Exploring chemical space in covalent and competitive glycosidase inhibitor design

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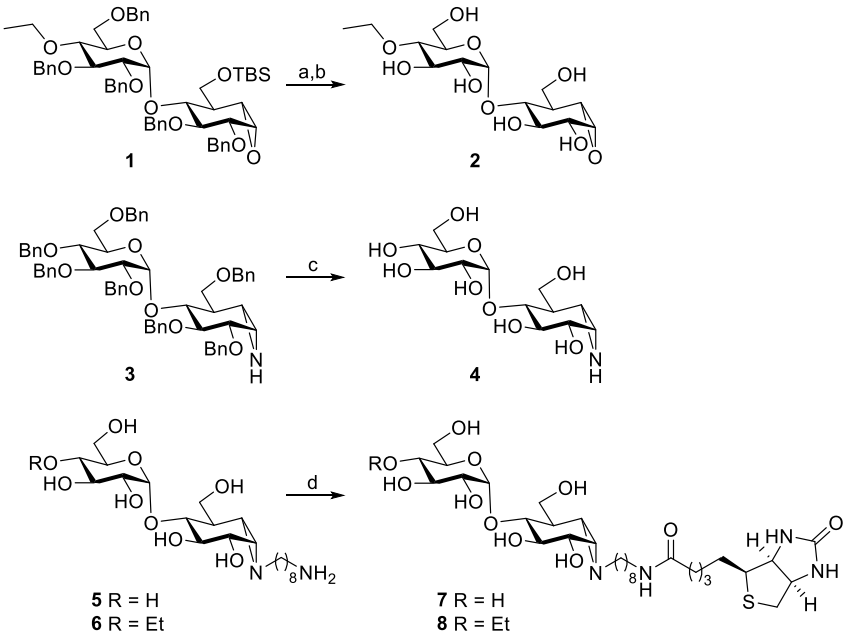
Summary and Future Prospects

The research described in this dissertation focus on the development and biochemical evaluation of covalent inhibitors and activity-based probes (ABPs) for retaining *endo*- and *exo*-glycosidases including starch-degrading enzymes and human lysosomal β-glucocerebrosidase (GBA), as well as the synthesis of a panel of uronic acid-type 1-*N*-iminosugars as potential competitive heparanase and β-glucuronidase inhibitors. The design of such covalent and competitive glycosidase inhibitors relies on the understanding of enzyme's function and mechanistic aspects. **Chapter 1** introduces the general mechanisms employed by retaining and inverting glycosidases during the hydrolysis of their substrates. The reaction itineraries of retaining α- and β-glucosidases are described and the design of covalent cyclophellitol-based inhibitors based on these itineraries discussed. Additionally, the design of competitive inhibitors based on transition state mimicry is described. Finally, an overview of the activity-based protein profiling (ABPP) workflow is presented.

The biodegradation of starch requires the synergistic action of a set of enzymes.[1] Amongst these, retaining α-amylases, which catalyze the hydrolysis of internal α-1,4-glycosidic linkages, are most extensively studied both in biomedicine[2] and biotechnology.[3] In **Chapter 2**, the synthesis of a set of *maltobiose*-configured *epi*-cyclophellitol derivatives as retaining α-amylase inhibitors and ABPs is presented. The key step in the synthesis route involves stereoselective α-1,4-glycosylation under proper pre-activation conditions, using acid-tolerant cyclohexene or *epi*-cyclophellitol cyclocarbonate acceptors. Following a series of chemical transformations the desired epoxide, aziridine and cyclosulfate warheads were obtained. Activities and efficiencies of the synthesized inhibitors and probes were substantiated by extensive biochemical analysis. Fluorescent labeling of recombinant human saliva α-amylase showed that the aziridine-based probe is less potent than the epoxide-based probe. This observation is in accordance with the kinetic studies on Taka-amylase where the aziridine inhibitor has the slowest rate of inhibition. X-ray crystallographic analysis of inhibitor-enzyme complexes showed that both epoxide and cyclosulfate inhibitors bind to the active site of Taka-amylase in a $^4C_1$ chair conformation, whilst the aziridine inhibitor adopts an unprecedented E_3 conformation, which might account for its lower potency. The epoxide probe was shown to be able to effectively label α-amylases in human saliva, murine tissue and fungal secretomes in a concentration-, pH-, time-, and temperature-dependent fashion, and the labeling could be competed with the non-tagged inhibitors, as well as the commercially available competitive inhibitor, acarbose. In addition, pull-down analysis with the biotinylated epoxide probe identified α-amylase in human saliva and fungal secretomes.

Starch polysaccharides are composed of linear amylose and branched amylopectin, the latter of which constitutes a major component of common starch.[4] In amylopectin, the branch points containing α-1,6-glucosidic linkages are resistant to the hydrolysis of α-1,4-specific amylases. As an extension of the maltobiose *epi*-cyclophellitol epoxide probes developed in Chapter 2, **Chapter 3** describes the synthesis of a panel of glucose-isomaltose (GIM) and isomaltose-glucose (IMG) configured *epi*-cyclophellitol probes, which mimic the branched parts of the amylopectin structure. Construction of the pseudotrisaccharide backbones was accomplished by stereoselective α-1,4- and α-1,6-glycosylations of cyclohexene acceptors under proper pre-activation conditions. After this, the epoxide functionality was introduced via a stereoselective epoxidation sequence, which was followed by global deprotection and amide-coupling with a reporter tag to afford the final probes after HPLC purification. The set of branched probes will be useful for the detection of industrially relevant starch-degrading enzymes that show preference for branched amylopectin-type polysaccharides.

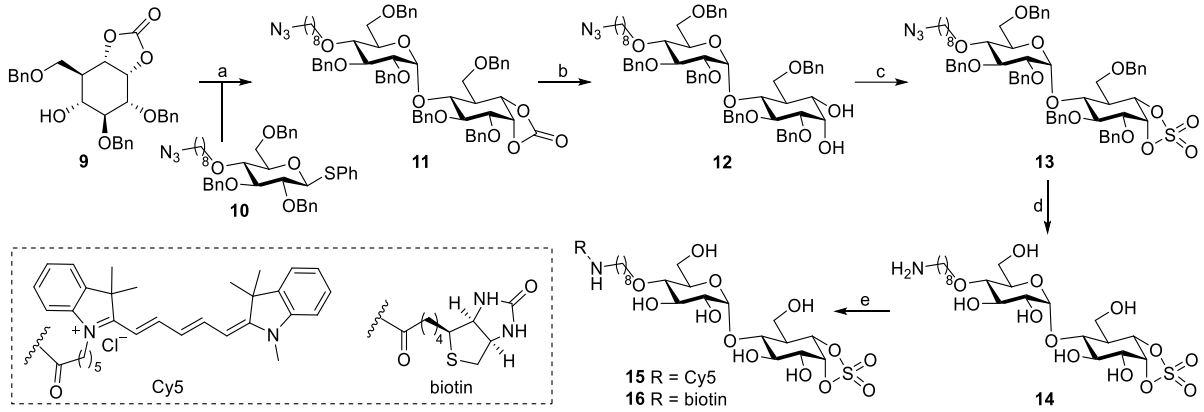
Besides the compounds presented in Chapter 2, several other pseudodisaccharide inhibitors and biotin probes were synthesized following similar strategies (Scheme 6.1). Desilylation of intermediate **1** (Chapter 2) followed by global debenzylation using Pearlman's catalyst under hydrogen atmosphere afforded O4' 'capped' inhibitor **2** in good yield. Preparation of aziridine inhibitor **4** was accomplished by deprotection of compound **3** (Chapter 2) under Birch debenzylation conditions. Amide coupling of the primary amine in **5** or **6** (Chapter 2) with biotin succinate ester (biotin-OSu) followed by HPLC purification provided maltobiose *epi*-cyclophellitol aziridine probes **7** and **8**.



Scheme 6.1. Synthesis of inhibitors **2** and **4**, and biotin probes **7** and **8**. Reagents and conditions: a) TBAF, THF, rt, 90%; b) $Pd(OH)_2/C$, H_2, $MeOH/H_2O$/dioxane (2/1/2), rt, 89%; c) Na, NH_3 (liq.), ^tBuOH, THF, -60 °C, 96%; d) biotin-OSu, DIPEA, DMF, rt, **7** 20%, **8** 44%.

Kinetic studies for the inhibition of Taka-amylase by the *maltobiose*-configured *epi*-cyclophellitols revealed that the cyclosulfate containing compound is 4-5 times more potent than its epoxide or aziridine counterparts while elongation at the nonreducing end O4' position of the maltobiose *epi*-cyclophellitol epoxide with an alkyl chain can increase its rate of inhibition nearly 10-fold (Chapter 2). Thus, it was envisioned that elongation at O4' of the maltobiose *epi*-cyclophellitol cyclosulfate inhibitor would yield more potent species. For this purpose, the corresponding maltobiose cyclosulfate probes bearing reporter entities at O4' position of the nonreducing end pyranose were synthesized (Scheme 6.2). The synthesis commenced with carbonate acceptor **9** (Chapter 2), which was glycosylated with O4-alkylated thiophenyl donor **10** (Chapter 2) under NIS/TfOH/DMF conditions, giving pseudodisaccharide **11** in good yield and stereoselectivity. The cyclic carbonate was then hydrolyzed under basic conditions affording diol **12**. Treatment of **12** with thionyl chloride resulted in the generation of a mixture of cyclosulfites, which was further oxidized to give cyclosulfate **13** in 88% yield over two steps. In the first instance, global debenzylation and concomitant azide reduction of **13** was attempted under Birch conditions which have been successfully applied for the deprotection of diverse cyclophellitol and cyclophellitol aziridine derivatives. However, in this case a complex mixture of products was obtained as indicated by NMR analysis (Table 6.1, entry 1). As an alternative, $Pd(OH)_2/C$ catalyzed hydrogenolysis was investigated. Treatment of **13** with an excess of Pearlman's catalyst in the presence of HOAc (10 eq.) for 16 h led to incomplete deprotection (entry 2 and 3). The azido group could be smoothly reduced to amine under these conditions while deprotection of the benzyl groups proved to be sluggish. Prolonged reaction time indeed led to the formation of desired product **14**, however an obvious side product of which the molecular weight is 94 Dalton less than that of **14** was detected by LC-MS analysis, indicating that the cyclosulfate ring may be hydrogenated as well to form a cyclohexane (entry 4). The slow rate of hydrogenolysis is attributed to catalyst poisoning by the emerging amine that has not been captured by the weak acetic acid (HOAc). This problem was circumvented by replacing HOAc with HCl to effectively capture the amine prior to catalyst poisoning. In this way the deprotection proceeded smoothly. Complete debenzylation was achieved after 5 h and the cyclosulfate functionality remained unaffected during the short reaction time, giving product **14** in good yield (entry 5). Amide coupling of the primary amine with the corresponding reporter tags gave ABPs **15** and **16** after HPLC purification eluting with

50 mM AcOH (note: HPLC purification by 50 mM NH_4HCO_3 resulted in nucleophilic opening of the cyclosulfate. See the experimental section for proposed structures and characterization of the rearranged products). Unfortunately, the fluorescent probe **15** was found unstable and decomposed during storage at -20 °C for months as revealed by LC-MS and NMR analyses, and thus could not be used for further ABPP experiments. In contrast, the biotin probe and 'untagged' inhibitors remain unaffected even after storing at -20 °C for more than one year.



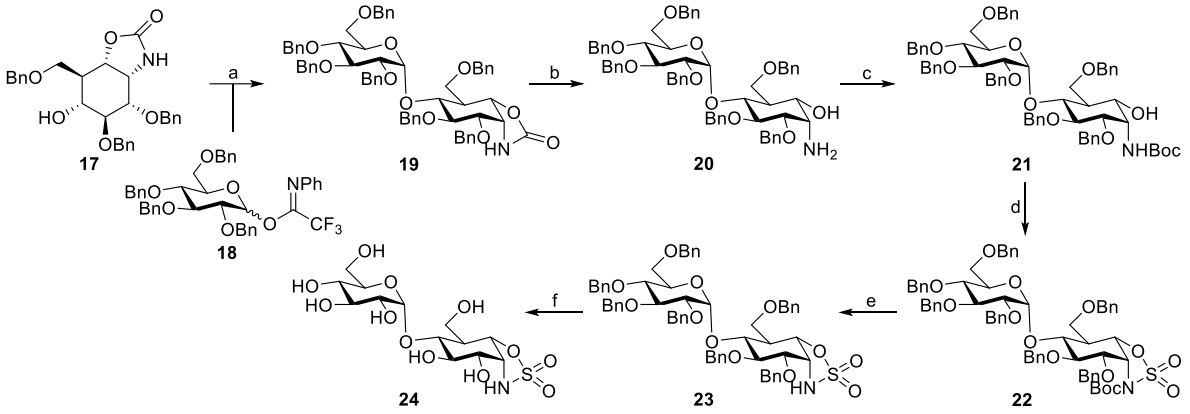
Scheme 6.2. Synthesis of maltobiose *epi*-cyclophellitol cyclosulfate probes. Reagents and conditions: a) NIS, TfOH, DMF, DCM, 4 Å MS, -20 °C to 0 °C, 90%; b) NaOMe, DCM/MeOH (1/1), rt, 94%; c) *i*) $SOCl_2$, TEA, DCM, 0 °C; *ii*) $RuCl_3.3H_2O$, $NaIO_4$, EtOAc/ACN/H_2O (2/2/1), 0 °C, 88%; d) $Pd(OH)_2/C$, H_2, HOAc, ^{t}BuOH/H_2O/dioxane (1/2/1), rt, 88%; e) for **15**: Cy5COOH, DIC, pentafluorophenol, Et_3N, DMF, rt, 11%; for **16**: biotin-OSu, DIPEA, DMF, rt, 17%.

Table 6.1. Azide reduction and global debenzylation of compound **13**.

Entry	Reagent	Solvent/Temperature	Time	Result
1	Na, ^{t}BuOH	NH_3 (liq.), THF, -60 °C	1 h	messy
2	$Pd(OH)_2/C$ (1.5 eq.), H_2 HOAc (10 eq.)	^{t}BuOH/H_2O/dioxane, rt (2/1/2, v/v/v)	16 h	azide→amine no benzyl deprotection
3	$Pd(OH)_2/C$ (1.5 eq.), H_2 HOAc (10 eq.)	^{t}BuOH/H_2O/dioxane, rt (1/2/1, v/v/v)	16 h	azide→amine partial debenzylatiom
4	$Pd(OH)_2/C$ (1.5 eq.), H_2 HOAc (10 eq.)	^{t}BuOH/H_2O/dioxane, rt (1/2/1, v/v/v)	2 days	product **14** (minor) cyclohexane side product (major)
5	$Pd(OH)_2/C$ (1.0 eq.), H_2 HCl (6 eq.)	^{t}BuOH/H_2O/dioxane, rt (1/2/1, v/v/v)	5 h	product **14** (88%)

Recently, α-D-Gal-cyclophellitol cyclosulfamidate has been developed as a Michaelis complex analog that stabilizes recombinant α-galactosidase A (α-GalA) both *in vitro* and *in*

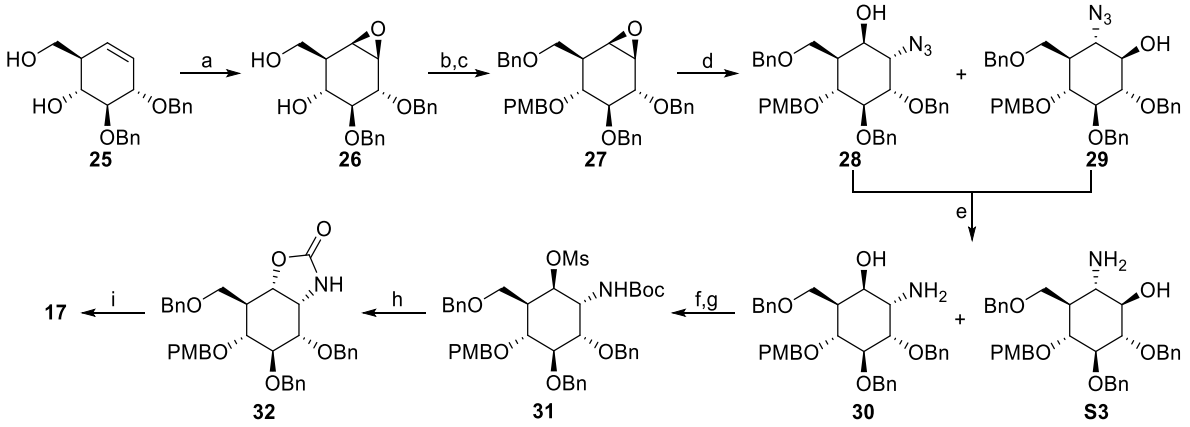
cellulo.[5] The design of this inhibitor is based on the replacement of the 'anomeric' oxygen of the parent cyclosulfate with a nitrogen atom. This replacement decreases its leaving group capacity, making the cyclosulfamidate a potent reversible inhibitor of α-GalA. It would be of interest to transfer the structural characteristic to *maltobiose*-configured *epi*-cyclophellitol cyclosulfate, which may provide a selective competitive inhibitor of retaining α-amylases. For this purpose, the maltobiose *epi*-cyclophellitol cyclosulfamidate was synthesized. The synthesis commenced with carbamate acceptor **17**, which was glycosylated with imidate donor **18** (Chapter 2) under TfOH/DMF pre-activation conditions, giving pseudodisaccharide **19**. Hydrolysis of the cyclic carbamate was accomplished by treatment with aqueous 1 M NaOH at elevated temperature to afford amino-alcohol **20**, of which the primary amine was protected with a *tert*-butyloxycarbonyl (Boc) group to provide **21**. Treatment of **21** with thionyl chloride followed by oxidation afforded fully protected cyclosulfamidate **22**. Removal of the Boc group with trifluoroacetic acid and subsequent global debenzylation using Pearlman's catalyst under hydrogen atmosphere provided final compound **24**.



Scheme 6.3. Synthesis of reversible maltobiose *epi*-cyclophellitol cyclosulfamidate inhibitor **24**. Reagents and conditions: a) TfOH, DMF, DCM, 4 Å MS, -20 °C to rt, 70%; b) 1 M NaOH, EtOH/THF (5/1), 80 °C, 95%; c) Boc_2O, Et_3N, DCM, rt, 95%; d) *i*) $SOCl_2$, TEA, imidazole, DCM, 0 °C; *ii*) $RuCl_3.3H_2O$, $NaIO_4$, EtOAc/ACN/H_2O (2/2/1), 0 °C, 90%; e) TFA, DCM, rt, 86%; f) $Pd(OH)_2/C$, H_2, MeOH/H_2O/dioxane (2/1/2), rt, quant.

Acceptor **17** was synthesized from published diol **25**[6] following adapted procedures from a paper previously reported by Artola *et al.* (Scheme 6.4).[5] Epoxidation of **25** with *meta*-chloroperoxybenzoic acid (*m*-CPBA) provided partially protected cyclophellitol **26** in 72% yield. The primary alcohol was selectively benzylated using 2-aminoethyl diphenylborinate as catalyst, followed by PMB protection of the remaining secondary alcohol to afford **27**. Nucleophilic opening of the epoxide with sodium azide in the presence of lithium perchlorate

at elevated temperature afforded an inseparable mixture of azido-alcohols **28** and **29**, which were directly reduced via platinum catalyzed hydrogenolysis resulting in the isolation of **30** in 46% yield. *N*-bocylation and subsequent mesylation of the secondary alcohol afforded **31**, which was heated to 120 °C in DMF to provide the cyclic carbamate **32** via intramolecular substitution of the mesylate group. The PMB ether was then removed by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to give acceptor **17** in good yield.



