, Me Ts), 2.12 (ddddd, *J* = 10.0, 8.2, 4.2, 4.2, 3.2 Hz, 1H, H-5), 2.07 (ddd, *J* = 14.4, 5.0, 3.9 Hz, 1H, H-7), 1.51 (ddd, *J* = 13.9, 10.2, 3.1 Hz, 1H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.5, 159.4, 159.2 , 143.5, 136.7, 130.6, 130.4, 130.4 ($C_{q\text{-}arom}$), 129.8, 129.5, 129.4, 129.3, 127.4, 114.2, 113.9, 113.9 (CH_{arom}), 81.7 (C-3), 79.2 (C-4), 74.3, 73.9, 72.8 (CH_2 PMB), 71.9 (C-2), 69.3 (C-6), 55.4, 55.4, 55.3 (OMe), 51.8 (C-1), 37.4 (C-5), 27.5 (C-7), 21.6 (Me Ts); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{38}H_{45}NO_9SNa$ 714.2713, found 714.2707.

Analytical data for **34**: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HH-NOESY, HSQC): δ 7.68 – 7.62 (m, 2H, CH_{arom}), 7.27 – 7.15 (m, 4H, CH_{arom}), 7.18 – 7.06 (m, 4H, CH_{arom}), 6.92 – 6.76 (m, 6H, CH_{arom}), 4.76 (d, *J* = 4.2 Hz, 1H, 2-NH), 4.71 (d, *J* = 11.3 Hz, 1H, C*H*H PMB), 4.64 (d, *J* = 10.5 Hz, 1H, C*H*H PMB), 4.51 (d, *J* = 11.3 Hz, 1H, CH*H* PMB), 4.43 – 4.28 (m, 3H, CH*H* PMB, C*H*H PMB, CH*H* PMB), 4.15 – 4.10 (m, 1H, H-1), 3.83 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.67 (dd, *J* = 9.0, 4.2 Hz, 1H, H-6), 3.55 (dd, *J* = 9.7, 8.6 Hz, 1H, H-3), 3.40 (dd, *J* = 10.1, 8.6 Hz, 1H, H-4), 3.34 (dd, *J* = 9.0, 2.9 Hz, 1H, H-6), 2.95 (ddd, *J* = 9.7, 4.4, 2.9 Hz, 1H, H-2), 2.41 (s, 3H, Me Ts), 2.26 (d, *J* = 3.2 Hz, 1H, 1-OH), 2.13 – 2.04 (m, 1H, H-5), 1.81 (ddd, *J* = 14.6, 4.0, 4.0 Hz, 1H, H-7), 1.57 (ddd, *J* = 14.9, 12.2, 2.7 Hz, 1H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.6, 159.3, 159.3, 143.7, 136.5, 130.6, 130.5, 130.3 ($C_{q\text{-}arom}$), 129.9, 129.6, 129.6, 129.5, 127.3, 114.3, 113.9, 113.9 (CH_{arom}), 81.1 (C-4), 79.7 (C-3), 74.5, 74.5, 72.9 (CH_2 PMB), 69.4 (C-6), 66.9 (C-1), 59.7 (C-2), 55.5, 55.4, 55.4 (OMe), 37.2 (C-5), 31.3 (C-7), 21.7 (Me Ts); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{38}H_{45}NO_9SNa$ 714.2713, found 714.2707.

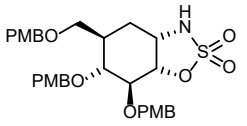
1,2-(*N*-(*p*-tolylsulfonyl),*O*)-sulfamidate-3,4,6-tri-*O*-(4-methoxybenzyl)-carba-α-D-glucose (S4).



Compound **33** (252 mg, 0.36 mmol) was co-evaporated with toluene and dissolved in anhydrous DCM (3.6 mL, 0.1 M). Triethylamine (0.2 mL, 1.4 mmol, 4.0 eq.) was added and the solution was cooled to –78 °C. SO_2Cl_2 (36 μL, 0.44 mmol, 1.2 eq.) was added dropwise, and the reaction mixture was allowed to slowly warm up to 5 °C over a period of 2-3 hours. Upon full conversion was observed (R_f 0.55 (EtOAc:pentane 4:6 v:v)), the reaction was quenched with sat. aq. $NaHCO_3$. The mixture was then extracted with EtOAc (3x), after which the combined organic layers were washed twice with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash column chromatography (20:80 → 40:60; EtOAc:pentane) yielded title compound **31** as a yellow oil (275 mg, 0.36 mmol, quant.). ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.85 – 7.79 (m, 2H, CH_{arom}), 7.32 – 7.27 (m, 2H, CH_{arom}), 7.25 – 7.19 (m, 4H, CH_{arom}), 7.15 – 7.10 (m, 2H, CH_{arom}), 6.90 – 6.81 (m, 6H, CH_{arom}), 4.75 – 4.68 (m, 3H, C*H*H PMB, CH*H* PMB, C*H*H PMB), 4.55 (dd, *J* = 7.5, 6.0

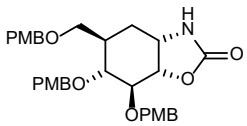
Hz, 1H, H-2), 4.40 (d, *J* = 10.8 Hz, 1H, CH*H* PMB), 4.36 (d, *J* = 11.3 Hz, 1H, C*H*H PMB), 4.27 (d, *J* = 11.3 Hz, 1H, CH*H* PMB), 4.15 – 4.07 (m, 2H, H-1, H-3), 3.80 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.59 (dd, *J* = 9.2, 3.2 Hz, 1H, H-6), 3.45 (dd, *J* = 9.5, 9.5 Hz, 1H, H-4), 3.32 (dd, *J* = 9.2, 2.6 Hz, 1H, H-6), 2.44 (s, 3H, Me Ts), 2.38 (ddd, *J* = 15.0, 5.1, 5.1 Hz, 1H, H-7), 2.01 – 1.93 (m, 1H, H-5), 1.87 (ddd, *J* = 14.4, 9.3, 3.7 Hz, 1H, H-7); ^{13}C NMR (214 MHz, $CDCl_3$, HSQC): δ 159.5, 159.4, 146.3, 132.7, 130.4, 130.3 ($C_{q\text{-}arom}$), 130.1 (CH_{arom}), 129.9 ($C_{q\text{-}arom}$), 129.8, 129.6, 129.5, 129.0, 114.0, 113.9, 113.9 (CH_{arom}), 84.0 (C-2), 81.0 (C-1), 76.8 (C-4), 74.7, 74.5, 72.9 (CH_2 PMB), 69.4 (C-6), 59.0 (C-3), 55.4, 55.4, 55.4 (OMe), 36.7 (C-5), 27.7 (C-7), 21.8 (Me Ts); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{38}H_{43}NO_{11}S_2Na$ 776.2175, found 776.2170.

1,2-(*N*,*O*)-Sulfamidate-3,4,6-tri-*O*-(4-methoxybenzyl)-carba-α-D-glucose (35).



An oven-dried round-bottom flask was equipped with a glass magnetic stirring bar. Naphthalene (4.4 gr, 34.4 mmol, 12 eq.) was added and dissolved in anhydrous THF (57 mL, 0.05 M), after which freshly cut sodium (656 mg, 28.5 mmol, 10 eq.) was added under a N_2 atmosphere. The reaction mixture was sonicated for 30 minutes to obtain a dark-green sodium naphthalenide solution which was subsequently cooled to –78 °C. Compound **S4** (1.97 gr, 2.85 mmol) was co-evaporated with toluene 3x and dissolved in anhydrous THF (2 mL) before its dropwise addition to the cooled sodium naphthalenide solution and stirring continued for 30 minutes. Upon full conversion was observed (R_f 0.3 (EtOAc:pentane 1:1 v:v)), the reaction was quenched at –78 °C with sat. aq. NH_4Cl until full discoloration of the mixture was observed. The product was allowed to attain to room temperature and extracted with EtOAc (3x), after which the combined organic layers were washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Flash column chromatography (30:70 → 50:50; EtOAc:pentane) yielded the title compound **35** as a yellow oil (1.3 gr, 2.15 mmol, 75%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.31 – 7.11 (m, 6H, CH_{arom}), 6.91 – 6.81 (m, 6H, CH_{arom}), 4.77 (m, 2H, C*H*H PMB, CH*H* PMB), 4.73 – 4.68 (m, 3H, C*H*H PMB, 1-NH, H-2), 4.42 (d, *J* = 10.6 Hz, 1H, CH*H* PMB), 4.39 – 4.33 (m, 2H, C*H*H PMB, CH*H* PMB), 4.24 (dddd, *J* = 6.5, 4.4, 4.4 3.2 Hz, 1H, H-1), 4.06 (dd, *J* = 9.4, 7.8 Hz, 1H, H-3), 3.80 (s, 6H, OMe, OMe), 3.79 (s, 3H, OMe), 3.58 (dd, *J* = 9.1, 4.6 Hz, 1H, H-6), 3.39 (dd, *J* = 9.0, 2.7 Hz, 1H, H-6), 3.36 (dd, *J* = 9.6, 9.6 Hz, 1H, H-4), 2.03 (ddd, *J* = 14.5, 3.3, 3.3 Hz, 1H, H-7), 2.03 – 1.94 (m, 1H, H-5), 1.81 (ddd, *J* = 15.2, 11.5, 4.2 Hz, 1H, H-7); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 159.5, 159.4, 159.4, 130.4, 130.2 ($C_{q\text{-}arom}$), 129.9, 129.7, 129.5, 114.0, 113.9, 113.9 (CH_{arom}), 88.6 (C-2), 82.0 (C-3), 77.8 (C-4), 75.0, 74.8, 73.0 (CH_2 PMB), 69.1 (C-6), 55.4, 55.4 (OMe, OMe, OMe), 36.7 (C-5), 26.9 (C-7); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{31}H_{37}NO_9SNa$ 622.2087, found 622.2081.

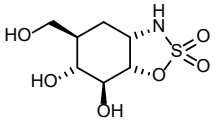
1,2-(*N*,*O*)-Carbamate-3,4,6-tri-*O*-(4-methoxybenzyl)-carba-α-D-glucose (36).



Compound **33** (2.0 g, 2.9 mmol) was co-evaporated with toluene and dissolved in anhydrous DCM (20 mL, 0.15 M) and cooled on ice. Pyridine (1.1 mL, 13 mmol, 4.5 eq.) and triphosgene (0.52 g, 1.7 mmol, 0.6 eq.) were added and after 10 minutes, the ice bath was removed, after which the reaction mixture was stirred at room temperature for 3 h. Upon full conversion was observed, the reaction was quenched with sat. aq. $NaHCO_3$. The mixture was extracted with EtOAc (3x), after which the combined organic layers were washed twice with brine, dried over

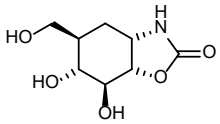
$MgSO_4$, filtered, and concentrated *in vacuo* to obtain the crude *N*-tosyl protected cyclic carbamate. An oven-dried round-bottom flask was equipped with a glass magnetic stirring bar. Naphthalene (4.5 g, 35 mmol, 12 eq.) was added and dissolved in anhydrous THF (60 mL, 0.05 M), after which freshly cut sodium (0.67 g, 29 mmol, 10 eq.) was added under a N_2 atmosphere. The reaction mixture was then sonicated for 10-15 minutes to obtain a dark-green Na/naphthalenide solution. The mixture was cooled to –78 °C and the crude *N*-Ts protected cyclic carbamate intermediate was co-evaporated with toluene (3x) and dissolved in anhydrous THF (3 mL) before its dropwise addition to the cooled Na/naphthalenide solution and stirred for 30 minutes. Upon full conversion was observed (R_f 0.2 (EtOAc:pentane 4:6 v:v)), the reaction was quenched at –78 °C with sat. aq. NH_4Cl until full discoloration of the mixture was observed. The product was allowed to warm up to room temperature and extracted with EtOAc (3x), after which the combined organic layers were washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. flash column chromatography (60:40 → 80:20; EtOAc:pentane) provided title compound **36** as a yellow oil (1.2 gr, 2.1 mmol, 71% over 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.32 – 7.12 (m, 6H, CH_{arom}), 6.85 (m, 6H, CH_{arom}), 5.57 (bs, 1H, 1-NH), 4.78 (d, *J* = 10.8 Hz, 1H, C*H*H PMB), 4.75 (d, *J* = 10.7 Hz, 1H, C*H*H PMB), 4.67 (d, *J* = 10.8 Hz, 1H, CH*H* PMB), 4.57 (dd, *J* = 7.1, 7.1 Hz, 1H, H-2), 4.41 (d, *J* = 10.7 Hz, 1H, CH*H* PMB), 4.39 – 4.32 (m, 2H, C*H*H PMB, CH*H* PMB), 4.14 (ddd, *J* = 8.0, 3.8, 3.8 Hz, 1H, H-1), 3.81 – 3.73 (m, 10H, OMe, OMe, OMe, H-3), 3.55 (dd, *J* = 9.0, 4.8 Hz, 1H, H-6), 3.40 (dd, *J* = 6.2, 2.7 Hz, 1H, H-6), 3.38 (dd, *J* = 6.7, 6.7 Hz, 1H, H-4), 2.00 (ddddd, *J* = 8.7, 8.7, 4.1, 4.1, 4.1 Hz, 1H, H-5), 1.91 (ddd, *J* = 15.2, 4.0, 4.0 Hz, 1H, H-7), 1.73 (ddd, *J* = 15.4, 11.2, 4.2 Hz, 1H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.7 (C=O), 159.3, 159.3, 159.3, 130.7, 130.4, 130.4 ($C_{q\text{-}arom}$), 129.7, 129.6, 129.4, 113.9, 113.9, 113.8 (CH_{arom}), 83.6 (C-3), 81.9 (C-2), 77.7 (C-4), 74.3, 74.0, 72.9 (CH_2 PMB), 69.8 (C-6), 55.4, 55.4 (OMe PMB), 52.1 (C-1), 36.5 (C-5), 28.3 (C-7); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{32}H_{37}NO_8Na$ 586.2417, found 586.2411.

1,2-(*N*,*O*)-Sulfamidate-carba-α-D-glucose (15).



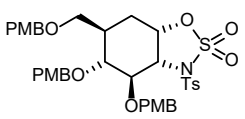
Compound **35** (26.4 mg, 44 μmol) was dissolved in DCM (1.5 mL, 0.03 M) and cooled on ice. Triethylsilane (42 μL, 0.26 mmol, 5.9 eq.) and TFA (34 μL, 0.44 mmol, 10 eq.) were added and the reaction mixture was stirred for 0.5-1 hour. Upon full conversion was observed (R_f 0.2 (MeOH/DCM 1:9 v:v), the reaction mixture was concentrated to dryness. Flash column chromatography (neutralized SiO_2, dry loading on Celite; (0:100 → 10:90; MeOH:DCM) and lyophilization yielded title compound **15** as a colorless transparent film (9.9 mg, 41.4 μmol, 94%). ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 4.55 (dd, *J* = 8.4, 5.7 Hz, 1H, H-2), 4.22 (ddd, *J* = 5.7, 4.5, 2.4 Hz, 1H, H-1), 3.86 (dd, *J* = 9.8, 8.4 Hz, 1H, H-3), 3.73 (dd, *J* = 10.9, 3.8 Hz, 1H, H-6), 3.65 (dd, *J* = 10.9, 5.9 Hz, 1H, H-6), 3.22 (dd, *J* = 10.5, 9.7 Hz, 1H, H-4), 2.08 (ddd, *J* = 15.3, 3.9, 2.4 Hz, 1H, H-7), 1.94 – 1.83 (m, 1H, H-5), 1.70 (ddd, *J* = 15.4, 12.7, 4.5 Hz, 1H, H-7); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 90.0 (C-2), 75.8 (C-3), 73.0 (C-4), 63.4 (C-6), 56.5 (C-1), 39.5 (C-5), 27.5 (C-7); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_7H_{13}NO_6SNa$ 262.0361, found 262.0356.

1,2-(*N*,*O*)-Carbamate-carba-α-D-glucose (20).



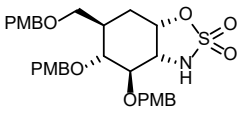
Compound **36** (86.1 mg, 0.15 mmol) was dissolved in DCM (2.0 mL, 0.08 M) and cooled on ice. TES (0.15 mL, 0.94 mmol, 6.0 eq.) and TFA (0.12 mL, 1.6 mmol, 10 eq.) were added and the reaction mixture was stirred for 1 h. Upon full conversion was observed (R_f 0.2 (acetone)), the reaction mixture was concentrated to dryness using a water aspirator. Flash column chromatography (neutralized SiO_2, dry loading on Celite; 10:90 → 90:10; acetone:DCM) yielded the title compound **20** as a white brittle foam (20.3 mg, 100 μmol, 67%). ^{1}H NMR (400 MHz, MeOD, HH-COSY, HSQC): δ 4.36 (dd, *J* = 7.3, 7.3 Hz, 1H, H-2), 4.16 (ddd, *J* = 7.2, 4.3, 2.8 Hz, 1H, H-1), 3.75 (dd, *J* = 10.8, 3.9 Hz, 1H, H-6), 3.63 (dd, *J* = 10.8, 6.1 Hz, 1H, H-6), 3.54 (dd, *J* = 9.6, 7.3 Hz, 1H, H-3), 3.22 (dd, *J* = 9.8, 9.8 Hz, 1H, H-4), 2.03 (ddd, *J* = 15.0, 3.4, 3.4 Hz, 1H, H-7), 1.77 (ddddd, *J* = 12.0, 10.1, 6.1, 4.0, 4.0 Hz, 1H, H-5), 1.58 (ddd, *J* = 15.0, 12.0, 4.4 Hz, 1H, H-7); ^{13}C NMR (101 MHz, MeOD, HSQC); δ 162.3 (C=O), 83.3 (C-2), 77.7 (C-3), 72.7 (C-4), 63.8 (C-6), 53.7 (C-1), 39.8 (C-5), 28.3 (C-7); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_8H_{13}NO_5Na$ 226.0691, found 226.0686.

1,2-(*O*,*N*-(*p*-tolylsulfonyl))-sulfamidate-3,4,6-tri-*O*-(4-methoxybenzyl)-carba-α-D-glucose (S5).



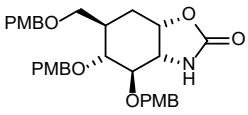
Compound **34** (246 mg, 0.36 mmol) was co-evaporated with toluene and dissolved in anhydrous DCM (3.6 mL, 0.1 M). Triethylamine (0.2 mL, 1.4 mmol, 4.0 eq.) was added, and the solution was cooled to –78 °C. SO_2Cl_2 (35 μL, 0.43 mmol, 1.2 eq.) was added dropwise, and the reaction mixture was allowed to slowly warm up to 5 °C over a period of 2-3 hours. Upon full conversion was observed (R_f 0.5 (EtOAc:pentane 4:6 v:v)), the reaction was quenched with sat. aq. $NaHCO_3$. The mixture was then extracted with EtOAc (3x), after which the combined organic layers were washed twice with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash column chromatography (30:70 → 40:60; EtOAc:pentane) yielded title compound **32** as an off-white foam (270 mg, 0.36 mmol, quant.). ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.98 – 7.91 (m, 2H, CH_{arom}), 7.44 – 7.31 (m, 4H, CH_{arom}), 7.18 – 7.09 (m, 4H, CH_{arom}), 6.91 – 6.80 (m, 6H, CH_{arom}), 4.99 (d, *J* = 9.8 Hz, 1H, C*H*H PMB), 4.81 (d, *J* = 10.6 Hz, 1H, C*H*H PMB), 4.78 (d, *J* = 9.9 Hz, 1H, CH*H* PMB), 4.43 (d, *J* = 10.6 Hz, 1H, CH*H* PMB), 4.36 (ddd, *J* = 3.0, 3.0, 3.0 Hz, 1H, H-1), 4.33 – 4.29 (m, 2H, C*H*H PMB, CH*H* PMB), 4.14 (dd, *J* = 8.9, 3.9 Hz, 1H, H-2), 4.06 (dd, *J* = 9.1, 9.1 Hz, 1H, H-3), 3.81 – 3.80 (m, 6H, OMe, OMe), 3.78 (s, 3H, OMe), 3.67 (dd, *J* = 9.1, 3.8 Hz, 1H, H-6), 3.43 (dd, *J* = 10.5, 9.4 Hz, 1H, H-4), 3.30 (dd, *J* = 9.1, 2.6 Hz, 1H, H-6), 2.45 (s, 3H, Me Ts), 2.07 (ddd, *J* = 15.7, 3.0, 3.0 Hz, 1H, H-7), 1.99 (ddddd, *J* = 14.0, 10.3, 3.4, 3.4, 3.4 Hz, 1H, H-5), 1.77 (ddd, *J* = 15.8, 12.7, 3.1 Hz, 1H, H-7); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.5, 159.4, 159.4, 146.1, 134.3, 130.6 ($C_{q\text{-}arom}$), 130.5 (CH_{arom}), 130.2 ($C_{q\text{-}arom}$), 130.1, 129.4, 129.4, 129.1, 113.9, 113.9, 113.9 (CH_{arom}), 82.6 (C-1), 81.9 (C-3), 78.7 (C-4), 76.4, 75.0, 72.9 (CH_2 PMB), 68.2 (C-6), 66.9 (C-2), 55.4, 55.4, 55.4 (OMe), 36.8 (C-5), 27.9 (C-7), 21.9 (Me Ts); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{38}H_{43}NO_{11}S_2Na$ 776.2175, found 776.2170.

1,2-(*O*,*N*)-Sulfamidate-3,4,6-tri-*O*-(4-methoxybenzyl)-carba-α-D-glucose (37).



An oven-dried round-bottom flask was equipped with a glass magnetic stirring bar. Naphthalene (1.5 gr, 11.7 mmol, 12 eq.) was added and dissolved in anhydrous THF (19 mL, 0.05 M), after which freshly cut sodium (221 mg, 9.6 mmol, 10 eq.) was added under a N_2 atmosphere. The reaction mixture was sonicated for 30 minutes to obtain a dark-green sodium naphthalenide solution which was subsequently cooled to –78 °C. Compound **S5** (664 mg, 0.96 mmol) was co-evaporated with toluene (3x) and dissolved in anhydrous THF (2 mL) before its dropwise addition to the cooled sodium naphthalenide solution and stirring continued for 30 minutes. Upon full conversion was observed (R_f 0.3 (EtOAc:pentane 1:1 v:v)), the reaction was quenched at –78 °C with sat. aq. NH_4Cl until full discoloration of the mixture was observed. The product was allowed to attain to room temperature and extracted with EtOAc (3x), after which the combined organic layers were washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Flash column chromatography (30:70 → 50:50; EtOAc:pentane) yielded the title compound **37** as an off-white solid (492 mg, 0.82 mmol, 85%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.26 – 7.13 (m, 6H, CH_{arom}), 6.92 – 6.82 (m, 6H, CH_{arom}), 4.94 (ddd, *J* = 3.6, 3.5, 3.5 Hz, 1H, H-1), 4.83 (d, *J* = 4.4 Hz, 1H, 2-NH), 4.80 (d, *J* = 11.2 Hz, 1H, C*H*H PMB), 4.72 (d, *J* = 10.7 Hz, 1H, C*H*H PMB), 4.64 (d, *J* = 11.1 Hz, 1H, CH*H* PMB), 4.48 (d, *J* = 10.7 Hz, 1H, CH*H* PMB), 4.42 – 4.35 (m, 2H, C*H*H PMB, CH*H* PMB), 3.88 (dd, *J* = 8.3, 8.3 Hz, 1H, H-3), 3.81 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.69 (dd, *J* = 9.2, 4.2 Hz, 1H, H-6), 3.63 (ddd, *J* = 8.7, 4.7, 4.7 Hz, 1H, H-2), 3.47 (dd, *J* = 9.0, 9.0 Hz, 1H, H-4), 3.38 (dd, *J* = 9.1, 3.1 Hz, 1H, H-6), 2.20 (ddd, *J* = 15.6, 3.4, 3.4 Hz, 1H, H-7), 2.07 (ddddd, *J* = 9.5, 9.5 3.7, 3.7, 3.7 Hz, 1H, H-5), 1.95 (ddd, *J* = 15.7, 12.2, 3.6 Hz, 1H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.7, 159.4, 159.4, 130.3, 130.2, 130.2 ($C_{q\text{-}arom}$), 129.9, 129.6, 129.5, 114.2, 114.0, 113.9 (CH_{arom}), 82.8 (C-1), 80.8 (C-3), 78.5 (C-4), 74.9, 74.4, 72.9 (CH_2 PMB), 68.8 (C-6), 62.0 (C-2), 55.4, 55.4, 55.4 (OMe), 36.8 (C-5), 27.8 (C-7); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{31}H_{37}NO_9SNa$ 622.2087, found 622.2081.

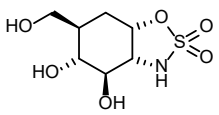
1,2-(*O*,*N*)-Carbamate-3,4,6-tri-*O*-(4-methoxybenzyl)-carba-α-D-glucose (38).



Compound **34** (0.75 g, 1.1 mmol) was co-evaporated with toluene and dissolved in anhydrous DCM (10 mL, 0.1 M) and cooled on ice. Pyridine (0.39 mL, 4.8 mmol, 4.5 eq.) and triphosgene (0.19 g, 0.65 mmol, 0.6 eq.) were added and after 10 minutes, the ice bath was removed, after which the reaction mixture was stirred at room temperature for 3 h. Upon full conversion was observed, the reaction was quenched with sat. aq. $NaHCO_3$. The mixture was extracted with EtOAc (3x), after which the combined organic layers were washed twice with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to obtain the crude *N*-tosyl protected cyclic carbamate. An oven-dried round-bottom flask was equipped with a glass magnetic stirring bar. Naphthalene (1.7 g, 13 mmol, 12 eq.) was added and dissolved in anhydrous THF (22 mL, 0.05 M), after which freshly cut sodium (0.25 g, 11 mmol, 10 eq.) was added under a N_2 atmosphere. The reaction mixture was then sonicated for 10-15 minutes to obtain a dark-green Na/naphthalenide solution. The mixture was cooled to –78 °C and the crude *N*-Ts protected cyclic carbamate intermediate was co-evaporated with toluene (3x) and dissolved in anhydrous THF (3 mL) before its dropwise addition to the cooled Na/naphthalenide solution and stirred for 30 minutes. Upon full conversion was observed (R_f 0.15 (EtOAc:pentane 1:1 v:v)), the reaction was quenched at –78

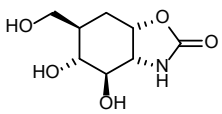
°C with sat. aq. NH_4Cl until full discoloration of the mixture was observed. The product was allowed to warm up to room temperature and extracted with EtOAc (3x), after which the combined organic layers were washed with sat. aq. $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. flash column chromatography (40:60 → 70:30; EtOAc:pentane) provided title compound **38** as an off-white solid (488 mg, 0.86 mmol, 79% over 2 steps). ^{1}H NMR (850 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.25 – 7.16 (m, 6H, CH_{arom}), 6.93 – 6.84 (m, 6H, CH_{arom}), 4.86 (d, *J* = 11.5 Hz, 1H, C*H*H PMB), 4.83 (s, 1H, 2-NH), 4.70 (d, *J* = 10.6 Hz, 1H, C*H*H PMB), 4.65 (ddd, *J* = 7.2, 3.5, 3.5 Hz, 1H, H-1), 4.50 (dd, *J* = 11.5, 11.5 Hz, 2H, CH*H* PMB, CH*H* PMB), 4.43 – 4.37 (m, 2H, C*H*H PMB, CH*H* PMB), 3.81 (s, 3H, OMe PMB), 3.80 (s, 3H, OMe PMB), 3.79 (s, 3H, OMe PMB), 3.68 (dd, *J* = 9.1, 4.0 Hz, 1H, H-6), 3.51 – 3.47 (m, 1H, H-2), 3.46 – 3.41 (m, 2H, H-3, H-4), 3.37 (dd, *J* = 9.2, 2.9 Hz, 1H, H-6), 2.14 (ddd, *J* = 15.4, 3.6, 3.6 Hz, 1H, H-7), 1.98 (dddd, *J* = 12.1, 8.6, 4.0, 4.0 Hz, 1H, H-5), 1.91 (ddd, *J* = 15.9, 11.7, 4.4 Hz, 1H, H-7); ^{13}C NMR (214 MHz, $CDCl_3$, HSQC): δ 159.7 (C=O), 159.4, 159.3, 159.2, 130.5, 130.4, 130.3 ($C_{q\text{-}arom}$), 129.7, 129.7, 129.5, 114.4, 114.0, 113.9 (CH_{arom}), 84.6 (C-3), 78.9 (C-4), 76.2 (C-1), 74.6, 74.3, 73.0 (CH_2 PMB), 69.5 (C-6), 58.2 (C-2), 55.5, 55.4, 55.4 (OMe PMB), 37.4 (C-5), 28.3 (C-7); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{32}H_{37}NO_8Na$ 586.2417, found 586.2411.

1,2-(*O*,*N*)-Sulfamidate-carba-α-D-glucose (16).



Compound **37** (31.7 mg, 53 μmol) was dissolved in DCM (1.3 mL, 0.04 M) and cooled on ice. Triethylsilane (50 μL, 0.32 mmol, 6 eq.) and TFA (41 μL, 0.53 mmol, 10 eq.) were added and the reaction mixture was stirred for 0.5-1 hour. Upon full conversion was observed (R_f 0.4 (MeOH/DCM 2:8 v:v), the reaction mixture was concentrated to dryness. Flash column chromatography (neutralized SiO_2, dry loading on Celite; (0:100 → 10:90; MeOH:DCM) and lyophilization yielded title compound **19** as a colorless transparent film (10.3 mg, 43.1 μmol, 81%). ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 4.98 (ddd, *J* = 4.9, 2.8, 2.8 Hz, 1H, H-1), 3.75 (dd, *J* = 9.4, 9.4 Hz, 1H, H-3), 3.73 (dd, *J* = 10.9, 3.6 Hz, 1H, H-6), 3.67 (dd, *J* = 10.9, 5.5 Hz, 1H, H-6), 3.52 (dd, *J* = 9.1, 4.5 Hz, 1H, H-2), 3.25 (dd, *J* = 9.8, 9.8 Hz, 1H, H-4), 2.30 (ddd, *J* = 15.2, 2.7, 2.7 Hz, 1H, H-7), 1.83 – 1.76 (m, 1H, H-5), 1.73 (ddd, *J* = 15.1, 12.9, 3.3 Hz, 1H, H-7); ^{13}C NMR (126 MHz, MeOD, HSQC); δ 85.9 (C-1), 75.9 (C-3), 73.5 (C-4), 64.6 (C-2), 63.2 (C-6), 39.6 (C-5), 29.0 (C-7); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_7H_{13}NO_6SNa$ 262.0361, found 262.0356.

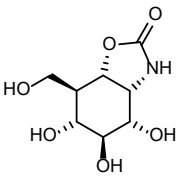
1,2-(*O*,*N*)-Sulfamidate-carba-α-D-glucose (21).



Compound **38** (77 mg, 0.14 mmol) was dissolved in DCM (1.7 mL, 0.08 M) and cooled on ice. TES (0.13 mL, 0.8 mmol, 6.0 eq.) and TFA (0.10 mL, 1.3 mmol, 10 eq.) were added and the reaction mixture was stirred for 1 h. Upon full conversion was observed (R_f 0.3 (acetone)), the reaction mixture was concentrated to dryness using a water aspirator. Flash column chromatography (neutralized SiO_2, dry loading on Celite; 10:90 → 70:30; acetone:DCM) yielded the title compound **21** as a white brittle foam (9.1 mg, 45 μmol, 32%).^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 4.72 (ddd, *J* = 6.3, 3.7, 2.4 Hz, 1H, H-1), 3.74 (dd, *J* = 10.9, 3.4 Hz, 1H, H-6), 3.66 (dd, *J* = 10.9, 5.4 Hz, 1H, H-6), 3.48 (dd, *J* = 7.9, 6.5 Hz, 1H, H-2), 3.34 (dd, *J* = 9.4, 7.9 Hz, 1H, H-3), 3.21 (dd, *J* = 9.5, 9.5 Hz, 1H, H-4), 2.24 (dd, *J* = 11.6, 2.4 Hz, 1H, H-7), 1.78 – 1.65 (m, 2H, H-5, H-7); ^{13}C NMR (126

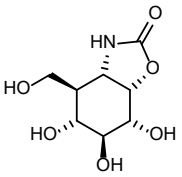
MHz, MeOD,: HSQC) δ 162.4 (C=O), 79.9 (C-3), 78.4 (C-1), 73.0 (C-4), 63.7 (C-6), 60.4 (C-2), 40.3 (C-5), 28.9 (C-7); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_8H_{13}NO_5Na$ 226.0691, found 226.0686.

1,7-(*S,S*)-(*N,O*)-Carbamate cyclophellitol alkane (10).



Carbamate **40** (23 mg, 50 µmol) was dissolved in anhydrous DCM (0.7 mL, 0.07 M) and cooled on ice. Subsequently TFA (30% v:v, 0.3 mL, 3.9 mmol, 78 eq.) and TES (48 µL, 0.3 mmol, 6.0 eq.) were added and the reaction was stirred on ice for 1 hour and subsequently at room temperature for another 1.5 hours.Upon full conversion was observed (R_f 0.1 (MeOH:DCM, 2:8, v:v)), the reaction was quenched by addition of Et_3N, concentrated at 30 °C. Flash column chromatography (dry loading, 0:100 MeOH:DCM → 17:83 MeOH:DCM) yielded the deprotected carbamate **10** as a colourless oil (8.0 mg, 37 µmol, 74%). ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 4.68 (dd, *J* = 9.2, 7.0 Hz, 1H, H-7), 4.20 (dd, *J* = 7.1, 3.2 Hz, 1H, H-1), 3.95 (dd, *J* = 11.1, 2.8 Hz, 1H, H-6), 3.66 (dd, *J* = 11.1, 3.1 Hz, 1H, H-6), 3.58 – 3.48 (m, 2H, H-2, H-3), 3.32 (dd, *J* = 5.7, 2.4 Hz, 1H, H-4), 1.73 (dddd, *J* = 11.9, 9.2, 2.9, 2.9 Hz, 1H, H-5); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 162.1 (C=O), 75.8, 75.6, 71.7 (C-2, C-3, C-7), 69.5 (C-4), 58.9 (C-6), 58.2 (C-1), 48.1 (C-5); HRMS (ESI) m/z: [M + H]$^+$ Calcd. for $C_8H_{13}NO_6$ 220.08156; Found 220.08144.

1,7-(*S,S*)-(*O,N*)-Carbamate cyclophellitol alkane (11).



Carbamate **41** (17 mg, 37 µmol) was dissolved in anhydrous DCM (0.65 mL, 0.07 M) and cooled on ice. Subsequently TFA (30% v:v, 0.3 mL, 3.9 mmol, 105 eq.) and TES-H (50 µL, 0.3 mmol, 8.0 eq.) were added and the reaction was stirred while attaining to room temperature for 1.5 hours. Upon full conversion was observed (R_f 0.1 (MeOH:DCM, 2:8, v:v)), the reaction was concentrated at 30 °C. Flash column chromatography (dry loading, 0:100 MeOH:DCM → 17:83 MeOH:DCM) yielded the deprotected carbamate **11** as a colourless oil (7.0 mg, 32 µmol, 86%). ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 4.69 (dd, *J* = 6.1, 4.1 Hz, 1H, H-1), 3.82 (dd, *J* = 11.0, 4.7 Hz, 1H, H-6), 3.78 – 3.75 (m, 1H, H-7), 3.73 (dd, *J* = 7.7, 3.3 Hz, 1H, H-6), 3.61 (dd, *J* = 9.3, 4.1 Hz, 1H, H-2), 3.53 (dd, *J* = 9.0, 9.0 Hz, 1H, H-3), 3.25 (dd, *J* = 11.3, 8.7 Hz, 1H, H-4), 1.57 (dddd, *J* = 11.3, 9.5, 4.7, 3.5 Hz, 1H, H-5); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 161.6 (C=O), 80.6 (C-1), 75.7 (C-3), 72.1 (C-2), 70.7 (C-4), 60.6 (C-6), 53.6 (C-7), 49.6 (C-5); HRMS (ESI) m/z: [M + H]$^+$ Calcd. for $C_8H_{13}NO_6$ 220.08156; Found 220.08141.

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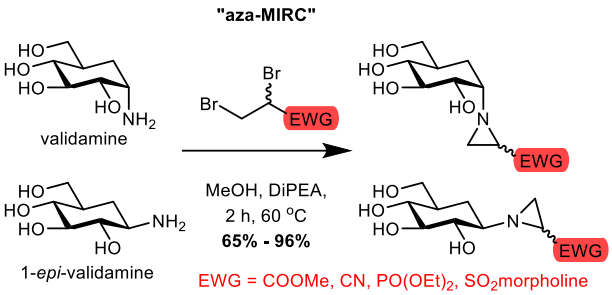
Chapter 4

Design and synthesis of exocyclic cyclitol aziridines as potential mechanism-based glycosidase inactivators



ABSTRACT Eight exocyclic aziridine cyclitols were synthesized, envisioned as putative covalent inhibitors of inverting glucosidases. The constructs, bearing a range of electron withdrawing moieties, were obtained efficiently *via* an *aza*-Michael initiated ring closure reaction (*aza*-MIRC) on validamine or 1-*epi*-validamine. The synthetic methodologies and inhibitor design presented here can fuel the future discovery of covalent inhibitors of inverting glycosidases.

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Introduction

In 1990, cyclophellitol (**1**, Figure 1B) was isolated from samples of the *Phellinus sp.* mushroom and shown to be a potent, irreversible inactivator of retaining β-glucosidases.[1,2] Structural elucidation revealed cyclophellitol to have a carba-glucose core, functionalized with a β-oriented epoxide spanning the C-1 and C-7 position.[3,4] This epoxide forces the carba-glucose backbone to adopt a half-chair conformation, mimicking the conformation of the oxocarbenium ion transition state of β-D-glucopyranosides during hydrolysis by retaining β-glucosidase enzymes.[5]

Retaining α- and β-glucosidases generally employ a Koshland double displacement mechanism (Figure 1A).[6–12] The acid/base residues of these enzymes are in close proximity, with a relative distance of roughly 5.5 Å.[9,13] Upon binding of a substrate molecule in the enzyme pocket, in a first nucleophilic substitution reaction the carboxylate residue acts as a nucleophile and displaces the substrate aglycon, which is activated through protonation by the acid-base residue leading to a covalent intermediate. Subsequently, the aglycon leaves the enzyme active site allowing water to enter and upon deprotonation it then engages in a second displacement reaction to deliver the glucopyranose product with net retention of stereochemistry at the anomeric center. The covalently bound intermediate formed during hydrolysis has inspired the design of mechanism-based inhibitors that react to form stable, covalent adducts, effectively incapacitating the enzyme.[14,15] This in turn has formed the basis for the design and synthesis of activity based probes (ABPs) as tools to study enzyme activities.[15–18]

In previous studies, it has been shown that equipping cyclophellitol **1** and its nitrogen congener cyclophellitol aziridine **2** with a tag (for instance, a fluorophore or biotin) allows for selective and sensitive profiling of β-glucosidases.[19] Subsequently, inhibitors and probes of the 1,7-epimers (Figure 1B, **3** and **4**) were constructed to selectively inhibit and probe retaining α-glucosidases, revealing **3** and **4** to be irreversible inactivators with micromolar to nanomolar potencies.[20]

In an alternative design, the incorporation of an *N*-2-bromoacetyl warhead on a β-glucose scaffold results in efficient, covalent inactivators of retaining β-glucosidases (Figure 1B, **5**).[21–23] In this case the electrophilic site is transpositioned from the anomeric center to the more distal α-bromo amide, which traps the catalytic acid/base residue through a nucleophilic substitution reaction of the bromide to form a stable ester linkage.[23–27]

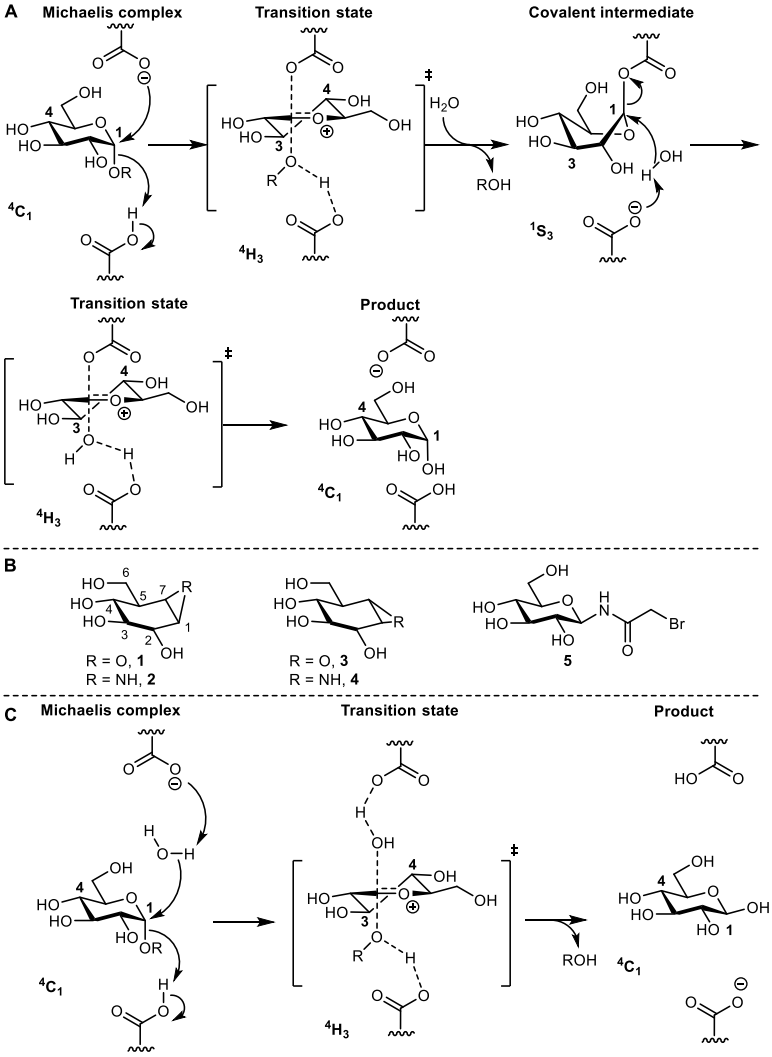


Figure 1. Conformational itinerary of inverting and retaining α-glucosidases *via* classic Koshland mechanisms, and potent, irreversible inhibitors **1** – **5**.[19,20,28,33] (A) Reaction itinerary of retaining α-glucosidases following a Koshland double displacement mechanism. (B) Potent, irreversible α- and β-glucosidase inhibitors; cyclophellitol **1**, cyclophellitol aziridine **2**, 1,7-*epi*-cyclophellitol **3**, 1,7-*epi*-cyclophellitol aziridine **4** and the structure of glucosyl-1-amine *N*-2-bromoacetyl **5**. (C) Reaction itinerary of inverting α-glucosidases following a Koshland single displacement mechanism.

Inverting glycosidases represent another large group of glycoside hydrolase (GH) and these hydrolases employ a different reaction mechanism than retaining glycosidases.[6,9,11,12] Inverting glycosidases employ a Koshland single displacement mechanism (Figure 1C).[9–11,28,29] The relatively large distance (6–12 Å) between the two catalytic side residues, which usually are two carboxylic acids, enables binding of the

substrate and a water molecule.[6,30–32] The active site carboxylate deprotonates the water molecule which concomitantly performs a nucleophilic substitution on the anomeric center expelling the aglycon, which is simultaneously protonated by the enzyme active site carboxylic acid. This results in net inversion of stereochemistry at the anomeric center of the thus produced glucopyranose.

Due to lack of a covalently bound intermediate during hydrolysis, the design of covalent inhibitors and probes for inverting glycosidases, in analogy to the *modus operandi* of cyclophellitol, is complicated. To date, this has led to an absence of covalent inhibitors and activity-based probes for selectively targeting inverting glycosidases.

In an attempt to identify such inhibitors, here a series of inhibitors is proposed based on 1-*epi*-validamine **6** and validamine **7**,[34–37] which are modified at the amine forming an exocyclic aziridine (Figure 2). This aziridine may act as a distal electrophile, for which it was reasoned there is enough space in the relatively large inverting glycosidase active site. It is hypothesized that the electrophile, further away from the anomeric position can bridge the relatively large distance between the carboxylic acid/carboxylate residues, allowing reaction with one of these – specifically, the one responsible for deprotonating the water molecule, which is replaced by the inhibitor in the enzyme pocket.

Here the synthesis of a panel of inhibitors **8** – **15** using an *aza*-Michael initiated ring closure reaction (*aza*-MIRC) as the key step is described.[38,39] Literature precedent has shown the *aza*-MIRC aziridine formation on primary amines to be high yielding and taking place under mild conditions.[38,39] To this end, validamine and 1-*epi*-validamine were considered suitable substrates for this transformation. A small series of dibromide coupling partners was composed, equipped with a diverse selection of electron withdrawing groups, all envisioned to be suitable for coupling under *aza*-MIRC conditions.

In turn, the inhibitor design and synthetic procedures presented here can fuel future design and synthesis of constructs to act on inverting glycosidases.

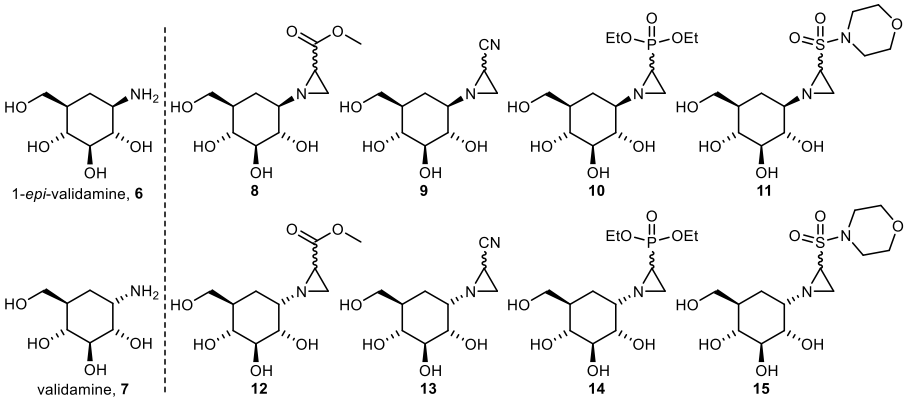


Figure 2. 1-*Epi*-validamine **6** and validamine **7** and eight 1-*N*-aziridine analogues **8** – **15** subject of the here-described studies.

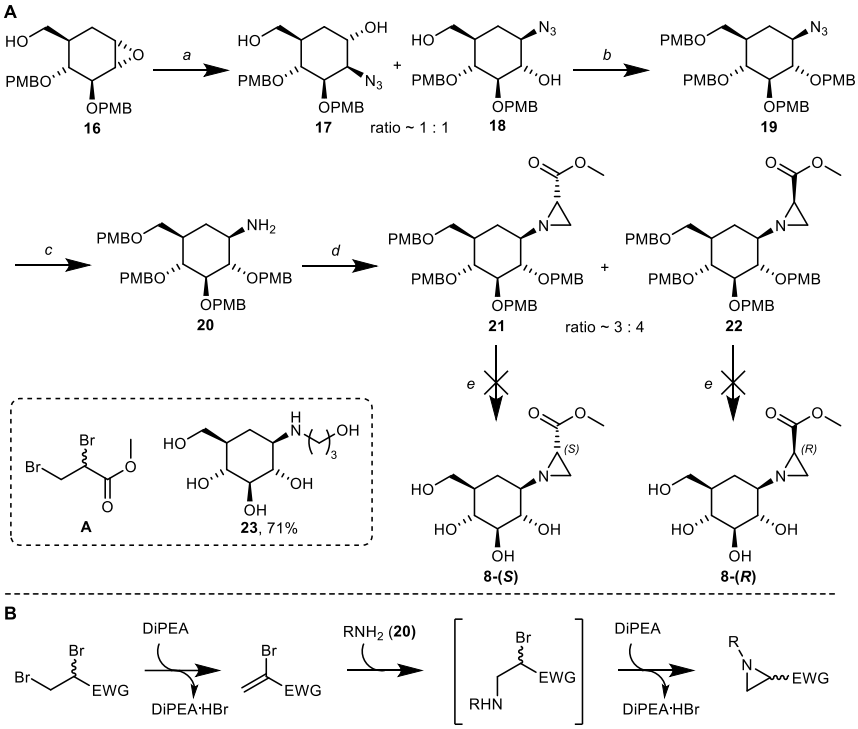
Results and discussion

The synthesis of the panel of target compounds as depicted in Figure 2 started with the preparation of 4-methoxybenzyl protected 1-*epi*-validamine **20**, which were envisioned to be suitably protected constructs to investigate the *aza*-MIRC reaction. To this end, epoxide **16**, the synthesis of which is part of the research described in chapter 3,[40] was treated with NaN_3 in DMF at elevated temperatures to yield a separable mixture of regioisomers **17** and **18** in a 1:1 ratio and an overall yield of 81% (Scheme 1A). Subsequent protection of the 2- and 6-OH in **18** under Williamson etherification conditions (NaH, PMBCl) yielded fully protected compound **19** in 77% yield. Reduction of the azide under Staudinger conditions (PMe_3, aq. NaOH, THF) transformed the azide into the corresponding primary amine **20** (74%).

With protected 1-*epi*-validamine **20** in hand, attention was then turned to the installation of the exocyclic aziridine. The protected 1-*epi*-validamine **20** was reacted with commercially available methyl 2,3-dibromopropanoate (**A**) in a polar, protic solvent (MeOH) using a non-nucleophilic base (DiPEA) to yield a separable mixture of diastereomers **21** and **22** in a 3:4 ratio, and an overall yield of 70%. Observed NOE interactions allowed for identification of both epimers.

The general mechanism of the efficient aziridine formation is shown in scheme 1B.[38,39] First, elimination of the primary bromide results in the *in situ* formation of the 2-bromovinyl intermediate which bears a Michael acceptor motive ready for a 1,4-addition of the primary amine of the protected 1-*epi*-validamine **20**. Subsequently, in an *aza*-Darzen reaction, the α-bromide is substituted by the resulting secondary amine to deliver the desired aziridine functionality.

Scheme 1. Attempted synthesis of two exocyclic aziridine epimers *via* an *aza*-MIRC reaction (A) and the mechanism of the *aza*-MIRC aziridine formation (B).

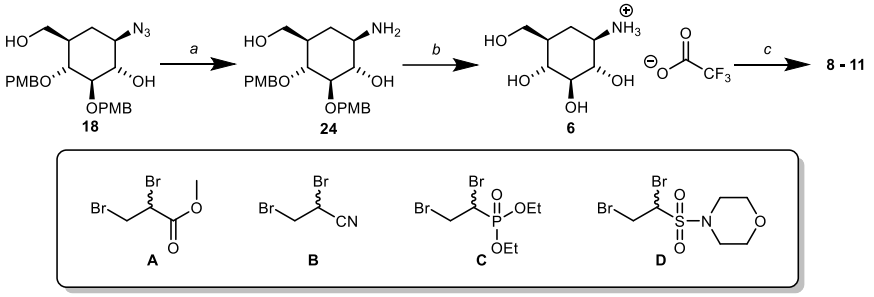


Reagents and conditions: *a)* NaN_3, DMF, 16 h, 130 °C, 39% (**17**), 42% (**18**); *b)* PMBCl, NaH, DMF, 16 h, rt (77%); *c)* PMe_3, NaOH, THF, H_2O, rt, 16 h (74%); *d)* methyl 2,3-dibromopropanoate, DiPEA, MeOH, 2 h, 60 °C, 30% (**21**), 40% (**22**); *e)* Na, NH_3, *t*-BuOH, 1 h, -60 °C (isolated **23** in 71%).

Unfortunately, all attempts to deprotect the exocyclic aziridines **21** and **22** resulted in degradation of the starting material, or led to undesired side reactions. Both reductive conditions (Pd/C, H_2) and acidic conditions (TFA, TES) resulted in complete degradation of material, while removal of the PMB ethers under Birch conditions led to the clean formation of compound **23**,[41] in which the reductive cleavage of the PMB groups was accompanied by reduction of the aziridine and methyl ester to from the *N*-propan-3-ol adduct.

Prompted by the robustness of the *aza*-MIRC reaction, it was hypothesized that the problematic PMB deprotection could be circumvented by the use of unprotected substrates.[39] Therefore, the use of unprotected 1-*epi*-validamine **6**, which was prepared from azide **18**, was explored (Scheme 2). Reduction of the azide under Staudinger conditions (PMe_3, NaOH, H_2O, THF) transformed the azide into the corresponding amine **24** (78%), of which the PMB protecting groups were removed under acidic conditions (TFA, TES, DCM) to yield 1-*epi*-validamine **6** as its TFA salt.

Scheme 2. Construction of target compounds **8** – **11** *via* an *aza*-MIRC reaction with 1-*epi*-validamine **6**.



Reagents and conditions: *a)* PMe_3, NaOH, THF, H_2O, rt, 16 h (78%); *b)* TFA, DCM, 1 h, 0 °C, (91%); *c)* DiPEA, dibromide (**A** – **D**), MeOH, 1 h, 80 °C, 96% (**8**), 82% (**9**), 73% (**10**), 79% (**11**).

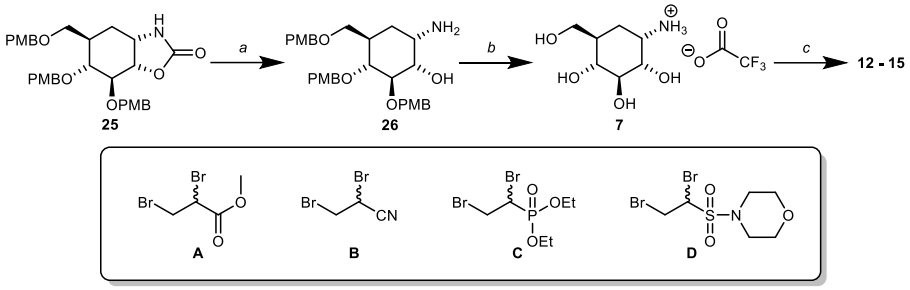
Next, 1-*epi*-validamine **6** was reacted, under the agency of DiPEA, with dibromides **A** – **D** (either commercially available (**A**) or easily accessible *via* known literature procedures (**B** – **D**),[42–44] see Scheme S1, Appendix), in MeOH at elevated temperatures. Gratifyingly, clean conversion towards the desired target structures was observed, yielding target compounds **8** – **11** in moderate to excellent yields after purification (73% – 96%).

With effective conditions in hand to generate the exocyclic aziridines, the assembly of the diasteroisomeric set of target compounds from validamine **7** was undertaken (Scheme 3).

To this end, compound **25**, previously described and synthesized in chapter 3, was considered as a suitable starting point.[40] The cyclic carbamate in **25** was hydrolyzed under alkaline conditions using NaOH in EtOH under elevated temperatures to afford the deprotected amino alcohol **26** (98%). Global deprotection using TFA and TES resulted in validamine **7** which was obtained as its TFA salt in quantitative yield.

Following the procedures applied to the 1-*epi*-validamine substrate **6**, validamine **7** was transformed into the set of target compounds **12** – **15** using dibromides **A** – **D**. Also these reactions proceeded uneventfully to cleanly provide **12** – **15** which were isolated in 65% to 87% yield.

Scheme 3. Construction of target compounds **12** – **15** *via* an *aza*-MIRC reaction with validamine **7**.



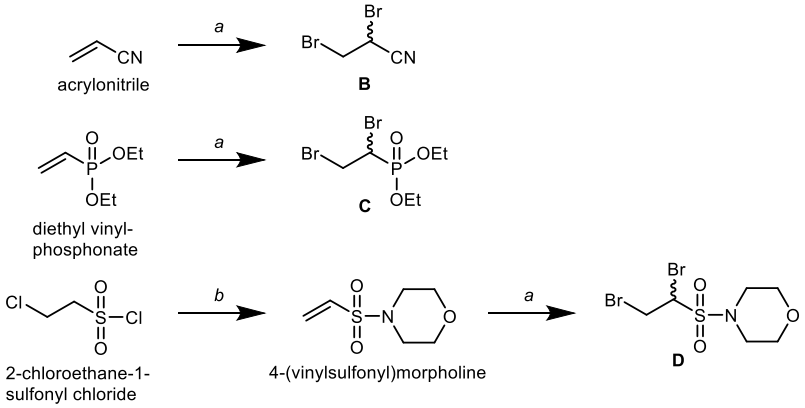
Reagents and conditions: *a)* NaOH, EtOH, 16 h, 80 °C (98%); *b)* TFA, DCM, 1 h, 0 °C, (quant.); *c)* DiPEA, dibromide (**A** – **D**), MeOH, 1 h, 80 °C, 85% (**12**), 82% (**13**), 65% (**14**), 87% (**15**).

Conclusion

In conclusion**,** this report describes the design and synthesis of functionalized validamines **8** – **15**, bearing an exocyclic aziridine motif as putative inhibitors of inverting glucosidases. These compounds were designed and synthesized on the premise that the exocyclic aziridine functionality can bridge the distance between the carboxylic acid/carboxylate residues in the enzyme pocket, potentially allowing for the formation of a covalent bond with the enzyme active site nucleophile, effectively incapacitating the enzyme. Key in the synthesis schemes has been an *aza*-Michael initiated ring closure (*aza*-MIRC) reaction, which in a single step converts unprotected 1-*epi*-validamine **6** and validamine **7** into target compounds **8** – **15**, proving the sturdiness and robustness of these aziridine forming reactions on complex, unprotected substrates. Suitable inhibition assays are currently being developed to probe whether this novel class of carbomimetics is capable of inhibiting inverting glucosidases. If so, the inhibitor design and synthetic procedures presented here, can fuel the future design and synthesis of constructs to effectively act on inverting glycosidases.

Appendix

Scheme S3. preparation of dibromide **B** – **D** *via* literature procedures.[42–44]



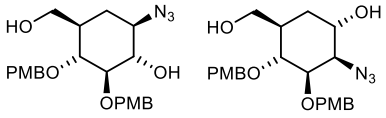
Reagents and conditions: *a)* Br_2, quant. (**B**), 68% (**C**); *b)* morpholine, Et_3N, DCM, 2 h, rt (78% over 2 steps).

Synthetic procedures.

General procedure A: *aza*-MIRC reaction of (1-*epi*)- validammonium trifluoroacetates (6 and 7) with corresponding dibromides.

Validammonium trifluoroacetate **6** or 1-*Epi*-validammonium trifluoroacetate **7** (29 mg, 0.1 mmol) was dissolved in MeOH (0.1 M). Subsequently, DiPEA (8.0 eq.) and the corresponding dibromide **A** – **D** (4.0 eq.) were added. The reaction mixture was stirred for 2 h at 60 °C after which full conversion was observed (MeOH:DCM, 2:8, v:v). The reaction mixture was concentrated under reduced pressure. Flash column chromatography (MeOH:DCM), and when mentioned followed by a second flash column (acetone:DCM), yielded the title compound as a mixture of diastereomers in roughly 1:1 ratios.

1-Deoxy-1-azido-3,4-di-*O*-(4-methoxybenzyl)-7-carba-β-D-glucose (18) and 2-Deoxy-2-azido-3,4-di-*O*-(4-methoxybenzyl)-7-carba-α-D-mannose (17).



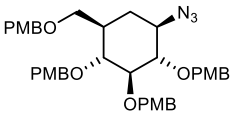
1,2-Anhydro-3,4-di-*O*-(4-methoxybenzyl)-7-carba-α-D-glucose (12.4 g, 31 mmol) was dissolved in DMF (310 mL, 0.1 M) followed by the addition of NaN_3 (20.1 g, 0.31 mol, 10 eq.). The reaction mixture was heated to 130 °C and stirring continued for 16 hours. Upon full conversion (R_f 0.8 and 0.4 for compound **18** and **17** respectively (EtOAc:pentane, 7:3, v:v)), the mixture was concentrated under reduced pressure to a quarter of its original volume and diluted with water. The aqueous layer was extracted with ethyl acetate (3x) followed by washing the combined organic layers with sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (50:50 EtOAc:pentane → 80:20 EtOAc:pentane) yielded title compounds **18** as a white solid (5.82 g, 13.1 mmol, 42%) and **17** as a colorless oil (5.40 g, 12.2 mmol, 39%).

Analytical data for **18**: ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.38 – 7.16 (m, 4H, CH_{arom}), 6.98 – 6.82 (m, 4H, CH_{arom}), 4.91 (d, *J* = 11.0 Hz, 1H, CH*H* PMB), 4.87 (d, *J* = 10.8 Hz, 1H, C*H*H PMB), 4.71 (d, *J* = 11.0 Hz, 1H, CH*H* PMB), 4.60 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 3.80 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.66 – 3.52 (m, 2H, H-6), 3.44 – 3.28 (m, 4H, H-1, H-2, H-3, H-4), 2.67 (s, 1H, 2-OH), 1.88 (ddd, *J* = 13.1, 3.8, 3.8 Hz, 1H, H-7), 1.79 (s, 1H, 6-OH), 1.65 (dddd, *J* = 17.5, 10.1, 4.0, 4.0 Hz, 1H, H-5), 1.30 – 1.21 (m, 1H, H-7); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 159.6, 159.5, 130.5, 130.1, 130.0 ($C_{q\text{-}arom}$), 129.7, 114.2, 114.2 (CH_{arom}), 86.2, 80.6, 76.6 (C-2, C-3, C-4), 75.4, 74.7 (CH_2 PMB), 63.6 (C-6), 62.5 (C-1), 55.4 (OMe), 41.3 (C-5), 29.6 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{23}H_{29}N_3NaO_6$ 466.2256; Found 466.1947.

Analytical data for **17**: ^{1}H NMR (300 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.40 – 7.14 (m, 4H, CH_{arom}), 6.96 – 6.79 (m, 4H, CH_{arom}), 4.78 (d, *J* = 10.9 Hz, 1H, CH*H* PMB), 4.69 – 4.59 (m, 2H, CH_2 PMB), 4.55 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 3.96 (m, 2H, H-1, H-3), 3.80 (m, 7H, OMe, OMe, H-2), 3.67 – 3.48 (m, 3H, H-4, H-6), 2.41 (bs, 1H, 6-OH), 2.36 (bs, 1H, 1-OH), 2.01 (dq, *J* = 9.3, 4.7 Hz, 1H, H-5), 1.62 – 1.53 (m, 2H, H-7); ^{13}C NMR (75 MHz, $CDCl_3$, HSQC): δ 159.5, 130.4, 130.1, 130.0 ($C_{q\text{-}arom}$), 129.7, 129.7, 129.7, 114.1, 114.0, 114.0 (CH_{arom}), 80.9 (C-1/C-3), 78.9 (C-4), 74.2, 72.8 (CH_2 PMB), 67.8

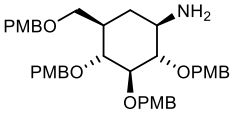
(C-1/C-3), 65.2 (C-6), 64.1 (C-2), 55.4, 55.4 (OMe), 38.9 (C-5), 30.1 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{23}H_{29}N_3NaO_6$ 466.2256; Found 466.1950.

1-Deoxy-1-azido-2,3,4,6-tetra-*O*-(4-methoxybenzyl)-7-carba-β-D-glucose (19).



Compound **18** (133 mg, 0.3 mmol) was dissolved in anhydrous DMF (3.0 mL, 0.1 M) and cooled on ice. PMBCl (0.10 mL, 0.75 mmol, 2.5 eq.) and NaH (60% in mineral oil, 60 mg, 1.5 mmol, 5.0 eq.) was subsequently added. The reaction mixture was allowed to attain to room temperature and stirring continued overnight. Upon full conversion (R_f 0.7 (EtOAc:pentane, 1:1, v:v)), the mixture was diluted with water. The aqueous layer was extracted with Et_2O (3x) followed by washing the combined organic layers with sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (10:90 EtOAc:pentane → 30:70 EtOAc:pentane) yielded the title compound **19** (159 mg, 0.23 mmol, 77%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.33 – 7.04 (m, 8H, CH_{arom}), 6.94 – 6.76 (m, 8H, CH_{arom}), 4.87 – 4.72 (m, 5H, CH*H* PMB, CH*H* PMB, CH*H* PMB, C*H*H PMB, C*H*H PMB), 4.45 – 4.32 (m, 3H, CH*H* PMB, C*H*H PMB, C*H*H PMB), 3.80 (s, 3H, OMe), 3.79 (s, 6H, OMe, OMe), 3.78 (s, 3H, OMe), 3.54 – 3.28 (m, 6H, H-1, H-2, H-3, H-4, H-6), 1.99 (ddd, *J* = 13.5, 4.1, 4.1 Hz, 1H, H-7), 1.75 – 1.64 (m, 1H, H-5), 1.40 (q, *J* = 12.9 Hz, 1H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.4, 159.3, 159.3, 159.2, 131.0, 130.7, 130.4, 130.3, 130.0 ($C_{q\text{-}arom}$), 129.7, 129.7, 129.4, 129.3, 129.3, 114.0, 114.0, 114.0, 113.9, 113.9, 113.8 (CH_{arom}), 86.6, 84.7, 80.3 (C-2, C-3, C-4), 75.5, 75.1, 72.9 (CH_2 PMB), 69.3 (C-6), 63.1 (C-1), 55.4, 55.4 (OMe), 40.0 (C-5), 30.7 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{39}H_{45}N_3NaO_8$ 706.3104; Found 706.3099.

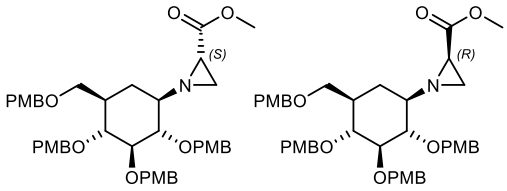
1-*Epi*-2,3,4,6-tetra-*O*-(4-methoxybenzyl)-validamine (20).



Compound **19** (159 mg, 0.23 mmol) was dissolved in THF (4.6 mL, 0.05 M) followed by the addition of aq. NaOH (1.0 M solution in water, 0.92 mL, 0.92 mmol, 4.0 eq.) and PMe_3 (0.92 mL, 0.92 mmol, 4.0 eq.). Stirring continued overnight upon which full conversion was observed (R_f 0.1 (EtOAc:pentane, 1:1, v:v)). The mixture was diluted with water and subsequently the aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (30:70 EtOAc:pentane → 100:0 EtOAc:pentane) yielded the title compound **20** (112 mg, 0.17 mmol, 74%).^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.34 – 7.08 (m, 8H, CH_{arom}), 6.90 – 6.79 (m, 8H, CH_{arom}), 4.93 (d, *J* = 10.8 Hz, 1H, CH*H* PMB), 4.86 – 4.76 (m, 3H, CH*H* PMB, CH*H* PMB, C*H*H PMB), 4.61 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 4.47 – 4.35 (m, 3H, CH*H* PMB, C*H*H PMB, C*H*H PMB), 3.79 (s, 3H, OMe), 3.79 (s, 6H, OMe, OMe), 3.78 (s, 3H, OMe), 3.56 – 3.40 (m, 4H, H-3, H-4, H-6), 3.10 (t, *J* = 9.3 Hz, 1H, H-2), 2.73 (ddd, *J* = 11.9, 9.4, 4.2 Hz, 1H, H-1), 1.87 (ddd, *J* = 13.3, 4.0, 4.0 Hz, 1H, H-7), 1.74 (ddd, *J* = 13.9, 13.0, 7.1 Hz, 1H, H-5), 1.29 (q, *J* = 12.7 Hz, 1H, H-7); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 159.4, 159.3, 159.2, 159.2, 131.1, 131.0, 130.9, 130.6 ($C_{q\text{-}arom}$), 129.7, 129.6, 129.4, 129.3 (CH_{arom}), 87.3 (C-3), 87.2 (C-2), 81.3 (C-4), 75.3, 75.3, 75.0, 72.8

(CH_2 PMB), 69.8 (C-6), 55.4, 55.3, 52.9 (OMe), 40.6 (C-5), 33.3 (C-7); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{39}H_{48}NO_8$ 658.3380; Found 658.3369.

Methyl (*S*)-1-(1-*epi*-validamine)aziridine-2-carboxylate (21) and methyl (*R*)-1-(1-*epi*-validamine)aziridine-2-carboxylate (22).

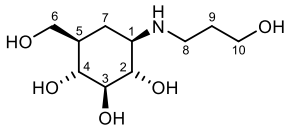


Compounds **21** and **22** were prepare according to **general procedure A** using **20** (133 mg, 0.2 mmol), DiPEA (0.28 mL, 1.6 mmol, 8.0 eq.) and methyl 2,3-dibromopropanoate (100 µL, 0.8 mmol, 4.0 eq.) in MeOH (2.0 mL, 0.1 M). Flash column chromatography (25:75 EtOAc:pentane → 40:60 EtOAc:pentane) yielded the title compounds **21** as a white solid (45 mg, 60 µmol, 30%) and **22** as a white solid (59 mg, 80 µmol, 40%). R_f 0.4 and 0.3 for compound **21** and **22** respectively (EtOAc:pentane, 7:3, v:v).

Analytical data for **21**: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HH-NOESY, HSQC): δ 7.33 – 7.02 (m, 8H, CH_{arom}), 6.91 – 6.75 (m, 8H, CH_{arom}), 4.90 – 4.71 (m, 5H, C*H*H PMB, C*H*H PMB, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.43 – 4.36 (m, 3H, C*H*H PMB, CH*H* PMB, CH*H* PMB), 3.79 (s, 3H, OMe), 3.79 (s, 6H, OMe, OMe), 3.78 (s, 3H, OMe), 3.74 (s, 3H, OMe), 3.66 (dddd, *J* = 10.2, 6.6, 3.3, 3.3 Hz, 1H, H-2), 3.53 – 3.47 (m, 2H, H-6), 3.43 – 3.38 (m, 2H, H-3, H-4), 2.31 (d, *J* = 3.2 Hz, 1H, H-8), 2.10 (d, *J* = 6.9 Hz, 1H, H-8), 2.03 (dd, *J* = 6.5, 3.3 Hz, 1H, H-9), 1.96 – 1.85 (m, 1H, H-7), 1.64 (m, 2H, H-5, H-7), 1.47 – 1.38 (m, 1H, H-1); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 171.8 (C=O), 159.2, 159.2, 159.2, 159.1, 131.1, 130.9, 130.8, 130.5 ($C_{q\text{-}arom}$), 129.6, 129.5, 129.3, 129.2, 113.9, 113.9, 113.8, 113.8 (CH_{arom}), 87.0 (C-3/C-4), 85.4 (C-2), 80.7 (C-3/C-4), 75.5, 75.3, 75.0, 72.8 (CH_2 PMB), 70.0 (C-1), 69.8 (C-6), 55.3, 55.3, 55.3, 52.4 (OMe), 40.2 (C-5), 36.1 (C-8), 34.6 (C-9), 31.2 (C-7); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{43}H_{52}NO_{10}$ 742.3591; Found 742.3582.

Analytical data for **22**: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HH-NOESY, HSQC): δ 7.32 – 7.06 (m, 8H, CH_{arom}), 6.91 – 6.74 (m, 8H, CH_{arom}), 4.83 (d, *J* = 10.6 Hz, 1H, C*H*H PMB), 4.79 – 4.75 (m, 3H, C*H*H PMB, C*H*H PMB, CH*H* PMB), 4.70 (d, *J* = 10.5 Hz, 1H, CH*H* PMB), 4.43 – 4.37 (m, 3H, C*H*H PMB, CH*H* PMB, CH*H* PMB), 3.80 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.78 (s, 6H, OMe, OMe), 3.63 (t, *J* = 9.2 Hz, 1H, H-2), 3.56 (s, 3H, OMe), 3.51 (dd, *J* = 8.8, 2.3 Hz, 1H, H-6), 3.47 – 3.37 (m, 3H, H-3, H-4, H-6), 2.56 (dd, *J* = 6.7, 3.2 Hz, 1H, H-9), 2.04 (d, *J* = 3.2 Hz, 1H, H-8), 1.91 – 1.85 (m, 1H, H-7), 1.72 – 1.62 (m, 2H, H-5, H-7), 1.59 (d, *J* = 6.8 Hz, 1H, H-8), 1.49 (ddd, *J* = 11.2, 9.2, 4.2 Hz, 1H, H-1); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 171.4 (C=O), 159.3, 159.2, 159.1, 159.0, 131.0 ($C_{q\text{-}arom}$), 130.9, 130.8, 130.5, 129.7, 129.2, 129.2, 128.9 (CH_{arom}), 86.9 (C-3/C-4), 85.6 (C-2), 80.9 (C-3/C-4), 75.5, 75.0, 72.8 (CH_2 PMB), 69.9 (C-6), 69.3 (C-1), 55.3, 52.1 (OMe), 40.3 (C-5), 39.0 (C-9), 31.1 (C-7), 30.8 (C-8); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{43}H_{52}NO_{10}$ 742.3591; Found 742.3575.

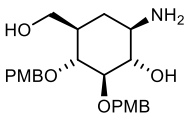
3-*N*-Propan-1-ol-(1-*epi*-2,3,4,6-tetra-*O*-(4-methoxybenzyl)-validamine) (23).



To liquid ammonia (3 mL) at -60 °C, sodium metal (74 mg, 3.2 mmol, 40 eq.) was added. This mixture was stirred for 30 minutes while maintaining a temperature of -60 °C. Subsequently, Compound **22** (60 mg, 81 µmol) was dissolved in THF (1.0 mL) followed by the addition *t*-BuOH (74 µL, 0.81 mmol, 10 eq.). This solution was added dropwise to

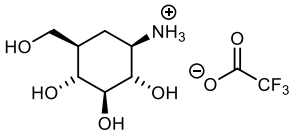
the flask containing ammonia. The solution was stirred for 1 hour while maintaining a temperature of -60 ºC. The reaction was quenched by addition of water (500 µL), let to attain to room temperature and concentrated under reduced pressure. The residue was purified by size exclusion chromatography over HW-40 eluted with water to obtain the title compound **23** as a colorless oil (5.0 mg, 10 µmol, 91%). Flash column chromatography (10:90 MeOH:DCM → 40:60 MeOH:DCM) yielded the title compound (14 mg, 58 µmol, 71%). (R_f 0.3 (MeOH:DCM, 2:8, v:v)); ^{1}H NMR (600 MHz, D_2O, HH-COSY, HSQC): δ 3.97 (dd, *J* = 11.4, 3.5 Hz, 1H, H-6), 3.96 – 3.90 (m, 2H, H-10), 3.87 (dd, *J* = 11.4, 5.9 Hz, 1H, H-6), 3.72 (dd, *J* = 10.4, 8.7 Hz, 1H, H-2), 3.61 – 3.45 (m, 4H, H-1, H-3, H-4, H-8), 3.39 (ddd, *J* = 12.5, 8.6, 6.3 Hz, 1H, H-8), 2.43 (ddd, *J* = 12.9, 4.0, 4.0 Hz, 1H, H-7), 2.25 – 2.08 (m, 2H, H-9), 1.92 (ddddd, *J* = 13.3, 9.6, 6.8, 3.6, 3.6 Hz, 1H, H-5), 1.65 (q, *J* = 12.7 Hz, 1H, H-7); ^{13}C NMR (151 MHz, D_2O, HSQC): δ 78.3 (C-3), 73.6 (C-2), 73.3 (C-4), 63.2 (C-6), 60.8 (C-10), 60.0 (C-1), 44.6 (C-8), 42.1 (C-5), 29.2 (C-9), 26.6 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{10}H_{22}NNaO_5$ 258.1317; Found 258.1504.

1-*Epi*-3,4-di-*O*-(4-methoxybenzyl)-validamine (24).



Compound **18** (0.22 g, 0.5 mmol) was dissolved in THF (10 mL, 0.05 M) followed by the addition of aq. NaOH (1.0 M solution, 2.0 mL, 2.0 mmol, 4.0 eq.) and PMe_3 (1.0 M solution in THF, 2.0 mL, 2.0 mmol, 4.0 eq.). The reaction mixture was stirred for 16 hours at room temperature. Upon full conversion (R_f 0.2 (MeOH:DCM, 1:9, v:v)), the mixture was diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (5:95 MeOH:DCM → 10:90 MeOH:DCM) yielded the title compound **24** (162 mg, 0.39 mmol, 78%). ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 7.35 – 7.17 (m, 4H, CH_{arom}), 6.95 – 6.78 (m, 4H, CH_{arom}), 4.88 – 4.83 (m, 1H, CH*H* PMB), 4.74 (m, 2H, CH*H* PMB, C*H*H PMB), 4.52 (d, *J* = 10.5 Hz, 1H, CH*H* PMB), 3.78 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.70 (dd, *J* = 10.7, 3.1 Hz, 1H, H-6), 3.58 (dd, *J* = 10.7, 5.9 Hz, 1H, H-6), 3.37 – 3.27 (m, 2H, H-3, H-4), 3.14 (ddd, *J* = 9.3, 6.8, 2.4 Hz, 1H, H-2), 2.61 (ddd, *J* = 11.9, 9.6, 4.3 Hz, 1H, H-1), 1.91 (ddd, *J* = 13.3, 4.0, 4.0 Hz, 1H, H-7), 1.62 (dddd, *J* = 12.7, 9.6, 3.3, 3.3 Hz, 1H, H-5), 1.17 (q, *J* = 12.9 Hz, 1H, H-7); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 160.8, 160.7, 132.5, 132.1 ($C_{q\text{-}arom}$), 130.6, 130.6, 114.7, 114.6 (CH_{arom}), 88.1, 82.1 (C-3, C-4), 79.9 (C-2), 76.2, 75.6 (CH_2 PMB), 63.2 (C-2), 55.7 (OMe), 54.3 (C-1), 43.5 (C-5), 33.1 (C-7); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{23}H_{32}NO_6$ 418.2230; Found 418.2225.

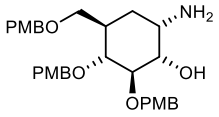
1-*Epi*-validammonium trifluoroacetate (6).



Compound **24** (162 mg, 0.39 mmol) was dissolved in anhydrous DCM (7.8 mL, 0.05M) and cooled on ice. Subsequently, TFA (0.30 mL, 3.9 mmol, 10 eq.) was added and the reaction was stirred for 1 hour while keeping on ice. Upon full conversion was observed (R_f 0.2 (MeOH:DCM, 1:1, v:v + 2% Et_3N)), the mixture was concentrated under reduced pressure and co-evaporated twice with water. The solid precipitate was filtered off using a cotton plug and rinsed with water. The filtrate was concentrated under reduced pressure to give the title compound **6** (103 mg, 0.35 mmol, 91%). ^{1}H NMR (500 MHz, D_2O, HH-COSY, HSQC): δ 3.71 (dd, *J* = 11.3, 3.4 Hz, 1H, H-6), 3.60 (dd, *J* = 11.3, 6.0 Hz, 1H, H-6), 3.35 (dd, *J* = 10.2, 9.3 Hz, 1H, H-2), 3.32

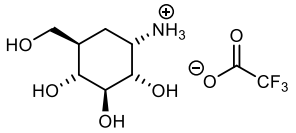
– 3.23 (m, 2H, H-3, H-4), 3.15 (ddd, *J* = 12.4, 10.1, 4.3 Hz, 1H, H-1), 2.06 (ddd, *J* = 13.0, 4.0, 4.0 Hz, 1H, H-7), 1.70 – 1.59 (m, 1H, H-5), 1.38 (q, *J* = 12.7 Hz, 1H, H-7); ^{13}C NMR (126 MHz, D_2O, HSQC): δ 76.9 (C-3/C-4), 73.1 (C-2), 71.9 (C-3/C-4), 61.5 (C-6), 52.4 (C-1), 40.8 (C-5), 27.6 (C-7); ^{19}F NMR (376 MHz, MeOD): δ -76.81; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_7H_{16}NO_4$ 178.1074; Found 178.1074.

3,4,6-Tri-*O*-(4-methoxybenzyl)-validamine (30).



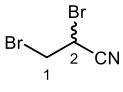
Compound **29** (1.0 g, 1.8 mmol) was dissolved in EtOH (18 mL, 0.1 M) after which NaOH (1.5 g, 37 mmol, 20 eq.) was added. The reaction was heated to 80 °C and stirred for 16 hours. Upon full conversion was observed (R_f 0.4 (MeOH:DCM, 1:9, v:v)), the mixture was diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (0:100 MeOH:DCM → 10:90 MeOH:DCM) yielded the title compound **30** (0.96 g, 1.8 mmol, 98%). ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.29 – 7.12 (m, 6H, CH_{arom}), 6.89 – 6.80 (m, 6H, CH_{arom}), 4.82 (d, *J* = 11.0 Hz, 1H, C*H*H PMB), 4.71 (d, *J* = 10.6 Hz, 1H, C*H*H PMB), 4.64 (d, *J* = 11.1 Hz, 1H, CH*H* PMB), 4.45 (d, *J* = 10.6 Hz, 1H, CH*H* PMB), 4.40 (d, *J* = 11.7 Hz, 1H, C*H*H PMB), 4.36 (d, *J* = 11.6 Hz, 1H, CH*H* PMB), 3.79 (d, *J* = 1.0 Hz, 6H, OMe), 3.78 (s, 3H, OMe), 3.73 – 3.65 (m, 1H, H-3), 3.64 – 3.58 (m, 1H, H-6), 3.53 (bs, 1H, H-2), 3.43 (m, 2H, H-4, H-6), 3.33 (bs, 1H, H-1), 2.72 (bs, 3H, 1-NH_2, 2-OH), 2.15 (m, 1H, H-5), 1.78 (d, *J* = 14.1 Hz, 1H, H-7), 1.65 (bs, 1H, H-7); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 159.4, 159.3, 159.2, 131.0, 130.8, 130.6 ($C_{q\text{-}arom}$), 129.6, 129.4, 114.1, 113.9, 113.9 (CH_{arom}), 82.6 (C-3), 80.3 (C-4), 74.7, 74.2 (CH_2 PMB), 73.8 (C-2), 72.8 (CH_2 PMB), 69.9 (C-6), 55.4 (OMe), 55.4 (OMe), 49.2 (C-1), 37.4 (C-5), 29.5 (C-7); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{31}H_{40}NO_7$ 538.2805; Found 538.2800.

Validammonium trifluoroacetate (7).



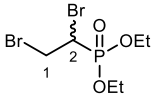
Compound **30** (0.32 g, 0.60 mmol) was dissolved in anhydrous DCM (12 mL, 0.05M) and cooled on ice. Subsequently, TFA (0.46 mL, 6.0 mmol, 10 eq.) was added and the reaction was stirred for 1 hour while keeping on ice. Upon full conversion was observed (R_f 0.2 (MeOH:DCM, 1:1, v:v + 2% Et_3N)), the mixture was concentrated under reduced pressure and co-evaporated twice with water. The solid precipitate was filtered off using a cotton plug and rinsed with water. The filtrate was concentrated under reduced pressure to give the title compound **7** (0.18 g, 0.60 mmol, quant.). Analytical data in full agreement with literature data.[34–37] ^{1}H NMR (500 MHz, D_2O, HH-COSY, HSQC): δ 3.78 – 3.68 (m, 3H, H-1, H-2, H-6), 3.65 (dd, *J* = 11.3, 5.2 Hz, 1H, H-6), 3.47 (t, *J* = 9.5 Hz, 1H, H-3), 3.31 (dd, *J* = 10.2, 9.2 Hz, 1H, H-4), 2.01 (dd, *J* = 11.7, 2.5 Hz, 1H, H-7), 1.77 – 1.66 (m, 2H, H-5, H-7); ^{13}C NMR (126 MHz, D_2O, HSQC): δ 73.8 (C-3), 72.1 (C-4), 70.0 (C-2), 61.5 (C-6), 51.3 (C-1), 38.0 (C-5), 26.0 (C-7); ^{19}F NMR (376 MHz, D_2O): δ -75.75; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_7H_{16}NO_4$ 178.1074; Found 178.1074.

2,3-Dibromopropanenitrile (B).



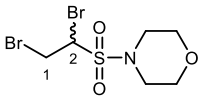
Prepared according to literature procedure.[44] Acrylonitrile (1.3 mL, 20 mmol) was dissolved in acetonitrile (10 mL, 2.0 M) and cooled on ice. Bromine (1.0 mL, 20 mmol, 1.0 eq.) was added dropwise and the reaction was allowed to attain to room temperature over a period of 2 hours after which the reaction was quenched by addition of sat. aq. $Na_2S_2O_3$ solution and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the title compound **B** as an inseparable mixture of stereoisomers (4.4 g, 20 mmol, quant.). Analytical data in full agreement with literature data.[44] ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.54 (ddd, *J* = 9.1, 6.4, 1.4 Hz, 1H, H-1), 3.79 (d, *J* = 3.4 Hz, 1H, H-2), 3.77 (d, *J* = 0.8 Hz, 1H, H-2).

Diethyl (1,2-dibromoethyl)phosphonate (C).



Prepared according to literature procedure.[42] diethyl vinylphosphonate (1.2 mL, 10 mmol) was dissolved in DCM (50 mL, 0.2 M) and cooled on ice. Bromine (0.77 mL, 15 mmol, 1.5 eq.) was added dropwise and the reaction was allowed to attain to room temperature over a period of 30 minutes after which the reaction was quenched by addition of sat. aq. $Na_2S_2O_3$ solution and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (40:60 EtOAc:pentane → 60:40 EtOAc:pentane) yielded the title compound **C** as an inseparable mixture of stereoisomers (2.0 g, 6.8 mmol, 68%). Analytical data in full agreement with literature data.[42] (R_f 0.1 (EtOAc:pentane 1:1 v:v)); ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 4.28 – 4.18 (m, 4H, CH_2 OEt, CH_2 OEt), 4.09 – 3.97 (m, 2H, H-1, H-2), 3.62 (tdd, *J* = 9.2, 7.8, 4.4 Hz, 1H, H-1), 1.40 – 1.31 (m, 6H, CH_3 OEt, CH_3 OEt); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 64.5, 64.5, 64.3, 64.2 (CH_2 OEt), 42.8, 41.6 (C-1), 32.1, 32.1 (C-2), 16.6, 16.5 (CH_3 OEt); ^{31}P NMR (202 MHz, $CDCl_3$) δ 17.26.

4-((1,2-Dibromoethyl)sulfonyl)morpholine (D).

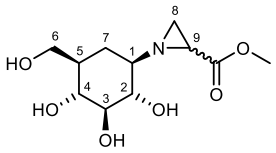


Prepared according to literature procedure.[43] 2-chloroethane-1-sulfonyl chloride (0.52 mL, 5.0 mmol) was dissolved in anhydrous DCM (10 mL, 0.5 M) and cooled on ice, Et_3N (2.1 mL, 15 mmol, 3.0 eq.) and morpholine (0.48 mL, 5.5 mmol, 1.1 eq.) were added subsequently and the reaction was allowed to attain to room temperature over a period of 2 hours upon which the reaction was quenched by addition of sat. aq. $NaHCO_3$ solution and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo.*

The crude intermediate was dissolved in acetonitrile (5.0 mL, 1.0 M) and cooled on ice. Bromine (0.26 mL, 5.0 mmol, 1.0 eq.) was added dropwise and the reaction was allowed to attain to room temperature over a period of 2 hours after which the reaction was quenched by addition of sat. aq. $Na_2S_2O_3$ solution and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine

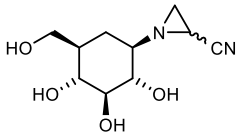
respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (10:90 EtOAc:pentane → 30:70 EtOAc:pentane) yielded the title compound **D** as an inseparable mixture of stereoisomers (1.3 g, 3.9 mmol, 78%). Analytical data in full agreement with literature data.[43] (R_f 0.3 (EtOAc:pentane 2:8 v:v)); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.94 (dd, *J* = 10.0, 3.1 Hz, 1H, H-2), 4.20 (dd, *J* = 11.5, 3.2 Hz, 1H, H-1), 3.77 – 3.66 (m, 5H, H-1, CH_2 morpholine, CH_2 morpholine), 3.51 – 3.41 (m, 4H, CH_2 morpholine, CH_2 morpholine).

Methyl 1-(1-*epi*-validamine)aziridine-2-carboxylate (8).



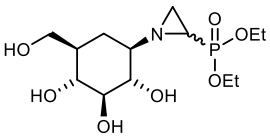
Compound **8** was prepared according to **general procedure A** using 1-*epi*-validamine **6** (29 mg, 0.1 mmol), DiPEA (0.14 mL, 0.8 mmol, 8.0 eq.) and methyl 2,3-dibromopropanoate **A** (50 µL, 0.4 mmol, 4.0 eq.) in MeOH (1.0 mL, 0.1 M). Flash column chromatography (0:100 MeOH:DCM → 20:80 MeOH:DCM) yielded the title compound **8** (25 mg, 96 µmol, 96%). R_f 0.3 (MeOH:DCM, 2:8, v:v); ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 3.79 – 3.70 (m, 8H, H-6, H-6', OMe, OMe'), 3.61 – 3.56 (m, 2H, H-6, H-6'), 3.46 – 3.36 (m, 2H, H-2, H-2'), 3.26 – 3.21 (m, 2H, H-4, H-4'), 3.18 – 3.13 (m, 2H, H-3, H-3'), 2.64 (dd, *J* = 6.7, 3.5 Hz, 1H, H-9), 2.30 – 2.25 (m, 2H, H-8', H-9'), 2.16 (dd, *J* = 6.0, 1.1 Hz, 1H, H-8'), 1.98 (dd, *J* = 3.5, 0.8 Hz, 1H, H-8), 1.91 (t, *J* = 3.9 Hz, 1H, H-7/H-7'), 1.88 (t, *J* = 3.9, Hz, 1H, H-7/H-7'), 1.75 (dd, *J* = 6.7, 0.8 Hz, 1H, H-8), 1.58 – 1.47 (m, 4H, H-1, H-1', H-5, H-5'), 1.38 – 1.24 (m, 2H, H-7, H-7'); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 173.2, 172.9 (C=O, C=O'), 79.4, 79.3 (C-3, C-3'), 78.0, 77.9 (C-2, C-2'), 74.7, 74.6 (C-4, C-4'), 70.6, 70.1 (C-1, C-1'), 64.2, 64.1 (C-6, C-6'), 52.8, 52.7 (OMe, OMe'), 42.9, 42.8 (C-5, C-5'), 39.6 (C-9), 36.6 (C-8'), 34.5 (C-9'), 31.3, 31.0 (C-7, C-7'), 30.8 (C-8); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{11}H_{19}NO_6$ 262.1291; Found 262.1285.

1-(1-*Epi*-validamine)aziridine-2-carbonitrile (9).



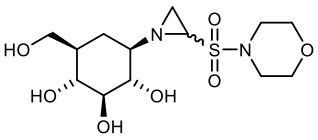
Compound **9** was prepared according to **general procedure A** using 1-*epi*-validamine **6** (29 mg, 0.1 mmol), DiPEA (0.14 mL, 0.8 mmol, 8.0 eq.) and 2,3-dibromopropanenitrile **B** (85 mg, 0.4 mmol, 4.0 eq.) in MeOH (1.0 mL, 0.1 M). Flash column chromatography (0:100 MeOH:DCM → 20:80 MeOH:DCM) and consecutively (50:50 acetone:DCM → 70:30 acetone:DCM) yielded the title compound **9** (19 mg, 82 µmol, 82%). R_f 0.3 (MeOH:DCM, 2:8, v:v); ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 3.80 – 3.71 (m, 2H, H-6, H-6'), 3.62 – 3.53 (m, 2H, H-6, H-6'), 3.46 – 3.37 (m, 2H, H-2, H-2'), 3.27 – 3.17 (m, 2H, H-4, H-4'), 3.14 – 3.06 (m, 2H, H-3, H-3'), 2.61 (dd, *J* = 6.6, 3.5 Hz, 1H, H-9), 2.39 – 2.32 (m, 2H, H-8', H-9'), 2.20 (d, *J* = 6.4 Hz, 1H, H-8'), 2.11 (d, *J* = 3.5 Hz, 1H, H-8), 1.97 (ddd, *J* = 12.6, 3.4, 3.4 Hz, 1H, H-7/H-7'), 1.87 (ddd, *J* = 13.3, 3.9, 3.9 Hz, 1H, H-7/H-7'), 1.81 (d, *J* = 6.6 Hz, 1H, H-8), 1.58 – 1.22 (m, 6H, H-1, H-1', H-5, H-5', H-7, H-7'); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 120.3, 120.1 (CN, CN'), 79.7, 79.4 (C-3, C-3'), 78.0, 77.9 (C-2, C-2'), 74.5 (C-4, C-4'), 70.8, 70.5 (C-1, C-1'), 64.1, 64.0 (C-6, C-6'), 43.0, 42.9 (C-5, C-5'), 36.5 (C-8'), 31.2, 31.0 (C-7, C-7'), 30.5 (C-8), 25.7 (C-9), 20.3 (C-9'); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{10}H_{17}N_2O_4$ 229.1188; Found 229.1183.

Diethyl 1-(1-*epi*-validamine)aziridine-2-phosphonate (10).



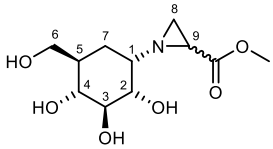
Compound **10** was prepared according to **general procedure A** using 1-*epi*-validamine **6** (29 mg, 0.1 mmol), DiPEA (0.14 mL, 0.8 mmol, 8.0 eq.) and diethyl (1,2-dibromoethyl)phosphonate **D** (130 mg, 0.4 mmol, 4.0 eq.) in MeOH (1.0 mL, 0.1 M). Flash column chromatography (0:100 MeOH:DCM → 20:80 MeOH:DCM) and consecutively (70:30 acetone:DCM → 95:5 acetone:DCM) yielded the title compound **10** (25 mg, 73 µmol, 73%). R_f 0.4 (MeOH:DCM, 2:8, v:v); ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 4.24 – 4.11 (m, 8H, CH_2 OEt, CH_2 OEt, CH_2 OEt', CH_2 OEt'), 3.76 (dd, *J* = 10.8, 4.0 Hz, 2H, H-6, H-6'), 3.56 (dd, *J* = 10.8, 6.3 Hz, 2H, H-6, H-6'), 3.42 (dd, *J* = 9.1, 9.0 Hz, 2H, H-2, H-2'), 3.22 (dd, *J* = 10.4, 9.0 Hz, 2H, H-4, H-4'), 3.13 (dd, *J* = 9.1, 9.0 Hz, 2H, H-3, H-3'), 2.13 (dd, *J* = 7.0, 3.9 Hz, 1H, H-9), 2.09 (dd, *J* = 7.0, 3.9 Hz, 1H, H-9'), 1.98 (ddd, *J* = 9.6, 3.9, 0.7 Hz, 2H, H-8, H-8'), 1.90 (ddd, *J* = 13.0, 3.7, 3.6 Hz, 2H, H-7, H-7'), 1.70 (ddd, *J* = 7.8, 6.9, 0.7 Hz, 2H, H-8, H-8'), 1.52 – 1.44 (m, 2H, H-5, H-5'), 1.43 – 1.26 (m, 16H, H-1, H-1', H-7, H-7', CH_3 OEt, CH_3 OEt, CH_3 OEt', CH_3 OEt'); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 79.5 (C-3, C-3'), 78.4 (C-2, C-2'), 74.7 (C-4, C-4'), 71.8, 71.8 (C-1, C-1'), 64.3, 64.2, 64.2 (C-6, C-6', CH_2 OEt, CH_2 OEt', CH_2 OEt, CH_2 OEt'), 43.0 (C-5, C-5'), 34.4 (C-9), 32.6 (C-9'), 31.4 (C-7, C-7'), 28.6, 28.6 (C-8, C-8'), 16.7, 16.7, 16.7, 16.7 (CH_3 OEt, CH_3 OEt', CH_3 OEt, CH_3 OEt'); ^{31}P NMR (202 MHz, MeOD): δ 23.82; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{13}H_{27}NO_7P$ 340.1525; Found 340.1519.

