Activity-based protein profiling of glucosidases, fucosidases and glucuronidases

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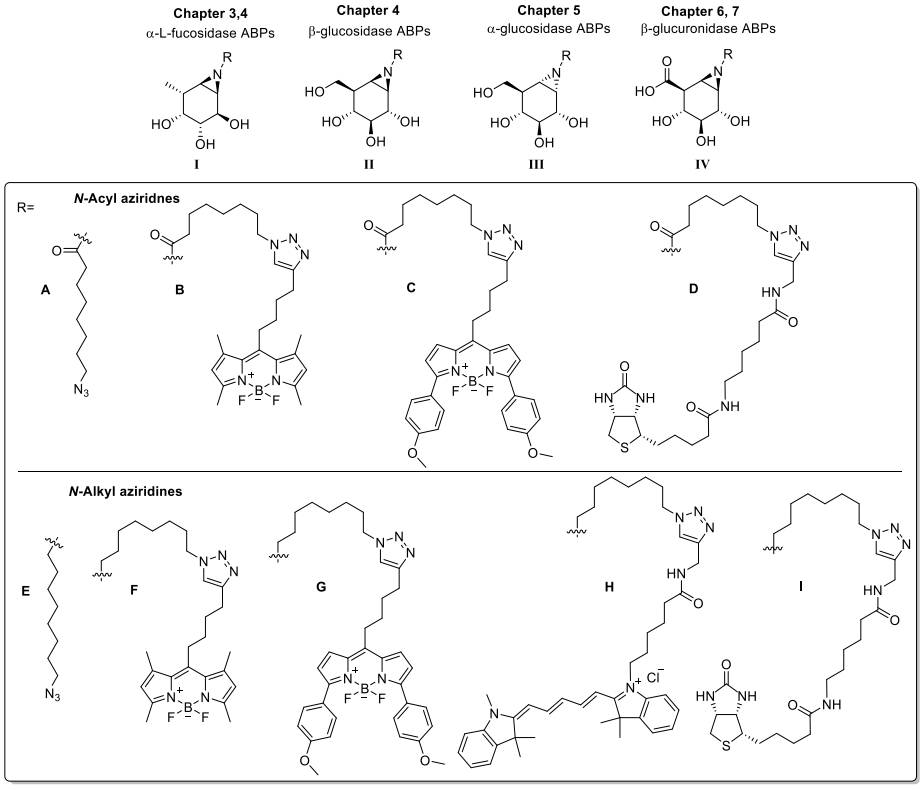
8

Summary and future prospects

8.1 Summary

This thesis describes the development of functional and configurational analogues of cyclophellitol aziridine as activity-based probes (ABPs) for various retaining glycoside hydrolases (GHs), namely α-L-fucosidases, β-glucosidases, α-glucosidases and β-glucuronidases (Figure 1). Attention is focused on the design and synthesis of the cyclophellitol aziridine derivatives and their application in chemical biology studies of various retaining GHs.

Chapter 1 introduces the research subject described in this thesis. Subjects introduced in this chapter include β-glucosidases classification, a description of the molecular mechanisms employed by these enzymes in the hydrolysis of their substrates, as well as the cyclophellitol aziridine-based ABPs targeting retaining β-glucosidases. The retaining glycosidases employ Koshland double displacement mechanism,[1] and on the basis of which the research of this thesis was formulated. **Chapter 2** reviews the literature on the synthesis of cyclitol aziridines as the scaffold of choice for the development of retaining GHs ABPs.



ABPs	A	B	C	D	E	F	G	H	I
I	JJB237	JJB256	JJB244	JJB243	JJB349	JJB350	JJB380	JJB381	JJB385
II	JJB103	JJB70	JJB75	JJB111	JJB339	JJB376	JJB343	JJB367	JJB377
III					CF022	JJB382	JJB347	JJB383	JJB384
IV	JJB133		JJB144	JJB249	JJB355	JJB395	JJB391	JJB392	JJB397

Figure 1. Activity-based glycosidase probes (ABPs) discussed in this thesis. **I**-**IV** are configurational and functional cyclophellitol aziridine isomers emulating the structure of the parent monosaccharides as indicated; **A**-**I** are acyl and alkyl functional tags.