Scheme 6.4. Synthesis of acceptor **17**. Reagents and conditions: a) *m*-CPBA, DCM, 0 °C, 72%; b) BnBr, KI, K_2CO_3, 2-aminoethyl diphenylborinate, MeCN, 60 °C, 86%; c) PMBCl, NaH, TBAI, DMF, 0 °C to rt, 75%; d) NaN_3, $LiClO_4$, DMF, 80 °C; e) PtO_2, H_2, THF, rt, **30** 46%, **S3** 44% over 2 steps; f) Et_3N, Boc_2O, DCM, rt, 93%; g) MsCl, *N*-methyl imidazole, Et_3N, $CHCl_3$, rt; h) DMF, 120 °C, 71% over 2 steps; i) DDQ, DCM/H_2O (19/1), rt, 85%.

Next, the inhibitory potency of cyclosufamidate **24** and parent cyclosulfate **33** (Chapter 2) toward α-amylases in human saliva were investigated by competitive ABPP. Pre-incubation of human saliva samples with competitors for 30 min at 37 °C prior to labeling with 5 µM ABP **34** (Chapter 2) for 1 h resulted in a concentration-dependent competition of fluorescent labeling in the range of 0 to 500 µM (Figure 6.1A). While pre-incubation with **24** did not fully abrogate labeling, pre-incubation with **33** was able to fully compete the signal at concentrations higher than 10 µM. In order to evaluate whether the inhibition by **24** is reversible, human saliva samples were pre-incubated with inhibitors for different time periods (15, 30, 60, 120, 180 and 240 min), followed by labeling with probe **34** for 1 h (Figure 6.1B). Pre-incubation with covalent inhibitor **33** resulted in a decrease of fluorescent signal with longer inhibition time, whereas an almost constant intensity of the signal was observed by pre-incubation with compound **24** for extended inhibition times, indicating that cyclosulfamidate **24** may be a reversible inhibitor. To further confirm that **24** is indeed a non-covalent, active-center-directed,

inhibitor of α-amylase, kinetic studies and X-ray crystallographic analysis could be performed in the future.

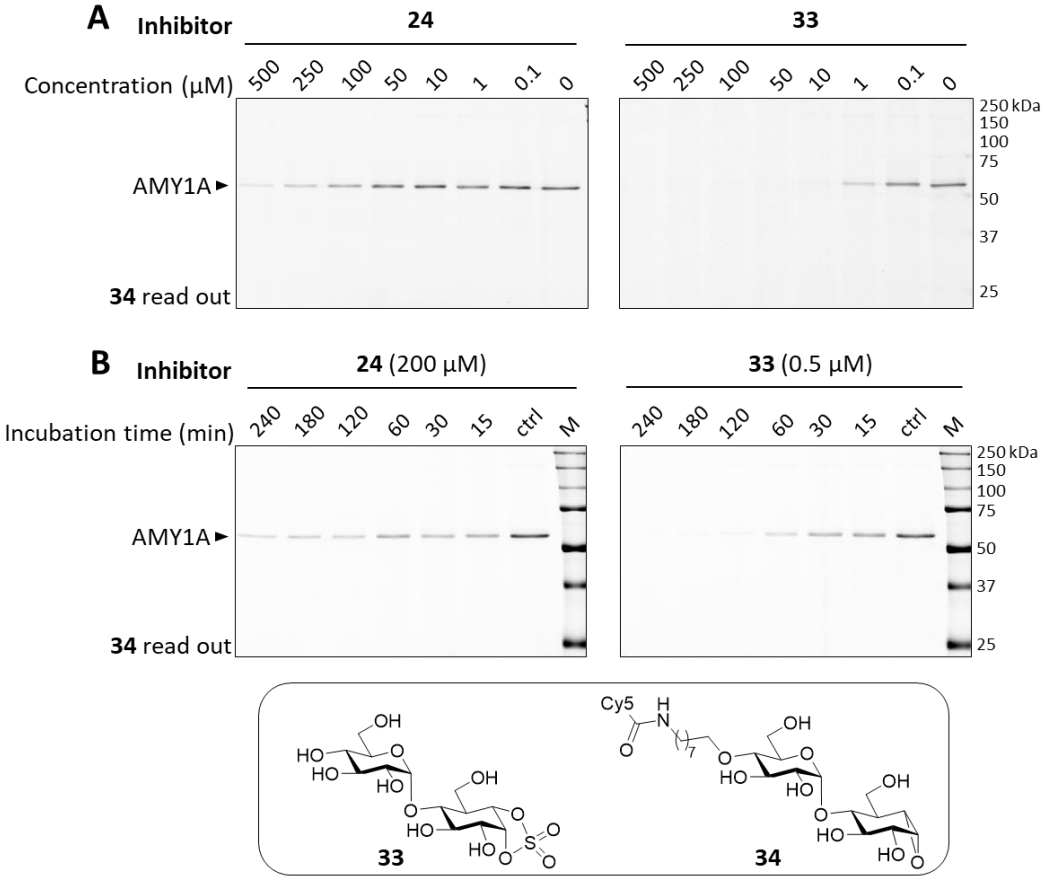
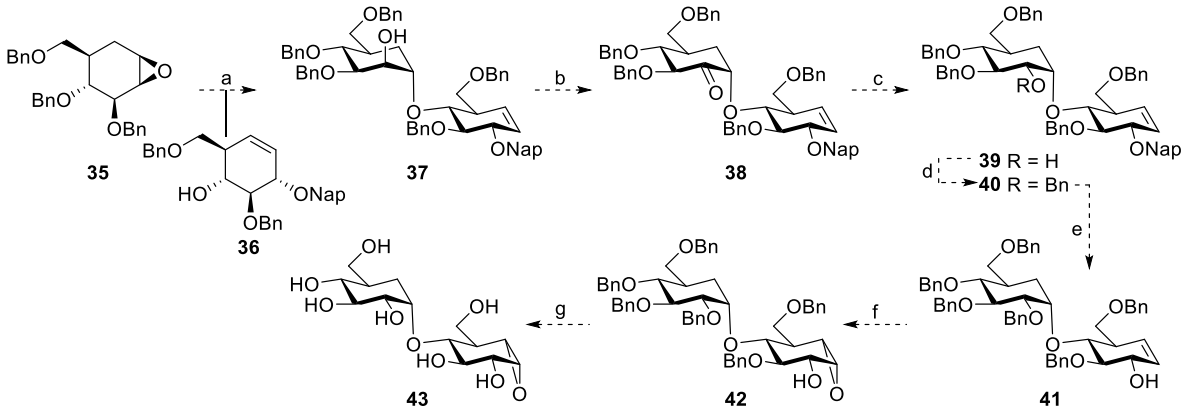


Figure 6.1. Competitive ABPP of α-amylase in human saliva with inhibitors **24** and **33**. A) The human saliva sample (10 μg total protein) was pre-incubated with **24** or **33** at varying concentrations (30 min, pH 5.0, 37 °C) prior to labeling with ABP **34** (5 μM, 1 h, pH 5.0, 37 °C); B) The human saliva sample (10 μM total protein) was pre-incubated with 200 μM of **24** or 0.5 μM of **33** for different time periods (pH 5.0, 37 °C) prior to labeling with ABP **34** (5 μM, 1 h, pH 5.0, 37 °C), "ctrl" means no pre-incubation with competitors.

The glycosidic linkage in an *endo*-acting 'disaccharide' inhibitor may be susceptible to hydrolysis by *exo*-glycosidases present in complex biological samples. One strategy to prevent the potential *exo*-glycosidase cleavage is to 'cap' O4 of the non-reducing end pyranose with an alkyl group (Chapter 2 and Chapter 3). An alternative strategy would be the substitution of the endocyclic oxygen of the non-reducing end pyranose with a methylene group to create stabilized *endo*-amylase inhibitors. For example, regioselective opening of epoxide **35**[7] with cyclohexene **36** (Chapter 3) in a *trans*-diaxial fashion under Lewis acid catalysis may provide alcohol **37** (Scheme 6.5). Oxidation of the hydroxy group with Dess-Martin periodate would afford C2-ulose derivative **38**. Sodium borohydride ($NaBH_4$) mediated reduction[8-10] of the

ketone may provide pseudodisaccharide **39** with a 1,2-*cis* linkage. Benzyl protection of the secondary hydroxy group in **39** followed by removal of the naphthyl group in **40** would afford allylic alcohol **41**. Stereoselective epoxidation of **41** could be achieved via an iodocarbonate intermediate to provide **42**, which after global debenzylation using Pearlman's catalyst under hydrogen atmosphere would provide stabilized *carba*-pseudodisaccharide **43**.



Scheme 6.5. Proposed synthetic scheme towards stabilized α-amylase inhibitor **43**. Reagents and conditions: a) $Cu(OTf)_2$, DCM; b) Dess-Martin periodinane, DCM; c) $NaBH_4$, MeOH/THF; d) BnBr, NaH, TBAI, DMF; e) DDQ, DCM/H_2O; f) *i*) Boc_2O, DMAP, THF; *ii*) NIS, AcOH, DCM; *iii*) NaOMe, MeOH, DCM; g) $Pd(OH)_2$/C, H_2, MeOH/H_2O/dioxane.

Chapter 4 describes the synthesis of a set of β-D-*gluco*-cyclophellitol aziridine inhibitors and probes, which are functionalized at both position C6 and aziridine nitrogen. X-ray crystallographic analysis revealed the structurally exclusive accommodation of the two functionalities by recombinant human β-glucocerebrosidase (rhGBA). Selectivity and potency of these bifunctional aziridines towards GBA were investigated both *in vitro* and *in situ*. The IC_{50} values for the inhibition of rhGBA showed that the new bifunctional cyclophellitol aziridine inactivators are around 10-15 times less potent than the corresponding C6-mono-functionalized cyclophellitol epoxides that were reported previously.[11] In addition, incubation of the bifunctional probes with mouse brain lysates indeed exhibited a selective labeling of GBA over GBA2, whilst a decreased selectivity was observed by *in situ* labeling of HEK293T cells containing endogenous GBA and overexpressed GBA2. While the bifunctional aziridines are less selective and potent than their mono-functionalized epoxide counterparts, they remain potent inactivators of GBA which can be used for the study of GBA in relation to Gaucher disease.

Siastatin B, a natural product originally isolated from a *Streptomyces* culture,[12] has previously been reported as a potent and effective inhibitor of β-D-glucuronidases.[13] However,

the molecular structure of siastatin B appears too bulky to be accommodated within a typical retaining β-glucuronidase active site pocket.[14-16] NMR analyses of trifluoroacetamide containing siastatin B derivatives have suggested that solvent mediated breakdown of these molecules can rapidly occur, releasing a hemiaminal/hydrated ketone that acts as the true inhibitor.[17,18] However, this rearrangement has not been demonstrated for siastatin B. In **Chapter 5**, X-ray crystallographic analysis of co-complexes between siastatin B and different enzymes including human heparanase (HPSE), *A. capsulatum* β-glucuronidase (AcGH79), *Burkholderia pseudomallei* (BpHEP), and *E. coli* β-glucuronidase (EcGusB) is presented, confirming that it is hemiaminal breakdown product **44** or hydrated ketone **45**, and not siastatin B, that binds to the enzyme active site (Figure 6.2). In order to understand the action of the breakdown products, a panel of *galacto*- and *gluco*-configured 1-*N*-iminosugar derivatives was synthesized. Preliminary structural analysis for enzyme inhibition by the synthetic *gluco*-configured iminosugars revealed that compound **49** binds to the active site of both HPSE and AcGH79 in a hydrated form, similar to **45** derived from siastatin B when soaked with HPSE. This result indicates that these synthetic iminosugars may be potential inhibitors of HPSE and β-D-glucuronidases. To evaluate the inhibitory properties of these compounds in depth, kinetic studies and competitive ABPP experiments can be performed in future studies.

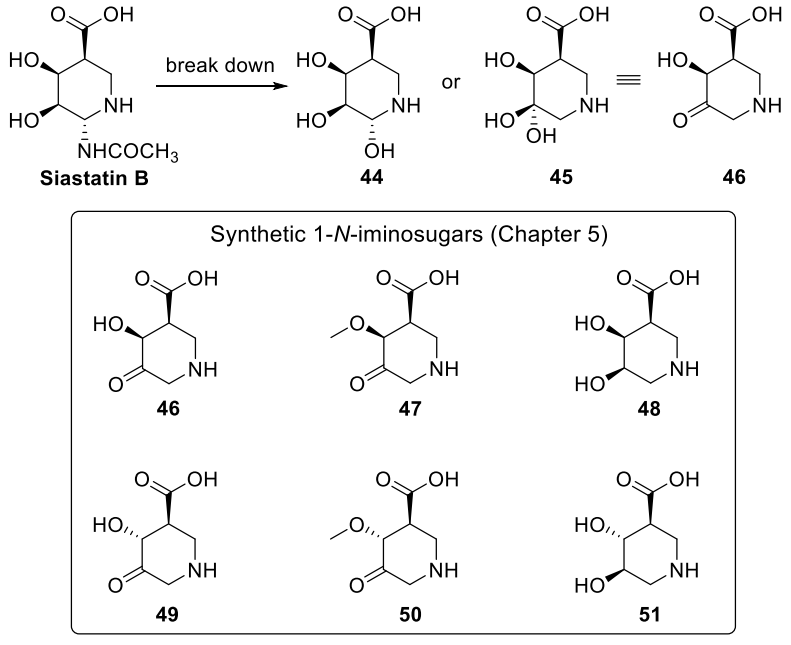


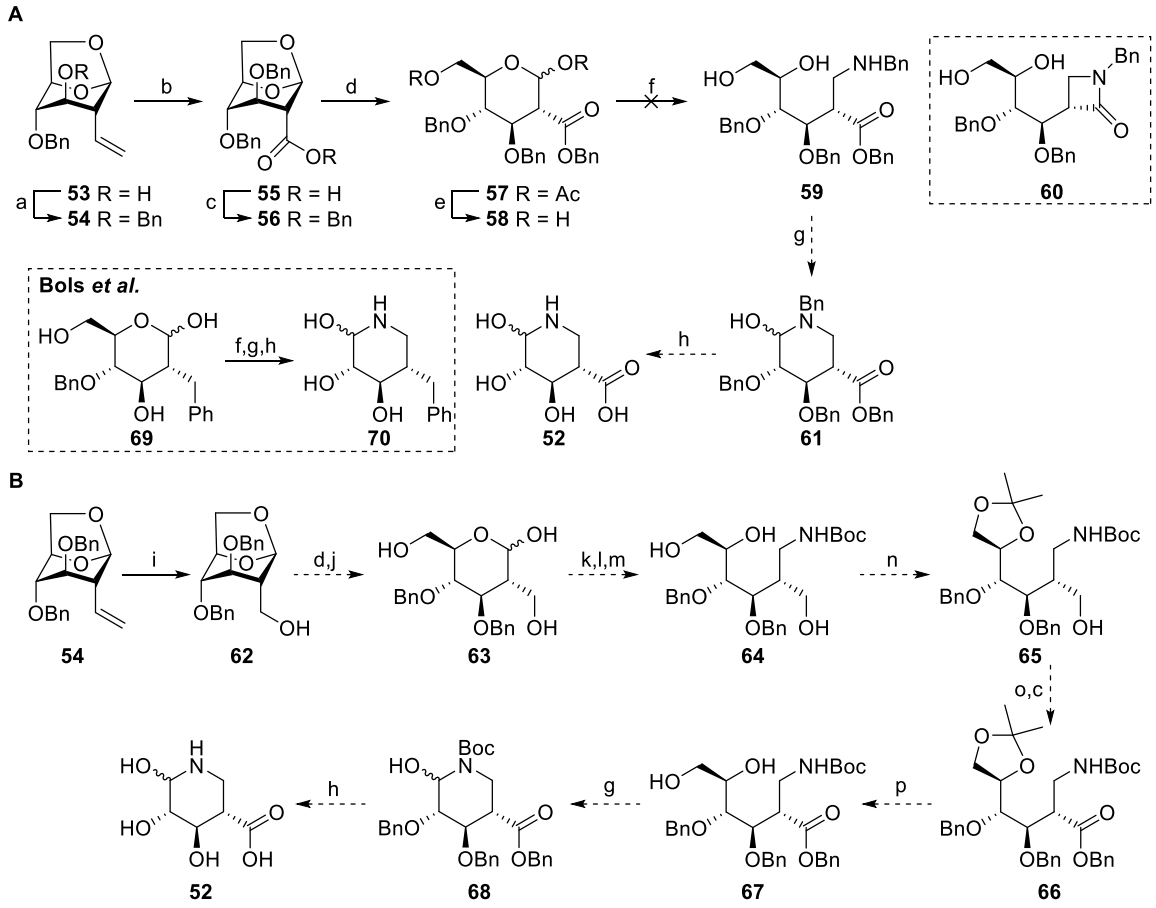
Figure 6.2. Chemical structures of siastatin B and its breakdown products as well as the synthetic *galacto*- and *gluco*-configured 1-*N*-iminosugar derivatives as described in chapter 5.

Since AcGH79, BpHEP and EcGusB all have hemiaminal **44** bound in the active site when treated with siastatin B, it would be of interest to synthesize compound **44** and its *gluco*-

configured analogue **52**. The synthesis of glucuronic acid-type noeuromycin **52** has been partially conducted (Scheme 6.6A). Compound **53** was prepared on a large scale from commercially available tri-*O*-acetyl-D-glucal according to reported procedures.[19,20] Benzyl protection of the secondary alcohol in **53** afforded compound **54**. Oxidative cleavage of the terminal alkene in **54** resulted in the formation of carboxylic acid **55**, which was condensed with benzyl alcohol under the activation of *N*,*N'*-diisopropylcarbodiimide (DIC), providing ester **56** in good yield. Direct hydrolysis of the 1,6-anhydride with aqueous 2 M HCl/dioxane (1/1, v/v) at elevated temperature (100 °C) for 4 h gave a complex mixture of products, presumably due to elimination of the 3-substituent.[21] In contrast, acetolysis[20] of the 1,6-anhydro bond in **56** was readily accomplished using trifluoracetic acid in acetic anhydride, providing diacetate **57** as a mixture of anomers (α/β = 1:2). Deacetylation of **57** could be promoted by *p*-toluenesulfonic acid (*p*-TsOH), affording lactol **58** in good yield. Transformation from **58** to product **52** was attempted by following the procedures reported by Bols and co-workers for the preparation of 6-deoxy-6-phenylnoeuromycin **70**.[21] The three-step sequence involves reductive amination of lactol **58** to provide vicinal diol **59**, followed by periodate cleavage to afford hemiaminal **61** which can be deprotected using Pearlman's catalyst under hydrogen atmosphere to provide final product **52**. However, reductive amination of **58** with benzylamine proved to be sluggish and an intractable mixture of products was obtained. This is attributed to the presence of the neighboring benzyl ester group which might be attacked intramolecularly by the amine to form lactam **60**, of which the molecular weight was detected by TLC-MS and LC-MS analyses.

Based on these results, it was envisioned that reductive amination of the lactol should be performed at an early stage. For this purpose, an alternative synthesis route was proposed (Scheme 6.6B). Oxidation of **54** with osmium tetroxide in the presence of sodium periodate gave the labile aldehyde, which was directly reduced with sodium borohydride to provide alcohol **62** in 84% yield over two steps. Acetolysis of the 1,6-anhydride followed by deacetylation would provide lactol **63**. Reductive amination of **63** with allylamine followed by deallylation using Wilkinson's catalyst[22] would provide a free amine intermediate which could be protected with *tert*-butyloxycarbonyl (Boc) group to afford **64**. The vicinal diol in **64** may then be regioselectively protected as an isopropylidene acetal. The remaining primary alcohol in **65** could be oxidized to a carboxylic acid, which is condensed with benzyl alcohol to afford ester **66**. Acidic hydrolysis of the isopropylidene acetal followed by periodate cleavage of the

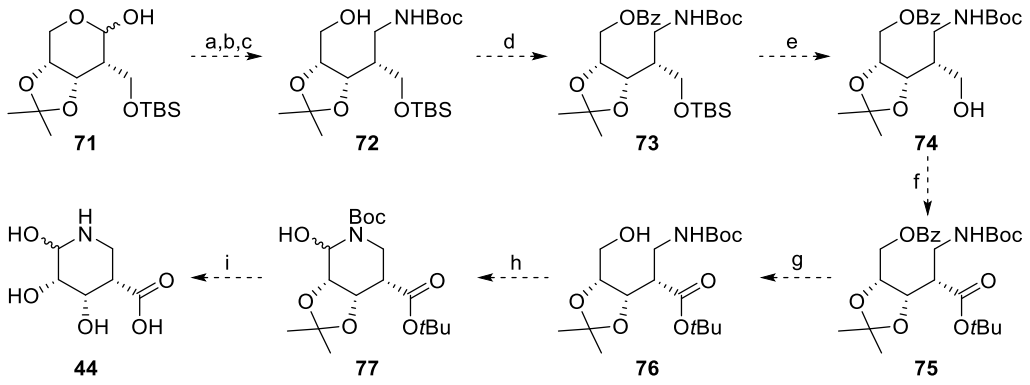
liberated vicinal diol in **67** would provide hemiaminal **68**, which after global deprotection under palladium catalyzed hydrogenolysis in the presence of HCl may provide product **52**.