Morpholino 1-(1-*epi*-validamine)aziridine-2-sulfonamide (11).



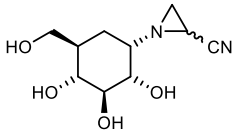
Compound **11** was prepared according to **general procedure A** using 1-*epi*-validamine **6** (29 mg, 0.1 mmol), DiPEA (0.14 mL, 0.8 mmol, 8.0 eq.) and 4-((1,2-dibromoethyl)sulfonyl)morpholine **D** (135 mg, 0.4 mmol, 4.0 eq.) in MeOH (1.0 mL, 0.1 M). Flash column chromatography (0:100 MeOH:DCM → 20:80 MeOH:DCM) and consecutively (70:30 acetone:DCM → 95:5 acetone:DCM) yielded the title compound **11** (28 mg, 79 µmol, 79%). R_f 0.5 (MeOH:DCM, 2:8, v:v); ^{1}H NMR (500 MHz, D_2O, HH-COSY, HSQC): δ 5.48 (dd, *J* = 8.9, 4.0 Hz, 1H, H-9), 5.42 (dd, *J* = 7.7, 4.6 Hz, 1H, H-9'), 3.83 – 3.76 (m, 10H, H-6, H-6', H-10, H-10'), 3.67 – 3.61 (m, 2H, H-6, H-6'), 3.59 – 3.46 (m, 10H, H-8, H-8', H-10, H-10'), 3.36 – 3.17 (m, 8H, H-2, H-2', H-3, H-3', H-4, H-4', H-8, H-8'), 2.75 – 2.56 (m, 2H, H-1, H-1'), 2.08 – 2.02 (m, 2H, H-7, H-7'), 1.68 – 1.58 (m, 2H, H-5, H-5'), 1.14 – 1.01 (m, 2H, H-7, H-7'); ^{13}C NMR (126 MHz, D_2O, HSQC): δ 77.6, 77.6 (C-3/C-4, C-3'/C-4'), 75.6, 75.5 (C-2, C-2'), 72.7, 72.7 (C-3/C-4, C-3'/C-4'), 66.6 (C-11, C-11'), 62.3 (C-6, C-6'), 61.1, 60.9 (C-9, C-9'), 57.8, 56.5 (C-1, C-1'), 48.1, 48.0, 47.1, 47.0 (C-8, C-8'), 46.8 (C-10, C-10'), 41.1, 41.0 (C-5, C-5'), 28.7, 28.2 (C-7, C-7'); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{13}H_{25}N_2O_7S$ 353.1383; Found 353.1376.

Methyl 1-(validamine)aziridine-2-carboxylate (12).



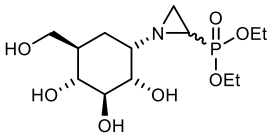
Compound **12** was prepared according to **general procedure A** using validamine **7** (29 mg, 0.1 mmol), DiPEA (0.14 mL, 0.8 mmol, 8.0 eq.) and commercially available methyl 2,3-dibromopropanoate **A** (40 µL, 0.4 mmol, 4.0 eq.) in MeOH (1.0 mL, 0.1 M). Flash column chromatography (0:100 MeOH:DCM → 20:80 MeOH:DCM) and consecutively (70:30 acetone:DCM → 95:5 acetone:DCM) yielded the title compound **12** (22 mg, 85 µmol, 85%). R_f 0.3 (MeOH:DCM, 2:8, v:v); ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 3.93 (t, *J* = 9.3 Hz, 1H, H-3/H-3'), 3.86 (t, *J* = 9.3 Hz, 1H, H-3/H-3'), 3.74 – 3.65 (m, 8H, H-6, H-6', OMe, OMe'), 3.63 – 3.55 (m, 2H, H-6, H-6'), 3.46 – 3.39 (m, 2H, H-2, H-2'), 3.24 – 3.16 (m, 2H, H-4, H-4'), 2.47 (dd, *J* = 6.4, 3.2 Hz, 1H, H-9), 2.26 (dd, *J* = 3.1, 1.1 Hz, 1H, H-8'), 2.23 – 2.14 (m, 1H, H-5/H-5'), 2.10 – 1.97 (m, 3H, H-5/H-5', H-8', H-9'), 1.94 – 1.87 (m, 3H, H-1, H-1', H-8), 1.86 – 1.78 (m, 2H, H-7, H-7'), 1.50 (dd, *J* = 6.5, 1.3 Hz, 1H, H-8), 1.38 – 1.28 (m, 2H, H-7, H-7'); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 173.8, 173.7 (C=O, C=O'), 76.5 (C-3, C-3'), 76.4, 76.2 (C-2, C-2'), 75.9, 75.8 (C-4, C-4'), 69.4, 69.0 (C-1, C-1'), 64.5, 64.5 (C-6, C-6'), 52.6, 52.5 (OMe, OMe'), 40.6, 40.5 (C-5, C-5'), 40.0 (C-9), 37.4 (C-8'), 34.2 (C-9'), 30.9, 30.6 (C-7, C-7'), 30.5 (C-8); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{11}H_{20}NO_6$ 262.1291; Found 262.1285.

1-(Validamine)aziridine-2-carbonitrile (13).



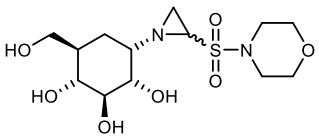
Compound **13** was prepared according to **general procedure A** using validamine **7** (29 mg, 0.1 mmol), DiPEA (0.14 mL, 0.8 mmol, 8.0 eq.) and 2,3-dibromopropanenitrile **B** (85 mg, 0.4 mmol, 4.0 eq.) in MeOH (1.0 mL, 0.1 M). Flash column chromatography (0:100 MeOH:DCM → 20:80 MeOH:DCM) and consecutively (50:50 acetone:DCM → 70:30 acetone:DCM) yielded the title compound **13** (18 mg, 82 µmol, 82%) as a 6:1 mixture of diastereoisomers. R_f 0.4 (MeOH:DCM, 2:8, v:v); data for the major stereoisomer: ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 3.89 (t, *J* = 9.2 Hz, 1H, H-3), 3.73 (dd, *J* = 10.7, 4.0 Hz, 1H, H-6), 3.60 (dd, *J* = 10.7, 6.1 Hz, 1H, H-6), 3.45 (dd, *J* = 9.6, 3.4 Hz, 1H, H-2), 3.22 (dd, *J* = 10.7, 8.9 Hz, 1H, H-4), 2.49 (dd, *J* = 6.4, 3.3 Hz, 1H, H-9), 2.15 – 2.06 (m, 1H, H-5), 2.05 (d, *J* = 3.2 Hz, 1H, H-8), 1.87 (td, *J* = 3.2, 3.1 Hz, 1H, H-1), 1.81 (ddd, *J* = 14.3, 3.6, 3.6 Hz, 1H, H-7), 1.62 (d, *J* = 6.2 Hz, 1H, H-8), 1.34 (ddd, *J* = 14.3, 12.8, 2.9 Hz, 1H, H-7); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 120.5 (CN), 76.8 (C-3), 76.0 (C-2), 75.5 (C-4), 69.1 (C-1), 64.4 (C-6), 40.7 (C-5), 30.5 (C-7), 30.2 (C-8), 26.0 (C-9); data for the minor stereoisomer: ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 3.82 – 3.75 (m, 1H, H-3), 3.53 (dd, *J* = 9.7, 3.6 Hz, 1H, H-2), 2.57 (dd, *J* = 5.3, 3.4 Hz, 1H, H-9), 2.40 (d, *J* = 5.3 Hz, 1H, H-8), 2.37 (d, *J* = 3.3 Hz, 1H, H-1), 2.02 (dd, *J* = 3.5, 3.4 Hz, 1H, H-7), 1.47 – 1.39 (m, 1H, H-7); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 118.9 (CN), 76.7 (C-3), 76.0 (C-2), 75.7 (C-4), 66.1 (C-1), 40.6 (C-5), 36.9 (C-8), 30.5 (C-7), 18.4 (C-9); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{10}H_{17}N_2O_4$ 229.1188; Found 229.1183.

Diethyl 1-(validamine)aziridine-2-phosphonate (14).



Compound **14** was prepared according to **general procedure A** using validamine **7** (29 mg, 0.1 mmol), DiPEA (0.14 mL, 0.8 mmol, 8.0 eq.) and diethyl (1,2-dibromoethyl)phosphonate **C** (130 mg, 0.4 mmol, 4.0 eq.) in MeOH (1.0 mL, 0.1 M). Flash column chromatography (0:100 MeOH:DCM → 15:85 MeOH:DCM) and consecutively (70:30 acetone:DCM → 90:10 acetone:DCM) yielded the title compound **14** (22 mg, 65 µmol, 65%). R_f 0.5 (MeOH:DCM, 2:8, v:v); ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 4.21 – 4.12 (m, 8H, CH_2 OEt, CH_2 OEt, CH_2 OEt', CH_2 OEt'), 3.87 (dd, *J* = 9.6, 8.9 Hz, 2H, H-3, H-3'), 3.73 (dd, *J* = 10.7, 4.0 Hz, 2H, H-6, H-6'), 3.59 (dd, *J* = 10.7, 6.1 Hz, 2H, H-6, H-6'), 3.40 (dd, *J* = 9.7, 3.3 Hz, 2H, H-2, H-2'), 3.22 (dd, *J* = 10.6, 8.8 Hz, 2H, H-4, H-4'), 2.15 – 2.07 (m, 2H, H-5, H-5'), 2.05 (dd, *J* = 6.7, 3.6 Hz, 1H, H-9), 2.01 (dd, *J* = 6.7, 3.6 Hz, 1H, H-9'), 1.97 – 1.93 (m, 2H, H-8, H-8'), 1.88 – 1.83 (m, 4H, H-1, H-1', H-7, H-7'), 1.57 (ddd, *J* = 7.7, 6.7, 1.0 Hz, 2H, H-8, H-8'), 1.42 – 1.27 (m, 14H, H-7, H-7', CH_3 OEt, CH_3 OEt, CH_3 OEt', CH_3 OEt'); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 77.0 (C-3, C-3'), 76.8 (C-2, C-2'), 75.8 (C-4, C-4'), 70.1, 70.1 (C-1, C-1'), 64.6, 64.6, 64.5, 64.2, 64.2 (C-6, C-6', CH_2 OEt, CH_2 OEt', CH_2 OEt, CH_2 OEt'), 40.7 (C-5, C-5'), 34.9 (C-9), 33.2 (C-9'), 31.0 (C-7, C-7'), 28.4, 28.4 (C-8, C-8'), 16.8, 16.8, 16.7, 16.7 (CH_3 OEt, CH_3 OEt', CH_3 OEt, CH_3 OEt'); ^{31}P NMR (202 MHz, MeOD): δ 25.71; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{13}H_{27}NO_7P$ 340.1525; Found 340.1519.

Morpholino 1-(validamine)aziridine-2-sulfonamide (15).



Compound **15** was prepared according to **general procedure A** using validamine **7** (29 mg, 0.1 mmol), DiPEA (0.14 mL, 0.8 mmol, 8.0 eq.) and 4-((1,2-dibromoethyl)sulfonyl)morpholine **D** (135 mg, 0.4 mmol, 4.0 eq.) in MeOH (1.0 mL, 0.1 M). Flash column chromatography (0:100 MeOH:DCM → 20:80 MeOH:DCM) and consecutively (50:50 acetone:DCM → 75:25 acetone:DCM) yielded the title compound **15** (31 mg, 87 µmol, 87%). R_f 0.5 (MeOH:DCM, 2:8, v:v); ^{1}H NMR (500 MHz, MeOD, HH-COSY, HSQC): δ 5.38 (dd, *J* = 8.3, 4.0 Hz, 1H, H-9), 5.32 (dd, *J* = 8.0, 4.4 Hz, 1H, H-9), 3.78 – 3.67 (m, 6H, H-6, H-6', H-11, H-11'), 3.65 – 3.55 (m, 4H, H-3, H-3', H-6, H-6'), 3.53 – 3.38 (m, 12H, H-2, H-2', H-8, H-8', H-10, H-10'), 3.24 – 3.15 (m, 3H, H-4, H-4', H-8/H-8'), 3.13 – 3.03 (m, 3H, H-1, H-1', H-8/H-8'), 2.03 – 1.85 (m, 4H, , H-5, H-5', H-7, H-7'), 1.32 – 1.19 (m, 2H, H-7, H-7'); ^{13}C NMR (126 MHz, MeOD, HSQC): δ 76.4, 76.4 (C-3, C-3'), 75.6, 75.6 (C-4, C-4'), 75.5, 75.4 (C-2, C-2'), 68.0, 68.0 (C-11, C-11'), 64.5 (C-6, C-6'), 63.7, 63.4 (C-9, C-9'), 58.0, 56.6 (C-1, C-1'), 51.0, 50.3 (C-8, C-8'), 48.3, 48.2 (C-10, C-10'), 39.7, 39.7 (C-5, C-5'), 28.9, 28.3 (C-7, C-7'); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{13}H_{25}N_2O_7S$ 353.1383; Found 353.1371.

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Chapter 5

Trapping inverting glucosidases with conjugate addition acceptor cyclitols

Introduction

As described in chapter 4, the relatively large enzyme pocket (6 – 12 Å) and the presence of water alongside the substrate glucoside, allows inverting α- and β-glucosidases to employ a Koshland single-displacement mechanism in the hydrolysis of glycosidic linkages (Figure 1).[1–5] During enzyme catalysis, no covalently bound substrate-enzyme adduct is formed,[1–3,6–9] while such a covalent intermediate is crucial for cyclophellitol-based inhibitors during inactivation of retaining α- and β-glucosidases.[10–16] As a result of the one-step hydrolysis mechanism, no effective mechanism-based, covalent and irreversible inhibitors for inverting glucosidases have been developed to date.

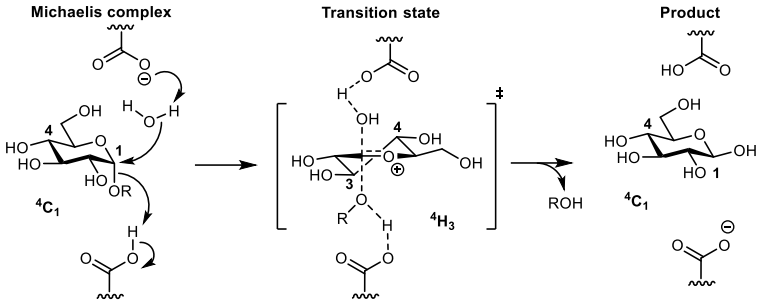


Figure 1. Conformational itinerary of an inverting α-glucosidases *via* a classic Koshland single displacement mechanism.[4]

Stick and Stubbs previously proposed implementing a conjugate addition warhead in their design of a glucosyl transferase inhibitor (Figure 2A, compound **1**).[17] The inhibitor they designed, was equipped with an anomeric vinyl moiety combined with an anomeric leaving group (UDP, in order to mimic the donor substrate of the glucosyl transferase). Unfortunately, due to the intrinsic lability of this compound, induced by the donating effect of the endocyclic oxygen, the synthesis of compound **1** proved troublesome and preparation of allyl phosphate **2** failed due to the elimination of the phosphate, yielding diene **3** instead (Figure 2B).

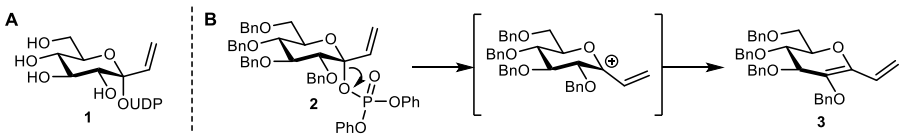


Figure 2. (A) Target compound **1** as proposed by Stick and Stubbs, who envisioned **1** as a putative glucosyl transferase inhibitor and (B) the observed side reaction resulting in compound **3**, troubling preparation of intermediate **2**.[17]

Prompted by Stick and Stubbs' design, a series of inhibitors bearing exotic chemical warheads can be envisioned that target inverting glucosidases. This chapter describes the synthetic endeavors towards this goal. In particular, structures **4** – **9** (Figure 3A), were considered as putative inhibitors targeting inverting glucosidases. Structures **4** – **9** exhibit a conjugate addition warhead, allowing for irreversible conjugate addition on the terminal side of the alkene. By extending the electrophile further from the anomeric center, the proposed inhibitors are envisioned to bridge the relatively large distance (6 – 12 Å) between the carboxylic acid/carboxylate residues in the enzyme active site pocket.[6] This may provoke an irreversible addition reaction with these more distal carboxylate residues to form a stable ester linkage, thereby effectively incapacitating the enzyme (Figure 3B).

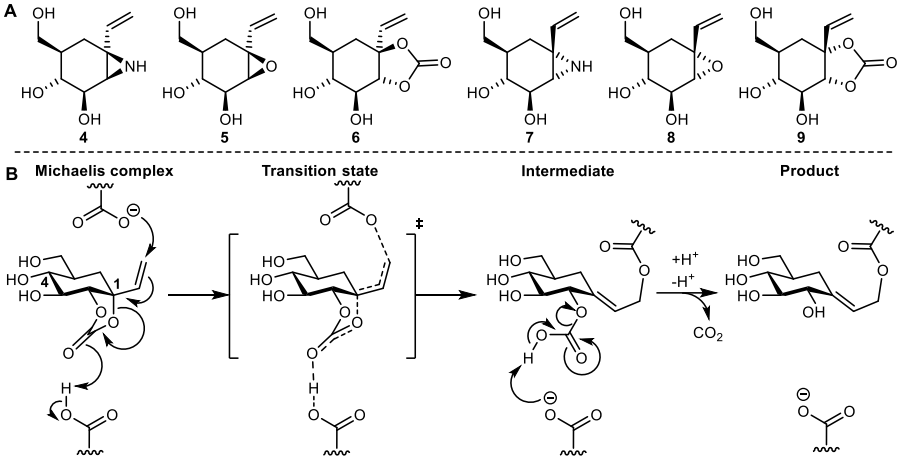


Figure 3. (A) Six proposed inhibitors of inverting α- or β-glucosidases bearing a conjugate addition warhead and (B) proposed mechanism of covalent inhibition of an inverting α-glucosidase by compound **9**.

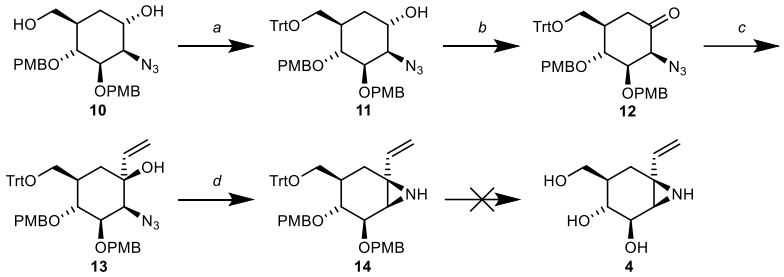
The intrinsic lability of vinylated constructs upon arming of the anomeric center with a leaving group, as in **2**, can likely be circumvented by switching to a carbasugar analogue. Lacking the endocyclic oxygen, the proposed constructs were hypothesized to be more stable and less prone to undesired side reactions when compared to analogues containing this endocyclic oxygen. In addition, previous work has shown the proposed chemical warheads to be suitable conjugate addition acceptors upon treatment with nucleophiles.[18–22] In this chapter, synthesis efforts towards these target molecules are described.

Results and discussion

Synthesis of 1-vinyl-β-aziridine 4. The synthesis scheme started from azido-alcohol **10**, envisioned as a suitably protected construct and a viable starting point towards 1-vinyl-β-aziridine **4** (Scheme 1). The preparation of azido-alcohol **10** is part of the research described in chapter 3. Regioselective protection of the primary hydroxyl as a trityl ether providing alcohol **11** in excellent yield (92%) under standard conditions. Oxidation with Dess-Martin periodinane and $NaHCO_3$ converted the secondary hydroxyl in **11** to ketone **12** in quantitative yield. Upon treatment of compound **12** with vinylmagnesium bromide at low temperature, the ketone was neatly converted into vinyl alcohol **13**, again in near-quantitative yield. The Grignard addition appeared to be very stereoselective as no formation of the C-1 epimer was observed. Proton NMR couplings ($^3J_{H\text{-}H}$ coupling, NOE

interactions), confirmed the assignment of the stereocenters in **13** (see appendix, Figure S1). The observed selectivity can be explained considering the Cornforth and Felkin-Ahn model, stating that addition to the carbonyl occurs following an trajectory *anti* with respect to a polar α-substituent (the azide), resulting only in bottom-side/axial attack.[23,24] With *syn* azido-alcohol **13** in hand, Staudinger cyclisation towards the anticipated 1-vinyl-β-aziridine **14** was investigated.[16,25] To this end, compound **13** was treated with triphenylphosphine in anhydrous acetonitrile at elevated temperature. Gratifyingly, clean conversion towards the desired 1-vinyl-β-aziridine **14** was observed in 45% yield. With the warhead in place, only global deprotection remained. Unfortunately, Birch reduction (Na, NH_3) did not yield target structure **4**. Instead, degradation of the vinyl aziridine was observed.

Scheme 1. Construction of target **4** from azido-alcohol **10**.



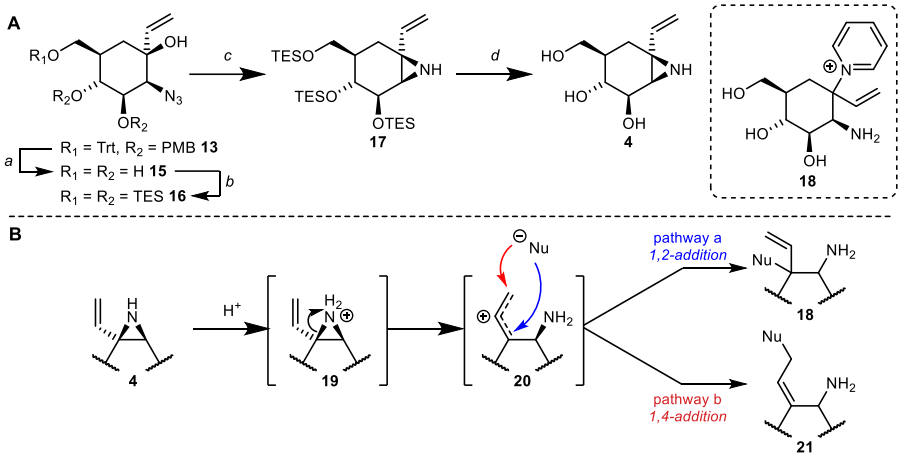
Reagents and conditions: *a*) TrtCl, DMAP, pyridine, DMF, rt, 16 h (92%); *b*) Dess-Martin periodinane, $NaHCO_3$, DCM, rt, 30 min. (quant.); *c*) vinylmagnesium bromide, THF, -78 °C to -30 °C, 2 h (99%); *d*) PPh_3, acetonitrile, 70 °C, 16 h, (45%).

All attempts to deprotect compound **14** under oxidative conditions (DDQ or CAN) proofed futile, again resulting in complete degradation of material.

Prompted by the robustness of the vinyl-aziridine formation aided by a Staudinger cyclisation, it was hypothesized that the problematic global deprotection could be circumvented using an alternative protecting group strategy. To this end, triethylsilyl ether (TES) protecting groups were considered, since it was hypothesized that the TES protecting groups could be removed readily under mild conditions using a fluoride source such as TBAF or $Et_3N{\cdot}HF$. To this end, intermediate **13** was deprotected under acidic conditions (TFA, TES) to yield compound **15** in 77% yield (Scheme 2A). The primary and secondary hydroxyls could be protected selectively under relatively mild conditions (TESCl, pyridine), giving compound **16** in a moderate yield (45%). Next up was the Staudinger cyclisation, which under identical conditions as described above yielded the desired vinyl aziridine **17** in good yield (52%). Global deprotection of compound **17** was first attempted using Olah's reagent (pyridine·HF), which is considered a very mild

fluoride source. However, upon treatment with this mild fluoride source, pyridine adduct **18** was isolated instead. The formation of this side product was rationalized by the slightly acidic nature of the pyridinium ion (Scheme 2B), which facilitates protonation of the relatively basic aziridine **4**. As a result, the aziridine opens, forming a relatively stable tertiary, allylic carbocation **20**.

Scheme 2. Preparation of aziridine **4** (A) and proposed mechanism of observed proton-induced side reaction upon global deprotection of **17** with Olah's reagent (B).



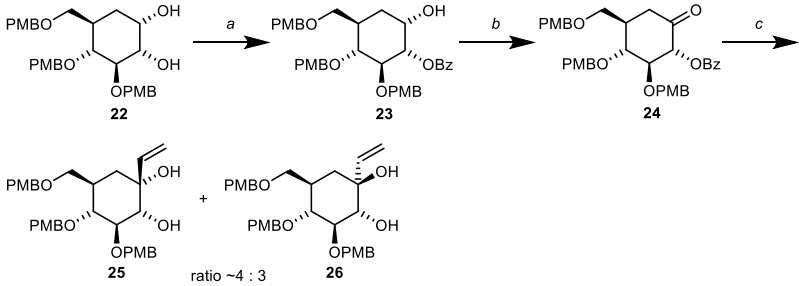
Reagents and conditions: *a*) TFA, TES, DCM, rt, 1 h (77%); *b*) TESCl, pyridine, rt, 16 h (45%); *c*) PPh_3, acetonitrile, 70 °C, 16 h (52%); *d*) TBAF, THF, 0 °C, 2 h, (70%).

A nucleophilic addition by pyridine on the C-1 position then results in the formation of compound **18**. In order to circumvent the acid-catalyzed aziridine opening, attention was shifted to the use of TBAF as an alkaline alternative.[26,27] Indeed, clean conversion was observed upon treatment of compound **17** with TBAF, obtaining target **4** in decent yield (70%). Proton NMR couplings ($^3J_{H\text{-}H}$ coupling), confirmed the correct assignment of the stereocenters in **4** (see appendix, Table S7).

Synthesis of 1-vinyl-β-epoxide 5 and 1-vinyl-β-carbonate 6. After successfully obtaining 1-vinyl-β-aziridine **4**, attention was turned to the preparation of 1-vinyl-β-epoxide **5** and *trans* 5,6-fused 1-vinyl-β-carbonate **6**, incorporating methodologies acquired during the previous synthesis of construct **4**. The synthesis started with the preparation of *syn*- and *anti*-diol **25** and **26**, respectively. *Syn*-diol **22** (Scheme 3), the synthesis of which is described in chapter 3, was regioselectively protected at the 2-OH as a benzoyl ester under mild conditions (BzCl, pyridine, -15 °C), according to literature procedures, to give compound **23** in quantitative yield.[28] Subsequently, oxidation of 1-OH under Dess-Martin oxidation conditions provided ketone **24** in near-quantitative yield (96%).[29,30] Treatment of **24** with excess vinylmagnesium bromide at low temperature (-78 °C) led

to the addition of the vinyl Grignard to the ketone within 15 minutes. Slowly increasing the temperature of the reaction mixture to 0 °C allowed the remaining Grignard reagent to perform an addition to the benzoyl carbonyl, thereby effectively liberating the 2-OH.

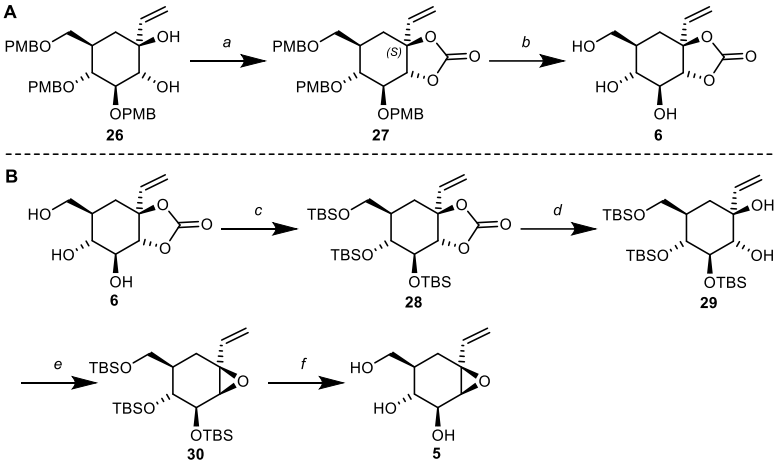
Scheme 3. Construction of *syn*- and *anti*-diol **25** and **26**.



Reagents and conditions: *a*) BzCl, pyridine, -15 °C, 1 h (quant.); *b*) Dess-Martin periodinane, $NaHCO_3$, DCM, rt, 1 h (96%); *c*) vinylmagnesium bromide, THF, -78 °C to 0 °C, 3 h, **25** (40%), **26** (32%).

The *syn*- and *anti*-diol **25** and **26** were obtained as a separable mixture in a 72% overall yield and in an 4:3 ratio, respectively. Proton NMR couplings ($^3J_{H\text{-}H}$ coupling, NOE interactions), confirmed the assignment of the stereocenters in **25** and **26** (see appendix, Figure S2 and S3). Protection of *anti*-diol **26** as the cyclic carbonate proceeded sluggishly and required elevated temperatures to get full consumption of the starting material as a result of the formation of a relatively strained trans 5,6-fused bicycle (Scheme 4). Attention was then turned to the global deprotection of the thus obtained compound **27** in acidic media (TFA, TES). This resulted in complete removal of all PMB ethers and the isolation of target compound **6** in 79% yield (See Figure S4 for the NMR spectra and assignment of the stereocenters in **6**). In addition, carbonate **6** was considered as a suitable intermediate to provide the 1-vinyl-β-epoxide **5**. Therefore, carbonate **6** was protected with silyl ethers (TBSOTf), in line with the methodology used during the preparation of 1-vinyl-β-aziridine **4**, to yield compound **28** in 56% yield. A transesterification attempt on intermediate **28** using NaOMe resulted in degradation of the starting material. However, removal of the carbonate protecting group proceeded smoothly under reductive conditions ($LiBH_4$), setting the stage for preparation of the 1-vinyl-β-epoxide warhead.

Scheme 4. Construction of *trans* 5,6-fused carbonate **6** (A) and 1-vinyl-β-epoxide **5** (B) from *anti*-diol **26**.



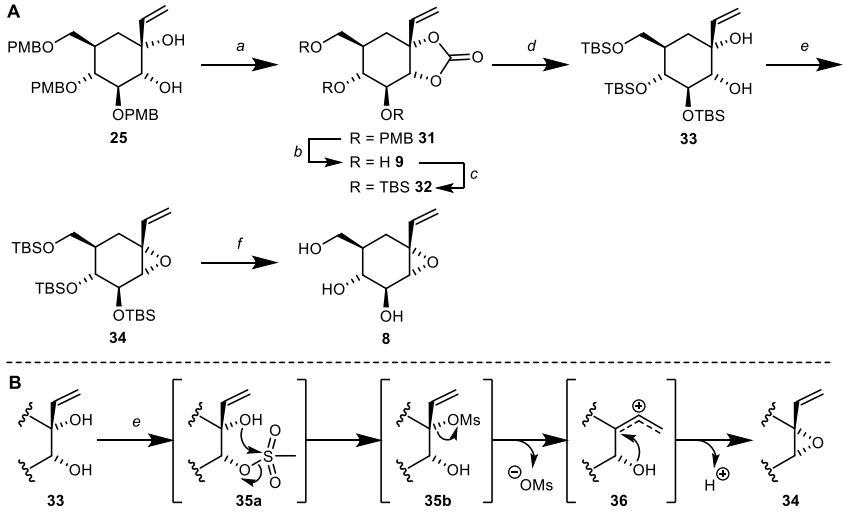
Reagents and conditions: *a*) CDI, DCE, 60 °C, 16 h (56%); *b*) TFA, TES, DCM, rt, 1 h (79%); *c*) TBSOTf, pyridine, DCM, 40 °C, 2 h (56%); *d*) $LiBH_4$, Et_2O, 0 °C to rt, 2 h (64%); *e*) MsCl, Et_3N, DCM, 0 °C, 30 min. (47%); *f*) silica bound TBAF, THF, rt, 2 h (63%).

It was anticipated that, due to the secondary hydroxyl being inherently more reactive than the tertiary hydroxyl, treatment with MsCl would allow for the selective mesylation of the 2-OH. Subsequently, the axial 1-OH is positioned perfectly to perform an intramolecular substitution to form the epoxide. Indeed, under mild conditions (MsCl, Et_3N, 0 °C) rapid formation of the desired epoxide was observed, which was isolated in 47% yield. The use of silica-bound TBAF resulted in clean removal of all protecting groups and in addition allowed for direct concentration and loading on a silica column for purification. This resulted in the isolation of target **5** in good yield (63%).

Synthesis of 1-vinyl-α-epoxide 8 and 1-vinyl-α-carbonate 9. With 1-vinyl-β-epoxide **5** and *trans* 5,6-fused carbonate **6** in hand, attention was then turned to the preparation of 1-vinyl-α-epoxide **8** and *cis* 1,2-fused 1-vinyl-α-carbonate **9** starting from *syn*-diol **25** (Scheme 5A). Following previously optimized methodology, diol **25** was protected as a cyclic carbonate. The reaction proceeded smoother in comparison with that on the corresponding *trans*-diol, as reflected by the lower operation temperature and the higher yield, since the product of this reaction is a less-strained *cis*-fused bicyclic construct. Subsequent removal of all PMB ethers under acidic conditions provided target **9** in excellent yield (91%). Proton NMR couplings ($^3J_{H\text{-}H}$ coupling), confirmed the correct assignment of the stereocenters in **9** (see appendix, Table S8). Compound **9** functioned as an intermediate towards the 1-vinyl-epoxide **8**, and for this, all hydroxyl functionalities were protected as silyl ethers (TBSOTf), followed by reductive cleavage

($LiBH_4$), as described above, of the cyclic carbonate to provide *syn*-diol **33**. Although *syn*-diol **33** bears an equatorially positioned 1-OH, not suited to perform an intramolecular substitution to form the desired epoxide, upon mesylation of *syn*-diol **33**, again, rapid formation of the desired epoxide was observed. It is hypothesized that, based on the inherent higher reactivity of secondary- over tertiary hydroxyls, mesylation occurs on the 2-OH to form intermediate **34** (Scheme 5B). Due to the *syn* orientation to the 1-OH, direct substitution and epoxide formation is considered to be non-viable. Instead, a migration of the mesylate group to the adjacent 1-OH is proposed, forming mesylated tertiary alcohol **35**. Due to the relatively stable carbocation that can form at the C-1 position, expulsion of the mesylate can take place, providing carbocation **36**. The 2-OH can then attack the C-1 cation to form the desired epoxide **34**. Exposure of compound **34** to silica bound TBAF let to removal of all silyl ethers and the isolation of target **8** in good yield (72%).

Scheme 5. Construction of epoxide **8** and *cis* 5,6-fused carbonate **9** from *syn*-diol **25** (A) and proposed mechanism of epoxide formation under mesylating conditions (B).



Reagents and conditions: *a*) CDI, DCM, 40 °C, 16 h (78%); *b*) TFA, TES, DCM, rt, 1 h (91); *c*) TBSOTf, pyridine, DCM, 0 °C to 40 °C, 2 h (77%); *d*) $LiBH_4$, Et_2O, 0 °C, 2 h (71%); *e*) MsCl, Et_3N, DCM, 0 °C, 30 min. (44%); *f*) silica bound TBAF, THF, 0 °C, 4 h (72%).

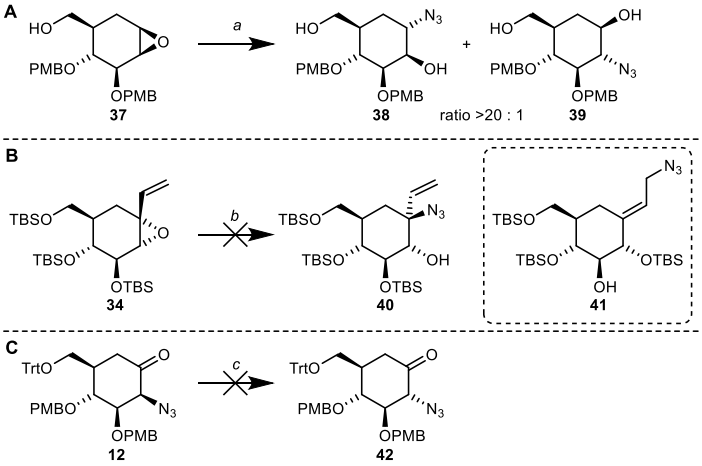
Synthesis of 1-vinyl-α-aziridine 7. Construction of the 1-vinyl-α-aziridine **7** was envisioned to follow identical chemical transformations as those leading to the aforementioned epimer **4**. To this end, β-epoxide **37**, synthesized in chapter 3, was treated with NaN_3 at elevated temperature (Scheme 6A). Unfortunately, opening of the β-epoxide proceeded following the Fürst-Plattner rule, through a favorable chair-like transition state,[31,32] resulting in a >20:1 ratio of the undesired compound **38** and the

desired compound **39**, respectively. Proton NMR couplings ($^3J_{H\text{-}H}$ coupling), confirmed the correct assignment of the stereocenters in **38** (see appendix, Table S9).

In an alternative attempt to synthesize target **7**, opening of the epoxide in compound **30** was envisioned to provide a suitable intermediate towards target **7** (Scheme 6B). For this, compound **34** was considered as an accessible substrate analogue to test the reaction (Scheme 6B). Exposure of **34** to NaN_3 at elevated temperature did not result in consumption of starting material. Turning to tetrabutylammonium azide, a commercially-available alternative known for its high solubility in organic solvents, conversion was observed at elevated temperatures. However, compound **41** was isolated instead in moderate yield (66%), being the product of an 1,4-addition. Although none of the desired azide **40** was obtained, the formation of the 1,4-addition-product can be seen as a proof of concept for these novel conjugate addition warheads.

In a final attempt to find a viable synthetic route towards the 1-vinyl-α-aziridine **7**, compound **12** was considered as a potential substrate to undergo a keto-enol epimerization (Scheme 6C). It was hypothesized that the inherent stability of equatorial substituents over axial ones would function as a driving force in this reaction. To this end, compound **12** was treated with a non-nucleophilic base (Cs_2CO_3) following literature procedures.[33] Although conversion towards the equatorial azide was observed by NMR, purification proved difficult because of degradation of the material in addition to the difficult separation of both C-2 epimers.

Scheme 6. Attempted construction of target **7**. Either following synthetic procedures as for **4** (A), *via* opening of epoxide **34** (B) or base-induced keto-enol C-2 epimerization of **12** (C).



Reagents and conditions: *a*) NaN_3, $LiClO_4$, DMF, 130 °C, 16 h, (72%); *b*) tetrabutylammonium azide, DMF, 100 °C, 2 h (66%); *c*) Cs_2CO_3, MeOH, rt, 16 h.

Conclusion

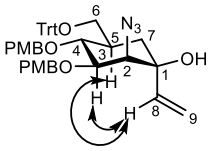
This chapter describes studies on the synthesis of a panel of putative inhibitors designed to inhibit inverting glucosidases. The inhibitors bear an anomeric vinyl functionality paired with bridged electrophiles spanning the C-1 and C-2 position. These bridged electrophiles included an aziridine (**4**), epoxide (**5** and **8**) or cyclic carbonate (**6** and **9**). The vinylogous warheads are envisioned to act as electrophiles, that can span the relatively large enzyme pocket of inverting glycosidases, and trap the catalytic machinery *via* a conjugated 1,4-addition. The use of silyl protecting groups appeared to be crucial during construction of the inhibitors and removal of the silyl protecting groups by TBAF proved imperative, because of its mild and alkaline nature, suppressing degradation and occurrence of side reactions. The synthetic challenges presented during preparation of the 2-deoxy-2-azido carba-glucose backbone, complicated the construction of 1-vinyl-α-aziridine **7**. Construction of a suitable carba-glucose intermediate for the consequent synthesis of 1-vinyl-α-aziridine **7** remains to be an active investigation. Suitably-designed inhibition assays have to show whether this novel class of inhibitors is capable of inhibiting inverting glucosidases.

Appendix

Structural proofs

Compound 13

Table S1. *^{1}H NMR H-H coupling constants*



H2: overlap with H4
H3: dd, *J* = 9.1 Hz ($H3_{ax}$-$H4_{ax}$), *J* = 3.0 Hz ($H3_{ax}$-$H2_{eq}$)
H4: overlap with H2
H5: ddddd, *J* = 14.0 Hz ($H5_{ax}$-H6), *J* = 8.8 Hz ($H5_{ax}$-$H4_{ax}$), *J* = 8.8 Hz ($H5_{ax}$-$H7_{ax}$), *J* = 2.9 Hz ($H5_{ax}$-$H7_{eq}$), *J* = 2.9 Hz ($H5_{ax}$-H6)
$H7_{ax}$: overlap with $H7_{eq}$
$H7_{eq}$: overlap with $H7_{ax}$

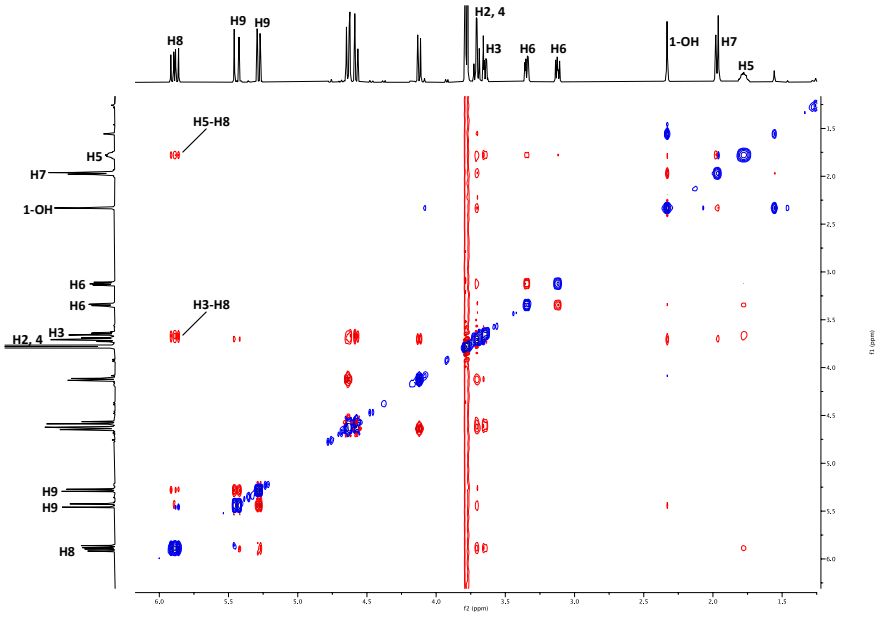
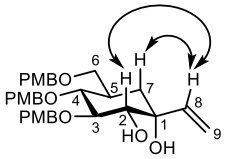


Figure S1. NOESY spectra of compound **13**. The key NOE interactions for **13** can be found between H3-H8 and H5-H8.

Compound 25

Table S2. *^{1}H NMR H-H coupling constants*



H2: d, *J* = 9.1 Hz ($H2_{ax}$-$H3_{ax}$)
H3: dd, *J* = 9.2 Hz ($H3_{ax}$-$H2_{ax}$), *J* = 9.2 Hz ($H3_{ax}$-$H4_{ax}$)
H4: dd, *J* = 9.3 Hz ($H4_{ax}$-$H3_{ax}$), *J* = 10.8 Hz ($H4_{ax}$-$H5_{ax}$)
H5: overlap with 2-OH
$H7_{ax}$: overlap with $H7_{eq}$
$H7_{eq}$: overlap with $H7_{ax}$

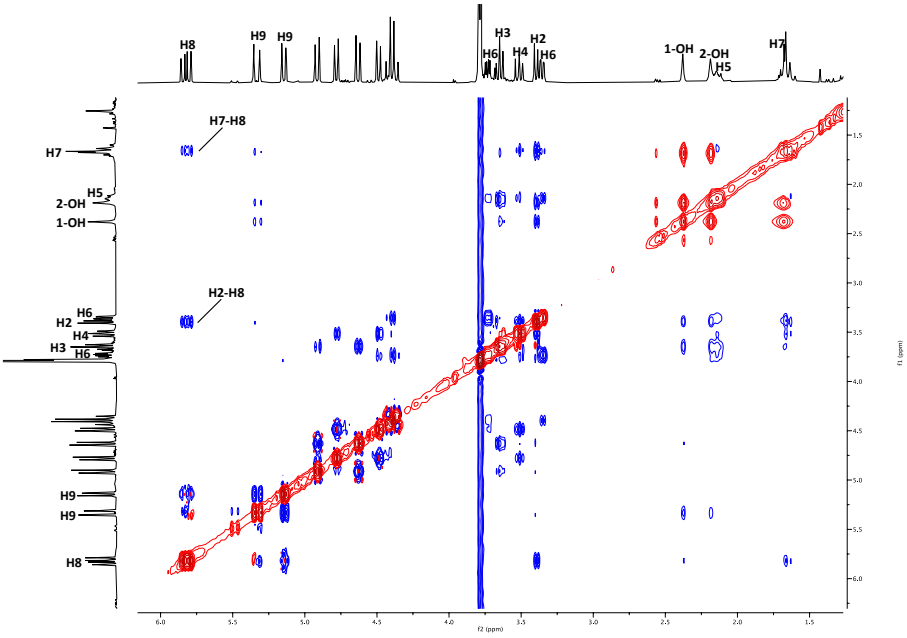
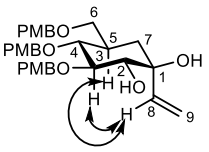


Figure S2. NOESY spectra of compound **25**. The key NOE interactions for **25** can be found between $H2_{ax}$-H8 and $H7_{ax}$-H8.

Compound 26

Table S3. *^{1}H NMR H-H coupling constants*



H2: overlap with H3 and H4
H3: overlap with H2 and H4
H4: overlap with H2 and H3
H5: overlap with $H7_{eq}$
$H7_{ax}$: overlap with H5
$H7_{eq}$: dd, *J* = 2.5 Hz ($H7_{eq}$-$H5_{ax}$), *J* = 11.3 Hz ($H7_{eq}$-$H7_{ax}$)

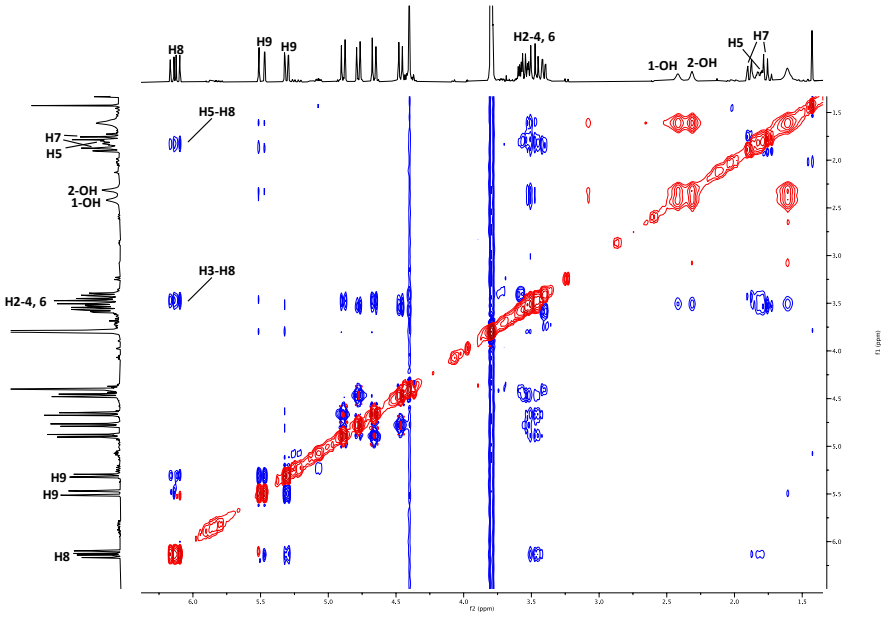
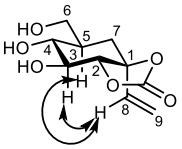


Figure S3. NOESY spectra of compound **26**. The key NOE interactions for **26** can be found between $H3_{ax}$-H8 and $H5_{ax}$-H8.

Compound 6

Table S4. *^{1}H NMR H-H coupling constants*



H2: d, *J* = 11.6 ($H2_{ax}$-$H3_{ax}$)
H3: dd, *J* = 11.6 Hz ($H3_{ax}$-$H2_{ax}$), 7.9 Hz ($H3_{ax}$-$H4_{ax}$)
H4: dd, *J* = 10.0 Hz ($H4_{ax}$-$H5_{ax}$), 7.9 Hz ($H4_{ax}$-$H3_{ax}$)
H5: overlap with $H7_{eq}$
$H7_{ax}$: d, *J* = 7.3 Hz ($H7_{ax}$-$H5_{ax}$), *J* = 10.8 Hz ($H7_{ax}$-$H7_{ax}$)
$H7_{eq}$: overlap with H5

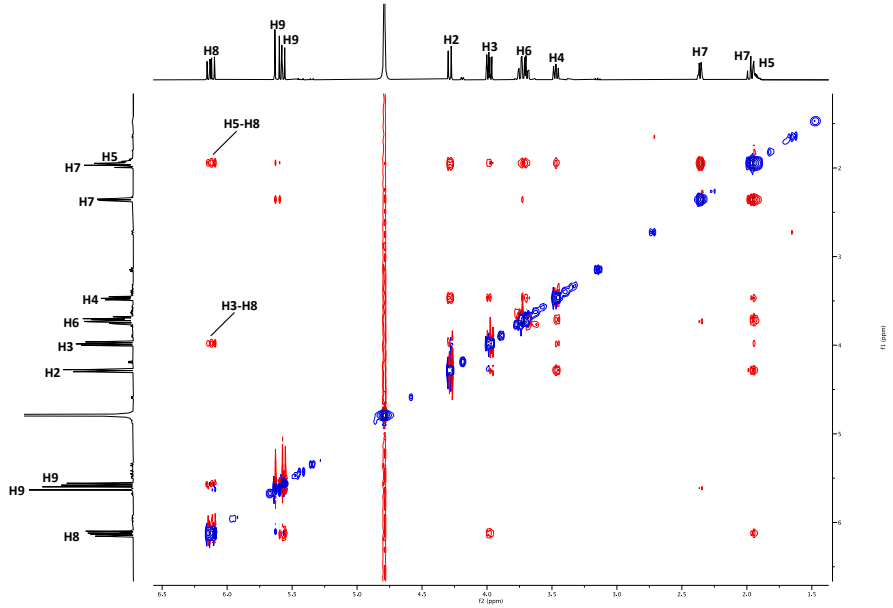
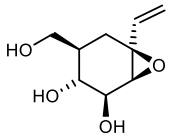


Figure S4. NOESY spectra of compound **6**. The key NOE interactions for **6** can be found between H3-H8 and H5-H8.

Compound 5

Table S5. *^{1}H NMR H-H coupling constants*



H2: d, *J* = 11.1 Hz ($H2_{p\text{-}eq}$-$H3_{p\text{-}ax}$)
H3: overlap with 6-OH
H4: multiplet
H5: multiplet
H7 $_{p\text{-}ax}$**:** dd, *J* = 12.7 Hz ($H7_{p\text{-}ax}$-$H5_{p\text{-}ax}$), *J* = 12.7 Hz ($H7_{p\text{-}ax}$-$H7_{p\text{-}eq}$)
H7 $_{p\text{-}eq}$**:** dd, *J* = 12.1 Hz ($H7_{p\text{-}eq}$-$H7_{p\text{-}ax}$), *J* = 4.4 Hz ($H7_{p\text{-}eq}$-$H5_{p\text{-}ax}$)

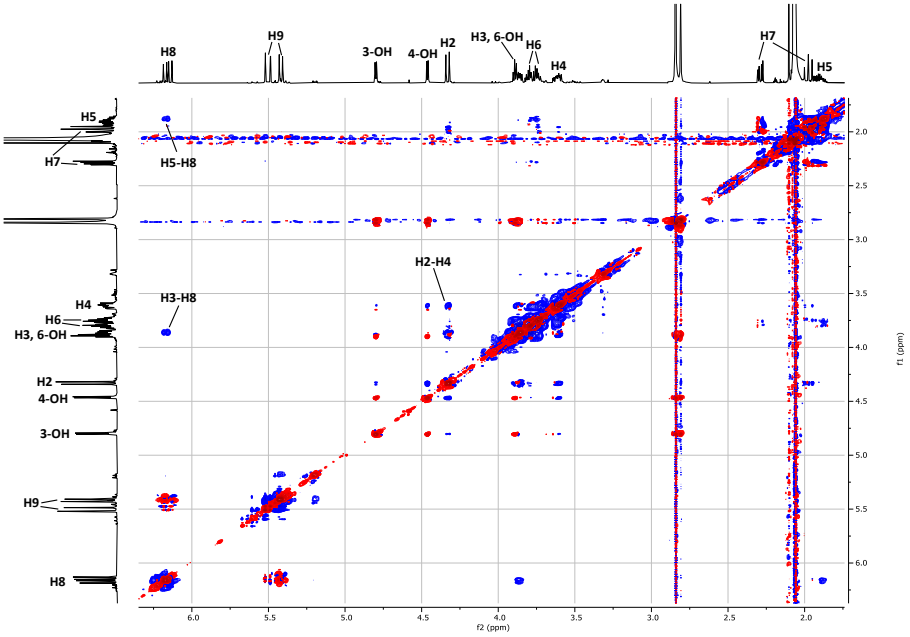
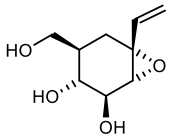


Figure S5. NOESY spectra of compound **5**. The key NOE interactions for **5** can be found between H3-H8 and H5-H8.

Compound 8

Table S6. *^{1}H NMR H-H coupling constants*



H2: d, *J* = 8.0 Hz ($H2_{p\text{-}ax}$-$H3_{p\text{-}ax}$)

H3: ddd, *J* = 9.7 Hz ($H3_{p\text{-}ax}$-$H4_{p\text{-}ax}$), *J* = 8.0 Hz ($H3_{p\text{-}ax}$-$H2_{p\text{-}ax}$), *J* = 4.4 Hz ($H3_{p\text{-}ax}$-3-OH)

H4: ddd, *J* = 10.0 Hz ($H4_{p\text{-}ax}$-$H3_{p\text{-}ax}$), *J* = 10.0 Hz ($H4_{p\text{-}ax}$-$H5_{p\text{-}ax}$), *J* = 3.9 Hz ($H4_{p\text{-}ax}$-4-OH)

H5: overlap with $H7_{ax}$

$H7_{ax}$: overlap with H5

$H7_{eq}$: d, *J* = 11.8 Hz ($H7_{eq}$-$H7_{ax}$)

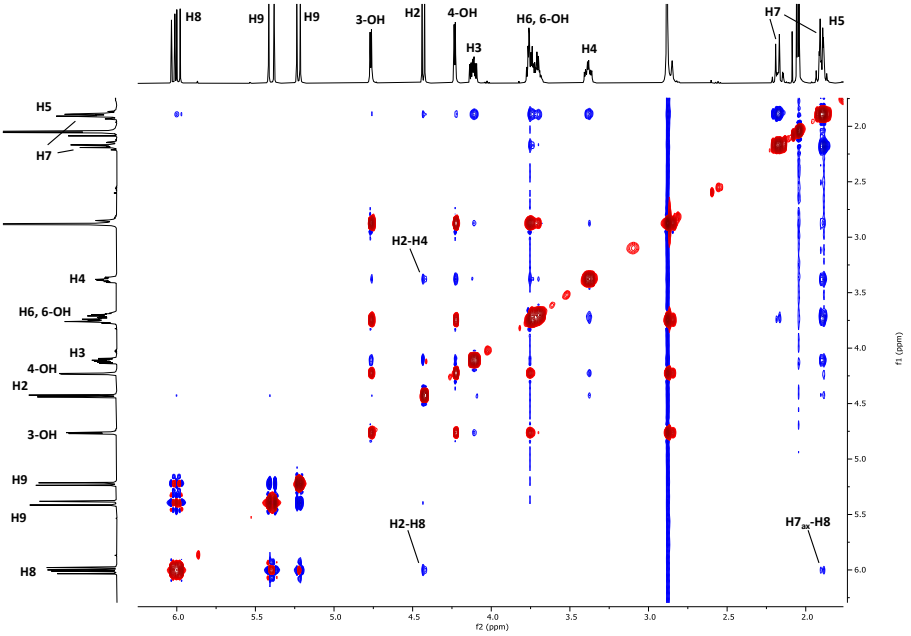
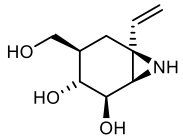


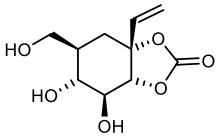
Figure S6. NOESY spectra of compound **8**. The key NOE interactions for **8** can be found between H2-H8 and $H7_{ax}$-H8.

Table S7. Compound **4**, *^{1}H NMR H-H coupling constants*



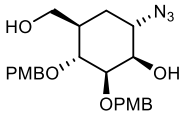
H2: bs
H3: bs
H4: dd, *J* = 11.1 Hz ($H4_{ax}$-$H5_{ax}$), *J* = 8.7 Hz($H4_{ax}$-$H3_{ax}$)
H5: dddd, *J* = 10.0 Hz ($H5_{ax}$-$H4_{ax}$), *J* = 10.0 Hz ($H5_{ax}$-$H7_{ax}$), *J* = 7.5 Hz ($H5_{ax}$-H6), *J* = 4.5 Hz ($H5_{ax}$-$H7_{eq}$)
$H7_{ax}$: dd, *J* = 14.5 Hz ($H7_{ax}$-$H7_{eq}$), *J* = 12.0 Hz ($H7_{ax}$-$H5_{ax}$)
$H7_{eq}$: dd, *J* = 14.6 Hz ($H7_{eq}$-$H7_{ax}$), *J* = 6.1 Hz ($H7_{eq}$-$H5_{ax}$)

Table S8. Compound **9**, *^{1}H NMR H-H coupling constants*



H2: d, *J* = 7.1 Hz ($H2_{ax}$-$H3_{ax}$)
H3: overlap with H6
H4: dd, *J* = 9.6 Hz ($H4_{ax}$-$H3_{ax}$), *J* = 9.6 Hz ($H4_{ax}$-$H5_{ax}$)
H5: overlap with $H7_{ax}$
$H7_{ax}$: overlap with H5
$H7_{eq}$: dd, *J* = 12.2 Hz ($H7_{eq}$-$H7_{ax}$), *J* = 1.3 Hz ($H7_{eq}$-$H5_{ax}$)

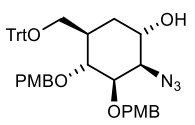
Table S9. Compound **38**, *^{1}H NMR H-H coupling constants*



H1: overlap with H2
H2: overlap with H1
H3: dd, *J* = 8.3 Hz ($H3_{ax}$-$H4_{ax}$), *J* = 3.0 Hz ($H3_{ax}$-$H2_{eq}$)
H4: overlap with H6
H5: multiplet
$H7_{ax}$: ddd, *J* = 14.4 Hz ($H7_{ax}$-$H7_{eq}$), *J* = 11.4 Hz ($H7_{ax}$-$H5_{ax}$), *J* = 3.2 Hz ($H7_{ax}$-$H1_{eq}$)
$H7_{eq}$: ddd, *J* = 14.0 Hz ($H7_{eq}$-$H7_{ax}$), *J* = 4.0 Hz ($H7_{eq}$-$H5_{ax}$), *J* = 4.0 Hz ($H7_{eq}$-$H1_{eq}$)

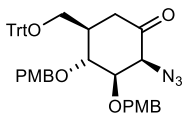
Synthetic procedures.

2-Deoxy-2-azido-3,4-di-*O*-(4-methoxybenzyl)-6-*O*-trityl-carba-α-D-mannose (11).



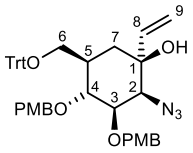
Compound **10** (5.4 g, 12 mmol) was dissolved in DMF (50 mL, 0.25 M) followed by the addition of pyridine (3.0 mL, 37 mmol, 3.0 eq.), DMAP (74 mg, 0.61 mmol, 0.05 eq.) and TrtCl (6.8 g, 24 mmol, 2.0 eq.). The solution was stirred for 16 hours on room temperature upon which TLC confirmed full conversion (R_f 0.3 (EtOAc:pentane, 3:7, v:v)). The reaction mixture was quenched by the addition of sat. aq. $NaHCO_3$ and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (10:90 → 40:60; EtOAc:pentane) yielded the title compound (7.7 g, 11 mmol, 92%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.46 – 7.18 (m, 19H, CH_{arom}), 6.95 – 6.69 (m, 4H, CH_{arom}), 4.62 – 4.54 (m, 3H, C*H*H PMB, C*H*H PMB, CH*H* PMB), 4.22 (d, *J* = 10.4 Hz, 1H, C*H*H PMB), 3.92 (dd, *J* = 4.0 Hz, 1H, H-1), 3.89 (dd, *J* = 8.0, 3.4 Hz, 1H, H-3), 3.79 (d, *J* = 5.8 Hz, 7H, OMe, OMe, H-2), 3.66 (dd, *J* = 8.4 Hz, 1H, H-4), 3.31 (dd, *J* = 8.9, 3.7 Hz, 1H, H-6), 3.21 (dd, *J* = 8.9, 6.4 Hz, 1H, H-6), 2.18 – 2.09 (m, 1H, H-5), 1.94 (ddd, *J* = 13.9, 10.6, 3.1 Hz, 1H, H-7), 1.80 (ddd, *J* = 14.5, 4.6, 4.6 Hz, 1H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.4, 159.2, 144.2, 130.7, 130.4, 129.7, 129.7, 129.0 ($C_{q\text{-}arom}$), 128.1, 127.9, 127.4, 127.1, 113.9, 113.7 (CH_{arom}), 86.5 (*C*(Ph)$_3$), 80.7 (C-3), 74.1 (C-4), 72.8 (CH_2 PMB), 68.0 (C-1), 64.6 (C-2), 63.3 (C-6), 55.4 (OMe), 37.9 (C-5), 30.8 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{42}H_{43}NaN_3O_6$ 708.30496; Found 708.30438.

2-*Epi*-2-deoxy-2-azido-3,4-di-*O*-(4-methoxybenzyl)-6-*O*-trityl-D-validone (12).



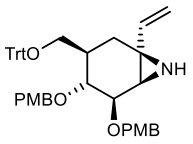
Compound **11** (1.7 g, 2.5 mmol) was dissolved in DCM (12.5 mL, 0.2 M) followed by the addition of $NaHCO_3$ (6.3 g, 75 mmol, 30 eq.) and Dess-Martin periodinane (2.1 g, 5.0 mmol, 2.0 eq.). After stirring the solution for 30 minutes at room temperature, TLC showed full conversion (R_f 0.3 (EtOAc:pentane, 3:7, v:v)). The reaction mixture was quenched by the addition of sat. aq. $Na_2S_2O_3$ and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (10:90 → 20:80; R_f 0.3 (EtOAc:pentane, 3:7, v:v)) yielded the title compound (1.7 g, 2.5 mmol, quant.) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.41 – 6.70 (m, 23H, CH_{arom}), 4.49 (d, *J* = 11.6 Hz, 1H, C*H*H PMB), 4.41 (d, *J* = 11.6 Hz, 1H, CH*H* PMB), 4.37 (d, *J* = 11.1 Hz, 1H, C*H*H PMB), 4.23 (d, *J* = 11.1 Hz, 1H, CH*H* PMB), 4.04 (d, *J* = 3.3 Hz, 1H, H-2), 3.97 (ddd, *J* = 3.3, 3.3, 0.8 Hz, 1H, H-3), 3.83 – 3.79 (m, 6H, OMe, OMe), 3.76 (dd, *J* = 5.5, 3.3 Hz, 1H, H-4), 3.26 – 3.13 (m, 1H, H-6), 2.71 – 2.59 (m, 1H, H-7), 2.27 (q, *J* = 6.2 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 204.1 (C-1), 159.6, 144.0, 129.8, 129.7, 129.6 ($C_{q\text{-}arom}$), 129.2, 128.8, 128.0, 127.2, 114.0, 114.0 (CH_{arom}), 86.9 (*C*(Ph)$_3$), 81.5 (C-3), 75.0 (C-4), 72.6, 72.1 (CH_2 PMB), 65.9 (C-2), 63.5 (C-6), 55.4, 55.4 (OMe), 40.4 (C-5), 38.8 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{42}H_{41}NaN_3O_6$ 706.28931; Found 706.28909.

1-(*S*)-1-Vinyl-1-hydroxyl-2-deoxy-2-azido-3,4-di-*O*-(4-methoxybenzyl)-6-*O*-trityl-carba-D-mannose (13).



Compound **12** (1.4 g, 2.0 mmol) was dissolved in anhydrous THF (8.0 mL, 0.25 M) and cooled to -78 °C. Subsequently, vinyl magnesium bromide (1.0 M solution in THF, 20 mL, 20 mmol, 10 eq.) was added dropwise. The solution was allowed to attain to -30 °C after which TLC confirmed full conversion (R_f 0.2 (pentane:EtOAc, 8:2, v:v)). The reaction mixture was quenched by the addition of sat. aq. $NaHCO_3$ and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (10:90 → 20:80; EtOAc:pentane) yielded the title compound (1.4 g, 2.0 mmol, 99%) as a colorless oil. ^{1}H NMR showed less than 5% of the C-1 epimer to be present. ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.46 – 7.16 (m, 17H, CH_{arom}), 6.95 – 6.64 (m, 6H, CH_{arom}), 5.89 (dd, *J* = 17.3, 10.9 Hz, 1H, H-8), 5.44 (dd, *J* = 17.3, 1.0 Hz, 1H, H-9), 5.28 (dd, *J* = 10.9, 1.0 Hz, 1H, H-9), 4.67 – 4.61 (m, 2H, CH_2 PMB), 4.58 (d, *J* = 11.2 Hz, 1H, CH*H* PMB), 4.12 (d, *J* = 10.1 Hz, 1H, C*H*H PMB), 3.78 (m, 6H, OMe, OMe), 3.74 – 3.68 (m, 2H, H-2, H-4), 3.65 (dd, *J* = 9.1, 3.0 Hz, 1H, H-3), 3.35 (dd, *J* = 8.6, 3.0 Hz, 1H, H-6), 3.12 (dd, *J* = 8.6, 5.8 Hz, 1H, H-6), 2.33 (s, 1H, 1-OH), 2.03 – 1.92 (m, 2H, H-7), 1.78 (dddd, *J* = 14.0, 8.8, 2.9, 2.9 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.6, 159.2, 144.1 ($C_{q\text{-}arom}$), 138.4 (C-8), 130.6, 130.0, 129.9 ($C_{q\text{-}arom}$), 129.0, 128.9, 127.9, 127.9, 127.9, 127.1 (CH_{arom}), 117.6 (C-9), 114.1, 113.7 (CH_{arom}), 86.4 ($C(Ph)_3$), 82.0 (C-3), 77.3 (C-2/C-4), 74.9 (CH_2 PMB), 72.8, 72.7 (CH_2 PMB, C-1), 69.8 (C-2/C-4), 63.2 (C-6), 55.4 (OMe), 39.1 (C-5), 36.1 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{44}H_{45}NaN_3O_6$ 734.32061; Found 734.32006.

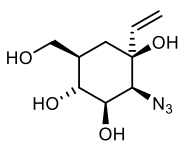
1-(*S*)-1-Vinyl-2-deoxy-1,2-azabicyclo[4.1.0]-3,4,-di-*O*-(4-methoxybenzyl)-6-*O*-trityl-carba-D-mannose (14).



Compound **13** (135 mg, 0.2 mmol) was co-evaporated with toluene (3x) and dissolved in anhydrous acetonitrile (2.0 mL, 0.1 M) under N_2 atmosphere. Triphenylphosphine (0.16 g, 0.6 mmol, 3.0 eq.) was added and the solution was stirred for 16 hours at 70 °C. TLC confirmed full conversion and minor hydrolyzed product to be formed (R_f 0.3 (EtOAc:pentane, 3:7, v:v)). The reaction mixture was quenched by the addition of sat. aq. $NaHCO_3$ and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (20:80 → 40:60; EtOAc:pentane) yielded the title compound (60 mg, 90 µmol, 45%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): 7.45 – 7.17 (m, 17H, CH_{arom}), 6.94 – 6.66 (m, 6H, CH_{arom}), 5.54 (dd, *J* = 17.2, 10.5 Hz, 1H, H-8), 5.18 (d, *J* = 17.1 Hz, 1H, H-9), 5.10 (d, *J* = 10.7 Hz, 1H, H-9), 4.73 – 4.60 (m, 3H, C*H*H PMB, C*H*H PMB, CH*H* PMB), 4.24 (d, *J* = 10.3 Hz, 1H, CH*H* PMB), 3.82 – 3.77 (m, 6H, OMe, OMe), 3.77 – 3.72 (m, 1H, H-3), 3.53 (dd, *J* = 11.1, 8.1 Hz, 1H, H-4), 3.38 (dd, *J* = 8.7, 3.1 Hz, 1H, H-6), 3.10 (dd, *J* = 8.6, 6.9 Hz, 1H, H-6), 2.46 (dd, *J* = 14.6, 6.2 Hz, 1H, H-7), 2.39 (d, *J* = 3.3 Hz, 1H, H-2), 1.91 (dd, *J* = 14.6, 11.8 Hz, 1H, H-7), 1.85 – 1.73 (m, 1H, H-5), 1.58 (s, 1H, NH); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 159.3, 159.1, 144.3 ($C_{q\text{-}arom}$), 142.3 (C-

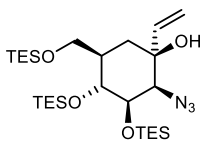
8), 131.0 ($C_{q\text{-}arom}$), 129.7, 129.5, 129.0, 127.9, 127.0, 113.9 (CH_{arom}), 113.7 (C-9), 86.5 ($C(Ph)_3$), 81.3 (C-3), 78.7 (C-4), 74.6, 72.0 (CH_2 PMB), 63.9 (C-6), 55.4 (OMe), 44.1 (C-2), 41.3 (C-5), 40.5 (C-7); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{44}H_{46}NO_5$ 668.33760; Found 668.33713.

1-(*S*)-1-Vinyl-1-hydroxyl-2-deoxy-2-azido-carba-D-mannose (15).



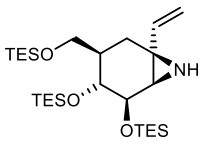
Compound **13** (0.71 g, 1.0 mmol) was dissolved in DCM (20 mL, 0.05 M) followed by the addition of TES (0.64 mL, 4.0 mmol, 4.0 eq.) and TFA (0.77 mL, 10 mmol, 10 eq.). After 1 hour, TLC confirmed full conversion (R_f 0.3 (MeOH:DCM, 1:9, v:v)). The reaction mixture was concentrated under reduced pressure to yield the crude product. Flash column chromatography (5:95 → 20:80; MeOH:DCM) yielded the title compound (0.18 g, 0.77 mmol, 77%) as a white solid. ^{1}H NMR (500 MHz, D_2O, HH-COSY, HSQC): δ 6.08 (dd, *J* = 17.5, 11.0 Hz, 1H, H-8), 5.50 (dd, *J* = 17.5, 0.5 Hz, 1H, H-9), 5.42 (dd, *J* = 11.0, 0.5 Hz, 1H, H-9), 3.91 (dd, *J* = 3.4, 1.8 Hz, 1H, H-2), 3.76 – 3.68 (m, 2H, H-3, H-6), 3.61 (dd, *J* = 11.3, 6.0 Hz, 1H, H-6), 3.50 (dd, *J* = 10.6, 9.6 Hz, 1H, H-4), 1.83 (ddd, *J* = 13.1, 4.2, 1.9 Hz, 1H, H-7), 1.73 (dd, *J* = 13.1, 13.1 Hz, 1H, H-7), 1.60 (dddd, *J* = 12.9, 8.3, 5.3, 2.9 Hz, 1H, H-5); ^{13}C NMR (126 MHz, D_2O, HSQC): δ 138.8 (C-8), 117.5 (C-9), 73.7 (C-1), 72.6 (C-3), 71.6 (C-2), 70.0 (C-4), 62.2 (C-6), 40.0 (C-5), 33.1 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_9H_{15}NaN_3O_4$ 252.09603; Found 252.09548.

1-(*S*)-1-Vinyl-1-hydroxyl-2-deoxy-2-azido-3,4,6-tri-*O*-triethylsilyl-carba-D-mannose (16).



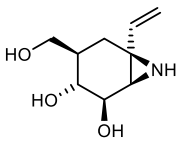
Compound **15** (0.18 g, 0.77 mmol) was dissolved in pyridine (15 mL, 0.05 M) followed by the addition of TESCl (0.78 mL, 4.6 mmol, 6.0 eq.). The solution was stirred for 16 hours on room temperature upon which TLC confirmed full conversion (R_f 0.4 (toluene:pentane, 1:1, v:v)). The reaction mixture was quenched by the addition of sat. aq. $NaHCO_3$ and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (30:70 → 90:10; toluene:pentane) yielded the title compound (200 mg, 0.35 mmol, 45%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 5.90 (dd, *J* = 17.1, 10.7 Hz, 1H, H-8), 5.52 (dd, *J* = 17.2, 1.4 Hz, 1H, H-9), 5.27 (dd, *J* = 10.7, 1.4 Hz, 1H, H-9), 4.06 (s, 1H, H-4), 3.92 (s, 2H, H-3, H-6), 3.60 (dd, *J* = 10.0, 6.0 Hz, 1H, H-6), 3.55 (s, 1H, H-2), 1.94 (dd, *J* = 14.1, 5.8 Hz, 1H, H-7), 1.77 (s, 1H, H-5), 1.64 (dd, *J* = 14.1, 5.9 Hz, 1H, H-7), 1.04 – 0.91 (m, 27H, $SiCH_2CH_3$), 0.76 – 0.54 (m, 18H, $SiCH_2CH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 140.9 (C-8), 115.7 (C-9), 76.5 (C-3), 70.8 (C-4), 64.3 (C-6), 43.2 (C-5), 36.0 (C-7), 7.3, 7.1, 7.0, 7.0 ($SiCH_2CH_3$), 5.4, 5.1, 4.9, 4.8, 4.6 ($SiCH_2CH_3$); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{27}H_{57}NaN_3O_4Si_3$ 594.35546; Found 594.35491.

1-(*S*)-1-Vinyl-2-deoxy-1,2-azabicyclo[4.1.0]-3,4,6-tri-*O*-triethylsilyl-carba-D-mannose (17).



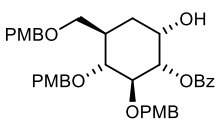
Compound **16** (200 mg, 0.35 mmol) was co-evaporated with toluene (3x) and dissolved in anhydrous acetonitrile (3.5 mL, 0.1 M) under N_2 atmosphere. Triphenylphosphine (0.18 g, 0.7 mmol, 2.0 eq.) was added and the solution was stirred for 16 hours at 70 °C. TLC confirmed full conversion and minor hydrolyzed product to be formed (R_f 0.3 (Et_2O:pentane, 1:9, v:v)). The reaction mixture was quenched by the addition of sat. aq. $NaHCO_3$ and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (50:50 toluene:pentane → 10:90; Et_2O:pentane) yielded the title compound (95 mg, 0.18 mmol, 52%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 5.53 (dd, *J* = 17.2, 10.6 Hz, 1H, H-8), 5.16 (dd, *J* = 17.2, 0.7 Hz, 1H, H-9), 5.07 (dd, *J* = 10.6, 0.6 Hz, 1H, H-9), 3.86 (s, 1H, H-3), 3.76 (d, *J* = 9.7 Hz, 1H, H-6), 3.51 (dd, *J* = 9.2, 6.9 Hz, 1H, H-4), 3.41 (dd, *J* = 9.7, 7.8 Hz, 1H, H-6), 2.41 – 2.34 (m, 1H, H-7), 2.27 (d, *J* = 3.9 Hz, 1H, H-2), 1.63 – 1.53 (m, 2H, H-5, H-7), 1.06 – 0.89 (m, 27H, $SiCH_2C\mathit{H}_3$), 0.75 – 0.52 (m, 18H, $SiC\mathit{H}_2CH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 142.7 (C-8), 113.4 (C-9), 75.0 (C-3), 73.0 (C-4), 64.5 (C-6), 45.9 (C-2), 43.1 (C-5), 28.5 (C-7), 7.3, 7.2, 7.2, 7.2, 7.0 ($SiCH_2\mathit{C}H_3$), 5.6, 5.5, 5.4, 4.6, 4.6 ($Si\mathit{C}H_2CH_3$); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{27}H_{58}NO_3Si_3$ 528.37245; Found 528.37190.

1-(*S*)-1-Vinyl-2-deoxy-1,2-azabicyclo[4.1.0]-carba-D-mannose (4).



Compound **17** (26 mg, 50 µmol) was dissolved in anhydrous THF (1.0 mL, 0.05 M). The reaction was cooled on ice and kept under inert atmosphere. TBAF (1.0 M solution in THF, 0.25 mL, 0.25 mmol, 5.0 eq.) was added and stirring continued for 2 hours while the reaction mixture was allowed to attain to room temperature. Upon full conversion (R_f 0.2 (MeOH:DCM, 2:8, v:v)), the mixture was concentrated at 25 °C under reduced pressure yielding the crude product. Flash column chromatography (10:90 → 70:30; acetone:DCM) yielded the title compound (6.5 mg, 35 µmol, 70%) as a colorless oil. ^{1}H NMR (500 MHz, D_2O, HH-COSY, HSQC): δ 5.62 (dd, *J* = 17.3, 10.7 Hz, 1H, H-8), 5.29 (d, *J* = 17.3 Hz, 1H, H-9), 5.18 (d, *J* = 10.7 Hz, 1H, H-9), 3.85 (bs, 1H, H-3), 3.71 (dd, *J* = 11.2, 3.7 Hz, 1H, H-6), 3.54 (dd, *J* = 11.2, 6.5 Hz, 1H, H-6), 3.37 (dd, *J* = 11.1, 8.7 Hz, 1H, H-4), 2.58 (bs, 1H, H-2), 2.41 (dd, *J* = 14.6, 6.1 Hz, 1H, H-7), 1.67 (dddd, *J* = 10.0, 10.0, 7.5, 4.5 Hz, 1H, H-5), 1.59 (dd, *J* = 14.5, 12.0 Hz, 1H, H-7); ^{13}C NMR (126 MHz, D_2O, HSQC): δ 140.8 (C-8), 114.5 (C-9), 72.9 (C-3), 71.4 (C-4), 62.5 (C-6), 45.0 (C-2), 40.8 (C-5), 27.8 (C-7); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_9H_{15}NO_3$ 186.11302; Found 186.11263.

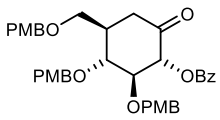
2-*O*-Benzoyl-3,4,6-tri-*O*-(4-methoxybenzyl)-carba-α-D-glucose (23).



Compound **22** (5.4 g, 10 mmol) was dissolved in pyridine (67 mL, 0.15 M) and cooled to -15 °C. BzCl (1.7 mL, 15 mmol, 1.5 eq.) was slowly added followed by stirring the reaction mixture for an hour while keeping the temperature at -15 °C. Upon full conversion (R_f 0.4 (EtOAc:pentane, 1:1, v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ solution and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined

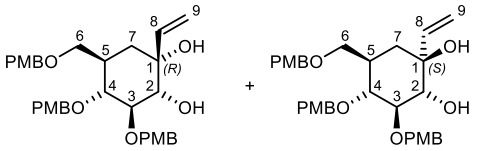
organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (20:80 → 40:60; EtOAc:pentane) yielded the title compound (6.4 g, 10 mmol, quant.) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 8.07 – 6.58 (m, 12H, CH_{arom}), 5.12 (dd, *J* = 10.0, 2.9 Hz, 1H, H-2), 4.79 (d, *J* = 10.5 Hz, 1H, C*H*H PMB), 4.76 (d, *J* = 10.7 Hz, 1H, CH*H* PMB), 4.65 (d, *J* = 10.7 Hz, 1H, C*H*H PMB), 4.46 (d, *J* = 10.5 Hz, 1H, CH*H* PMB), 4.43 (d, *J* = 11.6 Hz, 1H, C*H*H PMB), 4.38 (d, *J* = 11.6 Hz, 1H, CH*H* PMB), 4.25 (dd, *J* = 3.0 Hz, 1H, H-1), 4.07 (dd, *J* = 10.0, 9.1 Hz, 1H, H-3), 3.79 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.70 (s, 4H, OMe, H-6), 3.57 (dd, *J* = 10.9, 9.1 Hz, 1H, H-4), 3.43 (dd, *J* = 9.0, 2.6 Hz, 1H, H-6), 2.23 (dddd, *J* = 14.1, 6.5, 6.5, 3.4 Hz, 1H, H-5), 2.07 (bs, 1H, 1-OH), 1.95 (ddd, *J* = 14.6, 3.8, 3.8 Hz, 1H, H-7), 1.75 (ddd, *J* = 15.0, 13.0, 2.4 Hz, 1H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 165.7 (C=O ester), 159.3, 159.2, 159.1, 133.3, 131.0, 130.8 ($C_{q\text{-}arom}$), 130.7, 130.0, 129.8, 129.7, 129.6, 129.3, 128.6, 113.9, 113.9, 113.7 (CH_{arom}), 81.3 (C-3), 80.9 (C-4), 77.5 (C-2), 75.3, 75.0, 72.8 (CH_2 PMB), 69.4 (C-6), 68.0 (C-1), 55.4, 55.4, 55.3 (OMe), 36.9 (C-5), 31.0 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{38}H_{42}NaO_9$ 665.27265; Found 665.27164.

2-*O*-Benzoyl-3,4,6-tri-*O*-(4-methoxybenzyl)-D-validone (24).



Compound **23** (6.4 g, 10 mmol) was dissolved in DCM (100 mL, 0.1 M). $NaHCO_3$ (25 g, 300 mmol, 30 eq.) and Dess-Martin periodinane (8.5 g, 20 mmol, 2.0 eq.) were added respectively. After stirring for 1 hour, TLC showed full conversion (R_f 0.4 (EtOAc:pentane, 3:7, v:v)) and the reaction was quenched by the addition of sat. aq. $Na_2S_2O_3$ solution and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (20:80 → 30:70; EtOAc:pentane) yielded the title compound (6.1 g, 9.6 mmol, 96%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.63 – 6.64 (m, 12H, CH_{arom}), 5.55 (dd, *J* = 10.4, 1.1 Hz, 1H, H-2), 4.86 (d, *J* = 10.4 Hz, 1H, CH*H* PMB), 4.77 (s, 2H, CH_2 PMB), 4.52 (d, *J* = 10.4 Hz, 1H, C*H*H PMB), 4.43 (d, *J* = 11.6 Hz, 1H, CH*H* PMB), 4.39 (d, *J* = 11.6 Hz, 1H, C*H*H PMB), 4.00 (dd, *J* = 10.7, 9.1 Hz, 1H, H-4), 3.87 (dd, *J* = 10.3, 9.1 Hz, 1H, H-3), 3.81 – 3.74 (m, 7H, H-6, OMe, OMe), 3.72 (s, 3H, OMe), 3.38 (dd, *J* = 9.0, 2.6 Hz, 1H, H-6), 2.77 (ddd, *J* = 14.1, 14.1, 1.2 Hz, 1H, H-7), 2.48 (dd, *J* = 14.3, 4.2 Hz, 1H, H-7), 1.91 (dddd, *J* = 10.4, 10.4, 2.7, 2.7 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 200.8 (C-1), 165.5 (C=O ester), 159.4, 159.4, 159.4, 133.4, 130.4, 130.1 ($C_{q\text{-}arom}$), 130.0, 129.8, 129.7, 129.5, 129.4, 128.5, 113.9, 113.9 (CH_{arom}), 83.8 (C-3), 80.0 (C-2), 79.5 (C-4), 75.3, 75.3, 72.9 (CH_2 PMB), 68.2 (C-6), 55.4, 55.4, 55.3 (OMe), 39.9 (C-7), 39.3 (C-5); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{38}H_{40}NaO_9$ 663.25700; Found 663.25652.

1-(*R*)-1-Vinyl-1-hydroxyl-3,4,6-tri-*O*-(4-methoxybenzyl)-carba-D-glucose (25) and 1-(*S*)-1-vinyl-1-hydroxyl-3,4,6-tri-*O*-(4-methoxybenzyl)-carba-D-glucose (26).

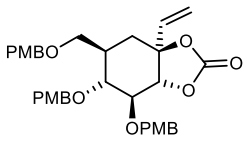


Compound **24** (6.1 g, 9.6 mmol) was dissolved in THF (96 mL, 0.1 M) and cooled to -78 °C. Vinyl magnesium bromide (1.0 M solution in THF, 72 mL, 72 mmol, 7.5 eq.) was added slowly. The reaction mixture was slowly allowed to attain to 0 °C and kept at this temperature for 3 hours. Upon full conversion was observed (R_f 0.2 and 0.1 for compound **25** and **26**, respectively (EtOAc:pentane, 7:3, v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ solution and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (30:70 → 50:50; EtOAc:pentane) yielded the title compounds as a separable mixture of *syn*- and *anti*-diols **25** and **26** in a ratio of 56:44 respectively. Yielding **25** (2.2 g, 3.8 mmol, 40%) and **26** (1.7 g, 3.1 mmol, 32%) both as a colorless oil.

Data of the major stereoisomer **25** (*syn*): ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.33 – 7.11 (m, 6H, CH_{arom}), 6.92 – 6.79 (m, 6H, CH_{arom}), 5.82 (dd, *J* = 17.2, 10.7 Hz, 1H, H-8), 5.33 (dd, *J* = 17.2, 1.3 Hz, 1H, H-9), 5.14 (dd, *J* = 10.7, 1.2 Hz, 1H, H-9), 4.91 (d, *J* = 11.3 Hz, 1H, C*H*H PMB), 4.78 (d, *J* = 10.4 Hz, 1H, CH*H* PMB), 4.63 (d, *J* = 11.3 Hz, 1H, C*H*H PMB), 4.49 (d, *J* = 10.4 Hz, 1H, CH*H* PMB), 4.42 (d, *J* = 11.5 Hz, 1H, C*H*H PMB), 4.37 (d, *J* = 11.6 Hz, 1H, CH*H* PMB), 3.80 – 3.77 (m, 9H, OMe, OMe, OMe), 3.73 (dd, *J* = 9.0, 4.0 Hz, 1H, H-6), 3.65 (dd, *J* = 9.2, 9.2 Hz, 1H, H-3), 3.51 (dd, *J* = 10.8, 9.3 Hz, 1H, H-4), 3.40 (d, *J* = 9.1 Hz, 1H, H-2), 3.35 (dd, *J* = 9.1, 2.5 Hz, 1H, H-6), 2.38 (s, 1H, 1-OH), 2.22 – 2.11 (m, 2H, 2-OH, H-5), 1.71 – 1.61 (m, 2H, H-7); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 159.5, 159.3, 159.3 ($C_{q\text{-}arom}$), 143.0 (C-8), 131.0, 130.9, 130.6 ($C_{q\text{-}arom}$), 129.7, 129.7, 129.5, 129.4, 129.3 , 114.3, 113.9, 113.9 (CH_{arom}), 113.8 (C-9), 84.2 (C-3), 80.7 (C-4), 76.1 (C-2), 75.0 (CH_2 PMB), 74.7 (CH_2 PMB), 74.3 (C-1), 72.9 (CH_2 PMB), 69.3 (C-6), 55.4, 55.4, 55.4 (OMe), 38.2 (C-5), 36.1 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{33}H_{40}NaO_8$ 587.26209; Found 587.26166.

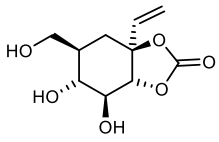
Data of the major stereoisomer **26** (*anti*): ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.34 – 6.73 (m, 12H, CH_{arom}), 6.13 (dd, *J* = 17.3, 10.9 Hz, 1H, H-8), 5.49 (dd, *J* = 17.3, 1.3 Hz, 1H, H-9), 5.31 (dd, *J* = 10.9, 1.4 Hz, 1H, H-9), 4.89 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 4.78 (d, *J* = 10.3 Hz, 1H, CH*H* PMB), 4.66 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 4.47 (d, *J* = 10.4 Hz, 1H, CH*H* PMB), 4.40 (m, 2H, CH_2 PMB), 3.84 – 3.75 (m, 9H, OMe, OMe, OMe), 3.63 – 3.35 (m, 5H, H-2, H-3, H-4, H-6), 2.42 (bs, 1H, 1-OH), 2.31 (bs, 1H, 2-OH), 1.89 (dd, *J* = 11.3, 2.5 Hz, 1H, H-7), 1.86 – 1.72 (m, 2H, H-5, H-7); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 159.4, 159.3 ($C_{q\text{-}arom}$), 138.2 (C-8), 130.8, 130.7, 130.5 ($C_{q\text{-}arom}$), 129.7, 129.7, 129.6, 129.5, 129.4 (CH_{arom}), 116.8 (C-9), 114.2, 114.0, 113.9, 113.9 (CH_{arom}), 84.6, 81.2, 79.1 (C-2, C-3, C-4), 75.1, 74.9 (CH_2 PMB), 74.5 (C-1), 72.9 (CH_2 PMB), 69.5 (C-6), 55.4, 55.4 (OMe), 39.1 (C-5), 37.4 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{33}H_{40}NaO_8$ 587.26209; Found 587.26182.

1-(*S*)-1-Vinyl-1,2-*trans*-*O*-carbonate-3,4,6-tri-*O*-(4-methoxybenzyl)-carba-D-glucose (27).



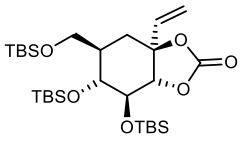
Compound **26** (1.7 g, 3.1 mmol) was dissolved in DCE (61 mL, 0.05 M) followed by the addition of CDI (3.0 g, 18 mmol, 6.0 eq.). The reaction was stirred at 60 °C for 16 hours upon which TLC confirmed full conversion (R_f 0.8 (EtOAc:pentane, 1:1, v:v)). The mixture was allowed to attain to room temperature and subsequently quenched by the addition of sat. aq. $NaHCO_3$ solution and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (20:80 → 30:70; EtOAc:pentane) yielded the title compound (1.0 g, 1.7 mmol, 56%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.36 – 6.76 (m, 12H, CH_{arom}), 5.98 (dd, *J* = 17.1, 11.1 Hz, 1H, H-8), 5.63 (d, *J* = 17.1 Hz, 1H, H-9), 5.47 (d, *J* = 11.0 Hz, 1H, H-9), 4.81 (m, 2H, CH_2 PMB), 4.57 (d, *J* = 10.8 Hz, 1H, C*H*H PMB), 4.42 – 4.34 (m, 2H, CH_2 PMB), 4.31 (d, *J* = 11.6 Hz, 1H, C*H*H PMB), 4.11 (dd, *J* = 11.3, 0.7 Hz, 1H, H-2), 3.96 (dd, *J* = 11.3, 7.5 Hz, 1H, H-3), 3.84 – 3.75 (m, 9H, OMe, OMe, OMe), 3.65 (dd, *J* = 10.3, 7.5 Hz, 1H, H-4), 3.55 (dd, *J* = 9.1, 4.2 Hz, 1H, H-6), 3.35 (dd, *J* = 9.1, 2.6 Hz, 1H, H-6), 2.20 – 2.06 (m, 2H, H-7), 2.03 – 1.92 (m, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.6, 159.5, 159.4 ($C_{q\text{-}arom}$), 155.1 (C=O), 131.4 (C-8), 130.3, 130.1, 130.0 ($C_{q\text{-}arom}$), 129.9, 129.8, 129.6 (CH_{arom}), 120.6 (C-9), 114.0, 113.9, 113.9 (CH_{arom}), 86.7 (C-2), 84.7 (C-1), 80.0 (C-4), 79.0 (C-3), 75.6, 73.1, 72.9 (CH_2 PMB), 68.7 (C-6), 55.4, 55.4 (OMe), 41.1 (C-5), 33.1 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{34}H_{38}NaO_9$ 613.24135; Found 613.24099.

1-(*S*)-1-Vinyl-1,2-*trans*-*O*-carbonate-carba-D-glucose (6).



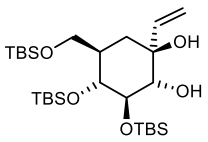
Compound **27** (1.0 g, 1.7 mmol) was dissolved in DCM (34 mL, 0.05 M) followed by the addition of TES (1.6 mL, 10 mmol, 6.0 eq.) and TFA (1.3 mL, 17 mmol, 10 eq.). After 1 hour, TLC confirmed full conversion (R_f 0.3 (MeOH:DCM, 1:9, v:v)). The reaction mixture was concentrated under reduced pressure to yield the crude product. Flash column chromatography (5:95 → 20:80; MeOH:DCM) yielded the title compound (0.31 g, 1.3 mmol, 79%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 6.13 (dd, *J* = 17.2, 11.1 Hz, 1H, H-8), 5.62 (d, *J* = 17.2 Hz, 1H, H-9), 5.57 (dd, *J* = 11.1, 0.7 Hz, 1H, H-9), 4.29 (d, *J* = 11.6, 1H, H-2), 3.98 (dd, *J* = 11.6, 7.9 Hz, 1H, H-3), 3.75 (dd, *J* = 11.4, 3.0 Hz, 1H, H-6), 3.69 (dd, *J* = 11.4, 5.1 Hz, 1H, H-6), 3.47 (dd, *J* = 10.0, 7.9 Hz, 1H, H-4), 2.39 – 2.36 (d, *J* = 7.3, 10.8 Hz, 1H) H-7), 2.02 – 1.89 (m, 2H, H-5, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.5 (C=O), 133.4 (C-8), 123.3 (C-9), 88.5 (C-1), 88.2 (C-2), 76.6 (C-4), 73.2 (C-3), 64.3 (C-6), 45.1 (C-5), 34.0 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{10}H_{14}NaO_6$ 253.06881; Found 253.06835.

1-(*S*)-1-Vinyl-1,2-*trans*-*O*-carbonate-3,4,6-tri-*O*-*tert*-butyldimethylsilyl-carba-D-glucose (28).



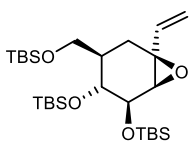
Compound **6** (0.83 g, 2.3 mmol) was dissolved in DCM (23 mL, 0.1 M). The mixture was cooled on ice followed by the addition of pyridine (3.7 mL, 46 mmol, 20 eq.) and TBSOTf (2.1 mL, 9.2 mmol, 4.0 eq.). The reaction mixture was heated to 40 °C and stirred for 2 hours upon which TLC confirmed full conversion (R_f 0.7 (Et_2O:pentane, 1:1, v:v)). Subsequent quenching by addition of sat. aq. $NaHCO_3$ solution and dilution with water followed. The aqueous layer was extracted with Et_2O (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (2:98 → 8:92; Et_2O:pentane) yielded the title compound (0.74 g, 1.3 mmol, 56%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 6.05 (dd, *J* = 17.2, 11.0 Hz, 1H, H-8), 5.61 (d, *J* = 17.2 Hz, 1H, H-9), 5.50 (d, *J* = 11.0 Hz, 1H, H-9), 3.94 (dd, *J* = 11.3, 6.6 Hz, 1H, H-3), 3.88 (d, *J* = 11.3 Hz, 1H, H-2), 3.64 (dd, *J* = 4.1, 1.5 Hz, 2H, H-6), 3.58 (dd, *J* = 8.9, 6.6 Hz, 1H, H-4), 2.20 (dd, *J* = 11.7, 4.4 Hz, 1H, H-7), 1.96 – 1.80 (m, 2H, H-5, H-7), 0.97 – 0.85 (m, 27H, $C(CH_3)_3$), 0.19 – 0.02 (m, 18H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 154.9 (C=O), 131.9 (C-8), 120.2 (C-9), 86.5 (C-2), 84.4 (C-1), 76.6 (C-4), 73.5 (C-3), 62.9 (C-6), 44.7 (C-5), 33.2 (C-7), 26.5, 26.3, 26.1 ($C(CH_3)_3$), 18.6, 18.4, 18.3 ($C(CH_3)_3$), -2.2, -2.4, -3.5, -4.0, -5.1, -5.4 ($SiCH_3$); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{28}H_{56}NaO_6Si_3$ 595.32824; Found 595.32778.