Retaining α-L-fucosidase (FUCA) is a member of the glycoside hydrolases family 29 (GH29). FUCA deficiency is at the basis of the rare lysosomal storage disorder, fucosidosis. **Chapter 3** describes an activity-based protein profiling (ABPP) study on GH29 α-L-fucosidases. L-Fucopyranose-configured cyclophellitol aziridines (JJB237, JJB243, JJB244 and JJB256), are applied as ABPs for selective *in vitro* and *in vivo* labeling of GH29 α-L-fucosidases from bacteria, mice and man. The chapter also reports on the synthesis of eight configurational L-fuconojirimycin isomers, which were screened as potential α-L-fucosidase inhibitors in a competitive ABPP setting. The covalent aziridine-enzyme adduct between the carbon

corresponding to the anomeric center in the substrate fucoside and the FUCA active site nucleophile was trapped in a co-crystal of *Bacteroides thetaiotaomicron* α-L-fucosidase treated with *N*-acetyl-L-*fuco*-cyclophellitol aziridine. The *trans*-diaxial skew boat conformation adopted by the covalently attached inhibitor also provides insight in the conformational changes substrate α-L-fucosides undergo during FUCA-mediated hydrolysis.

In order to improve the stability and availability of retaining GHs ABPs, a set of next generation probes, namely *N*-alkyl cyclophellitol aziridine ABPs, were investigated. The synthesis and evaluation of fluorescent *N*-alkyl probes directed at GH30 β-glucosidases (JJB339, JJB343) and GH29 α-L-fucosidases (JJB349, JJB380) are described in **Chapter 4**. In comparison with the corresponding acyl aziridine ABPs reported previously, the alkyl aziridine ABPs proved relatively easy to synthesize and are more stable in mildly acidic and basic media. The β-*gluco*-configured alkyl aziridine ABPs proved equally effective in inhibiting and labeling the lysosomal β-glucosidase (GBA) as its *N*-acyl counterparts. In contrast, the *N*-acyl aziridines targeting α-L-fucosidase outperform their *N*-alkyl counterparts. Therefore, *N*-alkyl cyclophellitol aziridines can be an attractive alternative in retaining GHs ABP design, but in targeting a new retaining glycosidase both *N*-alkyl and *N*-acyl aziridines are best considered at the onset of a new study.

Chapter 5 describes the development of ABPP technology to study GH31 α-glucosidases *in vitro* and *in situ*. To this end a comprehensive set of α-*gluco*-cyclophellitol aziridines bearing either a fluorescent group (JJB347, JJB382, JJB383) or a biotin (JJB384) was synthesized. α-Glucosidases are involved in diverse physiological processes in the human body, including carbohydrate assimilation in the gastrointestinal tract, glycoprotein processing in the endoplasmic reticulum (ER), and intra-lysosomal glycogen catabolism. Inherited deficiency of the lysosomal acid α-glucosidase (GAA) causes Pompe disease, a relatively common lysosomal glycogen storage disorder. The developed ABPs proved to be highly potent and irreversible inhibitors towards recombinant α-glucosidase as established by enzyme inhibition assays and X-ray crystallography analysis. Moreover the ABPs can specifically label distinct retaining GH31 α-glucosidases, notably, the lysosomal GAA and the ER α-glucosidase II and this labelling can be tuned by pH. The chapter further describes a direct diagnostic application in Pompe disease patient fibroblast cells, and reports on the analysis of intestinal dietary α-glucosidases, such as sucrase-isomaltase (Sis) and maltase-glycoamylase (MGAM).

Chapter 6 describes a synthesis strategy towards β-glucuronide-configured cyclophellitol, cyclophellitol aziridine and its derivatives (JJB133, JJB144, JJB249, JJB355, JJB391, JJB392, JJB395 and JJB397) as ABPs for GH2 and GH79 β-glucuronidases. The former enzyme is related to the glycosaminoglycan (GAG) storage disorder, Sly disease, and the latter enzyme is related to inflammation, tumour angiogenesis and cell migration. Uronic *N*-alkyl cyclophellitol

aziridines are easier to be prepared than uronic *N*-acyl cyclophellitol aziridines, and both alkyl and acyl inhibitors and probes proved equally effective in inhibition and labelling of β-glucuronidases, which is in line with the findings in **Chapter 4**. Crystallographical analysis on bacterial β-glucuronidase (AcaGH79) complexed with alkyl and acyl aziridines further confirmed the covalent modification of the enzyme nucleophile active site and showed a $^4C_1$ chair conformation for both enzyme-inhibitor adducts. Applications of the β-glucuronide-configured *N*-alkyl cyclophellitol aziridine ABPs are described in **Chapter 7** and include GH2 human lysosomal β-glucuronidase (GUSB) and GH79 human heparanase (HPSE) identification by activity-based proteomics. An interesting finding is that the *endo*-β-glucuronidase HPSE is effectively labeled and thus inhibited by cyclophellitol aziridine derivatives, which in essence are monosaccharide mimetics (thus expected to bind efficiently to *exo*-glycosidases, but not necessarily *endo*-glycosidases). Crystallographical analysis of the structures of recombinant *exo*-GUSB, *exo*-AcaGH79 and *endo*-HPSE as well as their corresponding nucleophile mutant (Glu to Ala) complexed with cyclophellitol aziridine JJB355, reveals the Koshland double displacement mechanism employed by these enzymes and $^1S_3$-$^4H_3$-$^4C_1$ itineraries substrate β-glucuronides undergo when processed.