Scheme 6.6. Proposed synthetic schemes for the preparation of glucuronic acid-type noeuromycin **52**. Regents and conditions: a) BnBr, NaH, TBAI, DMF, 0 °C to rt, 79%; b) $RuCl_3.3H_2O$, $NaIO_4$, EtOAc/ACN/H_2O (2/2/3), rt, 57%; c) BnOH, DIC, DMAP, DCM, 0 °C, 92%; d) TFA, Ac_2O, 0 °C, 93%; e) *p*-TsOH, THF/H_2O (9/1), 70 °C, 82%; f) $BnNH_2$, HOAc, $NaCNBH_3$, MeOH, 50 °C; g) $NaIO_4$, THF/H_2O, rt; h) Pd/C, H_2, HCl, EtOH/H_2O, rt; i) *i*) OsO_4, $NaIO_4$, dioxane/H_2O, rt; *ii*) $NaBH_4$, THF/isopropanol, 0 ^{0}C, 84% over two steps; j) NaOMe, MeOH; k) allylamine, HOAc, $NaCNBH_3$; l) $(Ph_3P)_3RhCl$, MeCN/H_2O, reflux; m) Boc_2O, TEA, DCM; n) *p*-TsOH, 2,2-dimethoxypropane; o) Jones reagent, acetone; p) 80% HOAc.

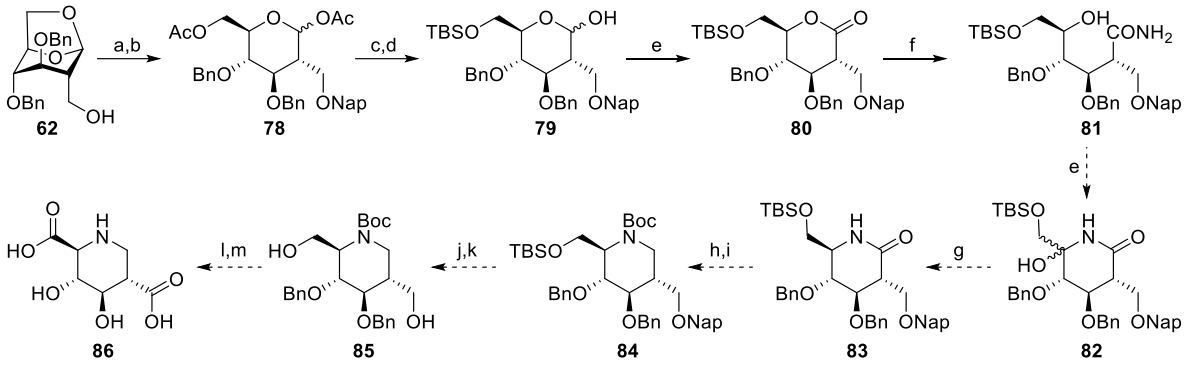
D-Galacturonic acid-type noeuromycin **44** can be prepared from known compound **71**[23] (Scheme 6.7). Reductive amination of **71** with allylamine followed by deallylation using Wilkinson's catalyst[22] and Boc-protection of the resulting free amine would afford alcohol **72**. Benzoylation of the primary hydroxy group in **72** followed by desilylation using tetrabutylammonium fluoride (TBAF) would provide compound **74**. Jones oxidation of the primary alcohol to the corresponding carboxylic acid followed by esterification with *tert*-butyl *N,N'*-diisopropylcarbamimidate in toluene may afford ester **75**. Debenzoylation of **75** under

Zémplen conditions would provide **76**. Alcohol **76** can then be oxidized with 2,2,6,6-tetramethylpiperidinyloxyl (TEMPO) and *meta*-chloroperbenzoic acid (*m*-CPBA) to provide hemiaminal **77**, which after global deprotection under acidic conditions may finally afford product **44**.



Scheme 6.7. Proposed synthetic scheme for the preparation of galacturonic acid-type noeuromycin **44**. Regents and conditions: a) allylamine, HOAc, $NaCNBH_3$; b) $(Ph_3P)_3RhCl$, $MeCN/H_2O$, reflux; c) Boc_2O, TEA, DCM; d) BzCl, Et_3N, DMAP, DCM; e) TBAF, THF; f) *i*) Jones reagent, acetone; *ii*) *tert*-butyl *N*,*N'*-diisopropylcarbamimidate, toluene; g) NaOMe, MeOH; h) TEMPO, *m*-CPBA, DCM; i) HCl, dioxane, H_2O.

Both compounds **44** and **52** may not be stable due to the presence of the 2-hydroxy group, which can undergo pH-dependent Amadori rearrangement[24,25] in aqueous solutions (Chapter 5). As a replacement, a 2-carboxy group can be introduced to increase the stability. The synthesis of the *gluco*-configured 2-carboxy derivative has been started (Scheme 6.8). The primary alcohol in **62** was protected as a naphthyl ether followed by acetolysis of the 1,6-anhydro bond to afford diacetate **78** as a mixture of anomers ($\alpha/\beta \approx 2:1$). Deacetylation under Zemplén conditions followed by selective protection of the primary alcohol with a *tert*-butyldimethylsilyl (TBS) group afforded lactol **79** in moderate yield. Oxidation of **79** under Albright-Goldman conditions[26] provided lactone **80**, which was treated with methanolic ammonium resulting in the isolation of hydroxy-amide **81** in 55% yield. To complete the synthesis several more steps could be executed. Dimethyl sulfoxide (DMSO)-acetic anhydride (Ac_2O) mediated oxidation of **81** may provide a diastereomeric mixture of hemiaminal **82**. Treatment of **82** with sodium cyanoborohydride under acidic conditions may allow the formation of **83**. Reduction of lactam **83** could be achieved by using borane-methyl sulfide complex[27] and the resulting amine may be protected with a Boc group to afford **84**. Removal of the naphthyl and silyl ether groups would provide diol **85**. Jones oxidation of the primary alcohols in **85** followed by global deprotection under palladium catalyzed hydrogenolysis conditions in the presence of HCl may finally provide inhibitor **86**.



Scheme 6.8. Proposed synthetic scheme for the preparation of **86**. Regents and conditions: a) NapBr, NaH, DMF, 0 °C to rt, 92%; b) TFA, Ac_2O, 0 °C, 80%; c) NaOMe, MeOH/DCM, rt; d) TBSCl, imidazole, DMAP, DCM, 0 °C to rt, 57% over two steps; e) DMSO, Ac_2O, 30 °C, 79%; f) 7 M NH_3 in MeOH, rt, 55%; g) $NaCNBH_3$, HCOOH, CH_3CN; h) BH_3Me_2S, DCM; i) Boc_2O, TEA, DCM; j) TBAF, THF; k) DDQ, DCM/H_2O; l) Jones reagent, acetone; m) Pd/C, H_2, HCl, dioxane/H_2O.

6.4 Experimental methods

6.4.1 Competitive ABPP experiments

Concentrated human saliva (10 µg total protein) was diluted with 150 mM McIlvaine buffer (pH 5.0, supplemented with 10 mM $CaCl_2$ and 10 mM NaCl) to a final 10 µL volume, pre-incubated with 2.5 µL inhibitor **24** or inhibitor **33** (diluted in McIlvaine buffer) at the indicated concentration at 37 °C for the indicated time period. These were followed by labeling with 2.5 µL ABP **34** (diluted in McIlvaine buffer) at a final ABP concentration of 5 µM at 37 °C for 1 h. Samples were then denatured with 4 µL sample buffer (5x Laemmli buffer, containing 50% (v/v) 1M Tris-HCl pH 6.8, 50% (v/v) glycerol, 10% (w/v) Dithiothreitol (DTT), 10% (w/v) sodium dodecyl sulphate (SDS), 0.01% bromophenol blue) and heated at 98 °C for 5 minutes. Proteins were resolved by electrophoresis in 10% SDS-PAGE gels, running at a constant of 90V for 30 minutes followed by 120V for 60 minutes. Wet slab gels were scanned on fluorescence using a Typhoon FLA9500 Imager (GE Healthcare) using λ_{EX} 635 nm; λ_{EM} > 665 nm and images were processed using ImageLab 5.2.1 (BioRad). Gels were subsequently extensively stained with Coomassie Brilliant Blue solution for assessing total protein amounts in each lane of sample.

6.4.2 Chemical synthesis

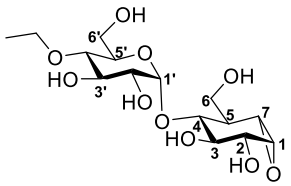
General experimental details

All reagents were of experimental grade and were used without further purification unless stated otherwise. Dichloromethane (DCM), tetrahydrofuran (THF) and toluene were stored over 3 Å molecular sieves and *N*,*N*-dimethylformamide (DMF) was stored over 4 Å molecular sieves, which were dried *in vacuo* before use. All reactions were performed under an Argon or N_2 atmosphere unless stated otherwise. Reactions were monitored by analytical thin layer chromatography (TLC) using Merck aluminum sheets pre-coated with silica gel 60 with detection by UV-absorption (254 nm) and by

spraying with a solution of $(NH_4)_6Mo_7O_{24}\cdot H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4\cdot H_2O$ (10 g/mL) in 10% sulfuric acid followed by charring at ~150 °C or by spraying with an aqueous solution of $KMnO_4$ (7%) and K_2CO_3 (2%) followed by charring at ~150 °C. Column chromatography was performed manually using Screening Device b.v. silica gel 60 (0.04-0.063 mm) in the indicated solvents. LC-MS analysis was performed on a LCQ Advantage Max (Thermo Finnigan) ion-trap spectrometer (ESI^+) coupled to a Surveyor HPLC system (Thermo Finnigan) equipped with a C18 column (Gemini, 4.6 mm x 50 mm, 5 µM particle size, Phenomenex). The applied buffers were A: H_2O, B: acetonitrile (MeCN) and C: 1% aqueous trifluoroacetic acid (TFA). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker AV-400 (400/101 MHz), Bruker AV-500 (500/126 MHz), and Bruker AV-850 (850/214 MHz) spectrometers in the given solvent. Chemical shifts (δ) are given in ppm relative to tetramethylsilane (TMS) as internal standard (^{1}H NMR in $CDCl_3$) or the residual signal of the deuterated solvent. Coupling constants (*J*) are given in Hz. All given ^{13}C-NMR spectra are proton decoupled. The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), Ar (aromatic), C_q (quaternary carbon). 2D NMR experiments (COSY, HSQC) were carried out to assign protons and carbons of the new structures. High-resolution mass spectrometry (HRMS) analysis was performed with a LTQ Orbitrap mass spectrometer (Thermo Finnigan), equipped with an electronspray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 mL/min, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150 – 2000) and dioctyl phthalate (m/z = 391.28428) as a "lock mass". The high-resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan).

Experimental Procedures and Characterization Data of Products

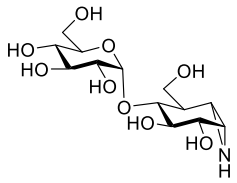
Compound 2



Compound **1** (108 mg, 0.116 mmol) was dissolved in dry THF (1.5 mL), TBAF (1.0 M in THF, 0.70 mL, 0.70 mmol) was added and the reaction was stirred at rt for 1 h. The reaction was quenched with sat. aq. NH_4Cl, extracted with DCM (2 x). The combined organic layers were washed with sat. aq. $NaHCO_3$, water and brine, dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (pentane/EtOAc 4:1→2:1) to afford the alcohol intermediate (85 mg, 0.10 mmol, 90%) as a white solid. The alcohol (29 mg, 35 µmol) was dissolved in a mixture of MeOH/H_2O/dioxane (2/1/2, 1 mL) under Argon and $Pd(OH)_2$/C (20 wt%, 38 mg, 53 µmol) was added. While stirring vigorously, the mixture was flushed with a H_2 balloon. After stirring for 3 h under H_2 atmosphere, the mixture was filtered over a small celite pad and evaporated to afford product **2** in high purity as a white solid (11.4 mg, 31.1 µmol, 89%) after lyophilization. ^{1}H NMR (400 MHz, MeOD) δ 5.01 (d, J = 3.9 Hz, 1H, H1'), 3.96 – 3.84 (m, 2H, H6a and 1CH*H* Et), 3.83 – 3.73 (m, 3H), 3.73 – 3.62 (m, 4H), 3.58 (dd, J = 9.8, 8.7 Hz, 1H), 3.44 (dd, J = 9.7, 3.9 Hz, 1H, H2'), 3.35 (d, J = 9.7 Hz, 1H, H4), 3.27 (dd, J = 4.0, 1.8 Hz, 1H, epoxide), 3.21 (d, J = 4.0 Hz, 1H, epoxide), 3.16 (t, J = 9.4

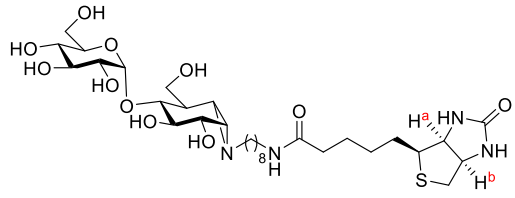
Hz, 1H), 2.04 (ddd, *J* = 9.1, 5.7, 3.0 Hz, 1H, H5), 1.18 (t, *J* = 7.0 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, MeOD) *δ* 103.1 (C1'), 83.0, 79.2, 75.1, 74.9, 74.0, 73.6, 72.7, 69.3 (CH_2 Et), 62.2 (C6'), 61.6 (C6), 57.9 (epoxide), 55.5 (epoxide), 45.2 (C5), 16.0 (CH_3). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{15}H_{26}O_{10}Na$ 389.1418, found 389.1422.

Compound 4



Ammonium (3.0 mL) was condensed in a dry flask at -60 °C under inert atmosphere. Sodium (50 mg, 2.2 mmol) was added and the resulting deep-blue solution was stirred for 15 min to dissolve all sodium completely. Aziridine **3** (30 mg, 31 µmol) and ^{t}BuOH (30 µL, 310 µmol) were taken up in dry THF (1 mL) and slowly added to the reaction mixture. After stirring for 1 h at -60 °C, the reaction was quenched carefully with H_2O. The mixture was allowed to warmed to rt and stirred until all ammonium had evolved. After sovent evaporation under reduced pressure the crude was re-dissolved in MilliQ-H_2O and eluted over a column packed with Amberlite CG-50 (NH_4^+) with 0.5M NH_4OH as eluent. The fractions containing product was concentrated *in vacuo*, affording compound **4** (10.1 mg, 29.7 µmol, 96%) as a white powder after lyophilization. ^{1}H NMR (500 MHz, D_2O) *δ* 5.12 (d, *J* = 4.0 Hz, 1H, H1'), 3.91 – 3.79 (m, 3H, H2, H6a and H6a'), 3.78 – 3.69 (m, 3H, H6b, H6b' and H5'), 3.69 – 3.63 (m, 1H, H3'), 3.59 – 3.52 (m, 2H, H3 and H2'), 3.42 – 3.33 (m, 2H, H4 and H4'), 2.57 (dd, *J* = 6.4, 3.5 Hz, 1H, H1), 2.36 (d, *J* = 6.4 Hz, 1H, H7), 2.02 (ddd, *J* = 9.4, 6.3, 3.0 Hz, 1H, H5). ^{13}C NMR (126 MHz, D_2O) *δ* 100.6 (C1'), 80.8, 74.0, 72.9 (C3'), 72.4 (C5'), 71.7, 70.9 (C2), 69.3, 61.6 (C6), 60.4 (C6'), 43.2 (C5), 35.4 (C1), 31.6 (C7). HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{13}H_{23}NO_9$ 360.1265, found 360.1267.

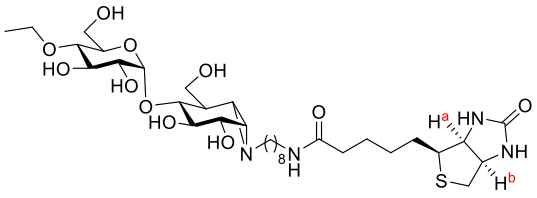
Compound 7



Compound **5** (4.6 mg, 10 µmol) was dissolved in dry DMF (0.5 mL). DIPEA (3.8 µL, 22 µmol) and biotin-OSu (3.8 mg, 11 µmol) were added and the mixture was stirred overnight at rt. Full conversion was observed by LC-MS analysis and the product was purified by semi-preparative reversed phase HPLC (linear gradient. Solution used: A: 50 mM NH_4HCO_3 in H_2O, B: MeCN). The fractions were concentrated under reduced pressure, co-evaporated with Milli-Q/MeCN (1/1, 3 x), dissolved in Milli-Q/^{t}BuOH (3/1) again and lyophilized to obtain product **7** (1.4 mg, 2.0 µmol, 20%) as a white powder. ^{1}H NMR (500 MHz, MeOD) *δ* 4.99 (d, *J* = 3.9 Hz, 1H, H1'), 4.50 (ddd, *J* = 8.0, 5.0, 0.9 Hz, 1H, H^b), 4.30 (dd, *J* = 7.9, 4.5 Hz, 1H, H^a), 3.89 (dd, *J* = 11.0, 3.0 Hz, 1H, H6a), 3.86 – 3.78 (m, 1H, H6a'), 3.70 (tdd, *J* = 7.3, 5.4, 3.6 Hz, 4H, H2, H5', H6b and H6'b), 3.61 (t, *J* = 9.3 Hz, 1H, H3'), 3.56 (dd, *J* = 9.9, 8.8 Hz, 1H, H3), 3.45 – 3.40 (m, 1H, H2'), 3.31-3.27 (m, 1H, H4'), 3.23 – 3.12 (m, 4H, H4, CHS and C*H*$_2$NHC=O), 2.93 (dd, *J* = 12.8, 5.0 Hz, 1H, CH*H*S), 2.71 (d, *J* = 12.7 Hz, 1H, CH*H*S), 2.35 (dt, *J* = 11.6, 7.2 Hz, 1H, CH*H*-N aziridine), 2.19 (t, *J* = 7.3 Hz, 2H, CH_2C=O), 2.19-

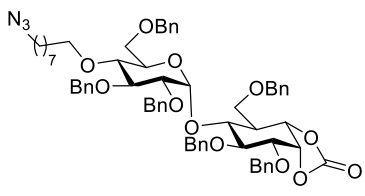
2.14 (m, 1H, CH*H*-N aziridine), 1.92 (ddd, *J* = 9.7, 6.6, 3.1 Hz, 1H, H5), 1.86 (dd, *J* = 6.5, 3.6 Hz, 1H, H1), 1.79 – 1.54 (m, 7H, H7 and 6CH*H* linker), 1.46 (dp, *J* = 23.1, 7.4 Hz, 4H, 4CH*H* linker), 1.34 (s, 8H, 8CH*H* linker). ^{13}C NMR (126 MHz, MeOD) δ 176.0, 103.2 (C1'), 84.1 (C4), 75.9 (C3), 75.0 (C3'), 74.4, 74.0 (C2'), 72.8, 71.5 (C4'), 63.4 (CH^{a}), 62.9 (C6), 62.7 (C6'), 62.2 (CH_2-N aziridine), 61.6 (CH^{b}), 57.0 (CHS), 45.9 (C1), 45.8 (C5), 42.2 (C7), 41.1 (CH_2S), 40.4 (CH_2NHC=O), 36.8 (CH_2NHC=O), 30.6, 30.5, 30.4, 30.3, 29.8, 29.5, 28.4, 28.0, 27.0 (9CH_2 linker). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{31}H_{55}N_4O_{11}S$ 691.3583, found 691.3581.