1-(*S*)-1-Vinyl-1-hydroxyl-3,4,6-tri-*O*-*tert*-butyldimethylsilyl-carba-D-glucose (29).



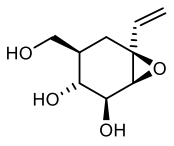
Compound **28** (85 mg, 0.15 mmol) was dissolved in anhydrous Et_2O (5.0 mL, 0.05 M) and cooled on ice. Subsequently, $LiBH_4$ (2.0 M solution in THF, 0.38 mL, 0.75 mmol, 5.0 eq.) was added and stirring continued for another 2 hours while attaining to room temperature. Upon full conversion (R_f 0.5 (Et_2O:pentane, 1:9, v:v)), The mixture was quenched by the addition of sat. aq. $NaHCO_3$ solution and diluted with water. The aqueous layer was extracted with Et_2O (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (10:90 → 20:80; Et_2O:pentane) yielded the title compound (53 mg, 96 µmol, 64%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 6.12 (ddd, *J* = 17.3, 10.8, 1.0 Hz, 1H, H-8), 5.39 (dd, *J* = 17.3, 2.0 Hz, 1H, H-9), 5.15 (dd, *J* = 10.8, 2.0 Hz, 1H, H-9), 4.23 (d, *J* = 2.8 Hz, 1H, H-2), 4.13 (d, *J* = 1.1 Hz, 1H, 1-OH), 4.12 – 4.01 (m, 3H, H-4, H-6, 2-OH), 3.64 (dd, *J* = 10.2, 5.0 Hz, 1H, H-6), 3.45 (ddd, *J* = 9.8, 3.1, 1.4, Hz, 1H, H-3), 2.23 (dd, *J* = 14.9, 7.1 Hz, 1H, H-7), 2.08 (ddd, *J* = 12.3, 8.6, 4.2 Hz, 1H, H-5), 1.52 (d, *J* = 14.9 Hz, 1H, H-7), 0.96 – 0.84 (m, 27H, $C(CH_3)_3$), 0.21 – 0.09 (m, 12H, $SiCH_3$), 0.07 – -0.04 (m, 6H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 143.3 (C-8), 113.3 (C-9), 74.9 (C-3), 73.9 (C-4), 73.9 (C-1), 71.2 (C-2), 65.6 (C-6), 43.9 (C-5), 29.9 (C-7), 26.1, 25.8 ($C(CH_3)_3$), 18.4, 18.1, 17.9 ($C(CH_3)_3$), -4.7, -4.8, -5.2 ($SiCH_3$); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{27}H_{58}NaO_5Si_3$ 569.34897; Found 569.34834.

1-(*S*)-1-Vinyl-1,2-anhydro-3,4,6-tri-*O-tert*-butyldimethylsilyl-carba-D-mannose (30).



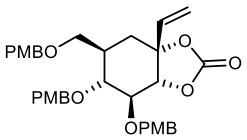
Compound **29** (55 mg, 0.1 mmol) was dissolved in DCM (2.0 mL, 0.05 M) and the mixture was cooled on ice. Subsequently, Et_3N (0.14 mL, 1.0 mmol, 10 eq.) and MsCl (16 µL, 0.2 mmol, 2.0 eq.) were added respectively. After stirring for 30 minutes, full conversion was observed (R_f 0.5 (toluene:pentane, 2:8, v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ solution and diluted with water. The aqueous layer was extracted with Et_2O (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (20:80 → 50:50; toluene:pentane) yielded the title compound (25 mg, 47 µmol, 47%) as a colorless oil.
^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 5.98 (dd, *J* = 17.1, 10.9 Hz, 1H, H-8), 5.46 (dd, *J* = 17.0, 0.7 Hz, 1H, H-9), 5.41 (ddd, *J* = 10.9, 0.7, 0.7 Hz, 1H, H-9), 4.32 (dd, *J* = 11.0, 0.6 Hz, 1H, H-2), 3.90 (dd, *J* = 10.9, 7.3 Hz, 1H, H-3), 3.72 (dd, *J* = 9.8, 3.4 Hz, 1H, H-6), 3.63 – 3.51 (m, 2H, H-4, H-6), 2.32 (dd, *J* = 12.4, 4.2 Hz, 1H, H-7), 1.88 (dd, *J* = 12.6, 12.6 Hz, 1H, H-7), 1.83 – 1.75 (m, 1H, H-5), 0.96 – 0.80 (m, 27H, $C(CH_3)_3$), 0.15 – -0.04 (m, 18H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 133.8 (C-8), 119.5 (C-9), 90.2 (C-1), 84.4 (C-2), 77.0 (C-4), 73.7 (C-3), 63.6 (C-6), 44.2 (C-5), 33.6 (C-7), 26.5, 26.3, 26.1 ($C(CH_3)_3$), 18.7, 18.4, 18.3($C(CH_3)_3$), -2.0, -2.1, -3.4, -3.7, -5.1, -5.3 ($SiCH_3$); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{27}H_{56}NaO_4Si_3$ 551.33841; Found 551.33786.

1-(*S*)-1-Vinyl-1,2-anhydro-carba-D-mannose (5).



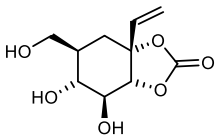
Compound **30** (31 mg, 59 µmol) was dissolved in anhydrous THF (2.4 mL, 0.025 M). The reaction was cooled on ice and kept under inert atmosphere. TBAF on silica gel (loading F^- ~1.5 mmol/g, 0.39 g, 0.59 mmol, 10 eq.) was added and stirring continued for 6 hours while the reaction mixture was allowed to attain to room temperature. Upon full conversion was observed (R_f 0.4 (acetone:DCM, 1:1, v:v)), the mixture was concentrated at 25 °C under reduced pressure yielding the crude product. Flash column chromatography (10:90 → 40:60; acetone:DCM) yielded the title compound (6.9 mg, 37 µmol, 63%) as a colorless oil. ^{1}H NMR (500 MHz, Acetone-d6, HH-COSY, HSQC): δ 6.16 (dd, *J* = 16.9, 10.9 Hz, 1H, H-8), 5.50 (dd, *J* = 16.9, 1.0 Hz, 1H, H-9), 5.42 (dd, *J* = 10.9, 0.9 Hz, 1H, H-9), 4.80 (d, *J* = 4.7 Hz, 1H, 3-OH), 4.46 (d, *J* = 5.0 Hz, 1H, 4-OH), 4.33 (d, *J* = 11.1 Hz, 1H, H-2), 3.91 – 3.84 (m, 2H, H-3, 6-OH), 3.83 – 3.78 (m, 1H, H-6), 3.75 (ddd, *J* = 10.5, 5.6, 5.6 Hz, 1H, H-6), 3.65 – 3.58 (m, 1H, H-4), 2.29 (dd, *J* = 12.1, 4.4 Hz, 1H, H-7), 1.98 (dd, *J* = 12.7, 12.7 Hz, 1H, H-7), 1.95 – 1.85 (m, 1H, H-5); ^{13}C NMR (126 MHz, Acetone-d6, HSQC): δ 135.5 (C-8), 119.1 (C-9), 91.5 (C-1), 85.4 (C-2), 76.9 (C-4), 72.5 (C-3), 63.9 (C-6), 43.3 (C-5), 34.3 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_9H_{14}NaO_4$ 209.07898; Found 209.07843.

1-(*R*)-1-Vinyl-1,2-*O*-carbonate-3,4,6-tri-*O*-(4-methoxybenzyl)-carba-D-glucose (31).



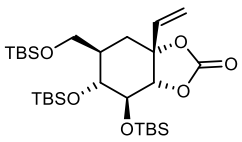
Compound **25** (2.2 g, 3.8 mmol) was dissolved in DCM (77 mL, 0.05 M) followed by the addition of CDI (1.9 g, 11.5 mmol, 3.0 eq.). The reaction was stirred at 40 °C for 16 hours upon which TLC confirmed full conversion (R_f 0.8 (EtOAc:pentane, 1:1, v:v)). The mixture was allowed to attain to room temperature and subsequently quenched by the addition of sat. aq. $NaHCO_3$ solution and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (20:80 → 30:70; EtOAc:pentane) yielded the title compound (1.8 g, 3.0 mmol, 78%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.33 – 6.76 (m, 12H, CH_{arom}), 5.81 (dd, *J* = 17.2, 10.8 Hz, 1H, H-8), 5.46 (d, *J* = 17.2 Hz, 1H, H-9), 5.28 (d, *J* = 10.8 Hz, 1H, H-9), 4.69 – 4.55 (m, 3H, CH*H* PMB, C*H*H PMB, CH*H* PMB), 4.47 – 4.33 (m, 4H, C*H*H PMB, CH*H* PMB, C*H*H PMB, H-2), 3.87 – 3.74 (m, 9H, OMe, OMe, OMe), 3.72 (dd, *J* = 7.1, 5.3 Hz, 1H, H-3), 3.61 (dd, *J* = 9.1, 4.4 Hz, 1H, H-6), 3.51 (dd, *J* = 8.9, 7.0 Hz, 1H, H-4), 3.36 (dd, *J* = 9.2, 3.5 Hz, 1H, H-6), 2.10 (dd, *J* = 15.3, 3.7 Hz, 1H, H-7), 2.01 (dddd, *J* = 8.8, 8.8, 4.2, 4.2 Hz, 1H, H-5), 1.87 (dd, *J* = 15.3, 12.8 Hz, 1H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.5, 159.3 ($C_{q\text{-}arom}$), 154.1 (C=O), 137.6 (C-9), 130.4, 130.3, 129.7 ($C_{q\text{-}arom}$), 129.7, 129.6, 129.4, 129.4 (CH_{arom}), 116.1 (C-8), 114.0, 113.9, 113.9 (CH_{arom}), 84.5 (C-1), 83.5 (C-2), 81.5 (C-3), 76.4 (C-4), 73.6, 73.4, 72.9 (CH_2 PMB), 69.5 (C-6), 55.4, 55.4 (OMe), 36.4 (C-5), 32.5 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{34}H_{38}NaO_9$ 613.24135; Found 613.24105.

1-(*R*)-1-Vinyl-1,2-*O*-carbonate-carba-D-glucose (9).



Compound **31** (0.3 g, 0.51 mmol) was dissolved in DCM (20 mL, 0.025 M) followed by the addition of TES (0.49 mL, 3.1 mmol, 6.0 eq.) and TFA (0.39 mL, 5.1 mmol, 10 eq.). After 1 hour, TLC confirmed full conversion (R_f 0.3 (MeOH:DCM, 1:9, v:v)). The reaction mixture was concentrated under reduced pressure to yield the crude product. Flash column chromatography (5:95 → 10:90; MeOH:DCM) yielded the title compound (107 mg, 0.46 mmol, 91%) as a colorless oil. ^{1}H NMR (300 MHz, D_2O, HH-COSY, HSQC): δ 5.96 (ddd, *J* = 17.2, 10.9, 1.0 Hz, 1H, H-8), 5.44 (dd, *J* = 17.2, 1.0 Hz, 1H, H-9), 5.36 (dd, *J* = 10.9, 1.0 Hz, 1H, H-9), 4.60 (d, *J* = 7.1 Hz, 1H, H-2), 3.80 – 3.63 (m, 3H, H-3, H-6), 3.46 (dd, *J* = 9.6 Hz, 1H, H-4), 2.27 (dd, *J* = 12.2, 1.0 Hz, 1H, H-7), 1.89 – 1.75 (m, 2H, H-5, H-7); ^{13}C NMR (75 MHz, $CDCl_3$, HSQC): δ 156.5 (C=O), 136.7 (C-8), 116.2 (C-9), 86.6 (C-1), 85.1 (C-2), 76.2 (C-3), 70.1 (C-4), 61.4 (C-6), 38.3 (C-5), 31.8 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{10}H_{14}NaO_6$ 253.06881; Found 253.06822.

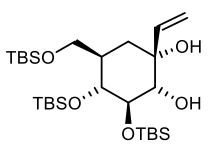
1-(*R*)-1-Vinyl-1,2-*O*-carbonate-3,4,6-tri-*O*-*tert*-butyldimethylsilyl-carba-D-glucose (32).



Compound **9** (107 mg, 0.46 mmol) was dissolved in DCM (9.2 mL, 0.05 M) and cooled on ice. Subsequently, pyridine (1.5 mL, 18 mmol, 40 eq.) and TBSOTf (1.1 mL, 4.6 mmol, 10 eq.) were added slowly. The reaction mixture was heated to 40 °C and stirred for 2 hours upon which TLC confirmed full conversion (R_f 0.5 (toluene:pentane, 1:1, v:v)). Subsequent quenching by addition of sat. aq. $NaHCO_3$ solution and dilution with water

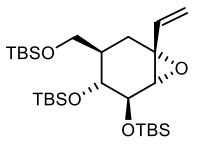
followed. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (40:60 → 60:40; toluene:pentane) yielded the title compound (202 mg, 0.35 mmol, 77%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 5.87 (dd, *J* = 17.2, 10.9 Hz, 1H, H-8), 5.44 (dd, *J* = 17.2, 0.5 Hz, 1H, H-9), 5.25 (dd, *J* = 10.9, 0.6 Hz, 1H, H-9), 4.26 (dd, *J* = 2.5, 1.6 Hz, 1H, H-2), 3.96 (dd, *J* = 2.5, 2.5 Hz, 1H, H-3), 3.73 (ddd, *J* = 3.5, 2.5, 1.6 Hz, 1H, H-4), 3.61 (dd, *J* = 9.7, 5.5 Hz, 1H, H-6), 3.54 (dd, *J* = 9.7, 6.6 Hz, 1H, H-6), 2.05 – 1.95 (m, 2H, H-5, H-7), 1.83 – 1.73 (m, 1H, H-7), 0.94 – 0.84 (m, 27H, $C(CH_3)_3$), 0.16 – 0.01 (m, 18H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 154.1 (C=O), 139.4 (C-8), 115.2 (C-9), 82.9 (C-1), 80.5 (C-2), 71.3 (C-3), 70.2 (C-4), 66.0 (C-6), 40.6 (C-5), 30.4 (C-7), 26.1, 25.8, 25.8 ($C(CH_3)_3$), 18.6, 18.1, 18.0 ($C(CH_3)_3$), -4.3, -4.5, -4.7, -4.8, -5.2 ($SiCH_3$); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{28}H_{56}NaO_6Si_3$ 595.32824; Found 595.32769.

1-(*R*)-1-Vinyl-1-hydroxyl-3,4,6-tri-*O*-*tert*-butyldimethylsilyl-carba-D-glucose (33).



Compound **32** (143 mg, 0.25 mmol) was dissolved in anhydrous Et_2O (12.5 mL, 0.02M) and cooled on ice. Subsequently, LiBH4 (2.0 M solution in THF, 0.15 mL, 0.3 mmol, 1.2 eq.) was added and stirring continued for another 15 minutes while keeping on ice. Upon full conversion was observed (R_f 0.5 (EtOAc:pentane, 1:1, v:v)) The mixture was quenched by the addition of sat. aq. $NaHCO_3$ solution and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (10:90 → 20:80; Et_2O:pentane) yielded the title compound (97 mg, 0.18 mmol, 71%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 5.91 (dd, *J* = 17.2, 10.8 Hz, 1H, H-8), 5.38 (dd, *J* = 17.2, 1.4 Hz, 1H, H-9), 5.14 (dd, *J* = 10.8, 1.4 Hz, 1H, H-9), 3.90 (ddd, *J* = 5.6, 4.8, 0.6 Hz, 1H, H-3), 3.76 (dd, *J* = 5.5, 5.5 Hz, 1H, H-4), 3.68 (dd, *J* = 9.7, 6.6 Hz, 1H, H-6), 3.57 (dd, *J* = 9.7, 6.1 Hz, 1H, H-6), 3.46 (s, 1H, 1-OH), 3.40 (dd, *J* = 6.2, 4.9 Hz, 1H, H-2), 2.81 (d, *J* = 6.2 Hz, 1H, 2-OH), 2.05 (ddd, *J* = 10.7, 5.5, 5.5 Hz, 1H, H-5), 1.87 (dd, *J* = 14.3, 5.8 Hz, 1H, H-7), 1.47 (dd, *J* = 14.3, 10.0 Hz, 1H, H-7), 0.99 – 0.82 (m, 27H, $C(CH_3)_3$), 0.20 – -0.00 (m, 18H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 143.0 (C-8), 113.6 (C-9), 77.6 (C-2), 76.9 (C-3), 73.1 (C-1), 72.7 (C-4), 64.7 (C-6), 42.2 (C-5), 34.0 (C-7), 26.2, 26.2, 26.1 ($C(CH_3)_3$), 18.5, 18.4, 18.2 ($C(CH_3)_3$), -3.4, -3.5, -4.0, -4.1, -5.2, -5.2 ($SiCH_3$); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{27}H_{58}NaO_5Si_3$ 569.34897; Found 569.34840.

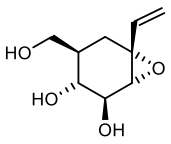
1-(*R*)-1-Vinyl-1,2-anhydro-3,4,6-tri-*O*-*tert*-butyldimethylsilyl-carba-D-glucose (34).



Compound **33** (27 mg, 50 µmol) was dissolved in DCM (5.0 mL, 0.01 M) and the mixture was cooled on ice. Subsequently, Et_3N (0.14 mL, 1.0 mmol, 20 eq.) and MsCl (50 µL, 0.50 mmol, 10 eq.) were added respectively. After stirring for 30 minutes, full conversion was observed (R_f 0.5 (toluene:pentane, 2:8, v:v)). The reaction was quenched by the addition of sat. aq. $NaHCO_3$ solution and diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with H_2O, sat. aq. $NaHCO_3$ and brine

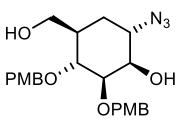
respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (10:90 → 20:80; toluene:pentane) yielded the title compound (12 mg, 22 µmol, 44%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 5.82 (dd, *J* = 17.1, 10.6 Hz, 1H, H-8), 5.44 (d, *J* = 17.0 Hz, 1H, H-9), 5.21 (d, *J* = 10.7 Hz, 1H, H-9), 4.23 (d, *J* = 5.2 Hz, 1H, H-2), 4.19 (dd, *J* = 5.1, 5.1 Hz, 1H, H-3), 3.73 (dd, *J* = 10.0, 3.6 Hz, 1H, H-6), 3.64 – 3.53 (m, 2H, H-4, H-6), 2.24 (dp, *J* = 9.0, 2.8 Hz, 1H, H-5), 2.14 (dd, *J* = 15.2, 3.3 Hz, 1H, H-7), 1.83 (dd, *J* = 15.2, 12.3 Hz, 1H, H-7), 0.95 – 0.85 (m, 27H, $C(CH_3)_3$), 0.18 – 0.02 (m, 18H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 139.6 (C-8), 115.3 (C-9), 90.8 (C-1), 87.9 (C-2), 77.0 (C-3), 73.1 (C-4), 63.5 (C-6), 39.7 (C-5), 33.0 (C-7), 26.3, 26.1, 26.1 ($C(CH_3)_3$), 18.4, 18.4, 18.2 ($C(CH_3)_3$), -3.1, -3.3, -3.6, -4.0, -5.2, -5.2 ($SiCH_3$); HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{27}H_{57}O_4Si_3$ 529.35646; Found 529.35602.

1-(*R*)-1-Vinyl-1,2-anhydro-carba-D-glucose (8).



Compound **34** (24 mg, 44 µmol) was dissolved in anhydrous THF (1.8 mL, 0.025 M). The reaction was cooled on ice and kept under inert atmosphere. TBAF on silica gel (loading F^- ~1.5 mmol/g, 147 mg, 0.22 mmol, 5.0 eq.) was added and stirring continued for 4 hours while the reaction mixture was allowed to attain to room temperature. Upon full conversion was observed (R_f 0.3 (acetone:DCM, 1:1, v:v)), the mixture was concentrated at 25 °C under reduced pressure yielding the crude product. Flash column chromatography (20:80 → 50:50; acetone:DCM) yielded the title compound (5.9 mg, 32 µmol, 72%) as a colorless oil. ^{1}H NMR (500 MHz, acetone-d6, HH-COSY, HSQC, NOESY): δ 6.01 (dd, *J* = 17.0, 10.7 Hz, 1H, H-8), 5.40 (dd, *J* = 17.0, 0.7 Hz, 1H, H-9), 5.23 (dd, *J* = 10.7, 0.7 Hz, 1H, H-9), 4.77 (d, *J* = 4.3 Hz, 1H, 3-OH), 4.43 (dd, *J* = 8.0, 0.6 Hz, 1H, H-2), 4.23 (d, *J* = 4.0 Hz, 1H, 4-OH), 4.12 (ddd, *J* = 9.7, 8.0, 4.4 Hz, 1H, H-3), 3.81 – 3.66 (m, 3H, H-6, 6-OH), 3.38 (ddd, *J* = 10.0, 10.0, 3.9 Hz, 1H, H-4), 2.18 (d, *J* = 11.8 Hz, 1H, H-7), 1.92 – 1.88 (m, 2H, H-5, H-7); ^{13}C NMR (126 MHz, acetone-d6, HSQC): δ 140.4 (C-8), 115.5 (C-9), 93.2 (C-1), 89.1 (C-2), 78.8 (C-3), 73.7 (C-4), 63.1 (C-6), 39.7 (C-5), 34.0 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_9H_{14}NaO_4$ 209.07898; Found 209.07835.

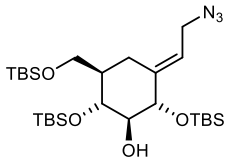
1-Deoxy-1-azido-3,4-di-*O*-(4-methoxybenzyl)-6-*O*-trityl-carba-α-D-mannose (38).



Compound **37** (0.58 g, 1.5 mmol) was dissolved in DMF (14 mL, 0.1 M) followed by the addition of $LiClO_4$ (0.8 g, 7.5 mmol, 5.0 eq.) and NaN_3 (488 mg, 7.5 mmol, 15 eq.). The reaction mixture was heated to 130 °C and stirring continued for 16 hours. Upon full conversion was observed (R_f 0.3 (EtOAc:pentane, 3:7 v:v)), the mixture was diluted with water. The aqueous layer was extracted with EtOAc (3x) followed by washing the combined organic layers with sat. aq. $NaHCO_3$ and brine respectively. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (40:60 → 50:50; EtOAc:pentane) yielded the title compound (0.22 g, 0.5 mmol, 72%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.34 – 7.18 (m, 4H, CH_{arom}), 6.97 – 6.74 (m, 4H, CH_{arom}), 4.79 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 4.65 (d, *J* = 11.1 Hz, 1H, C*H*H PMB), 4.59 (d, *J* = 11.1 Hz, 1H, CH*H* PMB), 4.55 (d, *J* = 10.9 Hz, 1H, CH*H* PMB), 3.94 – 3.87 (m, 2H, H-1, H-2), 3.81 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.72 (dd, *J* = 8.3, 3.0 Hz, 1H, H-3), 3.65 – 3.58 (m, 3H, H-4, H-6), 2.58 (d, *J* = 2.3 Hz, 1H, 2-OH), 2.04 (dd, *J* = 6.9, 4.5 Hz, 1H,

6-OH), 1.96 – 1.88 (m, 1H, H-5), 1.83 (ddd, *J* = 14.4, 11.4, 3.2 Hz, 1H, H-7), 1.67 (ddd, *J* = 14.0, 4.0, 4.0 Hz, 1H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.7, 159.5, 130.4 ($C_{q\text{-}arom}$), 129.9 (CH_{arom}), 129.9 ($C_{q\text{-}arom}$), 129.8, 114.2, 114.1 (CH_{arom}), 81.3 (C-3), 78.3 (C-4), 74.3 (CH_2 PMB), 72.7 (CH_2 PMB), 69.7 (C-2), 64.9 (C-6), 60.0 (C-1), 55.4 (OMe), 39.2 (C-5), 26.5 (C-7); HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{23}H_{29}N_3O_6Na$ 466.19541, found 466.1949.

1-(2-Azidoethylidene)-2,4,6-tri-*O*-*tert*-butyldimethylsilyl-carba-D-glucose (41).



Compound **34** (25 mg, 46 µmol) was dissolved in DMF (1.0 mL, 0.05 M) followed by the addition of tetrabutylammonium azide (0.13 g, 0.46 mmol, 10 eq.). The reaction mixture was heated to 100 °C and stirring continued for 2 hours. Upon full conversion was observed (R_f 0.5 (toluene:pentane, 3:7 v:v)), the mixture was diluted with water. The aqueous layer was extracted with Et_2O (3x) followed by washing the combined organic layers with brine. Subsequently, the organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product. Flash column chromatography (2:98 → 7:93; Et_2O:pentane) yielded the title compound (18 mg, 30 µmol, 66%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 5.69 (dd, *J* = 7.7, 7.7, 1.6, 1.6 Hz, 1H, H-8), 3.87 – 3.76 (m, 3H, H-2, H-9), 3.73 (dd, *J* = 9.8, 3.0 Hz, 1H, H-6), 3.63 (dd, *J* = 9.9, 6.0 Hz, 1H, H-6), 3.51 (dd, *J* = 10.1, 8.5 Hz, 1H, H-4), 3.16 (ddd, *J* = 8.8, 8.8, 2.2 Hz, 1H, H-3), 2.61 (dd, *J* = 13.9, 3.9 Hz, 1H, H-7), 2.25 (d, *J* = 2.2 Hz, 1H, 3-OH), 1.68 (dd, *J* = 13.5 Hz, 1H, H-7), 1.45 (ddd, *J* = 13.0, 6.4, 6.4, 3.5, 3.5 Hz, 1H, H-5), 0.99 – 0.85 (m, 27H, $C(CH_3)_3$), 0.17 – 0.04 (m, 18H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 143.9 (C-1), 113.9 (C-8), 81.2 (C-3), 76.4 (C-2), 73.7 (C-4), 63.5 (C-6), 47.3 (C-9), 46.4 (C-5), 27.3 (C-7), 26.2, 26.1 ($C(CH_3)_3$), 18.6, 18.5, 18.4 ($C(CH_3)_3$), -3.5, -4.4, -4.6, -4.6, -5.1, -5.3 ($SiCH_3$); HRMS (ESI) m/z: [M+Na]$^+$ Calcd for $C_{27}H_{57}N_3NaO_4Si_3$ 594.35546; Found 594.35491.

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Chapter 6

Synthesis of UDP-Glc and UDP-Gal mimetics as putative glycosyl transferase inhibitors

Introduction

Glycosyltransferases (GTs) catalyze the formation of glycosidic linkages by transferring a monosaccharide from an activated glycosyl donor to an acceptor substrate (Figure 1A).[1,2] GT acceptor substrates range from saccharides to lipids, proteins, DNA, and small molecules and GTs are therefore involved in the creation of a wide variety of complex carbohydrates, glycans, and glycoconjugates (*O*- and *N*-glycoproteins, glycolipids).[3,4] These biosynthetic products play pivotal roles in many biological processes, including cell growth and development, cell signaling, and host-pathogen interactions.[5,6] In addition, harmful pathogens manage to evade our immune system by decorating their exteriors through the action of GTs.[7,8] This, and deviations from normal glycosylation patterns commonly implicated in human disease, make GTs attractive medicinal targets in a vast variety of therapeutic areas, including, but not limited to, infection, inflammation, neuropathological disorders, and cancer.[9–11] Due to this significance, the development of GT inhibitors is a major area of interest within the fields of chemical biology and medicinal chemistry.

Universal glycoside donors utilized by GTs consist of a monosaccharide charged with an aglycon consisting of a mono- or diphosphate nucleotide, which make for a great leaving group during substitution (Figure 1B). Mammalian cells only use nine GT donor

substrates, one for each glycoside isostere incorporated by the mammalian biosynthesis machinery. Examples of mammalian donor substrates are α-UDP-Glu, β-CMP-Neu5NAc, β-GDP-Fuc and α-UDP-Gal.[12–14]

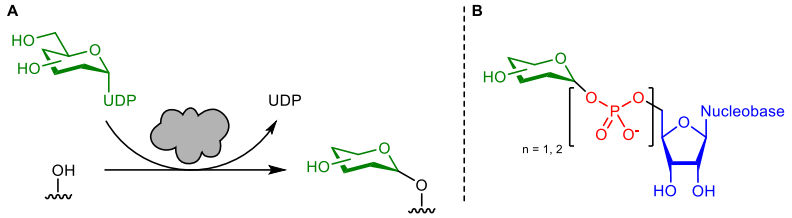


Figure 1. (A) Glycosyltransferases (GTs) catalyze glycosylation reactions with either inversion or retention of anomeric configuration of the glycosyl donor resulting in either α- or β-linkages. An α-linked UDP donor is exemplified here (B) Representation of generic glycosyltransferase donor substrate (In green = monosaccharide; in red = mono- or pyrophosphate; in blue = nucleotide).

GTs can be subdivided by the stereochemical outcome of the glycosyl transfer reaction, which can occur either with inversion or retention of the anomeric configuration with respect to the sugar donor.[15] Thus, GTs can be characterized as either inverting or retaining glycosyltransferases. These two classes of enzymes utilize different catalytic mechanisms giving rise to the different stereochemical outcomes.[1] Similar to inverting glycosidases, the itinerary of inverting GTs employ a single displacement pathway (S_N2) moving through a half-chair transition state carrying significant oxocarbenium ion character (Figure 2A). This reaction is facilitated by a carboxylate side chain in the active site (either Asp or Glu) which serves as a general base, deprotonating the hydroxyl of the acceptor substrate for direct nucleophilic displacement of the phosphate leaving group. In comparison to inverting GTs, retaining glycosyltransferases do not contain a correctly placed nucleophilic carboxylic acid residue to act as a nucleophile during catalysis.[16] Therefore, it is postulated that retaining GT's employ a front-side single displacement mechanism (Figure 2B). Here, the incoming acceptor attacks the anomeric center from the same face as the (pyro)phosphate aglycon departs (S_Ni-like). In addition, the (pyro)phosphate leaving group directly acts as an intermolecular base by deprotonating the incoming nucleophile. Also, these reactions proceed with significant oxocarbenium character. As a result, a net retention of the anomeric configuration is achieved. For this type of itinerary, the interactions between the aglycon and incoming acceptor by means of acid-base catalysis, forged by the enzymes' cavity, are crucial to facilitate this nucleophilic displacement.[17,18]

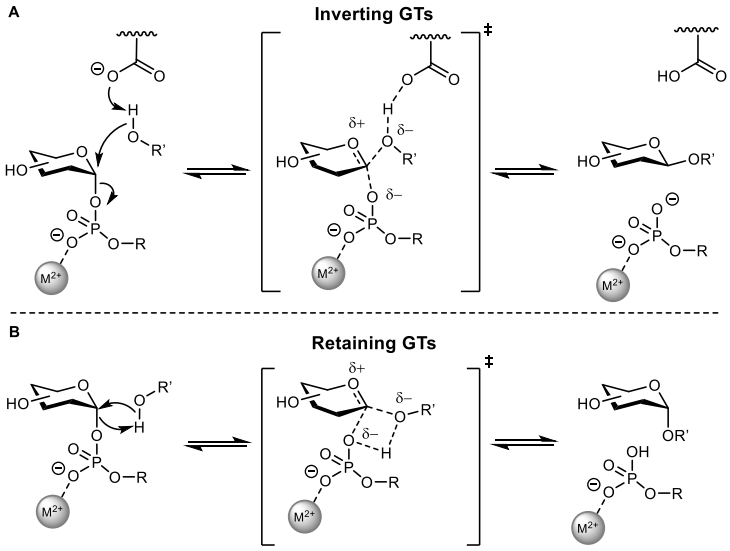


Figure 2. Proposed mechanisms for inverting and retaining metal ion-dependent glycosyltransferases. (A) Inverting GTs follow a single displacement pathway *via* an oxocarbenium ion-like transition state which leads to inversion of anomeric configuration. (B) Retaining GTs may follow a front-side single displacement (S_Ni-like) mechanism, in which the anomeric configuration of the donor substrate is retained.[1] Either phosphates of the pyrophosphate aglycon could act as an intermolecular base (here depicted with the β-phosphate).

Due to limited understanding of the reaction itineraries of glycosyltransferases, compared to that employed by glycosyl hydrolases, the rational design of GT inhibitors is complicated.[2] Design of inhibitors is further complicated by the enzymes' conformational plasticity, complex multicomponent transition states (carbohydrate donor, nucleotide, acceptor, (pyro)phosphate-chelating metal ion), weak natural substrate binding (usually in the mM range), and the limited availability of structural data.[19] Furthermore, and again in contrast to what is observed in the field of glycoside hydrolase inhibitor design (iminosugars, cyclophellitols), nature has yielded preciously little in terms of natural product GT inhibitor classes. Nevertheless, the past decades have seen some extensive systematic endeavors regarding the design and synthesis of suitable GT inhibitors.[20–24] The existing literature on the design of GT inhibitors particularly relies on donor substrate analogues, with a focus on the identification of modifications of the glycoside part of the donor.[20,25–27] These types of derivatives are highly attractive as their structural resemblance to regular sugars still allows for their recognition by biological systems while ensuring a higher stability towards endogenous carbohydrate-degrading enzymes.[28] In the context of GTs, this concept has translated into the development of a variety of carbohydrate mimetic-based GT inhibitors, mostly in the form of imino- and carbasugar nucleotide analogues.[29–31]

Due to the high degree of rotational freedom within the donor substrate, the binding mode in the enzymes' pocket diverge considerably.[32] In general, four distinct conformations have been observed, ranging from an extended, linear orientation to a "tucked under" orientation, each stabilized by different hydrogen interactions within the pocket. Adaptation of the correct conformation during binding appeared crucial for the enzymes' ability to accept donor mimetics.[32,33] For instance, during co-crystallization attempts with non-hydrolysable donor substrates as a mean to obtain insightful crystal structures.[33–36] Although the donor binding orientation of GTs is crucial for recognition, it has been ignored in contemporary studies aimed at the design of suitable inhibitors.[23,37–40] Strategically designing inhibitors to match the exact binding orientation within the binding pocket could, besides increase the binding affinity, increase the selectivity immensely, as GT's with different binding modes will show inferior binding.

The donor substrate binding orientation of several glucosyl- and galactosyl transferases have been identified. In several, the donor substrate (UDP-Glc and UDP-Gal, Figure 3A) adopts a "tucked under" conformation when bound to GTs.[32–36,41–43], Figure 3B depicts two crystal structures of UDP-Glc and UDP-Gal exhibiting this concaved orientation.

This chapter describes the design and synthesis of 8 putative GT inhibitors incorporating the above-described, distinct conformational characteristic of protein-bound donor substrates (**1** – **8**, Figure 3). In order to capitalize on the required conformational features, locking of the inhibitor molecules in the bioactive conformation was envisioned to be achieved through structure rigidification.[44,45] To this end, incorporation of a caged bicyclic scaffold, based on pyrophosphate mimetics developed by Montero *et al*.[46] and Grimes *et al*.[47] (Figure 3C), linked to a uridine monophosphate (UMP), positions the UMP below the glycoside. In this way, the concaved spatial arrangement of the GT-bound natural substrates could be mimicked.

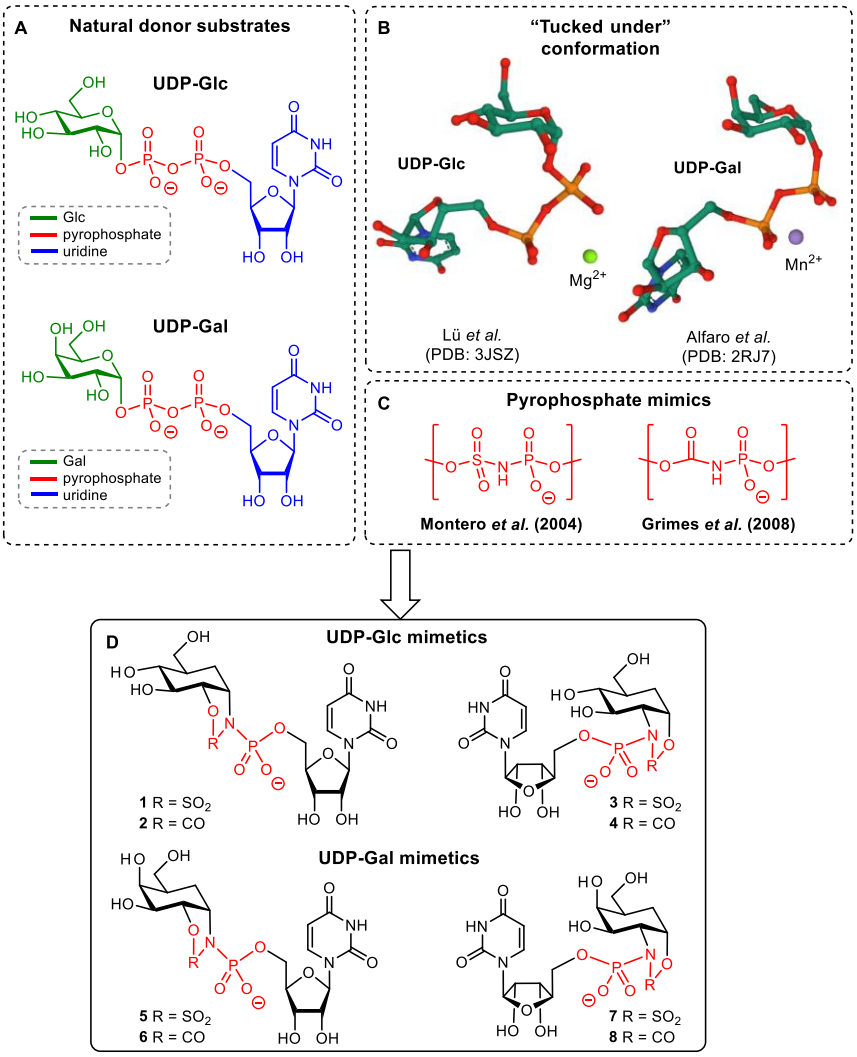


Figure 3. Overview of eight putative inhibitors proposed and synthesized in this chapter for UDP-glucosyl- and UDP galactosyl transferases, based on the binding orientation of their natural donor substrates. (A) UDP-Glc and UDP-Gal; the natural donor substrates (in green = monosaccharide; in red = pyrophosphate; in blue = nucleotide). (B) spatial orientation of UDP-Glc (left) and UDP-Gal (right), The former upon binding to glucosyltransferase Lgt1, one of three glucosylating toxins of *Legionella pneumophila*[43] and the latter upon binding to GTB/G176R, a human blood group A and B galactosyltransferase.[42] (C) *N*-sulfamidate- and *N*-carbamate phosphoramidate based pyrophosphate mimetics developed by Montero *et al*.[46] and Grimes *et al*.[47] (D) The four glucose configured and four galactose configured putative inhibitors, mimicking the tucked under donor substrate and envisioned to act on the corresponding GT's. In red the pyrophosphate mimicking entities.

From a synthetic point of view, compounds **9** – **12**, previously synthesized in chapter 3, were considered as suitable fragments ready to undergo coupling to a uridine phosphate fragment leading to the glucose configured targets (Figure 4A). In turn, the galactose configured targets were envisioned to be obtained after C-4 inversion of a carba-glucose intermediate. Although formation of the nitrogen-phosphorous linkage was considered to be a challenging transformation due to the electron depleted nature of the endocyclic nitrogen and the anticipated lability of the product, literature provides a vast array of coupling conditions (Figure 4B). Oxidative cross-coupling conditions have recently become attractive in phosphoramidate synthesis because of the mild, one-pot procedures which do not require pre-activation of a suitably protected H-phosphonate diester fragment (Figure 4B).[48–51] Alternatively, the Atherton-Todd reaction employs pre-activation of a H-phosphonate fragment *via* chlorination or bromination to allow for a subsequent nucleophilic substitution by a primary or secondary amine or amide (Figure 4B).[52–58]

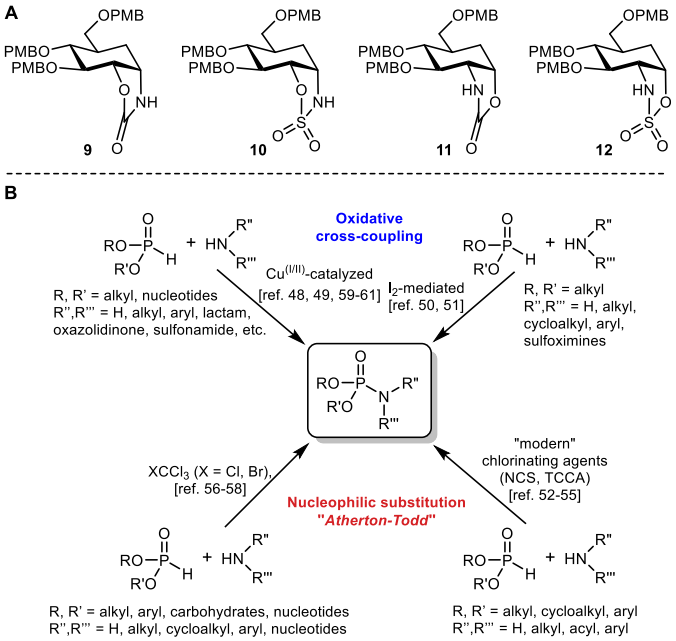
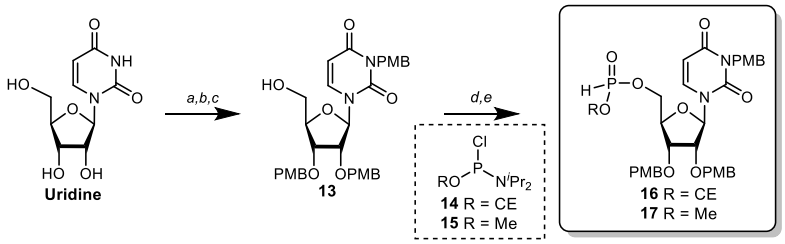


Figure 4. Formation of *N-P* linkages, envisioned to be the key transformation. (A) Four carba-glucose configured constructs previously described in chapter 3, considered to be suitable amide coupling fragments (B) Overview of most promising methods for synthesizing phosphoramidates, divided into oxidative cross-coupling reactions[48–51,59–61] (red) and Atherton-Todd type reactions[52–58] (blue).

Results and discussion

A suitably protected uridine H-phosphonate diester was advanced from commercially available uridine as follows (Scheme 1). The primary hydroxyl was regioselectively protected as a silyl ether (TBDPSCl, DMAP) allowing for a subsequent protection of the ribosyl 2'- and 3'-OH and the uracil amide with a PMB group. The PMB-protecting groups were selected to allow for a single, global deprotection step after coupling to the per-*O*-(4-methoxybenzyl)-protected carbamates and sulfamidates. Consecutive treatment with TBAF yielded intermediate **13** in 32% yield over three steps. The choice of phosphonate protecting group was anticipated to be crucial for the success of the subsequent *N*-*P* coupling and deprotection. To this end, two protecting group strategies were selected to broaden the range of compatible reaction conditions. In a two-step one-pot procedure, and depending on the commercially available phosphoramidite of choice, either the 2-cyanoethyl- or methyl- protected phosphodiesters were obtained in good to excellent yield (64% and 92%, for **16** and **17** respectively).

Scheme 1. Synthesis of PMB protected uridine H-phosphonate diester building blocks **16** and **17**.

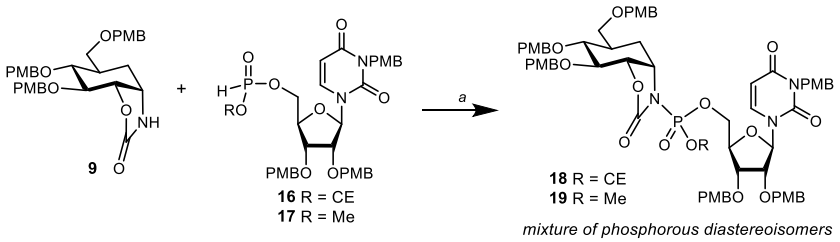


Reagents and conditions: a) TBDPSCl, DMAP, pyridine, rt, O/N; *b*) PMBCl, NaH, TBAI, DMF, 0 °C → rt, 2 days; *c*) TBAF, THF, rt, 2 h (32% over three steps); *d*) 2-cyanoethyl *N,N*-diisopropyl chlorophosphoramidite (**14**) or methyl *N,N*-diisopropyl chlorophosphoramidite (**15**), DiPEA, DCM, 0 °C, 15-30 min; *e*) ETT, H_2O, MeCN, rt, 15-30 min, 64% (**16**), 92% (**17**).

With both the H-phosphonate diester building blocks **16** and **17**, and the protected carbamates and sulfamidates in hand, *N*-*P* coupling conditions were investigated next. Preliminary coupling attempts between cyclic carbamate **9** and H-phosphonate diester **16** employing a myriad of oxidative cross-coupling reaction, either mediated by copper salts[49,59–61] or iodine[50,51], resulted in degradation of the H-phosphonate rather than product formation. No product formation was observed for H-phosphonate diester **17** when treated with identical conditions. Then attention was turned to the more classical approach to synthesize phosphoramidates by means of an Atherton-Todd reaction. Following procedures of Lowary and co-workers formation of the *N*-*P* bond was attempted *via* bromination of the H-phosphonate diester ($BrCCl_3$, DiPEA) followed by a nucleophilic substitution by carbamate **9** (Scheme 2).[58] Bromination of H-phosphonate

diester **16** indeed resulted in clean conversion into the bromophosphate intermediate according to ^{31}P NMR (two bromophosphate signals at δ = −7.05 and −7.08 ppm corresponding to P(V) diastereomers). Combining the bromophosphate with a solution of activated cyclic carbamate (KHMDS, THF) did not result in formation of the product **18**. Instead, degradation was observed, probably because of the lability of the cyanoethyl group towards the alkaline reaction conditions. However, switching to the base stable methyl-protected H-phosphonate diester **17** did result in product formation, as compound **19** was isolated as a mixture of phosphorous diastereomers in a yield of 72%. Structural elucidation *via* ^{13}C NMR confirmed product **19** indeed to be the *N*-phosphorylated product (see Appendix, Scheme S1).

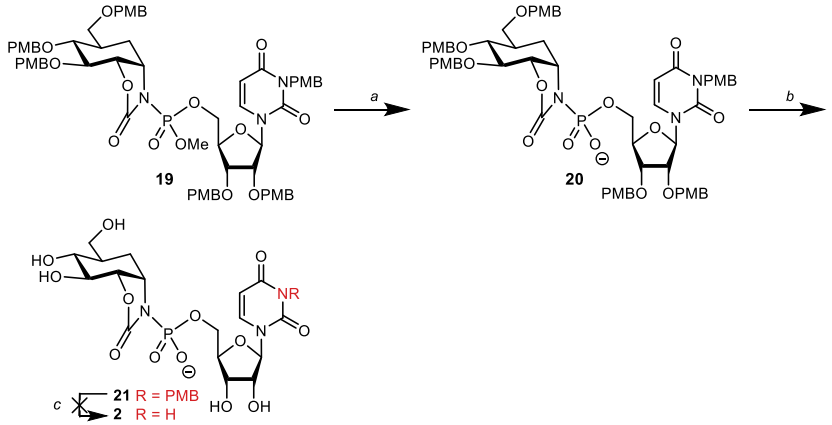
Scheme 2. Coupling attempts of PMB protected cyclic carbamate **9** and H-phosphonate diester fragments **16** or **17** employing a modified version of the Atherton-Todd reaction.



Reagents and conditions: *a*) *i.* **16** or **17**, $BrCCl_3$, DiPEA, DCM, 0 °C, 30 min; *ii.* **9**, NaH, 15-crown-5, THF, 0 °C → rt, 30 min; *then* added activated **16** or **17**, 0 °C, 2 h, 72% (**19**).

With a viable synthetic approach towards compound **19**, its global deprotection was studied next. Prior to acid catalyzed removal of the PMB ether, removal of the methyl phosphate protecting group was investigated. To this end, compound **19** was subjected to a mild nucleophile (PhSH, Et_3N, MeCN, 35 °C), which resulted in clean conversion to compound **20** as judged from the observed upfield shift in ^{31}P NMR as well as disappearance of diastereomeric signals (Scheme 3).[62] Unfortunately, due to the intrinsic stability of the uracil *N*-PMB group, as a result of the electron depleted nature induced by the two adjacent electron withdrawing carbonyl functionalities, removal of this protecting group under acidic conditions proved to be difficult and compound **21** was isolated instead.

Scheme 3. Demethylation of PMB protected *N*-phosphoramidate **19** followed by attempted global PMB-deprotection.



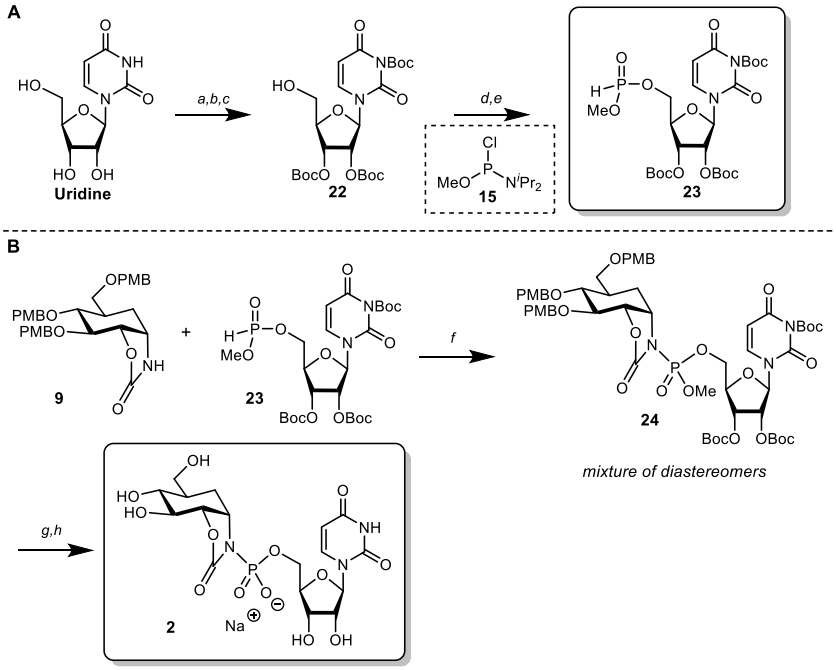
Reagents and conditions: *a*) PhSH:Et_3N:MeCN (1:2:2 v:v), 35 °C, 1 h (77%); *b*) TFA, TES, DCM, 0 °C, 16 h; *c*) Appendix Table S1.

Compound **21** was subjected to a myriad of deprotection conditions (acid, oxidative, reductive, Appendix Table S1) to obtain the target compound **2**, but none proved to be fruitful.

Prompted by the robustness of the phosphoramidate formation and its stability against acid conditions, it was hypothesized that the problematic uracil deprotection could be circumvented by the use of a different acid labile protecting group. To this end, focus was shifted to use of *tert*-butoxy carbonyl (Boc) protecting groups, since it was hypothesized the Boc protecting groups could be easily removed under the acidic conditions (20% TFA v:v) used for the PMB ether deprotection. In addition, the rate and stability of the Boc group during deprotection does not depend on the electronegativity of the adjacent heteroatom and should therefore result in clean conversion to the target compound.

Thus, commercially available uridine was protected at the 5'-OH as a TBDPS ether and subsequently, the 2'- and 3'-OH and uracil amide were protected with Boc groups (Boc_2O, DMAP, Scheme 4A). Lastly, the TBDPS ether was hydrolyzed under *aegis* of TBAF to yield compound **22** in an excellent yield of 92% over three steps. Again, in a two-step one-pot procedure, the commercially available phosphoramidite **15** was coupled to **22** and hydrolyzed affording the methyl protected H-phosphonate diester **23** in good yield (82% over two steps).

Scheme 4. Synthesis of Boc protected H-phosphonate diester **23** (A) and consequent synthesis of *N*-UMP-carba-1'',2''-(*N*,*O*)-carbamate **2** (B).

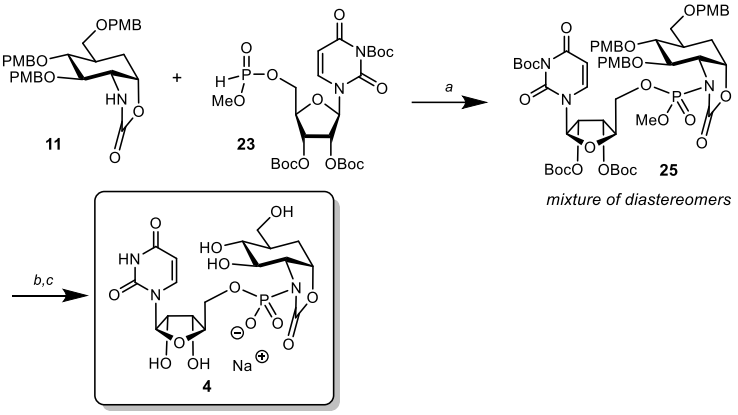


Reagents and conditions: a) TBDPSCl, DMAP, pyridine, rt, O/N; *b*) Boc_2O, Et_3N, DMAP, DCM, 0 °C → rt, 4 h; *c*) TBAF, THF, rt, O/N (92% over three steps); *d*) methyl *N*,*N*-diisopropyl chlorophosphoramidite (**15**), DiPEA, DCM, 0 °C, 15 min; *e*) ETT, H_2O, MeCN, rt, 15 min (82% over two steps); *f*) *i.* **23**, $BrCCl_3$, DiPEA, DCM, 0 °C, 30 min; *ii.* **9**, NaH, 15-crown-5, THF, 0 °C → rt, 30 min; *then* added activated **23**, 0 °C, 2 h (68%); *g*) 30% TFA/DCM (v:v), TES, 5 °C, 24 h; *h*) pyridine, 5 °C, 30 min., (72%).

Treatment of **23** with bromination conditions resulted in clean conversion to the bromophosphate intermediate according to ^{31}P NMR (Scheme 4B) and its subsequent addition to activated carbamate **9** (NaH, THF) led to clean formation of compound **24**, which could be isolated as a mixture of phosphorous diastereomers in good yield (68%). Removal of the PMB and Boc groups commenced by treating **24** with a solution of 30% TFA in DCM (v:v) and triethyl silane as cation scavenger. Full removal of all acid labile protecting groups was observed after 16 hours. Interestingly, quenching of the reaction with pyridine directly resulted in demethylation of the phosphoramidate methyl ester to provide the final compound **2**, as indicated by ^{31}P NMR. In this transformation pyridine acts as a mild nucleophile removing the methyl ester to yield the *N*-methyl pyridinium salt of **2**. After Dowex Na^+ ion exchange and lyophilization the *N*-UMP-carba-1'',2''-(*N*,*O*)-carbamate target **2** was successfully obtained as a Na^+ salt in good yield (72% over two steps).

After successfully obtaining of the *N*-UMP-carbaglucose-1'',2''-(*N*,*O*)-carbamate **2**, attention was turned to the 1'',2''-(*O*,*N*)-carbamate **4** to test the robustness of the novel three step sequence methodology (Scheme 5). Using the Atherton-Todd coupling conditions, compound **11** could be efficiently *N*-phosphorylated to give compound **25** in excellent yield, again as a mixture of phosphorous diastereomers (97%). Following the optimized two-step one-pot procedure, *i.e.* acid hydrolysis followed by demethylation under *aegis* of pyridine, neatly provided the target structure. Dowex Na^+ ion exchange and lyophilization led to the *N*-UMP-carbaglucose-1'',2''-(*O*,*N*)-carbamate target **4** as its Na^+ salt in 32% yield over two steps.

Scheme 5. Synthesis of *N*-UMP-carba-1'',2''-(*O*,*N*)-carbamate **4**.

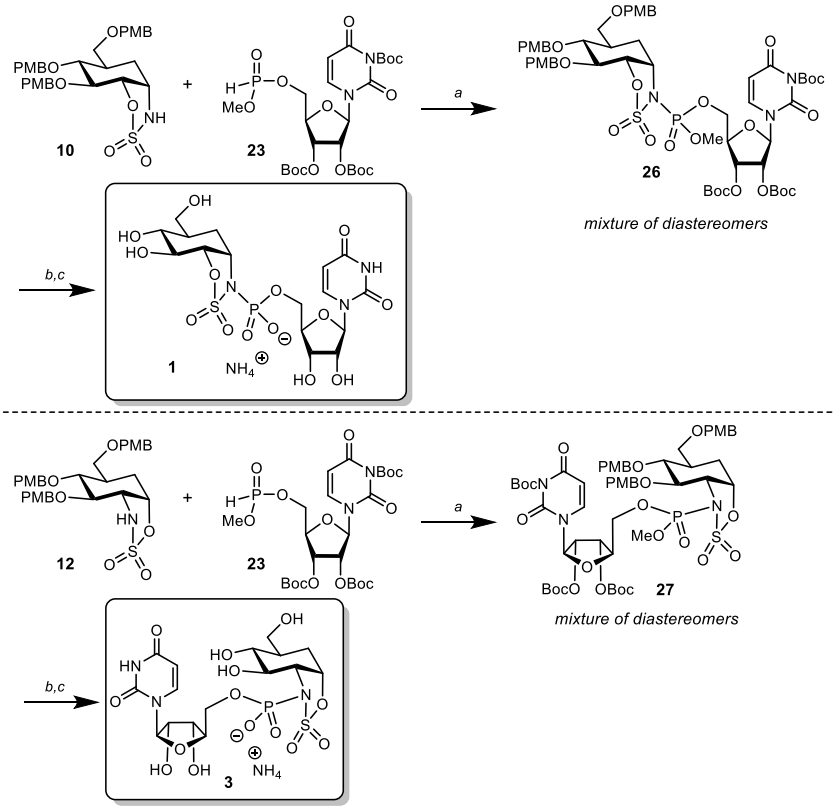


Reagents and conditions: *a*) *i.* **23**, $BrCCl_3$, DiPEA, DCM, 0 °C, 30 min; *ii.* **11**, NaH, 15-crown-5, THF, 0 °C → rt, 30 min; *then* added activated **23**, 0 °C, 2 h (97%); *b*) 30% TFA/DCM (v:v), TES, 5 °C, 24 h; *c*) pyridine, 0 °C, 30 min, (32%).

To further capitalize on the newly developed methodology, the scope was expanded towards the 1,2-cyclic sulfamidates **10** and **12** (scheme 6). Thus, treating cyclic sulfamidates **10** and **12** with H-phosphonate diester **23** under the standardized Atherton-Todd conditions yielded *N*-sulfonylphosphoramidates **26** and **27** as a mixture of phosphorous diastereomers in 53% and 77% yield for **26** and **27**, respectively. Global acidic deprotection of **26** and **27** using TFA/TES proceeded smoothly, after which the reaction mixture was neutralized with pyridine. Gratifyingly, ^{31}P NMR analysis revealed clean demethylation of the methyl ester, virtually without any trace of phosphorus-based by-products. However, upon conversion of the pyridinium salt into its Na^+ congener using Dowex Na^+ ion exchange, rapid degradation of the product was observed. The *N*-sulfonylphosphoramidates, **1** and **3**, were hypothesized to be relatively base labile and susceptible to hydrolysis under the slightly alkaline nature of the Dowex resin. By switching to a NH_4^+ counter ion the degradation could be circumvented. Thus, Dowex NH_4^+ ion exchange resin, prepared by treating Dowex resin with an aqueous 0.5

M NH_4OAc solution, followed by lyophilization yielded the target compounds **1** and **3**, as their NH_4^+ salt in good yield over two steps (77% and 74%, for **1** and **3** respectively).

Scheme 6. Synthesis of *N*-UMP-carba-1'',2''-sulfamidates **1** and **3**.



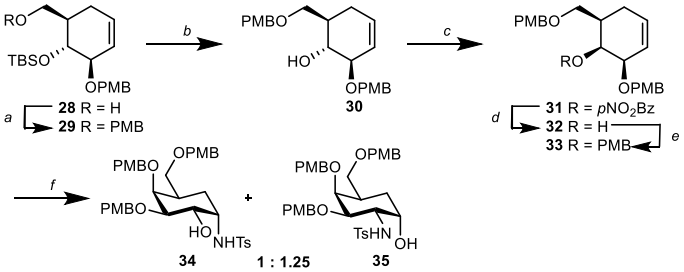
Reagents and conditions: a) *i.* **23**, $BrCCl_3$, DiPEA, DCM, 0 °C, 30 min, *ii.* cyclic sulfamidate **10** or **12**, NaH, 15-crown-5, THF, 0 °C → rt, 30 min; then added activated **23**, 2 h, 0 °C, **26** (53%), **27** (77%); *b*) 30% TFA/DCM (v:v), TES-H, 0 °C, 3 h; *c*) *i.* pyridine, 0 °C → rt, 16 h; *ii.* Dowex NH_4^+ cation exchange, **1** (77% over two steps), **3** (74% over two steps).

With robust synthetic methodologies towards glucose configured carbamate-*N*-phosphoramidates and sulfamidate-*N*-phosphoramidates, attention was shifted towards the galactose configured carbasugars. To this end, compound **28**, previously synthesized in chapter 2, was considered as a suitable starting point (Scheme 7). as the orthogonality between the protecting groups in **28** should allow for the regioselective manipulation of the C-4 substituent and provide access to its *galacto*-configured counterpart.

First the primary alcohol in compound **28** was protected as a PMB ether (Scheme 7). However, standard Williamson etherification conditions (PMBCl, NaH) led to partial

cleavage of the TBS ether.[63] Acid catalyzed PMB installation (PMB-2,2,2-trichloroacetimidate, *p*-TsOH) did prove successful and yielded compound **29** in a 57% yield. Regioselective deprotection of the 4-OH proceeded smoothly under *aegis* of TBAF to yield compound **30** in 67% yield. Initially, the C-4 inversion was investigated using an oxidation-reduction strategy. Thus, the 4-OH was oxidized to the corresponding ketone and consecutive reduction using $NaBH_4$, as described by Wei *et al*., provided the C-4-alcohol as an inseparable epimeric mixture in a 4:1 ratio between D-galactal and D-glucal configuration.[64] To prevent the formation of a diastereoisomeric mixture, Mitsunobu inversion conditions (4-nitrobenzoic acid, DEAD, TPP) were employed next on alcohol **30**, yielding D-galactal configured compound **31** in quantitative yield. Transesterification of the 4-nitrobenzoate (NaOMe, MeOH) followed by protection of the 4-OH as a PMB ether under standard Williamson etherification conditions then yielded intermediate **33** in 89% yield.[63]

Scheme 7. Synthesis of galactose configured α-*cis*-amino alcohols **34** and **35**.



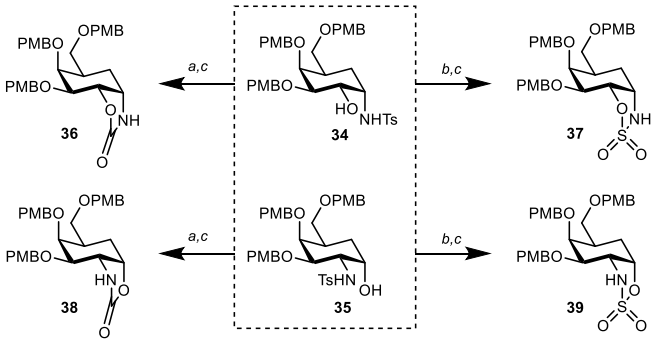
Reagents and conditions: a) PMB imidate, PPTS, DCM, rt, 16 h (57%); *b)* TBAF, THF, rt, 1 h (67%); *c)* PPh_3, *p*-nitrobenzoic acid, DEAD, THF, 60 °C, 4.5 h (*quant*.); *d)* NaOMe, DCM:MeOH (1:1 v:v), rt, 16 h (67%); *e)* PMBCl, NaH, DMF, rt, 16 h (89%); *f)* CAT·$3H_2O$, $K_2[OsO_2(OH)_4]$, TEBACl, $CHCl_3$:H_2O (1:1 v:v), 60 °C, 16 h (91%).

Next, a Sharpless amino hydroxylation stereospecifically provided the desired amino alcohols **34** and **35** in a regioisomeric ratio of 1:1.25 respectively, with an overall yield of 91%. The relative stereochemistry, *trans* with respect to the other ring substituents was anticipated based on results described in chapter 3.

With both *N*-tosylated *cis*-amino alcohols **34** and **35** in hand, the corresponding cyclic carbamates and sulfamidates **36** – **39** were prepared next (Scheme 8). Employing the methodologies described in chapter 3, both amino alcohols could be transformed in two-step procedures into their corresponding cyclic carbamates **36** and **38** and sulfamidates **37** and **39**. Treatment of **34** or **35** with triphosgene and subsequent reductive detosylation using a sodium naphthalenide solution, yielded cyclic carbamates **36** and **38** in 78% and 49% yield, respectively.[65] Alternatively, Treatment of **34** and **35** with sulfuryl chloride at -78 °C and subsequent reductive detosylation in a sodium

naphthalenide solution, yielded cyclic sulfamidates **37** and **39** in 34% and 55% yield, respectively.

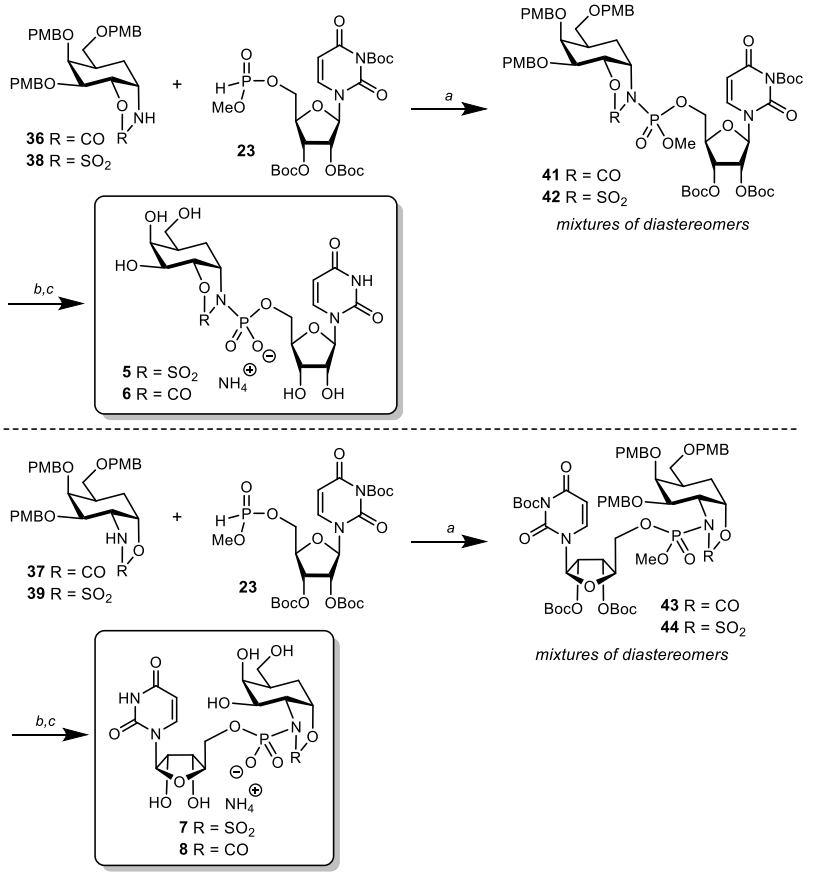
Scheme 8. Divergent synthesis towards cyclic carbamates **36** and **38** and cyclic sulfamidates **37** and **39**.



Reagents and conditions: a) triphosgene, pyridine, DCM, rt, 2 h; *b)* SO_2Cl_2, Et_3N, DCM, -78 °C → 5 °C, 2 h; *c)* naphthalene, Na, THF, -78 °C, 1 h, **36** (78%), **37** (34%), **38** (49%) and **39** (55%).

With all four coupling fragments **36** – **39** in hand, attempts were made to couple the galactose carbasugars to H-phosphonate diester **23** using the established Atherton-Todd conditions (Scheme 9). After formation of the bromophosphate, it was added to the anion of the carbamate or sulfamidate, generated using NaH in combination with 15-crown-5, to result in clean *N*-phosphorylation. The coupled products **41** – **44** were isolated as mixtures of phosphor-diastereomers in 54%, 27%, 42% and 33% yield, for **41**, **42**, **43** and **44**, respectively. All that remained now, was removal of the protecting groups in a two-step one-pot deprotection. To this end, compounds **41** – **44** were treated with a 30% TFA solution in DCM v:v with triethylsilane as a cation scavenger, which resulted in clean removal of all PMB and Boc protection groups according to ^{31}P NMR. Subsequent addition of pyridine freed the methylated P^V-phosphoramidate diesters giving rise to the four target structures, which after Dowex NH_4^+ ion exchange yielded the corresponding ammonium salts **5** – **8** in 55%, 30%, 22% and 54% yield over two steps respectively.

Scheme 9. Galactose configured target structures **5** – **8**, assembled *via N*-phosphorylation and subsequent global deprotection.



Reagents and conditions: a) i. **23**, $BrCCl_3$, DiPEA, DCM, 0 °C, 30 min, *ii.* cyclic sulfamidate or carbamates **36**, **37**, **38** or **39**, NaH, 15-crown-5, THF, 0 °C → rt, 30 min; then added activated **23**, 2 h, 0 °C, **41** (54%), **42** (27%), **43** (42%), **44** (33%); *b)* TES, TFA, DCM, 0 °C, 16 h; *c) i*. pyridine, 0 °C → rt, 16 h; *ii.* Dowex NH_4^+ cation exchange, **5** (55%), **6** (30%), **7** (22%) and **8** (54%).

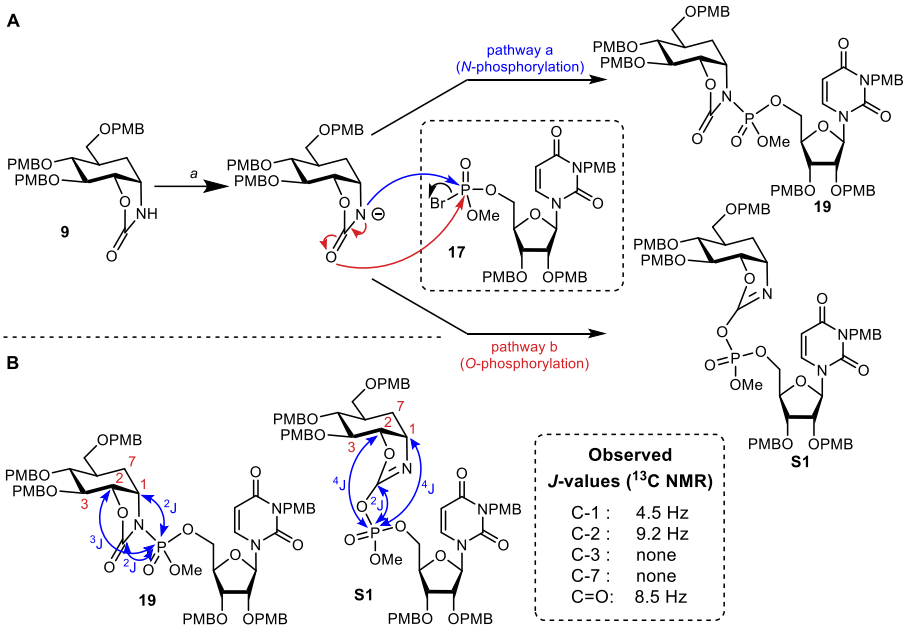
Conclusion

In conclusion, this chapter reports on the synthesis of UDP-glucose and UDP-galactose configured mimetics of high structural complexity as putative GT inhibitors. The design of these structures was based on crystallographic data, suggesting donor substrates to adopt a concaved kinked orientation upon forming a Michaelis complex with the enzyme. It was hypothesized that, by means of rigidification, the proposed structures could closely resemble the natural donor conformation in the Michaelis complexes, and therefore could act as strong enzyme binders. To this end, novel and robust methodology has been developed that allows for a divergent and efficient synthesis of targets **1** – **8**. Amongst the key transformations are the Atherton-Todd *N*-phosphorylation, providing a robust and efficient coupling between the cyclic carbamate or sulfamidate with a suitably protected H-phosphonate diester. In an efficient two-step one-pot deprotection method, the structures were successfully deprotected and isolated. The synthetic methodology described here can serve as a blueprint for future syntheses of complex phosphorylated structures. In addition, the described putative GT inhibitors can, upon screening for their inhibitory potencies in suitably designed assays, form a solid base to uncover potent and selective GT inhibitors.

Acknowledgements

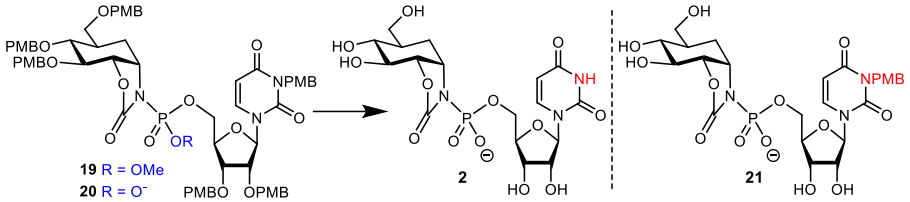
Roy Steneker and Duncan de Graaf are kindly acknowledged for their synthesis work in the context of their MSc internships. Pascal Balić is acknowledged for our insightful discussions in relation to the phosphorus chemistries.

Appendix



Scheme S4. (A) Two possible pathways under Atherton-Todd conditions, resulting either in *N*-phosphorylation or *O*-phosphorylation of cyclic carbamate **9**. (B) Structural proof the reaction merely occurs *via N*-phosphorylation (pathway I) to give desired phosphoramidate **19**, according to (^{31}P,^{13}C) *J*-values observed in the ^{13}C NMR spectrum of the isolated product.

Table S1. Attempted global deprotection of PMB protected compound **19** and **20**.



R-group	Conditions	Observations
R = Me	Pd/C, H_2, MeOH, rt, O/N	Reaction halted at compound **21** according to TLC-MS analysis
R = Me	DDQ (2.0 eq.), H_2O:DCM (1:2 v/v), 0 °C, 2 h	Degradation of starting material
R = H	HCl/HFIP (10 eq.), TES-H, DCM/HFIP (1:1 v/v), 0 °C, 30 h	Reaction halted at compound **21** according to TLC-MS analysis
R = H	20% TFA/DCM (v/v), TES-H, 0 °C, 3 h	Reaction halted at compound **21** according to TLC-MS analysis
R = H	MSA (10 eq.), TES-H, DCM, 0 °C, 1 h	Degradation of starting material
R = H	CAN (4.0 eq.), H_2O/MeCN (1:1 v/v), 3 days, 5 °C; then 65 °C, 3 h	Degradation of starting material

Synthetic procedures.

General procedure A: One-pot two-step H-phosphonate diester synthesis from protected uridine building blocks

The H-phosphonate diesters were prepared according to a modified literature procedure.[66] The protected uridine substrate was co-evaporated 3x with dry $CHCl_3$ or DCM under N_2 atmosphere and dissolved in anhydrous DCM (0.1-0.2 M). Anhydrous diisopropylethylamine (DiPEA; 2.5 eq.) was added and the solution was cooled on ice before the addition of the *N,N*-diisopropylchlorophosphoramidite (95%, 1.2 eq.). The reaction mixture was stirred for 15-30 min on ice, and after full conversion was confirmed by both TLC and ^{31}P NMR analysis (acetone-d_6 probe; $\delta \approx 150$ ppm), the mixture was concentrated to dryness under a N_2 atmosphere. The resulting crude product was redissolved in MeCN (0.05-0.1 M), after which demineralized H_2O (30 eq.) and 5-(ethylthio)-1*H*-tetrazole (ETT activator, 0.25 M in MeCN; 1.5-3.0 eq.) were added. The reaction mixture was stirred at room temperature for 15-30 minutes. After full conversion was confirmed by both TLC and ^{31}P NMR analysis (acetone-d_6 probe; ^1H gated decoupled: $\delta \approx$ 10-13 ppm; ^1H coupled: $\delta \approx$ 8-20 ppm and $^1J_{P,H} \approx$ 700-725 Hz), the reaction was quenched by the addition of a sat. aq. $NaHCO_3$ solution. The mixture was extracted with EtOAc (3x), after which the combined organic layers were washed once with a 1.0 M aq. HCl solution, once with sat. aq. $NaHCO_3$, and once with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Flash column chromatography of the crude material (SiO_2, EtOAc in pentane) yielded the H-phosphonate diester as a mixture of P(V) diastereomers.

General procedure B: Carbamylation and *N*-detosylation of *N*-Ts protected 1,2-*cis* amino-alcohols

The *N*-tosyl protected cyclic carbamates were prepared according to a modified literature procedure.[67] 1,2-*cis* amino-alcohol was co-evaporated with toluene, dissolved in anhydrous DCM (0.10 M) and cooled on ice. Pyridine (4.5 eq.) and triphosgene (0.60 eq.) were added and the ice bath was removed after 10 min. The reaction mixture was stirred for 2 h at room temperature. Upon full conversion on TLC, the reaction was quenched with sat. aq. $NaHCO_3$. The organic layer was separated, the aqueous layer was extracted thrice with EtOAc and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Subsequently, a second flask was prepared with a glass stirring bar, in which naphthalene (12 eq.) was dissolved in anhydrous THF (0.05 M). Na (10 eq.) was added to this solution. This mixture was sonicated for 15 min. at room temperature and stirred at room temperature, before being cooled to -78°C. The crude intermediate was co-evaporated thrice with toluene, dissolved in anhydrous THF and added dropwise to the Na-naphthalenide solution. The reaction mixture was stirred for 0.5-1 h at -78°C. Upon full conversion on TLC, the reaction was quenched with sat. aq. NH_4Cl at -78°C. The mixture was diluted with sat. aq. $NaHCO_3$, the organic layer was separated, the aqueous layer was extracted thrice with EtOAc and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash column chromatography (SiO_2, EtOAc in pentane) yielded the products.

General procedure C: Sulfamidation and *N*-detosylation of *N*-Ts protected 1,2-*cis* amino-alcohols

The *N*-tosyl protected cyclic sulfamidates were prepared according to a modified literature procedure.[68] 1,2-*cis* amino-alcohol substrate was co-evaporated with toluene and dissolved in anhydrous DCM (0.10 M). Et_3N (4.0 eq.) was added and the reaction mixture was cooled to -78°C. SO_2Cl_2 (1.3 eq.) was added dropwise. The reaction mixture was stirred for 2 h while allowing the reaction mixture to warm up from -78 °C to 0 °C. Upon full conversion on TLC, the reaction was quenched with sat. aq. $NaHCO_3$. The organic layer was separated, the aqueous layer was extracted thrice with Et_2O and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* at room temperature. Subsequently, a second flask was prepared with a glass stirring bar, in which naphthalene (12 eq.) was dissolved in anhydrous THF (0.05 M). Na (10 eq.) was added to this solution. This mixture was sonicated for 15 min. at room temperature and stirred at room temperature, before being cooled to -78°C. The crude intermediate was co-evaporated thrice with toluene, dissolved in anhydrous THF and added dropwise to the Na-naphthalene solution. The reaction mixture was stirred for 0.5-1 h at -78°C. Upon full conversion on TLC, the reaction was quenched with sat. aq. NH_4Cl at -78°C. The mixture was diluted with sat. aq. $NaHCO_3$, the organic layer was separated, the aqueous layer was extracted thrice with EtOAc and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash column chromatography (SiO_2, EtOAc in pentane) yielded the products.

General procedure D: *N*-phosphorylation of per-*O*-(4-methoxybenzyl) cyclic carbamates and sulfamidates using protected methyl H-phosphonate diesters

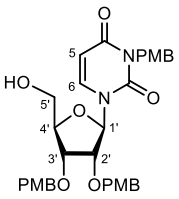
Per-*O*-(4-methoxybenzyl) cyclic carbamate or sulfamidate was co-evaporated with anhydrous $CHCl_3$ and added to an oven-dried round-bottom flask. The flask was connected to a Schlenk line and subjected to three vacuum-N_2 backfill cycles before dissolving in anhydrous THF (0.2-0.3 M). The solution was cooled on ice, after which 15-crown-5 ether (5.0 eq.) and NaH (60 wt% dispersion in mineral oil; 1.5 eq.) were added. After 5 minutes, the reaction mixture was removed from the ice bath and stirred at room temperature for 30 minutes. In parallel, another oven-dried round-bottom flask was charged with methyl H-phosphonate diester (2.0 eq.) and also put under a N_2 atmosphere using Schlenk setup before dissolving in anhydrous DCM (0.3-0.4 M). The solution was cooled on ice, after which anhydrous DiPEA (6.0 eq.) and bromotrichloromethane ($BrCCl_3$; 4.0 eq.) were added. The reaction mixture was stirred on ice for 15-30 minutes, after which full conversion to the bromophosphonate was confirmed by both TLC and ^{31}P NMR analysis (δ = –4.9, –5.1 ppm; 121 MHz, acetone-d_6 probe). The flask containing deprotonated cyclic carbamate or sulfamidate was cooled back on ice, after which the cooled bromophosphonate solution was transferred using a N_2-flushed syringe. The resulting reaction mixture was stirred on ice for 1.5-2 h. After full conversion was observed by TLC, the reaction was quenched on ice with sat. aq. $NaHCO_3$. The crude product was extracted with EtOAc (3x), after which the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash column chromatography of the crude material (SiO_2, EtOAc in pentane) followed by Sephadex™ LH-20 size exclusion chromatography yielded the protected *N*-acyl- or *N*-sulfonylphosphoramidate as a mixture of P(V) diastereomers.

Note: The P(V) diastereomers were partially separable using silica and size-exclusion column chromatography, making it possible to obtain clean NMR spectra of the individual diastereomers for easier structure elucidation. Aside from this, the phosphoramidate products were collected and subsequently deprotected as a mixture.

General procedure E: One-pot global deprotection and demethylation of protected *N*-acyl- or *N*-sulfonylphosphoramidate

Protected *N*-acyl- or *N*-sulfonylphosphoramidate compound (as a mixture of P(V) diastereomers) was dissolved in anhydrous DCM (0.05 M) and cooled on ice. TES (10 eq.) and TFA (30% v:v) were added, and the reaction mixture was stirred on ice for 3-16 h. After full conversion was observed by TLC-MS analysis (MeOH:DCM, 2:8 v:v), pyridine (20 eq. with respect to TFA) was added while stirring on ice. The resulting reaction mixture was stirred overnight at 25 °C to 35°C, during which the demethylation of the methyl phosphoramidates took place. After full conversion was observed by ^{31}P NMR analysis (acetone-d_6 probe), the mixture was concentrated to dryness under reduced pressure. Purification of the crude product by flash column chromatography (neutralized SiO_2, dry loading on Celite; distilled DCM then H_2O in MeCN) followed by Dowex 50WX4 NH_4^+ ion or Na^+ ion exchange (stored on 0.10 M NH_4OAc or 0.10 M NaOAc) and subsequent lyophilization yielded the *N*-UMP-carba-1'',2''-cyclic carbamates or sulfamidate target compounds as their NH_4^+ or Na^+ salts.

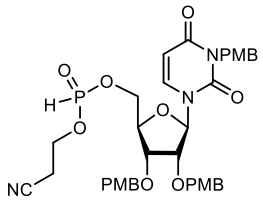
3-*N*-(4-Methoxybenzyl)-2',3'-di-*O*-(4-methoxybenzyl)uridine (13).



Uridine (3.66 gr, 15 mmol) was dissolved in pyridine (18 mL, 0.8 M). 4-Dimethylaminopyridine (DMAP; 183 mg, 1.5 mmol, 0.1 eq.) and *tert*-butyldiphenylchlorosilane (TBDPSCl; 4.3 mL, 16.6 mmol, 1.1 eq.) were added and the reaction mixture was stirred overnight under N_2 atmosphere according to literature procedure.[69] Upon full conversion (R_f 0.65 (MeOH:DCM, 1:9 v:v)), the reaction was quenched by the addition of MeOH, diluted with sat. aq. $NaHCO_3$ and brine and extracted with DCM (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product (colorless oil) was co-evaporated twice with toluene, dissolved in DMF (50 mL, 0.3 M) and cooled on ice. PMBCl (17 mL, 128 mmol, 8.5 eq.) was added followed by the addition of NaH (60 wt% dispersion in mineral oil; 5.1 gr, 128 mmol, 8.5 eq.) and TBAI (2.8 gr, 7.6 mmol, 0.5 eq.) while stirring on ice. The reaction mixture was slowly warmed to room temperature and stirred for 42 h. Upon full conversion (R_f 0.5 (EtOAc:pentane, 3:7 v:v)), the reaction mixture was carefully quenched using a sat. aq. $NaHCO_3$ solution while stirring on ice. The reaction mixture was extracted with EtOAc (3x), after which the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product (dark-brown oil) was dissolved in THF (80 mL), TBAF (1.0 M in THF; 20 mL, 20 mmol, 1.35 eq.) was added and the reaction mixture was stirred for 2 h. After full conversion was observed (R_f 0.15 (EtOAc:pentane, 1:1 v:v)), the mixture was diluted with sat. aq. $NaHCO_3$ solution and extracted with EtOAc (3x), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash column chromatography of the crude material (SiO_2, 50:50 EtOAc:pentane → 100:0 EtOAc:pentane)

followed by recrystallization from EtOAc/pentane yielded title compound **13** as a yellow solid (2.9 gr, 4.8 mmol, 32% over three steps). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.49 – 7.45 (m, 2H, CH_{arom}), 7.42 (d, *J* = 8.1 Hz, 1H, H-6), 7.22 – 7.17 (m, 4H, CH_{arom}), 6.89 – 6.81 (m, 4H, CH_{arom}), 6.78 – 6.73 (m, 2H, CH_{arom}), 5.71 (d, *J* = 3.8 Hz, 1H, H-1'), 5.65 (d, *J* = 8.1 Hz, 1H, H-5), 5.04 (d, *J* = 13.6 Hz, 1H, NC*H*H PMB), 4.95 (d, *J* = 13.6 Hz, 1H, NCH*H* PMB), 4.71 (d, *J* = 12.0 Hz, 1H, C*H*H PMB), 4.57 (d, *J* = 12.0 Hz, 1H, CH*H* PMB), 4.50 (d, *J* = 11.4 Hz, 1H, C*H*H PMB), 4.34 (d, *J* = 11.4 Hz, 1H, CH*H* PMB), 4.24 – 4.17 (m, 2H, H-2', H-4'), 4.01 (dd, *J* = 5.5, 5.5 Hz, 1H, H-3'), 3.93 (ddd, *J* = 12.6, 2.6, 2.6 Hz, 1H, H-5'), 3.80 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.67 (ddd, *J* = 12.3, 7.1, 2.2 Hz, 1H, H-5'), 2.50 (dd, *J* = 6.9, 3.6 Hz, 1H, 5'-OH); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 162.6 (C=O uracil), 159.7, 159.6, 159.3 ($C_{q\text{-}arom}$), 150.8 (C=O uracil), 139.5 (C-6), 131.1, 130.3 (CH_{arom}), 129.7 ($C_{q\text{-}arom}$), 129.6 (CH_{arom}), 129.4, 129.1 ($C_{q\text{-}arom}$), 114.0, 113.9, 113.8 (CH_{arom}), 102.0 (C-5), 92.4 (C-1'), 83.4 (C-4'), 77.7 (C-2'), 74.6 (C-3'), 72.2, 71.8 (CH_2 PMB), 61.6 (C-5'), 55.4, 55.4 (OMe), 43.6 (NCH_2 PMB). ^{1}H and ^{13}C NMR are consistent with literature data.[70] HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{33}H_{36}N_2O_9Na$ 627.2319, found 627.2313.

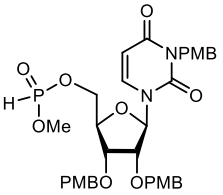
3-*N*-(4-Methoxybenzyl)-2',3'-di-*O*-(4-methoxybenzyl)uridine 2-cyanoethyl H-phosphonate (16).



Compound **16** was prepared according to **general procedure A** using **13** (1.2 gr, 1.9 mmol), 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite **14** (95%, 0.55 mL, 2.3 mmol, 1.2 eq.) and dry DiPEA (0.4 mL, 2.3 mmol, 1.2 eq.) in anhydrous DCM (20 mL, 0.1 M) in step 1; and ETT activator (0.25 M in MeCN; 23.2 mL, 5.8 mmol, 3.0 eq.), demineralized H_2O (1.1 mL, 61 mmol, 32 eq.) in MeCN (9.0 mL, 0.06 M) in step 2. Flash column chromatography of the crude product (SiO_2; 50:50 EtOAc:pentane → 80:20 EtOAc:pentane) yielded title compound **16** as a yellow oil (887 mg, 1.23 mmol, 64% over two steps). R_f 0.4 (EtOAc); The NMR data showed the presence of two P(V) diastereomers in a 1:1 ratio. Data for diastereomeric mixture: ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.74 (d, $^{1}J_{H,P}$ = 722.3 Hz, 1H, PH), 7.76 – 7.43 (m, 4H, CH_{arom}, CH_{arom}*), 7.31 (d, *J* = 8.2 Hz, 1H, H-6), 7.30 (d, *J* = 8.1 Hz, 1H, H-6*), 7.26 – 7.15 (m, 8H, CH_{arom}, CH_{arom}*), 6.90 – 6.82 (m, 8H, CH_{arom}, CH_{arom}*), 6.80 – 6.75 (m, 4H, CH_{arom}, CH_{arom}*), 5.92 (d, $^{1}J_{H,P}$ = 720.3 Hz, 1H, PH*), 5.92 (m, 1H, H-1'), 5.89 (d, *J* = 2.1 Hz, 1H, H-1'*), 5.71 (d, *J* = 8.1 Hz, 1H, H-5), 5.71 (d, *J* = 8.1 Hz, 1H, H-5*), 5.12 – 4.99 (m, 4H, NC*H*H PMB, NCH*H* PMB, NC*H*H PMB*, NCH*H* PMB*), 4.78 – 4.63 (m, 4H, C*H*H PMB, CH*H* PMB, C*H*H PMB*, CH*H* PMB*), 4.45 – 4.16 (m, 14H, H-4', H-4'*, H-5', H-5'*, C*H*H PMB, CH*H* PMB, C*H*H PMB*, CH*H* PMB*, CH_2CH_2CN, CH_2CH_2CN*), 3.98 – 3.93 (m, 2H, H-2', H-2'*), 3.85 (dd, *J* = 8.0, 5.3 Hz, 1H, H-3'), 3.81 – 3.77 (m, 19H, H-3'*, OMe, OMe, OMe, OMe*, OMe*, OMe*), 2.72 (t, *J* = 6.2 Hz, 2H, CH_2CH_2CN), 2.68 (t, *J* = 6.1 Hz, 2H, CH_2CH_2CN*); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 162.5, 162.5 (C=O uracil, C=O uracil*), 159.7, 159.7, 159.7, 159.3 ($C_{q\text{-}arom}$, $C_{q\text{-}arom}$*), 150.7, 150.7 (C=O uracil, C=O uracil*), 137.6 (C-6), 137.3 (C-6*), 131.0, 131.0, 130.5, 130.5, 129.7, 129.7 (CH_{arom}, CH_{arom}*), 129.2, 129.1, 129.1, 129.0 ($C_{q\text{-}arom}$, $C_{q\text{-}arom}$*), 114.1, 114.0, 113.8 (CH_{arom}, CH_{arom}*), 102.2 (C-5), 102.2 (C-5*), 90.8 (C-1'*) 90.5 (C-1'), 80.1 (d, $^{3}J_{C,P}$ = 6.6 Hz, C-4'), 80.0 (d, $^{3}J_{C,P}$ = 6.4 Hz, C-4'*), 77.5 (C-2'), 77.4 (C-2'*), 74.2 (C-3'), 74.2 (C-3'*), 72.1, 72.1, 71.6, 71.5 (CH_2 PMB, CH_2 PMB*), 64.4 (d, $^{2}J_{C,P}$ = 6.0 Hz, C-5'), 64.3 (d, $^{2}J_{C,P}$ = 5.9 Hz, C-5'*), 60.3 (d, $^{3}J_{C,P}$ = 5.3 Hz, CH_2CH_2CN), 60.2 (d, $^{3}J_{C,P}$ = 5.5 Hz, CH_2CH_2CN), 55.5, 55.4, 55.4 (OMe, OMe*), 43.7, 43.7 (NCH_2 PMB, NCH_2 PMB*), 20.1 (d, $^{2}J_{C,P}$ = 6.2 Hz, CH_2CH_2CN), 20.1 (d, $^{2}J_{C,P}$

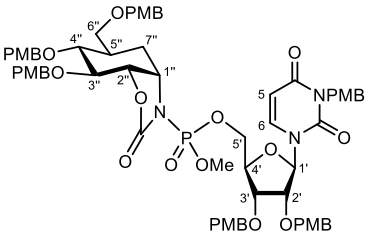
= 6.5 Hz, *C*H_2CH_2CN); ^{31}P NMR (162 MHz, $CDCl_3$, ^1H coupled): δ 9.31 ($^1J_{P,H}$ = 721.8 Hz; $^3J_{P,H}$ = 8.8 Hz), 8.69 ($^1J_{P,H}$ = 722.6 Hz; $^3J_{P,H}$ = 8.5 Hz); HRMS (ESI) m/z: [M+Na^+] calcd for $C_{36}H_{40}N_3O_{11}PNa$ 744.2298, found 744.2293.

3-*N*-(4-Methoxybenzyl)-2',3'-di-*O*-(4-methoxybenzyl)uridine methyl H-phosphonate (17).