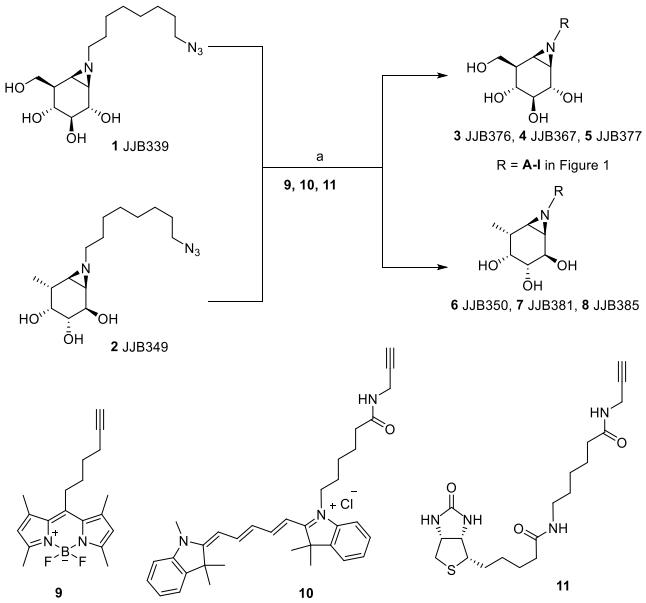
8.2 Future prospects

The research described in this thesis entails the design, synthesis and application of functional configurational cyclophellitol aziridine derivatives as ABPs selective for in-class GH family retaining glycosidases. Besides red BODIPY probes of β-glucosidases and α-L-fucosidases shown in Chapter 3, also green BODIPY probes **3** JJB376, **6** JJB350, blue Cy5 probes **4** JJB367, **7** JJB381 and biotin probes **5** JJB377, **8** JJB385 ABPs (Figure 1) can be prepared following a similar strategy (Scheme 1).[2]

By altering either configuration or substitution pattern, or both, selectivity of the resulting probes changes in a highly predictable manner. This bodes well for the development of ABPs directed at other retaining glycosidases. For instance, ABPs **12**-**14** (Figure 2) may target with good efficiency their respective underlying glycosidases: β-glucosidases (already a proven fact), β-galactosidases and β-mannosidases, which are of interest in the context of specific lysosomal storage disorders: Gaucher disease, GM1 gangliosidosis and mannosidosis respectively.[3] In comparison with ABPP technology developed for serine hydrolases and cysteine proteases, however, the in-class GH family selectivity displayed by the ABPs described in this thesis may also be considered a disadvantage. Cysteine protease probes and especially serine hydrolase probes have been reported that efficiently label a large number – sometimes hundreds of – related enzymes, which may be of advantage in for instance competitive ABPP experiments aimed at the discovery of selective inhibitors. Arguably, the nature (substitution pattern, configuration) of the highly functionalized cyclitol aziridines (Figure 1) determines their glycosidase specificity, and deleting some substituents may yield probes that, though less

potent, are also less selective, in other words, more broad-spectrum. For instance, diol cyclohexane aziridine **15** (Figure 2) may be developed as broad spectrum ABP for retaining β-glucosidases, β-galactosidases and β-mannosidases based on the idea that the hydroxyl at C2 (distinguishing β-glucose from β-mannose) and C4 (distinguishing β-glucose from β-galactose) are removed.

Scheme 1. Synthesis of alkyl aziridine ABPs for β-glucosidases and α-L-fucosidases.



Reagent and conditions: (a) $CuSO_4$(1.0 M in H_2O), sodium ascorbate (1.0 M in H_2O), DMF, **3** JJB376: 30%, **4** JJB367: 13%, **5** JJB377: 34%, **6** JJB350: 8%, **7** JJB381: 13%, **8** JJB385: 20%.