Compound 8



Compound **6** (6.5 mg, 13 µmol) was dissolved in dry DMF (0.5 mL). DIPEA (5.1 µL, 29 µmol) and biotin-OSu (5.4 mg, 16 µmol) were added and the mixture was stirred overnight at rt. Full conversion was observed by LC-MS and the product was purified by semi-preparative reversed phase HPLC (linear gradient. Solution used: A: 50 mM NH_4HCO_3 in H_2O, B: MeCN). The fractions were concentrated under reduced pressure, co-evaporated with Milli-Q/MeCN (1/1, 3 x), dissolved in Milli-Q/^{t}BuOH (3/1) again and lyophilized to obtain compound **8** (4.2 mg, 5.8 µmol, 44%) as a white powder. ^{1}H NMR (850 MHz, MeOD) δ 4.97 (d, *J* = 3.9 Hz, 1H, H1'), 4.50 (ddd, *J* = 7.9, 5.0, 0.9 Hz, 1H, H^{b}), 4.30 (dd, *J* = 7.9, 4.5 Hz, 1H, H^{a}), 3.92 – 3.85 (m, 2H, H6a and CH*H* Et), 3.77 (dd, *J* = 11.7, 2.0 Hz, 1H, H6'a), 3.73 – 3.62 (m, 6H, H2, H3', H5', H6b, H6'b and CH*H* Et), 3.55 (dd, *J* = 9.9, 8.8 Hz, 1H, H3), 3.42 (dd, *J* = 9.7, 3.9 Hz, 1H, H2'), 3.23 – 3.18 (m, 1H, CHS), 3.18 – 3.12 (m, 4H, H4, H4' and C*H*$_2$NHC=O), 2.93 (dd, *J* = 12.8, 5.0 Hz, 1H, CH*H*S), 2.71 (d, *J* = 12.7 Hz, 1H, CH*H*S), 2.34 (ddd, *J* = 11.6, 8.1, 6.3 Hz, 1H, CH*H*-N aziridine), 2.22 – 2.15 (m, 3H, CH_2C=O and CH*H*-N aziridine), 1.91 (ddd, *J* = 10.0, 6.9, 3.2 Hz, 1H, H5), 1.86 (dd, *J* = 6.4, 3.7 Hz, 1H, H1), 1.77 – 1.54 (m, 7H, H7 and 6CH*H* linker), 1.49 (h, *J* = 7.1 Hz, 2H, 2CH*H* linker), 1.46 – 1.41 (m, 2H, 2CH*H* linker), 1.38 – 1.30 (m, 8H, 8CH*H* linker), 1.18 (t, *J* = 7.0 Hz, 3H, CH_3). ^{13}C NMR (214 MHz, MeOD) δ 176.0, 166.1, 103.1 (C1'), 84.1, 79.1, 75.8 (C3), 75.1, 74.1 (C2'), 73.6, 72.7, 69.3 (CH_2 Et), 63.4 (CH^{a}), 62.9 (C6), 62.2 (C6'), 62.1 (CH_2-N aziridine), 61.6 (CH^{b}), 57.1 (CHS), 45.9 (C1), 45.8 (C5), 42.1 (C7), 41.1 (CH_2S), 40.4 (CH_2NHC=O), 36.8 (CH_2C=O), 30.6, 30.5, 30.4, 30.4, 29.8, 29.5, 28.4, 28.0, 27.0 (9CH_2 linker), 16.0 (CH_3). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{33}H_{59}N_4O_{11}S$ 719.3896, found 719.3892.

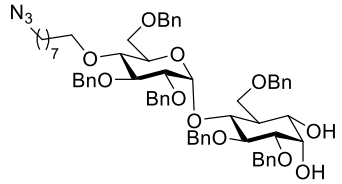
Compound 11



Donor **10** (313 mg, 0.45 mmol) was co-evaporated with toluene (3 x) and dissolved in dry DCM (9 mL) under nitrogen and stirred over fresh flame-dried 3 Å molecular sieves, after which DMF (0.55 mL, 7.2 mmol, 16 equiv. of donor) was added. After cooling to -20 °C, NIS (101 mg, 0.45 mmol) and TfOH (40 µL, 0.45 mmol) were added

successively. The reaction was pre-activated at -20 °C for 2 h. Then acceptor **9** (147 mg, 0.30 mmol) was added and the reaction mixture was placed in an ice bath. The mixture was stirred at 0 °C until TLC-analysis showed complete conversion of the acceptor. The reaction was then quenched with a mixture of sat. aq. $Na_2S_2O_3$ and sat. aq. $NaHCO_3$, stirred vigorously at rt until the brown color faded. The mixture was filtered over celite and diluted with DCM. The layers were separated and the organic layer was washed with water and brine, dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude was purified by size exclusion (DCM/MeOH = 1/1) giving the product as a single isomer (*dr* > 20/1, 300 mg) which was further purified by flash column chromatography (pentane/EtOAc 11:1 → 7:1) to obtain compound **11** (292 mg, 0.27 mmol, 90%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.16 (m, 28H, CH Ar), 7.15 – 7.06 (m, 2H, CH Ar), 5.11 (d, *J* = 3.6 Hz, 1H, H1'), 5.07 – 4.98 (t, *J* = 8.8 Hz, 1H, H7), 4.89 (d, *J* = 10.9 Hz, 1H, CH*H* Bn), 4.80 (d, *J* = 10.9 Hz, 1H, CH*H* Bn), 4.78 – 4.70 (m, 2H, CH*H* Bn and H1), 4.69 – 4.54 (m, 2H, CH*H* Bn), 4.56 – 4.37 (m, 4H, CH*H* Bn), 4.32 – 4.21 (m, 2H, CH*H* Bn), 4.17 (d, *J* = 11.5 Hz, 1H, CH*H* Bn), 3.98 – 3.81 (m, 5H, H2, H3, H4, H3' and H6a), 3.80 – 3.65 (m, 2H, CH*H*O linker and H5'), 3.63 (dd, *J* = 9.6, 2.5 Hz, 1H, H6b), 3.55 – 3.28 (m, 5H, H2', H4′, H6′ab and CH*H*O linker), 3.21 (t, *J* = 6.9 Hz, 2H, CH_2N_3), 2.79 (ddt, *J* = 11.8, 9.4, 2.4 Hz, 1H, H5), 1.61 – 1.50 (m, 2H, 2CH*H* linker), 1.50 – 1.38 (m, 2H, 2CH*H* linker), 1.39 – 1.11 (m, 8H, 8CH*H* linker). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.8 (C=O), 138.9, 138.4, 138.1, 138.0, 137.2, 137.1 (6C_q Ar), 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.4 (30CH Ar), 95.2 (C1'), 82.2, 80.8, 79.6 (C2'), 77.7 (C4'), 75.6 (Bn), 74.0, 74.0 (C1), 73.8 (C7), 73.7 (Bn), 73.5 (Bn), 73.2 (Bn), 73.1 (CH_2O linker), 73.0 (Bn), 72.0, 71.5 (Bn), 71.1(C5'), 68.1 (C6'), 65.6 (C6), 51.5 (CH_2N_3), 41.3 (C5), 30.5, 29.5, 29.2, 28.9, 26.8, 26.2 (6CH_2 linker). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{64}H_{73}N_3O_{12}Na$ 1098.5087, found 1098.5083.

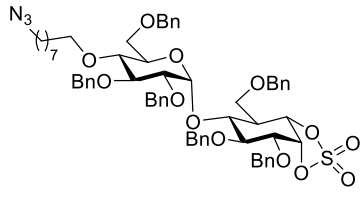
Compound 12



Compound **11** (284 mg, 0.26 mmol) was dissolved in a mixture of DCM/MeOH (1/1, 5.2 mL). NaOMe (30 wt% in MeOH, 30 µL, 0.16 mmol) was added and the reaction mixture was stirred at rt for 2 h. After which the reaction was diluted with DCM (5 mL) and neutralized with washed Amberlite IR-120 H^+ resin until pH ≈ 7. The mixture was filtered and the resin was washed with DCM (3 x 5 mL). The combined filtrates were concentrated *in vacuo* and the product was purified by flash column chromatography (pentane/acetone 9:1 → 6:1) to obtain compound **12** (260 mg, 0.244 mmol, 94%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.09 (m, 30H, CH Ar), 5.78 (d, *J* = 3.7 Hz, 1H, H1'), 4.97 (d, *J* = 11.9 Hz, 1H, CH*H* Bn), 4.85 (d, *J* = 10.9 Hz, 1H, CH*H* Bn), 4.76 (dd, *J* = 11.3, 2.0 Hz, 2H, CH*H* Bn), 4.63 – 4.39 (m, 7H, CH*H* Bn), 4.30 (d, *J* = 12.1 Hz, 1H, CH*H* Bn), 4.15 (t, *J* = 2.9 Hz, 1H), 4.04 (t, *J* = 9.0 Hz, 1H), 3.91 – 3.73 (m, 4H), 3.70 (ddt, *J* = 13.4, 10.0, 2.6 Hz, 3H), 3.50 – 3.37 (m, 5H), 3.36 – 3.30 (m, 1H, CH*H*O linker), 3.21 (t, *J* = 6.9 Hz, 2H, CH_2N_3), 3.00 (brs, 1H, OH), 2.64 (brs, 1H, OH), 2.37 – 2.25 (m, 1H, H5), 1.60 – 1.49

(m, 2H, 2CH*H* linker), 1.48 – 1.36 (m, 2H, 2CH*H* linker), 1.36 – 1.06 (m, 8H, 8CH*H* linker). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.0, 139.0, 138.2, 138.1, 138.1, 137.8 (6C_q Ar), 128.5, 128.4, 128.4, 128.3, 128.3, 128.1, 128.1, 128.0, 127.8, 127.8, 127.7, 127.6, 127.5, 127.5, 127.1, 126.5 (30CH Ar), 96.5 (C1'), 82.7, 82.0, 80.5, 79.4, 77.8, 75.4 (Bn), 73.9 (Bn), 73.6 (Bn), 73.4 (Bn), 73.1 (Bn), 73.0 (CH_2O linker), 72.5 (Bn), 71.2, 71.2, 69.7, 69.1, 68.4 (C6'), 67.7 (C6), 51.5 (CH_2N_3), 42.9 (C5), 30.5, 29.5, 29.2, 28.9, 26.8, 26.2 (6CH_2 linker). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{63}H_{75}N_3O_{11}Na$ 1072.5294, found 1072.5294.

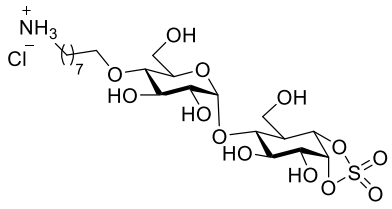
Compound 13



Compound **12** (0.28 g, 0.26 mmol) was dissolved in dry DCM (5 mL) and purged with nitrogen. After cooling to 0 °C, Et_3N (0.29 mL, 2.1 mmol) and thionyl chloride (57 µL, 0.78 mmol) were added successively. After stirring at 0 °C for 10 min, the reaction was diluted with DCM (100 mL), washed with sat. aq. $NaHCO_3$ (50 mL), H_2O (2 x 50 mL) and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* to give the crude cyclic sulfite as a yellow oil. The crude was directly dissolved in a mixture of EtOAc/MeCN (2.5 mL/2.5 mL) and cooled to 0 °C. A solution of $RuCl_3.3H_2O$ (0.5 M in H_2O, 5.2 µL, 2.6 µmol) and $NaIO_4$ (85 mg, 0.39 mmol) in H_2O (1.25 mL) was added and the mixture was stirred vigorously at 0 °C for 40 min. The reaction was quenched by addition of sat. aq. $Na_2S_2O_3$ (2 mL) and stirred vigorously at rt for 15 min. Then the mixture was diluted with H_2O (50 mL) and extracted with EtOAc (2 x 80 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude was purified by flash column chromatography (pentane/acetone 15:1 → 13:1) affording compound **13** (0.26 g, 0.23 mmol, 88%) as a clean oil. (*Note: oxidation with $RuCl_3$ and $NaIO_4$ should be monitored every 10 min by TLC analysis. The reaction should be quenched as soon as the conversion of the sulfite is complete. Otherwise an over-oxidized side product would be formed due to prolonged reaction times*). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.16 (m, 28H, CH Ar), 7.14 – 7.04 (m, 2H, CH Ar), 5.24 (d, *J* = 3.5 Hz, 1H, H1'), 5.22 – 5.18 (m, 1H, H7), 4.99 (dd, *J* = 6.9, 3.5 Hz, 1H, H1), 4.85 (d, *J* = 10.9 Hz, 1H, CH*H* Bn), 4.77 (d, *J* = 10.9 Hz, 1H, CH*H* Bn), 4.72 – 4.59 (m, 3H, CH*H* Bn), 4.59 – 4.43 (m, 3H, CH*H* Bn), 4.40 (d, *J* = 11.5 Hz, 2H, CH*H* Bn), 4.32 (dd, *J* = 13.7, 11.8 Hz, 2H, CH*H* Bn), 3.96 – 3.83 (m, 5H, H2, H3, H4, H3' and H6a), 3.74 (dt, *J* = 9.2, 6.5 Hz, 1H, CH*H*O linker), 3.67 (dt, *J* = 9.8, 2.7 Hz, 1H, H5'), 3.57 (dd, J = 9.7, 2.4 Hz, 1H, H6b), 3.52 – 3.41 (m, 3H, H2', H4' and H6'a), 3.41 – 3.30 (m, 2H, H6'b and CH*H*O linker), 3.22 (t, *J* = 6.9 Hz, 2H, CH_2N_3), 2.86 (td, *J* = 12.1, 2.3 Hz, 1H, H5), 1.63 – 1.50 (m, 2H, 2CH*H* linker), 1.47 – 1.38 (m, 2H, 2CH*H* linker), 1.27 (ddt, *J* = 21.3, 11.0, 7.1 Hz, 8H, 8CH*H* linker). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.9, 138.3, 138.0, 137.9, 137.3, 137.2 (6C_q Ar), 128.7, 128.6, 128.6, 128.6, 128.5, 128.5, 128.4, 128.3, 128.1, 128.0, 128.0, 128.0, 127.9, 127.9, 127.7, 127.5, 127.4 (30CH Ar), 96.0 (C1'), 82.1, 81.0, 79.6 (C2'), 79.4 (C1), 78.3 (C7), 77.7 (C4'), 75.6 (Bn), 74.3, 73.8 (Bn), 73.6 (Bn), 73.6 (Bn), 73.3 (Bn), 73.1 (CH_2O linker), 72.5 (Bn), 71.5, 71.3 (C5'), 68.3 (C6'),

64.9 (C6), 51.6 (CH_2N_3), 41.9(C5), 30.6, 29.6, 29.3, 29.0, 26.8, 26.2 (6CH_2 linker). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{63}H_{73}N_3O_{13}SNa$ 1134.4756, found 1134.4761.

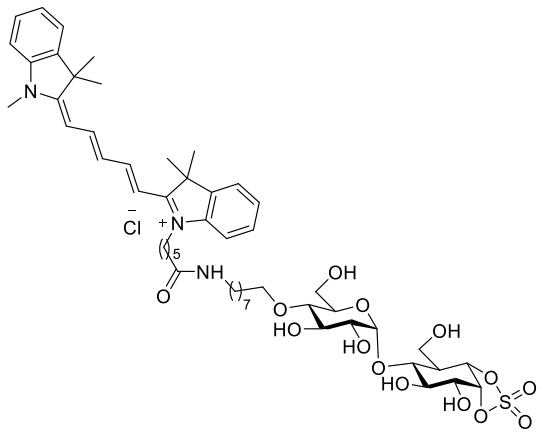
Compound 14



Compound **13** (64 mg, 58 µmol) was dissolved in a mixture of ^{t}BuOH/H_2O/dioxane (1/2/1, 2.4 mL). The solution was purged with nitrogen for 2 minutes under vigorous stirring. Then concentrated HCl (12 M, 28 µL, 0.34 mmol) and $Pd(OH)_2$/C (20 wt%, 64 mg) were added under nitrogen atmosphere. While stirring vigorously, the mixture was flushed with a H_2 balloon. After stirring for 5 h under H_2 atmosphere, the mixture was filtered over a small celite pad and evaporated to afford product **14** as a white solid (29.6 mg, 51 µmol, 88%) after lyophilization. ^{1}H NMR (500 MHz, D_2O) δ 5.48 (t, J = 3.8 Hz, 1H, H1), 5.31 – 5.28 (m, 2H, H1' and H7), 4.01 – 3.89 (m, 3H, H2, H3 and H6a), 3.86 – 3.72 (m, 6H), 3.70 – 3.55 (m, 3H, H2′, H4 and CH*H*O linker), 3.34 – 3.23 (m, 1H), 2.96 (t, J = 7.6 Hz, 2H, C*H*$_2$NH$_2$), 2.41 – 2.33 (m, 1H, H5), 1.60 (dt, J = 30.6, 7.2 Hz, 4H, 4CH*H* linker), 1.32 (d, J = 6.7 Hz, 8H, 8CH*H* linker). ^{13}C NMR (126 MHz, D_2O) δ 100.3 (C1'), 84.5 (C1), 82.2 (C7), 77.5, 76.4, 73.3 (CH_2O linker), 73.2, 72.9, 72.0, 71.9, 68.1, 60.2 (C6'), 56.5 (C6), 43.7 (C5), 39.6 (CH_2NH_2), 29.2, 28.2, 28.1, 26.7, 25.5, 25.1 (6CH_2 linker). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{21}H_{40}NO_{13}S$ 546.2215, found 546.2219.

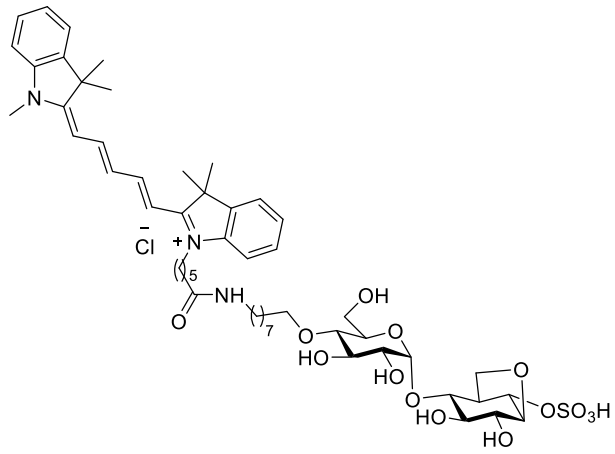
ABP 15 and proposed structure of the rearranged product

Cy5COOH (18 mg, 35 µmol) was dissolved in dry DMF (0.5 mL). 2,3,4,5,6-Pentaflurophenol (32 mg, 175 µmol), Et_3N (24 µL, 175 µmol) and DIC (8.1 µL, 52.5 µmol) were added successively and the mixture was stirred at rt for 3 h. Part of the stock solution (0.38 mL, 26.6 µmol) was added to the amine **14** (15.5 mg, 26.6 µmol) in dry DMF (0.2 mL) and Et_3N (3.7 µL, 26.6 µmol) was added. The reaction mixture was stirred at rt for 26 h until full conversion was observed by LC-MS analysis.



