

### Design and synthesis of next generation carbohydratemimetic cyclitols: towards deactivators of inverting glycosidases and glycosyl transferases

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### Citation

Ofman, T. P. (2024, March 28). *Design and synthesis of next generation* carbohydrate-mimetic cyclitols: towards deactivators of inverting glycosidases and glycosyl transferases. Retrieved from https://hdl.handle.net/1887/3729796

Version:	Publisher's Version
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Note: To cite this publication please use the final published version (if applicable).

# **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.<sup>[1]</sup> 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.<sup>[2,3]</sup> Moreover, specific glycans play key roles in many physiological processes by mediating cell-cell interaction and signal transduction processes amongst others.<sup>[1,4,5]</sup> 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<sup>[8]</sup> and are main factors in nutrient digestion and acquisition, protein post-translational modification and glycan turnover.<sup>[9,10]</sup>

Inhibitors, often designed to mimic the substrate structure of glycoprocessing enzymes (known as glycomimetics), have been widely used to study glycoprocessing enzymes.<sup>[11-</sup> <sup>23]</sup> 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.<sup>[16,21-24]</sup> 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.<sup>[12,13,22,25]</sup>

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 allcarbon six-membered ring motif is prominently featured in the natural mechanismbased retaining  $\beta$ -glucosidase inhibitor cyclophellitol, which has a remarkable structural similarity to  $\beta$ -glucose. Cyclophellitol adopts a half-chair conformation, resembling the transition state conformation that evolves during enzymatic hydrolysis of a  $\beta$ -glucosidic bond. Upon binding to a retaining  $\beta$ -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  $\beta$ -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.<sup>[26,27]</sup> 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.<sup>[28]</sup> The fundamental difference concerns the mechanistic itinerary displayed by these enzymes.



**Figure 1**. Catalytic itinerary of enzymatic hydrolysis of  $\beta$ -glucosides as mediated by  $\beta$ -glucosidases. **A**) In retaining  $\beta$ -glucosidases the first S<sub>N</sub>2 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  $\beta$ -glucose as the product. Both steps are mediated by the enzyme's catalytic acid/base residues (Asp/Glu). **B**) In inverting  $\beta$ -glucosidases, the aglycon is directly substituted in a single S<sub>N</sub>2 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  $\alpha$ -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).<sup>[29–31]</sup> 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  $\beta$ -glucosidase at hand) and concomitant S<sub>N</sub>2 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  $\beta$ -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).<sup>[32–35]</sup> 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.<sup>[32,36,37]</sup>

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.<sup>[28]</sup> 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  $\sigma^*$  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 leaving group  $\sigma^*$  (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  $\beta$ -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  $\alpha$ -glucosidases.  $[{}^{30,32,36-38]}$  In contrast, the conformational itinerary of inverting  $\beta$ -glucosidases is suggested to go through a  ${}^{2}S_{0} \rightarrow$ 

 $[^{2,5}B]^{\ddagger} \rightarrow {}^{4}C_{1}$  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.<sup>[45,46]</sup> Cyclophellitol is a potent irreversible inhibitor of retaining  $\beta$ -*exo*-glucosidases.<sup>[47]</sup> Upon binding in a transition-state mimicking <sup>4</sup>H<sub>3</sub> conformation to the active site of a retaining  $\beta$ -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  $\beta$ -*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.<sup>[48–50]</sup> 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<sup>[51]</sup> 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).<sup>[52]</sup> 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.*<sup>[51]</sup> started from sugar derivative **1A** and exploited an **1**,3-dipolar cycloaddition to build the carbacycle backbone. **B**) Fraser-Reid *et al.*<sup>[52]</sup> 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.*<sup>[53]</sup> 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, <sup>[53–56]</sup> all based on a common key synthetic transformation, as first exploited by Sato *et al*.<sup>[53]</sup> in their route towards cyclophellitol: the Ferrier carbocyclization (Figure 3C).<sup>[57]</sup> 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<sub>2</sub>, PdCl<sub>2</sub>) to neatly provide the desired carbocycles in high yields.<sup>[58]</sup> 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 (Dglucose, **1C**) into Ferrier precursor **3C**. The cyclic  $\alpha$ , $\beta$  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  $\alpha$ -D-glucopyranoside.<sup>[54]</sup>

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,<sup>[59]</sup> 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<sup>[60,61]</sup> 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<sub>3</sub>SiCH<sub>2</sub>MgCl, 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 1,7anhydro 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.*<sup>[62]</sup> 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 4position (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 1,7epoxide 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 coworkers in 1999.<sup>[63]</sup> 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.<sup>[64]</sup> Minor adjustments and modifications, which on the whole do not distort the scheme, have been made since.<sup>[21]</sup> 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.<sup>[65]</sup>



**Figure 5**. Synthetic scheme towards cyclophellitol **1** as reported by Madsen and co-workers,<sup>[64]</sup> 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.<sup>[66]</sup> The use of a smaller amount or the first-generation catalyst<sup>[67]</sup> led to lower yields and unidentified regioisomers.<sup>[21]</sup> 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.<sup>[11,13,15,16,19–21]</sup> In this way, inhibitors and probes targeting specific *endo*- or *exo*-glycosidases.<sup>[22,68]</sup> but also inhibitors to study broader ranges of glycosidases have been constructed and applied successfully.<sup>[24,69]</sup> 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.<sup>[70]</sup> 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  $\beta$ -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  $\alpha$ -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.

Chapter 1



**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  $\alpha$ - and  $\beta$ -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  $\alpha$ - and  $\beta$ -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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