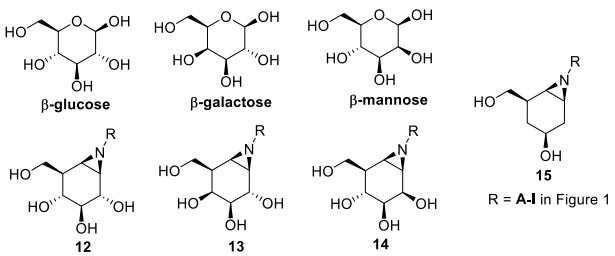
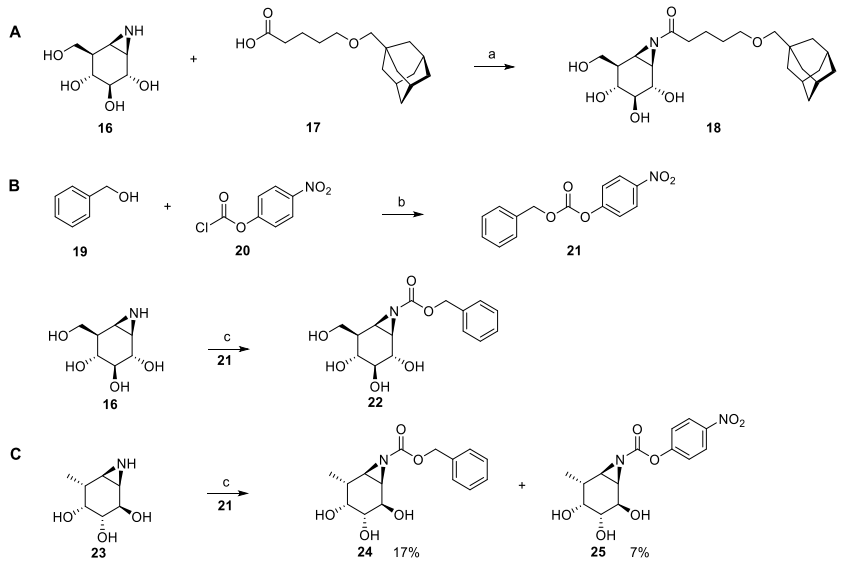


Figure 2. Chemical structures of β -glucose, β-galactose, β-mannose, ABPs **12-14** for β-glucosidases, β-galactosidase, β-mannosidase and broad spectrum ABP **15**.

Activity-based protein profiling is a powerful technique both to discover new enzyme activities (comparative ABPP) and to study their expression, activity and sensitivity in a tissue- and condition-dependent setting (competitive ABPP). The research described in this thesis focused predominantly on the latter, with probes designed with a specific GH glycosidase family in mind. Research in Chapter 5, however also demonstrates the potential of biotin-modified cyclophellitol aziridines to identify, by means of chemical proteomics, enzyme activities in a tissue-dependent fashion. In line with this, and underscored in a literature study on the application of β-glucopyranose-configured cyclophellitol aziridines to annotate *Arabidopsis* retaining β-glucosidases,[4] a comparative ABPP investigation can be envisaged in which all biotinylated cyclophellitol aziridine probes (Figure 1) are employed to screen tissue from various kingdoms and map their respective retaining glycosidase activities.

The research described in this thesis reveals that cyclophellitol aziridines are highly potent irreversible retaining glycosidase inhibitors, and that the inhibitory potency can be tuned by varying the nature of the aziridine *N*-substituent. For instance (see **Chapter 3**), *N*-acetyl-cyclophellitol aziridine (IC_{50} = 46.8 nM) inhibits recombinant FUCA1 about 8 times more potently than the corresponding *N*-benzoyl-cyclophellitol aziridine (IC_{50} = 371.6 nM). However, previously described in the literature, that *N*-alkylated derivatives of the natural product deoxynojirimycin (DNM) and in which the nitrogen substituent is large and hydrophobic, are considerably more potent GBA inhibitors than analogous derivatives that bear a small nitrogen substituent. For instance, *N*-(adamantanemethyloxypentyl)-deoxynojirimycin (AMP-DNM) is a considerably more potent GBA inhibitor than DNM as well as *N*-butyl-DNM.[5] In this light, it is of interest to explore cyclophellitol aziridine derivatives bearing as nitrogen substituent a variety of alkyl and acyl substituents. Scheme 2 describes the synthesis of several of these compounds, namely, *N*-adamantane-methoxypentanoyl aziridine **18** and *N*-carboxybenzyl aziridine **22** as potential GBA inhibitors and two *N*-substituted L-*fuco*-cyclophellitol aziridine inhibitors (Cbz-aziridine **24** and *p*-nitrophenyloxycarbonyl aziridine **25**) as potential FUCA inhibitors. Compound **18** was prepared with the appropriate acid under the agency of EEDQ as the condensation agent following a procedure as presented in **Chapter 3** to yield JJB237. Unprotected aziridines **16** and **23** were transformed in the corresponding carboxybenzyl derivatives via activated anhydride **21** yielding **22** and **24**. Interestingly, during the synthesis of fucose analogue **24**, compound **25** was also isolated after HPLC purification using a neutral eluent in a yield of 7%.

Scheme 2. Synthesis of acyl cyclophellitol aziridines **18**, **22, 24** and **25**.