The crude was purified by semi-preparative reversed phase HPLC with linear gradient, solutions A: 50 mM HOAc in H_2O and B: CH_3CN. The fractions were concentrated under reduced pressure, co-evaporated with Milli-Q/MeCN (1/1, 3 x), dissolved in Milli-Q/^{t}BuOH (1/1) again and lyophilized to obtain compound **15** (3.3 mg, 3.2 µmol, 11%) as a blue powder. ^{1}H NMR (500 MHz, MeOD) δ 8.24 (t, J = 13.0 Hz, 2H), 7.49 (d, J = 7.4 Hz, 2H), 7.45 – 7.38 (m, 2H), 7.33 – 7.23 (m, 4H), 6.63 (t, J = 12.4 Hz, 1H), 6.28 (dd, J = 13.7, 4.5 Hz, 2H), 5.28 (t, J = 4.0 Hz, 1H, H1), 5.21 (dd, J = 10.1, 4.4 Hz, 1H, H7), 5.13 (d, J = 3.9 Hz, 1H, H1'), 4.10 (t, J = 7.5 Hz, 2H, CH_2N^+), 4.03 (dd, J = 11.5, 2.4 Hz, 1H, H6a), 3.91 – 3.78 (m, 3H, H3, H6'a and CH*H*O linker), 3.78 – 3.69 (m,

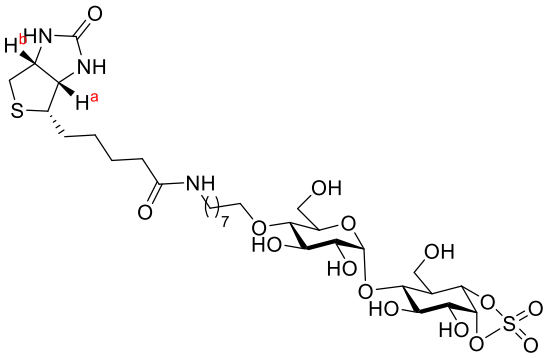
3H, H2, H3' and H5'), 3.68 – 3.60 (m, 5H, H6b, H6'b and CH_3N), 3.59 – 3.49 (m, 2H, CH*H*O linker and H4), 3.47 (dd, *J* = 9.8, 3.9 Hz, 1H, H2'), 3.14 – 3.10 (m, 3H, H4' and C*H*$_2$NHC=O), 2.23 – 2.14 (m, 3H, CH_2C=O and H5), 1.83 (q, *J* = 7.8 Hz, 2H, 2CH*H* linker), 1.73 (s, 14H, 4CH_3 and 2CH*H* linker), 1.61 – 1.41 (m, 4H, 4CH*H* linker), 1.40 – 1.20 (m, 10H, 10CH*H* linker). ^{13}C NMR (214 MHz, MeOD) δ 175.7, 175.4, 174.7, 155.5, 155.5, 144.3, 143.6, 142.7, 142.5, 129.8, 129.8, 126.6, 126.3, 126.3, 123.4, 123.3, 112.1, 111.9, 104.4, 104.3, 103.1 (C1'), 85.8 (C1), 82.8 (C7), 80.0 (C4), 79.4 (C4'), 75.2 (C3'), 74.7 (C3), 74.3 (C2'), 74.2 (C5'), 74.0 (CH_2O linker), 69.9 (C2), 62.4 (C6'), 56.8 (C6), 50.6 (C_q), 50.5 (C_q), 45.9 (C5), 44.8 (CH_2N^+), 40.4 (*C*H_2NHC=O), 36.4 (*C*H_2C=O), 31.4 (CH_2 linker), 30.7 (CH_3N), 30.5, 30.4, 30.3, 28.2 (4CH_2 linker), 28.0 (2CH_3), 27.9 (CH_2 linker), 27.8 (2CH_3), 27.4, 27.2, 26.6 (3CH_2 linker). LC-MS (ESI): R_t 6.03 min, linear gradient 30%→70% B in 10 min; m/z: $[M]^+$ calculated for $C_{53}H_{76}N_3O_{14}S$ 1010.50, observed 1010.83. HRMS (ESI) m/z: $[M]^+$ calculated for $C_{53}H_{76}N_3O_{14}S$ 1010.5043, found 1010.5049.



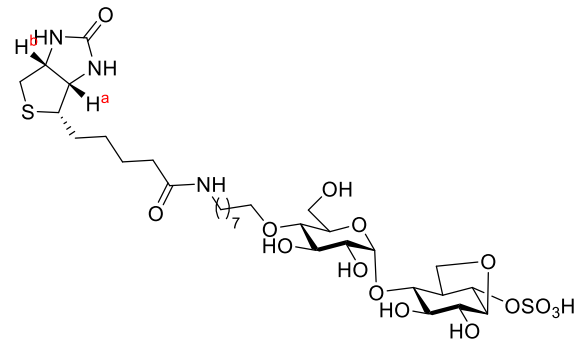
When the crude was purified by HPLC with linear gradient, solutions A: 50 mM NH_4HCO_3 in H_2O and B: CH_3CN, a rearranged product was obtained (2.1 mg, 2.0 µmol, 7%). ^{1}H NMR (500 MHz, MeOD) δ 8.24 (t, *J* = 13.0 Hz, 2H), 7.49 (dt, *J* = 7.4, 1.7 Hz, 2H), 7.45 – 7.38 (m, 2H), 7.32 – 7.23 (m, 4H), 6.65 (t, *J* = 12.4 Hz, 1H), 6.29 (dd, *J* = 13.7, 8.9 Hz, 2H), 4.91 (d, *J* = 3.8 Hz, 1H, H1'), 4.64 (dd, *J* = 8.9, 5.3 Hz, 1H, H1), 4.50 (d, *J* = 5.3 Hz, 1H, H7), 4.42 (t, *J* = 4.8 Hz, 1H, H3), 4.27 – 4.22 (m, 1H, H2,), 4.19 (t, *J* = 4.2 Hz, 1H, H4), 4.11 (t, *J* = 7.4 Hz, 2H, CH_2N^+), 3.88 – 3.78 (m, 2H, CH*H*O linker and H6'a), 3.77 – 3.68 (m, 2H, H5' and H3'), 3.67 – 3.58 (m, 5H, H6'b, NCH_3 and H6a), 3.56 – 3.49 (m, 2H, CH*H*O linker and H6b), 3.37 – 3.33 (m, 1H, H2'), 3.15 – 3.10 (m, 3H, H4' and C*H*$_2$NHC=O), 2.71 (td, *J* = 7.6, 3.7 Hz, 1H, H5), 2.21 (t, *J* = 7.2 Hz, 2H, CH_2C=O), 1.83 (t, *J* = 7.7 Hz, 2H, 2CH*H* linker), 1.73 (d, *J* = 0.7 Hz, 14H, 4CH_3 and 2CH*H* linker), 1.57 – 1.42 (m, 4H, 4CH*H* linker), 1.39 – 1.27 (m, 10H, 10CH*H* linker). ^{13}C NMR (214 MHz, MeOD) δ 175.8, 175.3, 174.7, 155.5, 144.3, 143.6, 142.7, 142.537, 129.8, 126.7, 126.2, 123.4, 123.3, 112.1, 111.9, 104.4, 100.0 (C1'), 83.9 (C7), 82.9 (C4), 79.2 (C4'), 76.5 (C3), 75.1 (C3'), 73.7 (C2'), 73.5 (CH_2O linker), 73.2 (C5'), 72.5 (C1), 72.3 (C2), 64.1 (C6), 62.3 (C6'), 50.6 (C_q), 50.5 (C_q), 45.4 (C5), 44.8 (CH_2N^+), 40.4 (*C*H_2NHC=O), 36.7 (*C*H_2C=O), 31.6 (NCH_3), 31.4, 30.5, 30.3, 30.3, 28.2 (5CH_2 linker), 28.0 (2CH_3), 27.9 (CH_2 linker), 27.8 (2CH_3), 27.3, 27.2, 26.6 (3CH_2 linker) ppm. LC-MS (ESI): R_t 5.31 min, linear gradient 30%→70% B in 10 min; m/z: $[M]^+$ calculated for $C_{53}H_{76}N_3O_{14}S$ 1010.50, observed 1010.17.

ABP 16 and proposed structure of the rearranged product

Compound **14** (9.6 mg, 16.5 µmol) was dissolved in dry DMF (0.5 mL). DIPEA (5.8 µL, 33 µmol) and biotin-OSu (8.5 mg, 25 µmol) were added and the reaction mixture was stirred at rt for 6 h until full conversion was observed by LC-MS analysis.



The crude was purified by semi-preparative reversed phase HPLC with linear gradient, solution A: 50 mM HOAc in H_2O and B: CH_3CN. The fractions were concentrated under reduced pressure, co-evaporated with Milli-Q/MeCN (1/1, 3 x), dissolved in Milli-Q/^{t}BuOH (1/1) again and lyophilized to obtain compound **16** (2.3 mg, 2.9 µmol, 17%) as a white powder. ^{1}H NMR (500 MHz, MeOD) δ 5.28 (dd, J = 4.4, 3.4 Hz, 1H, H1), 5.21 (dd, J = 10.1, 4.4 Hz, 1H, H7), 5.14 (d, J = 3.9 Hz, 1H, H1'), 4.49 (ddd, J = 7.9, 5.0, 0.9 Hz, 1H, H^b), 4.30 (dd, J = 7.9, 4.4 Hz, 1H, H^a), 4.04 (dd, J = 11.4, 2.3 Hz, 1H, H6a), 3.91 – 3.79 (m, 3H, H3, H6'a and CH*H*O linker), 3.78 – 3.70 (m, 3H, H2, H3' and H5'), 3.69 – 3.63 (m, 2H, H6b and H6'b), 3.61 – 3.49 (m, 2H, CH*H*O linker and H4), 3.47 (dd, J = 9.8, 3.9 Hz, 1H, H2'), 3.24 – 3.09 (m, 4H, CHS, H4' and CH_2NHC=O), 2.93 (dd, J = 12.8, 5.0 Hz, 1H, CH*H*S), 2.71 (d, J = 12.8 Hz, 1H, CH*H*S), 2.19 (dd, J = 9.1, 5.6 Hz, 3H, CH_2C=O and H5), 1.79 – 1.50 (m, 6H, 6CH*H* linker), 1.51 – 1.30 (m, 12H, 12CH*H* linker). ^{13}C NMR (126 MHz, MeOD) δ 176.0, 166.1 (2C=O), 103.0 (C1'), 85.8 (C1), 82.8 (C7), 79.8 (C4), 79.4 (C4'), 75.2 (C3'), 74.7 (C3), 74.2 (C2'), 74.2 (C5'), 74.0 (CH_2O linker), 70.0 (C2), 63.4 (CH^a), 62.4 (C6'), 61.6 (CH^b), 57.0 (CHS), 56.8 (C6), 45.9 (C5), 41.1 (CH_2S), 40.3 (*C*H_2NHC=O), 36.8 (CH_2C=O), 31.4, 30.5, 30.4, 30.3, 29.7, 29.5, 27.1, 27.1, 27.0 (9CH_2 linker). LC-MS (ESI): R_t 6.75 min, linear gradient 00%→50% B in 10 min; m/z: $[M+H]^+$ calculated for $C_{31}H_{54}N_3O_{15}S_2$ 772.30, observed 772.17. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{31}H_{54}N_3O_{15}S_2$ 772.2991, found 772.2995.

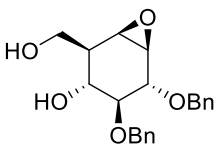


When the crude was purified by HPLC with linear gradient, solutions A: 50 mM NH_4HCO_3 in H_2O and B: CH_3CN, a rearranged product was obtained (1.8 mg, 2.3 µmol, 13%). ^{1}H NMR (850 MHz, MeOD) δ 4.68 – 4.60 (dd, J = 8.5, 5.1 Hz, 1H, H1), 4.52 – 4.48 (m, 2H, H^b and H7), 4.43 (t, J = 4.8 Hz, 1H, H3), 4.31 (dd, J = 7.9, 4.4 Hz, 1H, H^a), 4.25 (ddd, J = 9.0, 5.0, 1.5 Hz, 1H, H2), 4.20 (ddd, J = 5.0, 3.7, 1.5 Hz, 1H, H4), 3.85 (dt, J = 9.0, 6.4 Hz, 1H, CH*H*O linker), 3.81 (dd, J = 11.9, 2.2 Hz, 1H, H6'a), 3.75 – 3.70 (m, 2H, H3' and H5'), 3.66 (dd, J = 11.9, 4.9 Hz, 1H, H6'b), 3.62 (dd, J = 10.9, 7.4 Hz, 1H, H6a), 3.56 (dt, J = 9.1, 6.7 Hz, 1H, CH*H*O linker), 3.51 (dd, J = 11.0, 7.9 Hz, 1H, H6b), 3.37 (dd, J = 9.7, 3.9 Hz,

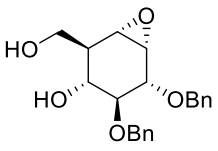
1H, H2'), 3.24 – 3.19 (m, 1H, CHS), 3.19 – 3.12 (m, 3H, C*H*$_2$NHC=O and H4'), 2.93 (dd, *J* = 12.8, 5.0 Hz, 1H, CH*H*S), 2.74 – 2.66 (m, 2H, CH*H*S and H5), 2.20 (td, *J* = 7.3, 1.7 Hz, 2H, CH$_2$C=O), 1.78 – 1.52 (m, 6H, 6CH*H* linker), 1.50 (t, *J* = 6.9 Hz, 2H, 2CH*H* linker), 1.46 – 1.42 (m, 2H, 2CH*H* linker), 1.42 – 1.32 (m, 8H, 8CH*H* linker). ^{13}C NMR (214 MHz, MeOD) δ 176.0, 166.2 (2C=O), 99.8 (C1'), 83.8 (C7), 82.5 (C4), 79.2 (C4'), 76.4 (C3), 75.1 (C3'), 73.6 (CH$_2$O linker), 73.6 (C2'), 73.2 (C5'), 72.5 (C1), 72.2 (C2), 64.1 (C6), 63.4 (CH^a), 62.2 (C6'), 61.6 (CH^b), 57.0 (CHS), 45.4 (C5), 41.1 (CH$_2$S), 40.4 (*C*H$_2$NHC=O), 36.8 (*C*H$_2$C=O), 31.4, 30.5, 30.4, 30.4, 29.8, 29.5, 27.9, 27.2, 27.0 (9CH$_2$ linker). LC-MS (ESI): R$_t$ 5.48 min, linear gradient 00%→50% B in 10 min; m/z: [M+H]$^+$ calculated for $C_{31}H_{54}N_3O_{15}S_2$ 772.30, observed 772.00.

Compound 26 and S1

Cyclohexene **25** (1.02 g, 3.00 mmol) was dissolved in dry DCM (30 mL) and cooled to 0 °C. *m*-CPBA (77 wt%, 4.03 g, 18.0 mmol) was added and the mixture was stirred at 0 °C for 2 days. The reaction was quenched by a mixture of sat. aq. $Na_2S_2O_3$ and sat. aq. $NaHCO_3$. The layers were separated and the aqueous layer was extracted with DCM (2 x). The combined organic layers were washed with sat. aq. $NaHCO_3$, H_2O and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The ratio of two isomers was determined as 1:8 (α/β) by crude ^{1}H NMR spectrum. Then the crude was purified by silica column chromatography (Pentane/EtOAc 7:1 → 3:1) to obtain β-epoxide **26** (770 mg, 2.16 mmol, 72%) and α-epoxide **S1** (86 mg, 0.24 mmol, 8%) as a white solid.

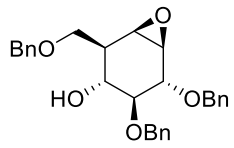


β-epoxide (**26**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 – 7.18 (m, 10H), 4.94 (d, *J* = 11.3 Hz, 1H), 4.81 (d, *J* = 11.4 Hz, 1H), 4.66 (dd, *J* = 11.3, 6.9 Hz, 2H), 4.00 (dd, *J* = 10.9, 6.7 Hz, 1H), 3.92 – 3.84 (m, 1H), 3.81 (dd, *J* = 8.0, 0.8 Hz, 1H), 3.47 (td, *J* = 9.8, 1.3 Hz, 1H), 3.38 (dd, *J* = 10.0, 7.9 Hz, 1H), 3.27 (dd, *J* = 3.9, 1.7 Hz, 1H), 3.15 (d, *J* = 3.7 Hz, 1H), 2.88 (d, *J* = 1.7 Hz, 1H), 2.72 (s, 1H), 2.19 – 2.10 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.3, 137.5, 128.7, 128.7, 128.6, 128.6, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 83.6, 79.5, 75.0, 72.7, 68.7, 64.1, 55.0, 53.1, 43.4. HRMS (ESI) m/z: [M+Na]$^+$ calculated for $C_{21}H_{24}O_5Na$ 379.1516, found 379.1510.



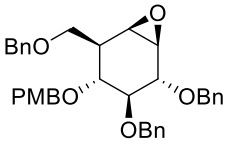
α-epoxide (**S1**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.18 (m, 10H), 5.00 (d, *J* = 11.2 Hz, 1H), 4.89 – 4.75 (m, 2H), 4.65 (d, *J* = 11.2 Hz, 1H), 3.99 – 3.77 (m, 3H), 3.58 (dd, *J* = 9.9, 8.0 Hz, 1H), 3.44 (t, *J* = 9.7 Hz, 1H), 3.37 (ddd, *J* = 3.9, 1.9, 0.6 Hz, 1H), 3.13 (d, *J* = 3.9 Hz, 1H), 2.76 (s, 1H), 2.19 (d, *J* = 4.0 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.4, 138.1, 128.8, 128.7, 128.6, 128.1, 128.1, 128.0 , 80.9, 79.6, 75.6, 72.2, 70.6, 63.0, 54.6, 54.2, 43.6. HRMS (ESI) m/z: [M+Na]$^+$ calculated for $C_{21}H_{24}O_5Na$ 379.1516, found 379.1514.

Compound S2



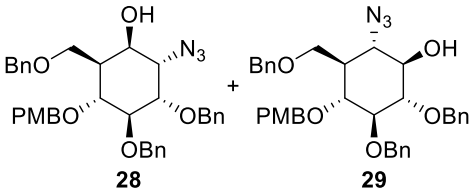
Compound **26** (693 mg, 1.94 mmol), 2-animoethyl diphenylborinate (44 mg, 0.19 mmol), KI (322.7 mg, 1.94 mmol) and K_2CO_3 (295 mg, 2.13 mmol) were dissolved in dry MeCN (20 mL). Then BnBr (346 µL, 2.91 mmol) was added. The reaction mixture was heated to 60 °C and stirred overnight. Most of the solvent was evaporated *in vacuo* and the residue was diluted with EtOAc, washed H_2O (2 x) and brine, dried over $MgSO_4$, filtrated, and concentrated *in vacuo*. The crude was purified by silica column chromatography (Pentane/EtOAc 11:1 → 7:1) to yield compound **S2** (744 mg, 1.67 mmol, 86%) as a yellow-white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 – 7.20 (m, 15H), 4.92 (d, J = 11.3 Hz, 1H), 4.81 (d, J = 11.4 Hz, 1H), 4.70 (d, J = 4.8 Hz, 1H), 4.68 (d, J = 4.8 Hz, 1H), 4.57 (d, J = 2.3 Hz, 2H), 3.89 (dd, J = 9.0, 5.0 Hz, 1H), 3.84 – 3.79 (m, 1H), 3.68 (t, J = 8.7 Hz, 1H), 3.43 (dd, J = 4.1, 1.7 Hz, 1H), 3.41 – 3.30 (m, 2H), 3.20 (d, J = 3.7 Hz, 1H), 2.72 (s, 1H), 2.26 (tdd, J = 8.7, 5.0, 1.8 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.5, 138.2, 137.6, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 127.8, 127.7, 127.7, 83.8, 79.4, 75.0, 73.6, 72.8, 70.0, 67.4, 54.9, 54.0, 42.2. HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{28}H_{30}O_5Na$ 469.1986, found 469.1982.