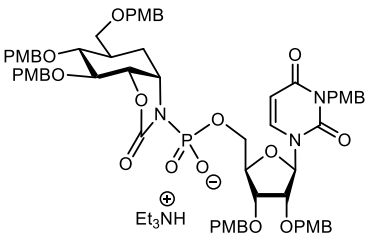
Compound **17** was prepared according to general procedure A, using **13** (4.6 gr, 7.6 mmol), *N,N*-diisopropylmethylphosphonamidic chloride **15** (95%, 1.9 mL, 9.3 mmol) and dry DiPEA (3.3 mL, 18.9 mmol, 2.5 eq.) in anhydrous DCM (40 mL, 0.2 M) in step 1; and ETT activator (0.25 M in MeCN; 92 mL, 23 mmol, 3.0 eq.), demineralized H_2O (4.1 mL, 230 mmol, 30 eq.) in MeCN (35 mL, 0.06 M) in step 2. Purification of the crude product by flash column chromatography (SiO_2; 60:40 EtOAc:pentane → 90:10 EtOAc:pentane) obtained title compound **17** as a colorless oil (4.8 gr, 7.0 mmol, 92% over two steps). R_f 0.35 (EtOAc); The NMR data showed the presence of two P(V) diastereomers in a 1:1 ratio. Data for diastereomeric mixture: ^1H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.65 (d, $^1J_{H,P}$ = 706.6 Hz, 1H, PH), 7.55 – 7.44 (m, 4H, CH_{arom}, CH_{arom}*), 7.41 (d, *J* = 8.2 Hz, 1H, H-6), 7.41 (d, *J* = 8.2 Hz, 1H, H-6*), 7.26 – 7.23 (m, 4H, CH_{arom}, CH_{arom}*), 7.20 – 7.12 (m, 4H, CH_{arom}, CH_{arom}*), 6.90 – 6.80 (m, 8H, CH_{arom}, CH_{arom}*), 6.80 – 6.74 (m, 4H, CH_{arom}, CH_{arom}*), 5.96 (d, *J* = 2.0 Hz, 1H, H-1'), 5.93 (d, *J* = 2.0 Hz, 1H, H-1'*), 5.83 (d, $^1J_{H,P}$ = 708.2 Hz, 1H, PH*), 5.68 (d, *J* = 8.2 Hz, 1H, H-5), 5.68 (d, *J* = 8.1 Hz, 1H, H-5*), 5.12 – 4.99 (m, 4H, NC*H* PMB, NCH*H* PMB, NC*H*H PMB*, NCH*H* PMB*), 4.78 – 4.67 (m, 4H, C*H*H PMB, C*H*H PMB*, CH*H* PMB, CH*H* PMB*), 4.42 – 4.28 (m, 6H, H-4', H-4'*, H-5', H-5'*, C*H*H PMB, C*H*H PMB*), 4.26 – 4.16 (m, 4H, H-5', H-5'*, CH*H* PMB, CH*H* PMB*), 3.94 – 3.89 (m, 2H, H-2', H-2'*), 3.84 (dd, *J* = 7.9, 5.1 Hz, 1H, H-3'), 3.79 – 3.78 (m, 13H, H-3'*, OMe, OMe, OMe*, OMe*), 3.78 (s, 3H, OMe), 3.78 (s, 3H, OMe*), 3.74 (d, $^3J_{H,P}$ = 12.0 Hz, 3H, P(O)OMe), 3.72 (d, $^3J_{H,P}$ = 11.9 Hz, 3H, P(O)OMe*); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 162.5, 162.5 (C=O uracil, C=O uracil*), 159.7, 159.7, 159.6, 159.3 ($C_{q\text{-}arom}$, $C_{q\text{-}arom}$*), 150.7, 150.7 (C=O uracil, C=O uracil*), 137.4 (C-6), 137.2 (C-6*), 131.0, 131.0, 130.5, 129.6, 129.6 (CH_{arom}, CH_{arom}*), 129.2, 129.1, 129.1, 129.1 ($C_{q\text{-}arom}$, $C_{q\text{-}arom}$*), 114.0, 113.9, 113.8 (CH_{arom}, CH_{arom}*), 102.0, 102.0 (C-5, C-5*), 90.2 (C-1'*), 90.0 (C-1'), 80.2 (d, $^3J_{C,P}$ = 6.2 Hz, C-4'), 80.2 (d, $^3J_{C,P}$ = 6.6 Hz, C-4'*), 77.5, 77.5 (C-2', C-2'*), 74.1 (C-3'), 74.0 (C-3'*), 72.2, 72.1, 71.4, 71.3 (CH_2 PMB, CH_2 PMB*), 63.8 (d, $^2J_{C,P}$ = 6.2 Hz, C-5'), 63.6 (d, $^2J_{C,P}$ = 6.2 Hz, C-5'*), 55.4, 55.4, 55.4 (OMe PMB, OMe PMB*), 52.4, 52.4 (P(O)OMe, P(O)OMe*), 43.6, 43.6 (NCH_2 PMB, NCH_2 PMB*); ^{31}P NMR (162 MHz, $CDCl_3$, ^1H coupled): δ 10.56 ($^1J_{P,H}$ = 707.1 Hz), 10.06 ($^1J_{P,H}$ = 705.6 Hz); HRMS (ESI) m/z: [M+Na^+] calcd for $C_{34}H_{39}N_2O_{11}PNa$ 705.2189, found 705.2184.

1'',2''-(*N*-(3-*N*-(4-Methoxybenzyl)-5'-*O*-methylphosphinyl-2',3'-di-*O*-(4-methoxybenzyl)uridinyl),*O*)-carbamate-3'',4'',6''-tri-*O*-(4-methoxybenzyl)-carba-α-D-glucopyranoside (19).



Compound **19** was prepared according to general procedure D using cyclic carbamate **9** (294 mg, 0.52 mmol), 15-crown-5 ether (0.52 mL, 2.6 mmol, 5.0 eq.) and NaH (60 wt% dispersion in mineral oil; 31.2 mg, 0.78 mmol, 1.5 eq.) in anhydrous THF (2.6 mL, 0.2 M); and H-phosphonate **17** (705 mg, 1.03 mmol, 2.0 eq.), dry DiPEA (0.54 mL, 3.1 mmol, 6.0 eq.) and $BrCCl_3$ (0.2 mL, 2.0 mmol, 3.9 eq.) in anhydrous DCM (3.4 mL, 0.3 M). Purification of the crude product by flash column chromatography (SiO_2; 50:50 EtOAc:pentane → 90:10 EtOAc:pentane) yielded title compound **19** as a yellow oil and a mixture of P(V) diastereomers (465 mg, 0.37 mmol, 72%). Data for first P(V) diastereomer: R_f 0.55 and 0.7 (EtOAc:pentane, 9:1 v:v); ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.50 (d, *J* = 8.1 Hz, 1H, H-6), 7.51 – 7.48 (m, 2H, CH_{arom}), 7.24 – 7.10 (m, 10H, CH_{arom}), 6.88 – 6.77 (m, 10H, CH_{arom}), 6.73 – 6.68 (m, 2H, CH_{arom}), 6.05 (d, *J* = 2.8 Hz, 1H, H-1'), 5.68 (d, *J* = 8.1 Hz, 1H, H-5), 5.09 – 5.01 (m, 2H, NC*H*H PMB, NCH*H* PMB), 4.68 – 4.65 (m, 3H, H-2'', C*H*H PMB, CH*H* PMB), 4.62 – 4.52 (m, 3H, C*H*H PMB, CH*H* PMB, C*H*H PMB), 4.49 – 4.41 (m, 1H, H-1''), 4.40 (ddd, *J* = 11.6, 4.4, 2.2 Hz, 1H, H-5'), 4.34 – 4.19 (m, 6H, H-5', CH*H* PMB, C*H*H PMB, CH*H* PMB, C*H*H PMB, CH*H* PMB), 4.20 – 4.13 (m, 1H, H-4'), 3.95 (dd, *J* = 7.1, 5.0 Hz, 1H, H-3'), 3.86 (dd, *J* = 5.1, 2.8 Hz, 1H, H-2'), 3.82 – 3.73 (m, 22H, H-3'', OMe, OMe, OMe, OMe, OMe, OMe, P(O)OMe), 3.45 (dd, *J* = 7.7, 6.2 Hz, 1H, H-4''), 3.33 (dd, *J* = 8.9, 3.5 Hz, 1H, H-6''), 3.29 (dd, *J* = 8.9, 4.8 Hz, 1H, H-6''), 2.13 (ddd, *J* = 13.6, 5.0, 5.0 Hz, 1H, H-7''), 1.97 – 1.85 (m, 2H, H-5, H-7''); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 162.6 (C=O uracil), 159.6, 159.6, 159.4, 159.4, 159.3, 159.2 ($C_{q\text{-}arom}$), 155.0 (d, $J_{C,P}$ = 8.5 Hz, C=O carbamate), 150.9 (C=O uracil), 137.5 (C-6), 131.0, 130.4 (CH_{arom}), 130.3, 130.3 ($C_{q\text{-}arom}$), 129.8 (CH_{arom}), 129.8, 129.7 ($C_{q\text{-}arom}$), 129.6, 129.5, 129.4 (CH_{arom}), 129.3, 129.3 ($C_{q\text{-}arom}$), 114.0, 113.9, 113.9, 113.9, 113.8, 113.8 (CH_{arom}), 102.0 (C-5), 88.9 (C-1'), 80.3 (d, $^3J_{C,P}$ = 9.1 Hz, C-4'), 78.7 (C-3''), 78.5 (d, $^3J_{C,P}$ = 9.2 Hz, C-2''), 78.1 (C-2'), 76.1 (C-4''), 74.6 (C-3'), 73.0, 73.0, 73.0, 72.2, 71.3 (CH_2 PMB), 71.0 (C-6''), 65.7 (d, $^2J_{C,P}$ = 5.3 Hz, C-5'), 55.4 (d, $^2J_{C,P}$ = 4.5 Hz, C-1''), 55.4 (OMe), 54.7 (d, $^2J_{C,P}$ = 5.7 Hz, P(O)OMe), 43.6 (NCH_2 PMB), 36.9 (C-5''), 28.0 (C-7''); ^{31}P NMR (202 MHz, $CDCl_3$): δ –1.60; HRMS (ESI) m/z: [M+Na^+] calcd for $C_{66}H_{74}N_3O_{19}PNa$ 1266.4552, found 1266.4546.

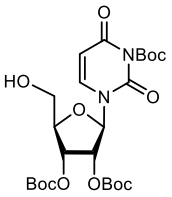
1'',2''-(*N*-(3-*N*-(4-Methoxybenzyl)-5'-*O*-phosphoryl-2',3'-di-*O*-(4-methoxybenzyl)uridinyl),*O*)-carbamate-3'',4'',6''-tri-*O*-(4-methoxybenzyl)-carba-α-D-glucopyranoside (20).



Compound **19** (79 mg, 64 μmol) was dissolved in a mixture of PhSH:Et_3N:MeCN (1:2:2 v:v, 0.6 mL, 0.1 M) and the reaction mixture was stirred at 35 °C according to a literature procedure.[62] After 1 h, ^{31}P NMR analysis (acetone-d_6 probe) indicated full demethylation of the starting material. The mixture was diluted with water, extracted with EtOAc (3x), after which the combined organic layers were washed with brine, dried over

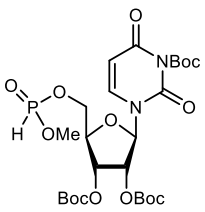
Na_2SO_4, filtered, and concentrated *in vacuo*. Flash column chromatography of (SiO_2, 0→10% MeOH/DCM) yielded title compound **20** as a colorless oil (61 mg, 49 μmol, 77%). Unfortunately, due to the amphiphilic nature of the demethylated compound, no interpretable ^1H or ^{13}C NMR spectra could be obtained. R_f 0.6 (MeOH:DCM, 1:9 v:v); ^{31}P NMR (121 MHz, acetone-d_6 probe): δ –7.68; HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{65}H_{72}N_3O_{19}PNa$ 1252.4395, found 1252.4390.

3-*N*-(*Tert*-butoxycarbonyl)-2',3'-di-*O*-(*tert*-butoxycarbonyl)uridine (22).



To a stirred solution of uridine (4.9 g, 20 mmol) in pyridine (25 mL, 0.8 M) were added DMAP (0.25 g, 2.0 mmol, 0.1 eq.) and TBDPSCl (6.3 mL, 24 mmol, 1.2 eq.). The reaction mixture was stirred overnight under N_2 atmosphere according to a literature procedure.[69] Upon full conversion was observed (R_f 0.65 (MeOH:DCM, 1:9 v:v)), the reaction was quenched by the addition of MeOH, diluted with sat. aq. $NaHCO_3$ and brine and extracted with EtOAc (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product (colorless oil) was co-evaporated twice with toluene and dissolved in DCM (100 mL, 0.2 M). Et_3N (17 mL, 120 mmol, 6.0 eq.) and DMAP (0.74 g, 6.0 mmol, 0.3 eq.) were added and the mixture was cooled on ice. Boc_2O (26.5 g, 120 mmol, 6.0 eq.) was added and the ice bath was removed after 5 minutes, after which the resulting reaction mixture was stirred at room temperature for 4 h. Upon full conversion was observed (R_f 0.8 (EtOAc:pentane, 1:1 v:v)), the reaction was concentrated to dryness. The crude product (yellow oily liquid) was dissolved in THF (75 mL), TBAF (1.0 M in THF; 80 mL, 80 mmol, 4.0 eq.) was added and the solution was stirred overnight. Upon full conversion (R_f 0.15 (EtOAc:pentane, 3:7 v:v), the reaction mixture was diluted with sat. aq. $NaHCO_3$ and brine and extracted with EtOAc (3x), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash column chromatography of the crude product (SiO_2, 30:70 EtOAc:pentane → 60:40 EtOAc:pentane) yielded title compound **22** as a white brittle foam (10.1 g, 18.6 mmol, 92% over three steps). ^1H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.60 (d, *J* = 8.2 Hz, 1H, H-6), 5.91 (d, *J* = 5.7 Hz, 1H, H-1'), 5.78 (d, *J* = 8.2 Hz, 1H, H-5), 5.41 (dd, *J* = 5.5, 5.5 Hz, 1H, H-2'), 5.34 (dd, *J* = 5.4, 3.9 Hz, 1H, H-3'), 4.28 (ddd, *J* = 4.1, 2.2, 2.2 Hz, 1H, H-4'), 3.97 (dd, *J* = 12.2, 2.1 Hz, 1H, H-5'), 3.84 (d, *J* = 12.3 Hz, 1H, H-5'), 2.52 (bs, 1H, OH-5'), 1.60 (s, 9H, $C(CH_3)_3$), 1.49 (s, 9H, $C(CH_3)_3$), 1.48 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 160.3 (C=O uracil), 152.5 (C=O carbamate), 152.1 (C=O uracil), 148.6, 147.5 (C=O carbonate), 140.6 (C-6), 102.9 (C-5), 89.5 (C-1'), 87.2, 83.8, 83.6 ($C(CH_3)_3$), 82.9 (C-4'), 75.0 (C-2'), 72.9 (C-3'), 61.9 (C-5'), 27.8, 27.8, 27.6 ($C(CH_3)_3$). HRMS (ESI) m/z: [M+Na]$^+$ Calcd. for $C_{24}H_{36}N_2NaO_{12}$ 567.2160; Found 567.2156.

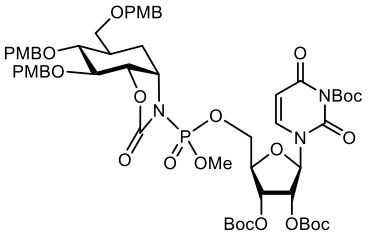
3-*N*-(*Tert*-butoxycarbonyl)-2',3'-di-*O*-(*tert*-butoxycarbonyl)uridine methyl H-phosphonate (23).



Compound **23** was prepared according to general procedure A using **22** (7.1 g, 13 mmol), *N,N*-diisopropylmethylphosphonamidic chloride **15** (95%, 3.2 mL, 15.7 mmol, 1.2 eq.) and dry DiPEA (5.6 mL, 32 mmol, 2.5 eq.) in anhydrous DCM (65 mL, 0.2 M) in step 1; and ETT activator (0.25 M in MeCN; 78 mL, 19.5 mmol, 1.5 eq.), demineralized H_2O (7.0 mL, 390 mol, 30 eq.) in MeCN (52 mL, 0.1 M) in step 2. Flash column chromatography of the crude product (SiO_2, 50:50 EtOAc:pentane →

100:0 EtOAc:pentane) yielded title compound **23** as a white brittle foam (6.6 g, 10.6 mmol, 82% over two steps). R_f 0.35 (EtOAc:pentane, 8:2 v:v); The NMR data showed the presence of two P(V) diastereomers in a 1:1 ratio. Data for diastereomeric mixture: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.60 (d, $^1J_{H,P}$ = 710.6 Hz, 1H, PH), 7.47 (d, *J* = 5.9 Hz, 1H, H-6), 7.45 (d, *J* = 5.9 Hz, 1H, H-6*), 6.90 (d, *J* = 713.2 Hz, 1H, PH), 6.89 (d, *J* = 709.5 Hz, 1H, PH*), 5.99 (s, 1H, H-1'), 5.93 (d, *J* = 4.5 Hz, 1H, H-1'*), 5.82 (d, *J* = 8.1 Hz, 1H, H-5), 5.79 (d, *J* = 8.2 Hz, 1H, H-5*), 5.37 – 5.14 (m, 4H, H-2', H-2'*, H-3', H-3'*), 4.49 – 4.17 (m, 6H, H-4', H-4'*, H-5', H-5'*), 3.84 (d, *J* = 12.0 Hz, 3H, P(O)OMe), 3.81 (d, *J* = 12.0 Hz, 3H, P(O)OMe*), 1.60 (s, 18H, $C(CH_3)_3$, $C(CH_3)_3$*), 1.50 (s, 18H, $C(CH_3)_3$, $C(CH_3)_3$*), 1.48 (s, 18H, $C(CH_3)_3$, $C(CH_3)_3$*); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 160.2, 160.2 (C=O uracil, C=O uracil*), 152.2 (C=O carbamate/ C=O carbamate*), 152.2 (C=O uracil/ C=O uracil*), 152.1 (C=O carbamate/C=O carbamate*), 152.1 (C=O uracil/C=O uracil*), 148.4, 148.4 (C=O carbonate/C=O carbonate*), 147.4, 147.4 (C=O carbonate/C=O carbonate*), 139.8, 139.4 (C-6, C-6*), 103.0 (C-5, C-5*), 89.0, 88.4 (C-1', C-1'*), 87.1 ($C(CH_3)_3$, $C(CH_3)_3$*), 84.0, 84.0, 83.9, 83.9 ($C(CH_3)_3$, $C(CH_3)_3$, $C(CH_3)_3$*, $C(CH_3)_3$*), 80.0 (d, *J* = 1.6 Hz), 80.0 (d, *J* = 5.8 Hz) (C-4', C-4'*), 74.8, 74.8 (C-2', C-2'*), 71.7, 71.5 (C-3', C-3'*), 64.1 (d, *J* = 5.3 Hz), 63.9 (d, *J* = 5.6 Hz) (C-5', C-5'*), 52.7 (d, *J* = 5,7 Hz), 52.6 (d, *J* = 5.8 Hz) (P(O)OMe, P(O)OMe*), 27.7 ($C(CH_3)_3$, ($C(CH_3)_3$*), 27.7 ($C(CH_3)_3$, ($C(CH_3)_3$*), 27.6 ($C(CH_3)_3$, ($C(CH_3)_3$*); ^{31}P NMR (202 MHz, $CDCl_3$, ^{1}H coupled): δ 10.63 ($^1J_{P,H}$ = 709.4 Hz), 10.02 ($^1J_{P,H}$ = 713.4 Hz). HRMS (ESI) m/z: $[M+Na]^+$ Calcd. for $C_{25}H_{39}N_2NaO_{14}P$ 645.2031; Found 645.2023.

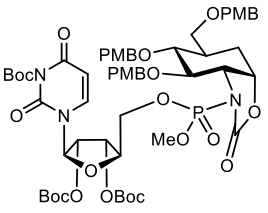
1'',2''-(*N*-(3-*N*-(*Tert*-butoxycarbonyl)-5'-*O*-(methylphosphinyl)-2',3'-di-*O*-(*tert*-butoxycarbonyl)uridinyl),*O*)-carbamate-3'',4'',6''-tri-*O*-(4-methoxybenzyl)-carba-*α*-D-glucopyranoside (24).



Compound **24** was prepared according to general procedure D using cyclic carbamate **9** (0.39 g, 0.69 mmol), 15-crown-5 ether (0.68 mL, 3.4 mmol, 5.0 eq.) and NaH (60 wt% dispersion in mineral oil; 41 mg, 1.0 mmol, 1.5 eq.) in anhydrous THF (3.4 mL, 0.2 M); and H-phosphonate **23** (0.86 g, 1.4 mmol, 2.0 eq.), dry DiPEA (0.72 mL, 4.1 mmol, 6.0 eq.) and $BrCCl_3$ (0.27 mL, 2.7 mmol, 3.9 eq.) in anhydrous DCM (4.6 mL, 0.3 M). Purification of the crude material by flash column chromatography (SiO_2; 30:70 EtOAc:pentane → 80:20 EtOAc:pentane) and Sephadex™ LH-20 size exclusion chromatography yielded the title compound **24** as a yellow oil and a mixture of P(V) diastereomers (0.56 g, 0.47 mmol, 68%).
Data for first P(V) diastereomer: R_f 0.75 (EtOAc:pentane, 8:2 v:v); ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.73 (d, *J* = 8.3 Hz, 1H, H-6), 7.25 – 7.13 (m, 6H, CH_{arom}), 6.90 – 6.81 (m, 6H, CH_{arom}), 6.16 (d, *J* = 5.8 Hz, 1H, H-1'), 5.87 (d, *J* = 8.2 Hz, 1H, H-5), 5.29 (dd, *J* = 5.5, 4.4 Hz, 1H, H-3'), 5.15 (dd, *J* = 5.6, 5.6 Hz, 1H, H-2'), 4.69 (dd, *J* = 8.3, 5.3 Hz, 1H, H-2''), 4.65 – 4.56 (m, 3H, C*H*H PMB, CH*H* PMB, C*H*H PMB), 4.52 – 4.42 (m, 2H, H-1'', H-5'), 4.42 – 4.26 (m, 5H, H-4', H-5', CH*H* PMB, C*H*H PMB, CH*H* PMB), 3.83 (dd, *J* = 5.7, 5.7 Hz, 1H, H-3''), 3.81 – 3.76 (m, 12H, OMe, OMe, OMe, P(O)OMe), 3.48 (dd, *J* = 8.0, 6.1 Hz, 1H, H-4''), 3.38 (qd, *J* = 8.9, 4.4 Hz, 2H, H-6''), 2.23 (ddd, *J* = 14.9, 5.4, 5.4 Hz, 1H, H-7''), 1.98 (dddd, *J* = 12.9, 8.7, 4.8, 4.8 Hz, 1H, H-5''), 1.90 (ddd, *J* = 13.9, 9.2, 4.4 Hz, 1H, H-7''), 1.59 (s, 9H, $C(CH_3)_3$), 1.49 (s, 9H, $C(CH_3)_3$), 1.47 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (126

MHz, $CDCl_3$, HSQC): δ 160.4 (C=O uracil), 159.6, 159.5, 159.3 ($C_{q\text{-}arom}$), 155.2 (d, $^2J_{C,P}$ = 8.3 Hz, C=O cyclic carbamate), 152.2 (C=O carbamate), 152.0 (C=O uracil), 148.8, 147.7 (C=O carbonate), 139.5 (C-6), 130.4, 130.4 ($C_{q\text{-}arom}$), 129.8 (CH_{arom}), 129.8 ($C_{q\text{-}arom}$), 129.7, 129.5, 114.1, 113.9, 113.9 (CH_{arom}), 103.3 (C-5), 86.9 ($C(CH_3)_3$), 86.3 (C-1'), 83.8, 83.7 ($C(CH_3)_3$), 80.0 (d, $^3J_{C,P}$ = 7.8 Hz, C-4'), 78.8 (C-3''), 78.5 (d, $^3J_{C,P}$ = 9.4 Hz, C-2''), 76.5 (C-4''), 74.7 (C-2'), 73.0, 73.0 (CH_2 PMB), 71.8 (C-3'), 70.9 (C-6''), 67.0 (d, $^2J_{C,P}$ = 5.9 Hz, C-5'), 55.5 (d, $^2J_{C,P}$ = 4.9 Hz, C-1''), 55.4, 55.4, 55.4 (OMe), 54.8 (d, $^2J_{C,P}$ = 5.8 Hz, P(O)OMe), 36.7 (C-5''), 27.8 (C-7''), 27.8, 27.8, 27.6 (C(CH_3)$_3$); ^{31}P NMR (202 MHz, $CDCl_3$): δ −2.15; HRMS (ESI) m/z: [M+Na^+] calcd for $C_{57}H_{74}N_3O_{22}PNa$ 1206.4399, found 1206.4394.
Data for second P(V) diastereomer: R_f 0.65 (EtOAc:pentane, 8:2 v:v); ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.65 (d, *J* = 8.3 Hz, 1H, H-6), 7.25 – 7.12 (m, 6H, CH_{arom}), 6.90 – 6.78 (m, 6H, CH_{arom}), 6.12 (d, *J* = 5.9 Hz, 1H, H-1'), 5.82 (d, *J* = 8.2 Hz, 1H, H-5), 5.25 (dd, *J* = 5.6, 4.2 Hz, 1H, H-3'), 5.14 (dd, *J* = 5.7, 5.7 Hz, 1H, H-2'), 4.71 – 4.63 (m, 1H, H-2''), 4.63 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 4.59 (d, *J* = 11.0 Hz, 1H, CH*H* PMB), 4.48 (ddd, *J* = 7.6, 7.6, 4.3 Hz, 1H, H-1''), 4.40 – 4.30 (m, 5H, H-5', C*H*H PMB, CH*H* PMB, C*H*H PMB), 4.25 (d, *J* = 11.4 Hz, 1H, CH*H* PMB), 4.20 (dddd, *J* = 4.3, 4.3, 2.4, 2.4 Hz, 1H, H-4'), 3.89 – 3.82 (m, 4H, H-3'', P(O)OMe), 3.80 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.49 (dd, *J* = 7.4, 7.4 Hz, 1H, H-4''), 3.42 – 3.31 (m, 2H, H-6''), 2.26 – 2.15 (m, 1H, H-7''), 1.99 (dddd, *J* = 17.6, 7.2, 3.9, 3.9 Hz, 1H, H-5''), 1.94 – 1.90 (m, 1H, H-7''), 1.58 (s, 9H, C(CH_3)$_3$), 1.47 (s, 9H, C(CH_3)$_3$), 1.44 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 160.3 (C=O uracil), 159.5, 159.4, 159.3 ($C_{q\text{-}arom}$), 155.0 (d, $^2J_{C,P}$ = 8.7 Hz, C=O cyclic carbamate), 152.1 (C=O carbamate), 151.9 (C=O uracil), 148.7, 147.6 (C=O carbonate), 139.4 (C-6), 130.5, 130.4, 129.9 ($C_{q\text{-}arom}$), 129.8, 129.6, 129.5, 114.0, 113.9, 113.8 (CH_{arom}), 103.1 (C-5), 86.9 ($C(CH_3)_3$), 86.5 (C-1'), 83.7, 83.6 ($C(CH_3)_3$), 80.0 (d, $^3J_{C,P}$ = 8.7 Hz, C-4'), 79.6 (C-3''), 79.2 (d, $^3J_{C,P}$ = 9.3 Hz, C-2''), 76.0 (C-4''), 74.7 (C-2'), 73.2, 72.9 (CH_2 PMB), 72.0 (C-3'), 71.0 (C-6''), 66.4 (d, $^2J_{C,P}$ = 5.5 Hz, C-5'), 55.6 (d, $^2J_{C,P}$ = 4.9 Hz, C-1''), 55.4, 55.4, 55.4 (OMe), 55.0 (d, $^2J_{C,P}$ = 6.0 Hz, P(O)OMe), 37.1 (C-5''), 28.0 (C-7''), 27.7, 27.7, 27.5 (C(CH_3)$_3$); ^{31}P NMR (202 MHz, $CDCl_3$): δ −1.47; HRMS (ESI) m/z: [M+Na^+] calcd for $C_{57}H_{74}N_3O_{22}PNa$ 1206.4399, found 1206.4394.

1'',2''-(*O*,*N*-(3-*N*-(*Tert*-butoxycarbonyl)-5'-*O*-(methylphosphinyl)-2',3'-di-*O*-(*tert*-butoxycarbonyl)uridinyl))-carbamate-3'',4'',6''-tri-*O*-(4-methoxybenzyl)-carba-α-D-glucopyranoside (25).

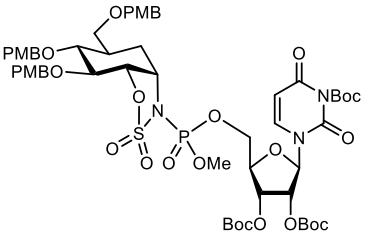


Compound **25** was prepared according to general procedure D using cyclic carbamate **11** (0.29 g, 0.51 mmol), 15-crown-5 ether (0.5 mL, 2.5 mmol, 5.0 eq.) and NaH (60 wt% dispersion in mineral oil; 31 mg, 0.76 mmol, 1.5 eq.) in anhydrous THF (2.5 mL, 0.2 M); and H-phosphonate **23** (0.67 g, 1.1 mmol, 2.0 eq.), dry DiPEA (0.57 mL, 3.3 mmol, 6.0 eq.) and $BrCCl_3$ (0.21 mL, 2.1 mmol, 4.0 eq.) in anhydrous DCM (3.6 mL, 0.3 M). Flash column chromatography of the crude product (SiO_2; 40:60 EtOAc:pentane → 70:30 EtOAc:pentane) and Sephadex™ LH-20 size exclusion chromatography yielded the title compound **25** as a pale-yellow oil and as a mixture of P(V) diastereomers (0.59 g, 0.50 mmol, 97%).
Data for first P(V) diastereomer: R_f 0.6 (EtOAc:pentane, 7:3 v:v); ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.56 (d, *J* = 8.3 Hz, 1H, H-6), 7.26 – 7.19 (m, 4H, CH_{arom}), 7.12 – 7.07 (m, 2H, CH_{arom}), 6.87 – 6.78 (m, 6H, CH_{arom}), 6.16 (d, *J* = 6.4 Hz, 1H, H-1'), 5.82 (d, *J* = 8.0 Hz, 1H, H-5), 5.35 (dd, *J* =

5.4, 3.4 Hz, 1H, H-3'), 5.06 (dd, *J* = 6.4, 5.3 Hz, 1H, H-2'), 4.93 (ddd, *J* = 7.8, 2.8, 2.8 Hz, 1H, H-1''), 4.67 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 4.60 (d, *J* = 10.9 Hz, 1H, CH*H* PMB), 4.48 – 4.37 (m, 5H, H-2'', C*H*H PMB, CH*H* PMB, C*H*H PMB, CH*H* PMB), 4.23 (ddd, *J* = 11.4, 4.4, 1.8 Hz, 1H, H-5'), 4.18 (dddd, *J* = 3.6, 3.6, 2.0, 2.0 Hz, 1H, H-4'), 4.15 (ddd, *J* = 11.3, 5.3, 2.4 Hz, 1H, H-5'), 3.95 (dd, *J* = 4.5, 4.5 Hz, 1H, H-3''), 3.79 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.67 – 3.61 (m, 4H, H-4'', P(O)OMe), 3.48 (dd, *J* = 9.1, 5.0 Hz, 1H, H-6''), 3.37 (dd, *J* = 9.1, 5.1 Hz, 1H, H-6''), 2.18 – 2.09 (m, 2H, H-5'', H-7''), 1.87 (ddd, *J* = 16.4, 13.7, 3.1 Hz, 1H, H-7''), 1.59 (s, 9H, $C(CH_3)_3$), 1.50 (s, 9H, $C(CH_3)_3$), 1.44 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 160.2 (C=O uracil), 159.4, 159.3, 159.3 ($C_{q\text{-}arom}$), 155.7 (d, $^2J_{C,P}$ = 8.8 Hz, C=O cyclic carbamate), 152.3 (C=O carbamate), 152.2 (C=O uracil), 148.7, 147.7 (C=O carbonate), 139.2 (C-6), 130.4, 130.3, 130.1 ($C_{q\text{-}arom}$), 129.7, 129.5, 129.5, 113.9, 113.8 (CH_{arom}), 103.4 (C-5), 87.0 ($C(CH_3)_3$), 85.7 (C-1'), 83.8, 83.6 ($C(CH_3)_3$), 80.2 (d, $^3J_{C,P}$ = 8.9 Hz, C-4'), 78.1 (C-3''), 77.1 (C-4''), 75.7 (d, $^3J_{C,P}$ = 8.7 Hz, C-1''), 74.8 (C-2'), 72.8, 72.7, 72.2 (CH_2 PMB), 72.2 (C-3'), 71.1 (C-6''), 66.3 (d, $^2J_{C,P}$ = 4.9 Hz, C-5'), 58.5 (d, $^2J_{C,P}$ = 3.9 Hz, C-2''), 55.5 (d, $^2J_{C,P}$ = 5.7 Hz, P(O)OMe), 55.4, 55.4, 55.4 (OMe), 34.8 (C-5''), 27.8, 27.7, 27.5 ($C(CH_3)_3$), 26.4 (C-7''); ^{31}P NMR (202 MHz, $CDCl_3$): δ −0.55; HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{57}H_{74}N_3O_{22}PNa$ 1206.4399, found 1206.4394.

Data for second P(V) diastereomer: R_f 0.5 (EtOAc:pentane, 7:3 v:v); ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.73 (d, *J* = 8.3 Hz, 1H, H-6), 7.23 – 7.19 (m, 4H, CH_{arom}), 7.10 – 7.05 (m, 2H, CH_{arom}), 6.87 – 6.83 (m, 4H, CH_{arom}), 6.79 – 6.75 (m, 2H, CH_{arom}), 6.09 (d, *J* = 6.3 Hz, 1H, H-1'), 5.86 (d, *J* = 8.1 Hz, 1H, H-5), 5.17 (dd, *J* = 5.2, 3.4 Hz, 1H, H-3'), 5.04 (dd, *J* = 6.3, 5.2 Hz, 1H, H-2'), 4.92 (ddd, *J* = 8.3, 2.8, 2.8 Hz, 1H, H-1''), 4.60 – 4.51 (m, 2H, C*H*H PMB, CH*H* PMB), 4.43 – 4.29 (m, 5H, H-2'', C*H*H PMB, CH*H* PMB, C*H*H PMB, CH*H* PMB), 4.09 (ddd, *J* = 11.8, 3.7, 1.9 Hz, 1H, H-5'), 4.05 (dd, *J* = 3.3, 3.3 Hz, 1H, H-3''), 3.95 (ddd, *J* = 11.8, 5.7, 2.4 Hz, 1H, H-5'), 3.89 – 3.84 (m, 4H, H-4', P(O)OMe), 3.80 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.76 (s, 3H, OMe), 3.64 (ddd, *J* = 6.1, 4.5, 2.3 Hz, 1H, H-4''), 3.45 (dd, *J* = 9.1, 5.1 Hz, 1H, H-6''), 3.36 (dd, *J* = 9.1, 5.7 Hz, 1H, H-6''), 2.19 – 2.06 (m, 2H, H-5'', H-7''), 1.84 (ddd, *J* = 15.3, 12.6, 2.9 Hz, 1H, H-7''), 1.58 (s, 9H, $C(CH_3)_3$), 1.50 (s, 9H, $C(CH_3)_3$), 1.44 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 160.5 (C=O uracil), 159.4, 159.3 ($C_{q\text{-}arom}$), 155.4 (d, $^2J_{C,P}$ = 8.2 Hz, C=O cyclic carbamate), 152.2 (C=O carbamate), 152.0 (C=O uracil), 148.8, 147.8 (C=O carbonate), 139.5 (C-6), 130.4, 130.1, 129.9 ($C_{q\text{-}arom}$), 129.8, 129.6, 129.4, 113.9, 113.9, 113.9 (CH_{arom}), 103.3 (C-5), 86.8 ($C(CH_3)_3$), 85.6 (C-1'), 83.6, 83.5 ($C(CH_3)_3$), 80.0 (d, $^3J_{C,P}$ = 9.3 Hz, C-4'), 76.5 (C-3''), 76.4 (C-4''), 75.0 (C-2'), 75.0 (d, $^3J_{C,P}$ = 8.7 Hz, C-1''), 72.8 (CH_2 PMB), 72.4 (C-3'), 72.2, 71.6 (CH_2 PMB), 71.5 (C-6''), 66.5 (d, $^2J_{C,P}$ = 5.1 Hz, C-5'), 57.5 (d, $^2J_{C,P}$ = 4.4 Hz, C-2''), 55.4, 55.4, 55.4 (OMe), 55.1 (d, $^2J_{C,P}$ = 5.9 Hz, P(O)OMe), 34.1 (C-5''), 27.8, 27.7, 27.6 ($C(CH_3)_3$), 26.1 (C-7''); ^{31}P NMR (202 MHz, $CDCl_3$): δ −1.45; HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{57}H_{74}N_3O_{22}PNa$ 1206.4399, found 1206.4393.

1'',2''-(*N*-(3-*N*-(*Tert*-butoxycarbonyl)-5'-*O*-(methylphosphinyl)-2',3'-di-*O*-(*tert*-butoxycarbonyl) uridinyl),*O*)-sulfamidate-3'',4'',6''-tri-*O*-(4-methoxybenzyl)-carba-*α*-D-glucopyranoside (26).



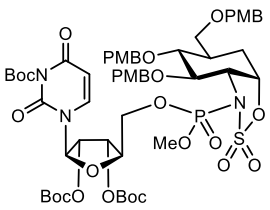
Compound **26** was prepared according to general procedure D using cyclic sulfamidate **10** (305 mg, 0.51 mmol), 15-crown-5 ether (0.50 mL, 2.52 mmol, 4.9 mmol) and NaH (60 wt% dispersion in mineral oil; 30.5 mg, 0.76 mmol, 1.5 eq.) in anhydrous THF (2.5 mL, 0.2 M); and H-phosphonate **23** (685 mg, 1.1 mmol, 2.2 eq.), dry DiPEA (0.58 mL, 3.33 mmol, 6.5 eq.) and $BrCCl_3$ (0.22 mL, 2.2 mmol, 4.3 eq.) in anhydrous DCM (3.6 mL, 0.3 M). Purification of the crude material by flash column chromatography (SiO_2, 30:70 EtOAc:pentane → 70:30 EtOAc:pentane) and Sephadex™ LH-20 size exclusion chromatography obtained title compound **26** as a yellow oil (which solidified upon standing) and as a mixture of P(V) diastereomers (330 mg, 0.27 mmol, 53%).

Data for first P(V) diastereomer: R_f 0.6 (EtOAc:pentane, 6:4 v:v); ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.58 (d, *J* = 8.3 Hz, 1H, H-6), 7.29 – 7.26 (m, 2H, CH_{arom}), 7.22 – 7.17 (m, 2H, CH_{arom}), 7.14 – 7.11 (m, 2H, CH_{arom}), 6.89 – 6.82 (m, 6H, CH_{arom}), 6.11 (d, *J* = 5.4 Hz, 1H, H-1'), 5.83 (d, *J* = 8.2 Hz, 1H, H-5), 5.26 (dd, *J* = 5.6, 4.7 Hz, 1H, H-3'), 5.16 (dd, *J* = 5.5, 5.5 Hz, 1H, H-2'), 4.78 (ddd, *J* = 7.1, 5.8, 1.1 Hz, 1H, H-2''), 4.76 – 4.67 (m, 2H, C*H*H PMB, CH*H* PMB), 4.53 – 4.43 (m, 3H, H-1'', H-5'), 4.43 – 4.35 (m, 3H, C*H*H PMB, CH*H* PMB, C*H*H PMB), 4.33 (ddd, *J* = 5.2, 2.6, 2.6 Hz, 1H, H-4'), 4.29 (d, *J* = 11.4 Hz, 1H, CH*H* PMB), 4.13 (dd, *J* = 8.6, 7.2 Hz, 1H, H-3''), 3.92 (d, $^3J_{H,P}$ = 11.8 Hz, 3H, P(O)OMe), 3.80 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.59 (dd, *J* = 9.1, 4.1 Hz, 1H, H-6''), 3.48 (dd, *J* = 9.6, 8.7 Hz, 1H, H-4''), 3.39 (dd, *J* = 9.1, 3.0 Hz, 1H, H-6''), 2.45 (ddd, *J* = 15.3, 4.7, 4.7 Hz, 1H, H-7''), 2.11 – 2.01 (m, 1H, H-5''), 1.91 (ddd, *J* = 14.4, 10.0, 4.1 Hz, 1H, H-7''), 1.59 (s, 9H, $C(CH_3)_3$), 1.49 (s, 9H, $C(CH_3)_3$), 1.47 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 160.3 (C=O uracil), 159.5, 159.4, 159.4 ($C_{q\text{-}arom}$), 152.1 (C=O carbamate), 152.0 (C=O uracil), 148.6, 147.6 (C=O carbonate), 139.3 (C-6), 130.4, 130.3, 129.9 ($C_{q\text{-}arom}$), 129.8, 129.8, 129.5, 114.0, 113.9, 113.9 (CH_{arom}), 103.2 (C-5), 87.0 ($C(CH_3)_3$), 86.9 (C-1'), 85.5 (d, $^3J_{C,P}$ = 9.4 Hz, C-2''), 83.8, 83.8 ($C(CH_3)_3$), 80.6 (C-3''), 79.9 (d, $^3J_{C,P}$ = 7.6 Hz, C-4'), 77.0 (C-4''), 74.8 (C-2'), 74.6, 74.5, 73.0 (CH_2 PMB), 71.6 (C-3'), 69.3 (C-6''), 67.2 (d, $^2J_{C,P}$ = 5.6 Hz, C-5'), 59.3 (d, $^2J_{C,P}$ = 3.3 Hz, C-1''), 55.9 (d, $^2J_{C,P}$ = 5.7 Hz, P(O)OMe), 55.4, 55.4, 55.4 (OMe), 36.9 (C-5''), 27.7, 27.7, 27.6 ($C(CH_3)_3$), 27.4 (C-7''); ^{31}P NMR (202 MHz, $CDCl_3$): δ −3.18; HRMS (ESI) m/z: [M+Na$^+$] calcd for $C_{56}H_{74}N_3O_{23}PSNa$ 1242.4069, found 1242.4064.

Data for second P(V) diastereomer: R_f 0.5 (EtOAc:pentane, 6:4 v:v); ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.47 (d, *J* = 8.3 Hz, 1H, H-6), 7.29 – 7.11 (m, 6H, CH_{arom}), 6.89 – 6.82 (m, 6H, CH_{arom}), 6.03 (d, *J* = 4.8 Hz, 1H, H-1'), 5.79 (d, *J* = 8.2 Hz, 1H, H-5), 5.26 (dd, *J* = 5.5, 5.5 Hz, 1H, H-3'), 5.21 (dd, *J* = 5.7, 4.8 Hz, 1H, H-2'), 4.79 (dd, *J* = 6.5, 6.5 Hz, 1H, H-2''), 4.73 – 4.65 (m, 2H, C*H*H PMB, CH*H* PMB), 4.51 (ddd, *J* = 7.2, 3.6, 1.6 Hz, 1H, H-1''), 4.47 (ddd, *J* = 11.5, 5.4, 2.3 Hz, 1H, H-5'), 4.41 – 4.34 (m, 4H, H-5', C*H*H PMB, CH*H* PMB, C*H*H PMB), 4.30 (dd, *J* = 5.3, 2.8 Hz, 1H, H-4'), 4.27 (d, *J* = 11.4 Hz, 1H, CH*H* PMB), 4.11 (dd, *J* = 8.5, 7.0 Hz, 1H, H-3''), 3.90 (d, $^3J_{H,P}$ = 11.9 Hz, 3H, P(O)OMe), 3.80 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.51 (dd, *J* = 8.9, 4.3 Hz, 1H, H-6''), 3.46 (dd, *J* = 9.0, 9.0 Hz, 1H, H-4''), 3.41 (dd, *J* = 9.2, 3.1 Hz, 1H, H-6''), 2.40 (ddd, *J* = 15.2, 5.1, 5.1 Hz, 1H, H-7''), 2.11 – 2.03 (m, 1H, H-5''), 1.89 (ddd, *J* = 14.5, 9.1, 3.9 Hz, 1H, H-7''), 1.59 (s, 9H, $C(CH_3)_3$),

1.48 (s, 9H, C(CH_3)$_3$), 1.45 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 160.2 (C=O uracil), 159.6, 159.4, 159.4 ($C_{q\text{-}arom}$), 152.0 (C=O carbamate), 151.9 (C=O uracil), 148.5, 147.5 (C=O carbonate), 139.5 (C-6), 130.4, 130.3, 129.9 ($C_{q\text{-}arom}$), 129.8, 129.7, 129.5, 114.0, 113.9, 113.9 (CH_{arom}), 103.0 (C-5), 87.9 (C-1'), 87.1 (*C*(CH_3)$_3$), 85.1 (d, $^3J_{C,P}$ = 9.4 Hz, C-2''), 83.8, 83.7 (*C*(CH_3)$_3$), 80.5 (C-3''), 79.5 (d, $^3J_{C,P}$ = 8.7 Hz, C-4'), 76.7 (C-4''), 74.8 (C-2'), 74.6, 74.3, 73.0 (CH_2 PMB), 71.5 (C-3'), 69.5 (C-6''), 66.5 (d, $^2J_{C,P}$ = 4.8 Hz, C-5'), 59.2 (d, $^2J_{C,P}$ = 3.0 Hz, C-1''), 55.8 (d, $^2J_{C,P}$ = 5.7 Hz, P(O)OMe), 55.4, 55.4, 55.4 (OMe), 37.1 (C-5''), 27.7, 27.7, 27.6 (C(*C*H_3)$_3$), 27.5 (C-7''); ^{31}P NMR (202 MHz, $CDCl_3$): δ −2.82; HRMS (ESI) m/z: [M+Na^+] calcd for $C_{56}H_{74}N_3O_{23}PSNa$ 1242.4069, found 1242.4064.

1'',2''-(*O*,*N*-(3-*N*-(*Tert*-butoxycarbonyl)-5'-*O*-(methylphosphinyl)-2',3'-di-*O*-(*tert*-butoxycarbonyl) uridinyl))-sulfamidate-3'',4'',6''-tri-*O*-(4-methoxybenzyl)-carba-*α*-D-glucopyranoside (27).

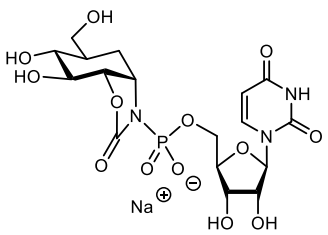


Compound **27** was prepared according to general procedure D using cyclic sulfamidate **12** (0.57 g, 0.94 mmol), 15-crown-5 ether (0.93 mL, 4.7 mmol, 5.0 mmol) and NaH (60 wt% dispersion in mineral oil; 57 g, 1.4 mmol, 1.5 eq.) in anhydrous THF (3.1 mL, 0.3 M); and H-phosphonate **23** (1.2 g, 1.9 mmol, 2.0 eq.), dry DiPEA (0.99 mL, 5.7 mmol, 6.0 eq.) and $BrCCl_3$ (0.22 mL, 2.2 mmol, 4.3 eq.) in anhydrous DCM (4.7 mL, 0.4 M). Flash column chromatography of the crude product (SiO_2, 30:70 EtOAc:pentane → 70:30 EtOAc:pentane) and Sephadex™ LH-20 size exclusion chromatography yielded title compound **27** as a white brittle foam and as a mixture of P(V) diastereomers (880 mg, 0.72 mmol, 77%).

Data for first P(V) diastereomer: R_f 0.45 (EtOAc:pentane, 1:1 v:v); ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.57 (d, *J* = 8.3 Hz, 1H, H-6), 7.41 – 7.34 (m, 2H, CH_{arom}), 7.22 – 7.15 (m, 2H, CH_{arom}), 7.13 – 7.06 (m, 2H, CH_{arom}), 6.89 – 6.79 (m, 6H, CH_{arom}), 6.22 (d, *J* = 6.6 Hz, 1H, H-1'), 5.84 (d, *J* = 8.3 Hz, 1H, H-5), 5.34 (dd, *J* = 5.2, 3.2 Hz, 1H, H-3'), 5.23 (ddd, *J* = 2.8, 2.8 2.3 Hz, 1H, H-1''), 5.05 (dd, *J* = 6.6, 5.2 Hz, 1H, H-2'), 4.91 (d, *J* = 10.4 Hz, 1H, C*H*H PMB), 4.82 (d, *J* = 10.3 Hz, 1H, CH*H* PMB), 4.75 (d, *J* = 10.6 Hz, 1H, C*H*H PMB), 4.45 (d, *J* = 10.6 Hz, 1H, CH*H* PMB), 4.37 (s, 2H, C*H*H PMB, CH*H* PMB), 4.35 – 4.26 (m, 3H, H-2'', H-5'), 4.22 (dddd, *J* = 3.8, 3.8, 2.0, 2.0 Hz, 1H, H-4'), 4.05 (dd, *J* = 9.3, 9.3 Hz, 1H, H-3''), 3.88 (d, $^3J_{H,P}$ = 11.8 Hz, 3H, P(O)OMe), 3.79 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.70 (dd, *J* = 9.2, 3.6 Hz, 1H, H-6''), 3.50 (dd, *J* = 9.7, 9.7 Hz, 1H, H-4''), 3.38 (dd, *J* = 9.1, 2.2 Hz, 1H, H-6''), 2.29 – 2.23 (m, 1H, H-7''), 2.12 – 1.99 (m, 2H, H-5'', H-7''), 1.59 (s, 9H, C(CH_3)$_3$), 1.48 (s, 9H, C(CH_3)$_3$), 1.43 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 160.2 (C=O uracil), 159.4, 159.3, 159.3 ($C_{q\text{-}arom}$), 152.3 (C=O carbamate), 152.3 (C=O uracil), 148.7, 147.7 (C=O carbonate), 139.0 (C-6), 130.5, 130.4, 130.3 ($C_{q\text{-}arom}$), 129.5, 129.4, 129.4, 113.9, 113.9 (CH_{arom}), 103.5 (C-5), 87.1 (*C*(CH_3)$_3$), 85.2 (C-1'), 84.7 (d, $^3J_{C,P}$ = 3.7 Hz, C-1''), 83.9, 83.7 (*C*(CH_3)$_3$), 82.2 (C-3''), 79.9 (d, $^3J_{C,P}$ = 9.6 Hz, C-4'), 79.3 (C-4''), 75.9 (CH_2 PMB), 74.9 (C-2'), 74.8, 72.9 (CH_2 PMB), 72.1 (C-3'), 68.3 (C-6''), 66.9 (d, $^2J_{C,P}$ = 5.3 Hz, C-5'), 66.4, 66.4 (d, $^2J_{C,P}$ = 1.9 Hz, C-2''), 56.0 (d, $^2J_{C,P}$ = 5.7 Hz, P(O)OMe), 55.4, 55.4, 55.4 (OMe), 37.1 (C-5''), 27.9 (C-7''), 27.8, 27.7, 27.6 (C(*C*H_3)$_3$); ^{31}P NMR (202 MHz, $CDCl_3$): δ −0.82; HRMS (ESI) m/z: [M+Na^+] calcd for $C_{56}H_{74}N_3O_{23}PSNa$ 1242.4069, found 1242.4064.

Data for second P(V) diastereomer: R_f 0.3 (EtOAc:pentane, 1:1 v:v); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.58 (d, *J* = 8.3 Hz, 1H, H-6), 7.33 – 7.29 (m, 2H, CH_{arom}), 7.22 – 7.19 (m, 2H, CH_{arom}), 7.07 – 7.04 (m, 2H, CH_{arom}), 6.87 – 6.78 (m, 6H, CH_{arom}), 6.13 (d, *J* = 6.3 Hz, 1H, H-1'), 5.85 (d, *J* = 8.2 Hz, 1H, H-5), 5.24 (dd, *J* = 5.4, 3.8 Hz, 1H, H-3'), 5.14 (ddd, *J* = 3.0, 3.0, 2.9 Hz, 1H, H-1''), 5.04 (dd, *J* = 6.3, 5.4 Hz, 1H, H-2'), 4.98 (d, *J* = 10.8 Hz, 1H, C*H*H PMB), 4.73 (d, *J* = 10.8 Hz, 1H, CH*H* PMB), 4.72 (d, *J* = 10.5 Hz, 1H, C*H*H PMB), 4.46 (d, *J* = 10.5 Hz, 1H, CH*H* PMB), 4.41 – 4.36 (m, 2H, C*H*H PMB, CH*H* PMB), 4.20 (dd, *J* = 5.5, 3.8 Hz, 1H, H-2''), 4.20 – 4.13 (m, 2H, H-5'), 4.11 (dddd, *J* = 2.9, 2.9, 2.9, 2.9 Hz, 1H, H-4'), 4.02 (dd, *J* = 9.3, 9.3 Hz, 1H, H-3''), 3.82 (d, $^3J_{H,P}$ = 11.8 Hz, 3H, P(O)OMe), 3.79 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.73 (dd, *J* = 9.1, 3.8 Hz, 1H, H-6''), 3.52 (dd, *J* = 9.9, 9.9 Hz, 1H, H-4''), 3.38 (dd, *J* = 9.2, 2.4 Hz, 1H, H-6''), 2.26 (ddd, *J* = 15.5, 2.7, 2.7 Hz, 1H, H-7''), 2.11 – 2.04 (m, 1H, H-5''), 2.00 (ddd, *J* = 15.9, 12.8, 3.1 Hz, 1H, H-7''), 1.59 (s, 9H, $C(CH_3)_3$), 1.49 (s, 9H, $C(CH_3)_3$), 1.45 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 160.3 (C=O uracil), 159.4, 159.4, 159.2 ($C_{q\text{-}arom}$), 152.2 (C=O carbamate), 152.1 (C=O uracil), 148.7, 147.7 (C=O carbonate), 139.2 (C-6), 130.5, 130.3, 130.2 ($C_{q\text{-}arom}$), 129.5, 129.5, 129.0, 114.0, 113.9, 113.9 (CH_{arom}), 103.5 (C-5), 87.0 ($C(CH_3)_3$), 85.8 (C-1'), 84.1 (d, $^3J_{C,P}$ = 3.9 Hz, C-1''), 83.8, 83.7 ($C(CH_3)_3$), 81.9 (C-3''), 79.7 (d, $^3J_{C,P}$ = 8.3 Hz, C-4'), 79.5 (C-4''), 75.2, 74.8 (CH_2 PMB), 74.7 (C-2'), 73.0 (CH_2 PMB), 71.9 (C-3'), 68.2 (C-6''), 66.9 (d, $^2J_{C,P}$ = 5.8 Hz, C-5'), 66.4 (d, $^2J_{C,P}$ = 0.9 Hz, C-2''), 56.0, 56.0 (d, $^2J_{C,P}$ = 5.9 Hz, P(O)OMe), 55.4, 55.4, 55.4 (OMe), 37.1 (C-5''), 28.1 (C-7''), 27.8, 27.7, 27.6 ($C(CH_3)_3$); ^{31}P NMR (202 MHz, $CDCl_3$): δ −2.08; HRMS (ESI) m/z: [M+Na^+] calcd for $C_{56}H_{74}N_3O_{23}PSNa$ 1242.4069, found 1242.4064.

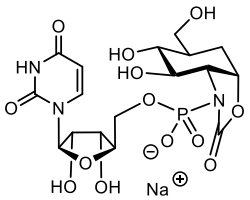
1'',2''-(*N*-(5'-*O*-Phosphoryluridinyl),*O*)-carbamate-carba-*α*-D-glucopyranoside (2).



Compound **2** was prepared according to general procedure E using **24** (81 mg, 69 μmol), TES (0.11 mL, 0.69 mmol, 10 eq.) and TFA (0.51 mL, 6.7 mmol, 30% v:v) in DCM (1.4 mL, 0.05 M). The reaction mixture was stirred at 5 °C for 24 h, after which full conversion was observed on TLC (R_f 0.25 MeOH:DCM, 2:8 v:v) and pyridine (10.8 mL, 134 mmol, 20:1 pyridine:TFA) was added. The reaction mixture was stirred overnight at 35°C, before full conversion was observed on TLC (R_f 0.25 (H_2O:MeCN, 1:9 v:v)) and ^{31}P NMR. Flash column chromatography (neutralized SiO_2, dry loading on Celite, 0:100 H_2O:ACN → 12:88 H_2O:ACN) followed by Dowex 50WX4 Na^+ ion exchange and lyophilization yielded the title compound **2** as a colorless transparent film (16.8 mg, 31.6 μmol, 72%). R_f 0.25 (H_2O:MeCN, 1:9 v:v); ^{1}H NMR (500 MHz, D_2O, HH-COSY, HSQC): δ 7.94 (d, *J* = 8.1 Hz, 1H, H-6), 5.95 (d, *J* = 4.7 Hz, 1H, H-1'), 5.92 (d, *J* = 8.1 Hz, 1H, H-5), 4.55 (dd, *J* = 7.6, 7.6 Hz, 1H, H-2''), 4.37 (ddd, *J* = 7.4, 7.1, 3.7 Hz, 1H, H-1''), 4.34 (dd, *J* = 5.0, 5.0 Hz, 1H, H-2'), 4.31 (dd, *J* = 4.9, 4.9 Hz, 1H, H-3'), 4.25 (dddd, *J* = 4.9, 2.6, 2.6, 2.6 Hz, 1H, H-4'), 4.23 – 4.11 (m, 2H, H-5'), 3.79 (dd, *J* = 9.5, 7.3 Hz, 1H, H-3''), 3.70 (dd, *J* = 11.4, 3.7 Hz, 1H, H-6''), 3.66 (dd, *J* = 11.4, 5.4 Hz, 1H, H-6''), 3.39 (dd, *J* = 9.0, 9.0 Hz, 1H, H-4''), 2.35 (qd, *J* = 8.6, 6.4 Hz, 1H, H-7''), 1.88 – 1.77 (m, 2H, H-5'', H-7''); ^{13}C NMR (126 MHz, D_2O, HSQC): δ 166.7 (C=O uracil), 158.7 (d, $^2J_{C,P}$ = 8.5 Hz, C=O carbamate), 152.1 (C=O uracil), 141.8 (C-6), 102.5 (C-5), 88.8 (C-1'), 83.0 (d, $^3J_{C,P}$ = 9.0 Hz, C-4'), 79.8 (d, $^3J_{C,P}$ = 8.1 Hz, C-2''), 74.5 (C-3''), 73.8 (C-2'), 69.9 (C-4''), 69.6 (C-3'), 64.7 (d, $^2J_{C,P}$ = 5.4 Hz,

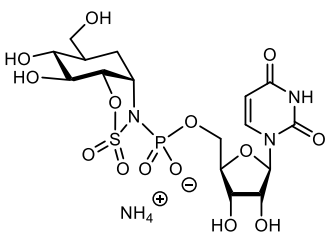
C-5'), 62.3 (C-6''), 56.0 (d, $^{2}J_{C,P}$ = 3.5 Hz, C-1''), 39.3 (C-5''), 26.2 (C-7''); ^{31}P NMR (202 MHz, D_2O): δ −5.58; HRMS (ESI) m/z: [M+H$^+$] calcd for $C_{17}H_{24}N_3O_{13}PNa$ 532.0944, found 532.0939.

1'',2''-(*O*,*N*-(5'-*O*-Phosphoryluridinyl))-carbamate-carba-*α*-D-glucopyranoside (4).



Compound **4** was prepared according to general procedure E using **25** (473 mg, 0.4 mmol), TES (0.63 mL, 4.0 mmol, 10 eq.) and TFA (2.8 mL, 36.8 mmol, 30% v:v) in DCM (4.6 mL, 0.05 M). The reaction mixture was stirred at 5 °C for 24 h, after which full conversion was observed on TLC (R_f 0.2 MeOH:DCM, 2:8 v:v) and pyridine (29 mL, 368 mmol, 10:1 pyridine:TFA) was added. The reaction mixture was stirred overnight at 35°C, before full conversion was observed on TLC (R_f 0.2 (H_2O:MeCN, 1:9 v:v)) and ^{31}P NMR. Flash column chromatography (neutralized SiO_2, dry loading on Celite, 0:100 H_2O:ACN → 12:88 H_2O:ACN) followed by Dowex 50WX4 Na^+ ion exchange and lyophilization yielded the title compound **4** as a colorless transparent film (67.5 mg, 0.13 mmol, 32% over two steps). ^{1}H NMR (500 MHz, D_2O, HH-COSY, HSQC): δ 7.94 (d, *J* = 8.2 Hz, 1H, H-6), 5.97 (d, *J* = 4.9 Hz, 1H, H-1'), 5.95 (d, *J* = 8.0 Hz, 1H, H-5), 4.86 (m, 1H, H-1''), 4.39 (dd, *J* = 5.0, 5.0 Hz, 1H, H-2'), 4.33 (dd, *J* = 4.9, 4.9 Hz, 1H, H-3'), 4.28 (ddd, *J* = 5.9, 2.1, 2.1 Hz, 1H, H-4'), 4.26 (dd, *J* = 4.2, 2.3 Hz, 1H, H-5'), 4.25 – 4.18 (m, 1H, H-5'), 4.03 (ddd, *J* = 7.9, 6.3, 1.8 Hz, 1H, H-2''), 3.78 (dd, *J* = 11.4, 3.6 Hz, 1H, H-6''), 3.72 (dd, *J* = 11.4, 5.6 Hz, 1H, H-6''), 3.66 (dd, *J* = 9.5, 7.8 Hz, 1H, H-3''), 3.38 (dd, *J* = 10.0, 10.0 Hz, 1H, H-4''), 2.30 (d, *J* = 15.3 Hz, 1H, H-7''), 1.82 (dddd, *J* = 16.0, 10.0, 3.3, 3.3 Hz, 1H, H-5''), 1.73 (ddd, *J* = 16.3, 13.0, 3.8 Hz, 1H, H-7''); ^{13}C NMR (126 MHz, D_2O, HSQC): δ 166.2 (C=O amide), 158.5 (d, $^{2}J_{C,P}$ = 8.1 Hz, C=O carbamate), 151.8 (C=O amide), 142.0 (C-6), 102.6 (C-5), 88.9 (C-1'), 82.9 (d, $^{3}J_{C,P}$ = 9.1 Hz, C-4'), 78.1 (C-3''), 77.4 (d, $^{3}J_{C,P}$ = 6.7 Hz, C-1''), 73.7 (C-2'), 71.2 (C-4''), 69.7 (C-3'), 65.1 (d, $^{2}J_{C,P}$ = 5.5 Hz, C-5'), 63.6 (d, $^{2}J_{C,P}$ = 3.2 Hz, C-2''), 61.8 (C-6''), 37.5 (C-5''), 27.0 (C-7''); ^{31}P NMR (202 MHz, D_2O): δ −4.88; HRMS (ESI) m/z: [M+H$^+$] calcd for $C_{17}H_{24}N_3O_{13}PNa$ 532.0944, found 532.0939.

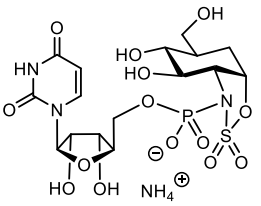
1'',2''-(*N*-(5'-*O*-Phosphoryluridinyl),*O*)-sulfamidate-carba-*α*-D-glucopyranoside (1).



Compound **1** was prepared according to general procedure E using protected *N*-sulfonylphosphoramidate **26** (78 mg, 64 μmol), TFA (0.39 mL, 5.1 mmol, 30% v:v), TES (0.10 mL, 0.63 mmol, 9.8 eq.) in anhydrous DCM (0.8 mL, 0.05 M). The reaction mixture was stirred for 3 h at 0°C, before full conversion was observed on TLC (R_f 0.1 (MeOH:DCM, 2:8 v:v)) and pyridine (8.2 mL, 101 mmol, 20:1 pyridine:TFA) was added. The reaction mixture was stirred overnight at room temperature, before full conversion was observed on TLC (R_f 0.2 (H_2O:ACN, 1:9 v:v)) and ^{31}P NMR. Flash column chromatography (neutralized SiO_2, dry loading on Celite, 0:100 H_2O:ACN → 12:88 H_2O:ACN) followed by NH_4^+-Dowex® 50WX4 ion exchange and lyophilization yielded the title compound **1** as a colorless transparent film (27.7 mg, 49.3 μmol, 77% over two steps). ^{1}H NMR (500 MHz, D_2O, HH-COSY): δ 7.91 (d, *J* = 8.1 Hz, 1H, H-6), 5.96 (d, *J* = 4.0 Hz, 1H, H-1'), 5.94 (d, *J* = 7.8 Hz, 1H, H-5), 4.76 (dd, *J* = 8.8, 5.7 Hz, 1H, H-2''), 4.49 (ddd, *J* = 6.0, 3.3, 3.3 Hz, 1H, H-1''), 4.36 (dd, *J* = 4.9, 4.9 Hz, 1H, H-2'), 4.32 (dd, *J* = 5.0, 5.0 Hz, 1H, H-3'), 4.30 – 4.23 (m, 3H, H-4', H-5'),

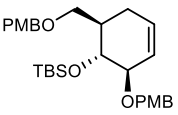
4.02 (dd, *J* = 9.8, 8.6 Hz, 1H, H-3''), 3.76 (dd, *J* = 11.5, 4.7 Hz, 1H, H-6''), 3.62 (dd, *J* = 11.5, 3.3 Hz, 1H, H-6''), 3.41 (dd, *J* = 10.1, 10.1 Hz, 1H, H-4''), 2.46 (ddd, *J* = 15.4, 3.2, 3.2 Hz, 1H, H-7''), 1.86 (dddd, *J* = 13.5, 10.6, 3.8, 3.8 Hz, 1H, H-5''), 1.77 (ddd, *J* = 15.9, 12.3, 3.8 Hz, 1H, H-7''); ^{13}C NMR (126 MHz, D_2O, HH-COSY, HSQC): δ 166.2, 151.8 (C=O uracil), 141.8 (C-6), 102.6 (C-5), 89.0 (C-1'), 85.4 (d, $^3J_{C,P}$ = 8.5 Hz, C-2''), 82.7 (d, $^3J_{C,P}$ = 8.5 Hz, C-4'), 73.8 (C-3'') 73.6 (C-2'), 70.6 (C-4''), 69.4 (C-3'), 65.1 (d, $^2J_{C,P}$ = 5.6 Hz, C-5'), 61.2 (C-6''), 58.9 (d, $^2J_{C,P}$ = 1.4 Hz, C-1''), 38.0 (C-5''), 25.9 (C-7''); ^{31}P NMR (202 MHz, D_2O): δ −7.77; HRMS (ESI) m/z: [M+H$^+$] calcd for $C_{16}H_{28}N_4O_{14}PS$ 563.1055, found 563.1055.

1'',2''-(*O*,*N*-(5'-*O*-Phosphoryluridinyl))-sulfamidate-carba-*α*-D-glucopyranoside (3).



Compound **3** was prepared according to general procedure E using protected *N*-sulfonylphosphoramidate **27** (70 mg, 57 μmol), TFA (0.35 mL, 4.6 mmol, 30% v:v), TES (91 μL, 66 mg, 0.57 mmol, 10.0 eq.) in anhydrous DCM (0.70 mL, 0.05 M). The reaction mixture was stirred for 3 h at 0°C, before full conversion was observed on TLC (R_f 0.1 (MeOH:DCM, 2:8 v:v)) and pyridine (7.4 mL, 92 mmol, 20:1 pyridine:TFA) was added. The reaction mixture was stirred overnight at room temperature, before full conversion was observed on TLC (R_f 0.25 (H_2O:ACN, 1:9 v:v)) and ^{31}P NMR. Flash column chromatography (neutralized SiO_2, dry loading on Celite, distilled DCM then 0:100 H_2O:ACN → 15:85 H_2O:ACN) followed by NH_4^+-Dowex® 50WX4 ion exchange and lyophilization yielded the title compound **3** as a colorless transparent film (23.9 mg, 42.5 μmol, 74% over two steps). ^{1}H NMR (500 MHz, D_2O, HH-COSY, HSQC): δ 7.87 (d, *J* = 8.1 Hz, 1H, H-6), 5.96 (d, *J* = 4.3 Hz, 1H, H-1'), 5.95 (d, *J* = 8.1 Hz, 1H, H-5), 5.22 (ddd, *J* = 4.6, 2.8, 2.8 Hz, 1H, H-1''), 4.40 – 4.35 (m, 2H, H-2', H-3'), 4.33 – 4.21 (m, 3H, H-4', H-5'), 3.99 (ddd, *J* = 8.4, 4.1, 4.1 Hz, 1H, H-2''), 3.88 (dd, *J* = 9.7, 8.5 Hz, 1H, H-3''), 3.76 (dd, *J* = 11.4, 3.6 Hz, 1H, H-6''), 3.71 (dd, *J* = 11.4, 5.6 Hz, 1H, H-6''), 3.35 (dd, *J* = 10.7, 9.6 Hz, 1H, H-4''), 2.36 (ddd, *J* = 16.0, 3.0, 3.0 Hz, 1H, H-7''), 1.88 (dddd, *J* = 13.9, 13.9, 6.1, 3.5 Hz, 1H, H-5''), 1.76 (ddd, *J* = 16.2, 13.1, 3.2 Hz, 1H, H-7''); ^{13}C NMR (126 MHz, D_2O, HSQC): δ 166.2, 151.8 (C=O uracil), 141.9 (C-6), 102.8 (C-5), 89.0 (C-1'), 82.7 (d, $^3J_{C,P}$ = 9.0 Hz, C-4'), 82.6 (d, $^3J_{C,P}$ = 5.6 Hz, C-1''), 76.1 (C-3''), 73.5 (C-2'), 71.2 (C-4''), 69.4 (C-3'), 66.5 (d, $^2J_{C,P}$ = 2.2 Hz, C-2''), 65.0 (d, $^2J_{C,P}$ = 5.7 Hz, C-5'), 61.3 (C-6''), 37.3 (C-5''), 27.1 (C-7''); ^{31}P NMR (202 MHz, D_2O): δ −6.76; HRMS (ESI) m/z: [M+H$^+$] calcd for $C_{16}H_{28}N_4O_{14}PS$ 563.1055, found 563.1055.

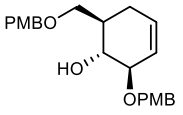
3,6-Di-*O*-(4-methoxybenzyl)-4-*O*-*tert*-butyldimethylsilyl-carba-D-glucal (29).



Compound **28** (1.9 g, 5.0 mmol) was dissolved in DCM (50 mL, 0.10 M). 4-methoxybenzyl-2,2,2-trichloroacetimidate (3.5 g, 13 mmol, 2.5 eq.) and PPTS (0.63 g, 2.5 mmol, 0.50 eq.) were added. The reaction mixture was stirred overnight at room temperature. Upon full conversion on TLC (R_f 0.7 (EtOAc:pentane, 1:9 v:v)), the reaction was quenched with sat. aq. $NaHCO_3$. The organic layer was separated, the aqueous layer was extracted thrice with Et_2O and the combined organic layers were washed with 1.0 M aq. HCl and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash column chromatography (SiO_2, dry loading on Celite, 3:97 Et_2O:pentane → 20:80 Et_2O:pentane) yielded the title compound **29** (1.4 g, 2.9 mmol, 57%). ^{1}H NMR (500 MHz, $CDCl_3$,

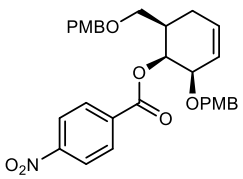
HH-COSY, HSQC): δ 7.30 – 7.23 (m, 4H, CH_{arom}), 6.91 – 6.85 (m, 4H, CH_{arom}), 5.81 – 5.71 (m, 1H, H-1), 5.65 (dddd, *J* = 10.1, 2.4, 2.3, 1.4 Hz, 1H, H-2), 4.55 (d, *J* = 11.3 Hz, 1H, C*H*H PMB), 4.51 (d, *J* = 11.3 Hz, 1H, CH*H* PMB), 4.47 (d, *J* = 11.7 Hz, 1H, C*H*H PMB), 4.40 (d, *J* = 11.6 Hz, 1H, CH*H* PMB), 3.86 (ddd, *J* = 4.6, 3.0, 1.3 Hz, 1H, H-3), 3.82 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.77 (dd, *J* = 9.1, 6.4 Hz, 1H, H-4), 3.64 (dd, *J* = 9.0, 3.4 Hz, 1H, H-6), 3.45 (dd, *J* = 9.0, 7.3 Hz, 1H, H-6), 2.39 – 2.24 (m, 1H, H-7), 2.12 – 1.99 (m, 2H, H-5, H-7), 0.89 (s, 9H, $C(CH_3)_3$), 0.07 (s, 3H, $SiCH_3$), 0.04 (s, 3H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.2, 159.1, 131.0, 131.0 ($C_{q\text{-}arom}$), 129.4, 129.2 (CH_{arom}), 128.6 (C-1), 125.5 (C-2), 113.8, 113.8 (CH_{arom}), 80.9 (C-3), 72.8 (CH_2 PMB), 72.5 (C-4), 71.1 (C-6), 70.7 (CH_2 PMB), 55.4, 55.4 (OMe), 40.6 (C-5), 28.3 (C-7), 26.2 ($C(CH_3)_3$), 18.4 ($C(CH_3)_3$), -3.8, -4.7 ($SiCH_3$); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{29}H_{42}NaO_5Si$ 521.2694; Found 521.2691.

3,6-Di-*O*-(4-methoxybenzyl)-carba-D-glucal (30).



Compound **29** (7.0 g, 14 mmol) was dissolved in THF (0.14 L, 0.10 M). TBAF (1.0 M solution in THF; 42 mL, 42 mmol, 3.0 eq.) was added. The reaction mixture was stirred for 1 h at room temperature. Upon full conversion on TLC (R_f 0.25 (EtOAc:pentane, 2:8 v:v)), the reaction was quenched with sat. aq. $NaHCO_3$. The organic layer was separated, the aqueous layer was extracted thrice with EtOAc and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash column chromatography (SiO_2, 20:80 EtOAc:pentane → 40:60 EtOAc:pentane) yielded the title compound **30** (3.6 g, 9.5 mmol, 67%). ^1H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.40 – 7.19 (m, 4H, CH_{arom}), 6.94 – 6.85 (m, 4H, CH_{arom}), 5.72 (dddd, *J* = 10.1, 4.3, 2.0, 2.0 Hz, 1H, H-2), 5.69 – 5.64 (m, 1H, H-1), 4.68 (d, *J* = 11.4 Hz, 1H, C*H*H PMB), 4.63 (d, *J* = 11.4 Hz, 1H, CH*H* PMB), 4.54 – 4.42 (m, 2H, C*H*H PMB, CH*H* PMB), 4.05 – 3.95 (m, 1H, H-3), 3.82 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.75 (ddd, *J* = 10.9, 7.6, 1.7 Hz, 1H, H-4), 3.64 (dd, *J* = 9.3, 6.0 Hz, 1H, H-6), 3.59 (dd, *J* = 9.3, 5.2 Hz, 1H, H-6), 3.26 (d, *J* = 1.8 Hz, 1H, 4-OH), 2.23 – 2.13 (m, 1H, H-7), 2.07 (dddd, *J* = 10.9, 10.9, 5.5, 5.5 Hz, 1H, H-5), 2.02 – 1.90 (m, 1H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.3, 159.3, 131.0, 130.2 ($C_{q\text{-}arom}$), 129.5, 129.3 (CH_{arom}), 127.9 (C-2), 126.3 (C-1), 113.9, 113.9 (CH_{arom}), 80.9 (C-3), 74.6 (C-4), 73.1, 72.5 (CH_2 PMB), 71.2 (C-6), 55.4 (OMe), 39.0 (C-5), 28.5 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{23}H_{28}NaO_5$ 407.1829; Found 407.1824.

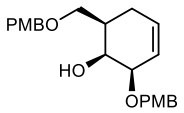
3,6-Di-*O*-(4-methoxybenzyl)-4-*O*-(4-nitrobenzoate)-carba-D-glucal (31).



Compound **30** (1.7 g, 4.5 mmol) was dissolved in anhydrous THF (45 mL, 0.10 M). PPh_3 (3.5 g, 13 mmol, 3.0 eq.), *p*-nitrobenzoic acid (2.2 g, 13 mmol, 3.0 eq.) and DEAD (2.1 mL, 13 mmol, 3.0 eq.) were added. The reaction mixture was stirred for 4.5 h at 60°C. Upon full conversion on TLC (R_f 0.45 (EtOAc:pentane, 2:8 v:v)), the reaction was quenched with sat. aq. $NaHCO_3$. The organic layer was separated, the aqueous layer was extracted thrice with EtOAc and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash column chromatography (SiO_2, dry loading on Celite, 10:90 Et_2O:pentane → 30:70 Et_2O:pentane) yielded the title compound **31** (2.3 g, 4.4 mmol, 98%). ^1H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 8.26 – 8.19 (m, 2H, CH_{arom}), 8.13 – 8.09 (m, 2H, CH_{arom}), 7.23 – 7.19 (m, 4H, CH_{arom}), 6.83 – 6.76 (m, 4H, CH_{arom}), 6.04 – 5.99 (m, 1H, H-4), 5.91 – 5.83 (m, 1H, H-1), 5.64 – 5.57 (m, 1H, H-2), 4.72 (d, *J* = 11.4 Hz, 1H,

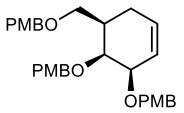
C*H*H PMB), 4.45 (d, *J* = 11.4 Hz, 1H, CH*H* PMB), 4.42 (d, *J* = 11.4 Hz, 1H, C*H*H PMB), 4.38 (d, *J* = 11.5 Hz, 1H, CH*H* PMB), 4.18 – 4.15 (m, 1H, H-3), 3.76 (s, 3H, OMe), 3.74 (s, 3H, OMe), 3.37 (s, 1H, H-6), 3.36 (s, 1H, H-6), 2.28 (dddd, *J* = 12.8, 12.8, 6.9, 1.4 Hz, 1H, H-7), 2.15 (ddddd, *J* = 17.9, 6.3, 4.3, 1.9, 1.9 Hz, 1H, H-5), 2.05 (dddd, *J* = 17.5, 11.0, 3.0, 3.0 Hz, 1H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 164.6, 159.3, 159.3, 150.5, 136.2 ($C_{q\text{-}arom}$), 130.9 (CH_{arom}), 130.3, 130.1 ($C_{q\text{-}arom}$), 129.6, 129.5 (CH_{arom}), 127.8 (C-1), 127.1 (C-2), 123.5, 113.9, 113.8 (CH_{arom}), 74.3 (C-3), 73.2, 70.8 (CH_2 PMB), 70.3 (C-6), 68.1 (C-4), 55.4, 55.3 (OMe), 37.0 (C-5), 24.8 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{30}H_{31}NNaO_8$ 556.1942; Found 556.1938.

3,6-Di-*O*-(4-methoxybenzyl)-carba-D-galactal (32).



Compound **31** (2.3 g, 4.4 mmol) was dissolved in DCM:MeOH (1:1, 88 mL, 0.05 M). NaOMe (0.94 g, 4.4 mmol, 4.0 eq.) was added on ice and the ice bath was removed after 5 min. The reaction mixture was stirred overnight at room temperature. Upon full conversion on TLC (R_f 0.2 (EtOAc:pentane, 2:8 v:v)), the reaction was quenched with sat. aq. $NaHCO_3$. The organic layer was separated, the aqueous layer was extracted thrice with EtOAc and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash column chromatography (SiO_2, 10:90 EtOAc:pentane → 40:60 EtOAc:pentane) yielded the title compound **32** (1.1 g, 3.0 mmol, 67%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.36 – 7.25 (m, 4H, CH_{arom}), 6.95 – 6.87 (m, 4H, CH_{arom}), 5.86 – 5.80 (m, 1H, H-1), 5.56 (ddd, *J* = 10.2, 1.8, 1.8 Hz, 1H, H-2), 4.66 (d, *J* = 11.4 Hz, 1H, C*H*H PMB), 4.56 (d, *J* = 11.4 Hz, 1H, CH*H* PMB), 4.53 (d, *J* = 11.5 Hz, 1H, C*H*H PMB), 4.46 (d, *J* = 11.5 Hz, 1H, CH*H* PMB), 4.29 (ddd, *J* = 3.9, 2.2 Hz, 1H, H-3), 4.04 (ddd, *J* = 4.1, 2.1, 2.1 Hz, 1H, H-4), 3.83 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.66 (dd, *J* = 9.0, 7.7 Hz, 1H, H-6), 3.46 (dd, *J* = 9.0, 5.5 Hz, 1H, H-6), 2.44 (d, *J* = 2.1 Hz, 1H, 4-OH), 2.04 – 1.97 (m, 3H, H-5, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.4, 159.3, 130.6, 130.3 ($C_{q\text{-}arom}$), 129.5, 129.4 (CH_{arom}), 129.2 (C-1), 125.3 (C-2), 114.0, 113.9 (CH_{arom}), 75.6 (C-4), 73.1 (CH_2 PMB), 71.5 (C-6), 70.3 (CH_2 PMB), 65.0 (C-3), 55.4 (OMe), 37.9 (C-5), 24.2 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{23}H_{28}NaO_5$ 407.1829; Found 407.1824.

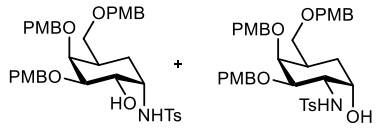
3,4,6-Tri-*O*-(4-methoxybenzyl)-carba-D-galactal (33).



Compound **32** (0.12 g, 0.31 mmol) was co-evaporated with toluene and dissolved in DMF (3.1 mL, 0.10 M). PMBCl (0.10 mL, 0.53 mmol, 1.7 eq.) and NaH (60 wt.% dispersion in mineral oil; 23 mg, 0.62 mmol, 2.0 eq.) were added on ice and the ice bath was removed after 5 min. The reaction mixture was stirred overnight at room temperature. Upon full conversion on TLC (R_f 0.45 (EtOAc:pentane, 2:8 v:v)), the reaction mixture was cooled on ice and quenched with sat. aq. $NaHCO_3$. The organic layer was separated, the aqueous layer was extracted thrice with EtOAc and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash column chromatography (SiO_2, dry loading on Celite, 10:90 Et_2O:pentane → 30:70 Et_2O:pentane) yielded the title compound **33** (0.14 g, 0.28 mmol, 89%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.31 – 7.18 (m, 6H, CH_{arom}), 6.91 – 6.78 (m, 6H, CH_{arom}), 5.75 (dddd, *J* = 10.1, 4.2, 2.2, 2.2 Hz, 1H, H-1), 5.69 – 5.64 (m, 1H, H-2), 4.84 (d, *J* = 11.6 Hz, 1H, C*H*H PMB), 4.61 (d, *J* = 11.7 Hz, 1H, C*H*H PMB), 4.57 (d, *J* = 11.7 Hz, 1H, CH*H* PMB), 4.51 (d, *J* = 11.6 Hz, 1H, CH*H* PMB), 4.33 – 4.27 (m, 2H, C*H*H PMB, CH*H* PMB), 4.07 (ddd, *J* = 3.3, 1.5, 1.5 Hz, 1H, H-3), 4.05 (dd, *J* = 3.7, 2.0 Hz, 1H, H-

4), 3.80 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.77 (s, 3H, OMe), 3.44 (dd, *J* = 8.9, 8.8 Hz, 1H, H-6), 3.26 (dd, *J* = 8.9, 5.2 Hz, 1H, H-6), 2.05 – 1.97 (m, 1H, H-5), 1.97 – 1.87 (m, 2H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.3, 159.1, 159.0, 131.9, 131.1, 130.6 ($C_{q\text{-}arom}$), 129.5, 129.5, 129.0 (CH_{arom}), 128.2 (C-1), 126.6 (C-2), 113.9, 113.8, 113.6 (CH_{arom}), 77.1 (C-4), 73.7 (CH_2 PMB), 72.9 (C-3), 72.9 (CH_2 PMB), 70.8 (C-6), 70.6 (CH_2 PMB), 55.4, 55.4, 55.3 (OMe), 38.1 (C-5), 25.3 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{31}H_{36}NaO_6$ 527.2404; Found 527.2401.

1-Deoxy-1-(*p*-toluenesulfonamido)-3,4,6-tri-*O*-(4-methoxybenzyl)-carba-α-D-galactopyranoside (34) and 2-Deoxy-(*p*-toluenesulfonamido)-3,4,6-tri-*O*-(4-methoxybenzyl)-carba-α-D-galactopyranoside (35).



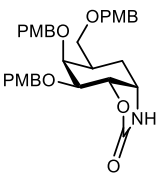
Compound **33** (2.9 g, 5.8 mmol) was dissolved in $CHCl_3$:H_2O (1:1, 58 mL, 0.10 M). Chloramine-T trihydrate (3.2 g, 12 mmol, 2.0 eq.), TEBACl (79 mg, 0.35 mmol, 0.06 eq.) and $K_2[OsO_2(OH)_4]$ (0.11 g, 0.28 mmol, 0.05 eq.) were added. The reaction mixture was stirred vigorously overnight at 60°C. Upon full conversion on TLC (R_f 0.4 and 0.2 for **34** and **35** respectively (EtOAc:pentane, 4:6 v:v)), the reaction was quenched with sat. aq. $Na_2S_2O_3$. The organic layer was separated, the aqueous layer was extracted thrice with Et_2O and the combined organic layers were washed thrice with 1.0 wt.% aq. NaOH and once with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash column chromatography (SiO_2, 30:70 EtOAc:pentane → 50:50 EtOAc:pentane) yielded the title compounds **34** (1.6 g, 2.3 mmol, 40%) and **35** (2.0 g, 2.9 mmol, 50%).

Analytical data for **34**: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.75 – 7.68 (m, 2H, CH_{arom}), 7.34 – 7.19 (m, 8H, CH_{arom}), 7.18 – 7.11 (m, 2H, CH_{arom}), 6.93 – 6.86 (m, 4H, CH_{arom}), 6.83 – 6.77 (m, 2H, CH_{arom}), 4.96 (s, 1H, 1-NH), 4.72 (d, *J* = 10.8 Hz, 1H, C*H*H PMB), 4.67 (d, *J* = 11.2 Hz, 1H, C*H*H PMB), 4.44 – 4.31 (m, 4H, C*H*H PMB, CH*H* PMB, CH*H* PMB, CH*H* PMB), 4.16 (d, *J* = 2.8 Hz, 1H, H-4), 3.95 (ddd, *J* = 9.9, 4.7, 1.3 Hz, 1H, H-2), 3.81 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.77 (s, 3H, OMe), 3.51 – 3.36 (m, 2H, H-3, H-6), 3.32 (ddd, *J* = 3.8, 3.6, 3.6 Hz, 1H, H-1), 3.22 (dd, *J* = 8.9, 4.8 Hz, 1H, H-6), 2.47 (s, 1H, 2-OH), 2.40 (s, 3H, CH_3 Ts), 2.28 – 2.18 (m, 1H, H-5), 1.90 (ddd, *J* = 14.2, 3.4, 3.4 Hz, 1H, H-7), 1.43 (ddd, *J* = 15.2, 12.8, 2.9 Hz, 1H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.6, 159.4, 159.2, 143.7, 135.8, 131.4, 130.5, 130.0 ($C_{q\text{-}arom}$), 129.8, 129.7, 129.6, 129.3, 127.6, 114.2, 114.0, 113.7 (CH_{arom}), 81.2 (C-3), 74.2, 72.9 (CH_2 PMB), 72.7 (C-4), 71.5 (CH_2 PMB), 69.9 (C-6), 69.0 (C-2), 55.4, 55.4, 55.4, 52.7 (C-1), 35.4 (C-5), 25.1 (C-7), 21.7 (CH_3 Ts); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{38}H_{45}NNaO_9S$ 714.2707; Found 714.2700.

Analytical data for **35**: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.69 – 7.62 (m, 2H, CH_{arom}), 7.25 – 7.21 (m, 2H, CH_{arom}), 7.17 – 7.13 (m, 2H, CH_{arom}), 7.10 – 7.05 (m, 2H, CH_{arom}), 7.00 – 6.93 (m, 2H, CH_{arom}), 6.89 – 6.83 (m, 4H, CH_{arom}), 6.80 – 6.76 (m, 2H, CH_{arom}), 4.90 (d, *J* = 3.3 Hz, 1H, 2-NH), 4.55 (d, *J* = 11.1 Hz, 1H, C*H*H PMB), 4.52 (d, *J* = 10.6 Hz, 1H, C*H*H PMB), 4.41 (d, *J* = 11.6 Hz, 1H, C*H*H PMB), 4.37 (d, *J* = 11.6 Hz, 1H, CH*H* PMB), 4.28 – 4.24 (m, 2H, CH*H* PMB, H-1), 4.22 (d, *J* = 11.1 Hz, 1H, CH*H* PMB), 4.12 (dd, *J* = 2.1, 2.1 Hz, 1H, H-4), 3.82 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.56 (dd, *J* = 10.6, 2.1 Hz, 1H, H-3), 3.45 (ddd, *J* = 10.6, 3.1, 3.1 Hz, 1H, H-2), 3.41 (dd, *J* = 9.3, 9.3 Hz, 1H, H-6), 3.22 (dd, *J* = 9.0, 4.9 Hz, 1H, H-6), 2.57 (bs, 1H, 1-OH), 2.39 (s, 3H, CH_3 Ts), 2.25 – 2.11 (m, 1H, H-5), 1.51 (d, *J* = 3.0 Hz, 1H, H-7), 1.49 (dd, *J* = 3.0, 2.9 Hz, 1H, H-7); ^{13}C NMR

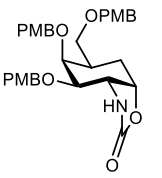
(126 MHz, $CDCl_3$, HSQC): δ 159.4, 159.3, 159.1, 143.3, 136.0, 131.3, 130.5, 129.8 ($C_{q\text{-}arom}$), 129.6, 129.5, 129.4, 129.1, 127.6, 114.0, 113.9, 113.6 (CH_{arom}), 78.1 (C-3), 73.9, 72.9 (CH_2 PMB), 72.6 (C-4), 71.0 (CH_2 PMB), 70.2 (C-6), 67.8 (C-1), 56.5 (C-2), 55.4, 55.4 (OMe), 35.2 (C-5), 28.0 (C-7), 21.7 (CH_3 Ts); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{38}H_{45}NNaO_9S$ 714.2707; Found 714.2701.

1,2-(*N*,*O*)-Carbamate-3,4,6-tri-*O*-(4-methoxybenzyl)-carba-α-D-galactopyranoside (36).



Compound **36** was prepared according to general procedure B using **34** (0.35 g, 0.50 mmol), pyridine (0.18 mL, 2.3 mmol, 4.5 eq.) and triphosgene (89 mg, 0.30 mmol, 0.60 eq.) in anhydrous DCM (3.1 mL, 0.10 M). Full conversion was observed on TLC (R_f 0.7 (EtOAc:pentane, 1:1 v:v)). The second step was performed using naphthalene (0.77 g, 6.0 mmol, 12 eq.) and Na (0.12 g, 5.0 mmol, 10 eq.) in anhydrous THF (6.2 mL, 0.05 M). Full conversion was observed on TLC (R_f 0.2 (EtOAc:pentane, 1:1 v:v)). Flash column chromatography (SiO_2, 30:70 EtOAc:pentane → 70:30 EtOAc:pentane) yielded the title compound **36** (0.21 g, 0.37 mmol, 75%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.35 – 7.28 (m, 2H, CH_{arom}), 7.24 – 7.18 (m, 2H, CH_{arom}), 7.17 – 7.11 (m, 2H, CH_{arom}), 6.92 – 6.84 (m, 4H, CH_{arom}), 6.85 – 6.79 (m, 2H, CH_{arom}), 5.51 – 5.30 (m, 1H, 1-NH), 4.80 (d, *J* = 11.0 Hz, 1H, C*H*H PMB), 4.75 – 4.69 (m, 2H, C*H*H PMB, H-2), 4.62 (d, *J* = 11.3 Hz, 1H, CH*H* PMB), 4.40 (d, *J* = 11.0 Hz, 1H, CH*H* PMB), 4.37 (d, *J* = 11.7 Hz, 1H, C*H*H PMB), 4.33 (d, *J* = 11.5 Hz, 1H, CH*H* PMB), 4.19 – 4.16 (m, 1H, H-1), 4.01 (dd, *J* = 2.0, 2.0 Hz, 1H, H-4), 3.80 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.58 (dd, *J* = 7.9, 2.2 Hz, 1H, H-3), 3.38 (dd, *J* = 8.8, 8.8 Hz, 1H, H-6), 3.23 (dd, *J* = 8.9, 5.6 Hz, 1H, H-6), 2.11 – 1.99 (m, 1H, H-5), 1.71 (ddd, *J* = 14.7, 12.6, 4.7 Hz, 1H, H-7), 1.60 (ddd, *J* = 14.8, 4.7, 2.0 Hz, 1H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 160.1 (C=O carbamate), 159.4, 159.3, 159.2, 131.1, 130.5, 130.2 ($C_{q\text{-}arom}$), 129.6, 129.6, 129.3, 114.0, 113.9, 113.7 (CH_{arom}), 82.0 (C-3), 81.0 (C-2), 74.2 (CH_2 PMB), 73.9 (C-4), 73.0, 72.4 (CH_2 PMB), 70.2 (C-6), 55.4, 55.4, 55.4 (OMe), 52.9 (C-1), 35.7 (C-5), 24.5 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{32}H_{37}NNaO_8$ 586.2411; Found 586.2407.

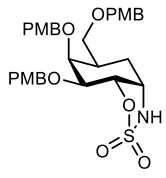
1,2-(*O*,*N*)-Carbamate-3,4,6-tri-*O*-(4-methoxybenzyl)-carba-α-D-galactopyranoside (38).



Compound **38** was prepared according to general procedure B using **35** (0.35 g, 0.50 mmol), pyridine (0.18 mL, 2.3 mmol, 4.5 eq.) and triphosgene (89 mg, 0.30 mmol, 0.60 eq.) in anhydrous DCM (1.2 mL, 0.10 M). Full conversion was observed on TLC (R_f 0.35 (EtOAc:pentane, 3:7 v:v)). The second step was performed using naphthalene (0.77 g, 6.0 mmol, 12 eq.) and Na (0.12 g, 5.0 mmol, 10 eq.) in anhydrous THF (2.4 mL, 0.05 M). Full conversion was observed on TLC (R_f 0.1 (EtOAc:pentane, 3:7 v:v)). Flash column chromatography (SiO_2, 30:70 EtOAc:pentane → 50:50 EtOAc:pentane) yielded the title compound **38** (0.14 g, 0.24 mmol, 49%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.27 – 7.22 (m, 4H, CH_{arom}), 7.17 – 7.13 (m, 2H, CH_{arom}), 6.93 – 6.86 (m, 4H, CH_{arom}), 6.85 – 6.82 (m, 2H, CH_{arom}), 5.26 (s, 1H, 2-NH), 4.75 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 4.70 (d, *J* = 10.9 Hz, C*H*H PMB), 4.68 – 4.66 (m, 1H, H-1), 4.46 – 4.40 (m, 2H, C*H*H PMB, CH*H* PMB), 4.38 (d, *J* = 11.6 Hz, 1H, CH*H* PMB), 4.33 (d, *J* = 11.0 Hz, 1H, CH*H* PMB), 4.18 (dd, *J* = 1.8, 1.8 Hz, 1H, H-4), 3.81 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.74 (dd, *J* = 8.7, 6.2 Hz, 1H, H-2), 3.43 (dd, *J* = 9.2, 9.2 Hz, 1H, H-6), 3.34 (dd, *J* = 8.8, 1.9 Hz, 1H, H-3), 3.30 (dd, *J* = 9.0, 5.0 Hz, 1H, H-6), 2.05 (dddd, *J* = 14.1, 9.5, 4.8, 1.5 Hz, 1H, H-5), 1.90 (dd, *J* = 14.9, 4.2 Hz,

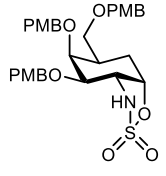
1H, H-7), 1.72 (ddd, *J* = 15.3, 13.3, 4.8 Hz, 1H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.7 (C=O carbamate), 159.7, 159.4, 159.3, 130.9, 130.2, 129.7 ($C_{q\text{-arom}}$), 129.6, 129.6, 129.5, 114.3, 114.0, 113.8 (CH_{arom}), 84.1 (C-3), 77.0 (C-1), 74.0, 72.9, 71.5 (CH_2 PMB), 71.0 (C-4), 70.0 (C-6), 55.9 (C-2), 55.4, 55.4, 55.4 (OMe), 36.3 (C-5), 24.2 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{32}H_{37}NNaO_8$ 586.2411; Found 586.2406.

1,2-(*N*,*O*)-Sulfamidate-3,4,6-tri-*O*-(4-methoxybenzyl)-carba-α-D-galactopyranoside (37).