Reagent and conditions: (a) EEDQ, DMF, 0 °C, 22%; (b) pyridine, DCM; (c) Et_3N, DMF, **22**: 42%, **24**: 17%, **25**: 7%.

The potency and selectivity of cyclophellitol and cyclophellitol aziridine as retaining β-glucosidase inhibitors can be attributed to their tight initial binding to the enzyme active site. Upon binding, the electrophilic carbon corresponding to C1 of the substrate glucoside is ideally positioned for reaction with the enzyme active site nucleophile, leading to covalent and irreversible modification of the enzyme active site. Glycosidase specificity is guided by the configuration and substitution pattern of the cyclophellitol derivative, as is amply demonstrated in this thesis. Looking at the conformation of cyclophellitol in comparison with that of a substrate β-glucoside, one could argue that initial binding happens, not so much as a substrate analogue, but as a transition analogue. Whereas β-glucopyranosides adopt a preferential $^4C_1$ chair conformation of Michaelis complex (Figure 3A), cyclophellitol prefers to adopt $^4H_3$ half-chair conformation (Figure 3B).

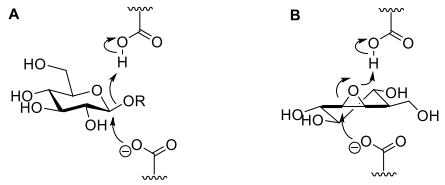
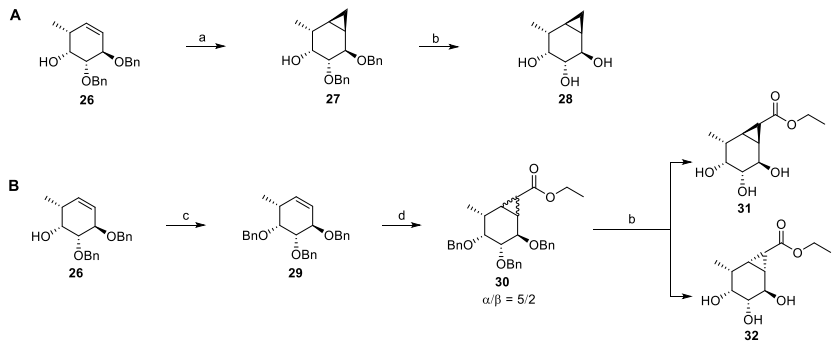


Figure 3. A) β-Glucopyranosides complex and B) cyclophellitol Michaelis complex conformations in enzyme catalysis.

Arguably, this conformation emulates that of an emerging oxocarbenium ion that may be formed as a transient intermediate in the enzyme active site and onto which the active site nucleophile will add. In a similar way, the configurational and substitutional isomers of cyclophellitol aziridine may resemble in conformation transition state oxocarbenium ions more than substrate glycosides of their underlying corresponding carbohydrates. Following this reason, cyclophellitol derivatives featuring the same substitution pattern and configuration, that are able to adopt a similar conformation but without an appropriate leaving group may turn out to be effective competitive inhibitors. With this reasoning in mind, *carba*-cyclophellitol analogues, with the epoxide oxygen substituted for methylene, can be proposed as a new class of glycosidase inhibitors.[6] Scheme 3 represents the synthesis of compounds **28**, **31** and **32**, being *carba*-cyclophellitol analogues featuring α-L-fucopyranose (**28**, **31**) and β-L-fucopyranose (**32**) configurations. Cyclopropanation of alkene **26** with diethylzinc/diiodomethane yielded cyclopropane **27** as the major stereoisomer, which was converted to α-cyclitol-cyclopropane **28** by palladium hydroxide catalyzed hydrogenolysis of the benzyl protective groups.

Scheme 3. Synthesis of L-*fuco*-carba-cyclophellitol compounds **28**, **31** and **32**

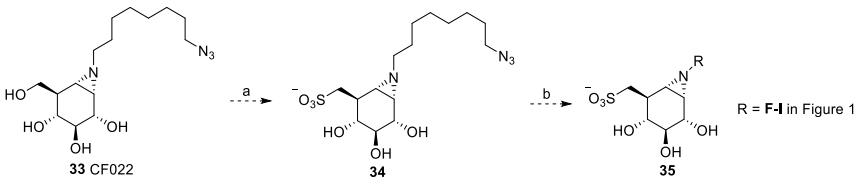


Reagent and conditions: (a) Et_2Zn, $BF_3{\cdot}O\,Et_2$, CH_2I_2, Et_2O, DCM, 83%; (b) $Pd(OH)_2/C$, H_2, MeOH, **28**: 99%, **31** 26%, **32** 11% over two steps; (c) BnBr, NaH, TBAI, DMF, 0 °C→20 °C, 95%; (d) $Cu(AcAc)_2$, Ethyl diazoacetate, EtOAc.