Compound 27



Compound **S2** (744 mg, 1.67 mmol) was dissolved in dry DMF (10 mL) and cooled to 0 °C. Then NaH (60% in mineral oil, 160 mg, 6.68 mmol) was added and the reaction mixture was stirred at 0 °C for 10 min and at rt for 20 min. After cooling to 0 °C again, 4-methoxybenzyl chloride (452 µL, 3.34 mmol) and TBAI (31 mg, 0.084 mmol) were added and the reaction mixture was stirred at rt for 4 h. The reaction was carefully quenched with H_2O at 0 °C, diluted with water, extracted with EtOAc (2 x). The combined organic layers were washed with water (2 x), brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/EtOAc 18:1 → 9:1) to obtain compound **27** (710 mg, 1.25 mmol, 75%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.20 (m, 15H), 7.09 – 7.01 (m, 2H), 6.82 – 6.74 (m, 2H), 4.84 (s, 2H), 4.80 – 4.66 (m, 3H), 4.51 (d, J = 2.6 Hz, 2H), 4.31 (d, J = 10.5 Hz, 1H), 3.85 (dd, J = 8.2, 0.8 Hz, 1H), 3.73 (s, 3H), 3.71 (d, J = 3.6 Hz, 1H), 3.59 – 3.49 (m, 2H), 3.45 (dt, J = 3.9, 1.0 Hz, 1H), 3.24 (t, J = 10.0 Hz, 1H), 3.17 (d, J = 3.7 Hz, 1H), 2.27 (dddd, J = 10.3, 8.7, 3.5, 1.6 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.2, 138.8, 138.3, 137.7, 130.4, 129.8, 128.5, 128.4, 128.3, 127.9, 127.9, 127.7, 127.7, 127.6, 127.6, 127.5, 113.8, 85.0, 79.8, 75.3, 75.0, 74.7, 73.2, 73.0, 68.6, 55.6, 55.2, 53.9, 42.5. HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{36}H_{38}O_6Na$ 589.2561, found 589.2558.

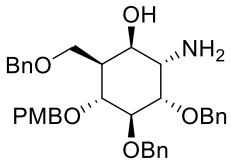
Compound 28 and 29



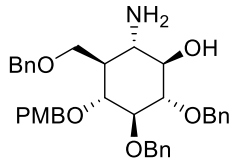
Compound **27** (671 mg, 1.18 mmol) was dissolved in dry DMF (15 mL) and cooled to 0 °C. NaN_3 (769 mg, 11.8 mmol) and $LiClO_4$ (2.50 g, 23.6 mmol) were added, then the mixture was heated to 80 °C and stirred overnight. After cooling to rt, the reaction was diluted with H_2O and extracted with EtOAc (2 x). The combined organic layers were washed with H_2O (3 x), brine, dried over $MgSO_4$, filtrated, concentrated *in vacuo*. Products **28** and **29** (680 mg) was obtained as an inseparable mixture and the crude was directly used for next step without further purification.

Compound 30 and **S3**

The inseparable mixture of compounds **28** and **29** (1.07 g, 1.76 mmol) was dissolved in anhydrous THF (35 mL) and purged with N_2 for 2 min under vigorous stirring. Then PtO_2 (0.16 g, 0.70 mmol) was added. While stirring vigorously, the mixture was flushed with a H_2 balloon. After stirring for 4 h under H_2 atmosphere, the reaction mixture was filtrated over celite and concentrated *in vacuo*. The crude residue was purified with silica column chromatography (DCM/MeOH 80:1 → 20:1) to obtain compound **30** (471 mg, 0.807 mmol, 46%) as a clean oil and compound **S3** (452 mg, 0.774 mmol, 44%) as a yellow-white solid.

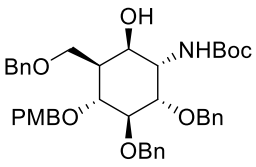


Amino alcohol **30**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.23 (m, 15H), 7.15 – 7.07 (m, 2H), 6.84 – 6.77 (m, 2H), 4.94 (d, J = 10.9 Hz, 1H), 4.84 (dd, J = 10.7, 8.7 Hz, 2H), 4.74 – 4.62 (m, 2H), 4.53 – 4.38 (m, 3H), 4.09 (dd, J = 9.1, 3.1 Hz, 1H), 4.02 (dd, J = 3.5, 2.1 Hz, 1H), 4.01 – 3.85 (m, 3H), 3.75 (s, 3H), 3.69 (dd, J = 9.1, 2.4 Hz, 1H), 3.47 (t, J = 3.6 Hz, 1H), 2.21 (dq, J = 10.9, 2.6 Hz, 1H), 1.69 – 1.07 (br s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.2, 139.3, 138.8, 137.4, 131.1, 129.6, 128.7, 128.4, 128.4, 128.1, 127.9, 127.8, 127.8, 127.6, 127.5, 113.8, 83.8, 80.7, 76.9, 75.6, 75.3, 75.0, 73.7, 72.6, 70.6, 55.3, 52.7, 40.9. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{36}H_{42}NO_6$ 584.3007, found 584.3004.



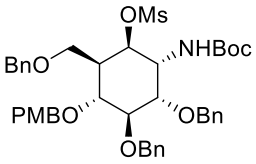
Amino alcohol **S3**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.23 (m, 15H), 7.15 – 7.08 (m, 2H), 6.87 – 6.77 (m, 2H), 4.97 – 4.84 (m, 3H), 4.80 (t, J = 10.5 Hz, 2H), 4.51 – 4.39 (m, 3H), 3.77 (s, 3H), 3.80 – 3.72 (m, 1H), 3.69 – 3.59 (m, 2H), 3.56 (t, J = 9.2 Hz, 1H), 3.38 (t, J = 9.1 Hz, 1H), 3.23 (t, J = 9.5 Hz, 1H), 2.79 (t, J = 10.4 Hz, 1H), 1.94 (brs, 3H), 1.38 (tt, J = 10.8, 2.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.3, 138.8, 128.7, 138.4, 130.8, 129.7, 128.7, 128.6, 128.6, 128.1, 127.9, 127.9, 127.8, 127.7, 113.9, 86.3, 83.9, 78.4, 76.8, 75.6, 75.5, 75.3, 73.1, 65.0, 55.4, 51.0, 46.9. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{36}H_{42}NO_6$ 584.3007, found 584.3002.

Compound S4



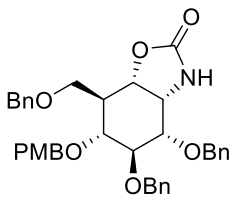
Compound **30** (471 mg, 0.807 mmol) was dissolved in anhydrous DCM (8 mL) and cooled to 0 °C. Then, Et_3N (563 µL, 4.04 mmol) and Boc_2O (212 mg, 0.968 mmol) were added at 0 °C and the reaction mixture was stirred overnight at rt. Next, the reaction was quenched with sat. aq. NH_4Cl and diluted with water. The aqueous layer was extracted with DCM (3 x) and the combined organic layers were washed with H_2O and brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/EtOAc 7:1 → 4:1) to obtain compound **S4** (512 mg, 0.749 mmol, 93%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.23 (m, 15H), 7.12 – 7.05 (m, 2H), 6.84 – 6.77 (m, 2H), 4.95 (d, J = 10.8 Hz, 1H), 4.86 – 4.73 (m, 3H), 4.66 (d, J = 11.1 Hz, 1H), 4.60 – 4.38 (m, 5H), 4.20 – 4.09 (m 2H), 4.03 (dd, J = 9.1, 3.2 Hz, 1H), 3.97 (dd, J = 11.3, 8.8 Hz, 1H), 3.78 (d, J = 3.7 Hz, 1H), 3.75 (s, 3H), 3.70 (dd, J = 9.2, 2.4 Hz, 1H), 3.54 (t, J = 9.0 Hz, 1H), 1.86 (d, J = 11.0 Hz, 1H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.2, 156.1, 139.0, 138.2, 137.3, 130.9, 129.6, 128.7, 128.5, 128.4, 128.2, 128.1, 127.9, 127.8, 127.8, 127.6, 113.9, 84.4, 79.8, 78.1, 76.1, 75.7, 75.2, 73.7, 72.1, 71.9, 70.1, 55.3, 51.9, 41.2, 28.4. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{41}H_{50}NO_8$ 684.3531, found 684.3526.

Compound 31



Compound **S4** (511 mg, 0.75 mmol) was dissolved in anhydrous $CHCl_3$ (7.5 mL) and cooled to 0 °C. Et_3N (521 µL, 3.75 mmol), *N*-methyl-imidazole (597 µL, 7.50 mmol) and MsCl (290 µL, 3.75 mmol) were added and the mixture was stirred at rt for 2 h. Then the reaction mixture was diluted with EtOAc, washed successively with diluted 1 M HCl solution, sat. aq. $NaHCO_3$, H_2O and brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The crude was directly used for next step without further purification.

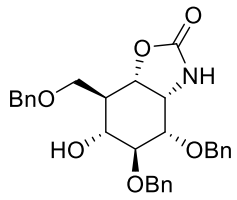
Compound 32



Crude compound **31** was dissolved in anhydrous DMF (37.5 mL) and the reaction was heated to 120 °C for 5 h until full conversion was observed by TLC-analysis. After cooling to rt, the reaction mixture was diluted with H_2O and extracted with EtOAc (2 x). The combined organic layers were washed H_2O (3 x), bine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/Acetone 9:1 → 4:1) to obtain carbamate **32** (329 mg, 0.53 mmol, 71% over 2 steps) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.26 (m, 15H), 7.13 – 7.08 (m, 2H), 6.84 – 6.78 (m, 2H), 5.22 (t, J = 5.5 Hz, 1H), 4.81 – 4.70 (m, 5H), 4.57 (d, J = 11.9 Hz, 1H), 4.53 – 4.41 (m, 3H), 4.05 (dd, J = 7.4, 4.4 Hz, 1H), 3.85 (dd, J = 9.2, 2.3 Hz, 1H), 3.81 (dd, J = 8.3, 7.4 Hz, 1H), 3.77 (s, 3H), 3.61 (dd, J = 9.2, 2.2 Hz, 1H), 3.56 (dd, J = 7.4, 4.3 Hz, 1H), 3.51 (dd, J = 11.6, 8.3 Hz, 1H), 2.14 (ddt, J = 11.5, 9.2, 2.2 Hz, 1H).^{13}C NMR (101 MHz, $CDCl_3$) δ 159.4, 158.9,

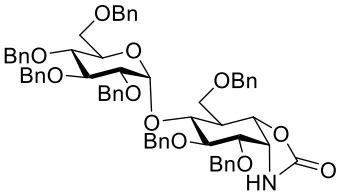
138.4, 138.1, 137.7, 130.6, 129.6, 128.7, 128.5, 128.5, 128.2, 128.1, 127.9, 127.9, 127.8, 113.9, 82.6, 77.4, 75.9, 74.7, 74.7, 74.2, 73.6, 73.5, 65.4, 55.4, 54.5, 44.6. HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{37}H_{39}NO_7Na$ 632.2619, found 632.2614.

Compound 17



Compound **32** (320 mg, 0.52 mmol) was dissolved in a mixture of DCM/H_2O (19/1, 10.6 mL). Then DDQ (144 mg, 0.64 mmol) was added and the reaction mixture was stirred at rt for 1.5 h. The reaction was diluted with DCM, washed with sat. aq. $NaHCO_3$ (3 x), H_2O and brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/Acetone 8:1 → 4:1) to obtain compound **17** (221 mg, 0.45 mmol, 85%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.24 (m, 15H), 5.61 (s, 1H), 4.88 (d, J = 11.3 Hz, 1H), 4.75 – 4.67 (m, 2H), 4.66 – 4.55 (m, 2H), 4.50 (d, J = 1.9 Hz, 2H), 4.08 (dd, J = 7.1, 4.2 Hz, 1H), 3.86 (dd, J = 9.3, 2.5 Hz, 1H), 3.69 – 3.50 (m, 4H), 2.78 (d, J = 2.4 Hz, 1H), 2.05 (ddt, J = 11.8, 9.2, 2.9 Hz, 1H. ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.0, 138.2, 138.1, 137.5, 128.8, 128.7, 128.5, 128.3, 128.1, 128.1, 128.0, 127.9, 127.7, 81.9, 77.8, 75.0, 74.0, 73.5, 73.3, 68.3, 66.3, 54.6, 44.7. HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{29}H_{31}NO_6Na$ 512.2044, found 512.2041.

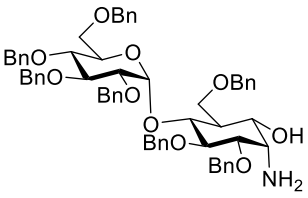
Compound 19



Imidate donor **18** (187 mg, 0.26 mmol) was co-evaporated with toluene (3 x) and dissolved in dry DCM (2.5 mL) under nitrogen and stirred over fresh flame-dried 3 Å molecular sieves, after which DMF (323 µL, 4.2 mmol) was added. The solution was cooled to -20 °C and TfOH (23 µL, 0.26 mmol) was added. After stirring at -20 °C for 1 h, the pre-activation was complete as indicated by TLC-analysis. Acceptor **17** (86 mg, 0.17 mmol) was dissolved with dry DCM (1.0 mL in total) and added to the solution. The reaction mixture was slowly warmed to rt and stirred at rt over-weekend. The reaction was then quenched with Et_3N, filtered and concentrated *in vacuo*. The crude was first purified by silica gel column chromatography (Pentane/EtOAc 7:1 → 2:1) to give an inseparable mixture of the product and unreacted acceptor. Then the mixture was further purified with size exclusion (MeOH/DCM = 1/1) to afford compound **19** (126 mg, 0.12 mmol, 70%) as a clean oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 – 7.19 (m, 29H), 7.16 (ddd, J = 7.6, 5.5, 2.7 Hz, 4H), 7.12 – 7.08 (m, 2H), 5.61 (s, 1H), 5.31 (d, J = 3.5 Hz, 1H), 4.95 – 4.85 (m, 2H), 4.81 (dd, J = 10.9, 4.5 Hz, 2H), 4.70 (d, J = 11.9 Hz, 1H), 4.65 (d, J = 12.0 Hz, 1H), 4.56 (d, J = 12.0 Hz, 1H), 4.48 (d, J = 12.1 Hz, 1H), 4.46 – 4.34 (m, 6H), 4.24 (d, J = 12.1 Hz, 1H), 4.01 – 3.91 (m, 3H), 3.89 – 3.83 (m, 2H), 3.79 (dt, J = 10.1, 2.5 Hz, 1H), 3.68 (dd, J = 10.1, 8.9 Hz, 1H), 3.65 – 3.59 (m, 2H), 3.55 (dd, J = 9.8, 3.5 Hz, 1H), 3.42 (dd, J = 10.8, 2.9 Hz, 1H), 3.30 (dd, J = 10.7, 2.0 Hz, 1H), 2.64 (ddt, J = 11.1, 8.9, 2.4 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.6, 138.8, 138.6, 138.4, 138.2, 138.0, 137.8, 137.6, 128.7, 128.5, 128.5,

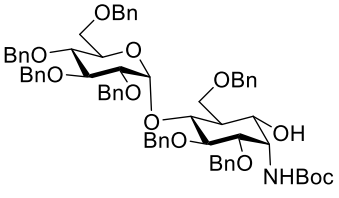
128.5, 128.4, 128.4, 128.4, 128.2, 128.2, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.6, 127.3, 95.8, 82.2, 81.4, 79.8, 77.8, 75.7, 75.5, 75.1, 74.6, 73.6, 73.5, 73.0, 72.8, 72.7, 72.2, 70.9, 68.2, 66.2, 52.6, 42.2. HRMS (ESI) m/z: $[M+NH_4]^+$ calculated for $C_{63}H_{69}N_2O_{11}$ 1029.4896, found 1029.4890.

Compound 20



Compound **19** (125 mg, 0.124 mmol) was dissolved in a mixture of EtOH/THF (5/1, 6 mL/1.2 mL). NaOH (1 M in H_2O, 1.8 mL, 1.8 mmol) was added and the reaction mixture was stirred at 70 °C for 8 h. TLC-analysis indicated the presence of starting material so more NaOH (1 M in H_2O, 1 mL) was added and the reaction mixture was further heated to 80 °C and stirred for another 7 h until full conversion was observed by TLC-analysis. The solvent was then evaporated *in vacuo* and the residue was diluted with EtOAc, washed with H_2O and brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (DCM/MeOH 100:0 → 50:1) to give compound **20** (116 mg, 0.118 mmol, 95%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.13 (m, 33H), 7.10 (dd, J = 6.9, 2.7 Hz, 2H), 5.76 (d, J = 3.6 Hz, 1H), 4.95 (d, J = 11.8 Hz, 1H), 4.87 (d, J = 10.8 Hz, 1H), 4.78 (dd, J = 10.9, 6.0 Hz, 2H), 4.72 (d, J = 11.8 Hz, 1H), 4.61 (d, J = 12.0 Hz, 1H), 4.57 – 4.48 (m, 4H), 4.41 (d, J = 11.4 Hz, 3H), 4.29 (d, J = 12.2 Hz, 1H), 4.02 (t, J = 8.5 Hz, 1H), 3.95 (t, J = 9.3 Hz, 1H), 3.84 (ddt, J = 8.3, 6.4, 3.2 Hz, 2H), 3.75 (dtd, J = 13.8, 10.1, 2.8 Hz, 3H), 3.65 (t, J = 9.4 Hz, 1H), 3.54 – 3.45 (m, 4H), 3.39 (dd, J = 10.7, 2.0 Hz, 1H), 2.29 (dp, J = 13.6, 4.3 Hz, 1H), 2.19 – 1.87 (brs, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.0, 138.9, 138.6, 138.2, 138.2, 138.1, 138.0, 128.5, 128.4, 128.4, 128.3, 128.2, 127.9, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.6, 127.4, 127.1, 126.6, 96.5, 82.0, 82.1, 81.1, 79.6, 77.9, 75.6, 75.0, 73.6, 73.6, 73.5, 72.9, 72.2, 71.9, 71.0, 69.3, 68.3, 68.2, 52.0, 42.8. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{62}H_{68}NO_{10}$ 986.4838, found 986.4830.

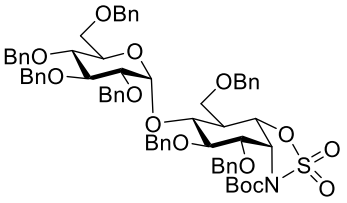
Compound 21



Compound **20** (116 mg, 0.118 mmol) was dissolved in dry DCM (2 mL) and cooled to 0 °C. Et_3N (82 µL, 0.59 mmol) and Boc_2O (31 mg, 0.14 mmol) were added at 0 °C and the mixture was stirred overnight at rt. Then the reaction was quenched with sat. aq. NH_4Cl, extracted with DCM (3 x). The combined organic layers were washed with sat. aq. $NaHCO_3$, H_2O and brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (Pentane/EtOAc 7:1 → 4:1) to give compound **21** (121 mg, 0.111 mmol, 95%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.12 (m, 33H), 7.12 – 7.04 (m, 2H), 5.47 – 5.37 (m, 1H), 5.23 (d, J = 6.8 Hz, 1H), 4.83 – 4.67 (m, 4H), 4.65 – 4.49 (m, 6H), 4.41 (d, J = 10.2 Hz, 4H), 4.27 (d, J = 12.2 Hz, 1H), 4.18 (s, 1H), 4.08 (t, J = 6.5 Hz, 1H), 4.00 (s, 1H, OH), 3.93 – 3.76 (m, 5H), 3.75 – 3.64 (m, 3H), 3.53 (ddd, J = 13.4, 10.2, 3.1 Hz, 2H), 3.42 (dd, J = 10.8, 1.9 Hz, 1H), 2.3

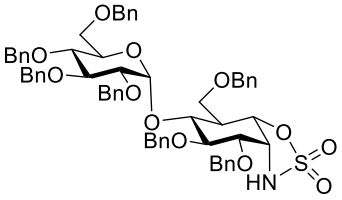
– 2.24 (m, 1H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.9, 138.7, 138.6, 138.2, 138.2, 138.0, 137.5, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.7, 127.6, 127.6, 127.4, 127.3, 127.2, 97.2, 82.6, 80.2, 79.8, 79.8, 78.3, 77.8, 75.6, 75.0, 73.5, 73.5, 72.9, 72.8, 72.2, 71.8, 70.9, 68.9, 68.2, 67.1, 50.5, 45.2, 28.5. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{67}H_{76}NO_{12}$ 1086.5362, found 1086.5355.