Compound **37** was prepared according to general procedure C using **34** (0.21 g, 0.31 mmol), Et_3N (0.17 mL, 1.2 mmol, 4.0 eq.) and SO_2Cl_2 (38 µL, 0.47 mmol, 1.3 eq.) in anhydrous DCM (3.1 mL, 0.10 M). Full conversion was observed on TLC (R_f 0.6 (EtOAc:pentane, 4:6 v:v)). The second step was prepared using naphthalene (0.48 g, 3.7 mmol, 12 eq.) and Na (71 mg, 3.1 mmol, 10 eq.) in anhydrous THF (6.2 mL, 0.05 M). Full conversion was observed on TLC (R_f 0.3 (EtOAc:pentane, 4:6 v:v)). Flash column chromatography (SiO_2, 20:80 EtOAc:pentane → 50:50 EtOAc:pentane) yielded the title compound **37** (64 mg, 0.11 mmol, 34%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.33 – 7.28 (m, 2H, CH_{arom}), 7.22 – 7.17 (m, 2H, CH_{arom}), 7.15 – 7.10 (m, 2H, CH_{arom}), 6.87 (m, 4H, CH_{arom}), 6.84 – 6.80 (m, 2H, CH_{arom}), 4.95 (dd, *J* = 8.8, 5.7 Hz, 1H, H-2), 4.92 (dd, *J* = 6.4, 2.8 Hz, 1H, 1-NH), 4.78 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 4.70 (d, *J* = 11.3 Hz, 1H, C*H*H PMB), 4.63 (d, *J* = 11.3 Hz, 1H, CH*H* PMB), 4.38 (d, *J* = 10.9 Hz, 1H, CH*H* PMB), 4.35 (d, *J* = 11.4 Hz, 1H, C*H*H PMB), 4.31 (d, *J* = 11.5 Hz, 1H, CH*H* PMB), 4.22 (dddd, *J* = 6.4, 6.4, 4.6, 1.9 Hz, 1H, H-1), 4.00 – 3.97 (m, 1H, H-4), 3.93 (dd, *J* = 8.8, 2.3 Hz, 1H, H-3), 3.78 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.77 (s, 3H, OMe), 3.37 (dd, *J* = 9.0, 8.9 Hz, 1H, H-6), 3.20 (dd, *J* = 9.0, 5.9 Hz, 1H, H-6), 2.05 (dddd, *J* = 11.9, 9.6, 6.5, 3.7 Hz, 1H, H-5), 1.77 (ddd, *J* = 14.9, 12.9, 4.7 Hz, 1H, H-7), 1.62 (ddd, *J* = 15.0, 4.4, 2.1 Hz, 1H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.4, 159.4, 159.3, 130.7, 130.2, 130.0 ($C_{q\text{-arom}}$), 129.7, 129.6, 129.5, 113.9, 113.9, 113.7 (CH_{arom}), 88.0 (C-2), 79.8 (C-3), 74.6 (C-4), 74.3, 73.0, 72.9 (CH_2 PMB), 69.9 (C-6), 55.8 (C-1), 55.4, 55.4, 55.3 (OMe), 35.5 (C-7), 24.0 (C-5); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{31}H_{37}NNaO_9S$ 622.2081; Found 622.2075.

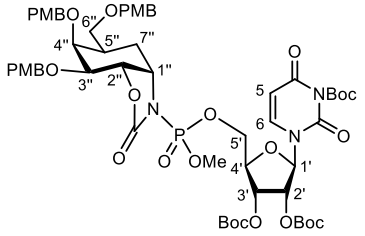
1,2-(*O*,*N*)-Sulfamidate-3,4,6-tri-*O*-(4-methoxybenzyl)-carba-α-D-galactopyranoside (39).



Compound **39** was prepared according to general procedure C using **35** (0.14 g, 0.20 mmol), Et_3N (0.11 mL, 0.80 mmol, 4.0 eq.) and SO_2Cl_2 (21 µL, 0.26 mmol, 1.3 eq.) in anhydrous DCM (2.0 mL, 0.10 M). Full conversion was observed on TLC (R_f 0.6 (EtOAc:pentane, 4:6 v:v)). The second step was prepared using naphthalene (0.30 g, 2.4 mmol, 12 eq.) and sodium metal (46 mg, 2.0 mmol, 10 eq.) in anhydrous THF (4.0 mL, 0.05 M). Full conversion was observed on TLC (R_f 0.5 (EtOAc:pentane, 4:6 v:v)). Flash column chromatography (SiO_2, 20:80 EtOAc:pentane → 50:50 EtOAc:pentane) yielded the title compound **39** (67 mg, 0.11 mmol, 56%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.31 – 7.27 (m, 2H, CH_{arom}), 7.25 – 7.21 (m, 2H, CH_{arom}), 7.16 – 7.12 (m, 2H, CH_{arom}), 6.92 – 6.87 (m, 4H, CH_{arom}), 6.85 – 6.81 (m, 2H, CH_{arom}), 5.00 (ddd, *J* = 4.0, 4.0, 2.0 Hz, 1H, H-1), 4.79 (d, *J* = 3.3 Hz, 1H, 2-NH), 4.77 (d, *J* = 10.8 Hz, 1H, C*H*H PMB), 4.70 (d, *J* = 11.2 Hz, 1H, C*H*H PMB), 4.53 (d, *J* = 11.2 Hz, 1H, CH*H* PMB), 4.41 (d, *J* = 10.9 Hz, 1H, CH*H* PMB), 4.41 (d, *J* = 11.6 Hz, 1H, C*H*H PMB) 4.36 (d, *J* = 11.6 Hz,

1H, CH*H* PMB), 4.11 (dd, *J* = 1.9, 1.9 Hz, 1H, H-4), 3.90 (ddd, *J* = 9.5, 4.3, 3.2 Hz, 1H, H-2), 3.82 – 3.76 (m, 10H, H-3, OMe, OMe, OMe), 3.40 (dd, *J* = 9.0, 9.0 Hz, 1H, H-6), 3.27 (dd, *J* = 9.0, 5.4 Hz, 1H, H-6), 2.13 (dddd, *J* = 10.7, 9.2, 6.3, 6.3 Hz, 1H, H-5), 1.95 (ddd, *J* = 15.6, 4.3, 2.0 Hz, 1H, H-7), 1.79 (ddd, *J* = 15.5, 13.2, 3.8 Hz, 1H, H-7); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.7, 159.5), 159.4, 130.8, 130.2, 129.9 ($C_{q\text{-arom}}$), 129.7, 129.6, 129.6, 114.2, 114.0, 113.8 (CH_{arom}), 83.8 (C-1), 80.3 (C-3), 74.3, 72.9 (CH_2 PMB), 72.7 (C-4), 72.6 (CH_2 PMB), 69.7 (C-6), 60.4 (C-2), 55.4, 55.4, 55.4 (OMe), 35.8 (C-5), 24.7 (C-7); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{31}H_{37}NNaO_9S$ 622.2081; Found 622.2077.

1'',2''-(*N*-(3-*N*-(*Tert*-butoxycarbonyl)-5'-*O*-(methylphosphinyl)-2',3'-di-*O*-(*tert*-butoxycarbonyl)uridinyl),*O*)-carbamate-3'',4'',6''-tri-*O*-(4-methoxybenzyl)-carba-α-D-galactopyranoside (41).



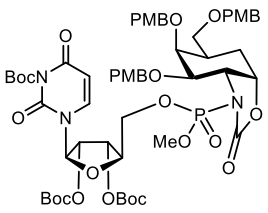
Compound **41** was prepared according to general procedure D using **36** (0.14 g, 0.24 mmol), 15-crown-5 ether (0.24 mL, 1.2 mmol, 5.0 eq.) and NaH (60 wt.% dispersion in mineral oil; 14 mg, 0.36 mmol, 1.5 eq.) in anhydrous THF (1.2 mL, 0.20 M); and **23** (0.30 g, 0.48 mmol, 2.0 eq.), DiPEA (distilled and stored over KOH; 0.17 mL, 1.0 mmol, 4.0 eq.) and $BrCCl_3$ (0.14 mL, 1.4 mmol, 6.0 eq.) in anhydrous DCM (1.6 mL, 0.30 M). Full conversion was observed on TLC (R_f 0.45 and 0.7 (EtOAc:pentane, 6:4 v:v)) and ^{31}P NMR. Flash column chromatography (SiO_2, 30:70 EtOAc:pentane → 70:30 EtOAc:pentane) and Sephadex™ LH-20 size exclusion chromatography yielded the title compound **41** as a mixture of P(V) diastereomers (0.15 g, 0.13 mmol, 54%).

Data for first P(V) diastereomer: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.64 (d, *J* = 8.3 Hz, 1H, H-6), 7.32 – 7.24 (m, 2H, CH_{arom}), 7.24 – 7.18 (m, 2H, CH_{arom}), 7.17 – 7.12 (m, 2H, CH_{arom}), 6.92 – 6.80 (m, 6H, CH_{arom}), 6.19 (d, *J* = 6.4 Hz, 1H, H-1'), 5.87 (d, *J* = 8.2 Hz, 1H, H-5), 5.32 – 5.27 (m, 1H, H-3'), 5.11 (dd, *J* = 6.4, 5.4 Hz, 1H, H-2'), 4.85 – 4.74 (m, 2H, H-2'', C*H*H PMB), 4.66 (d, *J* = 11.4 Hz, 1H, C*H*H PMB), 4.60 (d, *J* = 11.2 Hz, 1H, CH*H* PMB), 4.50 – 4.42 (m, 2H, H-1'', CH*H* PMB), 4.40 – 4.28 (m, 3H, C*H*H PMB, CH*H* PMB, H-5'), 4.27 – 4.21 (m, 2H, H-5', H4'), 4.09 – 4.05 (m, 1H, H-4''), 3.84 (s, 3H, P(O)OMe), 3.82 – 3.77 (m, 9H, OMe, OMe, OMe), 3.67 (dd, *J* = 7.3, 1.9 Hz, 1H, H-3''), 3.57 – 3.51 (m, 1H, H-6''), 3.36 – 3.29 (m, 1H, H-6''), 2.27 – 2.04 (m, 2H, H-7'', H-5''), 1.91 – 1.78 (m, 1H, H-7''), 1.61 – 1.42 (m, 9H, $C(CH_3)_3$, $C(CH_3)_3$, $C(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 160.3 (C=O uracil), 159.4, 159.4, 159.3 ($C_{q\text{-arom}}$), 155.2 (d, *J* = 8.5 Hz, C=O cyclic carbamate), 152.2 (C=O carbamate), 152.0 (C=O uracil), 148.8, 147.6 (C=O carbonate), 139.3 (C-6), 130.9, 130.3, 130.2, 130.1 ($C_{q\text{-arom}}$), 129.6, 129.5, 129.2, 113.9, 113.9, 113.7 (CH_{arom}), 103.4 (C-5), 86.9 ($C(CH_3)_3$), 85.8 (C-1'), 83.6, 83.5 ($C(CH_3)_3$), 81.6 (C-3''), 80.9 (d, *J* = 9.5 Hz, C-2''), 80.1 (d, *J* = 8.9 Hz, C-4'), 74.6 (C-2'), 74.4 (CH_2 PMB), 74.3 (C-4''), 73.0 (CH_2 PMB), 72.3 (C-3'), 72.2 (CH_2 PMB), 70.3 (C-6''), 66.4 (d, *J* = 5.3 Hz, C-5'), 56.9 (d, *J* = 4.7 Hz, C-1''), 55.4, 55.4, 55.3 (OMe), 55.0 (d, *J* = 5.8 Hz, P(O)OMe), 35.9 (C-5''), 27.7, 27.7, 27.5 ($C(CH_3)_3$), 24.4 (C-7''); ^{31}P NMR (202 MHz, $CDCl_3$): δ -0.83.

Data for second P(V) diastereomer: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.73 (d, *J* = 8.3 Hz, 1H, H-6), 7.32 – 7.24 (m, 2H, CH_{arom}), 7.24 – 7.18 (m, 2H, CH_{arom}), 7.17 – 7.12 (m, 2H, CH_{arom}), 6.92 – 6.80 (m, 6H, CH_{arom}), 6.15 (d, *J* = 5.8 Hz, 1H, H-1'), 5.89 (d, *J* = 8.2 Hz, 1H, H-5), 5.32 – 5.27

(m, 1H, H-3'), 5.16 (dd, *J* = 5.6 Hz, 1H, H-2'), 4.85 – 4.74 (m, 2H, H-2'', C*H*H PMB), 4.68 (d, *J* = 11.2 Hz, 1H, C*H*H PMB), 4.60 (d, *J* = 11.5 Hz, 1H, CH*H* PMB), 4.50 – 4.42 (m, 3H, H-1'', H-5', CH*H* PMB), 4.40 – 4.28 (m, 4H, H-5', C*H*H PMB, CH*H* PMB, H-4'), 4.09 – 4.05 (m, 1H, H-4''), 3.86 (s, 3H, P(O)OMe), 3.82 – 3.77 (m, 9H, OMe, OMe, OMe), 3.57 – 3.51 (m, 1H, H-3'') 3.48 (dd, J = 8.9, 8.9 Hz, 1H, H-6''), 3.36 – 3.29 (m, 1H, H-6''), 2.27 – 2.04 (m, 2H, H-7'', H-5''), 1.91 – 1.78 (m, 1H, H-7''), 1.61 – 1.42 (m, 9H, $C(CH_3)_3$, $C(CH_3)_3$, $C(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 160.3 (C=O uracil), 159.4, 159.3, 159.3 ($C_{q\text{-}arom}$), 155.5 (d, *J* = 4.4 Hz, C=O cyclic carbamate), 152.1 (C=O carbamate), 152.0 (C=O uracil), 148.7, 147.7 (C=O carbonate), 139.4 (C-6), 130.8, 130.2, 129.9 ($C_{q\text{-}arom}$), 129.6, 129.6, 129.3, 114.0, 113.9, 113.8 (CH_{arom}), 103.3 (C-5), 86.9 ($C(CH_3)_3$), 86.3 (C-1'), 83.7, 83.7 ($C(CH_3)_3$), 81.0 (C-3''), 80.8 (d, *J* = 9.7 Hz, C-2''), 80.0 (d, *J* = 7.9 Hz, C-4'), 74.7 (C-2'), 74.4 (CH_2 PMB), 73.9 (C-4''), 73.1, 72.0 (CH_2 PMB), 71.8 (C-3'), 69.9 (C-6''), 66.9 (d, *J* = 6.1 Hz, C-5'), 56.8 (d, *J* = 4.4 Hz, C-1''), 55.4, 55.4, 55.3 (OMe), 54.8 (d, *J* = 5.8 Hz, P(O)OMe), 36.0 (C-5''), 27.8, 27.7, 27.7 ($C(CH_3)_3$), 24.4 (C-7''); ^{31}P NMR (202 MHz, $CDCl_3$): δ -1.65; HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{57}H_{74}N_3NaO_{22}P$ 1206.4394; Found 1206.4383.

1'',2''-(*O*,*N*-(*N*-(*Tert*-butoxycarbonyl)-5'-*O*-(methylphosphinyl)-2',3'-di-*O*-(*tert*-butoxycarbonyl)uridinyl))-carbamate-3'',4'',6''-tri-*O*-(4-methoxybenzyl)-carba-α-D-galactopyranoside (43).



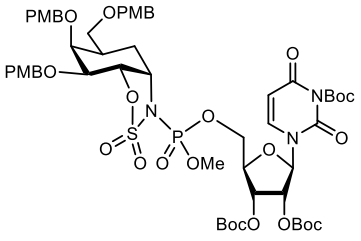
Compound **43** was prepared according to general procedure D using **37** (39 mg, 69 µmol), 15-crown-5 ether (69 µL, 0.35 mmol, 5.0 eq.) and NaH (60 wt.% dispersion in mineral oil; 8.3 mg, 0.21 mmol, 3.0 eq.) in anhydrous THF (0.35 mL, 0.20 M); and **23** (0.13 g, 0.21 mmol, 3.0 eq.) DiPEA (distilled and stored over KOH; 48 µL, 0.28 mmol, 4.0 eq.) and $BrCCl_3$ (41 µL, 0.42 mmol, 6.0 eq.) in anhydrous DCM (0.72 mL, 0.30 M). Full conversion was observed on TLC (R_f 0.6 (EtOAc:pentane, 6:4 v:v)) and ^{31}P NMR. Flash column chromatography (SiO_2, 30:70 EtOAc:pentane → 70:30 EtOAc:pentane) and Sephadex™ LH-20 size exclusion chromatography yielded the title compound **43** as a mixture of P(V) diastereomers (42 mg, 36 µmol, 42%).

Data for first P(V) diastereomer: ^1H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.65 (d, *J* = 8.3 Hz, 1H, H-6), 7.42 – 7.35 (m, 2H, CH_{arom}), 7.25 – 7.19 (m, 2H, CH_{arom}), 7.14 – 7.08 (m, 2H, CH_{arom}), 6.90 – 6.85 (m, 4H, CH_{arom}), 6.83 – 6.78 (m, 2H, CH_{arom}), 6.13 – 6.08 (m, 1H, H-1'), 5.82 (d, *J* = 8.3 Hz, 1H, H-5), 5.20 – 5.13 (m, 1H, H-3'), 5.11 (dd, *J* = 5.5, 5.5 Hz, 1H, H-2'), 4.83 – 4.71 (m, 3H, H-1'', C*H*H PMB, C*H*H PMB), 4.54 – 4.47 (m, 1H, CH*H* PMB), 4.43 – 4.31 (m, 4H, H-2'', C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.30 – 4.24 (m, 1H, H-5'), 4.19 – 4.16 (m, 3H, H-4'', H-4', H-5'), 3.81 – 3.69 (m, 12H, OMe, OMe, OMe, P(O)OMe), 3.50 – 3.44 (m, 1H, H-3''), 3.43 – 3.34 (m, 1H, H-6''), 3.31 – 3.24 (m, 1H, H-6''), 2.11 – 1.99 (m, 1H, H-5''), 1.97 – 1.87 (m, 1H, H-7''), 1.80 – 1.75 (m, 1H, H-7''), 1.59 (s, 9H, $C(CH_3)_3$), 1.48 (s, 9H, $C(CH_3)_3$), 1.45 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 160.3 (C=O uracil), 159.4, 159.3, 159.3($C_{q\text{-}arom}$), 155.7 (d, *J* = 8.6 Hz, C=O cyclic carbamate), 152.1 (C=O carbamate), 151.9 (C=O uracil), 148.7, 147.7 (C=O carbonate), 139.3 (C-6), 130.8, 130.2, 129.7 ($C_{q\text{-}arom}$), 129.6, 129.6, 129.4, 114.0, 113.9, 113.7 (CH_{arom}), 103.1 (C-5), 86.9 ($C(CH_3)_3$), 86.5 (C-1'), 83.7, 83.6 ($C(CH_3)_3$), 83.3 (C-3''), 80.1 (d, *J* = 8.4 Hz, C-4'), 78.4 (d, *J* = 7.9 Hz, C-2''), 74.8 (C-2'), 74.1, 72.9 (CH_2 PMB), 71.9 (C-3'), 71.4 (CH_2 PMB), 71.1 (C-4''), 69.8 (C-6''), 66.8 (d, *J* = 5.2 Hz, C-5'), 61.0 (d, *J*

= 2.7 Hz, C-1''), 55.4, 55.4, 55.4 (OMe), 55.2 (d, *J* = 5.5 Hz, P(O)OMe), 35.9 (C-5''), 27.7, 27.7, 27.5 (C(*C*H$_3$)$_3$), 24.1 (C-7''); ^{31}P NMR (202 MHz, CDCl$_3$): δ -0.78.

Data for second P(V) diastereomer: ^{1}H NMR (500 MHz, CDCl$_3$, HH-COSY, HSQC): δ 7.55 (d, *J* = 8.3 Hz, 1H, H-6), 7.42 – 7.35 (m, 2H, CH$_{arom}$), 7.25 – 7.19 (m, 2H, CH$_{arom}$), 7.14 – 7.08 (m, 2H, CH$_{arom}$), 6.90 – 6.85 (m, 4H, CH$_{arom}$), 6.83 – 6.78 (m, 2H, CH$_{arom}$), 6.13 – 6.08 (m, 1H, H-1'), 5.79 (d, *J* = 8.2 Hz, 1H, H-5), 5.23 (dd, *J* = 5.5, 4.4 Hz, 1H, H-3'), 5.20 – 5.13 (m, 1H, H-2'), 4.83 – 4.71 (m, 3H, H-1'', C*H*H PMB, C*H*H PMB), 4.54 – 4.47 (m, 1H, CH*H* PMB), 4.43 – 4.31 (m, 3H, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.30 – 4.24 (m, 3H, H-2'', H-5'), 4.24 – 4.19 (m, 2H, H-4', H-4''), 3.81 – 3.69 (m, 12H, OMe, OMe, OMe, P(O)OMe), 3.50 – 3.44 (m, 1H, H-3''), 3.43 – 3.34 (m, 1H, H-6''), 3.31 – 3.24 (m, 1H, H-6''), 2.11 – 1.99 (m, 1H, H-5''), 1.97 – 1.87 (m, 1H, H-7''), 1.80 – 1.75 (m, 1H, H-7''), 1.58 (s, 9H, C(CH$_3$)$_3$), 1.46 (s, 9H, C(CH$_3$)$_3$), 1.44 (s, 9H, C(CH$_3$)$_3$); ^{13}C NMR (126 MHz, CDCl$_3$, HSQC): δ 160.2 (C=O uracil), 159.4, 159.3, 159.3(C$_{q\text{-}arom}$), 155.8 (d, *J* = 8.6 Hz, C=O cyclic carbamate), 152.2 (C=O carbamate), 152.1 (C=O uracil), 148.6, 147.6 (C=O carbonate), 139.4 (C-6), 130.9, 130.2, 129.9 (C$_{q\text{-}arom}$), 129.5, 129.4, 129.0, 114.0, 113.9, 113.8 (CH$_{arom}$), 103.1 (C-5), 87.0 (*C*(CH$_3$)$_3$), 86.7 (C-1'), 83.8, 83.6 (*C*(CH$_3$)$_3$), 83.5 (C-3''), 80.0 (d, *J* = 7.2 Hz, C-4'), 78.3 (d, *J* = 7.9 Hz, C-2''), 74.8 (C-2'), 74.0, 72.9 (CH$_2$ PMB), 72.0 (C-3'), 71.3 (CH$_2$ PMB), 70.8 (C-4''), 69.9 (C-6''), 66.2 (d, *J* = 4.8 Hz, C-5'), 60.9 (d, *J* = 3.4 Hz, C-1''), 55.4 (d, *J* = 5.8 Hz, P(O)OMe), 55.4, 55.4, 55.3 (OMe), 35.8 (C-5''), 27.7, 27.7, 27.5 (C(*C*H$_3$)$_3$), 24.1 (C-7''); ^{31}P NMR (202 MHz, CDCl$_3$): δ -0.38; HRMS (ESI) m/z: [M+Na]$^+$ Calcd for $C_{57}H_{74}N_3NaO_{22}P$ 1206.4394; Found 1206.4380.

1'',2''-(*N*-(*N*-(*Tert*-butoxycarbonyl)-5'-*O*-(methylphosphinyl)-2',3'-di-*O*-(*tert*-butoxycarbonyl)uridinyl),*O*)-sulfamidate-3'',4'',6''-tri-*O*-(4-methoxybenzyl)-carba-α-D-galactopyranoside (42).

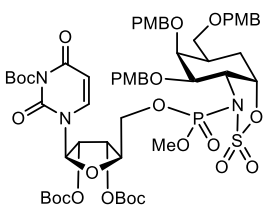


Compound **42** was prepared according to general procedure D using **38** (64 mg, 0.11 mmol), 15-crown-5 ether (0.11 mL, 0.55 mmol, 5.0 eq.) and NaH (60 wt.% dispersion in mineral oil; 6.8 mg, 0.17 mmol, 1.5 eq.) in anhydrous THF (0.55 mL, 0.20 M); and **23** (0.14 g, 0.22 mmol, 2.0 eq.) DiPEA (distilled and stored over KOH; 77 µL, 0.44 mmol, 4.0 eq.) and BrCCl$_3$ (65 µL, 0.66 mmol, 6.0 eq.) in anhydrous DCM (0.73 mL, 0.30 M). Full conversion was observed on TLC (R$_f$ 0.6 (EtOAc:pentane, 6:4 v:v)) and ^{31}P NMR. Flash column chromatography (SiO$_2$, 30:70 EtOAc:pentane → 70:30 EtOAc:pentane) and Sephadex™ LH-20 size exclusion chromatography yielded the title compound **42** as a mixture of P(V) diastereomers (36 mg, 30 µmol, 27%).

Data for first P(V) diastereomer: ^{1}H NMR (600 MHz, CDCl$_3$, HH-COSY, HSQC): δ 7.54 (d, *J* = 8.3 Hz, 1H, H-6), 7.32 – 7.25 (m, 2H, CH$_{arom}$), 7.21 – 7.12 (m, 4H, CH$_{arom}$), 6.92 – 6.81 (m, 6H, CH$_{arom}$), 6.22 (d, *J* = 7.4 Hz, 1H, H-1'), 5.87 (d, *J* = 8.3 Hz, 1H, H-5), 5.45 (dd, *J* = 5.1, 2.5 Hz, 1H, H-3'), 5.13 – 5.09 (m, 1H, H-2'), 5.05 – 4.99 (m, 1H, H-2''), 4.80 (d, *J* = 11.0 Hz, 1H, C*H*H PMB), 4.73 – 4.62 (m, 3H, H-1'', C*H*H PMB, CH*H* PMB), 4.46 (d, *J* = 10.9 Hz, 1H, CH*H* PMB), 4.34 – 4.29 (m, 1H, C*H*H PMB), 4.29 – 4.23 (m, 2H, H-3'', CH*H* PMB), 4.14 – 4.05 (m, 2H, H-4'', H-5'), 4.04 – 3.99 (m, 1H, H-4'), 3.98 – 3.87 (m, 1H, H-5'), 3.97 (d, *J* = 11.7 Hz, 3H, P(O)OMe), 3.83 – 3.78 (m, 9H, OMe, OMe, OMe), 3.57 (dd, *J* = 8.4, 5.6 Hz, 1H, H-6''), 3.35 (dd, *J* = 8.6, 8.6 Hz, 1H, H-6''), 2.52 (ddd, *J* = 15.7, 3.5, 3.5 Hz,

1H, H-7''), 2.30 – 2.16 (m, 1H, H-5''), 1.94 (ddd, *J* = 16.1, 12.2, 4.2 Hz, 1H, H-7''), 1.59 (s, 9H, $C(CH_3)_3$), 1.53 (s, 9H, $C(CH_3)_3$), 1.45 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (151 MHz, $CDCl_3$, HSQC): δ 160.3 (C=O uracil), 159.4, 159.3, 159.3 ($C_{q\text{-}arom}$), 152.2 (C=O carbamate), 152.2 (C=O uracil), 148.9, 147.7 (C=O carbonate), 139.3 (C-6), 130.6), 130.2, 130.2 ($C_{q\text{-}arom}$), 129.8, 129.7, 129.2, 113.9, 113.9, 113.8 (CH_{arom}), 103.6 (C-5), 87.0 ($C(CH_3)_3$), 86.2 (d, *J* = 9.1 Hz, C-2''), 84.7 (C-1'), 83.5, 83.4 ($C(CH_3)_3$), 79.8 (d, *J* = 7.9 Hz, C-4'), 79.5 (C-3''), 75.4 (C-4''), 74.3 (CH_2 PMB), 74.0 (C-2'), 73.2, 73.0 (CH_2 PMB), 72.4 (C-3'), 70.9 (C-6''), 66.5 (d, *J* = 4.5 Hz, C-5'), 60.6 (d, *J* = 4.1 Hz, C-1''), 57.0 (d, *J* = 5.5 Hz, P(O)OMe), 55.4, 55.4, 55.4 (OMe), 36.1 (C-5''), 27.9, 27.8, 27.5 ($C(CH_3)_3$), 24.1 (C-7''); ^{31}P NMR (121 MHz, $CDCl_3$): δ -1.69. Data for second P(V) diastereomer: ^{1}H NMR (600 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.66 (d, *J* = 8.3 Hz, 1H, H-6), 7.32 – 7.25 (m, 2H, CH_{arom}), 7.21 – 7.12 (m, 4H, CH_{arom}), 6.92 – 6.81 (m, 6H, CH_{arom}), 6.15 (d, *J* = 6.1 Hz, 1H, H-1'), 5.87 (d, *J* = 8.3 Hz, 1H, H-5), 5.31 (dd, *J* = 5.4, 4.0 Hz, 1H, H-3'), 5.13 – 5.09 (m, 1H, H-2'), 5.05 – 4.99 (m, 1H, H-2''), 4.79 (d, *J* = 10.7 Hz, 1H, C*H*H PMB), 4.73 – 4.62 (m, 2H, C*H*H PMB, CH*H* PMB), 4.52 (ddd, *J* = 4.4, 4.1, 4.1 Hz, 1H, H-1''), 4.42 (m, 2H, CH*H* PMB, H-5'), 4.36 (d, *J* = 11.1 Hz, 1H, C*H*H PMB), 4.34 – 4.29 (m, 1H, H-5'), 4.29 – 4.23 (m, 2H, H-4', CH*H* PMB), 4.14 – 4.05 (m, 2H, H-3'', H-4''), 3.92 (d, *J* = 11.8 Hz, 3H, P(O)OMe), 3.83 – 3.78 (m, 9H, OMe, OMe, OMe), 3.44 (dd, *J* = 8.2, 8.2 Hz, 1H, H-6''), 3.30 (dd, *J* = 8.7, 6.6 Hz, 1H, H-6''), 2.30 – 2.16 (m, 2H, H-7'', H-5''), 1.85 (ddd, *J* = 15.4, 11.6, 4.2 Hz, 1H, H-7''), 1.59 (s, 9H, $C(CH_3)_3$), 1.50 (s, 9H, $C(CH_3)_3$), 1.47 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (151 MHz, $CDCl_3$, HSQC): δ 160.3 (C=O uracil), 159.4, 159.4, 159.3 ($C_{q\text{-}arom}$), 152.1 (C=O carbamate), 152.0 (C=O uracil), 148.7, 147.7 (C=O carbonate), 139.3 (C-6), 130.6, 130.2, 130.0 ($C_{q\text{-}arom}$), 129.7, 129.7, 129.5, 114.0, 113.9, 113.8 (CH_{arom}), 103.4 (C-5), 86.9 ($C(CH_3)_3$), 86.0 (C-1'), 85.8 (d, *J* = 9.7 Hz, C-2''), 83.7, 83.7 ($C(CH_3)_3$), 79.8 (d, *J* = 7.9 Hz, C-4'), 79.0 (C-3''), 74.6 (C-4''), 74.6 (C-2'), 74.5, 73.1, 73.0 (CH_2 PMB), 71.7 (C-3'), 69.9 (C-6''), 67.1 (d, *J* = 5.5 Hz, C-5'), 60.1 (d, J = 2.8 Hz, C-1''), 55.9 (d, *J* = 5.7 Hz, P(O)OMe), 55.4, 55.4, 55.4 (OMe), 35.7 (C-5''), 27.8, 27.7, 27.5 ($C(CH_3)_3$), 24.0 (C-7''); ^{31}P NMR (121 MHz, $CDCl_3$): δ -2.50; HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{56}H_{74}N_3NaO_{23}PS$ 1242.4064; Found 1242.4057.

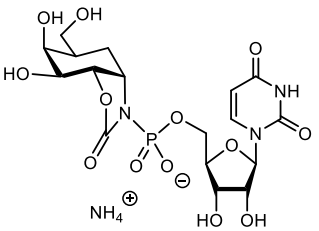
1'',2''-(*O*,*N*-(*N*-(*Tert*-butoxycarbonyl)-5'-*O*-(methylphosphinyl)-2',3'-di-*O*(*tert*-butoxycarbonyl)uridinyl))-sulfamidate-3'',4'',6''-tri-*O*-(4-methoxybenzyl)-carba-α-D-galactopyranoside (44).



Compound **44** was prepared according to general procedure D using **39** (67 mg, 0.11 mmol), 15-crown-5 ether (0.11 mL, 0.55 mmol, 5.0 eq.) and NaH (60 wt.% dispersion in mineral oil; 6.6 mg, 0.17 mmol, 1.5 eq.) in anhydrous THF (0.55 mL, 0.20 M); and **23** (0.14 g, 0.22 mmol, 2.0 eq.) DiPEA (distilled and stored over KOH; 77 µL, 0.44 mmol, 4.0 eq.) and $BrCCl_3$ (65 µL, 0.66 mmol, 6.0 eq.) in anhydrous DCM (0.73 mL, 0.30 M). Full conversion was observed on TLC (R_f 0.6 (EtOAc:pentane, 6:4 v:v)) and ^{31}P NMR. Flash column chromatography (SiO_2, 30:70 EtOAc:pentane → 70:30 EtOAc:pentane) and Sephadex™ LH-20 size exclusion chromatography yielded the title compound **44** as a mixture of P(V) diastereomers (44 mg, 36 µmol, 33%).
Data for first P(V) diastereomer: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.58 (d, *J* = 8.3 Hz, 1H, H-6), 7.42 – 7.37 (m, 2H, CH_{arom}), 7.23 – 7.17 (m, 2H, CH_{arom}), 7.15 – 7.07 (m, 2H, CH_{arom}), 6.92 – 6.85 (m, 4H, CH_{arom}), 6.84 – 6.77 (m, 2H, CH_{arom}), 6.16 (d, *J* = 5.9 Hz, 1H, H-1'), 5.81 (d, *J* = 8.2 Hz, 1H, H-5), 5.31 – 5.24 (m, 2H, H-3', H-1''), 5.20 – 5.12 (m, 1H, H-2'), 4.81 – 4.71 (m, 2H, C*H*H PMB,

C*H*H PMB), 4.62 (d, *J* = 11.3 Hz, 1H, CH*H* PMB), 4.59 – 4.52 (m, 2H, H-2'', CH*H* PMB), 4.47 – 4.29 (m, 6H, H-5', H-5', H-4', C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.18 – 4.11 (m, 1H, H-4''), 3.96 – 3.85 (m, 1H, H-3''), 3.80 – 3.74 (m, 12H, OMe, OMe, OMe, P(O)OMe), 3.41 – 3.33 (m, 1H, H-6''), 3.29 – 3.23 (m, 1H, H-6''), 2.17 – 2.08 (m, 1H, H-5''), 2.01 – 1.93 (m, 1H, H-7''), 1.90 – 1.81 (m, 1H, H-7''), 1.59 (s, 9H, $C(CH_3)_3$), 1.46 (s, 9H, $C(CH_3)_3$), 1.43 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 160.3 (C=O uracil), 159.4, 159.3, 159.3 ($C_{q\text{-}arom}$), 152.1 (C=O carbamate), 152.1 (C=O uracil), 148.6, 147.6 (C=O carbonate), 139.3 (C-6), 130.7, 130.1, 130.0 ($C_{q\text{-}arom}$), 129.8, 129.5, 128.8, 114.0, 114.0, 113.9 (CH_{arom}), 103.2 (C-5), 86.9 ($C(CH_3)_3$), 86.1 (C-1'), 85.0 (d, *J* = 4.4 Hz, C-2''), 83.8, 83.7 ($C(CH_3)_3$), 80.5 (C-3''), 79.8 (C-4'), 74.8 (C-2'), 74.2, 72.9 (CH_2 PMB), 72.2 (C-4''), 72.1 (CH_2 PMB), 71.7 (C-3'), 69.6 (C-6''), 66.5 (d, *J* = 5.4 Hz, C-5'), 64.6 (d, *J* = 1.4 Hz, C-1''), 55.5 (d, *J* = 5.8 Hz, P(O)OMe), 55.4, 55.4 (OMe), 35.6 (C-5''), 27.7, 27.7, 27.5 ($C(CH_3)_3$, 24.8 (C-7''); ^{31}P NMR (202 MHz, $CDCl_3$): δ -1.25. Data for second P(V) diastereomer: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.61 (d, *J* = 8.3 Hz, 1H, H-6), 7.37 – 7.31 (m, 2H, CH_{arom}), 7.23 – 7.17 (m, 2H, CH_{arom}), 7.15 – 7.07 (m, 2H, CH_{arom}), 6.92 – 6.85 (m, 4H, CH_{arom}), 6.84 – 6.77 (m, 2H, CH_{arom}), 6.14 (d, *J* = 5.9 Hz, 1H, H-1'), 5.85 (d, *J* = 8.2 Hz, 1H, H-5), 5.20 – 5.12 (m, 2H, H-3', H-1''), 5.06 (dd, *J* = 5.7, 5.7 Hz, 1H, H-2'), 4.81 – 4.71 (m, 2H, C*H*H PMB, C*H*H PMB), 4.47 – 4.29 (m, 4H, H-2'', C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.27 – 4.20 (m, 1H, H-5'), 4.18 – 4.11 (m, 3H, H-4'', H-4', H-5'), 3.96 – 3.85 (m, 1H, H-3''), 3.80 – 3.74 (m, 12H, OMe, OMe, OMe, P(O)OMe), 3.41 – 3.33 (m, 1H, H-6''), 3.29 – 3.23 (m, 1H, H-6''), 2.17 – 2.08 (m, 1H, H-5''), 2.01 – 1.93 (m, 1H, H-7''), 1.90 – 1.81 (m, 1H, H-7''), 1.59 (s, 9H, $C(CH_3)_3$), 1.48 (s, 9H, $C(CH_3)_3$), 1.45 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 160.3 (C=O uracil), 159.4, 159.4, 159.3 ($C_{q\text{-}arom}$), 152.1 (C=O carbamate), 151.9 (C=O uracil), 148.7, 147.7 (C=O carbonate), 139.3 (C-6), 130.6, 130.1, 129.7 ($C_{q\text{-}arom}$), 129.6, 129.5, 129.0, 114.0, 113.8, 113.7 (CH_{arom}), 103.4 (C-5), 86.9 ($C(CH_3)_3$), 85.9 (C-1'), 84.3 (d, *J* = 4.8 Hz, C-1''), 83.7, 83.6 ($C(CH_3)_3$), 80.4 (C-3''), 79.7 (d, *J* = 1.7 Hz, C-4'), 74.7 (C-2'), 74.3, 72.9 (CH_2 PMB), 72.2 (C-4''), 71.8 (C-3'), 71.8 (CH_2 PMB), 69.5 (C-6''), 66.5 (d, *J* = 6.0 Hz, C-5'), 64.5 (d, *J* = 2.4 Hz, C-2''), 55.78 (d, *J* = 5.8 Hz, P(O)OMe), 55.4, 55.3 (OMe), 35.6 (C-5''), 27.7, 27.5, 27.5 C($CH_3)_3$), 24.8 (C-7''); ^{31}P NMR (202 MHz, $CDCl_3$): δ -1.88; HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{56}H_{74}N_3NaO_{23}PS$ 1242.4064; Found 1242.4051.

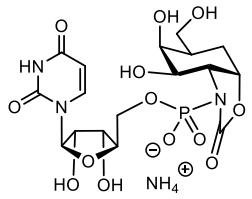
1'',2''-(*N*-(5'-*O*-Phosphoryluridinyl),*O*)-carbamate-carba-α-D-galactopyranoside (6).



Compound **6** was prepared according to general procedure E using **41** (77 mg, 65 µmol), TES (0.10 mL, 0.65 mmol, 10 eq.) and TFA (0.56 mL, 7.2 mmol, 30% v:v) in DCM (1.3 mL, 0.05 M). The reaction mixture was stirred overnight at 0°C, before full conversion was observed on TLC and pyridine (12 mL, 0.15 mol, 20:1 pyridine:TFA) was added. The reaction mixture was stirred overnight at 35°C, before full conversion was observed on TLC (R_f 0.5 (H_2O:ACN, 2:8 v:v)) and ^{31}P NMR. Flash column chromatography (neutralized SiO_2, dry loading on Celite, 0:100 H_2O:ACN → 15:85 H_2O:ACN), NH_4^+-Dowex® 50WX4 ion exchange and lyophilization yielded the title compound **6** (10 mg, 19 µmol, 30%). ^{1}H NMR (600 MHz, D_2O, HH-COSY, HSQC): δ 7.94 (d, *J* = 8.1 Hz, 1H, H-6), 5.92 (d, *J* = 4.7 Hz, 1H, H-1'), 5.89 (d, *J* = 8.1 Hz, 1H, H-5), 4.59 – 4.56 (m, 1H, H-2''), 4.41 (ddd, *J* = 7.7, 4.1, 4.1 Hz, 1H, H-1''), 4.32 (dd, *J* = 5.0, 5.0 Hz, 1H, H-2'), 4.28 (dd, *J* = 5.0, 5.0 Hz, 1H, H-3'), 4.25 – 4.21 (m, 1H, H-4'), 4.19 – 4.11 (m, 2H, H-5'), 4.07 (dd, *J* = 2.6, 2.6 Hz, 1H, H-4''), 3.73 (dd, *J* = 8.0, 2.8 Hz, 1H, H-3''), 3.59 (dd, *J* = 11.1, 8.2 Hz, 1H, H-

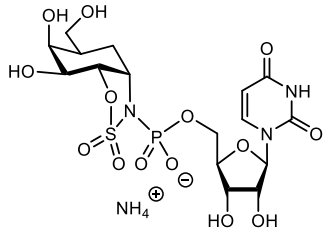
6''), 3.50 (dd, *J* = 11.1, 6.1 Hz, 1H, H-6''), 2.37 (ddd, *J* = 15.4, 4.2, 4.2 Hz, 1H, H-7''), 1.94 (dddd, *J* = 14.9, 10.8, 5.5, 2.3 Hz, 1H, H-5''), 1.67 (ddd, *J* = 15.9, 11.6, 4.8 Hz, 1H, H-7''); ^{13}C NMR (151 MHz, D_2O, HSQC): δ 167.2 (C=O uracil), 160.1 (d, *J* = 8.2 Hz, C=O carbamate), 152.7 (C=O uracil), 142.8 (C-6), 103.3 (C-5), 89.7 (C-1'), 83.8 (d, *J* = 9.0 Hz, C-4'), 80.2 (d, *J* = 8.3 Hz, C-2''), 74.7 (C-2'), 73.8 (C-3''), 70.4 (C-3'), 69.6 (C-4''), 65.6 (d, *J* = 3.1 Hz, C-5'), 62.8 (C-6''), 58.0 (d, *J* = 9.7 Hz, C-1''), 37.5 (C-5''), 23.1 (C-7''); ^{31}P NMR (121 MHz, D_2O): δ -5.07; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{17}H_{24}N_3NaO_{13}P$ 527.1385; Found 527.1381.

1'',2''-(*O*,*N*-(5'-*O*-Phosphoryluridinyl))-carbamate-carba-α-D-galactopyranoside (8).



Compound **8** was prepared according to general procedure E using **43** (35 mg, 30 µmol), TES (48 µL, 0.30 mmol, 10 eq.) and TFA (0.26 mL, 3.3 mmol, 30% v:v) in DCM (0.60 mL, 0.05 M). The reaction mixture was stirred overnight at 0°C, before full conversion was observed on TLC and pyridine (5.4 mL, 67 mmol, 20:1 pyridine:TFA) was added. The reaction mixture was stirred overnight at 35°C, before full conversion was observed on TLC (R_f 0.5 (H_2O:ACN, 2:8 v:v)) and ^{31}P NMR. Flash column chromatography (neutralized SiO_2, dry loading on Celite, 0:100 H_2O:ACN → 15:85 H_2O:ACN), NH_4^+-Dowex® 50WX4 ion exchange and lyophilization yielded the title compound **8** (8.5 mg, 16 µmol, 54%). ^{1}H NMR (500 MHz, D_2O,HH-COSY, HSQC): δ 7.96 (d, *J* = 8.1 Hz, 1H, H-6), 5.98 (d, *J* = 4.9 Hz, 1H, H-1'), 5.96 (d, *J* = 8.1 Hz, 1H, H-5), 4.92 (ddd, *J* = 6.2, 4.3, 1.8 Hz, 1H, H-1''), 4.39 (dd, *J* = 5.1, 5.1 Hz, 1H, H-2'), 4.34 (dd, *J* = 5.0, 5.0 Hz, 1H, H-3'), 4.30 – 4.27 (m, 1H, H-4'), 4.27 – 4.20 (m, 2H, H-5'), 4.09 (ddd, *J* = 8.2, 6.4, 1.6 Hz, 1H, H-2''), 4.07 – 4.06 (m, 2H, H-4''), 3.77 (dd, *J* = 8.4, 2.7 Hz, 1H, H-3''), 3.68 (dd, *J* = 11.1, 7.6 Hz, 1H, H-6''), 3.59 (dd, *J* = 11.1, 6.4 Hz, 1H, H-6''), 2.06 (dd, *J* = 15.5, 4.2 Hz, 1H, H-7''), 2.00 – 1.92 (m, 1H, H-5''), 1.77 (ddd, *J* = 15.5, 13.2, 4.4 Hz, 1H, H-7''); ^{13}C NMR (126 MHz, D_2O, HSQC): δ 166.3 (C=O uracil), 158.5 (d, *J* = 8.2 Hz, C=O carbamate), 151.8 (C=O uracil), 141.9 (C-6), 102.6 (C-5), 88.9 (C-1'), 83.0 (d, *J* = 9.1 Hz, C-4'), 77.6 (d, *J* = 6.8 Hz, C-1''), 75.2 (C-3''), 73.7 (C-2'), 69.7 (C-3'), 68.1 (C-4''), 65.1 (d, *J* = 5.6 Hz, C-5') 62.4 (C-6''), 61.4 (d, *J* = 3.3 Hz, C-2''), 36.3 (C-5''), 22.6 (C-7''); ^{31}P NMR (202 MHz, D_2O): δ -4.51; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{17}H_{24}N_3NaO_{13}P$ 527.1385; Found 527.1382.

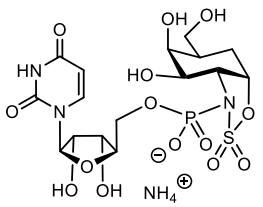
1'',2''-(*N*-(5'-*O*-Phosphoryluridinyl),*O*)-sulfamidate-carba-α-D-galactopyranoside (5).



Compound **5** was prepared according to general procedure E using **42** (36 mg, 30 µmol), TES (48 µL, 0.30 mmol, 10 eq.) and TFA (0.26 mL, 3.3 mmol, 30% v:v) in DCM (0.60 mL, 0.05 M). The reaction mixture was stirred for 3 h at 0°C, before full conversion was observed on TLC (R_f 0.1 (MeOH:DCM, 2:8 v:v)) and pyridine (5.4 mL, 67 mmol, 20:1 pyridine:TFA) was added. The reaction mixture was stirred overnight at room temperature, before full conversion was observed on TLC (R_f 0.7 (H_2O:ACN, 2:8 v:v)) and ^{31}P NMR. Flash column chromatography (neutralized SiO_2, dry loading on Celite, 0:100 H_2O:ACN → 15:85 H_2O:ACN), NH_4^+-Dowex® 50WX4 ion exchange and lyophilization yielded the title compound **5** (9.2 mg, 16 µmol, 55%). ^{1}H NMR (600 MHz, D_2O, HH-COSY, HSQC): δ 7.88 (d, *J* = 8.1 Hz, 1H, H-6), 5.93 (d, *J* = 4.5 Hz, 1H, H-1'), 5.91 (d, *J* = 8.1 Hz, 1H, H-5), 4.84 (dd, *J* = 8.1, 5.4 Hz, 1H, H-2''), 4.50 (ddd,

J = 6.0, 2.6, 2.6 Hz, 1H, H-1''), 4.33 (dd, *J* = 4.9, 4.9 Hz, 1H, H-2'), 4.29 (dd, *J* = 5.0, 5.0 Hz, 1H, H-3'), 4.26 – 4.21 (m, 3H, H-4', H-5'), 4.16 – 4.11 (m, 1H, H-4''), 4.12 (d, *J* = 2.9 Hz, 1H, H-3''), 3.54 (dd, *J* = 11.1, 8.7 Hz, 1H, H-6''), 3.44 (dd, *J* = 11.1, 5.5 Hz, 1H, H-6''), 2.19 (ddd, *J* = 15.6, 3.2, 3.2 Hz, 1H, H-7''), 1.99 (ddddd, *J* = 10.1, 7.5, 4.3, 4.3, 4.1 Hz, 1H, H-5''), 1.72 (ddd, *J* = 16.5, 13.0, 4.2 Hz, 1H, H-7''); ^{13}C NMR (151 MHz, D_2O, HSQC): δ 167.1, 152.6 (C=O uracil), 142.6 (C-6), 103.4 (C-5), 89.9 (C-1'), 85.8 (d, *J* = 8.5 Hz, C-2''), 83.5 (d, *J* = 8.6 Hz, C-4'), 74.5 (C-2'), 71.6 (C-3''), 70.2 (C-3'), 69.9 (C-4''), 65.9 (d, *J* = 5.6 Hz, C-5'), 62.4 (C-6''), 59.9 (d, *J* = 1.4 Hz, C-1''), 37.4 (C-5''), 23.3 (C-7''); ^{31}P NMR (202 MHz, D_2O): δ -6.88; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{16}H_{24}N_3NaO_{14}PS$ 563.1055; Found 563.1049.

1'',2''-(*O*,*N*-(5'-*O*-Phosphoryluridinyl))-sulfamidate-carba-α-D-galactopyranoside (7).



Compound **7** was prepared according to general procedure E using **44** (44 mg, 36 µmol), TES (58 µL, 0.36 mmol, 10 eq.) and TFA (0.31 mL, 4.0 mmol, 30% v:v) in DCM (0.72 mL, 0.05 M). The reaction mixture was stirred for 3 h at 0°C, before full conversion was observed on TLC and pyridine (6.4 mL, 80 mmol, 20:1 pyridine:TFA) was added. The reaction mixture was stirred overnight at room temperature, before full conversion was observed on TLC (R_f 0.6 (H_2O:ACN, 2:8 v:v)) and ^{31}P NMR. Flash column chromatography (neutralized SiO_2, dry loading on Celite, 0:100 H_2O:ACN → 15:85 H_2O:ACN), NH_4^+-Dowex® 50WX4 ion exchange and lyophilization yielded the title compound **7** (4.5 mg, 8.0 µmol, 22%). ^{1}H NMR (850 MHz, D_2O, HH-COSY, HSQC): δ 7.86 (d, *J* = 8.1 Hz, 1H, H-6), 5.94 (m, 2H, H-5, H-1'), 5.24 (ddd, *J* = 3.9, 3.9, 2.1 Hz, 1H, H-1''), 4.36 (dd, *J* = 5.4, 4.6 Hz, 1H, H-2'), 4.34 (dd, *J* = 5.1, 5.1 Hz, 1H, H-3'), 4.32 – 4.29 (m, H-5'), 4.26 – 4.24 (m, 1H, H-4'), 4.24 – 4.20 (m, 1H, H-5'), 4.07 (d, *J* = 2.4 Hz, 1H, H-4''), 4.06 – 4.03 (m, 1H, H-2''), 3.97 (dd, *J* = 9.0, 2.7 Hz, 1H, H-3''), 3.64 (dd, *J* = 11.1, 7.6 Hz, 1H, H-6''), 3.54 (dd, *J* = 11.1, 6.5 Hz, 1H, H-6''), 2.10 (ddd, *J* = 16.0, 3.2, 3.2 Hz, 1H, H-7''), 2.04 – 1.98 (m, 1H, H-5''), 1.80 (ddd, *J* = 15.9, 13.3, 3.6 Hz, 1H, H-7''); ^{13}C NMR (214 MHz, D_2O, HSQC): δ 166.8, 152.3 (C=O uracil), 142.2 (C-6), 103.3 (C-5), 89.5 (C-1'), 83.5 (d, *J* = 5.3 Hz, C-1''), 83.2 (d, *J* = 9.1 Hz, C-4'), 74.1 (C-2'), 73.7 (C-3''), 70.0 (C-3'), 68.8 (C-4''), 65.6 (d, *J* = 5.7 Hz, C-5'), 64.8 (C-2''), 62.7 (C-6''), 36.6 (C-5''), 23.7 (C-7''); ^{31}P NMR (202 MHz, D_2O): δ -6.51; HRMS (ESI) m/z: $[M+H]^+$ Calcd for $C_{16}H_{24}N_3NaO_{14}PS$ 563.1055; Found 563.1050.

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Chapter 7

Synthesis of UDP-Glc and UDP-Gal mimetics as putative glycosyl transferase inhibitors – Part II

Introduction

As described in chapter 6, glycosyltransferase (GT) donor substrates exhibit a high degree of rotational freedom.[1] In contrast, crystallographic data of donor substrates in Michaelis complex with corresponding GTs suggest donor substrates to be limited to just four distinct binding conformations when bound within a GT active site.[2–5] These binding orientations range from a linear to a "tucked under" conformation, each stabilized by different interactions within the pocket. Although the donor binding orientation appears crucial for the enzymes' ability to accept donor mimetics,[1,2] inducing conformational bias has been ignored in studies aimed at the design of GT inhibitors.[6–10] It has been postulated that the strategic design of inhibitors with the ability to match the exact binding orientation within the binding pocket could considerably increase enzyme active site binding. Besides an expected increase in the binding affinity, conformationally constrained donor substrate analogues may display GT selectivity as well, especially considering GTs that transfer the same glycosidase but employ different donor substrate binding modes.

Chapter 6 describes the design and synthesis of eight putative glucosyl- and galactosyl transferase inhibitors. These compounds are composed of a bicyclic scaffold with pyrophosphate mimetics related to the ones described by Montero *et al*.[11] and Grimes *et al*.[12], appended to the 1,2-position of the glucose- or galactose configured carbasugar backbone, linked to a uridine 5'monophosphate (UMP) (Figure 1A). In some of these structures the UMP moiety is forced in a tucked-under conformation, mimicking the concaved spatial arrangement observed for the natural substrates of several glucosyl- and galactosyl transferases.[1–5,13–15]

This chapter builds on the design and synthetic methodologies described in chapter 6 (Figure 1B) to expand on the set of putative glucosyltransferase- and galactosyltransferase inhibitors. It was envisioned that fusing the UDP-mimetic structural element at C1 and C7 (cyclophellitol numbering) of the carbasugar would yield a complementary set of putative inhibitors that still exhibit a concaved spatial arrangement.

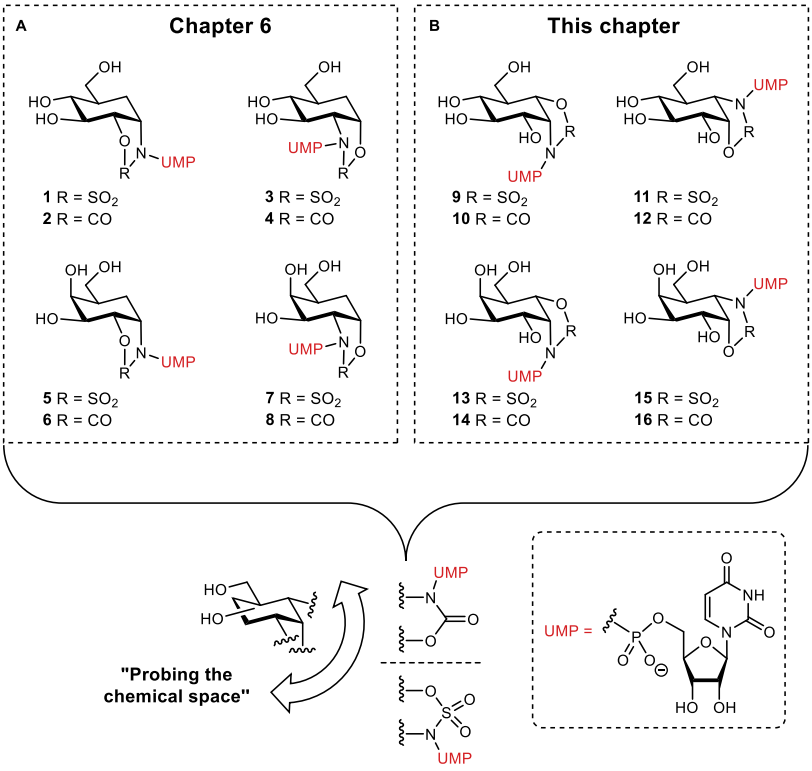


Figure 1. Overview of four glucose- and four galactose configured putative inhibitors (**1** – **8**) proposed and synthesized in chapter 6 for UDP-glucosyl- and UDP galactosyl transferases. (B) four

glucose configured and four galactose configured putative inhibitors (**9** – **16**) proposed in this chapter. In red the uridine 5'-monophosphate (UMP) moiety.

Following the synthetic methodology described in chapter 6, 1,7-cyclic carbamate and -sulfamidate carbasugars **17** – **24** are considered as viable constructs to undergo an Atherton-Todd coupling reaction (Figure 2).[16–22] In turn, the 1,7-cyclic carbamates and sulfamidates are envisioned to be accessible *via* the in chapter 6 described stereoselective Sharpless aminohydroxylation on the corresponding cyclohexene.[23,24] Ideally providing an equimolar regiomeric ratio of the desired α-*cis*-amino alcohols. The glucose- and galactose configured cyclohexenes are easily accessible *via* modified literature procedures.[25,26]

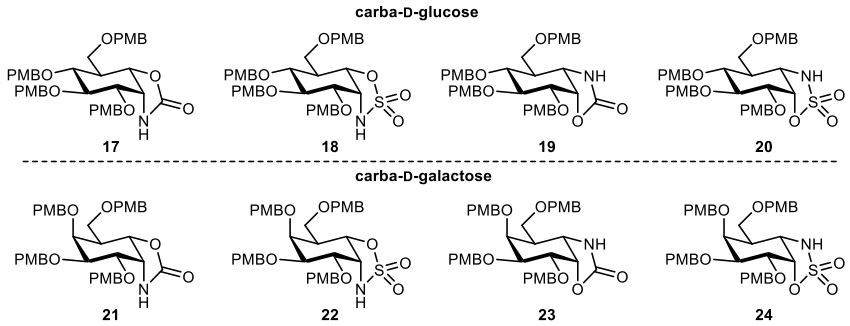


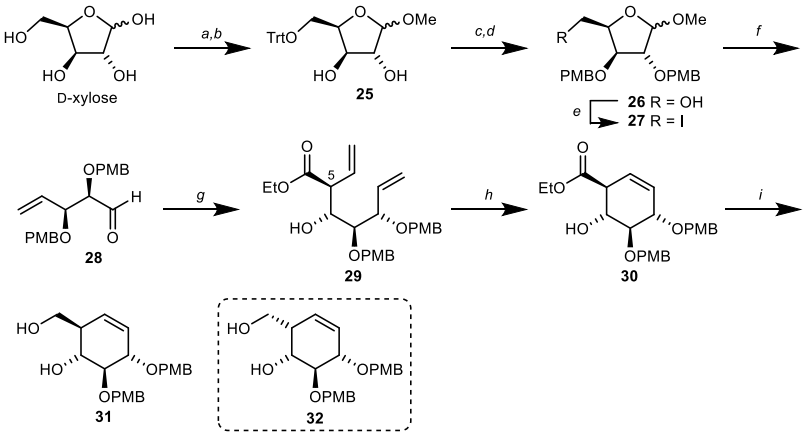
Figure 2. Four carba-glucose- and four carba-galactose configured constructs considered to be suitable amide coupling fragments in an Atherton-Todd type coupling reaction.

Results and discussion

The synthesis of the glucose configured constructs commenced from commercially available D-xylose (Scheme 1). Following modified literature procedures,[25] D-xylose was subjected to kinetic Fischer glycosylation conditions after which the primary hydroxyl of the obtained methyl-xylofuranoside intermediate was protected as a trityl ether to quantitatively yield compound **25**. The secondary hydroxyls in **25** were masked as PMB ethers under standard Williamson etherification conditions (PMBCl, NaH)[27] followed by *p*-TsOH-mediated removal of the trityl group to yield intermediate **26** in 73% yield. Compound **26** was then subjected to an Appel-like reaction to yield iodide **27** in near-quantitative yield (95%).[28] Compound **27** underwent Vasella fragmentation upon treatment with activated zinc and sonication to provide aldehyde **28** in 81% yield. Indium-mediated coupling with ethyl 4-bromocrotonate proceeded with high stereoselectivity and yielded **29** as major compound alongside trace amounts of the C-5 epimer which, at this stage, could not be separated. A ring-closing olefin metathesis, catalyzed by Grubbs-II ruthenium complex, provided glucuronic cyclohexene **30** in high yield (92%). At this stage removal of the C-5 epimeric side product (iduronic

cyclohexene) proved futile. However, after ester reduction using $LiBH_4$, glucose cyclohexene **31** could successfully be separated from idose cyclohexene **32.** In this way cyclohexene **31** was obtained in near quantitative yield together with trace amounts of L-ido congener **32**.

Scheme 1. Synthesis of PMB protected glucose cyclohexene **31** and idose configured cyclohexene **32**.



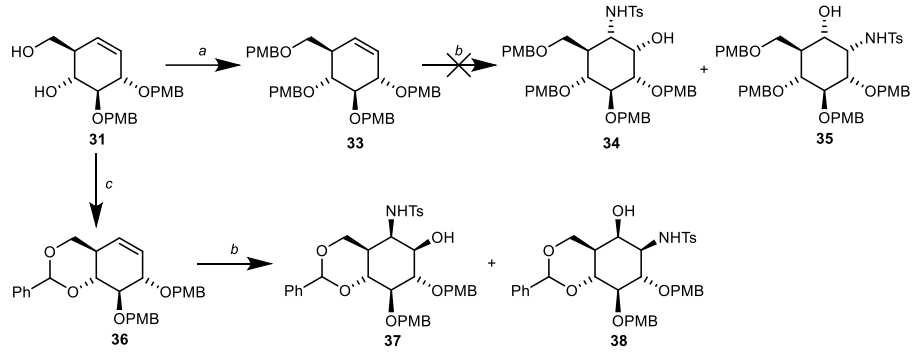
Reagents and conditions: a) AcCl, MeOH, rt, 6.5 h; *b)* TrCl, Et_3N, DMAP, DMF, 30 °C, 16 h (quant.); *c)* PMBCl, NaH, TBAI, DMF, rt, 16 h; *d) p*-TsOH, DCM:MeOH (1:1 v:v), rt, 16 h (73% over two steps); *e)* PPh_3, I_2, imidazole, THF, reflux, 3.5 h (95%); *f)* Zn, sonication, THF:H_2O (9:1 v:v), 40 °C, 12 h (81%); *g)* ethyl 4-bromocrotonate, In, $La(OTf)_3$, H_2O, rt, 5 days (89%); *h)* Grubbs-II, DCM, 38 °C, 2 days (92%); *i)* $LiBH_4$, THF, rt, 5 h, **31** (96%), **32** (2%).

With glucose cyclohexene **31** in hand, initial attempts on investigating the Sharpless aminohydroxylation could be undertaken (Scheme 2). Prior to this, the 4- and 6-hydroxyl were protected as PMB ethers under standard Williamson conditions which yielded fully protected cyclohexene **33** in 92% yield. Based on the results described in chapter 6, cyclohexene **33** was envisioned as a suitable substrate to undergo a Sharpless aminohydroxylation. However, exposure of cyclohexene **33** to aminohydroxylating conditions (chloramine-T hydrate, $K_2[OsO_2(OH)_4]$, 60 °C) did not result in any conversion.

In an alternative attempt, the 4- and 6-OH in cyclohexene **31** were protected as a benzylidene acetal, a known way to conformationally restrict the glucose moiety which could favorably influence the Sharpless aminohydroxylation. This influence can be attributed to the electronic, conformational, and steric effects induced by the rigid protecting group. In addition, benzylidene acetals are commonly removed under aqueous acid conditions, suggesting compatibility with the final deprotection sequences as described in chapter 6.[29–31] Although in low rates, indeed conversion was observed upon treatment of benzylidene protected cyclohexene **36** with the above

aminohydroxylating conditions. The reaction stagnated at roughly 30% conversion based on NMR analysis of the crude material. Purification of the crude resulted in the isolation of two compounds (**37** and **38**) of which NMR analysis allowed for assigning the newly introduced stereocenters to exhibit a *trans* orientation relative to C-2. Effectively, none of the desired *cis* product was observed.

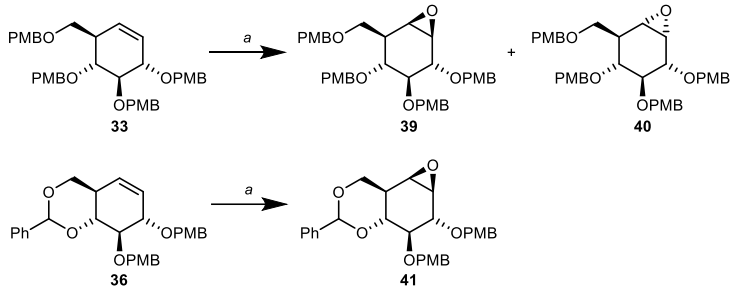
Scheme 2. Sharpless aminohydroxylation studies on glucose cyclohexene **33** and **36**.



Reagents and conditions: a) PMBCl, NaH, TBAI, DMF, rt, 16 h (92%); *b)* CAT·3H_2O, $K_2[OsO_2(OH)_4]$, TEBACl, $CHCl_3$:H_2O (1:1 v:v), 60 °C, 16 h, **37** (8.8%), **38** (5.2%); *c)* $PhCH(OMe)_2$, *p*-TsOH, DMF, rt, 4 h (85%).

In an alternative attempt towards the carbaglucose 1,7-α-*cis*-aminoalcohols, a methodology as described by Kok *et al*. was followed.[32] To this end, the formation of a cyclophellitol epoxide was required (Scheme 3). Treatment of cyclohexene **33** with *m*-CPBA resulted in sluggish product formation, stagnating at around 40% conversion. In addition, the epoxidation appeared not very stereoselective as both epoxides **39** and **40** were obtained as an inseparable mixture in 3:1 ratio. In contrast, treatment of the benzylidene protected cyclohexene **36** did not only increase reaction rate. In addition, the stereoselectivity of the reaction was drastically increased as cyclophellitol epoxide **41** was the only observed product.

Scheme 3. Epoxidation studies of glucose cyclohexenes **33** and **36**.

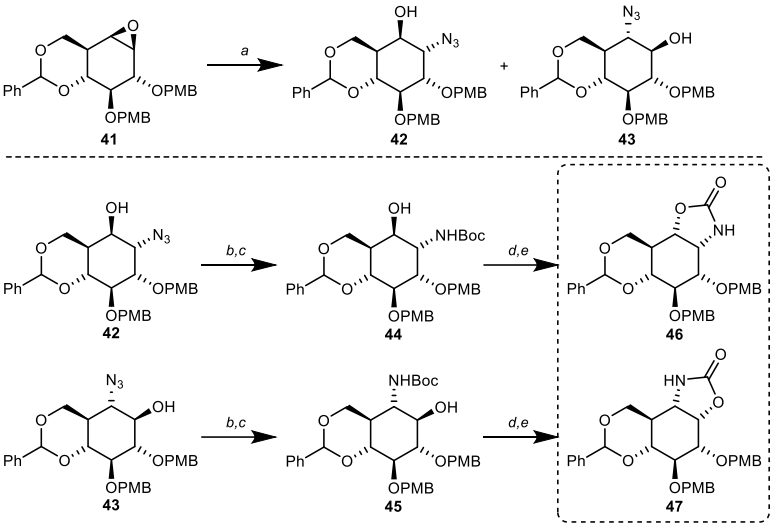


Reagents and conditions: a) m-CPBA, $NaHCO_3$, DCM, 5 °C, 4 days, **39** (32%), **40** (10%), **41** (56%, 70% based on recovered starting material).

The increased stereoselectivity could be attributed to the aforementioned conformational locking of the cyclohexene, effectively favoring the substrate for top-side attack.

Treatment of epoxide **41** with NaN_3 at elevated temperatures and aided by a Lewis acid ($LiClO_4$) resulted in clean conversion to a separable mixture of both regioisomers **42** and **43** in quantitative yield and in a 3:1 ratio respectively (Scheme 4). Both azides were subjected to Staudinger conditions to furnish the corresponding primary amines which were masked with a Boc protecting group to yield secondary carbamates **44** and **45** in a respective 66% and 48% yield over two steps. Mesylation of the secondary hydroxyls in these intermediates proceeded smoothly under standard mesylating conditions (MsCl, Et_3N) and aided by methyl imidazole acting as a nucleophilic catalyst. At elevated temperatures (130 °C), cyclic carbamate formation, induced by attack of the Boc carbonyl substituting the mesylate and subsequently expulsion of butene, was observed. This yielded cyclic carbamates **46** and **47** in 90% and 67% over two steps respectively.

Scheme 4. Synthesis of glucose configured carbamates **46** and **47**.

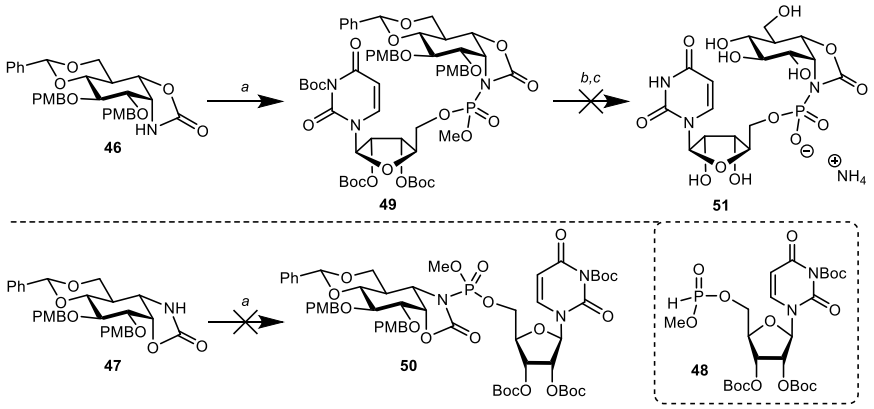


Reagents and conditions: a) NaN_3, $LiClO_4$, DMF, 100 °C, 16 h, **42** (75%), **43** (25%); *b)* TPP, THF/1 M aq. NaOH (1:1 v:v), rt, 16 h; *c)* Boc_2O, Et_3N, DCM, rt, 16 h, **44** (66% over two steps), **45** (48% over two steps); *d)* MsCl, Me-imidazole, Et_3N, DCM, rt, 16 h; *e)* DMF, 130 °C, 16 h, **46** (90% over two steps), **47** (67% over two steps).

Following optimized procedures as described in chapter 6, cyclic carbamates **46** was subjected to an Atherton-Todd phosphorylation using activated H-phosphonate diester **48** (Scheme 5). Sluggish conversion was observed for the carbamate resulting in isolation of phosphoramidate **49** as a mixture of diastereoisomers in only 22% yield. Atherton-Todd phosphorylation of cyclic carbamate **47** did not result in observable amounts of product and consequent recovery of starting material carbamate.

Global deprotection of phosphoramidate **49** proved unsuccesful, and any attempt resulted in degradation of material. Initially, deprotection conditions as optimized in chapter 6 were assessed (TFA, TES *then* pyridine). This resulted in a reductive opening of the benzylidene and the formation of a benzyl ether on either the 4''- and 6''-OH. In order to circumvent this reductive opening of the benzylidene protective group, an additional deprotection step was envisioned based on classical deprotection methods (*p*-TsOH, MeOH). The use of methanol in this deprotection step posed a potential problem as previous treatment of phosphoramidate triesters with methanol showed transesterification and thereby cleavage of the N-P linkage. The transformation to a phosphoramidate diester on the other hand, increases the intrinsic stability. Therefore, compound **49** was first treated with pyridine in order to remove the phosphoramidate methyl ester. However, subsequent treatment with a Brønsted acid (*p*-TsOH) in MeOH/DCM still resulted in degradation of material.

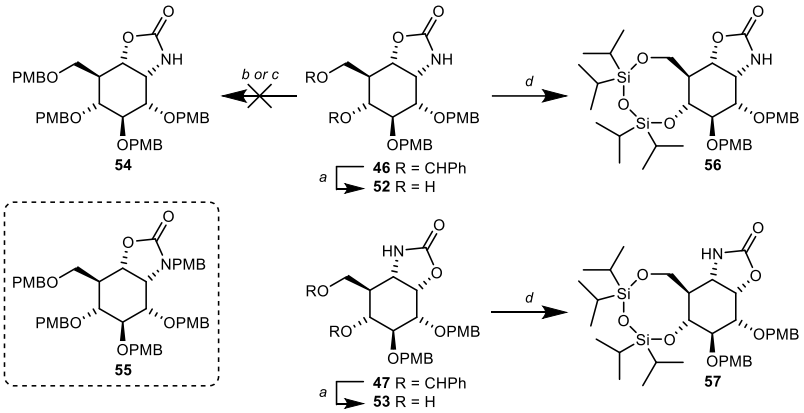
Scheme 5. Attempted Atherton-Todd reaction on cyclic carbamates **46** and **47**.



Reagents and conditions: a) i. **48**, $BrCCl_3$, DiPEA, DCM, 0 °C, 30 min, *ii.* cyclic carbamate **46** or **47**, NaH, 15-crown-5, THF, 0 °C → rt, 30 min; then added activated **48**, rt, 2 h, **49** (22%); *b)* 30% TFA/DCM (v:v), TES, 5 °C, 16 h; *c*) pyridine, 5 °C, 16 h.

In order to circumvent this troublesome global deprotection induced by the presence of the benzylidene protective group, alternative protective group schemes were studied next (Scheme 6). For this purpose, removal of the benzylidene functionality proceeded smoothly upon exposure to a Brønsted acid (*p*-TsOH) in MeOH yielding diols **52** and **53**. Attempts to protect the 4- and 6-OH as PMB ethers, either using standard Williamson etherification conditions or acid catalyzed conditions (PMB-trichloroacetimidate, CSA), proved futile as alkylation of the cyclic carbamate was observed (compound **55**). In order to selectively protect the 4- and 6-OH and prevent alkylation of the cyclic carbamate, the use of the 1,3-(1,1,3,3-tetraisopropyldisiloxanylidene) (TIPDS) protecting group was investigated. The TIPDS group has been found to be an useful protecting group of diols and in addition is easily removed in both acidic as fluoride-rich media,[33–36] suggesting its smooth deprotection during the envisioned two-step global deprotection sequence aided by TFA and TES. The 4- and 6-OH could be selectively protected over the cyclic carbamate under standard conditions, and full conversion was achieved within 30 minutes to yield both cyclic carbamates, **56** and **57**, in high yields (88% and 84% respectively).

Scheme 6. Alternative 4- and 6-OH protecting group schemes investigated.

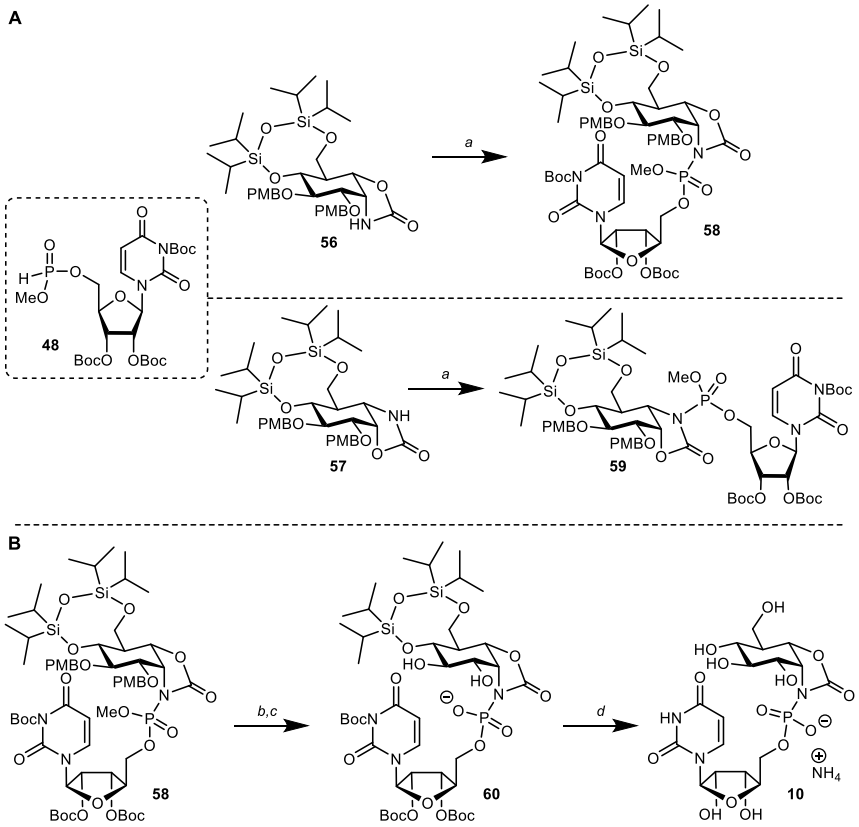


Reagents and conditions: a) p-TsOH, MeOH, 40 °C, 1 h, **52** (90%), **53** (74%); *b)* PMBCl, NaH, DMF, rt, 16 h; *c)* PMB-trichloroacetimidate, CSA, DCM, rt, 16 h; *d)* $TIPDSCl_2$, imidazole, DMF, rt, 30 min, **56** (88%), **57** (84%).

With both TIPDS protected carbamates available, their exposure to Atherton-Todd phosphorylation was examined (Scheme 7A). Relative clean and fast conversion was observed, resulting in the isolation of compounds **58** and **59** as a mixture of phosphorous diastereoisomers in 29% and 14% respectively.

Next, global deprotection of constructs **58** was examined to finalize the construction of target compound **10** (Scheme 7A). To this end, compound **58** was exposed to the in chapter 6 optimized two-step deprotection sequence (TFA/TES *then* pyridine) which allowed for the removal of all PMB and Boc protection groups prior to the removal of phosphorous methyl ester. However, the TIPDS protecting group proved stable under these conditions, as intermediate **60** was observed according to TLC-MS. At this stage, removal of the TIPDS moiety was considered viable because of the increased stability of the phosphoramidate diester by treatment of **60** with a fluoride source. Indeed, clean removal of the TIPDS protection group was observed upon treatment with TBAF, completing the synthesis of *N*-UMP-1'',7''-(*O,N*)-carbamate carba-α-D-glucopyranoside **10**. Due to limited amounts of construct **59**, no attempt to deprotect this construct was undertaken.

Scheme 7. Attempted Atherton-Todd reaction on cyclic carbamates **56** and **57** (A) and consequent global deprotection sequence towards target compound **10** (B).

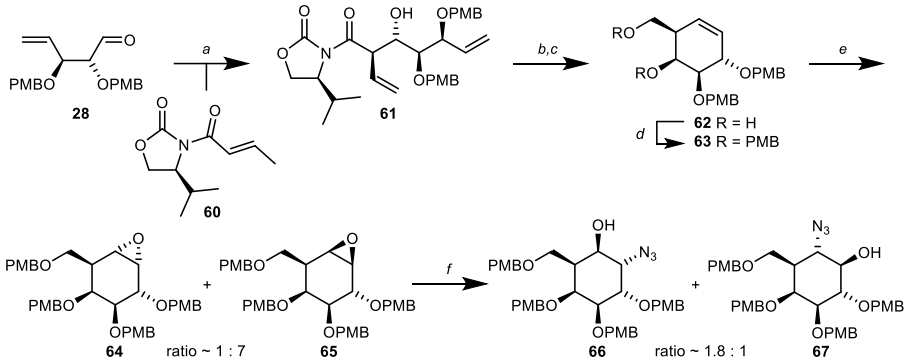


Reagents and conditions: a) i. **48**, $BrCCl_3$, DiPEA, DCM, 0 °C, 30 min, *ii.* cyclic carbamate **56** or **57**, NaH, 15-crown-5, THF, 0 °C → rt, 30 min; then added activated **48**, rt, 2 h, **58** (29%), **59** (14%, 34% brsm); *b)* TFA (30% v:v), TES, DCM, rt, 16 h; *c*) pyridine, 35 °C, 24 h; *d*) TBAF, THF, rt, 16 h (18% over three steps).

Upon successfully completing the synthesis towards *N*-UMP-1'',7''-(*O,N*)-carbamate carba-α-D-glucopyranoside **10**, attention was shifted to construction of the carba-galactoside targets **13** – **16**. The synthesis of the galactose configured constructs advanced from previously described intermediate **28** following modified procedures as described by Artola *et al*. (Scheme 8).[37] Aldehyde **28** readily underwent an asymmetric aldol condensation when treated with chiral Evans aldol **60** (prepared in four steps from commercially available Boc-L-valine, see Appendix Scheme S1)[38,39] and freshly prepared Bu_2BOTf (prepared in two steps from $BF_3{\cdot}OEt_2$, see Appendix Scheme S2)[40] to yield compound **61** in a stereoselective manner and near-quantitative yield (97%). Reductive cleavage of the auxiliary aided by $LiBH_4$ proceeded smoothly, giving rise to the primary hydroxyl. Treatment of the crude mixture to a Grubbs-II catalyzed ring-closing

metathesis provided galactose configured cyclohexene **62** in 66% yield over two steps. Protection of the 4- and 6-OH as a PMB ether proceeded sluggishly under standard Williamson etherification conditions and required elevated temperature in order to achieve full conversion to give compound **63**. Epoxidation of compound **63**, by treatment with *m*-CPBA, provided a separable mixture of epoxides **64** and **65** in modest stereoselectivity and yield in favor of the desired epoxide **65**.

Scheme 8. Construction of azide adducts **66** and **67** from pentenal **28**.

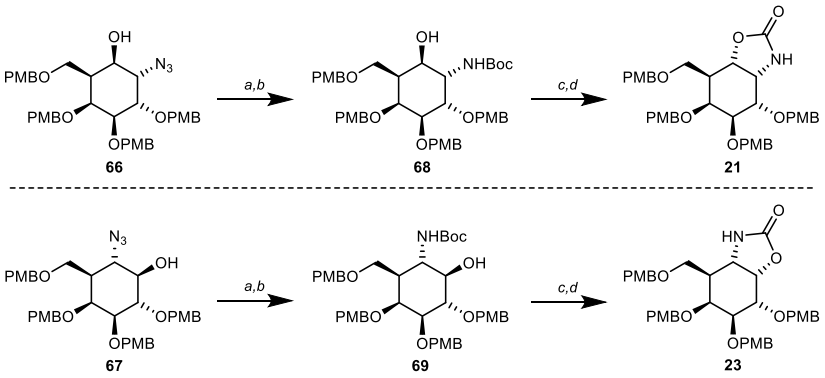


Reagents and conditions: a) **60**, Bu_2BOTf, Et_3N, 3Å molecular sieves, DCM, -78 °C → -30 °C, 16 h (98%); *b)* $LiBH_4$, THF:H_2O (9:1 v:v), 0 °C → rt, 1 h; *c)* Grubbs-II, DCM, 40 °C, 16 h (66% over two steps); *d)* PMBCl, NaH, TBAI, DMF, 45 °C, 16 h (73%); *e) m*-CPBA, $NaHCO_3$, DCM, 5 °C, 3 days, **64** (7%), **65** (43%); *f)* NaN_3, DMF, 140 °C, 16 h, **66** (45%), **67** (34%).

Treatment of compound **65** with NaN_3 at elevated temperatures neatly provided near-equimolar amounts of azide adducts **66** and **67** in 79% overall yield.

Each of the azides **66** and **67** were treated with Staudinger conditions (Scheme 9) to furnish the corresponding primary amines which were readily transformed into the *N*-Boc protected species to yield secondary carbamates **68** and **69** in near quantitative yields (96% and 95% over two steps respectively).

Scheme 9. Synthesis of galactose configured carbamates **21** and **23**.

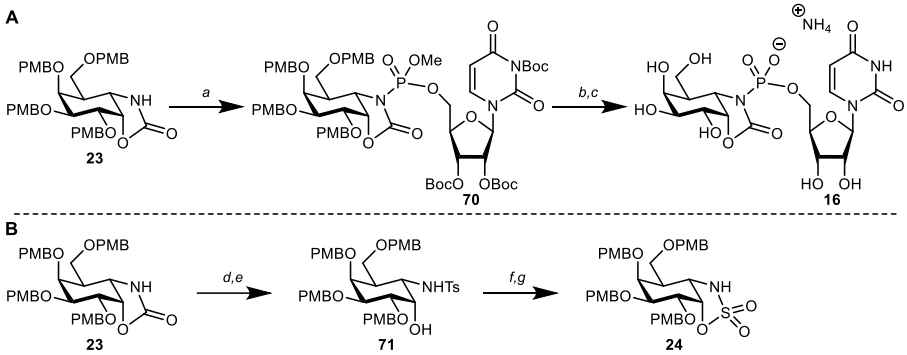


Reagents and conditions: a) TPP, THF : 1 M aq. NaOH (1:1 v:v), rt, 16 h; *b)* Boc_2O, Et_3N, DCM, rt, 16 h, **68** (96% over two steps), **69** (95% over two steps); *c)* MsCl, Me-imidazole, Et_3N, DCM, rt, 16 h; *d)* DMF, 130 °C, 16 h, **21** (59% over two steps), **23** (65% over two steps).

Mesylation of the secondary hydroxyl proceeded smoothly after which the crude mesylate was treated at elevated temperatures (130 °C) in order to induce cyclic carbamate formation. This provided cyclic carbamates **21** and **23** in 59% and 65% over two steps respectively, envisioned as suitable coupling partners in the upcoming Atherton-Todd reaction.

To this end, an Atherton-Todd phosphorylation of compound **23** (activation of H-phosphonate diester **48** with $BrCCl_3$ prior to addition to deprotected carbamate **23**) proceeded smoothly and showed almost full conversion towards the product after 2 hours at room temperature (Scheme 10A). Subsequent global deprotection using the two-step deprotection sequence, followed by Dowex NH_4^+ ion exchange and lyophilization afforded the *N*-UMP-1'',7''-(*O,N*)-carbamate carba-α-D-galactopyranoside target **16** as a NH_4^+ salt in good yield (63% over two steps).

Scheme 8. Atherton-Todd coupling of carbamate **23** and consequent deprotection towards target compound **16** (A) and preparation of cyclic sulfamidate **24** (B).

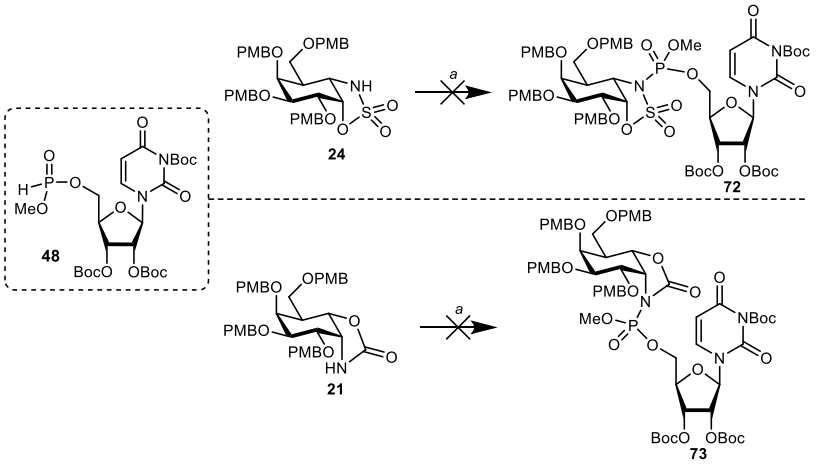


Reagents and conditions: a) i. **48**, $BrCCl_3$, DiPEA, DCM, 0 °C, 30 min, *ii.* cyclic carbamate **23**, NaH, 15-crown-5, THF, 0 °C → rt, 30 min; then added activated **48**, rt, 2 h (23%, 55% brsm); *b)* TFA (30% v:v), TES, DCM, rt, 24 h; *c*) pyridine, 35 °C, 24 h (63%); *d)* NaOH, EtOH:H_2O (4:1 v:v), 70 °C, 2 h; *e)* TsCl, Et_3N, THF, 0 °C, 1 h (70% over two steps); *f)* SO_2Cl_2, Et_3N, DCM, -78 °C → 0 °C, 2 h; g) Na, naphthalene, THF, -78 °C, 1 h (74% over two steps).

After successfully obtaining *N*-UMP-1'',7''-(*O,N*)-carbamate carba-α-D-galactopyranoside **16**, attention was then turned to create 1,7-(*O,N*)-sulfamidate **24**. Starting from cyclic carbamate **23**, alkaline induced hydrolysis of the cyclic carbamate at elevated temperatures neatly provided the primary amine which was readily protected as a tosylate (compound **71**, Scheme 10B). Following procedures as described in chapter 6, tosylate **71** was treated with SO_2Cl_2 at low temperature to provide the cyclic sulfamidate. Removal of the tosyl protecting group was effected by treatment of the intermediate with a sodium naphthalenide solution at low temperature, yielding cyclic sulfamidate **24** in 74% over two steps.

Subjecting of either cyclic carbamate **21** or sulfamidate **24** to an Atherton-Todd phosphorylation with H-phosphonate diester **48** did not result in product formation, but to full recovery of the starting materials (Scheme 11).

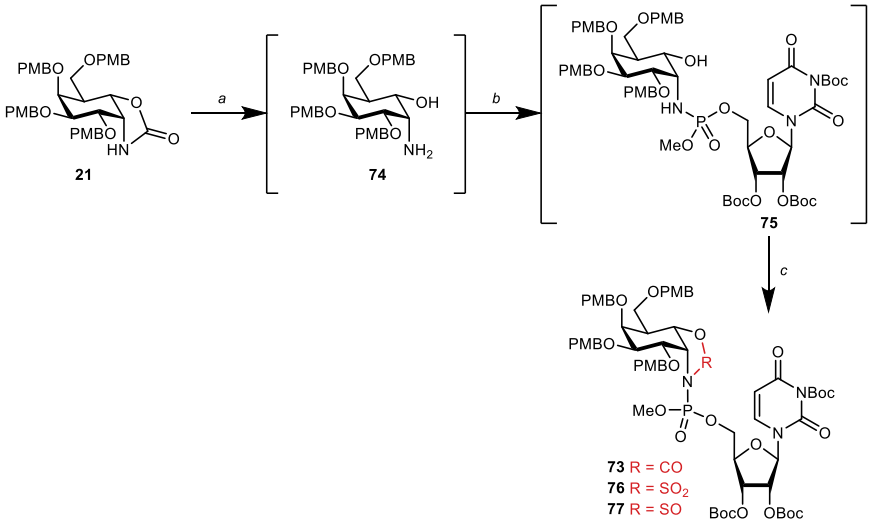
Scheme 9. Atherton-Todd reaction on cyclic sulfamidate **24** and cyclic carbamate **21**.



Reagents and conditions: a) i. **48**, $BrCCl_3$, DiPEA, DCM, 0 °C, 30 min, *ii.* cyclic sulfamidate **24** or carbamate **21**, NaH, 15-crown-5, THF, 0 °C → rt, 30 min; then added activated **48**, rt, 2 h.

With the inability to couple sulfamidate **24** or carbamate **21** to H-phosphonate diester **48** under Atherton-Todd conditions, an alternative synthesis route towards these constructs was sought for. Literature has shown primary amine phosphorylation to be selective over that of secondary hydroxyls and therefore an approach was taken to first install the phosphate on the primary amine and subsequently forge the cyclic carbamate or sulfamidate.[41–44] Indeed, reaction of crude amino-alcohol **74**, prepared by treatment of **21** with sodium hydroxide in ethanol/water (4:1) (Scheme 12), with H-phosphonate diester **48** under Atherton-Todd conditions led to clean and rapid conversion to phorsporamidate **75** as observed by ^{31}P NMR (signal at 20 ppm) and ^{1}H NMR. Upon prolonged reaction time (one day), migration of the UMP-moiety to the 7''-OH was observed as indicated by ^{31}P (signal at 14 ppm) and ^{1}H NMR.[43] Because of this observation, intermediate **75** was used without work-up and purification in the next step. Thus, crude **75** was subjected to a variety of different conditions to explore 1,7-(*N*,*O*)-bicycle formation (Table 1). Subjection of crude **75** to CDI, triphosgene or phosgene (Entries 1 – 3) all resulted in degradation of the starting material. The same holds true for treatment of crude **75** with SO_2Cl_2 (Entry 4): again, complete degradation was observed. In an attempt to minimize the degradation of the starting material *via* the aforementioned UMP migration, a two-step one-pot procedure was explored (Entries 5 and 6). Also in these instances degradation of the starting material was observed. Switching from SO_2Cl_2 to thionyl chloride ($SOCl_2$, Entry 7) in contrast allowed for the isolation of cyclic sulfamidite **77** in 37% yield over three steps.

Scheme 10. Alternative synthetic route towards *N-P* coupled carba-galactosides.



Reagents and conditions: a) NaOH, EtOH:H_2O (4:1 v:v), 70 °C, 2 h; *b)* **48**, $BrCCl_3$, DiPEA, DCM, 0 °C, 15 min; *c)* see Table 1.

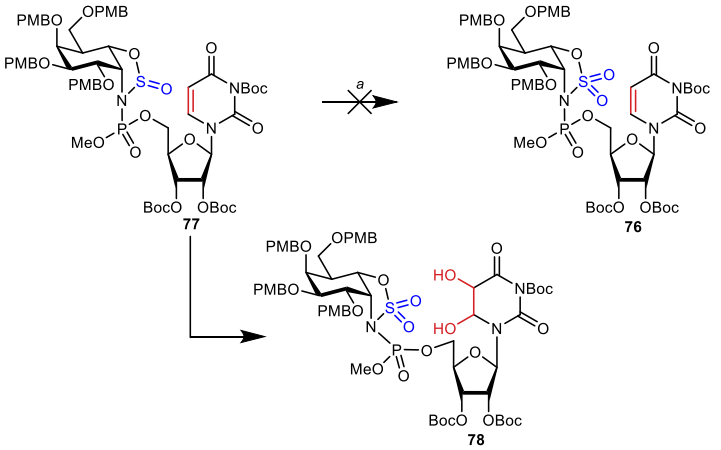
Table 1. Late stage 1,7-(*N*,*O*)-bicycle formation.

Entry	Conditions	R-group	Observations
1.	CDI, Et_3N, DCM, 0 °C, 2 h	R = CO (**73**)	Degradation of starting material
2.	Triphosgene, Et_3N, DCM, 0 °C, 2 h	R = CO (**73**)	Degradation of starting material
3.	$COCl_2$, DMAP, DBU, DCM, -78 °C → 0 °C, 2 h	R = CO (**73**)	Degradation of starting material
4.	SO_2Cl_2, Et_3N, DCM, 0 °C, 2 h	R = SO_2 (**76**)	Degradation of starting material
5.	$COCl_2$, one-pot with Atherton-Todd, -78 °C → 0 °C, 2 h	R = CO (**73**)	Degradation of starting material
6.	SO_2Cl_2, one-pot with Atherton-Todd, -40 °C → 0 °C, 16 h	R = SO_2 (**76**)	Degradation of starting material
7.	$SOCl_2$, one-pot with Atherton-Todd, -40 °C → 0 °C, 16 h	R = SO (**77**)	Clean conversion to **77** (37% over three steps)

Oxidation of the cyclic sulfamidite in **77** to the corresponding sulfamidate would yield target compound **76**. To this end, cyclic sulfamidite **77** was treated with standard S(IV) oxidation conditions ($RuCl_3$, $NaIO_4$).[45–48] Within 20 minutes, full conversion of the

starting material was observed. Unfortunately, NMR spectroscopy and MS revealed that overoxidation had occurred. Besides oxidation of the S(IV) centre, oxidation of the uracil moiety to the diol was observed (compound **78**, Scheme 13). Based on literature precedent, dihydroxylation of uracil is common occurrence when subjected to these harsh oxidation conditions ($RuCl_3$, $NaIO_4$).[49]

Scheme 11. Attempted oxidation of cyclic sulfamidite **77** towards cyclic sulfamidate **76**.



Reagents and conditions: a) $RuCl_3$, $NaIO_4$, EtOAc, CH_3CN, H_2O, 0 °C, 20 min, **78** (50%).

In order to prevent overoxidation of the uracil moiety, a milder S(IV) oxidation method was sought for (Table 2). Both *m*-CPBA and $KMnO_4$ have been used to accomplish the transformation of sulfamidites to sulfamidates,[47,48,50–52] and are known to be compatible with uracil bearing constructs. Sulfamidite oxidation by *m*-CPBA (Entry 1) proved unsuccessful as no conversion was observed. The use of $KMnO_4$ proved unsuccessful as well, this time resulting in slow degradation of the starting material without any indication of product formation (Entry 2). A last attempt was prompted by literature precedent stating that superior $KMnO_4$ oxidations were achieved when catalytic amounts (20%) of MnO_2 were added (Entry 3).[53] However, these conditions also resulted in complete degradation of the starting material.

Table 2. Attempted oxidation of cyclic sulfamidite **77** towards cyclic sulfamidate **76**.