Substituted cyclopropane derivatives **31** and **32** were prepared as follows. Benzylation of C4-OH in **26** yielded **29**, which was treated with copper (II) acetylacetonate ($Cu(AcAc)_2$) and diazoacetate giving **30** as a stereomeric mixture of cyclopropane products in a 5:2 ratio. Following debenzylation using Pearlman's catalyst and dihydrogen, cyclitol-cyclopropane **31** and **32** were isolated following silica gel column chromatography. Future studies will reveal whether these *carba*-cyclophellitol derivatives are able to inhibit FUCA1/2, and if so whether they do so by keeping a half-chair conformation within the enzyme active site.

Recently, Speciale *et al.* have identified a new sulfoquinovosidase in *Escherichia coli*, YihQ.[7] This enzyme is a GH31 α-glycosidase that cleaves the modified glucose derivative named sulfoquinovose (SQ) from sulfoquiovosyl diacylglyceride (SQDG) sulfolipids. It would be of interest to develop YihQ activity-based probes to identify its activity in SQDG sulfolipids metabolism in bacteria. In **Chapter 5**, *epi*-cyclophellitol aziridine CF022, JJB347, JJB382-384 have already been successfully synthesized and applied in ABPP of GH31 α-glucosidases. Introduction of a sulfite moiety on to this cyclitol aziridine scaffold would yield potential YihQ ABPs. A proposed synthesis approach for YihQ ABPs **35** is depicted in Scheme 4. O-sulfonation of primary alcohol with sulfur trioxide trimethylamine complex ($SO_3{\cdot}Et_3N$) would lead to the corresponding sulfate derivative **34**, following a sulfonaton protocol as previously reported.[8]

Scheme 4. Synthesis of sulfo-aziridine ABPs **35**.



Reagent and conditions: (a) $SO_3{\cdot}Et_3N$, DMF, 60 °C; (b) $CuSO_4$(1.0 M in H_2O), sodium ascorbate (1.0 M in H_2O), DMF, **9** or **10** or **11**.

Lysosomal β-glucuronidase (GUSB) is an *exo*-glucuronidase that removes D-glucuronic acid residues from the reducing end of glycosaminoglycans (GAGs), whereas heparanase (HPSE) is an *endo*-glucuronidase able to hydrolyse glycosidic linkages within heparin sulfate (HS) chains. Surprisingly, both of GUSB and HPSE are labeled by glucuronic acid emulating cyclophellitol aziridines JJB355, JJB392, JJB395 or JJB397 (compounds in **Chapter 6, 7**). In order to selectively modify HPSE *in vitro* and *in vivo*, it would be useful to have specific HPSE ABPs. Based on the chemical structure of HS and also the HPSE catalytic cleft spatial structure analysis recently performed by Wu *et al*.,[9] HPSE would prefer to recognize oligosaccharide substrate mimetics. Moreover, *exo*-glucuronidases would not cleave the internal linkage of oligosaccharide. Based on these considerations disaccharide derivatives **36**-**43** may turn out to be selective HPSE inhibitors and probes (Figure 4), also considering that the *exo*-glycosidase, GUSB, would not be able to fit these disaccharide-like compounds.