Compound 22



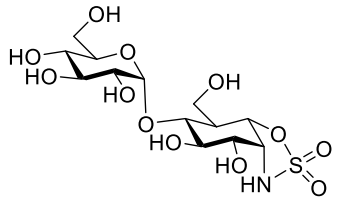
Compound **21** (121 mg, 0.111 mmol) was dissolved in dry DCM (2.0 mL) and purged with N_2. After cooling to 0 °C, Et_3N (163 µL, 1.17 mmol), imidazole (42 mg, 0.61 mmol) and thionyl chloride (49 µL, 0.66 mmol) were added successively. After stirring at 0 °C for 15 min, the reaction mixture was diluted with DCM, washed with diluted 1 M HCl solution, sat. aq. $NaHCO_3$, H_2O and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the crude sulfite as a pale-yellow oil. Then the crude was dissolved in a mixture of EtOAc/MeCN (1 mL/1 mL) and cooled to 0 °C. A solution of $RuCl_3.3H_2O$ (4 mg/mL stock solution in H_2O, 100 µL, 0.002 mmol) and $NaIO_4$ (35 mg, 0.16 mmol) in H_2O (0.4 mL) was added and the mixture stirred vigorously at 0 °C for 10 min. The reaction was quenched by addition of sat. aq. $Na_2S_2O_3$ (1 mL) and the mixture was stirred vigorously at rt for 15 min. Then the mixture was diluted with H_2O and extracted with EtOAc (2 x). The combined organic layers were washed with H_2O, brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (Pentane/Acetone, 15:1 → 11:1) to afford compound **22** (115 mg, 100 µmol, 90%) as a clean oil. (*Note: oxidation with $RuCl_3$ and $NaIO_4$ should be monitored every 10 min by TLC analysis. The reaction should be quenched as soon as the conversion of the sulfite was complete. Otherwise an over-oxidized side product would be formed due to prolonged reaction times*). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.07 (m, 35H), 5.24 (dd, *J* = 9.2, 7.4 Hz, 1H), 5.19 (d, *J* = 3.5 Hz, 1H), 4.88 (d, *J* = 10.9 Hz, 1H), 4.79 (dd, *J* = 10.9, 5.5 Hz, 2H), 4.72 (d, *J* = 11.8 Hz, 1H), 4.66 – 4.37 (m, 8H), 4.35 (t, *J* = 4.8 Hz, 1H), 4.24 (t, *J* = 12.6 Hz, 2H), 4.13 (d, *J* = 11.3 Hz, 1H), 4.01 – 3.81 (m, 4H), 3.78 – 3.63 (m, 2H), 3.57 (ddd, *J* = 15.3, 9.7, 3.0 Hz, 2H), 3.37 (dd, *J* = 10.8, 2.6 Hz, 1H), 3.25 (dd, *J* = 11.0, 1.9 Hz, 1H), 2.93 (ddt, *J* = 11.6, 9.0, 2.3 Hz, 1H), 1.48 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.6, 138.8, 138.5, 138.3, 138.0, 137.8, 137.4, 137.0, 128.6, 128.5, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 128.0, 128.0, 127.9, 127.9, 127.7, 127.7, 127.6, 127.6, 127.2, 95.0, 85.4, 82.2, 81.8, 79.8, 77.6, 75.6, 75.6, 75.0, 73.6, 73.4, 72.8, 72.8, 72.7, 71.2, 71.0, 70.9, 67.9, 64.8, 56.4, 40.4, 28.0. HRMS (ESI) m/z: $[M+NH_4]^+$ calculated for $C_{67}H_{77}N_2O_{14}S$ 1165.5090, found 1165.5089.

Compound 23



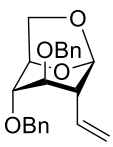
Compound **22** (105 mg, 91.5 µmol) was dissolved in dry DCM (4 mL). TFA (0.4 mL) was added and the mixture was stirred at rt for 2 h. Then the reaction was diluted with DCM, washed with sat. aq. $NaHCO_3$ (2 x), H_2O and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (Pentane/EtOAc, 11:1 → 7:1) to afford compound **23** (82 mg, 78 µmol, 86%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.16 (m, 31H), 7.12 (d, J = 6.7 Hz, 4H), 5.07 – 4.94 (m, 3H), 4.83 (dd, J = 22.3, 10.9 Hz, 2H), 4.69 (d, J = 11.1 Hz, 1H), 4.65 – 4.51 (m, 4H), 4.51 – 4.33 (m, 6H), 4.24 (dt, J = 11.4, 6.2 Hz, 1H), 4.17 – 3.98 (m, 4H), 3.87 (t, J = 10.0 Hz, 2H), 3.75 – 3.62 (m, 4H), 3.57 (dd, J = 9.9, 3.3 Hz, 1H), 3.49 (d, J = 10.5 Hz, 1H), 2.52 (t, J = 10.3 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.6, 138.3, 138.3, 137.9, 137.8, 137.1, 136.9, 128.8, 128.7, 128.6, 128.5, 128.5, 128.5, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.5, 127.4, 97.7, 82.0, 79.7, 79.1, 77.8, 75.4, 75.3, 75.1, 75.1, 74.5, 73.7, 73.3, 72.2, 71.9, 71.8, 71.5, 68.3, 65.6, 53.4, 42.1. HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{62}H_{65}NO_{12}SNa$ 1070.4120, found 1070.4118.

Compound 24



Compound **23** (41 mg, 40 µmol) was dissolved in a mixture of MeOH/H_2O/dioxane (2/1/2, 4 mL) under Argon and $Pd(OH)_2$/C (20 wt%, 41 mg, 60 µmol) was added. While stirring vigorously, the mixture was flushed with a H_2 balloon. After stirring for 5 h under H_2 atmosphere, the mixture was filtered over a small celite pad and evaporated to afford the product in high purity as a white powder (15.8 mg, quant) after lyophilization. ^{1}H NMR (400 MHz, MeOD) δ 5.13 (d, J = 3.8 Hz, 1H, H1'), 4.95 (ddd, J = 10.3, 5.3, 1.2 Hz, 1H, H7), 4.36 (t, J = 5.0 Hz, 1H, H1), 4.06 (dd, J = 11.4, 2.3 Hz, 1H, H6a), 3.94 – 3.83 (m, 2H, H3 and H6'a), 3.78 (ddd, J = 9.9, 6.1, 2.2 Hz, 1H, H5'), 3.72 – 3.60 (m, 4H, H2, H6b, H3' and H6'b), 3.55 – 3.43 (m, 2H, H4 and H2'), 3.26 (t, J = 9.4 Hz, 1H, H4'), 2.27 – 2.16 (m, 1H, H5). ^{13}C NMR (101 MHz, MeOD) δ 103.0 (C1'), 81.5 (C7), 80.4 (C4), 75.0, 75.0, 74.8 (C3, C3' and C5'), 74.1 (C2'), 71.6 (C4'), 70.5 (C2), 62.8 (C6'), 60.8 (C1), 57.1 (C6), 45.7 (C5). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{13}H_{23}NO_{12}SNa$ 440.0833, found 440.0832.

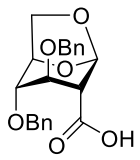
Compound 54



Compound **53** (2.49 g, 9.49 mmol) was dissolved in dry DMF (35 mL) and cooled to 0 °C. Then NaH (60% in mineral oil, 948 mg, 23.7 mmol) was added and the reaction mixture was stirred at 0 °C for 10 min and at rt for 20 min. After cooling to 0 °C again, BnBr (1.7 mL, 14.2 mmol) and TBAI (175 mg, 0.47 mmol) were added and the reaction mixture was stirred at rt for 2 h. The reaction was carefully quenched with H_2O at 0 °C, diluted with water and

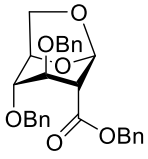
extracted with EtOAc (2 x). The combined organic layers were washed with water (2 x), brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/EtOAc 30:1 → 15:1) to obtain compound **54** (2.64 g, 7.50 mmol, 79%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.24 (m, 10H), 5.95 (dt, J = 17.2, 9.9 Hz, 1H), 5.37 (s, 1H), 5.20 – 5.09 (m, 2H), 4.63 – 4.51 (m, 4H), 4.47 (t, J = 11.7 Hz, 1H), 4.13 (d, J = 7.0 Hz, 1H), 3.73 (t, J = 6.4 Hz, 1H), 3.51 (t, J = 1.5 Hz, 1H), 3.39 (d, J = 1.9 Hz, 1H), 2.59 (d, J = 9.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.1, 137.9, 136.6, 128.6, 128.5, 127.9, 127.8, 127.8, 127.7, 117.4, 103.2, 76.8, 76.5, 74.2, 71.7, 71.1, 65.0, 48.7 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{22}H_{24}O_4Na$ 375.1567, found 375.1565.

Compound 55



Compound **54** (0.56 g, 1.6 mmol) was dissolved in a mixture of EtOAc/MeCN (10 mL/10 mL) and cooled to 0 °C. A solution of $RuCl_3.3H_2O$ (33 mg, 0.16 mmol) and $NaIO_4$ (1.7 g, 8.0 mmol) in H_2O (15 mL) was added at 0 °C and the mixture was stirred vigorously at rt for 1.5 h. The phases were separated and the organic phase was extracted with EtOAc (2 x 15 mL). To the combined organic layers was added isopropanol (1.0 mL) and the mixture was stirred at rt for 1 h. After which the mixture was washed with H_2O and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (DCM/EtOAc 12:1 → 4:1) to afford compound **55** (336 mg, 0.908 mmol, 57%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.47 (brs, 1H), 7.38 – 7.23 (m, 10H), 5.93 (s, 1H), 4.66 – 4.55 (m, 3H), 4.44 (dd, J = 12.2, 9.8 Hz, 2H), 4.23 (s, 1H), 4.13 (d, J = 7.1 Hz, 1H), 3.76 – 3.68 (m, 1H), 3.40 (s, 1H), 2.91 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.6, 137.6, 137.4, 128.6, 128.0, 128.0, 127.8, 99.7, 75.5, 74.8, 72.8, 72.0, 70.9, 65.2, 48.6 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{21}H_{22}O_6Na$ 393.1309, found 393.1307.

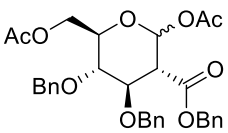
Compound 56



Compound **55** (336 mg, 0.908 mmol) was dissolved in dry DCM (9 mL) and cooled to 0 °C. Benzyl alcohol (281 µL, 2.72 mmol), *N*,*N*'-diisopropylcarbodiimide (168 µL, 1.09 mmol) and DMAP (33 mg, 0.27 mmol) were added at 0 °C and the mixture was stirred overnight at rt. The solvent was evaporated under reduced pressure and the resulting residue was dissolved with EtOAc, washed with diluted 1 M HCl solution, sat. aq. $NaHCO_3$, H_2O and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (pentane/EtOAc 13:1 → 7:1) to afford compound **56** (384 mg, 0.833 mmol, 92%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.22 (m, 15H), 5.96 (s, 1H), 5.23 – 5.09 (m, 2H), 4.55 (dd, J = 12.3, 3.3 Hz, 3H), 4.46 (d, J = 12.0 Hz, 1H), 4.38 (d, J = 12.3 Hz, 1H), 4.29 (s, 1H), 4.10 (d, J = 7.1 Hz, 1H), 3.71 (t, J = 6.4 Hz, 1H), 3.38 (s, 1H), 2.87 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$)

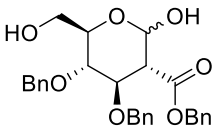
δ 169.6, 137.8, 137.7, 135.6, 128.6, 128.6, 128.5, 128.4, 128.3, 128.2, 127.9, 127.8, 127.8, 99.9, 76.0, 74.9, 73.0, 71.9, 70.8, 66.9, 65.3, 48.7 ppm.

Compound 57



TFA (230 µL, 3.01 mmol) was added to a cooled (0 °C) and stirred solution of **56** (252 mg, 0.547 mmol) in acetic anhydride (7.0 mL). After 5 h, the mixture was concentrated and the residue was co-evaporated with toluene (3 x 30 mL).The crude was purified with silica column chromatography (Pentane/acetone 15:1 → 7:1) to obtain compound **57** (α/β = 1:2, 286 mg, 0.508 mmol, 93%) as a colorless oil. ^{1}H NMR [α,β-anomers] (500 MHz, $CDCl_3$) *δ* 7.40 – 7.10 (m, 45H, Ar CH (α/β)), 6.38 (d, *J* = 3.7 Hz, 1H, H1 (α)), 5.80 (d, *J* = 9.0 Hz, 2H, H1 (β)), 5.18 – 5.03 (m, 6H, CH_2 ester (α,β)), 4.92 – 4.85 (m, 3H, CH*H* Bn (α/β)), 4.82 (d, *J* = 10.8 Hz, 2H, CH*H* Bn (α/β)), 4.73 (d, *J* = 10.9 Hz, 2H, CH*H* Bn (α/β)), 4.56 (td, *J* = 12.9, 10.8 Hz, 5H, CH*H* Bn (α/β)), 4.35 – 4.20 (m, 7H, H3 (α) and H6ab (α,β)), 4.06 (dd, *J* = 10.8, 8.7 Hz, 2H, H3 (β)), 3.94 (dt, *J* = 10.2, 3.2 Hz, 1H, H5 (α)), 3.69 (ddd, *J* = 9.8, 4.6, 2.2 Hz, 2H, H5 (β)), 3.61 (t, *J* = 9.6 Hz, 1H, H4 (α)), 3.55 (t, *J* = 9.3 Hz, 2H, H4 (β)), 3.07 (dd, *J* = 11.0, 3.7 Hz, 1H, H2 (α)), 2.93 (dd, *J* = 10.8, 9.0 Hz, 2H, H2 (β)), 2.03 (d, *J* = 3.5 Hz, 9H, CH_3 (α,β)), 1.90 (d, *J* = 5.8 Hz, 9H, CH_3 (α,β)). ^{13}C NMR [α,β-anomers] (126 MHz, $CDCl_3$) *δ* 170.7, 170.7, 169.2, 168.8, 168.7, 168.1 (6C=O (α,β)), 138.4, 137.8, 137.5, 137.5, 135.5, 135.4 (6C_q Ar (α,β)), 128.7, 128.7, 128.7, 128.7, 128.6, 128.6, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9 (CH Ar (α,β)), 92.3 (C1 (β)), 90.8 (C1 (α)), 81.3 (C3 (β)), 78.2 (C4 (α)), 77.8 (C3 (α)), 77.5 (C4 (β)), 75.7 (Bn (α)), 75.4 (Bn (α)), 75.2 (Bn (β)), 75.1 (Bn (β)), 74.1 (C5 (β)), 71.5 (C5 (α)), 67.2 (Bn ester (α)), 67.1 (Bn ester (β)), 62.7 (C6 (β)), 62.6 (C6 (α)), 54.0 (C2 (β)), 52.0 (C2 (α)), 21.0 (CH_3 (β)), 20.9 (CH_3 (α)), 20.7 (CH_3 (β)), 20.7 (CH_3 (α)) ppm. HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{32}H_{34}O_9Na$ 585.2095, found 585.2099.

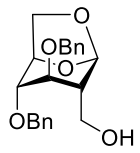
Compound 58



To a stirred solution of **57** (153 mg, 0.272 mmol) in THF/H_2O (9/1, 2.7 mL/0.3 mL) was added *p*-TsOH·H_2O (103 mg, 0.544 mmol) and the mixture was heated to 70 °C. After 7 h, the reaction mixture was cooled to rt and diluted with EtOAc, washed with sat. aq. $NaHCO_3$, H_2O and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (pentane/EtOAc 3:1 → 1:1) to afford compound **58** (α/β ≈ 1:1,107 mg, 0.223 mmol, 82%) as a colorless oil. ^{1}H NMR [α,β-anomers] (500 MHz, $CDCl_3$) *δ* 7.38 – 7.20 (m, 26H), 7.20 – 7.08 (m, 4H, CH_2 ester (α,β)), 5.48 (t, *J* = 2.9 Hz, 1H, H1 (α)), 5.25 – 5.04 (m, 4H), 4.93 (d, *J* = 8.6 Hz, 1H, H1 (β)), 4.87 – 4.75 (m, 4H, CH*H* Bn (α/β)), 4.70 (d, *J* = 11.0 Hz, 1H, CH*H* Bn (α/β)), 4.62 (t, *J* = 11.2 Hz, 2H, CH*H* Bn (α/β)), 4.47 (d, *J* = 11.0 Hz, 1H, CH*H* Bn (α/β)), 4.34 (dd, *J* = 10.9, 9.1 Hz, 1H, H3 (α)), 4.00 (ddd, *J* = 16.5, 9.3, 5.6 Hz, 2H, H5 (α) and H3 (β)), 3.82 (ddd, *J* = 14.5, 12.0, 2.4 Hz, 2H, H6a (α,β)), 3.66 (ddd, *J* = 15.1, 7.6, 4.3 Hz, 2H, H6b (α,β)), 3.54 (t, *J* = 9.3 Hz, 1H, H4 (β)), 3.51 – 3.46 (m, 1H, H4 (α)), 3.43 (ddd, *J* = 9.8, 4.7, 2.3 Hz, 1H,

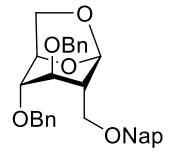
H5 (β)), 2.92 (dd, *J* = 10.9, 3.7 Hz, 1H, H2 (α)), 2.80 (ddd, *J* = 10.7, 8.5, 1.3 Hz, 1H, H2 (β)). ^{13}C NMR [α,β-anomers] (126 MHz, $CDCl_3$) *δ* 170.8, 169.8, 138.6, 138.0, 138.0, 137.9, 135.7, 135.4, 128.7, 128.7, 128.6, 128.6, 128.6, 128.5, 128.4, 128.4, 128.4, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 95.3, 91.8, 81.3, 79.3, 77.9, 77.7, 75.9, 75.5, 75.1, 75.1, 74.9, 71.6, 67.1, 67.0, 61.9, 61.6, 56.7, 53.5, 29.8 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{28}H_{30}O_7Na$ 501.1884, found 501.1883.

Compound 62



Compound **54** (1.36 g, 3.86 mmol) was dissolved in a mixture of dioxane/H_2O (5/2, 50 mL/20 mL). Osmium tetraoxide (0.1 M in H_2O, 2 mL, 0.2 mmol) and $NaIO_4$ (1.82 g, 8.50 mmol) were added and the reaction mixture was stirred at rt for 5 h. TLC-analysis indicated the presence of intermediate vicinal diol so more $NaIO_4$ (826 mg, 3.86 mmol) was added and the mixture was stirred at rt overnight. The mixture was then diluted with H_2O (50 mL) and extracted with EtOAc (3 x 80 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The resulting residue was dissolved in a mixture of THF/isopropanol (9/1, 45 mL/5 mL). The solution was cooled to 0 °C and $NaBH_4$ (294 mg, 7.72 mmol) was added. After 1 h, the reaction was quenched with sat. aq. NH_4Cl (50 mL) and extracted with EtOAc (3 x 80 mL). The combined organic layers were dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/EtOAc 4:1 → 2:1) to obtain compound **62** (1.16 g, 3.26 mmol, 84%) as a light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 7.40 – 7.26 (m, 10H), 5.55 (d, *J* = 1.3 Hz, 1H), 4.61 – 4.49 (m, 4H), 4.46 (d, *J* = 12.2 Hz, 1H), 4.15 – 4.09 (m, 1H), 3.71 (pd, *J* = 7.7, 7.2, 4.0 Hz, 3H), 3.52 (p, *J* = 1.5 Hz, 1H), 3.42 (dq, *J* = 2.4, 1.1 Hz, 1H), 2.24 (d, *J* = 8.1 Hz, 1H), 2.11 – 2.04 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) *δ* 138.1, 137.7, 128.6, 128.6, 128.1, 127.9, 127.9, 127.7, 101.6, 76.6, 74.4, 74.1, 71.5, 71.2, 64.7, 62.3, 45.6 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{21}H_{24}O_5Na$ 379.1516, found 379.1515.