Entry	Conditions	Observations
1.	*m*-CPBA, DCM, 0 °C → rt, 16 h	No conversion
2.	$KMnO_4$, DCM, 0 °C → rt, 16 h	Degradation of starting material
3.	$KMnO_4$, MnO_2, DCM, 0 °C → rt, 16 h	Degradation of starting material

Conclusions

In conclusion, this chapter describes the synthetic endeavors towards UMP linked glucose and galactose configured constructs, envisioned as putative inhibitors of UDP-Glc and UDP-Gal processing glycosyl transferases. Expanding on the 1,2-carbamate and -sulfamidate inhibitors described in chapter 6, this chapter is focused on the synthesis of constructs bearing cyclic carbamates and sulfamidates over the 1,7-position. By means of rigidification as induced by the annelated ring system, the set of compounds **9** – **16** are thought to resemble the concaved orientation often observed in crystallographic data of the donor substrate in Michaelis complexes with various GTs.

Initial attempts towards glucose configured cyclic carbamates and sulfamidates **9** – **12** focused on the synthetic methodology as described in chapter 6. Here, the key Sharpless aminohydroxylation of protected cyclophellitol cyclohexenes **31** and **36** resulted in sluggish transformations and with limited stereoselectivity and regioselectivity. Alternative methodologies provided access to the cyclic carbamates **46** and **47** as divergent building blocks in the construction of all four target structures **9** – **12**. The use of a TIPDS protecting group proved crucial during both the Atherton-Todd reaction and the global deprotection sequence and allowed for the isolation of the first target structure *N*-UMP-1'',7''-(*N,O*)-carbamate carba-α-D-glucopyranoside **10**. Following identical deprotection methodology, future deprotection of construct **59** should be feasible.

Following identical chemical transformations, both galactopyranose-configured cyclic carbamates **21** and **23** were efficiently synthesized. The Atherton-Todd coupling between H-phosphonate diester **48** and cyclic carbamate **23** followed by the global deprotection sequence provided target compound *N*-UMP-1'',7''-(*O,N*)-carbamate carba-α-D-galactopyranoside **16**. All attempted Atherton-Todd coupling reactions with either the sulfamidate analogue **24** or the carbamate 1,7-regioisomer **21** however resulted in full recovery of starting material. In a final attempt to obtain compounds **13** – **15**, carbamate **21** was hydrolyzed prior to exposure to Atherton-Todd conditions. This resulted in clean and fast conversion to phosphoramidate **75**. This intermediate was foreseen to be a divergent building block and could provide the cyclic carbamate and sulfamidate constructs upon exposure to carbamylating- or sulfamylating agents. Unfortunately, degradation of the starting material was observed, proving this synthesis route to be unfeasible.

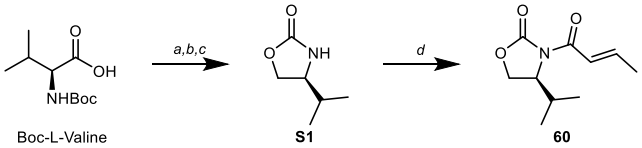
In sum, the here described synthetic methodologies and the identified synthetic pitfalls could in turn pave the way for construction of the remaining cyclic carbamate and cyclic sulfamidate constructs. The assessment of the synthesized compounds as GT inhibitors requires the establishment of suitable enzyme inhibition assays, which hopefully will be available in the near future.

Acknowledgements

Anne-mei Klein and Duncan de Graaf are kindly acknowledged for their synthesis work in the context of their MSc internships. Acknowledgments are due to Roy Steneker for our collaborative efforts in the preparation of dibutyl boron triflate, as well as for our insightful discussions. Pascal Balić is acknowledged for our insightful discussions in relation to the phosphorus chemistries.

Appendix

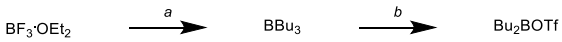
Scheme S1. Preparation of Evans auxiliary **60** from commercially available Boc-L-valine.



Boc-L-Valine **S1** **60**

Reagents and conditions: a) ethyl chloroformate, Et_3N, THF, 0 °C, 1 h; *b)* $NaBH_4$, H_2O, rt, 30 min; *c)* $SOCl_2$, THF, rt, 16 h (35% over three steps); *d) n*-BuLi, crotonyl chloride, THF, -78 °C → rt, 3 h (68%).

Scheme S2. Preparation of Bu_2BOTf from boron trifluoride etherate.



Reagents and conditions: a) n-BuLi, Et_2O, reflux, 2 h (66%); *b)* TfOH, 50 °C, 1 h (95%).

Synthetic procedures.

General procedure A: *N*-phosphorylation of per-*O*-(4-methoxybenzyl) cyclic carbamates and sulfamidates using protected methyl H-phosphonate diester

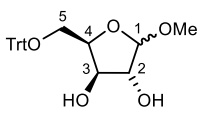
Per-*O*-(4-methoxybenzyl) cyclic carbamate or sulfamidate was co-evaporated with anhydrous $CHCl_3$ and added to an oven-dried round-bottom flask. The flask was connected to a Schlenk line and subjected to three vacuum-N_2 backfill cycles before dissolving in anhydrous THF (0.2-0.3 M). The solution was cooled on ice, after which 15-crown-5 ether (5.0 eq.) and NaH (60 wt% dispersion in mineral oil; 1.5 eq.) were added. After 5 minutes, the reaction mixture was removed from the ice bath and stirred at room temperature for 30 minutes. In parallel, another oven-dried round-bottom flask was charged with methyl H-phosphonate **48** (2.0 eq.) and also put under a N_2 atmosphere using a Schlenk line prior to dissolving in anhydrous DCM (0.3-0.4 M). The solution was cooled on ice, after which anhydrous DiPEA (6.0 eq.) and bromotrichloromethane ($BrCCl_3$; 4.0 eq.) were added. The reaction mixture was stirred on ice for 15-30 minutes, after which full conversion to the bromophosphate was confirmed by both TLC and ^{31}P NMR analysis (δ = –4.9, –5.1 ppm; 121 MHz, acetone-d_6 probe). The flask containing deprotonated cyclic carbamate or sulfamidate was cooled back on ice, after which the cooled bromophosphate solution was transferred using a N_2-flushed syringe. The resulting reaction mixture was stirred on ice for 1.5-2 h. After full conversion was observed by TLC, the reaction was quenched on ice with sat. aq. $NaHCO_3$. The crude product was extracted with EtOAc (3x), after which the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Flash column chromatography of the crude material (SiO_2, EtOAc in pentane) followed by Sephadex™ LH-20 size exclusion chromatography yielded the protected *N*-acyl- or *N*-sulfonylphosphoramidate as a mixture of P(V) diastereomers.

Note: The P(V) diastereomers were partially separable using silica and size-exclusion column chromatography, making it possible to obtain clean NMR spectra of the individual diastereomers for easier structure elucidation. Aside from this, the phosphoramidate products were collected and subsequently deprotected as a mixture.

General procedure B: One-pot global deprotection and demethylation of protected *N*-acyl- or *N*-sulfonylphosphoramidate

Protected *N*-acyl- or *N*-sulfonylphosphoramidate compound (as a mixture of P(V) diastereomers) was dissolved in anhydrous DCM (0.05 M) and cooled on ice. TES (10 eq.) and TFA (30% v:v) were added and the reaction mixture was stirred on ice for 3-16 h. After full conversion was observed by TLC-MS analysis (MeOH:DCM, 2:8 v:v), pyridine (20 eq. with respect to TFA) was added while stirring on ice. The resulting reaction mixture was stirred overnight at 25 °C to 35 °C, during which the demethylation of the methyl phosphoramidates took place. After full conversion was observed by ^{31}P NMR analysis (acetone-d_6 probe), the mixture was concentrated to dryness under reduced pressure. Purification of the crude product by flash column chromatography (neutralized SiO_2, dry loading on Celite; distilled DCM then H_2O in MeCN) followed by Dowex 50WX4 NH_4^+ ion or Na^+ ion exchange (stored on 0.10 M NH_4OAc or 0.10 M NaOAc) and subsequent lyophilization yielded the 1,7-UMP cyclic carbamates or sulfamidate target compounds as their NH_4^+ or Na^+ salts.

Methyl 6-*O*-trityl-α/β-D-xylofuranoside (25).

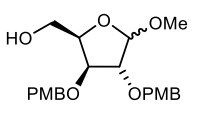


D-xylose (45 g, 300 mmol) was dissolved in MeOH (600 mL, 0.5 M) and cooled on ice. Acetyl chloride (10.7 mL, 150 mmol, 0.5 eq.) was added and the reaction mixture was stirred for 6.5 hours at room temperature to full conversion (R_f 0.5 (H_2O:MeCN, 1:9 v:v)). The reaction mixture was quenched with Et_3N to pH 8.0 and concentrated under reduced pressure. The crude, brown oil was co-evaporated with DMF (3x 120 mL) under N_2 atmosphere, dissolved in DMF (600 mL, 0.5 M) and flushed with N_2. Trityl chloride (100 g, 360 mmol, 1.2 eq.), Et_3N (66.7 mL, 480 mmol, 1.6 eq.) and DMAP (1.83 g, 15 mmol, 0.05 eq.) were added, and the reaction was stirred overnight at 30 °C. Upon full conversion (R_f 0.5 (EtOAc:pentane, 6:4, v:v)) the reaction was quenched with MeOH and concentrated *in vacuo*. The residue was dissolved in Et_2O and washed with H_2O, and the aqueous phase was extracted with Et_2O (2x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (40:60 EtOAc:pentane → 60:40 EtOAc:pentane) yielded **25** as a yellow oil and as anomeric mixture (132 g, 300 mmol, α:β ratio; 1.3:1, quant. over two steps). Spectroscopic data is in full agreement with literature data.[54]

Analytical data for the α-anomer: ^{1}H NMR (300 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.54 – 7.41 (m, 5H, CH_{arom}), 7.34 – 7.17 (m, 10H, CH_{arom}), 5.05 (d, *J* = 3.9 Hz, 1H, H-1), 4.33 – 4.12 (m, 3H, H-2, H-3, H-4), 3.53 – 3.24 (m, 4H, H-5, 1-OMe), 3.04 – 2.94 (m, 1H, 2-OH/3-OH), 2.86 (d, *J* = 6.3 Hz, 1H, 2-OH/3-OH); ^{13}C NMR (75 MHz, $CDCl_3$, HSQC): δ 143.4, 128.7, 128.5, 128.1, 127.9, 127.3, 127.0 ($C_{q\text{-}arom}$, CH_{arom}), 101.9 (C-1), 87.4 (C_q Trt), 79.8, 78.5, 77.7, 77.1 (C-2$_\beta$, C-2, C-3, C-4), 62.7 (C-5), 55.9 (1-OMe).

Analytical data for the β-anomer: ^{1}H NMR (300 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.54 – 7.41 (m, 5H, CH_{arom}), 7.34 – 7.17 (m, 10H, CH_{arom}), 4.86 (s, 1H, H-1), 4.49 (ddd, *J* = 6.5, 4.4, 4.4 Hz, 1H, H-4), 4.33 – 4.12 (m, 1H, H-2), 3.98 (ddd, *J* = 10.2, 4.5, 1.4 Hz, 1H, H-3), 3.53 – 3.24 (m, 4H, H-5, 1-OMe), 3.04 – 2.94 (m, 1H, 2-OH/3-OH), 2.77 (d, *J* = 4.3 Hz, 1H, 2-OH/3-OH); ^{13}C NMR (75 MHz, $CDCl_3$, HSQC): δ 143.4, 128.7, 128.5, 128.1, 127.9, 127.3, 127.0 ($C_{q\text{-}arom}$, CH_{arom}), 108.7 (C-1), 82.0 (C-4), 86.9 (C_q Trt), 79.8, 78.5, 77.7, 77.1 (C-2, C-2$_\alpha$, C-3$_\alpha$, C-4$_\alpha$), 76.8 (C-3), 63.6 (C-5), 55.3 (1-OMe); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{25}H_{26}NaO_5$ 429.1673; Found 429.1671.

Methyl 2,3-di-*O*-(4-methoxybenzyl)-α/β-D-xylofuranoside (26).



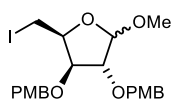
The furanose **25** (136 g, 334 mmol) was co-evaporated with toluene and dissolved in anhydrous DMF (557 mL, 0.6 M). Subsequently PMBCl (269 mL, 2.0 mol, 6.0 eq.) and TBAI (6.2 g, 17 mmol, 0.05 eq.) were added, and the reaction mixture was stirred and cooled to 0 °C. Then NaH (80 g (60% wt.), 2.0 mol, 6 eq.) was added portionwise, after which the reaction was allowed to warm to room temperature and stirred overnight. Upon full conversion (R_f 0.75 (EtOAc:pentane, 1:1 v:v)) the reaction was quenched with MeOH and concentrated under reduced pressure. The crude intermediate was dissolved in EtOAc and washed with H_2O. The aqueous layer was extracted with EtOAc (3x) and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*, providing a yellow oil. The crude intermediate was dissolved in a 1:1 mixture of DCM and MeOH (1.3 L, 0.25 M) and the pH was reduced to 2.0 as consequence of the addition of *p*-TsOH. The reaction was stirred overnight at room temperature. Upon full conversion

(R_f 0.25 (EtOAc:pentane, 7:3 v:v)) the reaction was quenched with Et_3N to pH 6.0 and concentrated under reduced pressure. The crude product was dissolved in EtOAc and washed with sat. aq. $NaHCO_3$ and extracted with EtOAc (3x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash column chromatography (33:67 EtOAc:pentane → 50:50 EtOAc:pentane) yielded **26** as an anomeric mixture (98.51 g, 244 mmol, α:β ratio; 1.44:1, 73% over two steps). ^{1}H NMR- and ^{13}C NMR-data correspond to literature data.[54]

Analytical data for the α-anomer: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.35 – 7.17 (m, 3H, CH_{arom}), 6.94 – 6.83 (m, 5H, CH_{arom}), 4.87 (d, *J* = 1.9 Hz, 1H, H-1), 4.62 – 4.37 (m, 4H, C*H*H PMB, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.27 (ddd, *J* = 6.9, 4.8, 4.8 Hz, 1H, H-4), 4.15 (dd, *J* = 6.9, 3.9 Hz, 1H, H-3), 4.06 (dd, *J* = 3.5, 1.8 Hz, 1H, H-2), 3.81 s, 6H, OMe), 3.78 – 3.66 (m, 1H, H-5) 3.40 (s, 3H, 1-OMe), 2.52 (dd, *J* = 6.6, 6.6 Hz, 1H, 5-OH); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.6, 159.6 ($C_{q\text{-}arom}$), 130.0 (CH_{arom}), 129.8, 129.7 ($C_{q\text{-}arom}$), 129.7, 129.6, 129.6, 114.0, 114.0, 114.0 (CH_{arom}), 108.1 (C-1), 87.0 (C-2), 82.7 (C-3), 80.6 (C-4), 72.5, 72.4, 72.2, 72.0 (CH_2 $PMB_{\alpha/\beta}$), 62.4 (C-5), 55.8 (1-OMe), 55.4 (OMe).

Analytical data for the β- anomer: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.35 – 7.17 (m, 3H, CH_{arom}), 6.94 – 6.83 (m, 5H, CH_{arom}), 4.77 (d, *J* = 4.2 Hz, 1H, H-1), 4.62 – 4.37 (m, 4H, C*H*H PMB, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.39 (d, *J* = 14.2 Hz, 1H, H-3), 4.21 – 4.16 (m, 1H, H-4), 4.01 (dd, *J* = 6.5, 4.2 Hz, 1H, H-2), 3.80 (s, 6H, OMe), 3.78 – 3.66 (m, 1H, H-5), 3.37 (s, 3H, 1-OMe), 2.41 (dd, *J* = 8.8, 5.0 Hz, 1H, 5-OH); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.6, 159.6 ($C_{q\text{-}arom}$), 130.0 (CH_{arom}), 129.8, 129.7 ($C_{q\text{-}arom}$), 129.7, 129.6, 129.6, 114.0, 114.0, 114.0 (CH_{arom}), 100.3 (C-1), 84.3 (C-2), 82.0 (C-3), 76.3 (C-4), 72.5, 72.4, 72.2, 72.0 (CH_2 $PMB_{\alpha/\beta}$), 62.4 (C-5), 55.4 (OMe), 55.2 (1-OMe); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{22}H_{28}NaO_7$ 427.1727; Found 427.1726.

Methyl 2,3-di-*O*-(4-methoxybenzyl)-5-deoxy-5-iodo-α/β-D-xylofuranoside (27).

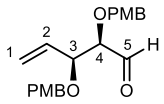


Alcohol **26** (98.6 g, 244 mmol) was co-evaporated with toluene (3x) and dissolved in anhydrous THF (750 mL, 0.3 M). Triphenylphosphine (95.9 g, 366 mmol, 1.5 eq.) and imidazole (33.2 g, 488 mmol, 2.0 eq.) were added, and the reaction was brought to reflux. I_2 (92.8 g, 366 mmol, 1.5 eq.) was dissolved in anhydrous THF (250 mL) and added dropwise to the reaction mixture, and the reaction was refluxed for another 3.5 hours. Upon full conversion was observed (R_f 0.4 (EtOAc:pentane, 2:8 v:v)), the reaction was allowed to attain to room temperature and concentrated *in vacuo*. The crude was dissolved in EtOAc and washed with sat. aq. $Na_2S_2O_3$, H_2O, and brine. The aqueous layers were extracted with EtOAc (2x), and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (5:95 EtOAc:pentane → 20:80 EtOAc:pentane) yielded iodide **27** as an anomeric mixture (120 g, 233 mmol, α:β ratio; 1.44:1, 95%).

Analytical data for the α-anomer: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.36 – 7.16 (m, 4H, CH_{arom}), 6.95 – 6.81 (m, 4H, CH_{arom}), 4.91 (s, 1H, H-1), 4.66 – 4.31 (m, 5H, H-4, C*H*H PMB, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.05 – 3.94 (m, 2H, H-2, H-3), 3.80 (s, 6H, OMe), 3.42 (s, 3H, 1-OMe), 3.39 – 3.29 (m, 2H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.6, 159.6, 159.4 ($C_{q\text{-}arom}$), 129.9, 129.8 (CH_{arom}), 129.7, 129.7 ($C_{q\text{-}arom}$), 129.6, 129.6, 114.0, 114.0, 113.9 (CH_{arom}), 108.5 (C-1), 86.5 (C-2), 82.1, 81.7, 81.5 (C-3, C-4, C-3_β), 72.4 (CH_2 PMB), 56.2 (1-OMe), 55.4 (OMe), 4.8 (C-5).

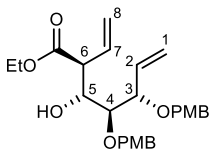
Analytical data for the β- anomer: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.36 – 7.16 (m, 4H, CH_{arom}), 6.95 – 6.81 (m, 4H, CH_{arom}), 4.82 (d, *J* = 4.3 Hz, 1H, H-1), 4.66 – 4.31 (m, 5H, H-4, C*H*H PMB, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.18 – 4.12 (m, 1H, H-3), 4.05 – 3.94 (m, 1H, H-2), 3.80 (s, 6H, OMe), 3.40 (s, 3H, 1-OMe), 3.39 – 3.29 (m, 1H, H-5), 3.18 (dd, *J* = 10.3, 7.4 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.6, 159.6, 159.5 ($C_{q\text{-}arom}$), 129.9, 129.8 (CH_{arom}), 129.7, 129.7 ($C_{q\text{-}arom}$), 129.7, 129.6, 114.0, 114.0, 113.9 (CH_{arom}), 101.0 (C-1), 83.6 (C-2), 82.1, 81.7, 81.5 (C-3, C-3$_\alpha$, C-4$_\alpha$), 77.7 (C-4), 71.9 (CH_2 PMB), 55.6 (1-OMe), 55.4 (OMe), 3.3 (C-5); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{22}H_{27}INaO_6$ 537.0745; Found 537.0747.

(2*R*,3*S*)-2,3-Di-*O*-(4-methoxybenzyl)-pent-4-enal (28).



27 (96.3 g, 187 mmol) was dissolved in THF/H_2O (9:1, 936 mL, 0.2 M) and purged with argon gas. Meanwhile, zinc dust was stirred in 3.0 M HCl (aq.) for 10 minutes. The acidic solution was removed by filtration and the activated zinc was consecutively washed with H_2O (3x), acetone (3x) and Et_2O (3x). The zinc was subsequently dried *in vacuo*. The activated zinc (3.4 mol, 218 g, 18 eq.) was added to the THF/H_2O solution of **27**, and the reaction mixture was purged with argon. The reaction was sonicated for 12 hours while maintaining a temperature of 40 °C. Upon full conversion was observed (R_f 0.6 (EtOAc:pentane, 3:7, v:v)), the suspension was filtered over a celite plug and rinsed with Et_2O (6x). The filtrate was concentrated under reduced pressure at 30 °C to remove THF. The crude mixture was dissolved in Et_2O and washed with H_2O (2x). The aqueous layer was extracted with Et_2O (3x), and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* at 30 °C. Flash column chromatography (10:90 EtOAc:pentane → 30:70 EtOAc:pentane) yielded **28** as a yellowish oil (54.2 g, 152 mmol, 81%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 9.61 (d, *J* = 1.6 Hz, 1H, H-5), 7.25 – 6.85 (m, 8H, CH_{arom}), 5.91 (ddd, *J* = 17.2, 10.4, 7.6 Hz, 1H, H-2), 5.33 (m, 2H, H-1), 4.65 (d, *J* = 11.8 Hz, 1H, C*H*H PMB), 4.61 – 4.51 (m, 2H, C*H*H PMB, CH*H* PMB), 4.27 (d, *J* = 11.7 Hz, 1H, CH*H* PMB), 4.12 (dddd, *J* = 7.6, 4.0, 1.0, 1.0 Hz, 1H, H-3), 3.80 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.77 (dd, *J* = 4.0, 1.6 Hz, 1H, H-4); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 203.0 (C-5), 159.7, 159.4 ($C_{q\text{-}arom}$), 134.1 (C-2), 130.0, 129.7 (CH_{arom}), 129.7, 129.2 ($C_{q\text{-}arom}$), 119.8 (C-1), 114.8, 113.4 (CH_{arom}), 85.0 (C-4), 79.7 (C-3), 73.3, 70.4 (CH_2 PMB), 55.4, 55.4 (OMe); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{21}H_{24}NaO_5$ 379.1516; Found 379.1513.

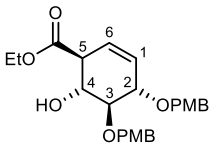
Ethyl (2*S*,3*R*,4*S*,5*S*)-3-hydroxy-4,5-bis((4-methoxybenzyl)oxy)-2-vinylhept-6-enoate (29).



28 (20 g, 56.1 mmol) was suspended in H_2O (280 mL, 0.2 M), and stirred vigorously. Consecutively, ethyl-4-bromocrotonate (42.9 mL, 252 mmol, 4.5 eq.), $La(OTf)_3$ (66.1 g, 112 mmol, 2 eq.) and indium powder (8.3 g, 73 mmol, 1.3 eq) were added. The reaction was stirred vigorously. At day 2 and 3 more indium powder was added (3.2 g, 28 mmol, 0.5 eq, each day). The reaction was stirred 5 days in total at room temperature to full conversion (R_f 0.55 (EtOAc:pentane, 3:7, v:v)). The suspension was filtered over a celite plug, and the residue was rinsed with Et_2O (6x). The filtrate was washed with sat. aq. $NaHCO_3$, and the aqueous phase was extracted with Et_2O (3x). The combined organic phases were washed once again with sat. aq. $NaHCO_3$, and the aqueous phase was extracted with Et_2O. All organic phases were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (dry loading, 5:95 EtOAc:pentane → 30:70 EtOAc:pentane) yielded glucose configured **29** as a colorless

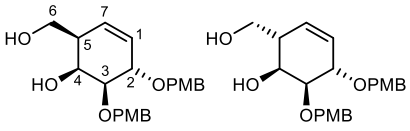
oil contaminated with an unidentifiable amount of L-idose configured diene (23 g, 50 mmol, 89%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.43 – 7.07 (m, 4H, CH_{arom}), 6.99 – 6.65 (m, 4H, CH_{arom}), 5.80 (ddd, *J* = 17.2, 10.3, 7.8 Hz, 1H, H-2), 5.69 (ddd, *J* = 17.2, 10.2, 9.3 Hz, 1H, H-7), 5.42 – 5.34 (m, 2H, H-1), 5.15 (ddd, *J* = 10.2, 1.3, 0.4 Hz, 1H, H-8), 5.06 (ddd, *J* = 17.2, 1.4, 0.8 Hz, 1H, H-8), 4.89 (d, *J* = 10.8 Hz, 1H, C*H*H PMB), 4.55 (d, *J* = 11.2 Hz, 1H, C*H*H PMB), 4.50 (d, *J* = 10.8 Hz, 1H, CH*H* PMB), 4.33 (d, *J* = 11.2 Hz, 1H, CH*H* PMB), 4.12 (m, 3H, H-3, CH_2 Et), 3.95 (ddd, *J* = 9.9, 9.2, 1.2 Hz, 1H, H-5), 3.81 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.50 (dd, *J* = 7.5, 1.2 Hz, 1H, H-4), 3.25 (dd, *J* = 9.2, 9.2 Hz, 1H, H-6), 2.66 (d, *J* = 9.8 Hz, 1H, 5-OH), 1.22 (t, *J* = 7.1 Hz, 3H, CH_3 Et); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 172.6 (C=O), 159.4, 159.3 ($C_{q\text{-}arom}$), 135.2 (C-2), 133.1 (C-7), 130.7, 130.6 ($C_{q\text{-}arom}$), 129.8, 129.5 (CH_{arom}), 119.9, 119.8 (C-1, C-8), 113.9 (CH_{arom}), 82.7 (C-3), 79.0 (C-4), 74.2 (CH_2 PMB), 72.2 (C-5), 70.6 (CH_2 PMB), 60.9 (CH_2 Et), 55.4, 55.4, 55.3 (C-6, OMe), 14.2 (CH_3 Et); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{27}H_{34}NaO_7$ 493.2197; Found 493.2194.

Ethyl (1*S*,4*S*,5*S*,6*R*)-6-hydroxy-4,5-bis((4-methoxybenzyl)oxy)cyclohex-2-ene-1-carboxylate (30).



Diene **29** (23 g, 50 mmol) was dissolved in anhydrous DCM (415 mL, 0.12 M) and purged in argon gas. Second generation Grubbs catalyst (1.7 g, 0.04 eq.) was added in the dark and the flask was purged again in argon gas. The reaction was stirred two days at 38 °C in the dark. Upon full conversion (R_f 0.3 (EtOAc:pentane, 3:7, v:v)) the reaction was concentrated under reduced pressure. Flash column chromatography (10:90 EtOAc:pentane → 30:70 EtOAc:pentane) yielded **30** as a brown oil contaminated with an unidentifiable amount of L-idose configured cyclohexene (20.2 g, 46 mmol, 92%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.29 – 7.22 (m, 4H, CH_{arom}), 6.90 – 6.84 (m, 4H, CH_{arom}), 5.76 (ddd, *J* = 10.2, 2.9, 2.2 Hz, 1H, H-6), 5.64 (ddd, *J* = 10.2, 2.2, 2.2 Hz, 1H, H-1), 4.87 (d, *J* = 11.0 Hz, 1H, C*H*H PMB), 4.70 (d, *J* = 11.0 Hz, 1H, C*H*H PMB), 4.62 – 4.58 (m, 2H, CH*H* PMB, CH*H* PMB), 4.18 (q, *J* = 7.1 Hz, 2H, CH_2 Et), 4.14 (dddd, *J* = 7.5, 3.3, 2.1, 2.1 Hz, 1H, H-2), 4.09 (ddd, *J* = 9.8, 8.9, 2.4 Hz, 1H, H-4), 3.79 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.60 (dd, *J* = 9.8, 7.5 Hz, 1H, H-3), 3.23 (dddd, *J* = 8.8, 3.1, 3.1, 2.5 Hz, 1H, H-5), 2.98 (d, *J* = 2.5 Hz, 1H, 4-OH), 1.26 (t, *J* = 7.1 Hz, 3H, CH_3 Et); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 172.0 ($C_{q\text{-}arom}$), 171.9 (C=O), 159.4, 130.6, 130.2 ($C_{q\text{-}arom}$), 129.6, 129.6 (CH_{arom}), 128.5 (C-6), 124.0 (C-1), 114.0, 113.9 (CH_{arom}), 82.2 (C-3), 79.0 (C-2), 74.5, 71.7 (CH_2 PMB), 70.4 (C-4), 61.3 (CH_2 Et), 55.3 (OMe), 50.2 (C-5), 14.3 (CH_3 Et); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{25}H_{30}NaO_7$ 465.1884; Found 465.1886.

2,3-Di-*O*-(4-methoxybenzyl)-cyclophellitol alkene (31) and 2,3-Di-*O*-(4-methoxybenzyl)-5-*epi*-cyclophellitol alkene (32).



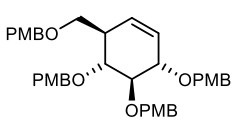
Cyclohexene **30** (20.2 g, 46 mmol) was co-evaporated once with toluene, dissolved in anhydrous THF (457 mL, 0.1 M), purged with N_2 gas and cooled to 0 °C. While stirring, $LiBH_4$ (4 M in THF, 28.6 mL, 2.5 eq.) was slowly added. The reaction was allowed to warm to room temperature and stirred for 5 hours. Upon full conversion was observed (R_f 0.2 and 0.3 for the D-glucose and L-idose configured diol respectively (EtOAc:pentane, 7:3, v:v)) the reaction was quenched with sat.

aq. NH_4Cl. The layers were separated and the green aqueous phase was extracted with EtOAc (3x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (10:90 EtOAc:pentane → 50:50 EtOAc:pentane) yielded the D-glucose and L-idose configured diol **31** and **32** as a colourless solid and brown oil respectively (17.6 g, 43.8 mmol, 96% for the glucose configuration and 0.37 g, 0.91 mmol, 2% for the idose configuration).

Analytical data for the glucose configured diol **31**: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.32 –6.84 (m, 8H, CH_{arom}), 5.75 (ddd, *J* = 10.2, 2.9, 2.1 Hz, 1H, H-7), 5.49 (ddd, *J* = 10.3, 2.0, 2.0 Hz, 1H, H-1), 4.94 (d, *J* = 11.0 Hz, 1H, C*H*H PMB), 4.70 – 4.61 (m, 2H, C*H*H PMB, CH*H* PMB), 4.58 (d, *J* = 11.1 Hz, 1H, CH*H* PMB), 4.16 (ddd, *J* = 7.1, 3.6, 1.7 Hz, 1H, H-2), 3.81 (s, 6H, OMe), 3.79 – 3.55 (m, 4H, H-3, H-4, H-6), 2.89 (d, *J* = 1.3 Hz, 1H, 4-OH), 2.53 – 2.46 (m, 1H, H-5), 2.43 (dd, *J* = 8.2, 2.9 Hz, 1H, 6-OH); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.5, 130.6, 130.3 ($C_{q\text{-}arom}$), 129.8, 129.7 (CH_{arom}), 127.7 (C-7), 127.3 (C-1), 114.1, 114.1 (CH_{arom}), 83.0 (C-3), 80.0 (C-2), 74.6 (CH_2 PMB), 73.0 (C-4), 71.3 (CH_2 PMB), 65.7 (C-6), 55.4 (OMe), 45.3 (C-5); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{23}H_{28}NaO_6$ 423.1778; Found 423.1778.

Analytical data for the idose configured diol **32** : ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.33 – 7.18 (m, 3H, CH_{arom}), 6.95 – 6.84 (m, 5H, CH_{arom}), 5.78 (ddd, *J* = 10.2, 2.7, 1.8 Hz, 1H, H-1), 5.63 (ddd, *J* = 10.2, 4.1, 1.6 Hz, 1H, H-7), 4.86 (d, *J* = 11.1 Hz, 1H, C*H*H PMB), 4.69 – 4.47 (m, 3H, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.08 – 4.00 (m, 2H, H-2, H-4), 3.81 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.80 – 3.76 (m, 2H, H-3, H-6), 3.67 (ddd, *J* = 11.7, 8.8, 3.2 Hz, 1H, H-6), 3.16 (d, *J* = 3.4 Hz, 1H, 4-OH), 3.13 (dd, *J* = 8.8, 3.6 Hz, 1H, 6-OH), 2.89 – 2.73 (m, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.6, 130.4, 130.0 ($C_{q\text{-}arom}$), 129.7, 129.7 (CH_{arom}), 127.6 (C-7), 127.1 (C-1), 114.2 114.1 (CH_{arom}), 78.2, 78.2 (C-2, C-3), 73.9 (CH_2 PMB), 71.3 (C-4), 71.3 (CH_2 PMB), 64.1 (C-6), 55.5, 55.4 (OMe), 41.9 (C-5); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{23}H_{28}NaO_6$ 423.1778; Found 423.1775.

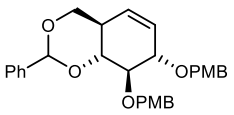
2,3,4,6-Per-*O*-(4-methoxybenzyl)-cyclophellitol alkene (33).



Cyclohexene diol **31** (3.2 g, 5.0 mmol) was co-evaporated with toluene (1x), dissolved in anhydrous DMF (20 mL, 0.25 M) and cooled on ice. Subsequently, PMBCl (1.1 mL, 20 mmol, 4.0 eq.), TBAI (0.02 g, 0.05 mmol, 0.01 eq.) and NaH (60% wt., 0.8 g, 20 mmol, 4.0 eq.) were added. The reaction was purged in N_2 and stirred overnight at room temperature. Upon full conversion (R_f 0.5 (EtOAc:pentane, 3:7 v:v)), the reaction was quenched with MeOH and concentrated under reduced pressure. The crude was dissolved in EtOAc, and washed with H_2O. The aqueous layer was extracted with EtOAc (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (10:90 EtOAc:pentane → 20:80 EtOAc:pentane) yielded cyclohexene **33** as a yellow oil (2.9 g, 4.6 mmol, 92%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.32 – 7.16 (m, 6H, CH_{arom}), 7.14 – 7.06 (m, 2H, CH_{arom}), 6.89 – 6.78 (m, 8H, CH_{arom}), 5.70 – 5.60 (m, 2H, H-1, H-7), 4.86 – 4.78 (m, 3H, C*H*H PMB, C*H*H PMB, C*H*H PMB), 4.63 – 4.59 (m, 2H, C*H*H PMB, CH*H* PMB), 4.41 (d, *J* = 11.9 Hz, 1H, CH*H* PMB), 4.36 – 4.30 (m, 2H, CH*H* PMB, CH*H* PMB), 4.20 (dddd, *J* = 7.5, 3.7, 1.8, 1.8 Hz, 1H, H-2), 3.80 (s, 6H, OMe), 3.79 (s, 3H, OMe), 3.76 (s, 3H, OMe), 3.73 (d, *J* = 7.9 Hz, 1H, H-3), 3.59 (dd, *J* = 9.8, 9.8 Hz, 1H, H-4), 3.47 (d, *J* = 4.1 Hz, 2H, H-6), 2.48 (dddd, *J* = 5.1, 5.1, 2.9, 2.9 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.3, 159.3, 159.3, 159.2, 131.3, 131.0, 130.8, 130.4 ($C_{q\text{-}arom}$), 129.8,

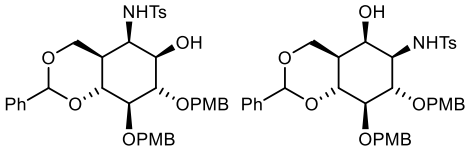
129.7, 129.6, 129.6 (CH_{arom}), 129.3, 127.1 (C-1, C-7), 113.9, 113.9, 113.9 (CH_{arom}), 85.3 (C-3), 80.8 (C-2), 78.3 (C-4), 75.1, 75.0, 72.8, 71.9 (CH_2 PMB), 68.9 (C-6), 55.4, 55.4 (OMe), 44.5 (C-5); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{39}H_{44}NaO_8$ 663.2928; Found 663.2924.

2,3-Di-*O*-((4-methoxybenzyl)-4,6-*O*-benzylidene-cyclophellitol alkene (36).



Diol **31** (3.6 g, 9.0 mmol) was dissolved in anhydrous DMF (18 mL, 0.5 M) after which benzaldehyde dimethyl acetal (2.7 mL, 18 mmol, 2.0 eq.) and *p*-TsOH (17 mg, 90 µmol, 0.01 eq.) were added. The mixture was rotated under reduced pressure (<20 mbar) at 25 °C for 4 hours. Upon full conversion (R_f 0.6 (EtOAc:pentane, 3:7, v:v)) the reaction was quenched with sat. aq. $NaHCO_3$ and extracted with Et_2O (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (10:90 EtOAc:pentane → 25:75 EtOAc:pentane) yielded **36** as a colorless oil (3.7 g, 7.7 mmol, 85%). ^1H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.59 – 7.45 (m, 2H, CH_{arom}), 7.43 – 7.13 (m, 3H, CH_{arom}), 6.94 – 6.70 (m, 8H, CH_{arom}), 7.59 – 7.45 (m, 1H, H-7), 5.62 (s, 1H, CHPh), 5.40 – 5.34 (m, 1H, H-1), 4.94 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 4.73 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 4.66 (d, *J* = 11.1 Hz, 1H, CH*H* PMB), 4.59 (d, *J* = 11.2 Hz, 1H, CH*H* PMB), 4.28 (dd, *J* = 10.8, 4.6 Hz, 1H, H-6), 4.24 (dddd, *J* = 5.4, 2.8, 2.8, 2.8 Hz, 1H, H-2), 3.96 (dd, *J* = 10.4, 6.9 Hz, 1H, H-3), 3.80 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.77 (d, *J* = 9.6 Hz, 1H, H-4), 3.64 (dd, *J* = 11.2, 11.2 Hz, 1H, H-6), 2.68 (dddd, *J* = 13.9, 8.3, 4.2, 4.2 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.3, 138.4, 131.1, 130.7 ($C_{q\text{-}arom}$), 129.9, 129.6 (CH_{arom}), 129.3 (C-7), 128.9, 128.3, 126.1 (CH_{arom}), 125.1 (C-1), 113.9, 113.9 (CH_{arom}), 101.7 (CHPh), 82.3 (C-4), 81.8 (C-3), 80.5 (C-2), 74.5, 72.1 (CH_2 PMB), 70.2 (C-6), 55.4 (OMe), 38.7 (C-5); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{30}H_{32}NaO_6$ 511.2091; Found 511.2090.

2,3,-Di-*O*-(4-methoxybenzyl)-4,6-di-*O*-benzylidene-7-(*R*)-(*p*-toluenesulfonamido)-1-(*R*)-ol cyclophellitol alkane (37) and 1-(*R*)-(*p*-toluenesulfonamido)-2,3-di-*O*-(4-methoxybenzyl)-4,6-di-*O*-benzylidene-7-(*R*)-ol cyclophellitol alkane (38).

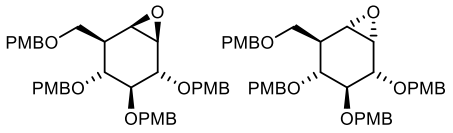


Cyclohexene **36** (0.12 g, 0.25 mmol) was dissolved in $CHCl_3$ (2.5 mL, 0.1 M) and flushed in N_2. Subsequently, chloramine-T hydrate (0.1 g, 0.5 mmol, 2.0 eq.) was added and the reaction was stirred vigorously. After 5 min, benzyltriethylammonium chloride (TEBACl, 0.003 g, 0.015 mmol, 0.06 eq.) was added and stirred vigorously. After 5 min, H_2O (2.5 mL) was added and stirred vigorously for 10 min. Finally, potassium osmate ($K_2[OsO_2(OH)_4]$) (0.005 g, 0.013 mmol, 0.05 eq.) was added and the reaction was stirred vigorously overnight at 60 °C. Upon partial conversion (R_f 0.8 and 0.6 for **37** and **38** respectively (EtOAc:pentane 1:1 v:v)), the reaction was cooled to room temperature, quenched with sat. aq. $Na_2S_2O_3$ and stirred for 30 min at 60 °C. The mixture was extracted with Et_2O (3x), and the organic layers were washed with 1% wt. NaOH (3x) and brine. The organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (dry loading, 20:80 EtOAc:pentane → 40:60 EtOAc:pentane) yielded the starting material (44 mg, 0.09 mmol) and regioisomers as colorless oils (15 mg, 22 µmol, 8.8% for **37** and 9 mg, 13 µmol, 5.2% for **38**, and 28 mg, 0.041 mmol, 16.5% mix fractions, 66% brsm). Analytical

data for **37**: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.78 – 7.31 (m, 8H, CH_{arom}), 7.31 – 7.10 (m, 5H, CH_{arom}), 6.95 – 6.72 (m, 4H, CH_{arom}), 5.54 (s, 1H, CHPh), 4.90 (d, *J* = 10.8 Hz, 1H, C*H*H PMB), 4.87 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 4.71 (d, *J* = 5.9 Hz, 1H, 7-NHTs), 4.68 (d, *J* = 10.8 Hz, 1H, CH*H* PMB), 4.56 (d, *J* = 10.9 Hz, 1H, CH*H* PMB), 4.16 – 4.03 (m, 2H, H-6), 3.97 (dd, *J* = 11.0, 9.1 Hz, 1H, H-4), 3.81 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.59 (dd, *J* = 9.1, 9.1 Hz, 1H, H-3), 3.57 – 3.52 (m, 2H, H-1, H-7), 3.34 (dd, *J* = 9.2, 9.2 Hz, 1H, H-2), 2.41 (s, 3H, $PhCH_3$), 2.18 (d, *J* = 1.9 Hz, 1H, 1-OH), 1.83 (dddd, *J* = 10.8, 10.8, 4.8, 2.5 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HH-COSY, HSQC): δ 159.6, 159.4, 143.9, 138.1, 136.4, 130.7, 130.4 ($C_{q\text{-}arom}$), 129.9, 129.8, 129.6, 128.9, 128.3, 127.9, 126.0, 114.1, 113.9 (CH_{arom}), 100.8 (CHPh), 82.7 (C-3), 80.8 (C-2), 79.2 (C-4), 75.4, 75.1 (CH_2 PMB), 71.6 (C-1), 68.9 (C-6), 55.4 (OMe), 52.8 (C-7), 37.8 (C-5), 21.8 ($PhCH_3$); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{37}H_{41}NNaO_9S$ 698.2394; Found 698.2396.

Analytical data for **38**: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.69 – 6.71 (m, 17H, CH_{arom}), 5.56 (s, 1H, CHPh), 4.89 (d, *J* = 10.4 Hz, 1H, C*H*H PMB), 4.78 (d, *J* = 11.2 Hz, 1H, C*H*H PMB), 4.63 (d, *J* = 10.6 Hz, 1H, CH*H* PMB), 4.59 (d, *J* = 3.1 Hz, 1H, 1-NHTs), 4.49 (d, *J* = 11.3 Hz, 1H, CH*H* PMB), 4.22 – 4.07 (m, 4H, H-4, H-6, H-7), 3.84 (s, 3H, OMe), 3.77 (s, 3H, OMe), 3.65 – 3.55 (m, 2H, H-2, H-3), 2.89 (ddd, *J* = 10.2, 3.0, 3.0 Hz, 1H, H-1), 2.55 (d, *J* = 3.0 Hz, 1H, 7-OH), 2.42 (s, 3H, $PhCH_3$), 1.77 (ddd, *J* = 10.8, 10.8, 6.9 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.75, 159.35, 144.07, 138.33, 135.72, 130.77, 130.04 ($C_{q\text{-}arom}$), 129.95, 129.91, 129.62, 128.87, 127.37, 126.12, 114.37, 113.83 (CH_{arom}), 100.81 (CHPh), 84.00 (C-2/C-3), 78.63 (C-4), 77.24 (C-2/C-3), 75.11, 74.95 (CH_2PMB), 68.31 (C-6), 67.23 (C-7), 59.94 (C-1), 55.44 (OMe), 39.13 (C-5), 21.73 ($PhCH_3$); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{37}H_{41}NNaO_9S$ 698.2394; Found 698.2393.

2,3,4,6-Tetra-*O*-(4-methoxybenzyl) cyclophellitol (39) and 1,7-*Epi*-2,3,4,6-tetra-*O*-(4-methoxybenzyl) cyclophellitol (40).

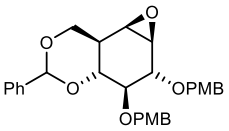


Per-PMB protected cyclohexene **33** (6.66 g, 10.4 mmol) was dissolved in anhydrous DCM (104 mL, 0.1 M) and cooled on ice. Subsequently, *m*-CPBA (3.9 g, 22.9 mmol, 2.2 eq.) was added and the reaction was purged with argon gas. The reaction was stirred overnight at room temperature. Upon partial conversion (R_f 0.32 and 0.26 for the β- and α-epoxide respectively (acetone:pentane, 2:8 v:v)) the reaction was quenched with sat. aq. $Na_2S_2O_4$. The aqueous layer was extracted with Et_2O (3x). The organic layers were combined and washed with sat. aq. $NaHCO_3$. The bicarb layer was extracted once with Et_2O and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Flash column chromatography (dry loading, 3:97 acetone:pentane → 15:85 acetone:pentane) yielded the epoxides as colourless oils (0.66 g, 1.0 mmol, 9.7% for α-epoxide **40** and 2.2 g, 3.4 mmol, 32% for β-epoxide **39**). Analytical data for α-epoxide **40**: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.36 – 7.03 (m, 8H, CH_{arom}), 6.94 – 6.77 (m, 8H, CH_{arom}), 4.80 – 4.70 (m, 5H, C*H*H PMB, C*H*H PMB, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.38 (d, *J* = 11.8 Hz, 1H, C*H*H PMB), 4.31 – 4.16 (m, 2H, CH*H* PMB, CH*H* PMB), 3.83 (dd, *J* = 8.5, 1.8 Hz, 1H, H-2), 3.78 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.76 (s, 3H, OMe), 3.75 (s, 3H, OMe), 3.66 (dd, *J* = 10.2, 8.5 Hz, 1H, H-3), 3.55 – 3.47 (m, 2H, H-6), 3.41 (dd, *J* = 10.0, 10.0 Hz, 1H, H-4), 3.27 (dd, *J* = 4.0, 1.8 Hz, 1H, H-1), 3.13 (d, *J* = 4.0 Hz, 1H, H-7), 2.16 (ddd, *J* = 10.0, 3.4, 3.4 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.3, 159.3, 159.2, 159.2, 131.1, 130.8, 130.7, 130.1 ($C_{q\text{-}arom}$), 129.6, 129.6,

129.5, 113.8, 113.8, 113.8 (CH_{arom}), 82.2 (C-3), 79.6 (C-2), 77.4 (C-4), 75.4, 74.9, 72.8, 72.5 (CH_2 PMB), 67.9 (C-6), 55.3, 55.3, 55.2, 54.8 (C-1, C-7, OMe), 42.9 (C-5); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{39}H_{44}NaO_9$ 679.2878; Found 679.2874.

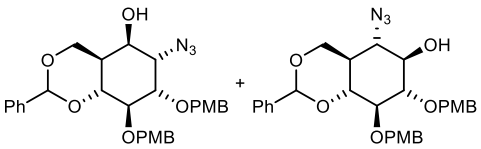
Analytical data for β-epoxide **39**: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.36 – 7.17 (m, 5H, CH_{arom}), 7.16 – 7.04 (m, 2H, CH_{arom}), 6.95 – 6.71 (m, 9H, CH_{arom}), 4.87 – 4.55 (m, 5H, C*H*H PMB, C*H*H PMB, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.45 (m, 2H, C*H*H PMB, CH*H* PMB), 4.30 (d, *J* = 10.6 Hz, 1H, CH*H* PMB), 3.82 (d, *J* = 0.9 Hz, 1H, H-2), 3.80 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.80 (s, 6H, OMe), 3.69 (dd, *J* = 8.8, 3.5 Hz, 1H, H-6), 3.56 – 3.39 (m, 3H, H-3, H-6, H-7), 3.19 (dd, *J* = 10.0, 10.0 Hz, 1H, H-4), 3.15 (d, *J* = 3.7 Hz, 1H, H-1), 2.23 (dddd, *J* = 10.2, 8.8, 3.5, 1.6 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.6, 159.4, 159.3, 159.3, 131.1, 130.6, 130.5, 130.0 ($C_{q\text{-}arom}$), 129.8, 129.8, 129.6, 129.4, 114.1, 113.9, 113.9, 113.9 (CH_{arom}), 84.9 (C-3), 79.8 (C-2), 75.2, 75.1 (CH_2 PMB), 75.0 (C-4), 73.0, 73.0 (CH_2 PMB), 68.4 (C-6), 55.8 (C-7), 55.4, 55.4, 55.4 (OMe), 54.1 (C-1), 42.7 (C-5); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{39}H_{44}NaO_9$ 679.2878; Found 679.2876.

2,3-Di-*O*-(4-methoxybenzyl)-4,6-*O*-benzylidene cyclophellitol (41).



Cyclohexene **36** (0.70 g, 1.3 mmol) was dissolved in anhydrous DCM (23 mL, 0.06 M). Subsequently, $NaHCO_3$ (1.1 g, 13 mmol, 10 eq.) and *m*-CPBA (0.6 g, 3.3 mmol, 2.5 eq.) were added. The reaction was stirred under N_2 atmosphere at 5 °C for 6 days. Upon partial conversion was observed (R_f 0.4 (EtOAc:pentane, 3:7, v:v)), the reaction was quenched with sat. aq. $Na_2S_2O_3$. The aqueous layer was extracted with Et_2O (3x) and the combined organic layers were subsequently washed with sat. aq. $NaHCO_3$. The organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (dry loading, 10:90 EtOAc:pentane → 30:70 EtOAc:pentane) yielded **41** as a single diastereoisomer (0.37 g, 0.74 mmol, 56%) and starting material (98 mg, 0.18 mmol, 14%) 70% brsm ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.56 – 7.16 (m, 9H, CH_{arom}), 6.96 – 6.77 (m, 4H, CH_{arom}), 5.58 (s, 1H, CHPh), 4.84 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 4.70 – 4.68 (m, 2H, C*H*H PMB, CH*H* PMB), 4.65 (d, *J* = 10.9 Hz, 1H, CH*H* PMB), 4.35 (dd, *J* = 11.0, 4.3 Hz, 1H, H-6), 4.00 (dd, *J* = 11.1, 11.1 Hz, 1H, H-6), 3.92 (d, *J* = 7.1 Hz, 1H, H-2), 3.83 (d, *J* = 10.0 Hz, 1H, H-4), 3.81 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.66 (dd, *J* = 10.4, 7.0 Hz, 1H, H-3), 3.15 (d, *J* = 3.8 Hz, 1H, H-1), 3.07 (d, *J* = 3.7 Hz, 1H, H-7), 2.36 (dddd, *J* = 11.2, 9.9, 4.2, 1.1 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.6, 159.3, 138.2, 130.9, 129.9 ($C_{q\text{-}arom}$), 129.9, 129.8, 128.9, 128.3, 126.1, 114.1, 113.8 (CH_{arom}), 101.4 (CHPh), 81.4 (C-3), 78.9 (C-2), 77.0 (C-4), 74.6, 73.3 (CH_2 PMB), 68.7 (C-6), 55.4, 55.4 (OMe), 53.2 (C-7), 53.0 (C-1) , 37.9 (C-5); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{30}H_{32}NaO_7$ 527.2040; Found 527.2041.

1-(*S*)-Azido-2,3-di-*O*-(4-methoxybenzyl)-4,6-*O*-benzylidene-7-(*R*)-ol cyclophellitol alkane (42) and 2,3-Di-*O*-(4-methoxybenzyl)-4,6-*O*-benzylidene-7-(*S*)-azido-1-(*R*)-ol cyclophellitol alkane (43).



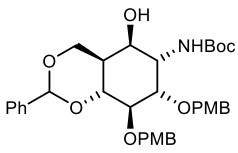
Epoxide **41** (6.1 g, 12 mmol) was dissolved in anhydrous DMF (121 mL, 0.1 M). Subsequently NaN_3 (16 g, 242 mmol, 20 eq.) and $LiClO_4$ (2.6 g, 24 mmol, 2.0 eq.) were added. The reaction was stirred under argon

atmosphere at 100 °C overnight. Upon full conversion was observed (R_f 0.5 and 0.7 for **42** and **43** respectively (EtOAc:pentane, 3.5:6.5, v:v)) the reaction was allowed to cool to room temperature and diluted with sat. aq. $NaHCO_3$. The aqueous layer was extracted with EtOAc (3x) and the combined organic layers were washed with brine. The organic layers were subsequently dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Flash column chromatography (10:90 EtOAc:pentane → 40:60 EtOAc:pentane) yielded the 1,7-(*N*,*O*) and –(*O*,*N*) regioisomers as a yellowish oil and solid respectively (5.0 g, 9.1 mmol, 75% for the 1,7-(*N*,*O*) isomer **42** and 1.7 g, 3.1 mmol, 25% for the 1,7-(*O*,*N*) isomer **43**).

Analytical data for the 1,7-(*N*,*O*) regioisomer **42**: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.56 – 7.44 (m, 2H, CH_{arom}), 7.42 – 7.27 (m, 7H, CH_{arom}), 6.91 – 6.80 (m, 4H, CH_{arom}), 5.54 (s, 1H, CHPh), 4.85 (d, *J* = 10.6 Hz, 1H, C*H*H PMB), 4.80 (d, *J* = 11.4 Hz, 1H, C*H*H PMB), 4.73 (d, *J* = 10.6 Hz, 1H, CH*H* PMB), 4.65 (d, *J* = 11.4 Hz, 1H, CH*H* PMB), 4.14 (dd, *J* = 10.8, 4.4 Hz, 1H, H-6), 4.09 – 3.82 (m, 5H, H-1, H-2, H-3, H-4, H-6), 3.81 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.78 – 3.71 (m, 1H, H-7), 2.13 (dddd, *J* = 11.0, 11.0, 4.4, 2.2 Hz, 1H, H-5), 1.57 (d, *J* = 4.4 Hz, 1H, 7-OH); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 138.2, 131.2, 130.5 ($C_{q\text{-}arom}$), 129.9, 129.7, 128.9, 128.3, 126.1, 114.0, 113.8 (CH_{arom}), 101.0 (CHPh), 80.7 (C-2), 78.9, 78.7 (C-3, C-4), 75.5, 73.7 (CH_2 PMB), 68.8 (C-1), 68.1 (C-6), 64.8 (C-7), 55.4 (OMe), 36.9 (C-5); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{30}H_{33}N_3NaO_7$ 570.2211; Found 570.2209.

Analytical data for the 1,7-(*O*,*N*) regioisomer **43**: ^{1}H NMR (500 MHz, $CDCl_3$ HH-COSY, HSQC): δ 7.53 – 7.20 (m, 8H, CH_{arom}), 6.92 – 6.81 (m, 5H, CH_{arom}), 5.54 (s, 1H, CHPh), 4.94 (d, *J* = 11.0 Hz, 1H, C*H*H PMB), 4.90 (d, *J* = 10.6 Hz, 1H, C*H*H PMB), 4.71 (d, *J* = 10.6 Hz, 1H, CH*H* PMB), 4.61 (d, *J* = 10.9 Hz, 1H, CH*H* PMB), 4.44 (dd, *J* = 11.1, 4.4 Hz, 1H, H-6), 3.81 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.72 – 3.64 (m, 3H, H-3, H-4, H-6), 3.62 (ddd, *J* = 9.4, 9.4, 2.2 Hz, 1H, H-1), 3.37 (ddd, *J* = 9.1, 6.6, 2.5 Hz, 1H, H-2), 3.09 (dd, *J* = 11.8, 9.5 Hz, 1H, H-7), 2.54 (d, *J* = 2.3 Hz, 1H, 1-OH), 1.77 (dddd, *J* = 10.8, 10.7, 10.7, 4.3 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 137.9, 130.6 ($C_{q\text{-}arom}$), 130.1, 129.9, 129.1, 128.4, 126.1, 114.3, 114.0 (CH_{arom}), 101.5 (CHPh), 82.4 (C-2), 82.1 , 81.0 (C-3, C-4), 76.5 (C-1), 75.5, 75.3 (CH_2 PMB), 69.5 (C-6), 61.1 (C-7), 55.4, 55.4 (OMe), 38.3 (C-5); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{30}H_{33}N_3NaO_7$ 570.2211; Found 570.2206.

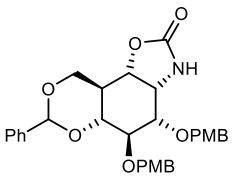
1-(*S*)-(*N*-(Tert-butoxycarbonyl)-2,3-di-*O*-(4-methoxybenzyl)-4,6-*O*-benzylidene-7-(*R*)-ol cyclophellitol alkane (44).



Azide **42** (0.27 g, 0.5 mmol) was dissolved in a 1:1 solution of THF and aq. 1.0 M NaOH (5.0 mL, 0.1 M). Subsequently triphenylphosphine (0.5 g, 2.0 mmol, 4.0 eq.) was added and the reaction was stirred vigorously overnight at room temperature. Upon full conversion of the starting material was observed, the reaction was diluted with sat. aq. $NaHCO_3$ and EtOAc. The aqueous phase was separated and subsequently extracted with EtOAc (3x), the organic layers were then washed with brine. The brine layer was extracted once with EtOAc, and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude amine was dissolved in anhydrous DCM (2.5 mL, 0.2 M) and cooled on ice. While stirring, Boc_2O (0.13 g, 0.6 mmol, 1.2 eq.) and Et_3N (0.35 mL, 2.5 mmol, 5.0 eq.) were added. The flask was flushed in N_2 and the reaction was stirred at room temperature for 22 hours until full conversion was observed (R_f 0.4 (EtOAc:pentane, 4:6, v:v)). The reaction was quenched with

sat. aq. NH_4Cl and the aqueous layer separated. Subsequently, the aqueous layer was extracted with EtOAc (3x), and the combined organic layers were washed with sat. aq. $NaHCO_3$. The organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (dry loading, 10:90 EtOAc:pentane → 50:50 EtOAc:pentane) yielded **44** as a white, brittle foam (0.2 g, 0.33 mmol, 66% over two steps). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.53 – 7.48 (m, 2H, CH_{arom}), 7.41 – 7.32 (m, 3H, CH_{arom}), 7.27 (m, 4H, CH_{arom}), 6.84 (m, 4H, CH_{arom}), 5.58 (s, 1H, CHPh), 4.90 (d, *J* = 3.9 Hz, 1H, 1-NHBoc), 4.82 (d, *J* = 10.8 Hz, 1H, C*H*H PMB), 4.71 (d, *J* = 10.8 Hz, 1H, C*H*H PMB), 4.56 (m, 2H, CH*H* PMB, CH*H* PMB), 4.22 – 4.12 (m, 3H, H-3, H-6, H-7), 4.09 (dd, *J* = 11.0, 11.0 Hz, 1H, H-6), 4.02 – 3.96 (m, 2H, H-1, H-4), 3.80 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.62 (dd, *J* = 8.7, 8.7 Hz, 1H, H-5), 2.13 (dd, *J* = 11.5, 11.5 Hz, 1H, H-2), 2.05 (s, 1H, 7-OH), 1.45 (s, 9H, $C(CH_3)_3$ Boc); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.7, 159.4 ($C_{q\text{-}arom}$), 138.5 (C=O Boc), 131.2, 130.2 ($C_{q\text{-}arom}$), 129.7, 129.7, 128.8, 128.3, 126.1, 114.0, 113.8 (CH_{arom}), 100.9 (CHPh), 80.6 (C-5), 80.3 (*C*$(CH_3)_3$) 78.6 (C-3, C-7), 76.7 (C-4), 75.1, 72.1 (CH_2 PMB), 68.5 (C-6), 55.4 (OMe), 54.8 (C-1), 36.8 (C-2), 28.1 (C(*C*$H_3)_3$); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{35}H_{43}NNaO_9$ 644.2830; Found 644.2831.

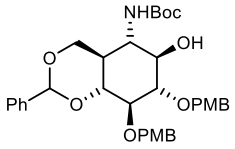
1,7-(*S*,*S*)-(*N*,*O*)-Carbamate-2,3-di-*O*-(4-methoxybenzyl)-4,6-*O*-benzylidene cyclophellitol alkane (46).



The Boc-protected amino alcohol **44** (6.0 g, 9.7 mmol) was co-evaporated with toluene, dissolved in anhydrous $CHCl_3$ (97 mL, 0.1 M) and cooled on ice. Subsequently Me-imidazole (7.8 mL, 97 mmol, 10 eq.), MsCl (3.8 mL, 49 mmol, 5.0 eq.) and Et_3N (6.8 mL, 49 mmol, 5.0 eq.) were added. The reaction was flushed with N_2 and stirred overnight at room temperature. Upon full conversion (R_f 0.6 (EtOAc:pentane, 4:6, v:v)) the reaction was diluted with EtOAc and washed with sat. aq. NH_4Cl. The aqueous layer was extracted with EtOAc (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude intermediate was dissolved in anhydrous DMF (487 mL, 0.02 M), flushed with argon gas and stirred at 120 °C overnight. Upon full conversion (R_f 0.7 (EtOAc:pentane, 9:1, v:v)) the reaction was cooled to room temperature and concentrated to a fifth of its original volume. The residue was diluted with EtOAc and washed with H_2O and consequently brine. The aqueous layers were back-extracted with EtOAc (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (dry loading, 50:50 EtOAc:pentane → 90:10 EtOAc:pentane) yielded the carbamate **46** as a yellowish, brittle foam (3.3 g, 6.0 mmol, 68% over two steps). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.51 – 7.47 (m, 2H, CH_{arom}), 7.39 (m, 3H, CH_{arom}), 7.24 – 7.17 (m, 4H, CH_{arom}), 6.91 – 6.80 (m, 4H, CH_{arom}), 5.51 (s, 1H, CHPh), 5.28 (s, 1H, 1-NH), 4.70 (d, *J* = 11.1 Hz, 1H, C*H*H PMB), 4.67 (d, *J* = 11.7 Hz, 1H, C*H*H PMB), 4.60 (d, *J* = 11.1 Hz, 1H, CH*H* PMB), 4.54 (dd, *J* = 11.1, 4.9 Hz, 1H, H-6), 4.43 (d, *J* = 11.8 Hz, 1H, CH*H* PMB), 4.22 (dd, *J* = 9.4, 7.6 Hz, 1H, H-7), 3.95 (dd, *J* = 7.7, 4.1 Hz, 1H, H-1), 3.86 (dd, *J* = 7.3, 5.1 Hz, 1H, H-3), 3.80 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.70 (d, *J* = 11.0 Hz, 1H, H-6), 3.61 (dd, *J* = 11.9, 7.3 Hz, 1H, H-4), 3.54 (dd, *J* = 4.6, 4.6 Hz, 1H, H-2), 2.61 (dddd, *J* = 15.7, 10.9, 4.8, 4.8 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.7 ($C_{q\text{-}arom}$), 159.5 (C=O), 158.9, 137.8, 129.9 ($C_{q\text{-}arom}$), 129.8, 129.8 (CH_{arom}), 129.4 ($C_{q\text{-}arom}$), 129.1, 128.4, 126.2, 114.2, 114.0 (CH_{arom}), 101.2 (CHPh), 78.7, 78.5 (C-3, C-4), 76.3

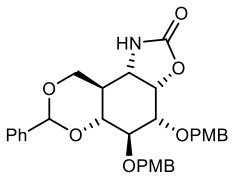
(C-5), 74.6 (C-7), 73.6, 72.9 (CH_2 PMB), 70.1 (C-6), 55.4 (OMe), 53.6 (C-1), 37.3 (C-2); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{31}H_{33}NNaO_8$ 570.2098; Found 570.2098.

2,3-Di-*O*-(4-methoxybenzyl)-4,6-*O*-benzylidene-7-(*S*)-(*N*-(*tert*-butoxycarbonyl)-1-(*R*)-ol cyclophellitol alkane (45).



Azide **43** (1.53 g, 2.8 mmol) was dissolved in a 1:1 solution of THF and aq. 1.0 M NaOH (28 mL, 0.1 M). Subsequently, triphenylphosphine (2.9 g, 11.2 mmol, 4.0 eq.) was added and the reaction was stirred vigorously overnight at room temperature. Upon full conversion of the starting material the reaction was washed with sat. aq. $NaHCO_3$. The aqueous phase was extracted with EtOAc (3x) and the organic layers were then washed with brine. The brine layer was extracted with EtOAc and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude amine was dissolved in anhydrous DCM (14 mL, 0.2 M) and cooled on ice. While stirring, Boc_2O (0.73 g, 3.4 mmol, 1.2 eq.) and Et_3N (1.9 mL, 14 mmol, 5.0 eq.) were added. The flask was flushed with N_2 and the reaction was stirred at room temperature for 19 hours until full conversion was observed (R_f 0.4 (EtOAc:pentane, 4:6, v:v)). The reaction was quenched with sat. aq. NH_4Cl. The aqueous phase was extracted with EtOAc (3x), and the combined organic layers were washed with sat. aq. $NaHCO_3$. The organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (dry loading, 10:90 EtOAc:pentane → 40:60 EtOAc:pentane) yielded **45** as a colourless solid (0.83 g, 1.34 mmol, 48% over two steps). ^1H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.53 – 7.46 (m, 2H, CH_{arom}), 7.41 – 7.32 (m, 3H, CH_{arom}), 7.31 – 7.14 (m, 4H, CH_{arom}), 7.04 – 6.72 (m, 4H, CH_{arom}), 5.54 (s, 1H, CHPh), 4.97 – 4.87 (m, 2H, C*H*H PMB, C*H*H PMB), 4.71 (d, *J* = 10.6 Hz, 1H, CH*H* PMB), 4.66 (d, *J* = 10.9 Hz, 1H, CH*H* PMB), 4.45 (d, *J* = 8.1 Hz, 1H, 7-NHBoc), 4.27 (dd, *J* = 11.4, 4.4 Hz, 1H, H-6), 3.84 – 3.79 (m, 1H, H-6), 3.79 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.72 (dd, *J* = 9.3, 9.3 Hz, 1H, H-4), 3.68 – 3.63 (m, 1H, H-3), 3.52 – 3.14 (m, 3H, H-1, H-2, H-7), 2.56 (s, 1H, 1-OH), 1.78 (dddd, *J* = 10.8, 10.8, 10.8, 4.7 Hz, 1H, H-5), 1.44 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.6 (C=O Boc), 159.4, 156.4, 138.1, 130.7, 130.6 ($C_{q\text{-}arom}$), 130.0, 129.9, 129.0, 128.4, 126.1, 114.2, 113.9 (CH_{arom}), 101.2 (CHPh), 83.1 (C-2), 82.4 (C-3), 81.5 (C-4), 80.3 ($C(CH_3)_3$) 75.5, 75.3 (CH_2 PMB), 75.1 (C-1), 68.4 (C-6), 55.4 (OMe), 51.5 (C-7), 40.0 (C-5), 28.4 ($C(CH_3)_3$ Boc); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{35}H_{43}NNaO_9$ 644.2830; Found 644.2829.

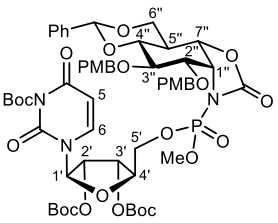
1,7-(*S*,*S*)-(*O*,*N*)-Carbamate-2,3-di-*O*-(4-methoxybenzyl)-4,6-*O*-benzylidene cyclophellitol alkane (47).



The boc-protected amino alcohol **45** (0.9 g, 1.46 mmol) was co-evaporated with toluene, dissolved in anhydrous $CHCl_3$ (14.6 mL, 0.1 M) and cooled to 0 °C. Subsequently Me-imidazole (1.1 mL, 14.6 mmol, 10 eq.), MsCl (0.56 mL, 7.3 mmol, 5.0 eq.) and Et_3N (1.0 mL, 7.3 mmol, 5.0 eq.) were added. The reaction was flushed in N_2 and stirred overnight at room temperature. Upon full conversion (R_f 0.6 (EtOAc:pentane, 4:6, v:v)) the reaction was diluted with EtOAc and washed with sat. aq. NH_4Cl. The aqueous layer was extracted with EtOAc (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude intermediate was dissolved in anhydrous DMF

(73 mL, 0.02 M), flushed in argon gas and stirred at 130 °C overnight. Upon full conversion (R_f 0.7 (EtOAc:pentane, 9:1, v:v)) the reaction was cooled to room temperature, diluted with EtOAc and washed with H_2O and brine. The aqueous layer was extracted with EtOAc (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (dry loading, 50:50 EtOAc:pentane → 90:10 EtOAc:pentane) yielded the carbamate **47** as a yellowish, brittle foam (0.54 g, 1.0 mmol, 67% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.53 – 7.14 (m, 9H, CH_{arom}), 6.89 – 6.77 (m, 4H, CH_{arom}), 6.67 (s, 1H, 7-NH), 5.48 (s, 1H, CHPh), 4.73 (dd, *J* = 8.7, 3.0 Hz, 1H, H-1), 4.64 – 4.44 (m, 4H, C*H*H PMB, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.49 – 4.36 (m, 1H, H-6), 3.84 – 3.81 (m, 2H, H-2, H-4), 3.79 (s, 3H, OMe), 3.73 (s, 3H, OMe), 3.66 – 3.42 (m, 4H, C-3, C-6, C-7), 2.57 (dddd, *J* = 10.8, 10.8, 10.8, 4.8 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.7, 159.5 ($C_{q\text{-}arom}$), 159.5 (C=O), 137.9, 130.0 ($C_{q\text{-}arom}$), 129.8, 129.6, 129.0, 128.3, 126.2, 113.9 (CH_{arom}), 101.1 (CHPh), 79.6 (C-3), 79.0 (C-4), 76.4 (C-2), 75.1 (C-1), 73.2, 72.6 (CH_2 PMB), 70.5 (C-6), 55.4 (OMe), 51.5 (C-7), 37.2 (C-5); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{31}H_{33}NNaO_8$ 570.2098; Found 570.2110.

1'',7''-(*N,O*-(3-*N*-(*Tert*-butoxycarbonyl)-5'-*O*-(methylphosphinyl)-2',3'-di-*O*-(*tert*-butoxycarbonyl)uridinyl))-carbamate-2'',3''-di-*O*-(4-methoxybenzyl)-4'',6''-di-*O*-benzylidene cyclophellitol alkane (49).

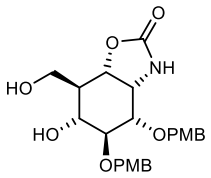


Compound **49** was prepared according to general procedure A using carbamate **46** (0.16 g, 0.3 mmol), 15-crown-5 (0.3 mL, 1.5 mmol, 5.0 eq.), anhydrous THF (1.5 mL, 0.2 M), NaH (60% wt., 0.02 g, 0.45 mmol, 1.5 eq.); and H-phosphonate **48** (0.47 g, 0.75 mmol, 2.5 eq.), anhydrous DCM (2.5 mL, 0.3 M), anhydrous DiPEA (0.4 mL, 0.75 mmol, 7.5 eq.) and $BrCCl_3$ (0.15 mL, 1.5 mmol, 5.0 eq.). Flash column chromatography (30:70 EtOAc:pentane → 70:30 EtOAc:pentane) and size exclusion yielded a P(V) diastereomeric mixture (ratio 1:1.3) of **49** as a colourless film (77 mg, 66 µmol, 22%, R_f 0.3 and 0.4 (EtOAc:pentane, 1:1 v:v)). Data for the first diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.71 (d, *J* = 8.3 Hz, 1H, H-6), 7.52 – 7.11 (m, 9H, CH_{arom}), 6.90 – 6.78 (m, 4H, CH_{arom}), 6.10 (d, *J* = 6.0 Hz, 1H, H-1'), 5.79 (d, *J* = 8.2 Hz, 1H, H-5), 5.52 (s, 1H, CHPh), 5.21 (dd, *J* = 5.2, 5.2 Hz, 1H, H-3'), 5.15 (ddd, *J* = 5.6, 5.4, 5.4 Hz, 1H, H-2'), 4.64 – 4.37 (m, 6H, H-1'', H-7'', C*H*H PMB, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.31 (dd, *J* = 6.6, 2.4 Hz, 2H, H-5'), 4.19 (dddd, *J* = 5.2, 5.2, 2.5, 2.5 Hz, 1H, H-4'), 4.15 (d, *J* = 2.0 Hz, 1H, H-3''), 3.90 (s, 3H, P(O)OMe), 3.90 (d, *J* = 9.4 Hz, 1H, H-2''), 3.80 (s, 6H, OMe), 3.79 – 3.64 (m, 3H, H-4'', H-6''), 2.93 – 2.77 (m, 1H, H-5''), 1.60 (s, 9H, $C(CH_3)_3$), 1.48 (s, 18H, $C(CH_3)_3$); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 160.3 (C=O uracil), 159.5, 159.4 ($C_{q\text{-}arom}$), 155.3 (d, J = 8.8 Hz, C=O cyclic carbamate), 152.2 (C=O carbamate), 152.1 (C=O uracil), 148.7, 148.6 (C=O carbonate), 139.4 (C-6), 137.7, 137.6 ($C_{q\text{-}arom}$), 129.8 (CH_{arom}), 129.5, 129.5, 129.3 ($C_{q\text{-}arom}$), 129.2, 129.1, 128.6, 128.5, 128.3, 126.1, 126.1, 114.0, 113.9, 113.9 (CH_{arom}), 103.4 (C-5), 101.2 (CHPh), 86.9 ($C(CH_3)_3$), 86.2 (C-1'), 83.8, 83.7 ($C(CH_3)_3$), 80.0 (C-4'), 78.8 (C-4''), 77.5 (C-3''), 76.5 (C-2''), 74.8, 74.7 (C-1'', C-2'), 72.1, 71.9 (CH_2PMB), 71.8 (C-3'), 70.6 (C-6''), 67.00 (d, *J* = 6.1 Hz, C-5'), 56.65 (d, *J* = 5.0 Hz, C-7''), 55.4 (OMe), 55.1 (P(O)OMe), 35.0 (C-5''), 27.7, 27.7, 27.5 ($C(CH_3)_3$); ^{31}P NMR (162 MHz, $CDCl_3$): δ -2.39.

Data for the second diastereomer: ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.59 (d, *J* = 8.2 Hz, 1H, H-6), 7.52 – 7.11 (m, 9H, CH_{arom}), 6.90 – 6.78 (m, 4H, CH_{arom}), 6.14 (d, *J* = 5.9 Hz, 1H, H-1'),

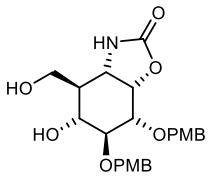
5.85 (d, *J* = 8.2 Hz, 2H, H-5), 5.52 (s, 1H, CHPh), 5.28 (dd, *J* = 5.3, 5.3 Hz, 1H, H-3'), 5.15 (ddd, *J* = 5.6, 5.4, 5.4 Hz, 1H, H-2'), 4.64 – 4.37 (m, 7H, H-1'', H-5', H-7'', C*H*H PMB, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.35 (dd, *J* = 4.7, 2.5 Hz, 1H, H-4'), 4.11 (d, *J* = 4.4 Hz, 1H, H-3''), 3.90 (d, *J* = 9.4 Hz, 1H, H-2''), 3.87 (s, 3H, P(O)OMe), 3.76 (s, 6H, OMe), 3.79 – 3.64 (m, 3H, H-4'', H-6''), 2.93 – 2.77 (m, 1H, H-5''), 1.49 (s, 9H, $C(CH_3)_3$), 1.46 (s, 18H, $C(CH_3)_3$); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 160.2 (C=O uracil), 159.5, 159.3 ($C_{q\text{-}arom}$), 155.1 (d, J = 8.9 Hz, C=O cyclic carbamate), 152.0 (C=O carbamate), 151.9 (C=O uracil), 147.6, 147.6 (C=O carbonate), 139.4 (C-6), 137.7, 137.6 ($C_{q\text{-}arom}$), 129.8 (CH_{arom}), 129.5, 129.5, 129.3 ($C_{q\text{-}arom}$), 129.2, 129.1, 128.6, 128.5, 128.3, 126.1, 126.1, 114.0, 113.9, 113.9 (CH_{arom}), 103.1 (C-5), 101.1 (CHPh), 87.1, ($C(CH_3)_3$), 86.7 (C-1'), 83.8, 83.6 ($C(CH_3)_3$), 79.9 (C-4'), 78.8 (C-4''), 77.3 (C-3''), 76.4 (C-2''), 74.7 74.6 (C-1'', C-2'), 71.8, (C-3'), 71.8, 71.7 (CH_2PMB), 70.5 (C-6''), 66.40 (d, *J* = 5.3 Hz, C-5'), 56.55 (d, *J* = 5.2 Hz, C-7''), 55.3 (OMe), 55.0 (P(O)OMe), 34.9 (C-5''), 27.7, 27.7, 27.5 ($C(CH_3)_3$); ^{31}P NMR (162 MHz, $CDCl_3$): δ -1.85; HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{56}H_{70}N_3NaO_{22}P$ 1190.4081; Found 1190.4086.

1,7-(*S,S*)-(*N,O*)-Carbamate-2,3-di-*O*-(4-methoxybenzyl) cyclophellitol alkane (52).



Carbamate **46** (0.55 g, 1.0 mmol) was dissolved in MeOH (40 mL, 0.025 M) and *p*-TsOH (0.1 g, 0.5 mmol, 0.5 eq.) was added. The reaction was stirred at 40 °C for 1 hour until full conversion was observed (R_f 0.3 (EtOAc:pentane, 9:1, v:v)). The reaction was quenched with Et_3N to neutral pH and concentrated under reduced pressure. Flash column chromatography (dry loading, 70:30 EtOAc:pentane → 100:0 EtOAc:pentane) yielded **52** as a white, brittle foam (0.41 g, 0.9 mmol, 90%). ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.43 – 7.14 (m, 4H, CH_{arom}), 6.96 – 6.76 (m, 4H, CH_{arom}), 5.63 (s, 1H, 1-NH), 4.81 (d, *J* = 11.0 Hz, 1H, C*H*H PMB), 4.65 (d, *J* = 11.5 Hz, 1H, C*H*H PMB), 4.62 (d, *J* = 11.1 Hz, 1H, CH*H* PMB), 4.52 (d, *J* = 11.5 Hz, 1H, CH*H* PMB), 4.46 (dd, *J* = 9.4, 7.0 Hz, 1H, H-7), 4.04 (dd, *J* = 7.1, 4.4 Hz, 1H, H-1), 4.00 – 3.91 (m, 1H, H-6), 3.79 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.69 (dd, *J* = 10.9, 3.9 Hz, 1H, H-6), 3.61 (dd, *J* = 8.3, 8.3 Hz, 1H, H-3), 3.49 (dd, *J* = 8.0, 4.5 Hz, 1H, H-2), 3.41 (dd, *J* = 11.5, 8.7 Hz, 1H, H-4), 3.21 (d, *J* = 2.7 Hz, 1H, 4-OH), 2.80 (s, 1H, 6-OH), 1.97 (ddd, *J* = 16.1, 8.3, 4.1 Hz, 1H, H-5); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 159.7 (C=O), 159.6, 159.1, 130.3 ($C_{q\text{-}arom}$), 129.8, 129.7 (CH_{arom}), 129.6 ($C_{q\text{-}arom}$), 114.2, 114.1 (CH_{arom}), 81.5 (C-3), 77.6 (C-2), 74.7 (CH_2 PMB), 74.0 (C-7), 73.0 (CH_2 PMB), 68.8 (C-4), 59.8 (C-6), 55.4 (OMe), 54.7 (C-1), 45.6 (C-5); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{24}H_{29}NNaO_8$ 482.1785; Found 482.1783.

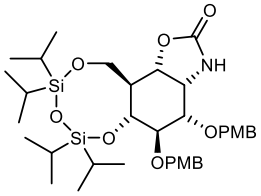
1,7-(*S,S*)-(*O,N*)-Carbamate-2,3-di-*O*-(4-methoxybenzyl) cyclophellitol alkane (53).



Carbamate **47** (27 mg, 50 µmol) was dissolved in MeOH (2.0 mL, 0.025 M) and *p*-TSOH (5.0 mg, 25 µmol, 0.5 eq.) was added. The reaction was rotated at 40 °C for 1 hour to full conversion (R_f 0.1 (EtOAc:pentane, 9:1, v:v)). The reaction was quenched with Et_3N till neutral pH was reached and concentrated under reduced pressure. Flash column chromatography (dry loading, 70:30 EtOAc:pentane → 100:0 EtOAc:pentane) yielded **53** as a white, brittle foam (17 mg, 37 µmol, 74%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.34 – 7.15 (m, 4H, CH_{arom}), 6.98 – 6.81 (m, 4H, CH_{arom}), 6.40 (s, 1H, 7-NH), 4.73 (d, *J* = 11.1 Hz, 1H, C*H*H PMB), 4.70 – 4.66 (m, 2H, H-1, C*H*H PMB), 4.58 (d, *J* = 11.4 Hz, 1H, CH*H* PMB), 4.52 (d, *J* = 11.1 Hz,

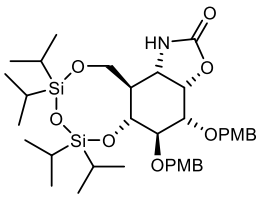
1H, CH*H* PMB), 3.86 (dd, *J* = 11.0, 4.5 Hz, 1H, H-6), 3.78 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.69 – 3.55 (m, 4H, H-2, H-3, H-6, H-7), 3.27 (dd, *J* = 11.3, 7.4 Hz, 1H, H-4), 3.03 – 2.93 (m, 2H, 4-OH, 6-OH), 2.02 – 1.90 (m, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.6 (C=O), 159.4, 130.2 ($C_{q\text{-}arom}$), 129.8 (CH_{arom}), 129.7 ($C_{q\text{-}arom}$), 114.1, 114.1 (CH_{arom}), 81.4 (C-3), 76.0 (C-1, C-2), 74.0, 72.6 (CH_2 PMB), 70.2 (C-4), 62.7 (C-6), 55.4 (OMe), 53.4 (C-7), 46.6 (C-5); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{24}H_{29}NNaO_8$ 482.1785; Found 482.1785.

1,7-(*S*,*S*)-(*N*,*O*)-Carbamate-2,3-di-*O*-(4-methoxybenzyl)-4,6-di-*O*-diisopropyldisiloxane cyclophellitol alkane (56).



Diol **52** (0.29 g, 0.64 mmol) was dissolved in anhydrous DMF (3.2 mL, 0.2 M). $TIPDSiCl_2$ (0.31 mL, 0.96 mmol, 1.5 eq.) and imidazole (0.17 g, 2.6 mmol, 4 eq.) were added after which the flask was purged with N_2. The reaction was stirred at room temperature for 30 min, upon which full conversion was observed (R_f0.2 (EtOAc:pentane, 3:7 v:v)). The mixture was dissolved in sat. aq. $NaHCO_3$ and the aqueous layer was extracted with EtOAc (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (dry loading, 20:80 EtOAc:pentane → 50:50 EtOAc:pentane) yielded carbamate **56** as a white foam (0.39 g, 0.56 mmol, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 – 7.17 (m, 4H, CH_{arom}), 6.91 – 6.79 (m, 4H, CH_{arom}), 5.78 (s, 1H, CHPh), 4.77 (dd, *J* = 9.7, 7.5 Hz, 1H, H-7), 4.64 (d, *J* = 11.1 Hz, 1H, C*H*H PMB), 4.60 (d, *J* = 11.5 Hz, 1H, C*H*H PMB), 4.50 (d, *J* = 11.2 Hz, 1H, CH*H* PMB), 4.46 (d, *J* = 11.6 Hz, 1H, CH*H* PMB), 4.04 (d, *J* = 11.2 Hz, 1H, H-6), 3.99 – 3.92 (m, 1H, H-1), 3.87 (d, *J* = 11.2 Hz, 1H, H-6), 3.80 (s, 3H, OMe), 3.77 (s, 3H, OMe), 3.69 – 3.56 (m, 2H, H-3, H-4), 3.50 (d, *J* = 4.4 Hz, 1H, H-2), 2.08 (ddd, *J* = 9.5, 9.4, 1.8 Hz, 1H, H-5), 1.13 – 0.95 (m, 28H, C*H*(CH_3)$_2$, CH(C*H*$_3$)$_2$); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 159.6 ($C_{q\text{-}arom}$), 159.4 (C=O), 159.2, 130.5, 129.8 ($C_{q\text{-}arom}$), 129.6, 129.2, 114.1, 113.8 (CH_{arom}), 83.1 (C-3), 77.1 (C-2), 73.9 (CH_2PMB), 73.6 (C-7), 72.9 (CH_2PMB), 68.7 (C-4), 57.1 (C-6), 55.4 (OMe), 53.8 (C-1), 45.8 (C-5), 17.6, 17.6, 17.5, 17.5, 17.4 (CH(*C*H$_3$)$_2$), 13.5, 13.4, 13.0, 12.9 (*C*H(CH$_3$)$_2$); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{36}H_{55}NNaO_9Si_2$ 724.3308; Found 724.3301.

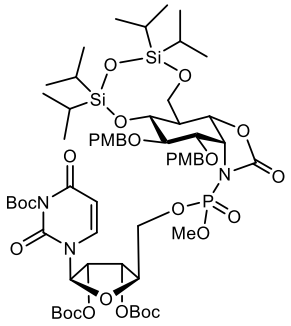
1,7-(*S*,*S*)-(*O*,*N*)-Carbamate-2,3-di-*O*-(4-methoxybenzyl)-4,6-di-*O*-diisopropyldisiloxane cyclophellitol alkane (57).



Diol **53** (0.27 g, 0.59 mmol) was dissolved in anhydrous DMF (2.95 mL, 0.2 M). $TIPSDSiCl_2$ (0.28 mL, 0.89 mmol, 1.5 eq.) and imidazole (0.16 g, 2.4 mmol, 4.0 eq.) were added after which the flask was purged with N_2. The reaction was stirred at room temperature for 30 min, upon which full conversion was observed (R_f 0.6 (EtOAc:pentane, 4:6 v:v)). The mixture was dissolved in sat. aq. $NaHCO_3$ and the aqueous layer was extracted with EtOAc (3x). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (dry loading, 15:85 EtOAc:pentane → 40:60 EtOAc:pentane) yielded carbamate **57** (0.57 g, 0.82 mmol, 84%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.50 – 7.08 (m, 4H, CH_{arom}), 6.95 – 6.73 (m, 4H, CH_{arom}), 6.01 (s, 1H, 7-NH), 4.70 (dd, *J* = 8.7, 3.7 Hz, 1H, H-1),

4.65 (d, *J* = 11.4 Hz, 1H, C*H*H PMB), 4.55 – 4.48 (m, 2H, C*H*H PMB, CH*H* PMB), 4.35 (d, *J* = 11.1 Hz, 1H, CH*H* PMB), 4.14 (ddd, *J* = 9.8, 8.6, 1.1 Hz, 1H, H-7), 4.08 (dd, *J* = 11.9, 2.1 Hz, 1H, H-6), 3.80 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.78 – 3.74 (m, 1H, H-2), 3.66 – 3.56 (m, 3H, H-3, H-4, H-6), 2.10 (dddd, *J* = 11.9, 9.9, 2.1, 2.1 Hz, 1H, H-5), 1.14 – 0.81 (m, 28H, C*H*(CH_3)$_2$, CH(C*H*$_3$)$_2$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.5, 159.5, 159.3, 130.3, 130.0 ($C_{q\text{-}arom}$), 129.8, 129.3, 113.9, 113.8 (CH_{arom}), 84.1 (C-3), 76.4 (C-2), 75.2 (C-1), 73.2, 72.9 (CH_2PMB), 69.8 (C-4), 57.7 (C-6), 55.4, 55.4 (OMe), 49.8 (C-7), 45.4 (C-5), 17.6, 17.5, 17.5, 17.5, 17.4, 17.4 (CH(*C*H$_3$)$_2$), 13.5, 13.4, 12.9, 12.8 (*C*H(CH_3)$_2$); HRMS (ESI) m/z: $[M + H]^+$ Calcd. for $C_{36}H_{55}NO_9Si_2$ 702.3488; Found 702.3486.

1'',7''-(*S,S*)-(*N,O*-(3-*N*-(T*ert*-butoxycarbonyl)-5'-*O*-(methylphosphinyl)-2',3'-di-*O*-(*tert*-butoxy carbonyl)uridinyl))-carbamate-2'',3''-di-*O*-(4-methoxybenzyl)-4'',6''-di-*O*- diisopropyldisiloxane cyclophellitol alkane (58).

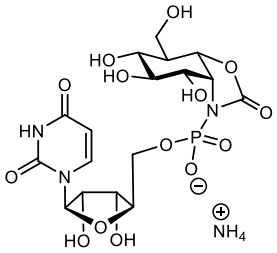


Compound **58** was prepared according to general procedure A using carbamate **56** (0.21 g, 0.3 mmol), 15-crown-5 (0.3 mL, 1.5 mmol, 5.0 eq.), anhydrous THF (1.5 mL, 0.2 M), NaH (60% wt., 0.02 g, 0.45 mmol, 1.5 eq.); and H-phosphonate **48** (0.56 g, 0.9 mmol, 3.0 eq.), anhydrous DCM (3 mL, 0.3 M), anhydrous DiPEA (0.47 mL, 2.7 mmol, 9.0 eq.) and $BrCCl_3$ (0.18 mL, 1.8 mmol, 6.0 eq.). Flash column chromatography (dry loading, 20:80 EtOAc:pentane → 40:60 EtOAc:pentane) and size exclusion yielded the P(V) diastereoisomeric mixture (ratio 1:1.39) of **58** as a colourless oil (113 mg, 86 µmol, 29%, R_f 0.3 and 0.4 (EtOAc:pentane, 4:6 v:v)). Data for the first P(V) diastereoisomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (d, *J* = 8.3 Hz, 1H, H-6), 7.35 – 7.10 (m, 4H, CH_{arom}), 6.97 – 6.75 (m, 4H, CH_{arom}), 6.15 (d, *J* = 5.4 Hz, 1H, H-1'), 5.82 (d, *J* = 8.2 Hz, 1H, H-5), 5.32 (dd, *J* = 5.2, 5.2 Hz, 1H, H-3'), 5.17 (ddd, *J* = 9.0, 5.5, 5.5 Hz, 1H, H-2'), 4.96 (dd, *J* = 9.0, 9.0 Hz, 1H, H-7''), 4.63 (d, *J* = 10.8 Hz, 1H, C*H*H PMB), 4.53 (d, *J* = 10.8 Hz, 1H, C*H*H PMB), 4.52 – 4.38 (m, 4H, H-5', CH*H* PMB, CH*H* PMB), 4.36 – 4.31 (m, 2H, H-1'', H-4'), 4.10 (d, *J* = 3.3 Hz, 1H, H-2''), 4.03 (ddd, *J* = 11.3, 9.1, 2.1 Hz, 1H, H-6''), 3.88 (s, 3H, P(O)OMe), 3.87 – 3.82 (m, 1H, H-6''), 3.76 (s, 6H, OMe), 3.78 – 3.66 (m, 2H, H-3'', H-4''), 2.42 (ddd, *J* = 11.5, 9.6, 1.9 Hz, 1H, H-5''), 1.59 (s, 9H, C(CH_3)$_3$), 1.55 – 1.38 (m, 18H, C(CH_3)$_3$), 1.15 – 0.91 (m, 28H, CH(CH_3)$_2$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 160.3 (C=O uracil), 159.5, 159.4 ($C_{q\text{-}arom}$), 155.3 (d, *J* = 8.5 Hz, C=O cyclic carbamate), 152.0 (C=O carbamate), 151.9 (C=O uracil), 148.7, 147.6 (C=O carbonate), 139.5 (C-6), 129.8 (CH_{arom}), 129.6, 129.5 ($C_{q\text{-}arom}$), 128.5, 128.4, 114.0, 114.0, 113.9 (CH_{arom}), 103.1 (C-5), 86.9 (*C*(CH_3)$_3$), 86.7 (C-1'), 83.7, 83.7 (*C*(CH_3)$_3$), 82.2 (C-3''), 80.0 (d, *J* = 8.5 Hz, C-4'), 76.7 (C-2''), 74.8 (C-2'), 73.6 (d, *J* = 8.7 Hz, C-7''), 72.1, 72.1, 72.0 (CH_2PMB), 71.8 (CH_2PMB), 71.6 (C-3'), 69.3 (C-4''), 66.1 (d, *J* = 5.5 Hz, C-5'), 57.0 (C-6''), 56.4 (d, *J* = 4.9 Hz, C-1''), 55.4 (OMe), 55.0 (P(O)OMe), 43.4 (C-5''), 27.7, 27.5 (C(*C*H$_3$)$_3$), 17.5, 17.5, 17.4, 17.4 (CH(*C*H$_3$)$_2$), 13.4, 12.8, 12.8, 12.7 (*C*H(CH_3)$_2$); ^{31}P NMR (202 MHz, $CDCl_3$) δ -1.92.

Data for the second P(V) diastereoisomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, *J* = 8.2 Hz, 1H, H-6), 7.35 – 7.10 (m, 4H, CH_{arom}), 6.97 – 6.75 (m, 4H, CH_{arom}), 6.11 (d, *J* = 5.9 Hz, 1H, H-1'), 5.77 (d, *J* = 8.2 Hz, 1H, H-5), 5.22 (dd, *J* = 5.5, 4.1 Hz, 1H, H-3'), 5.17 (ddd, *J* = 9.0, 5.5, 5.5 Hz, 1H, H-2'), 4.99 (dd, *J* = 9.0, 9.0 Hz, 1H, H-7''), 4.64 (d, *J* = 11.2 Hz, 1H, C*H*H PMB), 4.55 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 4.52 – 4.38 (m, 2H, CH*H* PMB, CH*H* PMB), 4.36 – 4.31 (m, 2H, H-5'), 4.29 (dd, *J* = 8.8, 3.0 Hz,

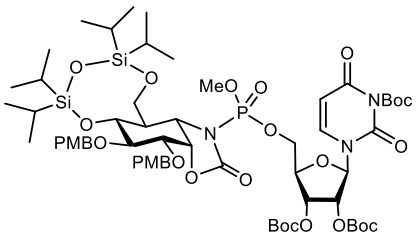
1H, H-1''), 4.19 (dddd, *J* = 5.1, 2.5 Hz, 1H, H-4'), 4.14 (d, *J* = 3.2 Hz, 1H, H-2''), 4.03 (ddd, *J* = 11.3, 9.1, 2.1 Hz, 1H, H-6''), 3.90 (s, 3H, P(O)OMe), 3.87 – 3.82 (m, 1H, H-6''), 3.80 (s, 6H, OMe), 3.78 – 3.66 (m, 2H, H-3'', H-4''), 2.42 (ddd, *J* = 11.5, 9.6, 1.9 Hz, 1H, H-5''), 1.59 (s, 9H, $C(CH_3)_3$), 1.55 – 1.38 (m, 18H, $C(CH_3)_3$), 1.15 – 0.91 (m, 28H, $CH(CH_3)_2$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 160.3 (C=O uracil), 159.5, 159.4 ($C_{q\text{-}arom}$), 155.6 (d, *J* = 8.9 Hz, C=O cyclic carbamate), 152.2 (C=O carbamate), 151.9 (C=O uracil), 148.7, 147.7 (C=O carbonate), 139.5 (C-6), 129.7 (CH_{arom}), 129.6, 129.5 ($C_{q\text{-}arom}$), 128.5, 128.4, 114.0, 114.0, 113.9 (CH_{arom}), 103.1 (C-5), 86.8 ($C(CH_3)_3$), 86.0 (C-1'), 83.7, 83.6 ($C(CH_3)_3$), 82.4 (C-3''), 80.0 (d, *J* = 7.3 Hz, C-4'), 76.7 (C-2''), 74.6 (C-2'), 73.6 (d, *J* = 8.7 Hz, C-7''), 72.1, 72.1, 72.0 (CH_2PMB), 71.9 (C-3'), 71.8 (CH_2PMB), 69.3 (C-4''), 67.0 (d, *J* = 6.3 Hz, C-5'), 57.0 (C-6''), 56.5 (d, *J* = 4.5 Hz, C-1''), 55.4 (OMe), 54.9 (P(O)OMe), 43.4 (C-5''), 27.7, 27.5 ($C(CH_3)_3$), 17.5, 17.5, 17.4, 17.4 ($CH(CH_3)_2$), 13.4, 12.8, 12.8, 12.7 ($CH(CH_3)_2$); ^{31}P NMR (202 MHz, $CDCl_3$) δ -2.13; HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{61}H_{92}N_3NaO_{23}PSi_2$ 1344.5900; Found 1344.5295.