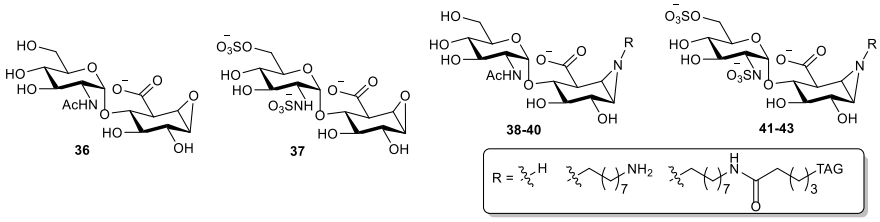
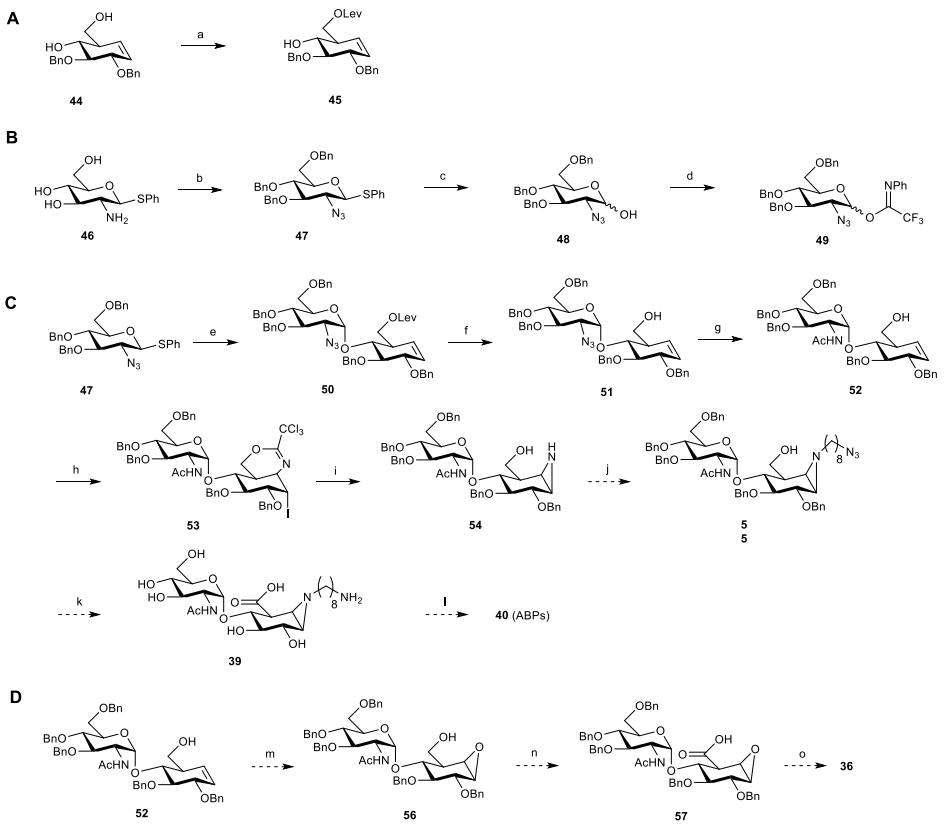


Figure 4. Proposed disaccharide cyclophellitol epoxide and aziridine inhibitors and probes for HPSE.

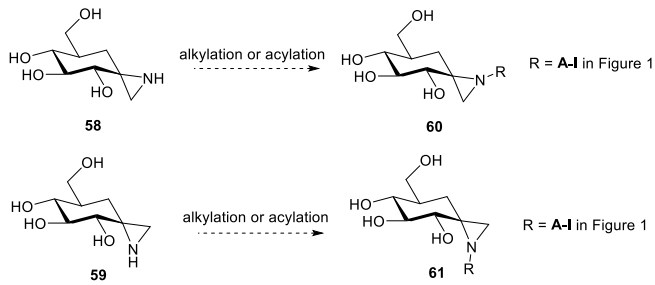
Scheme 5. Synthesis of HPSE inhibitors and probes.



Reagent and conditions: (a) Levulinic acid, DIC, DMAP, DCM, 78%; (b) i) imidazole-1-sulfonyl azide, H_2O, $CuSO_4{\cdot}5H_2O$; ii) BnBr, TBAI, NaH, DMF, 0 °C-20 °C, 43%; (c) *N*-iodosuccinimide (NIS), TFA. DCM/H_2O (v/v, 10/1), 94%; (d) $CF_3C(NPh)Cl$, Cs_2CO_3, acetone/H_2O (20/1), 68%; (e) *N*-formylmorpholine (NFM), NIS, TMSOTf, DCM, molecular sieves 3Å, **45**, -20 °C, 28%; (f) NH_2NH_2, AcOH, pyridine/AcOH (4/1), 64%; (g) AcSH, pyridine, $CHCl_3$, 70%; (h) i) Trichloroacetonitrile, DCM; ii) I_2, H_2O, $NaHCO_3$, 62%; (i) i) 1.0 M aq. HCl, MeOH; ii) $NaHCO_3$, MeOH, 58%; (j) 1-azido-8-iodooctane, K_2CO_3, DMF, 50 °C; (k) i) TEMPO/BAIB, DCM/H_2O (v/v, 2/1), 0 °C; ii) Li, NH_3, THF, -60 °C; (l) BODIPY/Cy5/Biotin-OSu; (m) *m*CPBA, DCM, 40 °C; (n) TEMPO/BAIB, DCM/H_2O (v/v, 2/1), 0 °C; (o) Pd/C, H_2, MeOH.