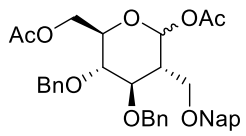
Compound S5



NaH (60% in mineral oil, 326 mg, 8.15 mmol) was added to a cooled (0 °C) and stirred solution of compound **62** (1.16 g, 3.26 mmol) in dry DMF (30 mL). NapBr (1.08 g, 4.89 mmol) was added and the reaction mixture was stirred at rt for 2.5 h. After which the reaction was carefully quenched with H_2O at 0 °C, diluted with water (60 mL) and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with water (2 x 100 mL), brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/EtOAc 15:1 → 7:1) to obtain compound **S5** (1.49 g, 3.00 mmol, 92%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.80 (dd, *J* = 9.1, 6.7 Hz, 3H), 7.72 (d, *J* = 1.7 Hz, 1H), 7.51 – 7.36 (m, 3H), 7.35 – 7.15 (m, 10H), 5.58 (s, 1H), 4.67 (d, *J* = 12.1 Hz, 1H), 4.60 – 4.49 (m, 3H), 4.48 – 4.38 (m, 3H), 4.16 – 4.07 (m, 1H), 3.71 (t, *J* = 6.5 Hz, 1H), 3.65 (dd, *J* = 9.3, 7.2 Hz, 1H), 3.56 – 3.47 (m, 2H), 3.35 (d, *J* = 1.9 Hz, 1H), 2.30 – 2.22 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 138.2, 137.9,

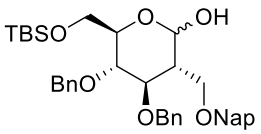
135.8, 133.3, 133.1, 128.6, 128.5, 128.2, 128.0, 127.9, 127.8, 127.8, 127.8, 127.7, 126.5, 126.2, 126.0, 125.9, 101.2, 76.9, 74.5, 73.4, 73.4, 71.3, 71.0, 69.2, 64.8, 43.8 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{32}H_{32}O_5Na$ 519.2142, found 519.2145.

Compound 78



TFA (1.26 mL, 16.4 mmol) was added to a cooled (0 °C) and stirred solution of **S5** (1.48 g, 2.98 mmol) in acetic anhydride (42 mL). After 45 min, the mixture was concentrated and the residue was co-evaporated with toluene (3 x 50 mL). The crude was purified with silica column chromatography (Pentane/EtOAc 11:1 → 4:1) to obtain compound **78** (α/β ≈ 2:1, 1.43 g, 2.39 mmol, 80%) as a white solid. ^{1}H NMR [α, β-anomers] (500 MHz, $CDCl_3$) δ 7.81 (ddd, *J* = 10.7, 8.6, 5.0 Hz, CH Ar (α,β)), 7.70 (d, *J* = 3.9 Hz, CH Ar (α,β)), 7.52 – 7.44 (m, CH Ar (α,β)), 7.41 – 7.16 (m, CH Ar (α,β)), 6.37 (d, *J* = 3.0 Hz, H1 (α)), 5.87 (d, *J* = 9.2 Hz, H1 (β)), 4.92 – 4.80 (m, CH*H* Bn (α,β)), 4.69 – 4.55 (m, CH*H* Bn (α,β)), 4.47 (dd, *J* = 20.0, 12.2 Hz, CH*H* Bn (α,β)), 4.34 – 4.24 (m, H6ab (α,β)), 3.98 (dd, *J* = 10.9, 8.8 Hz, H3 (β)), 3.90 (d, *J* = 9.8 Hz, H5 (α)), 3.79 (dd, *J* = 11.2, 8.8 Hz, H3 (α)), 3.73 – 3.59 (m, H7a (α), H7a (β), H5 (β) and H4 (α)), 3.56 (t, *J* = 9.3 Hz, H4 (β)), 3.47 – 3.37 (m, 2H, H7b (α) and H7b (β)), 2.40 – 2.31 (m, H2 (α)), 2.04 (d, *J* = 6.2 Hz, CH_3 (α,β)), 1.88 (d, *J* = 1.5 Hz, H2 (β) and CH_3 (α,β)). ^{13}C NMR [α,β-anomers] (126 MHz, $CDCl_3$) δ 170.9, 170.9, 169.2, 169.2, 138.3, 138.0, 137.8, 137.7, 135.5, 135.4, 133.3, 133.3, 133.1, 133.1, 128.7, 128.7, 128.6, 128.6, 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 126.8, 126.6, 126.3, 126.3, 126.1, 126.1, 126.0, 125.8, 92.2, 91.9, 79.2, 78.9, 78.8, 78.4, 75.5, 75.3, 75.3, 75.0, 73.7, 73.4, 73.3, 71.5, 66.5, 63.7, 63.2, 63.0, 47.3, 45.8, 21.0, 21.0, 21.0, 21.0 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{36}H_{38}O_8Na$ 621.2459, found 621.2457.

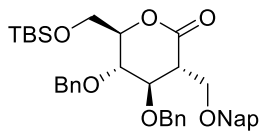
Compound 79



Sodium methoxide (4.373 M in MeOH, 165 µL, 0.72 mmol) was added to a solution of compound **78** (1.43 g, 2.39 mmol) in DCM/MeOH (1/1, 24 mL/24 mL). After 45 min, the reaction mixture was neutralized with washed Amberlite IR120 (H^+), filtered and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/EtOAc 3:1 → 1:1) to obtain the target product which was contaminated with an unknown byproduct. The mixture was directly dissolved in dry DCM (24 mL) and imidazole (0.41 g, 6.0 mmol) was added. After stirring at rt until imidazole completely dissolved, the reaction mixture was further stirred at 0 °C for another 1 h. Then *tert*-butyldimethylsilyl chloride (488 mg, 3.24 mmol) and DMAP (29 mg, 0.24 mmol) were added at 0 °C and the mixture was stirred at rt for 4 h. After which the mixture was diluted with DCM, washed with sat. aq. NH_4Cl, H_2O and brine, dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/EtOAc 15:1 → 5:1) to obtain compound **79** (α/β ≈ 2.5:1, 874 mg, 1.39 mmol, 57% over two steps) as a clean oil. ^{1}H NMR [α,β-anomers] (500 MHz, $CDCl_3$) δ 7.84 – 7.76 (m), 7.75 – 7.71 (m), 7.51

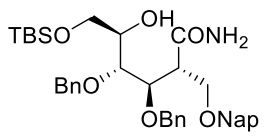
– 7.44 (m), 7.42 (ddd, *J* = 8.4, 3.8, 1.7 Hz), 7.36 – 7.25 (m), 7.24 – 7.15 (m), 7.12 (ddd, *J* = 6.6, 3.5, 1.6 Hz), 5.39 (t, *J* = 2.7 Hz, H1 (α)), 4.89 (dd, *J* = 8.5, 5.3 Hz, H1 (β)), 4.85 (dt, *J* = 10.9, 3.2 Hz), 4.75 (dd, *J* = 18.1, 10.9 Hz), 4.69 – 4.48 (m), 4.00 – 3.86 (m), 3.85 – 3.76 (m), 3.76 – 3.61 (m), 3.50 (d, *J* = 5.3 Hz), 3.36 (ddd, *J* = 9.5, 3.3, 2.4 Hz), 2.05 (ddt, *J* = 11.2, 6.1, 3.0 Hz, H2 (α)), 1.77 – 1.67 (m, H2 (β)), 0.90 (s, *t*-Bu (α,β)), 0.10 – 0.03 (m, CH_3 (α,β)). ^{13}C NMR [α,β-anomers] (126 MHz, $CDCl_3$) δ 138.8, 138.6, 138.5, 138.4, 135.6, 135.0, 133.3, 133.2, 133.1, 128.6, 128.6, 128.5, 128.5, 128.3, 128.1, 128.0, 128.0, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 126.9, 126.6, 126.4, 126.3, 126.2, 126.0, 125.9, 95.6, 93.9, 79.7, 79.4, 79.3, 77.4, 76.3, 75.4, 75.4, 74.9, 74.8, 73.7, 73.5, 72.3, 67.2, 65.9, 62.6, 62.3, 49.8, 46.8, 26.1, 18.5, 18.5, -4.8, -5.2 ppm. HRMS (ESI) m/z: $[M+NH_4]^+$ calculated for $C_{38}H_{52}O_6SiN$ 646.3558, found 646.3558.

Compound 80



Acetic anhydride (1.83 mL, 19.4 mmol) was added to a solution of compound **79** (553 mg, 0.881 mmol) in anhydrous DMSO (8.0 mL). The mixture was stirred at 30 °C for 16 h, after which it was diluted with Et_2O (150 mL), washed with aq. $NaHCO_3$ (10%, 3 x 15 mL) and brine (40 mL). The organic layer was dried over $MgSO_4$, filtrated and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/EtOAc 25:1 → 9:1) to obtain compound **80** (440 mg, 0.703 mmol, 79%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (td, *J* = 5.4, 4.9, 2.6 Hz, 3H), 7.72 (s, 1H), 7.50 – 7.20 (m, 11H), 7.13 (dt, *J* = 7.6, 1.8 Hz, 2H), 4.91 – 4.75 (m, 3H), 4.68 (d, *J* = 12.1 Hz, 1H), 4.53 (dt, *J* = 11.3, 3.0 Hz, 2H), 4.20 – 3.95 (m, 4H), 3.90 (d, *J* = 1.8 Hz, 2H), 3.71 (dt, *J* = 8.9, 2.2 Hz, 1H), 2.65 (dt, *J* = 9.3, 2.4 Hz, 1H), 0.89 (d, *J* = 1.8 Hz, 9H), 0.08 (d, *J* = 1.8 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 138.0, 138.0, 135.4, 133.3, 133.1, 128.7, 128.6, 128.6, 128.3, 128.1, 128.1, 128.0, 128.0, 127.9, 127.8, 126.7, 126.2, 126.0, 126.0, 79.3, 76.8, 76.7, 74.9, 74.9, 73.6, 66.6, 61.4, 48.8, 26.0, 18.3, -5.1, -5.3 ppm. $[M+NH_4]^+$ calculated for $C_{38}H_{50}O_6SiN$ 644.3402, found 644.3400.

Compound 81



Compound **80** (432 mg, 0.690 mmol) was dissolved in methanolic ammonia (7 M, 7.0 mL). After stirring at rt for 1.5 h, the reaction mixture was concentrated and traces of ammonia were removed by co-evaporation with toluene (15 mL). The crude was purified with silica column chromatography (Pentane/EtOAc 4:1 → 1:1) to obtain product **81** (245 mg, 0.381 mmol, 55%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 – 7.67 (m, 4H), 7.56 – 7.36 (m, 3H), 7.37 – 7.21 (m, 10H), 6.43 (s, 1H), 5.36 (s, 1H), 4.74 – 4.52 (m, 6H), 4.38 (dd, *J* = 7.0, 3.0 Hz, 1H), 3.90 (tt, *J* = 7.4, 4.4 Hz, 3H), 3.77 (dd, *J* = 10.2, 3.5 Hz, 1H), 3.72 – 3.59 (m, 2H), 3.06 (dd, *J* = 9.3, 3.8 Hz, 1H), 2.75 (d, *J* = 5.9 Hz, 1H), 0.89 (s, 9H), 0.05 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.2, 138.4, 138.1, 135.3, 133.3, 133.1, 128.5, 128.4, 128.2,

128.2, 128.0, 127.9, 127.8, 127.8, 126.8, 126.3, 126.1, 125.9, 79.2, 77.6, 74.4, 74.0, 73.7, 71.4, 68.4, 64.1, 47.7, 26.0, 18.4, -5.2, -5.2 ppm. $[M+H]^+$ calculated for $C_{38}H_{50}NO_6Si$ 644.3402, found 644.3402.

6.5 References

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Nederlandse Samenvatting

Glycosidasen zijn wijdverspreide enzymen in alle uithoeken van de natuur en zijn belangrijke biokatalysatoren die de hydrolyse van glycoside-bindingen in oligo/polysacchariden, glycoproteïnen en glycolipiden met een enorme efficiëntie katalyseren. Abnormale glycosidase-activiteit is nauw verbonden met een verscheidenheid aan menselijke ziekten. Neem bijvoorbeeld de overexpressie van heparanase die betrokken is bij bijna alle onderzochte soorten kanker. Deze overexpressie correleert met verhoogde tumorgrootte, tumor angiogenese, een toename in uitzaaiingen en een slechte prognose voor de patiënt. Specifieke remmers van glycosidasen zijn van grote waarde. Niet alleen omdat ze kunnen dienen als nuttige biologische hulpmiddelen om de katalytische machinerie, het mechanisme en zijn verschillende stadia te bestuderen door analyse van de kristalstructuur van (covalente) remmer-enzym complexen, maar ook omdat ze kunnen dienen als aanknopingspunten voor de ontwikkeling van geneesmiddelen voor de behandeling van glycosidase-gemedieerde ziekten. Bovendien is aangetoond dat covalent bindende remmers gebruikt kunnen worden als basis voor de ontwikkeling van activity-based probes (ABPs) die het indentificeren van glycosidasen in complexe biologische systemen mogelijk maken.

Dit proefschrift beschrijft de ontwikkeling en biochemische evaluatie van covalente remmers en ABPs voor stereochemie behoudende *endo*- en *exo*-glycosidasen waaronder zetmeel afbrekende enzymen en humaan lysosomaal β-glucocerebrosidase (GBA), als ook de synthese van een groep uronzuur achtige 1-*N*-iminosuikers als potentiële competitieve heparanase remmers. Het ontwerp van dergelijke covalente en competitieve glycosidase remmers is afhankelijk van het begrijpen van de functie en het mechanisme van deze enzymen. **Hoofdstuk 1** introduceert de algemene mechanismen die gebruikt worden door stereochemie behoudende en inverterende β-glycosidasen voor de hydrolyse van hun substraten. De verschillende stappen en tussenproducten van stereochemie behoudende α- en β-glucosidasen worden beschreven en het ontwerp van covalente cyclophellitol-achtige remmers op basis van deze routes wordt besproken. Daarnaast wordt het ontwerp van competitieve remmers, die de mechanistische overgangstoestanden nabootsen, beschreven. Tenslotte wordt een overzicht gegeven van de activity-based protein profiling (ABPP) workflow.

De biologische afbraak van zetmeel vereist de synergetische werking van een reeks enzymen. Van deze enzymen zijn de α-amylasen, die de hydrolyse van de interne α-1,4-glucosidebindingen katalyseren het meest uitgebreid bestudeerd, zowel binnen de geneeskunde als in de biotechnologie. **Hoofdstuk 2** rapporteert over het ontwerp en de synthese van een selectie 1,6-*epi*-cyclophellitol-gebaseerde pseudo-disachariden uitgerust met een reeks van reporter entiteiten en hun gebruik in ABPP van stereochemie behoudende amylasen in complexe biologische monsters. De activiteit en efficiëntie van de gesynthetiseerde remmers

en sondes werden aangetoond door uitgebreide biochemische analyse. De selectiviteit voor amylasen boven verwante endoglycosidasen werd gevalideerd door structurele studies. De maltobiose *epi*-cyclophellitol ABPs bleken effectief α-amylasen te kunnen labelen in menselijk speeksel, muizenweefsel en schimmel secretomen op een concentratie-, pH-, tijd- en temperatuurafhankelijke wijze. De labeling kon beconcurreerd worden met de niet-gemerkte remmers, evenals met de commercieel verkrijgbare concurrerende remmer acarbose. Deze ABPs kunnen worden gebruikt bij de ontdekking van nieuwe en doeltreffende humane amylase remmers. Deze remmers zouden kunnen bijdragen aan de behandeling van type 2-diabetes en bij de ontdekking van nieuwe microbiële amylasen met potentieel voordelige eigenschappen voor biotechnologische toepassingen.

Zetmeelpolysachariden zijn samengesteld uit lineaire amylose en vertakte amylopectine, waarvan de laatstgenoemde een belangrijk bestanddeel van standaard zetmeel vormt. In amylopectine zijn de vertakkingspunten die α-1,6-glucosidebindingen bevatten resistent tegen hydrolyse door α-1,4-specifieke amylasen. Als een uitbreiding van de maltobiose *epi*-cyclophellitol sondes behandeld in Hoofdstuk 2, beschrijft **Hoofdstuk 3** de synthese van een set glucose-isomaltose (GIM) en isomaltose-glucose (IMG) geconfigureerde *epi*-cyclophellitol sondes die de vertakte delen van de amylopectine structuur nabootsen. De centraal staande pseudo-trisachariden werden geconstrueerd door stereoselectieve α-1,4- en α-1,6-glycosyleringen van cyclohexeen acceptoren onder de juiste preactivatie condities. Hierna werd de epoxide functionaliteit aangebracht via een stereoselectieve epoxidatie, die werd gevolgd door globale ontscherming en een amide-koppeling met een reportertag om de uiteindelijke chemische sondes te verkrijgen na HPLC-zuivering. De reeks vertakte sondes zal bruikbaar zijn voor de detectie van industrieel relevante zetmeel-afbrekende enzymen die een voorkeur vertonen voor vertakte amylopectine achtige polysachariden.

Hoofdstuk 4 beschrijft de synthese van een set β-D-*gluco*-cyclophellitol aziridine remmers en ABPs, die gefunctionaliseerd zijn op zowel de C6 positie als de endocyclische stikstof van de aziridine. Röntgen kristallografische analyse toonde aan dat recombinant humaan β-glucocerebrosidase (rhGBA) de twee functionaliteiten kon accommoderen. De selectiviteit en potentie van deze bifunctionele aziridines voor GBA werden zowel *in vitro* als *in situ* onderzocht. De IC_{50} waarden voor de remming van rhGBA toonden aan dat de nieuwe bifunctionele cyclophellitol aziridine verbindingen ongeveer 10-15 keer minder potent waren dan de eerder gerapporteerde C6-mono-functionele cyclophellitol epoxides. Bovendien vertoonde incubatie van muis hersenlysaten met de bifunctionele verbindingen inderdaad een selectief markeringspatroon voor GBA boven GBA2, terwijl een verminderde selectiviteit werd waargenomen bij *in situ* markering van HEK293T cellen die endogeen GBA en tot overexpressie gebracht GBA2 bevatten. Hoewel de bifunctionele aziridines minder selectief en potent zijn dan hun C6-monogefunctionaliseerde epoxide tegenhangers, blijven ze nanomolair

inactiverende stoffen voor GBA die gebruikt kunnen worden voor de studie van GBA in relatie tot de ziekte van Gaucher.

Siastatine B is eerder gerapporteerd als een krachtige en effectieve remmer van β-D-glucuronidasen. De moleculaire structuur van siastatine B blijkt echter te volumineus om te passen in het katalytische centrum van een glucuronidase. NMR-analyse van 2-trifluoraceetamide bevattende siastatine B derivaten suggereerde dat oplosmiddel gemedieerde afbraak van deze moleculen snel kan plaatsvinden, waarbij een hemiaminal/hydraat keton vrijkomt dat als de echte remmer fungeert. Deze omlegging is echter niet aangetoond voor siastatine B. In **Hoofdstuk 5** wordt een röntgen-kristallografische analyse gepresenteerd van co-complexen tussen siastatine B en verschillende *exo*- en *endo*-β-glucuronidases, die bevestigen dat het de hemiaminale of het gehydrateerde keton is en niet siastatine B, die zich bindt in het katalytische centrum van het enzym. Om de werking van de afbraakproducten te begrijpen, werd een selectie van *galacto*- en *gluco*-geconfigureerde 1-*N*-iminosuikerderivaten gesynthetiseerd en werd een voorlopige structurele analyse voor enzym inhibitie door de synthetische *gluco*-geconfigureerde iminosuikers onderzocht. Om de remmende eigenschappen van deze verbindingen grondig te evalueren, kunnen in de toekomst kinetische studies en competitieve ABPP-experimenten worden uitgevoerd.

Tenslotte wordt in **Hoofdstuk 6** een meer gedetailleerde samenvatting gegeven van de, in dit proefschrift beschreven bevindingen, in combinatie met voorstellen voor vervolgonderzoek.