1'',7''(*S,S*)-(*N,O*-(5'-*O*-Phosphoryluridinyl))-carbamate cyclophellitol alkane (10).



1,7-UMP carbamate **58** (28 mg, 21 µmol) was dissolved in DCM (0.28 mL, 0.08 M), cooled to 0 °C and purged with N_2. Subsequently, TFA (30% v:v, 0.13 mL, 1.65 mmol, 79 eq.) and TES (10 µL, 63 µmol, 3 eq.) were added. The reaction was stirred overnight at room temperature. Upon full conversion (R_f 0.2 (MeOH:DCM, 1:9 v:v)) the reaction was quenched with pyridine and heated to 35 °C. The reaction was stirred for 27 hours until ^{31}P NMR showed full conversion of the starting material. The reaction was concentrated under reduced pressure and purified by flash column chromatography (dry loading, neutralized silica, 0:100 H_2O:acetonitrile → 15:85 H_2O:acetonitrile) yielded the TIPDS-protected intermediate as an insoluble amphiphile **60** (6 mg, 8 µmol). Intermediate **60** was redissolved in anhydrous THF (1.0 mL, 0.008 M) and TBAF (1.0 M in THF, 16 µL, 16 µmol, 2.0 eq.) was added. The reaction was stirred overnight at room temperature. Upon full conversion (R_f 0.4 (H_2O:ACN, 2:8 v:v)), the reaction was concentrated under reduced pressure. Flash column chromatography (dry loading, neutralized silica, 0:100 H_2O:acetonitrile → 15:85 H_2O:acetonitrile) and subsequent gel filtration (NH_4HCO_3 in H_2O, ACN) yielded the title compound **10** as colourless oil (2 mg, 3.8 µmol, 18%). ^{1}H NMR (850 MHz, D_2O, HH-COSY, HSQC): δ 7.94 (d, *J* = 8.1 Hz, 1H, H-6), 5.97 (d, *J* = 5.0 Hz, 1H, H-1'), 5.94 (d, *J* = 8.0 Hz, 1H, H-5), 4.81 (ddd, *J* = 8.8, 4.5, 4.5 Hz, 1H, H-4''), 4.38 (dd, *J* = 5.1, 5.1 Hz, 1H, H-2'), 4.32 (dd, *J* = 5.0, 5.0 Hz, 1H, H-3'), 4.29 (dd, *J* = 4.7, 2.4 Hz, 1H, H-5'), 4.28 – 4.27 (m, 1H, H-4'), 4.23 (ddd, *J* = 8.3, 3.2, 1.1 Hz, 1H, H-3''), 4.21 – 4.18 (m, 1H, H-5'), 4.08 (dd, *J* = 2.3, 2.3 Hz, 1H, H-2''), 3.88 – 3.81 (m, 3H, H-1'', H-6''), 3.46 (dd, *J* = 12.4, 6.4 Hz, 1H, H-7''), 2.37 (ddd, *J* = 12.0, 8.7, 4.3 Hz, 1H, H-5''); ^{13}C NMR (214 MHz, D_2O, HSQC): δ 167.1 (C=O uracil), 159.8 (C=O carbamate), 152.7 (C=O uracil), 142.8 (C-6), 103.4 (C-5), 89.7 (C-1'), 83.9 (d, *J* = 8.8 Hz, C-4'), 78.6 (C-1''), 76.3 (C-4''), 74.6 (C-2'), 73.2 (C-2''), 70.9 (C-7''), 70.6 (C-3'), 65.9 (d, *J* = 5.4 Hz, C-5'), 60.0 (C-6''), 58.1 (C-3''), 43.2 (C-5''); ^{31}P NMR (202 MHz, D_2O) δ -6.57; HRMS (ESI) m/z: $[M + H + NH_4]^+$ Calcd. for $C_{17}H_{23}N_3NaO_{14}P$ 543.1334; Found 543.1335.

1'',7''-(*S,S*)-(*O,N*-(3-*N*-(*Tert*-butoxycarbonyl)-5'-*O*-(methylphosphinyl)-2',3'-di-*O*-(*tert*-butoxy carbonyl)uridinyl))-carbamate-2'',3''-di-*O*-(4-methoxybenzyl)-4'',6''-di-*O*-diisopropyl disiloxane cyclophellitol alkane (59).

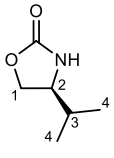


Compound **59** was prepared according to general procedure A using carbamate **57** (0.21 g, 0.3 mmol), 15-crown-5 (0.3 mL, 1.5 mmol, 5.0 eq.), anhydrous THF (1.5 mL, 0.2 M), NaH (60% wt., 0.02 g, 0.45 mmol, 1.5 eq.); and H-phosphonate **48** (0.56 g, 0.9 mmol, 3.0 eq.), anhydrous DCM (3 mL, 0.3 M), anhydrous DiPEA (0.47 mL, 2.7 mmol, 9.0 eq.) and $BrCCl_3$ (0.18 mL, 1.8 mmol, 6.0 eq.). Flash column chromatography (dry loading, 20:80 EtOAc:pentane → 40:60 EtOAc:pentane) and size exclusion yielded the starting material (41 mg, 58 µmol, 20%) and a P(V) diastereoisomeric mixture (ratio 1:1.74) of **59** as a colourless oil (57 mg, 43 µmol, 14%, R_f 0.3 and 0.4 (EtOAc:pentane, 4:6 v:v), 34% brsm). Data for the first P(V) diastereoisomer: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.71 (d, *J* = 8.2 Hz, 1H, H-6), 7.35 – 7.09 (m, 4H, CH_{arom}), 6.97 – 6.78 (m, 4H, CH_{arom}), 6.12 (d, *J* = 3.9 Hz, 1H, H-1'), 5.86 (d, *J* = 8.2 Hz, 1H, H-5), 5.24 (dd, *J* = 10.2, 5.0 Hz, 1H, H-3'), 5.17 (dd, *J* = 5.6, 5.6 Hz, 1H, H-2'), 4.77 (d, *J* = 10.7 Hz, 1H, C*H*H PMB), 4.70 (m, 3H, H-1'', C*H*H PMB, CH*H* PMB), 4.65 – 4.57 (m, 1H, CH*H* PMB), 4.39 – 4.29 (m, 4H, H-4', H-5', H-7''), 4.18 – 4.01 (m, 2H, H-6''), 3.85 (s, 3H, P(O)OMe), 3.80 (s, 6H, OMe), 3.74 – 3.60 (m, 2H, H-3'', H-4''), 3.56 (ddd, *J* = 8.4, 7.9, 3.8 Hz, 1H, H-2''), 1.90 (dd, *J* = 10.5, 10.5 Hz, 1H, H-5''), 1.60 (s, 9H, $C(CH_3)_3$), 1.48 (s, 18H, $C(CH_3)_3$), 1.09 – 0.93 (m, 28H, $C(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 160.3 (C=O uracil), 159.6, 159.1 ($C_{q\text{-}arom}$), 155.1 (d, *J* = 4.0 Hz, C=O cyclic carbamate), 152.0 (C=O carbamate), 148.7, 147.7 (C=O carbonate), 139.5 (C-6), 130.7 ($C_{q\text{-}arom}$), 129.9 (CH_{arom}), 129.7 ($C_{q\text{-}arom}$), 129.2, 114.0, 113.7 (CH_{arom}), 103.2 (C-5), 87.0 ($C(CH_3)_3$), 86.8 (C-1'), 83.7, 83.6 ($C(CH_3)_3$), 81.6 (C-3''), 79.9 (d, *J* = 7.1 Hz, C-4'), 77.5 (d, *J* = 7.1 Hz, C-1''), 77.4 (C-2''), 74.7 (C-2'), 74.5, 73.0 (CH_2PMB), 71.7 (C-3'), 68.8 (C-4''), 66.8 (d, *J* = 6.0 Hz, C-5'), 57.0 (C-6''), 55.8 (d, *J* = 3.8 Hz, C-7''), 55.4, 55.4 (OMe), 54.8 (P(O)OMe), 47.6 (C-5''), 27.7, 27.7, 27.5 ($C(CH_3)_3$), 17.7, 17.6, 17.5, 17.4 ($CH(CH_3)_2$), 13.5, 13.5, 13.4, 13.1, 13.1, 13.0, 12.9 ($CH(CH_3)_2$); ^{31}P NMR (202 MHz, $CDCl_3$) δ -0.83.

Data for the second P(V) diastereoisomer: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.62 (d, *J* = 8.2 Hz, 1H, H-6), 7.35 – 7.09 (m, 4H, CH_{arom}), 6.97 – 6.78 (m, 4H, CH_{arom}), 6.11 (d, *J* = 3.8 Hz, 1H, H-1'), 5.81 (d, *J* = 8.2 Hz, 1H, H-5), 5.24 (dd, *J* = 10.2, 5.0 Hz, 1H, H-3'), 5.17 (dd, *J* = 5.6, 5.6 Hz, 1H, H-2'), 4.77 (d, *J* = 10.7 Hz, 1H, C*H*H PMB), 4.70 (m, 3H, H-1'', C*H*H PMB, CH*H* PMB), 4.65 – 4.57 (m, 1H, CH*H* PMB), 4.52 – 4.39 (m, 2H, H-5'), 4.39 – 4.29 (m, 1H, H-7''), 4.29 (dd, *J* = 3.5, 3.5 Hz, 1H, H-4'), 4.18 – 4.01 (m, 2H, H-6''), 3.83 (s, 3H, P(O)OMe), 3.80 (s, 6H, OMe), 3.74 – 3.60 (m, 2H, H-3'', H-4''), 3.56 (ddd, *J* = 8.4, 7.9, 3.8 Hz, 1H, H-2''), 1.83 (dd, *J* = 10.2 Hz, 1H, H-5''), 1.60 (s, 9H, $C(CH_3)_3$), 1.47 (s, 18H, $C(CH_3)_3$), 1.09 – 0.93 (m, 28H, $C(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 160.2 (C=O uracil), 159.5, 159.1 ($C_{q\text{-}arom}$), 155.0 (d, *J* = 4.6 Hz, C=O cyclic carbamate), 152.1 (C=O carbamate), 148.6, 147.6 (C=O carbonate), 139.4 (C-6), 130.8 ($C_{q\text{-}arom}$), 129.8 (CH_{arom}), 129.5 ($C_{q\text{-}arom}$), 129.2, 113.9, 113.6 (CH_{arom}), 103.1 (C-5), 87.0 ($C(CH_3)_3$), 86.7 (C-1'), 83.8, 83.7 ($C(CH_3)_3$), 81.6 (C-3''), 80.0 (d, *J* = 8.2 Hz, C-4'), 77.9 (d, *J* = 7.2 Hz, C-1''), 77.4 (C-2''), 75.0 (CH_2PMB), 74.5 (C-2'), 72.9 (CH_2PMB), 71.9 (C-3'), 68.8 (C-4''), 67.2 (d, *J* = 5.2 Hz, C-5'), 56.9 (C-6''), 56.0 (d, *J* = 3.4 Hz, C-7''), 55.4, 55.3 (OMe), 54.8 (P(O)OMe), 47.6 (C-5''), 27.7, 27.7, 27.5 ($C(CH_3)_3$), 17.7, 17.6, 17.5, 17.4

(*C*H(CH$_3$)$_2$), 13.5, 13.5, 13.4, 13.1, 13.1, 13.0, 12.9 (CH(*C*H$_3$)$_2$); ^{31}P NMR (202 MHz, CDCl$_3$) δ -0.26; HRMS (ESI) m/z: [M + Na]$^+$ Calcd. for $C_{61}H_{92}N_3NaO_{23}PSi_2$ 1344.5900; Found 1344.5289.

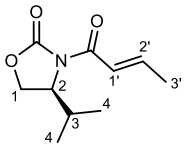
(*S*)-4-Isopropyloxazolidin-2-one (S1).



Boc-L-valine (43 g, 0.20 mol) was dissolved in THF (0.50 L, 0.40 M). Et$_3$N (31 mL, 0.22 mol, 1.1 eq.) and ethyl chloroformate (25 mL, 0.26 mol, 1.3 eq.) were added on ice. The reaction mixture was stirred for 1 h at 0°C. Upon precipitate formation, the reaction mixture was cooled and filtered off, while rinsing with THF. The filtrate was carefully added to a solution of NaBH$_4$ (13 g, 0.34 mol, 1.7 eq.) in H$_2$O (0.13 L, 1.5 M). The reaction mixture was stirred for 1 h at room temperature, before adding additional NaBH$_4$ (4.0 g, 0.1 mol, 0.50 eq.) portion-wise. The reaction mixture was stirred for 30 min. at room temperature, before quenching with 1.0 M aq. HCl until pH 2 was reached. The mixture was diluted with sat. aq. NaHCO$_3$, the organic layer was separated, the aqueous layer was extracted thrice with EtOAc and the combined organic layers were washed with brine, dried over MgSO$_4$, filtered and concentrated *in vacuo*.

The crude intermediate was dissolved in THF (0.75 L, 0.27 M). SOCl$_2$ (36 mL, 050 mol, 2.5 eq.) was added on ice. The reaction mixture was stirred overnight at room temperature. Upon full conversion on TLC (R$_f$ 0.6 (1:1, EtOAc:pentane v:v)), the reaction mixture was concentrated *in vacuo* and co-evaporated with toluene. Flash column chromatography (30:70 EtOAc:pentane → 70:30 EtOAc:pentane) yielded the title compound **S1** (9.2 g, 71 mmol, 35% over three steps). Analytical data in full agreement with literature data.[38] 1H NMR (500 MHz, CDCl3, HH-COSY, HSQC): δ 5.73 (bs, 1H, NH), 4.45 (dd, J = 8.6, 8.6 Hz, 1H, H-1), 4.11 (dd, J = 8.7, 6.3 Hz, 1H, H-1), 3.61 (dddd, J = 8.4, 7.2, 6.3, 1.1 Hz, 1H, H-2), 1.74 (dq, *J* = 13.5, 6.8 Hz, 1H, H-3), 0.96 (d, *J* = 6.7 Hz, 3H, H-4), 0.91 (d, *J* = 6.7 Hz, 3H, H-4); ^{13}C NMR (126 MHz, CDCl$_3$, HSQC): δ 68.7 (C-1), 58.4 (C-2), 32.8 (C-3), 18.2, 17.8 (C-4); HRMS (ESI) m/z: [M+H]$^+$ Calcd. for $C_6H_{12}NO_2$ 130.0863; Found 130.0862.

(*S*,*E*)-3-(But-2-enoyl)-4-isopropyloxazolidin-2-one (60).



Compound **S1** (9.2 g, 71 mmol) was dissolved in anhydrous THF (0.35 mL, 0.20 M). *n*-BuLi (2.5 M solution in THF; 31 mL, 78 mmol, 1.1 eq.) was added at -78°C. The reaction mixture was stirred for 15 min. at -78°C, before adding crotonyl chloride (10 mL, 92 mmol, 1.3 eq.) at -78°C. The reaction mixture was stirred for 30 min. at -78°C and 3 h at room temperature. Upon full conversion on TLC (R$_f$ 0.6 (EtOAc:pentane, 2:8 v:v)), the reaction was quenched with sat. aq. NH$_4$Cl. The mixture was diluted with sat. aq. NaHCO$_3$, the organic layer was separated, the aqueous layer was extracted thrice with EtOAc and the combined organic layers were washed with brine, dried over MgSO$_4$, filtered and concentrated *in vacuo*. Flash column chromatography (5:95 Et$_2$O:pentane → 40:60 Et$_2$O:pentane) yielded the title compound **60** (9.4 g, 48 mmol, 68%). Analytical data in full agreement with literature data.[39] ^{1}H NMR (400 MHz, CDCl$_3$, HH-COSY, HSQC): δ 7.28 (dd, *J* = 15.2, 1.6 Hz, 1H, H-1'), 7.13 (dq, *J* = 15.2, 6.7 Hz, 1H, H-2'), 4.50 (ddd, *J* = 8.3, 3.5, 3.5 Hz, 1H, H-2), 4.32 (dd, *J* = 8.7, 8.7 Hz, 1H, H-1), 4.23 (dd, *J* = 9.1, 3.1 Hz, 1H, H-1), 2.39 (pd, *J* = 7.0, 3.9 Hz, 1H, H-3), 1.96 (dd, *J* = 6.8, 1.6 Hz, 3H, H-3'), 0.93 (d, *J* = 7.1 Hz, 3H, H-4), 0.88 (d, *J* = 7.0 Hz, 3H, H-4); ^{13}C NMR (101 MHz, CDCl$_3$, HSQC): δ 164.5 (C=O, oxazolidinone), 153.8 (C=O,

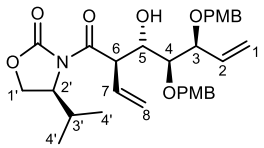
amide), 146.0 (C-2'), 121.6 (C-1'), 63.1 (C-1), 58.2 (C-2), 28.2 (C-3), 18.1 (C-3'), 17.6, 14.3 (C-4); HRMS (ESI) m/z: $[M+H]^+$ Calcd. for $C_{10}H_{16}NO_3$ 198.1125; Found 198.1122.

Preparation of dibutylboron triflate.

Following modified literature procedures,[55] a flame-dried round-bottom flask under argon atmosphere was filled with *n*-BuLi (2.5 M in hexanes; 720 mL, 1.8 mol, 3.0 eq.). In a separate flask, $BF_3{\cdot}OEt_2$ (74 mL, 600 mmol, 1.0 eq.) was dissolved in dry Et_2O (400 mL) and the solution was cannulated into a dropping funnel. While stirring, the solution of $BF_3{\cdot}OEt_2$ was carefully added to the *n*-BuLi at allowing the reaction mixture to slowly warm up to a gentle boil. Reflux continued for another 2 hours. The mixture was cooled to room temperature and carefully quenched with 3 M HCl (400 mL) followed by the addition of water (400 mL). The organic layer was separated and transferred to a round-bottom flask containing anhydrous Na_2SO_4 after which the supernatant was transferred to a second round bottom flask and solvents were removed under reduced pressure yielding crude tri-*n*-butylborane. Vacuum distillation of the crude (bp 50 °C, 3.0 mbar) yielded tri-*n*-butylborane as a colorless oil (72 gr, 393 mmol, 66%) and stored under protective atmosphere.

According to modified literature procedures,[40] the freshly prepared tri-*n*-butylborane was placed in an oil bath while kept under argon atmosphere. While stirring vigorously, trifluoromethanesulfonic acid (34.5 mL, 393 mmol, 1.0 eq.) was added dropwise while closely monitoring of the temperature. Subsequently, the reaction mixture was heated to 50 °C for an hour after which the reaction mixture was cooled to room temperature. Vacuum distillation of the crude (bp 60 °C, 2-3 mbar) yielded Bu_2BOTf as a yellowish liquid (103 gr, 374 mmol, 95%) and store at -25 °C under argon atmosphere.

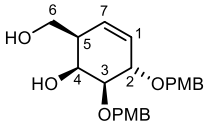
(*S*)-3-[(2*S*,3*S*,4*S*,5*S*)-4,5-Di-*O*-(4-methoxybenzyl)-3-hydroxy-2-vinyl-hept-6-enoyl]-4-isopropyl-oxazolidin-2-one (61).



Compound **60** (2.5 g, 12 mmol, 1.8 eq.) was dissolved in anhydrous DCM (21 mL, 0.60 M) and 3 Å molecular sieve rods were added to the solution. Freshly prepared Bu_2BOTf (1.0 M solution in DCM; 12 mL, 12 mmol, 1.8 eq.) was added at -78°C and after 10 min. Et_3N (1.9 mL, 14 mmol, 2.0 eq.) was added. The reaction mixture was stirred for 1 h at -78°C and 15 min. at 0°C. Compound **28** (2.5 g, 6.9 mmol), dissolved in anhydrous DCM (12 mL, 0.6 M), was added to the reaction mixture at -78°C. The reaction mixture was stirred for 2 h while allowing the reaction mixture to warm up to -30°C and was kept at this temperature overnight. Upon full conversion on TLC (R_f 0.4 (3:7, EtOAc:pentane v:v)), the reaction was quenched with a pH 7.4 aq. phosphate buffer and H_2O_2 (30% w/w in H_2O). The mixture was diluted with sat. aq. $NaHCO_3$, the organic layer was separated, the aqueous layer was extracted thrice with DCM and the combined organic layers were washed with sat. aq. $Na_2S_2O_3$ and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash column chromatography (10:90 EtOAc:pentane → 40:60 EtOAc:pentane) yielded the title compound **61** (3.7 g, 6.7 mmol, 97%). ^1H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.25 – 7.21 (m, 4H, CH_{arom}), 6.87 – 6.83 (m, 4H, CH_{arom}), 6.01 (ddd, *J* = 17.2, 10.6, 7.1 Hz, 1H, H-2), 5.90 (ddd, *J* = 17.2, 10.2, 8.9 Hz, 1H, H-7), 5.41 – 5.38 (m, 2H, H-1, H-8), 5.37 (ddd, *J* = 3.5, 1.6, 1.0 Hz, 1H, H-1), 5.28 (dd, *J* = 10.2, 1.5 Hz, 1H, H-8), 4.96

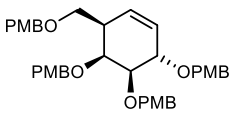
(dd, *J* = 8.9, 7.0 Hz, 1H, H-6), 4.59 (m, 2H, C*H*H PMB, C*H*H PMB), 4.43 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 4.41 – 4.38 (m, 1H, H-5), 4.34 (d, *J* = 11.4 Hz, 1H, C*H*H PMB), 4.23 (dddd, *J* = 7.1, 4.0, 1.1, 1.1 Hz, 1H, H-3), 4.08 (ddd, *J* = 8.6, 3.9, 3.1 Hz, 1H, H-2'), 3.91 (dd, *J* = 8.9, 3.1 Hz, 1H, H-1'), 3.80 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.51 (dd, *J* = 8.3, 3.9 Hz, 1H, H-4), 3.45 (dd, *J* = 8.8, 8.8 Hz, 1H, H-1'), 3.36 (d, *J* = 2.3 Hz, 1H, 5-OH), 2.23 (pd, *J* = 7.0, 3.8 Hz, 1H, H-3'), 0.79 (d, *J* = 7.1 Hz, 3H, H-4'), 0.74 (d, *J* = 6.9 Hz, 3H, H-4'); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 172.8 (C=O, oxazolidinone), 159.4, 159.3 ($C_{q\text{-}arom}$), 153.6 (C=O, amide), 134.7 (C-2), 133.6 (C-7), 130.3, 130.0 ($C_{q\text{-}arom}$), 129.7, 129.2 (CH_{arom}), 120.6 (C-8), 119.1 (C-1), 113.9, 113.8 (CH_{arom}), 81.5 (C-4), 79.6 (C-3), 72.8, 70.9 (CH_2 PMB), 70.8 (C-5), 62.6 (C-1'), 58.1 (C-3'), 55.5, 55.4 (OMe), 50.1 (C-6), 28.2 (C-2'), 17.9, 14.6 (C-4'); HRMS (ESI) m/z: $[M+Na]^+$ Calcd. for $C_{31}H_{39}NNaO_8$ 576.2568; Found 576.2561.

2,3-Di-*O*-(4-methoxybenzyl)-4-*epi*-cyclophellitol alkene (62).



Compound **61** (3.7 g, 6.7 mmol) was dissolved in THF:H_2O (9:1, 93 mL, 0.07 M). $LiBH_4$ (2.0 M solution in THF; 8.7 mL, 17 mmol, 2.6 eq.) was added on ice. The reaction mixture was stirred for 30 min. at 0 °C and 1 h at room temperature. Upon full conversion on TLC (R_f 0.3 (EtOAc:pentane, 6:4 v:v)), the reaction was quenched with 2.0 M aq. NaOH (100 mL). The mixture was diluted with sat. aq. $NaHCO_3$, the organic layer was separated, the aqueous layer was extracted thrice with EtOAc and the combined organic layers were washed with sat. aq. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash column chromatography (SiO_2, 20:80 EtOAc:pentane → 45:55 EtOAc:pentane) yielded the crude intermediate.
The crude intermediate was dissolved in anhydrous DCM (34 mL, 0.20 M). 2nd generation Grubbs catalyst (0.11 g, 0.13 mmol, 0.02 eq.) was added. The reaction mixture was purged with N_2 for 10 min. and stirred for 24 h at 40 °C. A second portion of 2nd generation Grubbs catalyst (0.11 g, 0.13 mmol, 0.02 eq.) was added and the reaction mixture was purged with N_2 for 10 min. and stirred overnight at 40 °C. Upon full conversion on TLC (R_f 0.4 (EtOAc:pentane, 8:2 v:v)), the reaction was concentrated *in vacuo*. Flash column chromatography (65:35 EtOAc:pentane → 90:10 EtOAc:pentane) yielded the title compound **62** (1.8 g, 4.4 mmol, 66% over two steps). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.31 – 7.25 (m, 4H, CH_{arom}), 6.89 – 6.81 (m, 4H, CH_{arom}), 5.79 (ddd, *J* = 10.2, 2.6, 2.6 Hz, 1H, H-7), 5.53 – 5.47 (m, 1H, H-1), 4.66 – 4.56 (m, 4H, C*H*H PMB, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.26 (m, 2H, H-2, H-4), 3.77 (s, 3H, OMe), 3.77 (s, 3H, OMe), 3.75 – 3.73 (m, 2H, H-6), 3.59 (dd, *J* = 7.7, 2.2 Hz, 1H, H-3), 3.27 – 3.15 (m, 2H, 4-OH, 6-OH), 2.40 (dddd, *J* = 6.4, 3.3, 2.9, 2.9 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.3, 159.1, 130.7, 130.2 ($C_{q\text{-}arom}$), 129.5, 129.4 (CH_{arom}), 127.6 (C-7), 126.7 (C-1), 113.8, 113.7 (CH_{arom}), 81.6 (C-3), 76.5 (C-2), 71.9, 71.8 (CH_2 PMB), 69.8 (C-4), 63.4 (C-6), 55.2 (OMe), 42.1 (C-5); HRMS (ESI) m/z: $[M+Na]^+$ Calcd. for $C_{23}H_{28}NaO_6$ 423.1778; Found 423.1771.

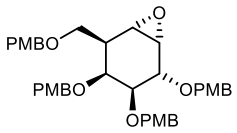
4-*Epi*-2,3,4,6-tetra-*O*-(4-methoxybenzyl)- cyclophellitol alkene (63).

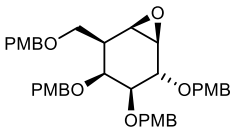


Compound **63** (2.0 g, 5.0 mmol) was co-evaporated with toluene twice and dissolved in DMF (10 mL, 0.5 M). Subsequently, TBAI (37 mg, 0.1 mmol, 0.02 eq.), PMBCl (2.7 mL, 20 mmol, 4.0 eq.) and NaH (60 wt.% dispersion in mineral oil; 0.8 g, 20 mmol, 4.0 eq.) were added on ice. The reaction mixture was kept stirring at 0°C for 30 min. after which the reaction mixture was

heated to 45 °C for 16 hours. Upon full conversion was observed (R_f 0.5 (EtOAc:pentane, 2:8 v:v)), the reaction mixture was cooled to 0°C, quenched with MeOH and concentrated *in vacuo*. The crude intermediate was dissolved in Et_2O and diluted with sat. aq. $NaHCO_3$. The organic layer was separated, the aqueous layer was extracted thrice with Et_2O and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash column chromatography (5:95 EtOAc:pentane → 25:75 EtOAc:pentane) yielded the title compound **63** as a yellow oil (2.4 g, 3.7 mmol, 73%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.36 – 7.19 (m, 8H, CH_{arom}), 6.94 – 6.80 (m, 8H, CH_{arom}), 5.75 (ddd, *J* = 10.1, 2.6, 2.6 Hz, 1H, H-7), 5.47 (d, *J* = 10.2 Hz, 1H, H-1), 4.85 (d, *J* = 11.2 Hz, 1H, C*H*H PMB), 4.73 – 4.61 (m, 4H, C*H*H PMB, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.58 (d, *J* = 11.2 Hz, 1H, CH*H* PMB), 4.46 – 4.38 (m, 3H, C*H*H PMB, CH*H* PMB, H-2), 4.15 (d, *J* = 2.2 Hz, 1H, H-4), 3.83 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.71 (dd, *J* = 7.9, 1.8 Hz, 1H, H-3), 3.56 (dd, *J* = 8.7, 8.7 Hz, 1H, H-6), 3.45 (dd, *J* = 8.8, 6.4 Hz, 1H, H-6), 2.66 (ddd, *J* = 6.0, 6.0, 3.0 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.3, 159.2, 159.1, 159.1, 131.4, 131.1, 131.0, 130.4 ($C_{q\text{-}arom}$), 129.6, 129.5, 129.5, 129.2 (CH_{arom}), 128.7 (C-7), 127.7 (C-1), 114.2, 114.1, 113.9, 113.8, 113.8, 113.7 (CH_{arom}), 83.1 (C-3), 77.4 (C-2), 74.9 (C-4), 73.7, 73.0, 72.1, 71.9 (CH_2 PMB), 70.1 (C-6), 55.4, 55.4, 55.3 (OMe), 42.0 (C-5); HRMS (ESI) m/z: $[M+Na]^+$ Calcd. for $C_{39}H_{44}NaO_8$ 663.2934; Found 663.2943.

1,4,7-*Epi*-2,3,4,6-tetra-*O*-(4-methoxybenzyl) cyclophellitol (64) and 4-*epi*-2,3,4,6-tetra-*O*-(4-methoxybenzyl) cyclophellitol (65).



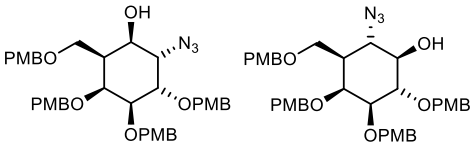


Compound **63** (0.94 g, 1.5 mmol) was dissolved in anhydrous DCM (15 mL, 0.1 M). Subsequently, $NaHCO_3$ (1.2 g, 15 mmol, 10 eq.) and *m*-CPBA (0.64 g, 3.7 mmol, 2.5 eq.) were added. The reaction was stirred under N_2 atmosphere at 5 °C for 3 days. Upon partial conversion was observed (R_f 0.5 and 0.3 for **64** and **65** respectively (EtOAc:pentane, 3:7, v:v)), the reaction was quenched with sat. aq. $Na_2S_2O_3$. The aqueous layer was extracted with Et_2O (3x), and the combined organic layers were subsequently washed with sat. aq. $NaHCO_3$. The organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (dry loading, 15:85 EtOAc:pentane → 25:75 EtOAc:pentane) yielded **64** (66 mg, 0.1 mmol, 7%), **65** (0.41 g, 0.63 mmol, 43%) and starting material (0.18 g, 0.28 mmol, 19%), 69% brsm.

Analytical data for α-epoxide **64**: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.37 – 7.10 (m, 8H, CH_{arom}), 6.91 – 6.77 (m, 8H, CH_{arom}), 4.81 – 4.71 (m, 3H, C*H*H PMB, C*H*H PMB, CH*H* PMB), 4.62 (d, *J* = 11.3 Hz, 1H, C*H*H PMB), 4.59 (d, *J* = 11.3 Hz, 1H, CH*H* PMB), 4.47 – 4.38 (m, 3H, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.19 (dd, *J* = 8.5, 2.5 Hz, 1H, H-2), 3.85 (ddd, *J* = 3.3, 1.5, 1.5 Hz, 1H, H-4), 3.80 – 3.80 (m, 9H, OMe), 3.79 (s, 3H, OMe), 3.56 (dd, *J* = 8.6, 1.4 Hz, 1H, H-3), 3.54 – 3.51 (m, 2H, H-6), 3.31 (dd, *J* = 3.9, 2.5 Hz, 1H, H-1), 2.91 (dd, *J* = 4.0, 1.5 Hz, 1H, H-7), 2.24 (ddd, *J* = 7.9, 3.4 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.4, 159.2, 159.2, 131.1, 131.0, 131.0, 130.2 ($C_{q\text{-}arom}$), 129.7, 129.6, 129.6, 129.3, 114.0, 113.8, 113.8, 113.7 (CH_{arom}), 81.0 (C-3), 76.5 (C-2), 75.5 (C-4), 74.0, 73.1, 72.8, 72.5 (CH_2 PMB), 68.5 (C-6), 55.5, 55.4 (OMe), 55.4 (C-1), 54.6 (C-7), 41.0 (C-5); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{39}H_{44}NaO_9$ 679.2878; Found 679.2884.

Analytical data for β-epoxide **65**: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.35 – 7.17 (m, 8H, CH_{arom}), 6.95 – 6.78 (m, 8H, CH_{arom}), 4.79 – 4.68 (m, 3H, C*H*H PMB, C*H*H PMB, CH*H* PMB), 4.66 – 4.59 (m, 2H, C*H*H PMB, CH*H* PMB), 4.51 (d, *J* = 11.7 Hz, 1H, CH*H* PMB), 4.45 – 4.37 (m, 2H, C*H*H PMB, CH*H* PMB), 4.09 (d, *J* = 8.7 Hz, 1H, H-2), 3.88 (dd, *J* = 1.7, 1.7 Hz, 1H, H-4), 3.83 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.67 (dd, *J* = 9.0, 6.9 Hz, 1H, H-6), 3.57 (dd, *J* = 9.0, 7.3 Hz, 1H, H-6), 3.41 (dd, *J* = 8.8, 2.2 Hz, 1H, H-3), 3.21 (d, *J* = 3.8 Hz, 1H, H-1), 3.16 (dd, *J* = 3.0, 3.0 Hz, 1H, H-7), 2.31 – 2.22 (m, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.4, 159.3, 159.2, 159.1, 131.1, 130.8, 130.4, 130.3 ($C_{q\text{-}arom}$), 129.6, 129.6, 129.2, 113.9, 113.9, 113.8, 113.6 (CH_{arom}), 82.4 (C-3), 76.0 (C-2), 73.9, 73.2, 73.1 (CH_2 PMB), 72.5 (C-4), 72.4 (CH_2 PMB), 68.8 (C-6), 55.4, 55.3, 55.3 (OMe), 54.7 (C-1), 52.6 (C-7), 40.4 (C-5); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{39}H_{44}NaO_9$ 679.2878; Found 679.2880.

4-*Epi*-1-(*S*)-azido-2,3,4,6-tetra-*O*-(4-methoxybenzyl)-7-(*R*)-ol cyclophellitol alkane (66) and 4-*Epi*-2,3,4,6-tetra-*O*-(4-methoxybenzyl)-7-(*S*)-azido-1-(*R*)-ol cyclophellitol alkane (67).



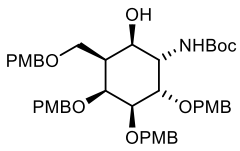
Epoxide **65** (3.9 g, 5.9 mmol) was dissolved in anhydrous DMF (59 mL, 0.1 M). Subsequently NaN_3 (7.6 g, 0.12 mol, 20 eq.) were added. The reaction was stirred under N_2 atmosphere at 140 °C for 16 hours. Upon full conversion was observed (R_f 0.5 and 0.45 for **66** and **67** respectively (EtOAc:pentane, 3:7, v:v)) the reaction was allowed to cool to room temperature and diluted with sat. aq. $NaHCO_3$. The aqueous layer was extracted with Et_2O (3x) and the combined organic layers were washed with brine. The organic layers were subsequently dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Flash column chromatography (15:85 EtOAc:pentane → 25:75 EtOAc:pentane) yielded regioisomer **66** (1.9 g, 2.7 mmol, 45%) and regioisomer **67** (1.4 g, 2.0 mmol, 34%) in an overall yield of 79%.

Analytical data for **66**: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.38 – 7.18 (m, 6H, CH_{arom}), 7.13 – 7.04 (m, 2H, CH_{arom}), 6.93 – 6.73 (m, 8H, CH_{arom}), 4.84 (d, *J* = 10.5 Hz, 1H, C*H*H PMB), 4.77 – 4.60 (m, 4H, C*H*H PMB, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.42 (d, *J* = 11.5 Hz, 1H, C*H*H PMB), 4.37 – 4.31 (m, 2H, C*H*H PMB, CH*H* PMB), 4.22 (dd, *J* = 9.9, 3.5 Hz, 1H, H-3), 4.18 (s, 1H, H-4), 4.03 (d, *J* = 3.7 Hz, 1H, H-1), 3.91 (d, *J* = 10.0 Hz, 1H, 7-OH), 3.81 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.77 – 3.74 (m, 1H, H-7) 3.70 (dd, *J* = 10.0, 2.5 Hz, 1H, H-2), 3.62 (dd, *J* = 9.2, 9.2 Hz, 1H, H-6), 3.50 (dd, *J* = 7.4, 7.4 Hz, 1H, H-6), 2.02 – 1.92 (m, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.5, 159.4, 159.3, 159.2, 131.0, 130.5, 130.2 ($C_{q\text{-}arom}$), 129.8, 129.7, 129.5, 129.2, 128.8, 113.9, 113.9, 113.9 (CH_{arom}), 80.6 (C-2), 78.0 (C-4), 76.4 (C-3), 75.5, 73.3, 73.1, 73.0 (CH_2 PMB), 71.8 (C-7), 67.6 (C-6), 65.0 (C-1), 55.4, 55.4 (OMe), 38.7 (C-5); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{39}H_{45}NaO_9$ 722.3054; Found 722.3057.

Analytical data for **67**: ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.37 – 7.09 (m, 8H, CH_{arom}), 6.92 – 6.80 (m, 8H, CH_{arom}), 4.92 (d, *J* = 11.0 Hz, 1H, C*H*H PMB), 4.88 (d, *J* = 10.6 Hz, 1H, C*H*H PMB), 4.69 (d, *J* = 11.2 Hz, 1H, C*H*H PMB), 4.64 – 4.56 (m, 2H, CH*H* PMB, CH*H* PMB), 4.47 – 4.32 (m, 3H, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.16 (dd, *J* = 2.2, 2.2 Hz, 1H, H-4), 3.83 – 3.78 (m, 13H, OMe, OMe, OMe, OMe, H-2), 3.62 (dd, *J* = 8.7, 4.0 Hz, 1H, H-6), 3.55 (dd, *J* = 10.2, 8.8 Hz, 1H, H-6), 3.47 (ddd, *J* = 9.2, 9.2, 2.2 Hz, 1H, H-1), 3.44 – 3.34 (m, 2H, H-3, H-7), 2.50 (d, *J* = 2.3 Hz, 1H, 1-OH), 1.61

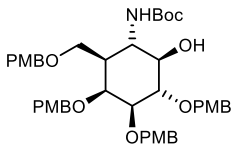
(dddd, *J* = 10.0, 10.0, 4.0, 2.2 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.4, 159.4, 159.3, 159.2, 131.2, 130.7, 130.5 ($C_{q\text{-}arom}$), 129.8, 129.7, 129.4, 129.2, 114.1, 114.0, 113.9, 113.7 (CH_{arom}), 83.5 (C-3), 80.9 (C-2), 77.0 (C-1), 75.2, 74.8 (CH_2 PMB), 73.3 (C-4), 73.2, 72.3 (CH_2 PMB), 67.5 (C-6), 61.7 (C-7), 55.4, 55.4 (OMe), 42.4 (C-5); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{39}H_{45}NaO_9$ 722.3054; Found 722.3058.

4-*Epi*-1-(*S*)-(*N*-(tert-butoxycarbonyl)-2,3,4,6-tetra-*O*-(4-methoxybenzyl)-7-(*R*)-ol cyclophellitol alkane (68).



Azide **66** (1.9 g, 2.7 mmol) was dissolved in a 1:1 solution of THF and aq. 1.0 M NaOH (27 mL, 0.1 M). Subsequently triphenylphosphine (2.8 g, 11 mmol, 4.0 eq.) was added and the reaction was stirred vigorously overnight at room temperature. Upon full conversion of the starting material was observed, the reaction was diluted with sat. aq. $NaHCO_3$ and EtOAc. The aqueous phase was separated and subsequently extracted with EtOAc (3x), the organic layers were then washed with brine. The brine layer was extracted once with EtOAc and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude amine was dissolved in anhydrous DCM (13.5 mL, 0.2 M) and cooled on ice. While stirring, Et_3N (1.9 mL, 13.5 mmol, 5.0 eq.) and subsequently Boc_2O (0.93 mL, 4.1 mmol, 1.5 eq.) were added. The flask was flushed with N_2 and the reaction was stirred at room temperature for 16 hours until full conversion was observed (R_f 0.5 (EtOAc:pentane, 4:6, v:v)). The reaction was quenched with sat. aq. NH_4Cl and the aqueous layer separated. Subsequently, the aqueous layer was extracted with EtOAc (3x), and the combined organic layers were washed with sat. aq. $NaHCO_3$. The organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (10:90 EtOAc:pentane → 30:70 EtOAc:pentane) yielded **68** as a white, brittle foam (2.0 g, 2.6 mmol, 96% over two steps). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.31 – 7.04 (m, 8H, CH_{arom}), 6.91 – 6.74 (m, 8H, CH_{arom}), δ 4.89 – 4.81 (m, 1H, C*H*H PMB), 4.79 – 4.72 (m, 2H, C*H*H PMB, CH*H* PMB), 4.64 – 4.57 (m, 2H, C*H*H PMB, CH*H* PMB), 4.55 – 4.48 (m, 1H, CH*H* PMB), 4.43 (d, *J* = 11.4 Hz, 1H, C*H*H PMB), 4.37 – 4.30 (m, 1H, CH*H* PMB), 4.28 – 4.18 (m, 3H, H-1, H-3, H-4), 4.05 (s, 1H, H-7), 3.93 – 3.87 (m, 2H, 1-NH, 7-OH), 3.79 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.77 – 3.76 (m, 7H, OMe, OMe, H-6), 3.54 (s, 1H, H-6), 3.43 (s, 1H, H-2), 1.95 (s, 1H, H-5), 1.44 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.3, 159.2, 159.1 ($C_{q\text{-}arom}$), 156.2 (C=O), 130.9, 130.4, 130.2 ($C_{q\text{-}arom}$), 129.7, 129.6, 129.0, 113.8, 113.8, 113.7 (CH_{arom}), 86.9 ($C(CH_3)_3$), 80.4 (C-2), 77.5 (C-3/C-4), 75.5 (CH_2 PMB), 74.4 (C-3/C-4), 73.2, 73.0, 71.5 (CH_2 PMB), 71.3 (C-7), 68.0 (C-6), 55.3, 55.2, 54.2 (OMe), 45.4 (C-1), 38.6 (C-5), 28.4 ($C(CH_3)_3$); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{44}H_{55}NNaO_{11}$ 796.3673; Found 796.3675.

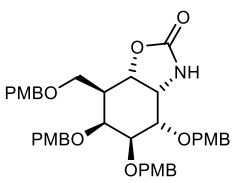
4-*Epi*-2,3,4,6-tetra-*O*-(4-methoxybenzyl)-7-(*S*)-(*N*-(*tert*-butoxycarbonyl)-1-(*R*)-ol cyclophellitol alkane (69).



Azide **67** (1.4 g, 2.0 mmol) was dissolved in a 1:1 solution of THF and aq. 1.0 M NaOH (20 mL, 0.1 M). Subsequently triphenylphosphine (2.1 g, 8.0 mmol, 4.0 eq.) was added and the reaction was stirred vigorously overnight at room temperature. Upon full conversion of the starting material was observed, the reaction was diluted with sat. aq. $NaHCO_3$

and EtOAc. The aqueous phase was separated and subsequently extracted with EtOAc (3x), the organic layers were then washed with brine. The brine layer was extracted once with EtOAc and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude amine was dissolved in anhydrous DCM (10 mL, 0.2 M) and cooled on ice. While stirring, Et_3N (1.4 mL, 10 mmol, 5.0 eq.) and subsequently Boc_2O (0.69 mL, 3.0 mmol, 1.5 eq.) were added. The flask was flushed with N_2 and the reaction was stirred at room temperature for 16 hours until full conversion was observed (R_f 0.3 (EtOAc:pentane, 4:6, v:v)). The reaction was quenched with sat. aq. NH_4Cl and the aqueous layer separated. Subsequently, the aqueous layer was extracted with EtOAc (3x), and the combined organic layers were washed with sat. aq. $NaHCO_3$. The organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (10:90 EtOAc:pentane → 40:60 EtOAc:pentane) yielded **69** as a white, brittle foam (1.5 g, 1.9 mmol, 95% over two steps). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.33 – 7.13 (m, 8H, CH_{arom}), 6.91 – 6.77 (m, 8H, CH_{arom}), 4.91 – 4.82 (m, 2H, C*H*H PMB, C*H*H PMB), 4.76 – 4.69 (m, 2H, 7-NH, CH*H* PMB), 4.68 – 4.59 (m, 2H, C*H*H PMB, CH*H* PMB), 4.43 (d, *J* = 11.0 Hz, 1H, CH*H* PMB), 4.37 – 4.28 (m, 2H, C*H*H PMB, CH*H* PMB), 4.06 (s, 1H, H-4), 3.88 – 3.82 (m, 1H, H-2), 3.79 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.77 (s, 3H, OMe), 3.71 – 3.67 (m, 1H, H-7), 3.55 – 3.45 (m, 2H, H-6), 3.36 (ddd, *J* = 11.6, 5.8, 5.8 Hz, 2H, H-1, H-3), 3.08 (s, 1H, 1-OH), 1.72 – 1.64 (m, 1H, H-5), 1.42 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HH-COSY, HSQC): δ 159.4, 159.3, 159.2, 159.1 ($C_{q\text{-}arom}$), 157.0 (C=O), 131.3, 131.2, 130.8, 130.2 ($C_{q\text{-}arom}$), 129.8, 129.7, 129.4, 129.4, 129.1, 113.9, 113.9, 113.6 (CH_{arom}), 83.5 (C-1/C-3), 81.9 (C-2), 79.8 ($C(CH_3)_3$), 77.2 (C-1/C-3), 75.2, 74.4 (CH_2 PMB), 74.1 (C-4), 73.1, 72.5 (CH_2 PMB), 69.0 (C-6), 55.3, 55.3 (OMe), 52.5 (C-7), 43.1 (C-5), 28.4 ($C(CH_3)_3$); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{44}H_{55}NNaO_{11}$ 796.3673; Found 796.3676.

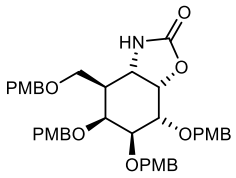
4-*Epi*-1,7-(*S*,*S*)-(*N*,*O*)-carbamate-2,3,4,6-tetra-*O*-(4-methoxybenzyl) cyclophellitol alkane (21).



Compound **68** (2.0 g, 2.6 mmol) was co-evaporated with toluene, dissolved in anhydrous $CHCl_3$ (26 mL, 0.1 M) and cooled on ice. Subsequently Me-imidazole (2.1 mL, 26 mmol, 10 eq.), Et_3N (1.8 mL, 13 mmol, 5.0 eq.) and MsCl (1.0 mL, 13 mmol, 5.0 eq.) were added. The reaction was flushed with N_2 and stirred overnight at room temperature. Upon full conversion (R_f 0.6 (EtOAc:pentane, 3:7, v:v)) the reaction was diluted with EtOAc and washed with sat. aq. NH_4Cl. The aqueous layer was extracted with EtOAc (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude intermediate was dissolved in anhydrous DMF (87 mL, 0.03 M), flushed with argon gas and stirred at 130 °C overnight. Upon full conversion (R_f 0.3 (EtOAc:pentane, 1:1, v:v)) the reaction was cooled to room temperature and concentrated to a fifth of its original volume. The residue was diluted with EtOAc and washed with H_2O and consequently brine. The aqueous layers were back-extracted with EtOAc (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (20:80 EtOAc:pentane → 60:40 EtOAc:pentane) yielded carbamate **21** as a brittle foam (1.2 g, 1.7 mmol, 65% over two steps). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.35 – 7.05 (m, 8H, CH_{arom}), 6.92 – 6.74 (m, 8H, CH_{arom}), 5.37 (s, 1H, 1-NH), 4.78 (d, *J* = 10.9 Hz, 1H, C*H*H PMB), 4.75 (d, *J* = 11.4 Hz, 1H, C*H*H PMB), 4.68 (d, *J* = 11.2 Hz, 1H, C*H*H PMB), 4.64 (d, *J* =

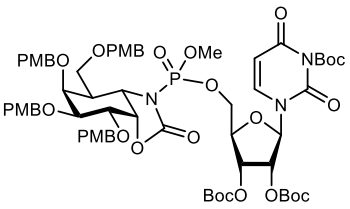
11.2 Hz, 1H, CH*H* PMB), 4.57 (d, *J* = 11.3 Hz, 1H, C*H*H PMB), 4.42 (d, *J* = 11.4 Hz, 1H, C*H*H PMB), 4.36 (d, *J* = 10.8 Hz, 1H, CH*H* PMB), 4.31 (d, *J* = 11.4 Hz, 1H, CH*H* PMB), 4.19 – 4.14 (m, 2H, H-4, H-7), 4.11 (dd, *J* = 6.0, 6.0 Hz, 1H, H-1), 3.99 (dd, *J* = 9.5, 5.1 Hz, 1H, H-2), 3.80 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.78 – 3.77 (m, 6H, OMe, OMe), 3.68 (dd, *J* = 9.6, 1.8 Hz, 1H, H-3), 3.60 – 3.52 (m, 2H, H-6), 2.05 (dddd, *J* = 9.6, 9.6, 4.6, 1.9 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.5, 159.4, 159.3, 159.3 ($C_{q\text{-}arom}$), 158.5 (C=O), 130.8, 130.6, 130.3, 130.1 ($C_{q\text{-}arom}$), 129.7, 129.6, 129.2, 114.0, 113.9, 113.9, 113.7 (CH_{arom}), 81.2 (C-3), 75.6 (C-7), 75.5 (C-2), 74.4, 73.8 (CH_2 PMB), 73.1 (C-4), 73.0, 72.5 (CH_2 PMB), 67.1 (C-6), 55.5 (C-1), 55.3, 55.3, 55.3 (OMe), 43.4 (C-5); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{40}H_{45}NNaO_{10}$ 722.2941; Found 722.2945.

4-*Epi*-1,7-(*S*,*S*)-(*O*,*N*)-carbamate-2,3,4,6-tetra-*O*-(4-methoxybenzyl) cyclophellitol alkane (23).



Compound **69** (1.5 g, 2.0 mmol) was co-evaporated with toluene, dissolved in anhydrous $CHCl_3$ (20 mL, 0.1 M) and cooled on ice. Subsequently Me-imidazole (1.6 mL, 20 mmol, 10 eq.), Et_3N (1.4 mL, 10 mmol, 5.0 eq.) and MsCl (0.8 mL, 10 mmol, 5.0 eq.) were added. The reaction was flushed with N_2 and stirred overnight at room temperature. Upon full conversion was observed(R_f 0.6 (EtOAc:pentane, 3:7, v:v)), the reaction was diluted with EtOAc and washed with sat. aq. NH_4Cl. The aqueous layer was extracted with EtOAc (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude intermediate was dissolved in anhydrous DMF (65 mL, 0.03 M), flushed with argon gas and stirred at 130 °C overnight. Upon full conversion (R_f 0.2 (EtOAc:pentane, 1:1, v:v)) the reaction was cooled to room temperature and concentrated to a fifth of its original volume. The residue was diluted with EtOAc and washed with H_2O and consequently brine. The aqueous layers were back-extracted with EtOAc (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (30:70 EtOAc:pentane → 60:40 EtOAc:pentane) yielded carbamate **23** (0.82 g, 1.2 mmol, 59% over two steps). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.38 – 7.06 (m, 8H, CH_{arom}), 6.95 – 6.79 (m, 8H, CH_{arom}), 5.48 (s, 1H, 7-NH), 4.82 – 4.69 (m, 5H, C*H*H PMB, C*H*H PMB, C*H*H PMB, CH*H* PMB, H-1), 4.63 (d, *J* = 11.4 Hz, 1H, CH*H* PMB), 4.39 – 4.34 (m, 3H, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.13 (dd, *J* = 9.6, 4.5 Hz, 1H, H-2), 3.84 (s, 3H, OMe), 3.83 – 3.83 (m, 6H, OMe, OMe), 3.82 (s, 3H, OMe), 3.75 (dd, *J* = 9.6, 2.1 Hz, 1H, H-3), 3.72 (dd, *J* = 2.1, 2.1 Hz, 1H, H-4), 3.56 (dd, *J* = 9.4, 6.0 Hz, 1H, H-7), 3.48 (dd, *J* = 9.8, 8.8 Hz, 1H, H-6), 3.39 (dd, *J* = 8.8, 5.1 Hz, 1H, H-6), 1.88 (dddd, *J* = 9.7, 9.7, 5.1, 2.0 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.6, 159.4, 159.4, 159.3 ($C_{q\text{-}arom}$), 159.1 (C=O), 130.7, 130.5, 130.4 ($C_{q\text{-}arom}$), 129.8, 129.7, 129.6, 129.3, 114.1, 113.9, 113.8, 113.8 (CH_{arom}), 80.6 (C-3), 77.1 (C-1), 75.0 (C-4), 74.8 (C-2), 74.0, 73.4, 73.3 (CH_2 PMB), 72.6 (C-6), 55.4, 55.4, 55.3 (OMe, C-7), 43.9 (C-5); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{40}H_{45}NNaO_{10}$ 722.2941; Found 722.2943.

4''-*Epi*-1'',7''-(*S*,*S*)-(*O*,*N*-(3-*N*-(*tert*-butoxycarbonyl)-5'-*O*-(methylphosphinyl)-2',3'-di-*O*-(*tert*-butoxy carbonyl)uridinyl))-carbamate-2'',3'',4'',6''-tetra-*O*-(4-methoxybenzyl) cyclophellitol alkane (70).



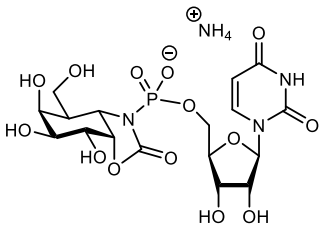
Compound **70** was prepared according to general procedure A using cyclic carbamate **23** (0.14 g, 0.2 mmol), 15-crown-5 ether (0.2 mL, 1.0 mmol, 5.0 eq.) and NaH (60 wt% dispersion in mineral oil; 12 mg, 0.3 mmol, 1.5 eq.) in anhydrous THF (1.0 mL, 0.2 M); and H-phosphonate **48** (0.25 g, 0.4 mmol, 2.0 eq.), dry DiPEA (0.21 mL, 1.2 mmol, 6.0 eq.) and $BrCCl_3$ (79 µL, 0.8 mmol, 4.0 eq.) in anhydrous DCM (1.3 mL, 0.3 M). Flash column chromatography of the crude product (20:80 EtOAc:pentane → 60:40 EtOAc:pentane) and Sephadex™ LH-20 size exclusion chromatography yielded the title compound **70** as a pale-yellow oil and as a mixture of P(V) diastereomers (84 mg, 64 µmol, 32%) and starting material (32 mg, 46 µmol, 23%), 55% brsm.

Data for first P(V) diastereomer: R_f 0.6 (EtOAc:pentane, 1:1 v:v); ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.69 (d, *J* = 8.3 Hz, 1H, H-6), 7.32 – 7.29 (m, 4H, CH_{arom}), 7.25 – 7.22 (m, 2H, CH_{arom}), 7.14 – 7.10 (m, 2H, CH_{arom}), 6.92 – 6.82 (m, 8H, CH_{arom}), 6.17 (d, *J* = 6.1 Hz, 1H, H-1'), 5.91 (d, *J* = 8.2 Hz, 1H, H-5), 5.28 (dd, *J* = 5.4, 4.2 Hz, 1H, H-3'), 5.13 (dd, *J* = 5.8, 5.8 Hz, 1H, H-2'), 4.83 – 4.64 (m, 6H, H-1'', C*H*H PMB, C*H*H PMB, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.45 – 4.30 (m, 5H, H-4', H-5', C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.27 – 4.13 (m, 2H, H-5', H-2''), 4.01 – 3.94 (m, 1H, H-7''), 3.83 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.78 – 3.67 (m, 5H, P(O)OMe, H-4'', H-6''), 3.57 (dd, *J* = 9.8, 9.8 Hz, 1H, H-6''), 2.01 – 1.93 (m, 1H, H-5''), 1.61 (s, 9H, $C(CH_3)_3$), 1.50 (s, 9H, $C(CH_3)_3$), 1.48 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 160.3 (C=O uracil), 159.5, 159.3, 159.3 ($C_{q\text{-}arom}$), 154.7 (d, $^2J_{C,P}$ = 7.5 Hz, C=O cyclic carbamate), 152.1 (C=O carbamate), 152.0 (C=O uracil), 148.7, 147.7 (C=O carbonate), 139.4 (C-6), 130.7, 130.7, 130.1 ($C_{q\text{-}arom}$), 129.8, 129.7, 129.2, 114.0, 113.9, 113.9, 113.7 (CH_{arom}), 103.4 (C-5), 86.9 (*C*$(CH_3)_3$), 86.1 (C-1'), 83.8, 83.7 (*C*$(CH_3)_3$), 80.3 (C-4''), 80.0 (d, $^3J_{C,P}$ = 7.8 Hz, C-4'), 79.3 (d, $^3J_{C,P}$ = 6.6 Hz, C-1''), 74.5 (C-2'), 74.4 (CH_2 PMB), 74.2 (C-3''), 73.5 (C-2''), 73.1, 73.1, 73.0 (CH_2 PMB), 71.7 (C-3'), 66.9 (d, $^3J_{C,P}$ = 6.1 Hz, C-5'), 66.7 (C-6''), 57.6 (d, $^3J_{C,P}$ = 4.4 Hz, C-7''), 55.4 (OMe), 55.1 (d, $^2J_{C,P}$ = 6.5 Hz, P(O)OMe), 44.5 (C-5''), 27.7, 27.7, 27.5 (C(*C*$H_3)_3$).

Data for second P(V) diastereomer: R_f 0.3 (EtOAc:pentane, 1:1 v:v); ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.67 (d, *J* = 8.3 Hz, 1H, H-6), 7.34 – 7.20 (m, 6H, CH_{arom}), 7.17 – 7.08 (m, 2H, CH_{arom}), 6.94 – 6.79 (m, 8H, CH_{arom}), 6.16 (d, *J* = 6.1 Hz, 1H, H-1'), 5.82 (dd, *J* = 8.3, 0.4 Hz, 1H, H-5), 5.26 (dd, *J* = 5.5, 3.8 Hz, 1H, H-3'), 5.19 (dd, *J* = 5.8, 5.8 Hz, 1H, H-2'), 4.83 – 4.65 (m, 6H, H-1'', C*H*H PMB, C*H*H PMB, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.44 – 4.30 (m, 3H, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.28 – 4.13 (m, 4H, H-4', H-5', H-5', H-2''), 4.01 – 3.93 (m, 1H, H-7''), 3.83 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.81 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.78 – 3.67 (m, 5H, P(O)OMe, H-4'', H-6''), 3.58 (dd, *J* = 9.8 Hz, 1H, H-6''), 2.12 – 2.06 (m, 1H, H-5''), 1.60 (s, 9H, $C(CH_3)_3$), 1.49 (s, 9H, $C(CH_3)_3$), 1.46 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.3 (C=O uracil), 159.5, 159.4, 159.3, 159.3 ($C_{q\text{-}arom}$), 154.6 (d, $^2J_{C,P}$ = 7.3 Hz, C=O cyclic carbamate), 152.1 (C=O carbamate), 151.9 (C=O uracil), 148.7, 147.6 (C=O carbonate), 139.6 (C-6), 130.8, 130.7, 130.3, 130.1 ($C_{q\text{-}arom}$), 129.9, 129.8, 129.7, 129.2, 113.9, 113.9, 113.9, 113.7 (CH_{arom}), 103.1 (C-5), 86.8 (*C*$(CH_3)_3$), 86.3 (C-1'), 83.6, 83.6 (*C*$(CH_3)_3$), 80.5 (C-4''), 80.0 (d, $^3J_{C,P}$ = 8.2 Hz, C-4'), 79.3 (d, $^3J_{C,P}$ = 5.7 Hz, C-1''), 74.5 (CH_2 PMB), 74.4

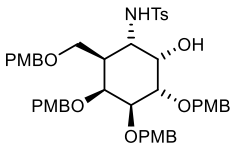
(C-2'), 74.2 (C-3''), 73.7 (C-2''), 73.1, 73.1, 73.0 (CH_2 PMB), 72.1 (C-3'), 66.9 (C-6''), 66.5 (d, $^3J_{C,P}$ = 5.9 Hz, C-5'), 57.7 (d, $^3J_{C,P}$ = 4.4 Hz, C-7''), 55.4 (OMe), 54.7 (d, $^2J_{C,P}$ = 5.9 Hz, P(O)OMe), 44.2 (C-5''), 27.7, 27.7, 27.5 ($C(CH_3)_3$); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{65}H_{82}N_3NaO_{24}P$ 1342.4924; Found 1342.4920.

4''-*Epi*-1'',7''-(*S*,*S*)-(*N*,*O*-(5'-*O*-phosphoryluridinyl))-carbamate cyclophellitol alkane (16).



Compound **16** was prepared according to general procedure B using **70** (84 mg, 64 μmol), TES (0.10 mL, 0.64 mmol, 10 eq.) and TFA in DCM (30% v:v, 1.4 mL, 0.05 M). The reaction mixture was stirred at 5 °C for 24 h, after which full conversion was observed on TLC (R_f 0.5 MeOH:DCM, 3:7 v:v). Pyridine (20:1 pyridine:TFA, 5.0 mL, 62 mmol) was added and the reaction mixture was stirred overnight at room temperature. Upon full conversion was observed on TLC (R_f 0.3 (H_2O:MeCN, 1:9 v:v)) and ^{31}P NMR. Flash column chromatography (0:100 H_2O:ACN → 15:85 H_2O:ACN) followed by Dowex 50WX4 Na^+ ion exchange and lyophilization yielded the title compound **16** as a transparent film (21 mg, 40 μmol, 63%). ^1H NMR (850 MHz, D_2O, HH-COSY, HSQC): δ 7.91 (d, *J* = 8.1 Hz, 1H, H-6), 5.90 (d, *J* = 4.6 Hz, 1H, H-1'), 5.87 (d, *J* = 8.1 Hz, 1H, H-5), 4.86 (dd, *J* = 4.8, 4.8 Hz, 1H, H-1''), 4.30 – 4.27 (m, 2H, H-2'', H-3''), 4.22 (dddd, *J* = 5.0, 2.6, 2.6, 2.6 Hz, 1H, H-4'), 4.17 – 4.11 (m, 3H, H-5', H-4''), 4.07 (dd, *J* = 10.4, 4.3 Hz, 1H, H-2''), 3.98 (dd, *J* = 11.5, 3.9 Hz, 1H, H-6''), 3.95 (ddd, *J* = 10.4, 5.4, 2.2 Hz, 1H, H-7''), 3.74 (dd, *J* = 10.4, 2.6 Hz, 1H, H-3''), 3.69 (dd, *J* = 11.5, 9.9 Hz, 1H, H-6''), 1.87 (dddd, *J* = 10.1, 10.1, 3.9, 2.2 Hz, 1H, H-5''); ^{13}C NMR (214 MHz, D_2O, HSQC): δ 167.2 (C=O uracil), 158.9 (d, *J* = 7.7 Hz, C=O carbamate), 152.7(C=O uracil), 142.8 (C-6), 103.3 (C-5), 89.5 (C-1'), 83.8 (d, *J* = 9.4 Hz, C-4'), 81.2 (d, *J* = 5.7 Hz, C-1''), 74.7 (C-2'/C-3'), 71.9 (C-3''), 70.4 (C-2'/C-3'), 69.5 (C-4''), 68.2 (C-2'), 65.7 (d, *J* = 5.3 Hz, C-5'), 59.9 (C-6''), 57.9 (d, *J* = 2.5 Hz, C-7''), 46.3 (C-5''); ^{31}P NMR (162 MHz, D_2O) δ -5.59; HRMS (ESI) m/z: $[M + H]^+$ Calcd. for $C_{17}H_{28}N_4O_{14}P$ 543.1340; Found 543.1345.

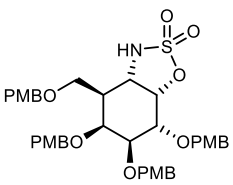
4-*Epi*-2,3,4,6-tetra-*O*-(4-methoxybenzyl)-7-(*S*)-(*p*-toluenesulfonamido)-1-(*S*)-ol cyclophellitol alkane (71).



Compound **23** (0.62 g, 0.9 mmol) was dissolved in EtOH (14 mL, 0.02 M) followed by the addition of aq. NaOH (3.0 M, 3.6 mL, 11 mmol, 12 eq.). The reaction mixture was heated to 70 °C and stirred for 2 hours after which full conversion was observed (R_f 0.3 (MeOH:DCM, 1:9, v:v)). The reaction was diluted with EtOAc and washed with sat. aq. $NaHCO_3$. The aqueous layer was extracted with EtOAc (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude intermediate was dissolved in anhydrous THF (9.0 mL, 0.1 M). The reaction mixture was cooled on ice followed by the addition of Et_3N (0.49 mL, 3.6 mmol, 4.0 eq.) and TsCl (0.22 g, 1.2 mmol, 1.3 eq.). Stirring at this temperature continued for 1 hour after which full conversion was observed (R_f 0.6 (EtOAc:pentane, 1:1, v:v)). The reaction was diluted with EtOAc and washed with sat. aq. $NaHCO_3$. The aqueous layer was extracted with EtOAc (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (20:80 EtOAc:pentane → 70:30 EtOAc:pentane) yielded **71** (0.52 g, 0.63 mmol, 70% over two steps). ^1H

NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.70 (d, *J* = 8.3 Hz, 2H, CH_{arom}), 7.29 – 7.06 (m, 10H, CH_{arom}), 6.89 – 6.79 (m, 8H, CH_{arom}), 5.26 (d, *J* = 9.5 Hz, 1H, 7-NH), 4.77 (d, *J* = 10.7 Hz, 1H, C*H*H PMB), 4.62 – 4.54 (m, 3H, C*H*H PMB, C*H*H PMB, CH*H* PMB), 4.43 (d, *J* = 11.0 Hz, 1H, CH*H* PMB), 4.36 – 4.27 (m, 2H, C*H*H PMB, CH*H* PMB), 4.17 (d, *J* = 11.3 Hz, 1H, CH*H* PMB), 4.02 (dd, *J* = 2.3, 2.3 Hz, 1H, H-4), 3.81 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.74 – 3.70 (m, 2H, H-1, H-2), 3.59 (dd, *J* = 9.4, 2.4 Hz, 1H, H-3), 3.47 – 3.34 (m, 2H, H-6, H-7) 3.26 (dd, *J* = 9.2, 9.2 Hz, 1H, H-6), 2.50 (d, *J* = 1.8 Hz, 1H, 1-OH), 2.44 (s, 3H, Me Ts), 2.07 (dddd, *J* = 11.4, 9.2, 4.5, 2.0 Hz, 1H, H-5); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 159.3, 159.1, 159.1, 143.4, 138.2, 131.4, 131.0, 130.4, 130.2 ($C_{q\text{-}arom}$), 129.8, 129.7, 129.6, 129.5, 129.5, 129.4, 129.1, 127.2, 113.9, 113.9, 113.8, 113.6 (CH_{arom}), 80.1 (C-3), 78.2 (C-1/C-2), 74.6 (CH_2 PMB), 74.1 (C-4), 73.0, 72.6, 72.6 (CH_2 PMB), 69.5 (C-1/C-2), 68.4 (C-6), 55.4 (OMe), 52.3 (C-7), 40.3 (C-5), 21.7 (Me Ts); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{46}H_{53}NNaO_{11}S$ 850.3237; Found 850.3240.

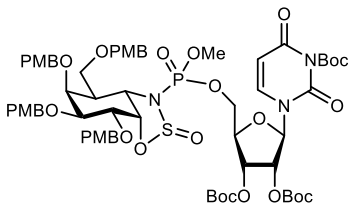
4-*Epi*-1,7-(*S,S*)-(*O,N*)-sulfamidate-2,3,4,6-tetra-*O*-(4-methoxybenzyl) cyclophellitol alkane (24).



Compound **71** (0.52 g, 0.63 mmol) was dissolved in anhydrous DCM (6.3 mL, 0.1 M) and kept under inert atmosphere. Et_3N (0.35 mL, 2.5 mmol, 4.0 eq.) was added and the reaction mixture was cooled to -78 °C followed by the dropwise addition of SO_2Cl_2 (61 µL, 0.76 mmol, 1.2 eq.). The reaction was allowed to attain to 0 °C over the course of 2 hours after which full conversion was observed (R_f 0.6 (EtOAc:pentane, 3:7, v:v)). The reaction was quenched with sat. aq. NH_4Cl at -78 °C and subsequently diluted with EtOAc and washed with sat. aq. $NaHCO_3$. The aqueous layer was extracted with EtOAc (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. In a separate flask, naphthalene (0.97 g, 7.6 mmol, 12 eq.) was dissolved in anhydrous THF (12.5 mL, 0.05 M) followed by the addition of sodium metal (0.15 g, 6.3 mmol, 10 eq.). The mixture was sonicated for 15 min. at room temperature before being cooled to -78 °C. The crude intermediate was co-evaporated thrice with toluene, dissolved in anhydrous THF (1 mL) and added dropwise to the Na-naphthalene solution. The reaction mixture was stirred for 1 hour at -78 °C. Upon full conversion on TLC (R_f 0.2 (EtOAc:pentane, 3:7, v:v)), the reaction was quenched with sat. aq. NH_4Cl at -78 °C. The mixture was diluted with sat. aq. $NaHCO_3$, the organic layer was separated, the aqueous layer was extracted thrice with EtOAc and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Flash column chromatography (30:70 EtOAc:pentane → 70:30 EtOAc:pentane) yielded **24** (0.34 g, 0.47 mmol, 74% over two steps). ^1H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.36 – 7.05 (m, 8H, CH_{arom}), 6.95 – 6.79 (m, 8H, CH_{arom}), 5.43 (d, *J* = 3.1 Hz, 1H, 7-NH), 5.00 (dd, *J* = 4.0, 4.0 Hz, 1H, H-1), 4.80 (d, *J* = 10.8 Hz, 1H, C*H*H PMB), 4.77 – 4.72 (m, 3H, C*H*H PMB, C*H*H PMB, CH*H* PMB), 4.65 (d, *J* = 11.3 Hz, 1H, CH*H* PMB), 4.43 – 4.35 (m, 3H, C*H*H PMB, CH*H* PMB, CH*H* PMB), 4.13 (dd, *J* = 9.4, 3.7 Hz, 1H, H-2), 3.92 (dd, *J* = 2.2, 2.2 Hz, 1H, H-4), 3.84 (s, 3H, OMe), 3.83 – 3.82 (m, 10H, OMe, OMe, OMe, H-3), 3.65 (ddd, *J* = 10.5, 3.5, 3.5 Hz, 1H, H-7), 3.57 (dd, *J* = 9.0, 7.7 Hz, 1H, H-6), 3.46 (dd, *J* = 9.0, 6.5 Hz, 1H, H-6), 2.37 (dddd, *J* = 10.1, 7.9, 6.4, 2.0 Hz, 1H, H-5); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.6, 159.6, 159.4, 159.4, 130.5, 130.4 ($C_{q\text{-}arom}$), 129.9, 129.8, 129.7, 129.7, 129.6, 129.3, 114.1, 114.0, 114.0, 113.8 (CH_{arom}), 83.1 (C-1), 80.1 (C-3), 74.7 (C-4), 74.5 (C-2), 74.5, 73.3, 73.2,

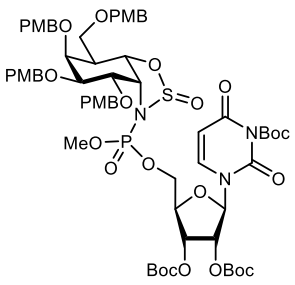
73.1 (CH_2 PMB), 70.0 (C-6), 58.6 (C-7), 55.4, 55.4 (OMe), 40.9 (C-5); HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{39}H_{45}NNaO_{11}S$ 758.2611; Found 758.2608.

4''-*Epi*-1'',7''-(*S*,*S*)-(*O*,*N*-(3-*N*-(*tert*-butoxycarbonyl)-5'-*O*-(methylphosphinyl)-2',3'-di-*O*-(*tert*-butoxy carbonyl)uridinyl))-sulfamidite-2'',3'',4'',6''-tetra-*O*-(4-methoxybenzyl) cyclophellitol alkane (S2).



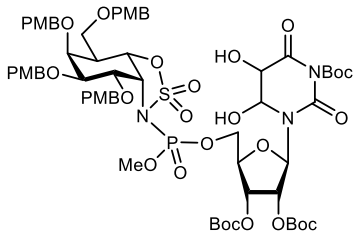
Compound **23** (70 mg, 0.1 mmol) was dissolved in EtOH (2.0 mL, 0.05 M) followed by the addition of aq. NaOH (3.0 M, 1.2 mL, 2.0 mmol, 20 eq.). The reaction mixture was heated to 80 °C and stirred for 2 hours after which full conversion was observed (R_f 0.3 (MeOH:DCM, 1:9, v:v)). The reaction was diluted with EtOAc and washed with sat. aq. $NaHCO_3$. The aqueous layer was extracted with EtOAc (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude intermediate was dissolved in anhydrous $CHCl_3$ (2.0 mL, 0.05 M). The reaction mixture was cooled on ice followed by the addition of H-phosphonate **48** (93 mg, 0.15 mmol, 1.5 eq.), DiPEA (0.13 mL, 0.8 mmol, 8.0 eq.) and consequently $BrCCl_3$ (20 µL, 0.2 mmol, 2.0 eq.). Stirring continued for 15 minutes while kept on ice after which full conversion was observed (R_f 0.4 and 0.5 corresponding to both phosphor diastereomers (EtOAc:pentane, 8:2, v:v)). The reaction mixture was cooled to -40 °C followed by the addition of $SOCl_2$ (15 µL, 0.2 mmol, 2.0 eq.). Stirring continued overnight while allowing the mixture to attain to 5 °C. Upon full conversion was observed (R_f 0.3 (EtOAc:pentane, 1:1, v:v)), the reaction was diluted with EtOAc and washed with sat. aq. $NaHCO_3$. The aqueous layer was extracted with EtOAc (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (20:80 EtOAc:pentane → 50:50 EtOAc:pentane) yielded **S2** as a mixture of S(IV) and P(V) diastereomers (23 mg, 17 µmol, 17% over three steps). ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.64 (d, *J* = 8.3 Hz, 1H, H-6), 7.49 – 7.36 (m, 2H, H-6), 7.34 – 7.07 (m, 32H, CH_{arom}), 6.94 – 6.78 (m, 23H, CH_{arom}), 6.15 – 5.91 (m, 3H, H-5), 5.84 – 5.69 (m, 3H, H-1'), 5.29 – 5.08 (m, 7H, H-2', H-3'), 4.84 – 4.52 (m, 17H, H-3', CH_2 PMB, CH_2 PMB, CH_2 PMB), 4.43 – 4.10 (m, 16H, H-4', H-5', H-1'', H-2'', H-3'', H-4'', CH_2 PMB), 4.03 – 3.43 (m, 49H, H-6'', H-7'', OMe, OMe, OMe, OMe, P(O)OMe), 2.68 – 2.52 (m, 3H, H-5''), 1.65 – 1.52 (m, 27H, $C(CH_3)_3$), 1.54 – 1.40 (m, 56H, $C(CH_3)_3$, $C(CH_3)_3$); ^{13}C NMR (101 MHz, $CDCl_3$, HH-COSY, HSQC): δ 160.2, 160.2, 160.0 (C=O uracil), 159.5, 159.4, 159.3, 159.3, 159.2 ($C_{q\text{-}arom}$), 152.1, 152.0 (C=O cyclic carbamate), 148.6, 148.3, 147.6, 147.4 (C=O carbonate), 139.4, 139.3, 130.9, 130.8, 130.7, 130.7, 130.0, 130.0 ($C_{q\text{-}arom}$), 129.9, 129.9, 129.8, 129.7, 129.6, 129.2, 129.2, 113.9, 113.9, 113.9, 113.8 (CH_{arom}), 103.0, 102.8 (C-5), 89.0, 88.6, 88.3, 87.6 ($C(CH_3)_3$), 87.1, 87.1, 86.9, 86.4 (C-1'), 83.9, 83.8, 83.7, 83.6, 82.3, 82.2 ($C(CH_3)_3$), 80.6, 80.5, 80.2, 80.1, 79.9, 79.8, 77.5, 74.9, 74.8, 74.7, 74.6, 74.5, 74.1, 74.0, 73.8, 73.5, 73.3, 73.1, 73.1, 73.0, 72.6, 71.9, 71.5, 71.2 (C-1'', C-2', C-3', C-4', C-2'', C-3'', C-4'', CH_2 PMB, CH_2 PMB, CH_2 PMB CH_2 PMB), 66.6, 66.4, 66.1, 65.6 (C-5', C-6''), 59.4, 57.0, 55.4, 55.0, 54.9, 54.8, 54.7 (C-1'', OMe, OMe, OMe, OMe, P(O)OMe), 44.5, 43.8 (C-5''), 29.8, 28.7, 28.5, 27.7, 27.5 ($C(CH_3)_3$, $C(CH_3)_3$, $C(CH_3)_3$); ^{31}P NMR (162 MHz, $CDCl_3$): δ 2.17, 2.09, 1.87, 1.80; HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{64}H_{82}N_3NaO_{24}S$ 1362.4644; Found 1362.4647.

4''-*Epi*-1'',7''-(*S*,*S*)-(*N*,*O*-(3-*N*-(*tert*-butoxycarbonyl)-5'-*O*-(methylphosphinyl)-2',3'-di-*O*-(*tert*-butoxy carbonyl)uridinyl))-sulfamidite-2'',3'',4'',6''-tetra-*O*-(4-methoxybenzyl) cyclophellitol alkane (76).



Compound **21** (0.14 g, 0.2 mmol) was dissolved in EtOH (4.0 mL, 0.05 M) followed by the addition of aq. NaOH (3.0 M, 2.4 mL, 4.0 mmol, 20 eq.). The reaction mixture was heated to 80 °C and stirred for 2 hours after which full conversion was observed (R_f 0.3 (MeOH:DCM, 1:9, v:v)). The reaction was diluted with EtOAc and washed with sat. aq. $NaHCO_3$. The aqueous layer was extracted with EtOAc (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude intermediate was dissolved in anhydrous $CHCl_3$ (4.0 mL, 0.05 M). The reaction mixture was cooled on ice followed by the addition of H-phosphonate **48** (0.19 g, 0.3 mmol, 1.5 eq.), DiPEA (0.27 mL, 1.6 mmol, 8.0 eq.) and consequently $BrCCl_3$ (40 µL, 0.4 mmol, 2.0 eq.). Stirring continued for 15 minutes while kept on ice after which full conversion was observed (R_f 0.3 and 0.5 corresponding to both phosphor diastereomers of **74** (EtOAc:pentane, 8:2, v:v)). The reaction mixture was cooled to -40 °C followed by the addition of $SOCl_2$ (29 µL, 0.4 mmol, 2.0 eq.). Stirring continued overnight while allowing the mixture to attain to 5 °C. Upon full conversion was observed (R_f 0.3 (EtOAc:pentane, 1:1, v:v)), the reaction was diluted with EtOAc and washed with sat. aq. $NaHCO_3$. The aqueous layer was extracted with EtOAc (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (20:80 EtOAc:pentane → 50:50 EtOAc:pentane) yielded **76** as a mixture of P(V) diastereomers (99 mg, 74 µmol, 37% over three steps). ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.58 (d, *J* = 8.3 Hz, 1H, H-6), 7.52 (d, *J* = 8.3 Hz, 1H, H-6*), 7.22 – 7.03 (m, 8H, CH_{arom}), 6.90 – 6.75 (m, 9H, CH_{arom}), 6.08 (d, *J* = 5.1 Hz, 1H, H-5), 6.02 (d, *J* = 5.2 Hz, 1H, H-5*), 5.82 (m, 2H, H-1', H-1'*), 5.31 – 5.09 (m, 4H, H-2, H-3, H-2*, H-3*), 4.57 – 4.18 (m, 64H, H-4', H-5', H-7'', H-2'', H-3'', H-4'', H-4'*, H-5'*, H-7''*, H-2''*, H-3''*, H-4''*, CH_2 PMB, CH_2 PMB, CH_2 PMB, CH_2 PMB, CH_2 PMB*, CH_2 PMB*, CH_2 PMB*, CH_2 PMB*), 4.09 – 4.02 (m, 1H, H-1'', H-1''*), 3.88 – 3.68 (m, 32H, H-6'', H-6''*, OMe, OMe, OMe, OMe, P(O)Me, OMe*, OMe*, OMe*, OMe*, P(O)Me*), 3.66 – 3.59 (m, 2H, H-6'', H-6''*), 2.73 (m, 2H, H-5'', H-5''*), 1.62 – 1.56 (m, 18H, $C(CH_3)_3$, $C(CH_3)_3$*), 1.51 – 1.42 (m, 36H, $C(CH_3)_3$, $C(CH_3)_3$, $C(CH_3)_3$*, $C(CH_3)_3$*); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 160.2, 160.2 (C=O uracil), 159.4, 159.4, 159.3, 159.3, 159.3 ($C_{q\text{-}arom}$), 152.1, 152.0, 152.0, 151.9 (C=O cyclic carbamate), 148.5, 148.5, 147.6, 147.5 (C=O carbonate), 139.3, 138.9, 130.4, 130.3, 130.3, 130.2, 130.0, 130.0 ($C_{q\text{-}arom}$), 129.9, 129.9, 129.6, 129.6, 129.3, 129.2, 128.9, 128.7, 113.9, 113.8, 113.8 (CH_{arom}), 103.1, 103.0 (C-5, C-5*), 87.2, 86.9 ($C(CH_3)_3$), 86.9, 86.8 (C-1', C-1'*), 83.8, 83.8, 83.7, 83.6 ($C(CH_3)_3$), 79.8, 79.8, 79.7, 77.5, 75.9, 75.7, 75.2, 74.9, 74.7, 73.3, 72.9, 72.8, 72.7, 72.7, 72.5, 72.1, 71.7, 71.6, 71.5, 71.5 (C-2', C-3', C-4', C-7'', C-2'', C-3'', C-4'', CH_2 PMB, CH_2 PMB, CH_2 PMB CH_2 PMB), 67.1, 65.7, 65.6 (C-5', C-6''), 57.9, 57.9, 57.6, 55.4, 55.3, 54.8, 54.7, 54.6, 54.5 (C-1'', OMe, OMe, OMe, OMe, P(O)OMe) 39.7 (C-5''), 27.7, 27.5 ($C(CH_3)_3$, $C(CH_3)_3$, $C(CH_3)_3$); ^{31}P NMR (162 MHz, $CDCl_3$): δ 0.58, 0.51; HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{64}H_{82}N_3NaO_{24}S$ 1362.4644; Found 1362.4649.

4''-E*pi*-1'',7''-(*O,N*-(3-*N*-(*tert*-butoxycarbonyl)-5'-*O*-(methylphosphinyl)-2',3'-di-*O*-(*tert*-butoxy carbonyl)-5,6-dihydroxydihydro-uridinyl))-sulfamidate-2'',3'',4'',6''-tetra-*O*-(4-methoxybenzyl) cyclophellitol alkane (77).



Compound **76** (40 mg, 30 µmol) was dissolved in a 1:1 mixture of EtOAc and acetonitrile (0.6 mL, 0.05 M) and cooled on ice. In a separate flask, $RuCl_3$ (2.0 mg, 7.5 µmol, 0.25 eq.) and $NaIO_4$ (16 mg, 75 µmol, 2.5 eq.) were dissolved in H_2O and subsequently added to the substrate solution. After stirring for 20 minutes, TLC indicated full conversion (R_f 0.2 (EtOAc:pentane, 1:1, v:v)) and the reaction was quenched with sat. aq. $Na_2S_2O_3$. The aqueous layer was extracted with Et_2O (3x), and the combined organic layers were subsequently washed with sat. aq. $NaHCO_3$. The organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (30:70 EtOAc:pentane → 50:50 EtOAc:pentane) yielded **77** as a mixture of P(V) diastereomers (20 mg, 15 µmol, 50%). ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.30 – 7.04 (m, 9H), 6.91 – 6.79 (m, 8H), 6.01 (d, *J* = 5.2 Hz, 1H), 5.48 – 5.20 (m, 2H), 4.96 (dd, *J* = 35.3, 5.2 Hz, 1H), 4.78 – 4.53 (m, 1H), 4.50 – 4.18 (m, 8H), 4.10 (dd, *J* = 6.2, 3.8 Hz, 1H), 4.05 – 3.86 (m, 3H), 3.84 – 3.74 (m, 12H), 3.72 – 3.52 (m, 2H), 3.37 (d, *J* = 5.8 Hz, 0H), 2.66 (d, *J* = 9.3 Hz, 1H), 1.59 – 1.51 (m, 7H), 1.51 – 1.38 (m, 18H); ^{13}C NMR (101 MHz, $CDCl_3$, HSQC): δ 167.9, 167.6, 159.5, 159.4, 159.3, 152.5, 152.2, 152.1, 149.6, 149.0, 147.4, 130.2, 129.6, 129.4, 129.0, 114.0, 113.9, 86.6, 86.4, 86.2, 83.6, 83.5, 81.7, 78.8, 76.8, 76.1, 75.6, 74.1, 73.8, 73.5, 73.2, 73.1, 72.9, 72.2, 72.0, 71.9, 71.5, 71.3, 69.5, 69.3, 68.0, 67.2, 66.3, 60.0, 59.8, 56.2, 56.1, 55.7, 55.4, 39.9, 39.8, 27.7, 27.5; ^{31}P NMR (162 MHz, $CDCl_3$) δ -2.82, -3.06, -3.98; HRMS (ESI) m/z: $[M + Na]^+$ Calcd. for $C_{64}H_{84}N_3NaO_{27}PS$ 1412.4648; Found 1412.4652.

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Chapter 8

Summary and future prospects

Cyclophellitol and its analogues are established inactivators of retaining glycosidases (GHs) often showing excellent potencies and selectivities.[1–6] Over the years, understanding of their mode of action has allowed for the design of analogues tailored towards predetermined glycosidases and for specific purposes.[2,3,7–11] In this way, inhibitors and probes targeting specific *endo*- or *exo*-glycosidases,[12,13] but also reagents to study broader ranges of glycosidases have been constructed and applied successfully.[14,15] The work described in this thesis focusses on novel designs and the development of synthetic methodologies in order to widen the palette of inhibitors and probes targeting glycol-processing enzymes. Putative inhibitors are described envisioned as covalently binding deactivators of inverting glycosidases, enzyme families as large and diverse as retaining ones, but for which no covalent and irreversible inhibitors exist to date. In addition, the design and synthesis of a new class of conformationally constrained glycosyl transferase (GT) donor analogues, envisioned as putative GT inhibitors, is presented. In all, the work presented expands on the growing list of retaining GH inhibitors and activity-based probes, presents the first steps towards

the complementary inverting GH ones and reports on the design of a new class of potential, conformationally biased, competitive GT inhibitors.

1 | **Chapter 1** provides a global overview of literature precedents regarding the synthesis of carbasugar motifs. The main focus lies on the developed synthetic methodologies towards carbaglucose and carbagalactose backbones – substructures that also feature as discriminating motifs in most of the experimental work of this thesis.

2 | A new route towards an orthogonal cyclophellitol building block from commercially available tri-*O*-acetyl-D-glucal is described in **Chapter 2**. The key transformation of this synthesis route relies on a Claisen rearrangement, effectively transforming D-glucal to carba-D-glucal. Subsequent transposition of the 1,2-alkene to the 1,7-position afforded a key orthogonal building block in an overall yield of 19% over 12 steps. Due to the full orthogonality of all four hydroxyl protecting groups in this glucopyranose-configured cyclohexene, regioselective manipulations are feasible and gave rise to a small series of α-(1,3)-linked di- and trisaccharide nigerose and dextran mimetics. As well, making judicious use of neighboring group participation potential (utilizing the allylic secondary alcohol, available by selective deprotection) allowed for the stereoselective introduction of both epoxide and aziridine warheads to emulate α-glucopyranosides.

The set of established di- and trisaccharide structures are, besides a demonstration of the ease of use of the orthogonal building block, envisioned as potential inhibitors of the corresponding nigerase and dextranase enzymes. To further capitalize on the novel methodology described in this chapter, and to study these relatively underexplored enzyme families, a set of probes is proposed as shown in figure 1. The proposed set of probes cover three different glucoside-backbones, consisting of α-(1,3)-linked glucosides (**1** – **4**), α-(1,6)-linked glucosides (**5** – **8**) and a combination of the two (**9** – **12**), relating to nigerose, pullulan and dextran respectively. The proposed nigerase, pullulanase and dextranase probes could find use in studying and identifying glycosidases involved in the degradation of common cell wall polysaccharides of fungi and bacteria. An important component of cell wall polysaccharides of numerous eukaryotic and bacterial organisms is repeating α-(1,3)-linked glucan (also called mutan) with a high degree of branching (for instance, α-(1,6)-linked glucosides forming dextran).[16–21] These polysaccharides are widely found in both fungi (for instance, *Aspergillus niger*, *Agrocybe cylinducea* and *Piptoporus betulinus*) and bacteria (for instance, *Streptococci*, the main causal agent of dental plaques). A high degree of branching is crucial for organismal survival, since it correlates to low substrate

recognition by glycosidases.[16,22,23] As a result, low turn-over rates are generally observed by host enzymes, effectively making the cell wall impenetrable. In contrast, some microorganisms produce *exo*- and *endo*-mutan and dextran processing enzymes, which allows them to use these polysaccharides as their main carbon source.[22,24–26] Knock-out strains, lacking enzymes competent in processing α-(1,3)-linked glucans, generally show poor growth and are less or not viable.[23,26] This has marked these enzymes as interesting therapeutic targets. To this end, the probes described here could assist in the development of much needed novel antibiotics targeting these glycan cell wall processing enzymes. As aforementioned, crippling of this pathway will constitute serious limitations for the pathogenic micro-organisms.

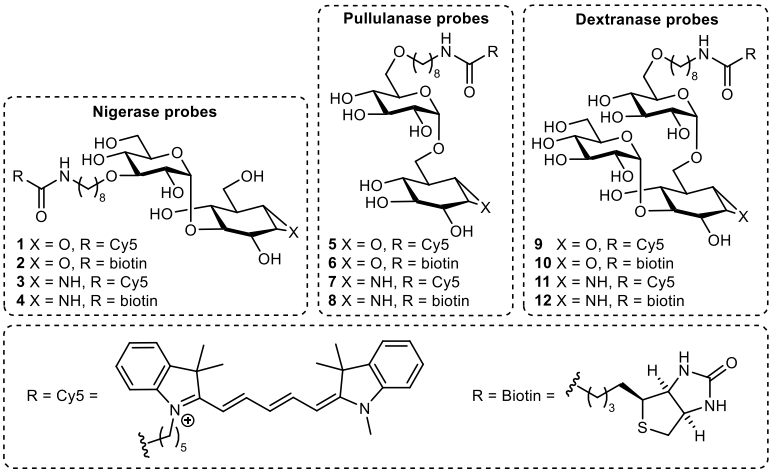
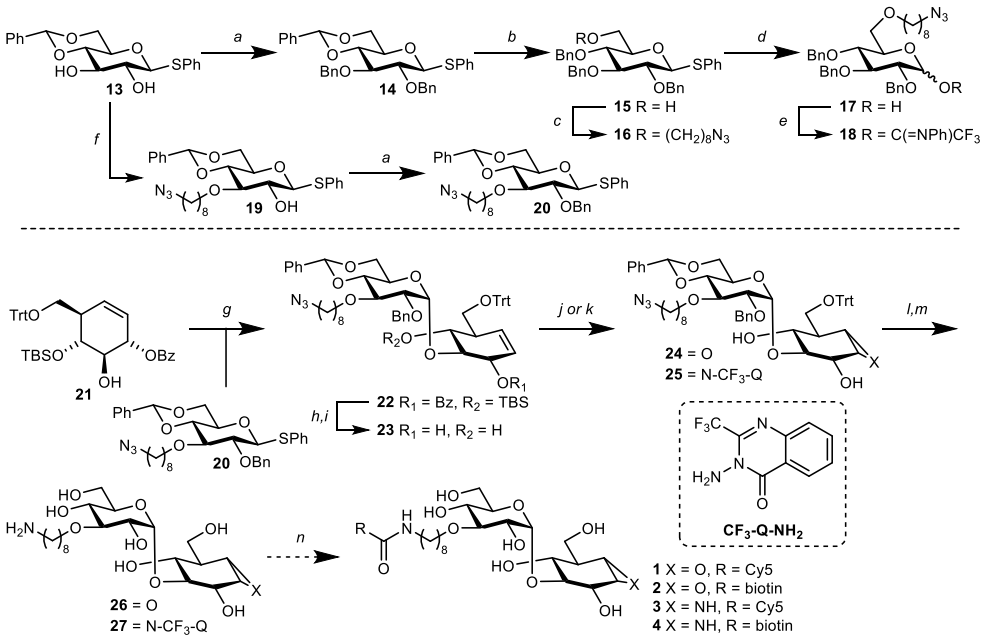


Figure 1. Library of twelve probes, envisioned as suitable mimetics of nigerose (**1** – **4**), pullulan (**5** – **8**) and dextran (**9** – **12**). The probes are either equipped with a fluorescent Cy5 tag for visualisation, or equipped with a biotin tag, allowing for pull-down and isolation.

Initial studies towards these aims have been conducted and comprised the synthesis of disaccharide ABPs having a nigerose, pullulan or dextran configuration with reporter tags installed at the appropriate non-reducing alcohol (Figure 1). The synthesis of these compounds commenced with preparation of donor glucosides **18** and **20** (Scheme 1) that allow the introduction of the reporter entities (fluorophore or biotin) in later stages of the synthesis schemes. To this end, the 2- and 3-OH of thiophenol donor **13** were protected as benzyl ethers[27] after which the benzylidene was reductively opened aided by cobalt chloride and a small hydride donor (BH_3·THF) in quantitative yield.[28] Alkylation of the 6-OH using 1-azido-8-iodooctane, prepared in two steps from commercially available 8-chloro-octanol,[14] and NaH afforded azide **16** in 80% yield. Subsequent hydrolysis of the thiophenol aglycon under the aegis of trichloroisocyanuric acid (TCCA) in aqueous acetone afforded construct **17** as a mixture of α/β anomers in 74% yield.

Treatment of compound **17** with 2,2,2-trifluoro-*N*-phenylacetimido-yl chloride in aqueous acetone and aided by a mild base (Cs_2CO_3) yielded donor **18** in near-quantitative yield. In parallel, compound **13** was regioselectively equipped with an azide spacer *via* an one-pot two-step procedure. Here, installation of a tin ketal over the 2- and 3-position provided the necessary regioselectivity to yield compound **19** upon exposure to 1-azido-8-iodooctane and cesium fluoride in 61% yield. Subsequent protection of the 2-OH as a benzyl ether under standard Williamson etherification conditions (BnBr, NaH) afforded donor **20** in 81% yield.[29]

Scheme 1. Preparation of donor glucosides **18** and **20** and proposed construction of nigerase probes **1** – **4**.

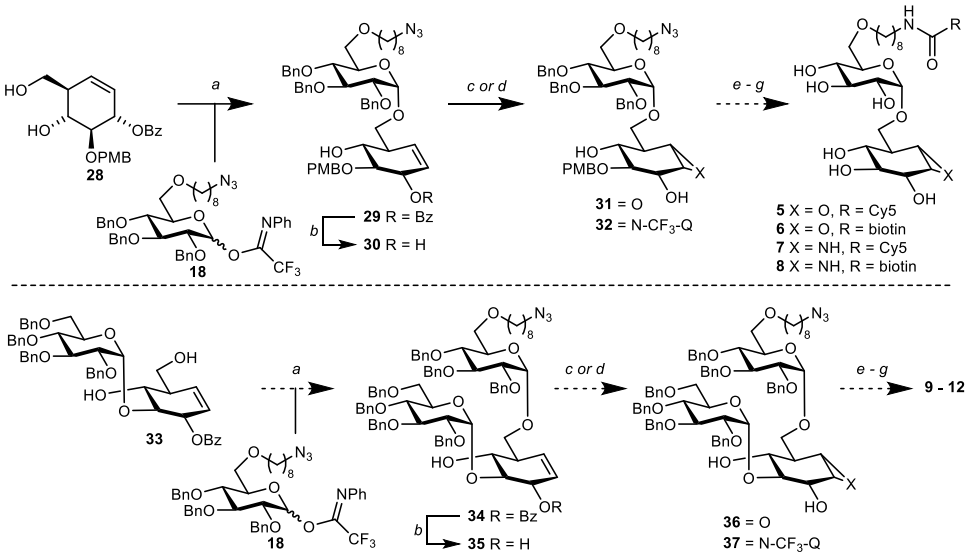


Reagents and conditions: a) BnBr, NaH, DMF, rt, 16 h, **14** (83%), **20** (81%); *b)* $CoCl_2$, BH_3·THF, rt, 15 min (quant.); *c)* 1-azido-8-iodooctane, NaH, DMF, rt, 16 h (80%); *d)* TCCA, acetone:H_2O (4:1 v:v), rt, 2 h (74%); *e)* 2,2,2-trifluoro-*N*-phenylacetimido-yl chloride, Cs_2CO_3, acetone:H_2O (50:1 v:v), rt, 16 h (96%); *f) i.* $(n\text{-Bu})_2SnO$, toluene, reflux 4 h, *ii.* 1-azido-8-iodooctane, CsF, DMF, rt, 3 days (61%); *g)* TTBP, Ph_2SO, donor **20**, 3Å molecular rods, DCM, -78 °C, *then* **21**, 2 h, -78 °C to -10 °C (57%); *h)* TBAF, THF, 1 h, rt; *i)* NaOMe, DCM, MeOH, 16 h, rt (77% over two steps); *j) m*-CPBA, $NaHCO_3$, DCM, 16 h, rt, **24** (96%); *k)* BAIB, CF_3-Q-NH_2, DCM, 48 h, -40 °C to rt; *l)* PtO_2, H_2, THF, 4h, rt; *m)* Na, *t*-BuOH, NH_3, 2 h, -60 °C, **26** (96%); *n)* Cy5-COOH *or* biotin-COOH, COMU, DIPEA, DMF, 4 h, rt.

Initial glycosylation experiments between donor **20** and acceptor **21** resulted in clean conversion to compound **22** with the 1,2-*cis*-linked product as the only observable isomer formed. Liberation of the 2- and 4-OH, aided by TBAF and subsequent treatment with methanolic NaOMe, yielded construct **23** in 77% yield over two steps. Construct **23**

could be used to produce, in parallel fashion and aided by neighbouring group participation of the 2-OH, both epoxide **24** and aziridine **25**. The former was synthesized by subjecting alkene **23** to *m*-CPBA, affording solely epoxide **24** in near-quantitative yields. An azide reduction catalysed by PtO_2 under H_2 atmosphere followed by a global deprotection under Birch condition[30] afforded disaccharide **26** in near-quantitative yield. All that remains to do comprises condensation with either Cy5-COOH or biotin-COOH utilizing a suitable coupling reagent[31] to afford probes **1** and **2**. In addition, aziridine formation (compound **25**) aided by CF_3-Q-NH_2 and BAIB,[32] followed by identical transformations as for the epoxide probes, the construction of the aziridine probes **3** and **4** should be feasible.

Scheme 2. Proposed construction of pullulanase probes **5** – **8** and dextranase probes **9** – **12**.



Reagents and conditions: a) donor **18**, PPh_3O, TMSI, 3Å molecular rods, DCM, 48 h, rt, **29** (68%); *b)* NaOMe, MeOH, DCM, 16 h, rt, **30** (86%); *c) m*-CPBA, $NaHCO_3$, DCM, 48 h, 5 °C, **31** (86%); *d)* BAIB, CF_3-Q-NH_2, DCM, 48 h, -40 °C to rt; *e)* PtO_2, H_2, THF, 4 h, rt; *f)* Na, *t*-BuOH, NH_3, 2 h, -60 °C; *g)* Cy5-COOH *or* biotin-COOH, COMU, DIPEA, DMF, 4 h, rt.

In line with the synthetic methodology described in chapter 2, a synthesis of pullulanase probes is proposed in scheme 2. Initial studies to regio- and stereoselectively couple imidate donor **18** with acceptor **28**, modulated by PPh_3O and TMSI, yielded disaccharide **29** in 68% yield. Hydrolysis of the benzoyl protecting group of the 2-position, followed by a stereoselective epoxidation afforded epoxide **31** in 86% yield. In a divergent manner, compound **31** is expected to undergo stereoselective aziridination with CF_3-Q-NH_2 and BAIB. A PtO_2 catalysed azide reduction followed by global deprotection would then result, after coupling to either Cy5-COOH or biotin-COOH, in epoxide and aziridine

probes **5** – **8**. Putative dextranase probes are in turn considered accessible when disaccharide **33** is used as acceptor in the PPh_3O/TMSI modulated glycosylation with imidate donor **18**. Following identical transformations as for the pullulanase probes, the construction of the dextranase probes **9** – **12** should be feasible.

3 | **Chapter 3** describes a study exploring the use of 1,2- and 1,7-cyclophellitols as potential α-glucosidase inhibitors. To this end, a series of twenty configurational and functional cyclophellitol analogues, featuring a systematic array of electrophiles were synthesized and studied in *in vitro* assays for their inhibitory potencies against human acid α-glucosidase (GAA) and ER α-glucosidase II (ER-II). Subsequently, the conformational free energy landscapes of the most active compounds were mapped. Although no potent inhibitors were found, low micromolar affinity was observed for some of the cyclophellitols. A systematic shift in lowest energy conformation of the 1,2-cyclophellitols in contrast to their 1,7-counterparts was observed during metadynamic simulations. As a result of this shift, the conformation does not resemble that of either the Michaelis complex or transition state during hydrolysis. This conformational shift may explain the overall reduction in observed inhibitory potency of the 1,2-cyclophellitols in comparison to their 1,7-counterparts.

In order to further study the effect of conformational change of the inhibitors on the binding in the enzyme, it would be of interest to obtain crystal structures of the most prominent 1,2-cyclitols. Covalent binding within the active site, combined with the binding interactions within the enzyme active site could shed light on the binding mode of the 1,2-cyclitols. This may allow for the identification of novel binding interactions which can be exploited in future inhibitor designs.

4 | Eight exocyclic aziridine cyclitols were synthesized in **Chapter 4**, envisioned to be selective deactivators of inverting α- and β-glycosidases, enzymes for which no mechanism-based, covalent and irreversible inhibitors exist to date. It was hypothesized that by transpositioning the electrophilic site from the anomeric center to a more distal position through the appendage of an exocyclic aziridine, covalent bond formation could be evoked with the more distal nucleophilic acid/base residue that characterizes inverting GH active site pockets when compared to retaining ones. The key step in the synthesis route employed a divergent *aza*-Michael initiated ring closure reaction (*aza*-MIRC) between unprotected validamine or 1-*epi*-validamine and a small series of dibromide coupling partners bearing a diverse selection of electron withdrawing functionalities. In this fashion, all eight foreseen deactivators were obtained in excellent yield proving the mildness and robustness of the aziridine forming reactions on complex, unprotected substrates.

It is hypothesized that the developed methodology can be easily extended to various substrates bearing primary amines. An interesting substrate for this would be valiolamine (**38**, Figure 2) which is a potent, competitive inhibitor of ER-I and ER-II α-glucosidases with an IC_{50} value of 12 µM for both glucosidases.[33] In addition, *N*-substitution of valiolamine is widely accepted by the glucosidases as nanomolar potencies have been reported.[34–37] This has even resulted in the admission of the α-glucosidase inhibitor Voglibose (**39**, Figure 2), as a drug to treat diabetes mellitus type 2.[38–40] *Via* the described *aza*-MIRC reaction, employing a series of dibromides, valiolamine could be converted in a divergent manner to a series of putative irreversible inhibitors of ER-I (**40** – **43**, Figure 2).

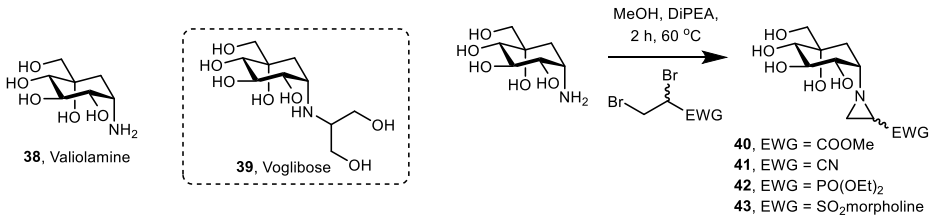
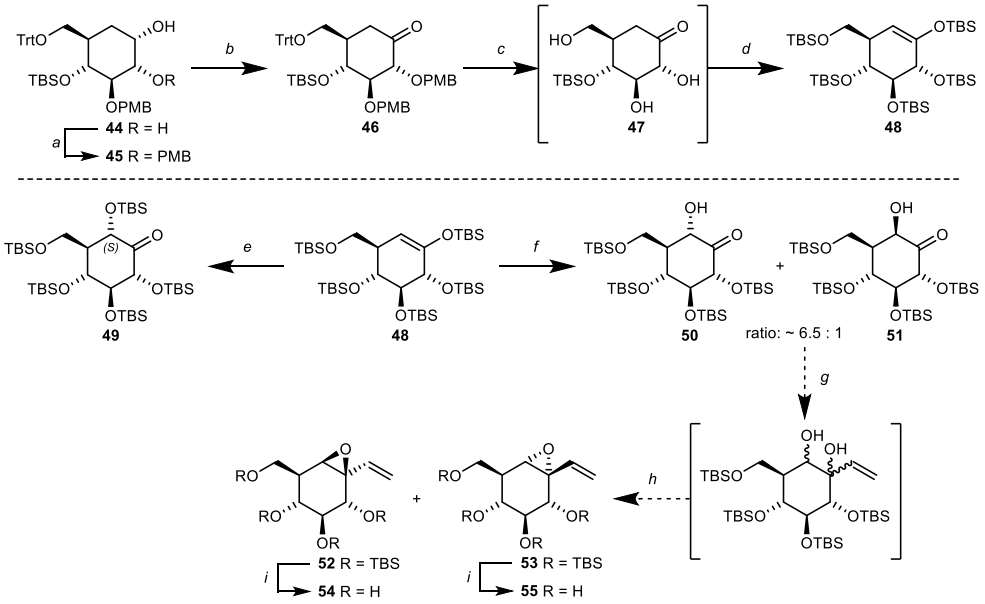


Figure 2. Valiolamine **38**, a potent, reversible inhibitor of ER-I and ER-II, and Voglibose **39**, an α-glucosidase inhibitor used as a drug to treat diabetes mellitus type 2. Proposed putative covalent inhibitors **40** – **43** of ER-I bearing the valiolamine backbone accessible *via* the in chapter 5 described *aza*-MIRC reaction.

5 | As an extension of the studies towards putative inhibitors of inverting α- and β-glucosidases subject of Chapter 4, **Chapter 5** describes the synthesis of a small series of compounds bearing an anomeric vinyl moiety in addition to a range of electrophilic warheads spanning the 1,2-position. It was hypothesized that *via* a conjugated addition the terminal side of the alkene could, due its size and distance from the anomeric center, be attacked by the distal acid/base residue in the enzyme active site, thereby effectively incapacitating the enzyme. The constructs were synthesized in a divergent manner from carbaglucose derivatives that were synthesized as described in Chapter 3. Subjection of the validone analogues with vinyl Grignard reagent neatly provided the vinyl adducts. Warhead installation proceeded smoothly as mesylation or carbonylation of diol intermediates provided the vinyl-epoxides or -carbonates. A Staudinger induced ring closure on the azido-alcohol allowed for isolation of the desired vinyl-aziridine. Due to the intrinsic reactivity of the proposed warheads, and the consequent synthetic challenges that arose during the global deprotection step, the use of silyl protection groups proved crucial allowing the use of a mild fluoride based deprotection step.

To further capitalize on the developed synthetic methodology and inhibitor design, it was hypothesized that transposition of the warhead to the 1,7-position will yield putative potential inhibitors bearing a conjugate addition warhead while closely resembling cyclophellitol. To this end, carbaglucose **44**, previously synthesized in Chapter 2, was transformed in two steps into protected validone **46** (Scheme 3). Here, tin-ketal chemistry provided the necessary regioselectivity during PMB protection of the 2-OH after which a Dess-Martin oxidation yielded construct **46** in quantitative yield.[41] Acid treatment (TFA, TES) removed all acid labile protecting groups to provide partially protected validone **47**. This intermediate was directly subjected to strong silylating conditions (TBSOTf, 2,6-lutidine). Here, not only protection of the primary and secondary hydroxyls was achieved, but formation of the silyl enolate was observed. The regioselectivity was remarkable, as the desired 1,7-silylenolate was the only observed product, yielding **48** in 88% yield. The regioselectivity could be explained by the better accessibility of H-7 by the sterically hindered base, resulting in the formation of the kinetic 1,7-enolate.[42] Subsequent oxidation of the enolate towards the α-hydroxy ketone was attempted. To this end, enolate **48** was subjected to a range of epoxidation conditions (*m*-CPBA, oxone, DMDO), all of which resulted in the formation of α-hydroxy ketone **49** as the single diastereomer. Here, migration of the silyl moiety towards the newly introduced hydroxyl was observed. This would prevent the foreseen orthogonal functionalization of the 7-OH. Migration of the silyl functionality could be circumvented by subjecting silylenolate **48** to dihydroxylating conditions (OsO_4, NMO), providing a diastereomeric mixture of the α-hydroxy ketones **50** and **51** as an inseparable mixture. It was foreseen that subsequent exposure of the α-hydroxy ketones **50** and **51** to the vinyl Grignard conditions described in Chapter 5 would provide an anomeric mixture of the vinyl adducts.

Scheme 3. Proposed synthetic scheme for the preparation of putative inverting α- and β-glucosidase inhibitors.



Reagents and conditions: a) i. (n-Bu)$_2$SnO, toluene, 4 h, reflux; *ii.* PMBCl, CsF, DMF, 16 h, rt (33%); *b)* DMP, NaHCO$_3$, DCM, 2 h, rt (quant.); *c)* TFA, TES, DCM, 3.5 h, 0 °C (93%); *d)* TBSOTf, 2,6-lutidine, DCM, 16 h, rt (88%); *e)* *m*-CPBA, DCM, 2 h, 0 °C (53%); *f)* OsO$_4$, NMO, acetone, 20 h, 75 °C (50%, 79% brsm); *g)* vinyl Grignard, THF, 20 h, -78 °C → 0 °C; *h)* MsCl, Et$_3$N, DCM, 1 h, 0 °C; *i)* TBAF, THF, 2 h, rt.

In a convergent manner, a subsequent intramolecular substitution aided by MsCl would provide a separable mixture of both the α- and β- epoxide, **52** or **53**. Subsequent removal of the silyl protecting groups would yield target structures **54** and **55**.

6 | **Chapter 6** describes the design and synthesis of a series of eight conformational donor mimetics of glucosyl and galactosyl transferases. The design of these structures is based on crystallographic data, suggesting the UDP-glucose or UDP-galactose donor substrates to adopt a concave orientation upon formation of a Michaelis complex with some glucosyl- or galactosyl transferases. Key to the design is the conformational restriction induced by a cyclic carbamate or -sulfamidate functionality, bridging the C1 and C2 position. In addition, the amide is further functionalized with a uridine 5'-monophosphate moiety. Amongst the key transformations are a Sharpless aminohydroxylation, which appeared completely stereoselective on protected carba-D-glucal and carba-D-galactal and yielded almost equimolar quantities of both α-cis-aminohydroxylated regio-isomers in excellent yield. In a divergent and parallel manner, the regio-isomers were transformed to both the cyclic carbamates and sulfamidates. In the next step, Atherton-Todd *N*-phosphorylation

allowed for the efficient condensation of the cyclic carbamate or sulfamidate with a suitably protected H-phosphonate diester. Finally, and using a mild and efficient two-step one-pot deprotection method, all eight complex target structures were successfully deprotected and isolated.

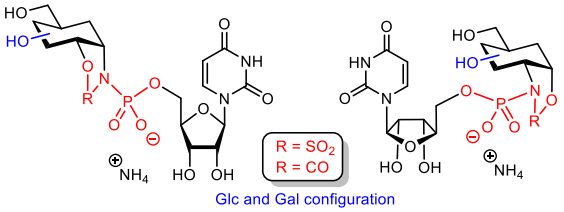
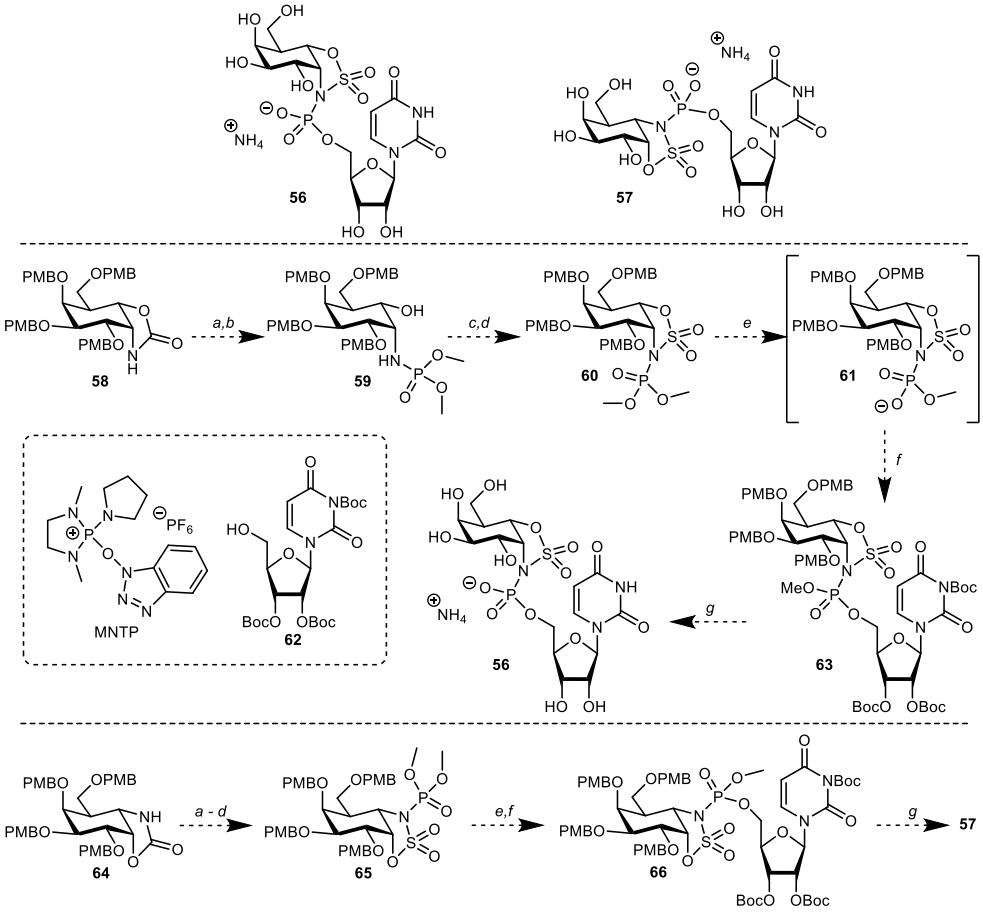


Figure 3. Library of eight complex glucosyl and galactosyl transferase donor mimetics, the synthesis of which is described in Chapter 6.

7 | To further expand on the 1,2-carbamate and sulfamidate inhibitors described in Chapter 6, **Chapter 7** describes synthetic studies towards the corresponding regio-isomers in which the cyclic carbamate or sulfamidate occupies the 1,7-position. It was hypothesized that, by translocating the bicycle over the carba-backbone, a larger chemical space can be probed. In contrast to what was observed in Chapter 6, Sharpless aminohydroxylation proved abortive when attempted on cyclophellitol cyclohexenes. Therefore, somewhat lengthy literature procedures were followed to gain access to the cyclic carbamate building blocks. The use of a TIPDS protecting group on the 4- and 6-position of the carbaglucose analogues proved key for subsequent productive execution of both the Atherton-Todd reaction and the global deprotection sequence, allowing for the isolation of the first target structure. In parallel, both regio-isomers of the carbagalactose carbamate were synthesized following the aforementioned procedures. Atherton-Todd phosphorylation of the first cyclic carbamate and a subsequent global deprotection yielded the first carbagalactose target structure. Atherton-Todd phosphorylation attempts with the second carbamate, as well as with the sulfamidate analogue, proved unsuccessful. Therefore, new routes towards the remaining carbagalactose-configured cyclic carbamate and cyclic sulfamidate constructs are required.

Scheme 4. Proposed synthesis of carbagalactose cyclic sulfamidate constructs **56** and **57**.



Reagents and conditions: a) NaOH, EtOH:H_2O (4:1 v:v), 70 °C, 2 h; *b)* dimethyl phosphite, $BrCCl_3$, DiPEA, DCM, 0 °C, 15 min; *c)* $SOCl_2$, -40 °C → 0 °C, 16 h; *d)* $RuCl_3$, $NaIO_4$, EtOAc, CH_3CN, H_2O, 0 °C, 20 min; *e)* Et_3N, CH_3CN, 70 °C, 2 h; *f)* MNTP, 2,6-lutidine, CH_3CN, rt, 2 h; *g)* TFA (30% v:v), TES, DCM, rt, 24 h *then* pyridine, 35 °C, 24 h.

Taken all developed methodology and the identified pitfalls into account, an alternative synthesis route towards carbagalactose cyclic sulfamidate construct **56** and **57** is proposed (Scheme 4). As previously observed in Chapter 7, hydrolysis of carbamate **58** under alkaline conditions produces an amino-alcohol that can be easily converted into the corresponding phosphoramidate through an Atherton-Todd phosphorylation.

It is postulated that dimethyl phosphite would be a suitable coupling partner, yielding the dimethyl phosphoramidate intermediate **59**.[43] Following procedures as described in chapter 7, treatment of crude **59** with thionyl chloride would provide the corresponding cyclic sulfamidite. Here, an $RuCl_3/NaIO_4$ oxidation is expected to result in

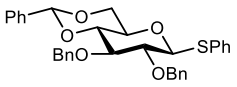
the formation of cyclic sulfamidate **60**.[44,45] After the S(IV) oxidation, the uridine moiety is envisioned to be installed *via* hydrolysis of a single phosphoramidate methyl ester.[46] A subsequent coupling using a phosphonium-type condensing agent (for instance, MNTP)[47] allows for coupling with the primary hydroxyl of protected uridine **62**, previously synthesized in Chapter 6, to provide sulfamidate **63**. The one-pot two-step global deprotection procedure (TFA, TES *then* pyridine), optimized in Chapter 6, is expected to remove all acid labile protecting groups (PMB and Boc) allowing the subsequent nucleophilic removal of the final phosphoramidate methyl ester to yield *N*-UMP-1'',7''-(*N,O*)-sulfamidate carba-α-D-galactopyranoside **56**. Following identical transformations, the synthesis of the 1,7-regioisomer could be feasible, starting from cyclic carbamate **64**, to yield *N*-UMP-1'',7''-(*O,N*)-sulfamidate carba-α-D-galactopyranoside **57**.

Acknowledgements

I extend my acknowledgments to Florian Küllmer for our joint efforts in the partial synthesis of di- and trisaccharide probes, as well as in developing conjugate addition-type inverting glycosidase inhibitors. Our invaluable discussions greatly contributed to these endeavours. I also wish to express gratitude to Madouc Bergers for her synthetic work during her BSc internship.

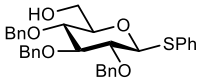
Synthetic procedures.

Phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranose (14).



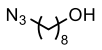
Compound **13** (1.1 g, 3.0 mmol) was dissolved in anhydrous DMF (10 mL, 0.3 M) and cooled on ice. Subsequently, benzyl bromide (1.1 mL, 9.0 mmol, 3.0 eq.) and NaH (60 wt% in mineral oil, 0.36 g, 9.0 mmol, 3.0 eq.) were added respectively. The reaction was stirred overnight while allowed to attain to room temperature. Upon full conversion was observed (R_f 0.6 (EtOAc:pentane, 1:9, v:v)), the reaction was cooled on ice and quenched with water and subsequently diluted further with Et_2O. The aqueous layer was extracted with Et_2O (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (10:90 Et_2O:pentane → 30:70 Et_2O:pentane) yielded the donor **14** as a white solid (1.35 g, 2.5 mmol, 83%). Analytical data was determined to be in full agreement with literature data.[27]

Phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranose (15).



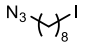
Compound **14** (0.54 g, 1.0 mmol) was combined with $CoCl_2$ (0.39 g, 3.0 mmol, 3.0 eq.) prior to the addition of BH_3·THF (1.0 M, 6.0 mL, 6.0 mmol, 6.0 eq.) and the reaction mixture was stirred for 15 minutes upon TLC confirmed full conversion (R_f 0.2 (Et_2O:pentane, 2:8, v:v)). The mixture was diluted with sat. aq. $NaHCO_3$ and the aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (10:90 Et_2O:pentane → 30:70 Et_2O:pentane) yielded compound **15** (0.55 g, 1.0 mmol, quant.). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.62 – 7.16 (m, 20H, CH_{arom}), 4.98 – 4.86 (m, 4H, C*H*H Bn, C*H*H Bn, CH*H* Bn, H-1), 4.78 (m, 4H, C*H*H Bn, CH*H* Bn), 4.69 (d, *J* = 11.0 Hz, 1H, CH*H* Bn), 3.91 (ddd, *J* = 12.1, 6.1, 2.6 Hz, 1H, H-6), 3.80 – 3.69 (m, 2H, H-3, H-6), 3.62 (dd, *J* = 9.3, 9.3 Hz, 1H, H-4), 3.53 (dd, *J* = 10.1, 8.7 Hz, 1H, H-2), 3.43 (ddd, *J* = 9.7, 4.8, 2.0 Hz, 1H, H-5), 2.00 (s, 1H, 6-OH); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 138.4, 138.0, 137.9, 133.6, 131.9 ($C_{q\text{-}arom}$), 129.2, 128.6, 128.6, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.8 (CH_{arom}), 87.7 (C-1), 86.7 (C-3), 81.2 (C-2), 79.4 (C-5), 77.7 (C-4), 75.9, 75.7, 75.2 (CH_2Bn), 62.2 (C-6); HRMS (ESI) m/z: [M+Na]+ Calcd for $C_{33}H_{34}NaO_5S$ 565.2025; found 565.2036.

8-Azido-octanol.



8-Chlorooctanol (24 g 148 mmol) was dissolved in DMSO (37 mL, 4.0 M). NaN_3 (14 g, 222 mmol, 1.5 eq.) was added and the reaction mixture was stirred for 17 hours at 80 °C. After TLC-MS confirmed full conversion, the reaction was diluted with EtOAc and H_2O. The organic layer was washed with water ten times and subsequently dried over $MgSO_4$, filtered and concentrated. Yielding 8-azido-octanol as an colourless oil and was used in the next step without further purification (26 g, 150 mmol quant.). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 3.66 – 3.55 (m, 2H, C*H*$_2$OH), 3.26 (m, 3H, CH_2N_3, OH), 1.67 – 1.47 (m, 4H, spacer), 1.43 – 1.30 (m, 8H, spacer); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): *δ* 62.7 (CH_2OH), 51.4 (CH_2N_3), 32.6, 29.2, 29.1, 28.8, 26.6, 25.6.

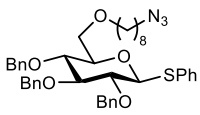
1-Azido-8-iodooctane.



8-azido-octanol (8.5 g, 50 mmol) was dissolved in DCM (150 mL, 0.3 M) and Et_3N (11 mL, 79 mmol, 1.6 eq.) was added. The mixture was cooled on ice and MsCl (5.7 mL, 74 mmol, 1.5 eq.) was added dropwise to the cooled reaction mixture. The reaction mixture was stirred for 3 hours, while allowing to attain room temperature. TLC-MS confirmed full conversion (R_f 0.3 (MeOH:DCM, 1:99, v:v). The reaction mixture was quenched with H_2O and further diluted with DCM. The mixture was washed three times with 1 M HCl followed by aqueous saturated $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure.

The crude intermediate was then diluted in anhydrous DMF (300 mL, 0.2 M) and KI (12 g, 74 mmol, 1.5 eq.) was added. The reaction was stirred for 10 hours at 70 °C. Upon full conversion was observed (R_f 0.3 (Pentane)), the reaction mixture was concentrated to a fifth of its original volume and diluted with H_2O. The aqueous layer was extracted three times with Et_2O. The organic layer was washed twice with aq. sat. $Na_2S_2O_3$ and subsequently brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography (pentane). Obtaining 1-azido-8-iodooctane as a pale-yellow oil (7.4 g, 26 mmol, 53% over two steps).

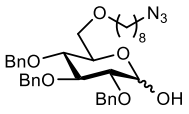
Phenyl 2,3,4-tri-*O*-benzyl-6-*O*-(8-azido-octane)-1-thio-β-D-glucopyranose (16).



Compound **15** (0.54 g, 1.0 mmol) was dissolved in anhydrous DMF (10 mL, 0.1 M) and cooled on ice. 1-azido-8-iodooctane (0.42 g, 1.5 mmol, 1.5 eq.) and NaH (60 wt% in mineral oil, 80 mg, 2.0 mmol, 2.0 eq.) were added respectively. The reaction was stirred overnight while allowed to attain to room temperature. Upon full conversion was observed (R_f 0.7 (Et_2O:pentane, 2:8, v:v)), the reaction was diluted with Et_2O and washed with sat. aq. NH_4Cl. The aqueous layer was extracted with Et_2O (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (10:90 Et_2O:pentane → 30:70 Et_2O:pentane) yielded the donor **16** as a colourless oil (0.55 g, 0.8 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.66 – 7.22 (m, 20H, CH_{arom}), 4.98 – 4.83 (m, 4H, C*H*H Bn, C*H*H Bn, CH*H* Bn, H-1), 4.76 (d, *J* = 10.2 Hz, 1H, CH*H* Bn), 4.70 – 4.65 (m, 2H, C*H*H Bn, CH*H* Bn), 3.77 – 3.63 (m, 4H, H-3, H-4, H-6), 3.56 – 3.41 (m, 4H, H-2, H-5, CH_2O spacer), 3.26 (t, *J* = 7.0 Hz, 2H, CH_2N_3 spacer), 1.67 – 1.53 (m, 4H, spacer), 1.44 – 1.29 (m, 8H, spacer); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 138.5, 138.3, 138.1, 134.1, 131.9 ($C_{q\text{-}arom}$), 128.9, 128.6, 128.5, 128.3, 128.0, 127.9, 127.8, 127.4 (CH_{arom}),

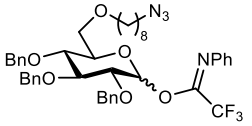
87.6 (C-1), 86.8 (C-3), 80.9 (C-2), 79.2 (C-5), 78.0 (C-4), 76.0, 75.5, 75.1 (CH_2Bn), 71.7 (CH_2O spacer), 69.7 (C-6), 51.5 (CH_2N_3 spacer), 29.9, 29.5, 29.2, 28.9, 26.8, 26.2 (spacer); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{41}H_{49}N_3NaO_5S$ 718.3291; Found 718.3305.

2,3,4-Tri-*O*-benzyl-6-*O*-(8-azido-octane)-α/β-D-glucopyranose (17).



Compound **16** (0.55 g, 0.8 mmol) was dissolved in a mixture of acetone and water (4:1, 10 mL, 0.08M) after which TCCA (0.19 g, 0.8 mmol, 1.0 eq.) was added. The reaction mixture was stirred for 2 hours at room temperature after which TLC confirmed full consumption of the starting material (R_f 0.5 (Et_2O:pentane, 1:1, v:v)). The mixture was diluted with sat. aq. $Na_2S_2O_3$ and Et_2O, the aqueous layer was extracted three times with Et_2O. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (40:60 Et_2O:pentane → 50:50 Et_2O:pentane) yielded compound **17** as an anomeric mixture in a 1:1 ratio (0.36 g, 0.59 mmol, 74%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.44 – 7.06 (m, 30H, CH_{arom}), 5.24 (dd, *J* = 3.1, 3.1 Hz, 1H, H-1β), 4.99 – 4.61 (m, 13H, CH_2Bn, CH_2Bn, CH_2Bn, CH_2Bn, CH_2Bn, CH_2Bn, H-1α), 4.07 – 3.94 (m, 2H, H-3α, H-3β), 3.72 – 3.56 (m, 6H, H-2α/β, H-4α/β, H-6α, H-6β), 3.55 – 3.47 (m, 6H, 1-OHα, H-4α/β, CH_2N_3 spacer), 3.45 – 3.35 (m, 4H, H-2α/β, H-5α, H-5β), 3.25 (m, 4H, CH_2O spacer), 3.11 – 3.00 (m, 1H, 1-OHβ), 1.68 – 1.52 (m, 8H, spacer), 1.40 – 1.26 (m, 16H, spacer); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 138.8, 138.6, 138.5, 138.4, 138.3, 137.9 ($C_{q\text{-}arom}$), 128.6, 128.6, 128.5, 128.5, 128.5, 128.2, 128.2, 128.1, 128.1, 127.9, 127.9, 127.8, 127.7 (CH_{arom}), 97.6 (C-1α), 91.4 (C-1β), 84.7, 83.3 (C-3), 81.9, 80.1 (C-2) , 77.9, 77.8 (C-5), 75.9, 75.8 (C-4), 74.8, 73.0, 71.9, 71.8 (CH_2Bn), 70.8 (CH_2O spacer), 69.7, 68.9 (C-6), 51.5 (CH_2N_3 spacer), 29.6, 29.4, 29.2, 28.9, 27.4, 26.1 (spacer); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{35}H_{45}N_3NaO_6$ 626.3206; Found 626.3212.

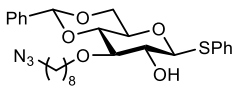
2,2,2-Trifluoro-*N*-phenylacetimido-yl 2,3,4-tri-*O*-benzyl-6-*O*-(8-azido-octane)-α/β-D-glucopyranose (18).



Compound **17** (0.36 g, 0.59 mmol) was dissolved in a mixture of acetone and water (50:1, 6.0 mL, 0.1 M) and cooled on ice. Subsequently, Cs_2CO_3 (0.31 g, 0.95 mmol, 1.6 eq.) and 2,2,2-Trifluoro-*N*-phenylacetimido-yl chloride (0.20 g, 0.95 mmol, 1.6 eq.) were added respectively. The reaction was stirred overnight while allowed to attain to room temperature. Upon full conversion was observed (R_f 0.2 (Et_2O:pentane, 1:9, v:v)), the reaction was diluted with Et_2O and washed with sat. aq. NH_4Cl. The aqueous layer was extracted with Et_2O (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography (10:90 Et_2O:pentane → 20:80 Et_2O:pentane) yielded the donor **18** as an anomeric mixture in a 1:1 ratio (0.44 g, 0.56 mmol, 96%). ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.41 – 7.23 (m, 40H, CH_{arom}), 6.54 (bs, 1H, H-1α/β), 5.66 (bs, 1H, H-1α/β), 5.04 – 4.61 (m, 12H, CH_2Bn, CH_2Bn, CH_2Bn, CH_2Bn, CH_2Bn, CH_2Bn), 4.06– 3.62 (m, 12H, H-2α, H-3α, H-4α, H-5α, H-6α, H-2β, H-3β, H-4β, H-5β, H-6β), 3.54 (m, 2H, CH_2O spacer), 3.47 – 3.36 (m, 2H, CH_2O spacer), 3.25 (m, 4H, CH_2N_3 spacer), 1.67 – 1.52 (m, 8H, spacer), 1.35 (m, 16H, spacer); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): ^{13}C NMR (126 MHz, $CDCl_3$) δ 143.8, 143.6 (C=N), 138.7, 138.5, 138.3, 137.9, 137.8 ($C_{q\text{-}arom}$), 128.8, 128.6, 128.5, 128.5, 128.3, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7

(CH_{arom}), 81.6, 81.0, 79.4, 77.4, 76.9 (C-2, C-3, C-4, C-5), 75.9, 75.4, 75.3, 73.4, 73.2 (CH_2Bn), 71.8, 71.8 (CH_2O spacer), 69.5, 68.9 (C-6), 51.5 (CH_2N_3 spacer), 29.8, 29.7, 29.4, 29.2, 28.9, 26.8, 26.2 (spacer); HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{43}H_{49}F_3N_4NaO_6$ 797.3502; Found 797.3514.

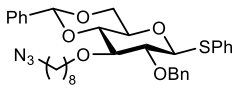
Phenyl 3-*O*-(8-azido-octane)-4,6-*O*-benzylidene-1-thio-β-D-glucopyranose (19).



Compound **13** (18 mmol, 6.4 g) was dissolved in anhydrous toluene (0.1 M, 170 mL) and Bu_2SnO (5.3 g, 21 mmol, 1.2 eq.) was added. The reaction mixture refluxed for 4 hours, equipped with a Dean-Stark apparatus, after which the residual solvent was removed under reduced pressure.

1-azido-8-iodooctane (6.0 g, 21 mmol, 1.2 eq.) was added to the crude intermediate and co-evaporated three times with toluene. The mixture was dissolved in anhydrous DMF (50 mL, 0.4 M) and CsF (27 mmol, 4.1 g, 1.5 eq.) was added. The reaction mixture was stirred for 72 hours at room temperature. While there was still starting material present (R_f 0.3 (EtOAc:pentane, 1:9, v:v)) the reaction mixture was diluted with CH_2Cl_2 and sat. aq. $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 twice and the combined organic layers were washed with brine (400 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography (5:95 Et_2O:pentane → 50:50 Et_2O:pentane) to obtain the title compound as an off-white solid (5.5 g, 11 mmol, 61% over two steps). ^1H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.61 – 7.41 (m, 4H, CH_{arom}), 7.41 – 7.27 (m, 6H, CH_{arom}), 5.54 (s, 1H, CHPh), 4.64 (d, J = 9.1 Hz, 1H, H-1), 4.38 (dd, J = 10.4, 4.3 Hz, 1H, H-4), 3.94 – 3.60 (m, 3H, H-2, H-6), 3.60 – 3.39 (m, 4H, spacer, H-2, H-5), 3.22 (t, J = 6.9 Hz, 2H, N_3-CH_2 spacer), 2.61 (d, J = 1.9 Hz, 1H, 2-OH), 1.57 (m, 4H, spacer), 1.35 (m, 2H, spacer), 1.27 (m, 6H, spacer). HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{27}H_{35}N_3NaO_5S$ 536.2195; Found 536.2208.

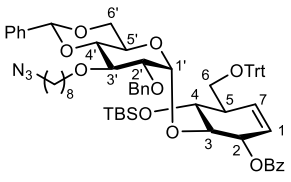
Phenyl 2-*O*-benzyl-3-*O*-(8-azido-octane)-4,6-*O*-benzylidene-1-thio-β-D-glucopyranose (20).



NaH (60 wt% in mineral oil, 0.3 g, 6.1 mmol, 1.8 eq.) was dissolved in anhydrous DMF (17 mL, 0.2 M) under a nitrogen atmosphere and cooled on ice. Subsequently, compound **19** (1.8 g, 3.5 mmol) was dissolved in anhydrous DMF (9.0 mL) and added dropwise to the NaH suspension. The reaction mixture was stirred for 10 min, after which benzyl bromide (0.6 mL, 5.3 mmol, 1.5 eq.) was added dropwise. The reaction was stirred for 2 hours while attaining to room temperature. Upon full conversion (R_f 0.6 (EtOAc:pentane, 1:9, v:v)), the reaction was again cooled to 0 °C and quenched by the addition of MeOH. The solvent was removed under reduced pressure and followed by diluting the reaction mixture with Et_2O and ice-cold water. The solution was transferred to a separation funnel and was further diluted with H_2O and Et_2O. The aqueous layer was extracted with Et_2O twice, and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was purified using column chromatography (1:99 Et_2O:pentane → 15:85 Et_2O:pentane) and compound **20** was obtained as a pale-yellow oil (1.7 g, 2.8 mmol, 81%). ^1H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.56 – 7.27 (m, 15H, CH_{arom}), 5.55 (s, 1H, CHPh), 4.91 – 4.78 (m, 2H, CH_2 Bn), 4.73 (d, J = 9.8 Hz, 1H, H-1), 4.37 (dd, J = 10.5, 5.0 Hz, 1H, H-4), 3.94 – 3.79 (m, 1H, H-2), 3.79 – 3.69 (m, 2H, H-6), 3.69 – 3.53 (m, 2H, H-1, H-5), 3.53 – 3.37 (m, 2H, spacer), 3.21 (t, J = 6.9 Hz, 2H, N_3-CH_2 spacer), 1.56 (m, 4H, spacer), 1.25 (m, 8H, spacer); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 132.3, 129.0, 128.9 ($C_{q\text{-}arom}$), 128.4, 128.2, 128.1, 127.9, 127.8, 126.0

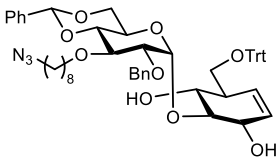
(CH_{arom}), 101.1 (C-7), 88.3 (C-1), 83.3 (C-3), 81.3 (C-2), 80.6 (C-3), 75.8 (CH_2Bn), 73.5 (C-5), 70.3 (C-6), 68.7 (C-4), 51.4, 30.3, 29.3, 29.1, 26.7, 26.1 (spacer). HRMS (ESI) m/z: $[M+Na]^+$ Calcd for $C_{34}H_{41}N_3NaO_5S$ 626.2665; Found 626.2678.

2-*O*-Benzoyl-3-*O*-(2-*O*-benzyl-3-*O*-(8-azido-octane)-4,6-*O*-benzylidene-α-D-glucopyranose)-4-*O*-*tert*-butyldimethylsilyl-6-*O*-trityl-cyclophellitol alkene (22).



To compound **20** (0.54 g, 0.89 mmol, 2.0 eq.) was added TTBP (0.87 g, 3.3 mmol, 7.5 eq.) and Ph_2SO (0.23 g, 1.2 mmol, 2.6 eq.) and co-evaporated three times with toluene. The mixture was dissolved in anhydrous DCM (9.0 mL, 0.05 M) and 3 Å molecular rods were added under argon atmosphere. The reaction mixture was cooled down to -78 °C and Tf_2O (0.16 mL, 0.97 mmol, 2.2 eq.) was added. The reaction mixture was warmed-up to -60 °C and was stirred for 15 minutes. Subsequently, the solution was cooled back to -78 °C, and compound **21** (0.28 g 0.44 mmol), dissolved in anhydrous DCM (1.0 mL) was added dropwise. The mixture was stirred for 2 hours, while attaining to -10 °C, after which full conversion (R_f 0.3 (Et_2O:pentane, 2:8, v:v)) was observed. The reaction was quenched by adding sat. aq. $NaHCO_3$ and the mixture were diluted with H_2O and Et_2O. The aqueous layer was extracted twice with Et_2O. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The reaction was purified using flash column chromatography (10:90 Et_2O: pentane → 20:80 Et_2O:pentane), followed by size exclusion chromatography over HW-40 eluted with DCM/MeOH (1:1) to obtain the title compound as a colourless oil (0.28 g, 0.25 mmol, 57%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.99 – 7.90 (m, 2H, CH_{arom}), 7.64 – 7.12 (m, 32H, CH_{arom}), 6.24 – 6.18 (m, 1H, H-1), 5.75 (ddd, J = 10.2, 2.9, 2.9 Hz, 1H, H-7), 5.48 (m, 2H, CHPh, H-2), 4.83 (d, J = 3.9 Hz, 1H, H-1'), 4.71 (d, J = 12.5 Hz, 1H, C*H*H Bn), 4.55 (d, J = 12.5 Hz, 1H, CH*H* Bn), 4.30 (dd, J = 10.3, 4.9 Hz, 1H, H-4'), 3.90 (dd, J = 6.0, 3.9 Hz, 1H, H-3), 3.85 – 3.44 (m, 7H, H-4, H-5, H-3', H-4', H-5', H-6'), 3.44 – 3.27 (m, 1H, H-6), 3.22 (t, J = 7.0 Hz, 2H, N_3-CH_2 spacer), 3.14 (d, J = 9.2 Hz, 1H, H-6), 2.59 (s, 1H, H-5), 1.56 (m, 6H, spacer), 1.31 (m, 8H, spacer), 0.74 (s, 9H, $C(CH_3)_3$), 0.01 (s, 3H, $SiCH_3$), -0.16 (s, 3H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 165.9 (C=O Bz), 144.3, 132.8, 131.6, 130.4 ($C_{q\text{-}arom}$), 129.7, 128.7, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.0, 126.1 (C_{arom}), 122.6 (C-1), 101.0 (CHPh), 99.9 (C-1'), 82.0 (C-3') , 81.7 (C-3), 79.2 (C-2'), 78.4 (C-5'), 73.4 (CH_2Bn), 73.1 (C-6'), 69.4 (C-4'), 68.7 (C-2), 65.3 (C-6), 63.3 (C-4), 51.5 (CH_2N_3 spacer), 44.4 (C-5), 29.4, 29.2, 28.8, 26.7, 26.1 (spacer), 25.9 ($C(CH_3)_3$), -3.6, -3.6 ($C(CH_3)_3$); HRMS (ESI) m/z: [M+Na]+ Calcd for $C_{67}H_{79}N_3NaO_{10}Si$ 1136.5432; found 1136.5439.

3-*O*-(2-*O*-Benzyl-3-*O*-(8-azido-octane)-4,6-*O*-benzylidene-α-D-glucopyranose)-6-*O*-trityl-cyclophellitol alkene (23).

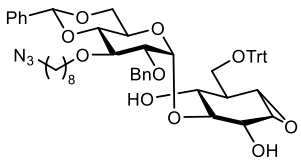


Compound **22** (32 mg, 28 µmol) was dissolved in THF (1.5 mL, 0.02 M) and cooled on ice. TBAF (1 M solution in THF, 56 µL, 56 µmol, 2.0 eq.) was added and the reaction was stirred for 1 hour at room temperature. Upon full conversion was observed (R_f 0.3 (Et_2O:pentane, 3:7, v:v)), the reaction was quenched by the addition of $NaHCO_3$ and diluted with H_2O and Et_2O. The organic layer was separated from the

aqueous layer and the aqueous layer was extracted with Et_2O twice. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated.

The crude was dissolved in a 1:1 mixture of MeOH:DCM (1.1 mL, 0.025 M), and NaOMe (36 mg, 0.56 mmol, 20 eq.) was added. The reaction was stirred for 16 hours at room temperature, after which full conversion was observed (R_f 0.2 (Et_2O: pentane, 1:1, v:v)). The reaction was quenched by the addition of sat. aq. $NaHCO_3$ and diluted with H_2O and Et_2O. The aqueous layer was extracted with Et_2O twice. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The reaction was purified using flash column chromatography (30:70 Et_2O:pentane → 60:40 Et_2O:pentane), obtaining compound **23** as a white brittle foam (20 mg, 22 µmol, 77%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.50 – 7.46 (m, 2H, CH_{arom}), 7.45 – 7.41 (m, 6H, CH_{arom}), 7.39 – 7.26 (m, 14H, CH_{arom}), 7.23 – 7.20 (m, 2H, CH_{arom}), 5.69 – 5.55 (m, 2H, H-1, H-7), 5.53 (s, 1H, CHPh), 4.90 – 4.83 (m, 2H, H-1', C*H*H Bn), 4.73 (d, J = 11.8 Hz, 1H, CH*H* Bn), 4.30 – 4.22 (m, 2H, H-4', H-2), 4.06 (ddd, J = 10.0, 10.0, 4.9 Hz, 1H, H-3), 3.93 – 3.83 (m, 3H, H-4, H-3', H-5'), 3.78 – 3.67 (m, 3H, H-6', 2-OH), 3.54 (ddd, J = 9.5, 6.8, 2.9 Hz, 1H, H-2'), 3.40 – 3.30 (m, 2H, H-6), 3.28 – 3.18 (m, 3H, H-4, N_3-CH_2 spacer), 2.94 (s, 1H, 4-OH), 2.55 – 2.46 (m, 1H, H-5), 1.65 – 1.50 (m, 4H, spacer), 1.43 – 1.17 (m, 10H, spacer); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 144.2, 137.6, 137.4 ($C_{q\text{-}arom}$), 129.1 (CH_{arom}), 128.8 (C-1), 128.7, 128.5, 128.3, 128.3, 127.9 (CH_{arom}), 127.5 (C-7), 127.0, 126.1, 125.6 (CH_{arom}), 101.4 (CHPh), 101.3 (C-1'), 86.5 (CPh_3), 82.0 (C-3'), 78.9 (C-2'), 78.6 (C-5'), 74.5 (CH_2Bn), 73.5 (C-6'), 71.2 (C-2), 69.8 (C-4), 68.8 (C-4'), 63.7 (C-3), 63.3 (C-6), 51.5 (CH_2N_3 spacer), 44.4 (C-5), 30.5, 29.4, 29.2, 28.9, 26.8, 26.1 (spacer); HRMS (ESI) m/z: [M+Na]+ Calcd for $C_{54}H_{61}N_3NaO_9$ 918.4306; found 918.4310.

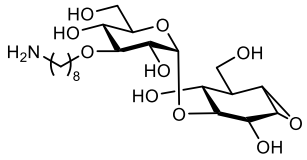
3-*O*-(2-*O*-Benzyl-3-*O*-(8-azido-octane)-4,6-*O*-benzylidene-α-D-glucopyranose)-6-*O*-trityl-1,7-*epi*-cyclophellitol (24).



Compound **23** (24 mg, 27 µmol) was dissolved in anhydrous DCM (1.0 mL, 0.025 M). $NaHCO_3$ (30 mg, 0.35 mmol, 13 eq.) and *m*-CPBA (15 mg, 81 µmol, 3.0 eq.) were added and the reaction mixture was stirred for 36 hours at 5 °C. TLC confirmed full conversion (R_f 0.3 (Et_2O:pentane, 6:4, v:v)). The reaction was quenched by the addition of sat. aq. $NaHCO_3$ and sat. aq. $Na_2S_2O_3$ and diluted further with H_2O and DCM. The aqueous layer was extracted twice with DCM, and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude was purified using flash column chromatography (40:60 Et_2O:pentane → 60:40 Et_2O:pentane). The title compound was obtained as a colourless oil (24 mg, 26 µmol, 97%). ^{1}H NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): *δ* 7.48 (m, 2H, CH_{arom}), 7.45 – 7.41 (m, 5H, CH_{arom}), 7.39 – 7.32 (m, 7H, CH_{arom}), 7.32 – 7.26 (m, 7H, CH_{arom}), 7.24 – 7.21 (m, 3H, CH_{arom}), 5.53 (s, 1H, CHPh), 4.91 – 4.79 (m, 1H, C*H*H Bn), 4.77 (d, J = 4.0 Hz, 1H, H-1'), 4.75 – 4.65 (m, 1H, C*H*H Bn), 4.26 (dd, J = 10.3, 6.1 Hz, 1H, H-4'), 4.05 (dd, J = 10.0, 4.9 Hz, 1H, H-3'), 3.98 – 3.80 (m, 4H, H-2, H-7, H-5', 2-OH), 3.80 – 3.63 (m, 3H, H-4, H-6'), 3.58 – 3.29 (m, 4H, H-6, CH_2O spacer), 3.26 – 3.16 (m, 2H, CH_2N_3), 3.15 – 3.08 (m, 1H, H-3), 2.89 (d, J = 4.0 Hz, 1H, 4-OH), 2.22 (ddd, J = 8.9, 5.1, 3.3 Hz, 1H, H-5), 1.66 – 1.50 (m, 6H, spacer), 1.43 (s, 1H, spacer), 1.40 – 1.18 (m, 8H, spacer); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): *δ* 144.2, 144.0, 137.4 ($C_{q\text{-}arom}$), 129.1, 128.8, 128.7, 128.5, 128.4, 128.3, 128.0, 127.9, 127.2, 127.1, 126.1 (CH_{arom}), 101.6 (CHPh), 101.4 (C-1'), 87.6 (CPh_3), 81.9 (C-3'), 79.0 (C-2'), 78.4 (C-5'), 74.7 (CH_2Bn), 73.5 (C-6'), 71.0 (C-2), 69.3 (C-

4), 68.8 (C-4'), 63.6 (C-3), 61.9 (C-6), 56.6 (C-1), 55.1 (C-7), 51.5 (CH_2N_3 spacer), 42.7 (C-5), 30.5, 30.4, 29.4, 29.2, 28.9, 26.8, 26.1 (spacer); HRMS (ESI) m/z: [M+Na]+ Calcd for $C_{54}H_{61}N_3NaO_{10}$ 934.4255; found 934.4256.

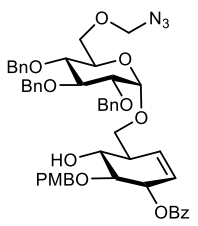
3-*O*-(3-*O*-(8-Azido-octane)-α-D-glucopyranose)-1,7-*epi*-cyclophellitol (26).



Compound **24** (24 mg, 26 µmol,) was co-evaporated two times with anhydrous chloroform and dissolved in anhydrous THF (1.0 mL, 0.025 M). PtO_2 (2.6 mg, 11 µmol, 0.4 eq.) was added and the mixture was purged with N_2, followed by purging with H_2 and kept under a positive H_2 atmosphere for 4 hours. The reaction mixture was filtered over celite, rinsed with THF and concentrated.

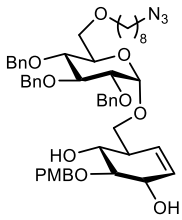
In a separate flask, sodium metal (25 mg, 1.1 mmol, 40 eq.) was dissolved in condensed NH_3 (3.0 mL) at -60 °C. This mixture was stirred for 30 minutes at -60 °C. The crude mixture was dissolved in THF (1.0 mL) and *t*-BuOH (25 µL, 0.26 mmol, 10 eq.) was added. The THF mixture was added dropwise to the liquid NH_3 and was stirred for 1 hour at -60 °C. The reaction was quenched by the addition of 500 µL H_2O and was allowed to attain to room temperature. The mixture was concentrated under reduced pressure and desalted using a C_{18} column chromatography (5:95 MeCN:H_2O → 20:80 MeCN: H_2O), obtaining compound **26** as a colourless oil (12 mg, 25 µmol, 96%).

2-*O*-Benzoyl-3-*O*-(4-methoxybenzyl)-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-(8-azido-octane)-α-D-glucopyranose)-cyclophellitol alkene (29).



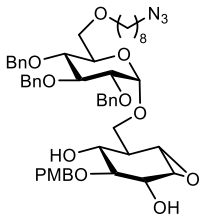
Acceptor **28** (0.14 g, 0.37 mmol) and trifluoro-imidate donor **18** (0.44 g, 0.56 mmol, 1.5 eq.), were combined and co-evaporated twice with toluene. PPh_3O (1.7 g, 5.9 mmol, 16 eq.), activated 3Å molecular rods and anhydrous DCM (7.4 mL, 0.05 M) were added and kept under N_2 atmosphere. Subsequently, TMSI (0.11 mL, 0.74 mmol, 2.0 eq.) was added dropwise and the reaction mixture was stirred overnight at room temperature. Upon full conversion (R_f 0.5 (Et_2O:pentane, 1:1 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ followed by diluting the reaction mixture with water and Et_2O. The organic layer was separated and the aqueous layer was extracted twice with Et_2O. The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (30:70 Et_2O:pentane → 40:60 Et_2O:pentane) to obtain the title compound as a colorless oil (0.24 g, 0.25 mmol, 68%).

3-*O*-(4-Methoxybenzyl)-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-(8-azido-octane)-α-D-glucopyranose)-cyclophellitol alkene (30).



Compound **29** (0.14 g, 0.15 mmol) was dissolved in a 1:1 mixture of DCM and MeOH (4.0 mL, 0.05 M) followed by the addition of NaOMe (98 mg, 1.5 mmol, 10 eq.). The reaction was stirred overnight at room temperature. Upon full conversion (R_f 0.4 (Et_2O:pentane, 8:2 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ followed by diluting the reaction mixture with water and Et_2O. The organic layer was separated and the aqueous layer was extracted twice with Et_2O. The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (60:40 Et_2O:pentane → 80:20 Et_2O:pentane) to obtain the title compound as a colorless oil (0.11 g, 0.13 mmol, 86%). NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC, HMBC-GATED): δ 7.45 – 7.26 (m, 17H, CH_{arom}), 6.94 – 6.88 (m, 2H, CH_{arom}), 5.66 (ddd, *J* = 10.1, 2.4, 2.4 Hz, 1H, H-7), 5.48 (ddd, *J* = 10.2, 2.2, 2.2 Hz, 1H, H-1), 4.98 (m, 2H, C*H*H Bn, C*H*H Bn), 4.92 (d, *J* = 10.9 Hz, 1H, C*H*H Bn), 4.87 – 4.75 (m, 4H, C*H*H PMB, CH*H* PMB, CH*H* Bn, H-1'), 4.68 (d, *J* = 11.9 Hz, 1H, CH*H* Bn), 4.64 (d, *J* = 10.9 Hz, 1H, CH*H* Bn), 4.28 – 4.24 (m, 1H, H-2), 3.98 (dd, *J* = 9.2, 9.2 Hz, 1H, H-4'), 3.88 (dd, *J* = 9.5, 5.3 Hz, 1H, H-6), 3.82 (s, 3H, OMe), 3.81 – 3.73 (m, 2H, H-4, 4-OH), 3.70 – 3.57 (m, 5H, H-2', H-3', H-5', H-6'), 3.55 – 3.47 (m, 2H, H-3, CH_2O spacer), 3.41 – 3.34 (m, 2H, H-6, CH_2O spacer), 3.25 (dd, *J* = 7.0, 7.0 Hz, 2H, CH_2N_3 spacer), 2.66 (ddddd, *J* = 8.4, 5.5, 5.5, 2.7, 2.7 Hz, 1H, H-5), 2.20 (d, *J* = 3.5 Hz, 1H, 2-OH), 1.66 – 1.54 (m, 4H, spacer), 1.41 – 1.26 (m, 8H, spacer); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC, HMBC-GATED): δ 138.8, 138.5, 138.2, 131.2 ($C_{q\text{-}arom}$), 129.8 (CH_{arom}), 129.7 (C-7), 128.6, 128.6, 128.5, 128.2, 128.1, 128.0, 127.9, 127.9, 127.7 (CH_{arom}), 126.0 (C-1), 114.1 (CH_{arom}), 97.9 (C-1'), 84.8 (C-3), 82.2 (C-4'), 80.0, 77.7 (C-2'/C-3'/C-5'/C-6'), 75.8, 75.2, 74.6 (CH_2 Bn/PMB), 74.0 (C-4), 73.5 (CH_2 Bn/PMB), 71.9 (C-2), 71.8 (CH_2O spacer), 71.5 (C-6), 70.6, 69.3 (C-2'/C-3'/C-5'/C-6'), 55.4 (OMe), 51.5 (CH_2N_3 spacer), 44.6 (C-5), 29.7, 29.4, 29.2, 28.9, 26.8, 26.2 (spacer); HRMS (ESI) m/z: [M+Na]+ Calcd for $C_{50}H_{63}N_3NaO_{10}$ 888.4411; found 888.4425.

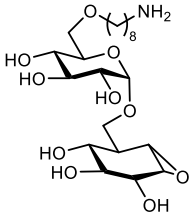
3-*O*-(4-Methoxybenzyl)-6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-(8-azido-octane)-α-D-glucopyranose)-1,7-*epi*-cyclophellitol (31).



Compound **30** (55 mg, 64 µmol) was dissolved in anhydrous DCM (1.5 mL, 0.04 M) followed by the addition of $NaHCO_3$ (27 mg, 0.32 mmol, 5.0 eq.). The solution was cooled on ice and *m*-CPBA (33 mg, 0.19 mmol, 3.0 eq.) were added and the reaction was stirred overnight at 4 °C. Upon full conversion (R_f 0.2 (Et_2O:pentane, 8:2 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ and sat. aq. $Na_2S_2O_3$ followed by diluting the reaction mixture with water and Et_2O. The organic layer was separated and the aqueous layer was extracted twice with Et_2O. The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (70:30 Et_2O:pentane → 90:10 Et_2O:pentane) to obtain the title compound as a colorless oil (49 mg, 56 µmol, 86%). NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC, NOESY): 7.36 – 7.24 (m, 17H, CH_{arom}), 6.90 – 6.85 (m, 2H, CH_{arom}), 4.93 (d, *J* = 10.9 Hz, 1H, C*H*H Bn), 4.88 (d, *J* = 10.9 Hz, 1H, C*H*H Bn), 4.83 – 4.74 (m, 5H, C*H*H Bn, CH*H* Bn, C*H*H PMB, CH*H* PMB, H-1'), 4.64 – 4.57 (m, 2H,

CH*H* Bn, CH*H* Bn), 3.98 – 3.87 (m, 3H, H-2, H-6, H-3'), 3.79 (s, 3H, OMe), 3.69 (ddd, *J* = 10.0, 3.7, 2.0 Hz, 1H, H-5'), 3.65 – 3.54 (m, 4H, H-2', H-4', H-6'), 3.52 – 3.43 (m, 3H, H-4, H-6, CH_2O spacer), 3.42 – 3.30 (m, 3H, H-1, H-3, CH_2O spacer), 3.21 (t, *J* = 7.0 Hz, 2H, CH_2N_3 spacer), 3.10 (d, *J* = 3.9 Hz, 1H, H-7), 3.05 (s, 1H, 4-OH), 2.31 – 2.26 (m, 1H, H-5), 2.25 – 2.20 (bs, 1H, 2-OH), 1.59 – 1.51 (m, 4H, spacer), 1.35 – 1.23 (m, 8H, spacer); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.6, 138.8, 138.4, 138.3, 130.7 ($C_{q\text{-}arom}$), 129.8, 128.6, 128.6, 128.5, 128.1, 128.1, 128.0, 127.9, 127.8, 114.2 (CH_{arom}), 97.7 (C-1'), 82.1, 82.1(C-3, C-3'), 80.2 (C-4'), 77.7 (C-2'), 75.8, 75.3, 75.2 (CH_2 Bn/PMB), 73.4 (C-2), 72.5 (CH_2 Bn/PMB), 71.9 (CH_2O spacer), 71.4 (C-4), 70.7 (C-5'), 69.3 (C-6'), 68.3 (C-6), 56.7 (C-1), 55.4 (OMe), 54.2 (C-7), 51.6 (CH_2N_3 spacer), 42.6 (C-5), 29.7, 29.4, 29.2, 28.9, 26.8, 26.2 (spacer); HRMS (ESI) m/z: [M+Na]+ Calcd for $C_{50}H_{63}N_3NaO_{11}$ 904.4360; found 904.4373.

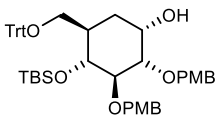
6-*O*-(6-*O*-(8-Azido-octane)-α-D-glucopyranose)-1,7-*epi*-cyclophellitol (S1).



Compound **31** (25 mg, 28 µmol,) was co-evaporated two times with anhydrous chloroform and dissolved in anhydrous THF (1.0 mL, 0.03 M). PtO_2 (2.6 mg, 11 µmol, 0.4 eq.) was added and the mixture was purged with N_2, followed by purging with H_2 and kept under a positive H_2 atmosphere for 4 hours. The reaction mixture was filtered over celite, rinsed with EtOAc and concentrated.

In a separate flask, sodium metal (26 mg, 1.1 mmol, 40 eq.) was dissolved in condensed NH_3 (3.0 mL) at -60 °C. This mixture was stirred for 30 minutes at -60 °C. The crude mixture was dissolved in THF (1.0 mL) and *t*-BuOH (27 µL, 0.28 mmol, 10 eq.) was added. The THF mixture was added dropwise to the liquid NH_3 and was stirred for 1 hour at -60 °C. The reaction was quenched by the addition of 500 µL H_2O and was allowed to attain to room temperature. The mixture was concentrated under reduced pressure and desalted using a C_{18} column chromatography (5:95 MeCN:H_2O → 20:80 MeCN: H_2O), obtaining compound **S1** as a colourless oil (14 mg, 28 µmol, quant.). NMR (500 MHz, D_2O, HH-COSY, HSQC, NOESY): δ 4.82 (d, *J* = 3.7 Hz, 1H, H-1'), 3.85 – 3.80 (m, 2H, H-3, H-6), 3.68 – 3.51 (m, 6H, H-6, H-3', H-4', H-5', H-6'), 3.50 – 3.37 (m, 3H, H-2', CH_2O spacer), 3.36 – 3.20 (m, 5H, H-1, H-2, H-4, H-7), 2.48 (t, *J* = 7.1 Hz, 2H, CH_2N_3 spacer), 2.09 (ddd, *J* = 9.6, 6.5, 3.4 Hz, 1H, H-5), 1.50 – 1.27 (m, 4H, spacer), 1.20 (d, *J* = 5.3 Hz, 8H, spacer); ^{13}C NMR (126 MHz, D_2O, HSQC): δ 98.2 (C-1'), 73.1 (C-2/C-4), 73.0 (C-3'/C-4'/C-5'), 71.9 (CH_2O spacer), 71.2 (C-3), 70.6, 69.8 (C-3'/C-4'/C-5'), 69.5 (C-2/C-4), 69.1 (C-6'), 66.6 (C-6), 57.5 (C-1), 55.1 (C-7), 42.2 (C-5), 40.5 (CH_2N_3 spacer), 31.4, 28.5, 28.4, 28.4, 25.9, 25.1 (spacer); HRMS (ESI) m/z: [M+H]+ Calcd for $C_{21}H_{40}NO_{10}$ 466.2652; found 466.2658.

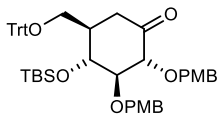
2,3-Di-*O*-(4-methoxybenzyl)-4-*O*-*tert*-butyldimethylsilyl-6-*O*-trityl-carba-α-D-glucose (45).



Compound **44** (1.3 g, 2.0 mmol) was dissolved in anhydrous toluene (30 mL, 0.067 M) and Bu_2SnO (0.56 g, 2.3 mmol, 1.15 eq.) was added. The reaction mixture was stirred at reflux for 4 hours under N_2 atmosphere after which the reaction mixture was allowed to cool to room temperature and concentrated under reduced pressure. The crude was dissolved in anhydrous DMF (20 mL, 0.1 M) followed by the addition of CsF (0.52 g, 3.4 mmol, 1.8 eq.) and PMBCl (0.4 mL, 3.0 mmol, 1.5 eq.). The mixture was continued stirring for 16 hours at room temperature after which TLC confirmed full conversion (R_f 0.3 (Et_2O:pentane, 2:8, v:v)). The reaction was quenched

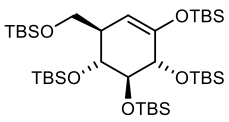
by the addition of sat. aq. $NaHCO_3$ and diluted further with H_2O and Et_2O. The aqueous layer was extracted twice with Et_2O, and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude was purified using flash column chromatography (10:90 Et_2O:pentane → 40:60 Et_2O:pentane). The title compound was obtained as a colourless oil (0.59 g, 0.67 mmol, 33%). In addition, starting material was recovered (1.0 g, 1.5 mmol, 67%). NMR (500 MHz, $CDCl_3$, HH-COSY, HSQC): δ 7.61 – 7.29 (m, 19H, CH_{arom}), 7.01 – 6.94 (m, 4H, CH_{arom}), 4.97 (d, *J* = 11.0 Hz, 1H, C*H*H PMB), 4.79 (d, *J* = 11.0 Hz, 1H, CH*H* PMB), 4.70 (s, 2H, CH*H* PMB, CH*H* PMB), 4.33 – 4.20 (m, 1H, H-1), 3.94 (s, 6H, OMe, OMe), 3.78 – 3.68 (m, 2H, H-3, H-6), 3.49 (dd, *J* = 9.4, 3.1 Hz, 1H, H-2), 3.28 (dd, *J* = 10.2, 8.6 Hz, 1H, H-4), 2.71 – 2.64 (m, 2H, H-6, H-7), 2.59 – 2.48 (m, 1H, H-5), 1.41 (dd, *J* = 7.2, 7.2 Hz, 1H, 1-OH), 1.15 (ddd, *J* = 14.9, 12.5, 2.5 Hz, 1H, H-7), 0.81 (s, 9H, $C(CH_3)_3$), 0.00 (s, 3H, $SiCH_3$), -0.25 (s, 3H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 159.3, 158.6, 144.4, 131.6, 130.2 ($C_{q\text{-}arom}$), 129.6, 128.8, 128.7, 127.7, 126.8, 113.9, 113.4 (CH_{arom}), 86.4 (CPh_3), 83.6 (C-2), 82.6 (C-3), 74.9 (C-4), 74.8, 72.5 (CH_2 PMB), 66.1 (C-1), 65.9 (C-6), 55.3, 55.2 (OMe), 38.4 (C-5), 30.5 (C-7), 26.0 ($C(CH_3)_3$), 17.9 ($C(CH_3)_3$), -3.4, -4.5 ($SiCH_3$); HRMS (ESI) m/z: [M+Na]+ Calcd for $C_{48}H_{58}NaO_7Si$ 797.3850; found 797.3855.

2,3-Di-*O*-(4-methoxybenzyl)-4-*O*-*tert*-butyldimethylsilyl-6-*O*-trityl-D-validone (46).



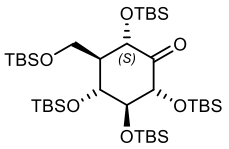
Compound **45** (2.2 g, 2.8 mmol) was dissolved in anhydrous DCM (22 mL, 0.1 M) and cooled on ice. Subsequently, $NaHCO_3$ (1.9 g, 23 mmol, 8.0 eq.) and DMP (2.4 g, 5.7 mmol, 2.0 eq.) were added and the reaction mixture was stirred for 2 hours while attaining to room temperature. Upon full conversion (R_f 0.7 (Et_2O:pentane, 2:8 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ and sat. aq. $Na_2S_2O_3$ followed by diluting the reaction mixture with water and Et_2O. The organic layer was separated and the aqueous layer was extracted twice with Et_2O. The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (0:100 Et_2O:pentane → 20:80 Et_2O:pentane) to obtain the title compound as a colorless oil (2.2 g, 2.8 mmol, quant.). NMR (500 MHz, D_2O, HH-COSY, HSQC): δ 7.42 – 7.11 (m, 19H, CH_{arom}), 6.87 – 6.77 (m, 4H, CH_{arom}), 4.83 (d, *J* = 10.8 Hz, 1H, C*H*H PMB), 4.76 (d, *J* = 10.9 Hz, 1H, CH*H* PMB), 4.56 (d, *J* = 10.8 Hz, 1H, C*H*H PMB), 4.41 (d, *J* = 10.9 Hz, 1H, CH*H* PMB), 4.00 (d, *J* = 9.2 Hz, 1H, H-2), 3.80 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.63 (dd, *J* = 8.7, 3.3 Hz, 1H, H-6), 3.57 (dd, *J* = 9.2, 8.0 Hz, 1H, H-4), 3.46 (dd, *J* = 9.3, 8.1 Hz, 1H, H-3), 2.91 (d, *J* = 10.0 Hz, 1H, H-7), 2.73 (dd, *J* = 8.7, 8.7 Hz, 1H, H-6), 2.15 – 2.00 (m, 2H, H-5, H-7), 0.67 (s, 9H, $C(CH_3)_3$), -0.12 (s, 3H, $SiCH_3$), -0.33 (s, 3H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 206.1 (C-1), 159.5, 158.9, 144.1, 131.4, 130.1 ($C_{q\text{-}arom}$), 129.8, 129.1, 128.8, 128.0, 127.2, 113.9, 113.6 (CH_{arom}), 86.9 (CPh_3), 85.7 (C-3), 85.6 (C-2), 74.8 (CH_2 PMB), 73.9 (C-4), 73.1 (CH_2 PMB), 66.4 (C-6), 55.9 (OMe), 41.4 (C-5), 40.2 (C-7), 26.0 ($C(CH_3)_3$), 17.1 ($C(CH_3)_3$), -3.4, -4.5 ($SiCH_3$); HRMS (ESI) m/z: [M+Na]+ Calcd for $C_{48}H_{56}NaO_7Si$ 795.3693; found 795.3701.

1,2,3,4,6-Penta-*O-tert*-butyldimethylsilyl-cyclophellitol alk-1-enyl ether (48).



Compound **46** (2.2 g, 2.8 mmol) was dissolved in anhydrous DCM (56 mL, 0.05 M) and cooled on ice. Subsequently, TES (1.4 mL, 8.5 mmol, 3.0 eq.) and TFA (2.6 mL, 34 mmol, 12 eq.) were added and stirring continued for 3.5 hours at this temperature. Upon full conversion (R_f 0.2 (Et_2O:pentane, 1:1 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ followed by diluting the reaction mixture with water and Et_2O. The organic layer was separated and the aqueous layer was extracted twice with Et_2O. The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (30:70 Et_2O:pentane → 70:30 Et_2O:pentane) to obtain the deprotected intermediate as a colorless oil (0.75 g, 2.6 mmol, 93%). The purified intermediate was dissolved in anhydrous DCM (50 mL, 0.05 M) under argon atmosphere and cooled on ice. Subsequently, 2,6-lutidine (3.6 mL, 31 mmol, 12 eq.) was added followed by the dropwise addition of TBSOTf (4.8 mL, 21 mmol, 8.0 eq.). The reaction mixture was allowed to warm to room temperature and stirring continued at this temperature overnight. Upon full conversion was observed (R_f 0.3 (DCM:pentane, 1:9 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ followed by diluting the reaction mixture with water and Et_2O. The organic layer was separated and the aqueous layer was extracted twice with Et_2O. The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (1:99 DCM:pentane → 20:80 DCM:pentane) to obtain the title compound as a colorless oil (1.7 g, 2.3 mmol, 88%). NMR (500 MHz, D_2O, HH-COSY, HSQC): δ 4.88 (dd, *J* = 4.4, 0.7 Hz, 1H, H-7), 3.88 (ddd, *J* = 2.7, 1.8, 0.6 Hz, 1H, H-4), 3.82 (ddd, *J* = 2.6, 1.1, 1.1 Hz, 1H, H-3), 3.75 (dd, *J* = 2.0, 0.9 Hz, 1H, H-2), 3.59 (dd, *J* = 9.3, 7.6 Hz, 1H, H-6), 3.44 (dd, *J* = 9.3, 7.3 Hz, 1H, H-6), 2.47 (ddd, *J* = 7.0, 7.0, 3.8 Hz, 1H, H-5), 0.96 – 0.83 (m, 45H, $C(CH_3)_3$), 0.20 – -0.03 (m, 30H, $SiCH_3$); ^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 149.6 (C-1), 103.9 (C-7), 76.8 (C-4), 73.5 (C-2), 69.6 (C-3), 66.1 (C-6), 46.7 (C-5), 26.4, 26.3, 26.2, 26.1, 25.9 ($C(CH_3)_3$), 18.7, 18.6, 18.5, 18.5, 18.0 ($C(CH_3)_3$), -3.7, -3.7, -4.0, -4.1, -4.3, -4.3, -4.5, -4.6, -5.2 ($SiCH_3$); HRMS (ESI) m/z: [M+Na]+ Calcd for $C_{37}H_{82}NaO_5Si_5$ 769.4906; found 769.4912.

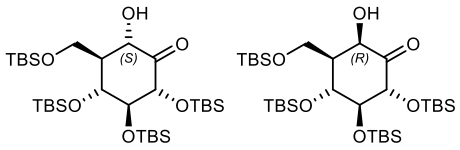
2,3,4,6,7-Penta-*O-tert*-butyldimethylsilyl-D-7-(*S*)-hydroxyvalidone (49).



Compound **48** (0.11 g, 0.15 mmol) was dissolved in anhydrous DCM (7.0 mL, 0.02 M) and cooled on ice. Subsequently, *m*-CPBA (63 mg, 0.37 mmol, 2.5 eq.) was added and the reaction mixture was stirred for 2 hours while kept on ice. TLC confirmed full consumption of the starting material (R_f 0.6 (DCM:pentane, 2:8 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ and sat. aq. $Na_2S_2O_3$ followed by diluting the reaction mixture with water and Et_2O. The organic layer was separated and the aqueous layer was extracted twice with Et_2O. The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (1:99 DCM:pentane → 20:80 DCM:pentane) to obtain the title compound as a colorless oil (60 mg, 79 µmol, 53%). NMR (500 MHz, D_2O, HH-COSY, HSQC): δ 4.17 (dd, *J* = 8.2, 1.0 Hz, 1H, H-3), 4.12 (dd, *J* = 3.7, 1.5 Hz, 1H, H-6), 4.01 (ddd, *J* = 3.7, 1.3 Hz, 1H, H-6), 3.95 (d, *J* = 1.1 Hz, 1H, H-2), 3.91 – 3.79 (m, 2H, H-4, H-7), 2.12 (dddd, *J* = 9.6, 8.5, 5.6, 1.5 Hz, 1H, H-5), 0.97 – 0.79 (m, 45H, $C(CH_3)_3$), 0.22 – 0.00 (m, 30H, $SiCH_3$);

^{13}C NMR (126 MHz, $CDCl_3$, HSQC): δ 206.9 (C-1), 82.7 (C-2), 81.0 (C-3), 72.1 (C-4), 68.8 (C-7), 63.9 (C-6), 58.2 (C-5), 26.2, 26.2, 26.1, 25.9 (C(CH_3)$_3$), 18.6, 18.5, 18.1, 18.0 (*C*(CH_3)$_3$), -3.6, -4.0, -4.3, -4.5, -4.9, -5.1, -5.2, -5.7 ($SiCH_3$); HRMS (ESI) m/z: [M+Na]+ Calcd for $C_{37}H_{82}NaO_6Si_5$ 785.4855; found 785.4864.

2,3,4,6-Tetra-*O-tert*-butyldimethylsilyl-D-7-(*S*)-hydroxyvalidone (50) and 2,3,4,6-tetra-*O-tert*-butyldimethylsilyl-D-7-(*R*)-hydroxyvalidone (51).



Compound **48** (0.30 g, 0.40 mmol) was dissolved in acetone (15 mL, 0.025 M) followed by the addition of NMO (50 wt% in H_2O, 0.38 mL, 1.6 mmol, 4.0 eq.), OsO_4 (2 wt% in H_2O, 5.1 mL, 0.4 mmol, 1.0 eq.). The reaction mixture was stirred vigorously at 75 °C for 20 hours upon almost full conversion was observed. (R_f 0.5 (DCM:pentane, 3:7 v:v)), the reaction was quenched by the addition of sat. aq. $NaHCO_3$ and sat. aq. $Na_2S_2O_3$ followed by diluting the reaction mixture with water and Et_2O. The organic layer was separated and the aqueous layer was extracted twice with Et_2O. The combined organic layers were subsequently dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography (5:95 DCM:pentane → 30:70 DCM:pentane) to obtain the title compounds as a mixture of C-7 epimers in a 6.5:1 ratio of *S*:*R* respectively (0.13 g, 0.2 mmol, 50%). In addition, starting material was recovered (87 mg, 0.12 mmol, 29%).

Data of the major isomer **50**: ^{1}H NMR (600 MHz, DMSO, HH-COSY, HSQC): δ 4.90 (d, *J* = 5.6 Hz, 1H, 7-OH), 4.08 (dd, *J* = 3.4, 2.3 Hz, 1H, H-4), 3.96 – 3.94 (m, 1H, H-3), 3.94 – 3.90 (m, 2H, H-2, H-7), 3.85 – 3.79 (m, 2H, H-6), 1.97 – 1.91 (m, 1H, H-5), 0.95 – 0.72 (m, 36H, C(CH_3)$_3$), 0.18 – -0.11 (m, 24H, $SiCH_3$); ^{13}C NMR (151 MHz, DMSO, HSQC): δ 210.0 (C-1), 82.7 (C-3), 80.8 (C-2/C-7), 70.8 (C-2/C-7), 69.9 (C-4), 64.1 (C-6), 57.3 (C-5), 27.1, 27.1, 27.0 (C(*C*H_3)$_3$), 19.5, 19.3, 19.0, 18.9 (*C*(CH_3)$_3$), -2.6, -3.4, -3.6, -3.8, -4.0, -4.1, -4.1, -4.4 ($SiCH_3$).

Data of the minor isomer **51**: ^{1}H NMR (600 MHz, DMSO, HH-COSY, HSQC): δ 5.18 (dd, *J* = 6.8, 5.5 Hz, 1H, H-7), 4.90 (d, *J* = 5.8 Hz, 1H, 7-OH), 4.20 (q, *J* = 2.0 Hz, 1H, H-4), 3.94 – 3.92 (m, 1H, H-3), 3.85 – 3.83 (m, 1H, H-2), 3.75 (dd, *J* = 10.3, 4.9 Hz, 1H, H-6), 3.51 (dd, *J* = 10.7, 10.7 Hz, 1H, H-6), 2.50 – 2.45 (m, 1H, H-5), 0.92 – 0.68 (m, 36H, C(CH_3)$_3$), 0.15 – -0.09 (m, 24H, $SiCH_3$); ^{13}C NMR (151 MHz, DMSO, HSQC): δ 210.3 (C-1), 81.2 (C-2), 81.0 (C-3), 72.4 (C-4), 69.6 (C-7), 60.1 (C-6), 57.1 (C-5), 27.0, 26.9, 26.8 (C(*C*H_3)$_3$), 19.2, 19.2, 19.2, 18.9 (*C*(CH_3)$_3$), -3.3, -3.6, -3.7, -4.0, -4.0 ($SiCH_3$); HRMS (ESI) m/z: [M+Na]+ Calcd for $C_{31}H_{68}NaO_6Si_4$ 671.3991; found 671.4000.

References

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Nederlandse samenvatting

Cyclophellitol en zijn vele analoga zijn veel gebruikte remmers van retentie-glycosidasen (GH's) en vertonen vaak uitstekende potenties en selectiviteit binnen deze enzym familie. In de loop der jaren heeft een beter begrip van hun werkingsmechanisme geleid tot het ontwerp van analogen die zijn afgestemd op specifieke glycosidasen en voor specifieke doeleinden. Op die manier zijn er onder meer remmers en probes ontwikkeld voor specifieke endo- of exo-glycosidasen, maar ook reagentia om juist een breder scala aan glycosidasen te bestuderen zijn succesvol geconstrueerd en toegepast. Het onderzoek beschreven in dit proefschrift richt zich op nieuwe ontwerpen en de ontwikkeling van synthetische methodologie om het palet aan remmers en probes van glycaan verwerkende enzymen uit te breiden. Zo worden er mogelijke irreversibele remmers beschreven van inverterende glycosidasen, een enzymfamilie zo groot en divers als retentie-enzymen maar waarvoor tot op heden geen covalente remmers bestaan. Daarnaast wordt het ontwerp en de synthese van een nieuwe klasse van glycosyltransferase (GT) donoranalogen gepresenteerd, die als potentiële remmers worden beschouwd. Al met al borduurt het gepresenteerde werk voort op de groeiende lijst van remmers en probes van retentie GH's, zet het de eerste stappen richting de complementaire inverterende GH's, en rapporteert het over het ontwerp van een nieuwe klasse van potentiële, conformatie gedreven GT-remmers.

Hoofdstuk 2 beschrijft een nieuwe syntheseroute naar een orthogonaal beschermde cyclophellitol bouwsteen uitgaande van commercieel verkrijgbaar tri-*O*-acetyl-D-glucal. De sleuteltransformatie van de synthese berusten op een Claisen-omlegging, waarmee D-glucal effectief kan worden omgezet in carba-D-glucal. De daaropvolgende verplaatsing van de 1,2-alkeen naar de 1,7-positie levert de orthogonaal beschermde cyclophellitol bouwsteen op met een rendement van 19% over 12 stappen. Vanwege de volledige orthogonaliteit van alle vier de hydroxyl beschermgroepen, zijn regio selectieve manipulaties mogelijk. De brede inzetbaarheid van de bouwsteen is vervolgens aangetoond in de synthese van een kleine reeks aan α-(1,3)-gekoppelde di- en trisacharide-nigerose- en dextraan-mimetica. Cruciaal in de synthese van de mimetica is het inspelen op het naburige groepsparticipatiepotentieel van het allylische, secundaire alcohol, wederom beschikbaar dankzij regioselectieve ontscherming. De stereoselectieve introductie van zowel epoxide- als aziridine functionaliteiten werd vervolgens mogelijk.

Hoofdstuk 3 behelst een studie naar de conformationele en elektronische effecten van 1,2- en 1,7-cyclophellitolen op hun inhibitie potentie van menselijke α-glucosidasen. Hiertoe zijn een reeks van twintig configuratieve en functionele cyclophellitol-analogen, met een systematische reeks van elektrofielen, gesynthetiseerd en bestudeerd in *in vitro*

experimenten op hun remmende potenties van menselijke zure α-glucosidase (GAA) en ER α-glucosidase II (ER-II). Hoewel er in vergelijking met bestaande remmers geen potentere remmers werden gevonden, werden voor sommige verbindingen laag-micromolaire affiniteiten waargenomen. Vervolgens zijn de conformationele vrije energielandschappen van de meest actieve verbindingen in kaart gebracht. Er werd een systematische 60° verschuiving in de laagste energieconformatie van het 1,2-cyclophellitol waargenomen in vergelijking met hun 1,7-tegenhangers. Als gevolg van deze verschuiving lijkt de laagste energieconformatie niet op die van het Michaelis-complex of die van transitietoestand tijdens hydrolyse. Deze cruciale conformatie verandering kan de algemene vermindering in waargenomen remmende potenties van de 1,2-cyclophellitolen ten opzichte van hun 1,7-tegenhangers verklaren.

Hoofdstuk 4 beschrijft de synthese van acht cyclitol verbindingen uitgerust met buiten de ring verkerende aziridines. De cyclitol verbindingen zijn potentieel selectieve remmers van inverterende α- en β-glucosidasen - een enzym groep waarvoor tot op heden geen, op mechanisme gebaseerde, covalente remmers bestaan. De hypothese is dat door het buiten de ring plaatsen van de elektrofiele positie, een covalente binding aan kan worden gegaan met het ver weg gelegen nucleofiel, kenmerkend voor inverterende glycosidasen. De sleutelstap in de synthese was een divergente aza-Michael-geïnitieerde ringsluiting reactie (aza-MIRC) tussen onbeschermde validamine of 1-*epi*-validamine en een kleine serie van dibromo verbindingen bestaande uit een diverse selectie van elektronen zuigende functionaliteiten. Op deze manier werden alle acht beoogde remmers verkregen in uitstekende rendementen, wat de mildheid en robuustheid van deze aziridine-vormende reacties op complexe, onbeschermde substraten aantoont.
Als uitbreiding op de in hoofdstuk 4 beschreven zoektocht naar covalente remmers van inverterende α- en β-glucosidasen, beslaat **Hoofdstuk 5** de synthese van een kleine reeks verbindingen met een anomere vinylgroep in combinatie met verschillende elektrofielen over de 1,2-positie. De hypothese is dat het terminale deel van de alkeen via een 1,4-Michael-additie, wederom een irreversibele additie reactie zou kunnen aangaan met het verafgelegen nucleofiel. De verbindingen zijn op divergente wijze gesynthetiseerd vanuit carbaglucose-derivaten die zijn gesynthetiseerd zoals beschreven in hoofdstuk 3. Vanwege de intrinsieke instabiliteit en reactiviteit van de voorgestelde elektrofielen, en de daaruit voortvloeiende synthetische uitdagingen tijdens de globale ontscherming, bleek het gebruik van silyl-beschermgroepen gecombineerd met een fluoride ontschermingsstap cruciaal.

Hoofdstuk 6 beschrijft het ontwerp en de synthese van een reeks complexe, conformationele donor-mimetica van glucosyl- en galactosyl transferasen. Het ontwerp

van deze complexe structuren is gebaseerd op kristallografische data. Hieruit blijkt dat de donorsubstraten (UDP-glucose of UDP-galactose, respectievelijk) een sterk gebogen oriëntatie aannemen bij het vormen van een Michaelis-complex met sommige glucosyl- of galactosyl transferasen. Sleutel tot het ontwerp is de conformationele beperking veroorzaakt door de aanwezigheid van een cyclische carbamaat- of -sulfamidaat groep, die de C1- en C2-positie overbrugt. Bovendien is het amide verder gefunctionaliseerd met een uridine 5'-monofosfaatgroep. Een van de sleutel transformaties was een Sharpless aminohydroxylering, die volledig stereoselectief bleek te zijn op zowel carba-D-glucal als carba-D-galactal en bijna equimolaire hoeveelheden van beide regio-isomeren opleverde met een uitstekend rendement. Op een divergente en parallelle wijze werden de regio-isomeren omgezet in zowel de cyclische carbamaten als sulfamidaten. In de volgende stap maakte een Atherton-Todd *N*-fosforylering een robuuste en efficiënte condensatie mogelijk van het cyclische carbamaat of sulfamidaat met een beschermd H-fosfonaat. Uiteindelijk, en met behulp van een milde en efficiënte één-pot-tweestaps ontschermings methode, konden alle acht complexe doelstructuren succesvol gesynthetiseerd en geïsoleerd worden.

Om verder in te gaan op de 1,2-carbamaat- en sulfamidaat donormimetica uit hoofdstuk 6, beschrijft **Hoofdstuk 7** de synthetische studie naar de overeenkomstige isomeren waarin de cyclisch carbamaat en sulfamidaat de 1,7-positie overbruggen. Er werd verondersteld dat door het verplaatsen van de bicyclische structuur, een grotere chemische ruimte onderzocht kan worden. In tegenstelling tot wat werd waargenomen in hoofdstuk 6, bleek de Sharpless-aminohydroxylatie niet te gaan wanneer uitgevoerd op cyclophellitol-cyclohexenen. Een relatief lange synthese route was nodig om tot de cyclische carbamaten te komen. Het gebruik van een TIPDS-bescherm groep op de 4- en 6-positie van de carbaglucose-analogen bleek essentieel voor de daaropvolgende Atherton-Todd-reactie en de globale ontschermingsstap, wat resulteerde in de isolatie van het eerste doelmolecuul. Parallel hieraan werden volgens de eerder genoemde procedures beide regio-isomeren van de carbagalactose carbamaten gesynthetiseerd. Een Atherton-Todd-fosforylering van het eerste cyclische carbamaat en daaropvolgende globale ontscherming leverde de eerste doelmolecuul van carbagalactose op. Het bleek niet mogelijk om, middels de Atherton-Todd methode fosforylering van het tweede carbamaat, alsook het cyclosulfamidaat, te bewerkstelligen. Om die reden zijn nieuwe synthetische routes nodig in de synthese van de overgebleven cyclische carbamaat- en cyclische sulfamidaat constructen.

Ten slotte beslaat **Hoofdstuk 8** een samenvatting van de verkregen resultaten met enkele suggesties voor vervolgonderzoek.

List of publications

Conformational and electronic effects of 1,2- and 1,7-cyclophellitols on α-glucosidase activities

Ofman, T. P.*; Heming, J. J. A.; Nin-Hill, A.; Moran, E.; Küllmer, F.; Steneker, R.; Klein, A.-M.; Ruijgrok, G.; Kok, K.; Aerts, J. M. F. G.; van der Marel, G. A.; Rovira, C.; Davies, G. J.; Artola, M.; Codée, J. D. C.; Overkleeft, H. S.

Manuscript in preparation.

Cyclic sulphate inhibitor of ER α-glucosidase II activity blocks replication of SARS-CoV-2 and other coronaviruses

Thaler, M.*; Ofman, T. P.*; Kok, K.; Heming, J. J. A.; Salgado-Benvindo, C.; Leijs, A.; Snijder, E. J.; Artola, M.; Overkleeft, H. S.; van Hemert, M. J.

Manuscript in preparation.

Design and synthesis of exocyclic cyclitol aziridines as potential mechanism-based glycosidase inactivators

Ofman, T. P.; van der Marel, G. A.; Codée, J. D. C.; Overkleeft, H. S.

Eur. J. Org. Chem. **2023**, *26*, e202300186.

An orthogonally protected cyclitol for the construction of nigerose- and dextran-mimetic cyclophellitols

Ofman, T. P.; Küllmer, F.; van der Marel, G. A.; Codée, J. D. C.; Overkleeft, H. S.

Org. Lett. **2021**, *23*, 9516–9519.

Reactivity–stereoselectivity mapping for the assembly of *Mycobacterium marinum* lipooligosaccharides

Hansen, T.*; Ofman, T. P.*; Vlaming, J. G. C.*; Gagarinov, I. A.; van Beek, J.; Goté, T. A.; Tichem, J. A.; Ruijgrok, G.; Overkleeft, H. S.; Filippov, D. V.; van der Marel, G. A.; Codée, J. D. C.

Angew. Chem. Int. Ed. **2021**, *60*, 937–945.

*equal contribution

Structure-activity relationship studies of α-ketoamides as inhibitors of the phospholipase A and acyltransferase enzyme family

Zhou, J.*; Mock, E. D.*; Al Ayed, K.; Di, X.; Kantae, V.; Burggraaff, L.; Stevens, A. F.; Martella, A.; Mohr, F.; Jiang, M.; van der Wel, T.; Wendel, T. J.; Ofman, T. P.; Tran, Y.; de Koster, N.; van Westen, G. J. P.; Hankemeier, T.; van der Stelt, M.

J. Med. Chem. **2020**, *63*, 9340–9359.

Curriculum Vitae

Tim Ofman was born on October 1st, 1994, in Zaandam, The Netherlands. He attended high school at the Sint Michael College in Zaandam. In 2013, he graduated with the specialization “Natuur en Techniek”. His academic journey started at the Leiden University and Delft University of Technology, where he pursued a bachelor's degree in Molecular Science and Technology.

After the minor “Modern Drug Discovery”, he completed his major with a thesis on the synthesis of novel α- and β-glucosidase inhibitors bearing cycling C-phosphonates. This pivotal phase of his education was conducted under the guidance of dr. Sybrin Schröder, Prof. dr. Jeroen Codée and Prof. dr. Gijs van der Marel at the Bio-organic synthesis group in Leiden.

With his bachelor's degree in hand by 2016, Tim embarked on the Chemistry master's program at Leiden University, specializing in Chemical Biology. As part of this program, he performed a master’s internship at the Bio-organic synthesis group in Leiden under supervision of dr. Thomas Hansen, Prof. dr. Jeroen Codée and Prof. dr. Gijs van der Marel. Research focused on the total synthesis of LOS-IV fragments, a vital constituent of the cell wall of *mycobacterium marinum*. In the fall of 2018, he obtained his master’s degree (*cum laude*).

Subsequently, Tim transitioned into the next phase of academic exploration. Beginning in 2018, within the same group, he commenced the research presented in this Thesis, during which he was guided by Prof. dr. Hermen Overkleeft and Prof. dr. Jeroen Codée. Parts of the research described here were presented either as oral presentations or on posters at the Annual ABPP meeting 2019 in Leuven, Belgium, NWO Chains 2019 in Velthoven, The Netherlands, and the Annual ERC synergy symposium in Leiden, The Netherlands (2020 and 2022) and Barcelona, Spain (2022).

Tim is currently continuing his research in the group of Prof. dr. Hermen Overkleeft as a postdoctoral fellow.