Compounds **36**, **40** can be synthesized via the strategy proposed in Scheme 5. Firstly, building blocks **45** ('acceptor') and **47**, **49** ('donor') are prepared from diol **44** and thiophenyl **46**, respectively. The primary alcohol in **44**[10] is selectively protected as the levulinoyl ester yielding acceptor **45**. The free amine in **46** is converted into the azide by treatment with Stick's reagent, followed by benzylation of the remaining hydroxyl groups. The glycosylation step proved a major limiting step during these initial synthesis studies, because it involves selective α-1,4-glycosylation linkage formation. Literature procedures towards the installation of related 1,2-*cis*-glycosidic linkages make use of activation strategies including nucleophile modulators, such as dimethylacetamide (DMA),[11] *N*-formylmorpholine (NFM)[12] and diphenyl sulfoxide (DPSO).[13] Herein, NFM was used as the modulator for α-selectivity of glycosylation from **47** to **50**, and after the reaction, 28% α-glycosyl product **50** was isolated. The use of *N*-phenyl-trifluoroacetimidate **49** as donor could be an alternative option to increase the efficiency and α-selectivity in the desired glycosylation event. Disaccharide aziridine **54** was successfully synthesized using the intramolecular aziridination protocol described in Chapter 6. *N*-alkylation of the aziridine nitrogen in **54** followed by global deprotection and oxidation of the cyclophellitol aziridine primary hydroxyl would require some carefully designed synthesis strategies, but seems feasible based on methodology described in this thesis. The disaccharide cyclophellitol epoxide **36** could be prepared from intermediate **52** by *m*CPBA epoxidation, TEMPO/BAIB oxidation and debenzylation depicted in Scheme 5D.

Scheme 6. Synthesis of proposed new type aziridine ABPs **60** and **61** for inverting GHs.

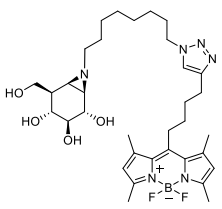


Finally, carbasugar-derived *spiro*-aziridines compounds **58** and **59** (Scheme 6) were reported by Vasella and co-workers group in 2003.[14] These aziridines, structurally related to the cyclophellitol aziridines described in this thesis, proved to be moderately weak inhibitors of *Caldocellum saccharrolyticum* β-glucosidase and yeast α-glucosidase. One might consider investigating whether *spiro*-aziridines **58** and **59** would be useful scaffolds for the development of a new generation of glycosidase activity-based probes. Possibly, the exocyclic aziridine would be able to expel water from the active site of inverting glycosidases to next react with the catalytic base. In case valid, this would lead to the discovery of inverting glycosidase ABPs, although it can not be excluded that compounds **58** and **59** also irreversibly

inhibit retaining glycosidases. The proposed ABPs **60** and **61** could be developed from *spiro*-aziridines **58** and **59** respectively via alkylation or acylation of the nitrogen on the aziridine ring and conjugation of reporter groups.

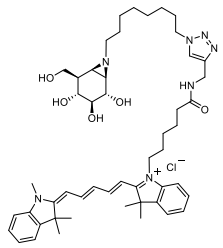
8.3 Experimental section

All reagents were of a commercial grade and were used as received unless stated otherwise. Dichloromethane (DCM), tetrahydrofuran (THF) and *N*, *N*-dimethylformamide (DMF) were stored over 4 Å molecular sieves, which were dried *in vacuo* before use. All reactions were performed under an argon atmosphere unless stated otherwise. Solvents used for flash column chromatography were of pro analysis quality. Reactions were monitored by TLC analysis using Merck aluminium sheets pre-coated with silica gel 60 with detection by UV absorption (254 nm) and by spraying with a solution of $(NH_4)_6Mo_7O_{24}.H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4.H_2O$ (10 g/L) 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 using either Baker or Screening Device silica gel 60 (0.04 - 0.063 mm) in the indicated solvents. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker AV-850 (850/214 MHz), Bruker DMX-600 (600/150 MHz) and Bruker AV-400 (400/100 MHz) spectrometer in the given solvent. Chemical shifts are given in ppm relative to the chloroform residual solvent peak or tetramethylsilane (TMS) as internal standard. Coupling constants are given in Hz. All given ^{13}C spectra are proton decoupled. High-resolution mass spectra were recorded with a LTQ Orbitrap (Thermo Finnigan). Optical rotations were measured on Propol automatic polarimeter (Sodium D-line, λ = 589 nm). IR spectra were recorded on a Shimadzu FT-IR 83000 spectrometer. LC-MS analysis was performed on an LCQ Advantage Max (Thermo Finnigan) ion-trap spectrometer (ESI+) coupled to a Surveyor HPLC system (Thermo Finnigan) equipped with a C_{18} column (Gemini, 4.6 mm x 50 mm, 3 µm particle size, Phenomenex) equipped with buffers A: H_2O, B: acetonitrile (MeCN) and C: 1% aqueous TFA or 50 mM NH_4HCO_3 in H_2O, For reversed-phase HPLC purifications an Agilent Technologies 1200 series instrument equipped with a semi preparative Gemini C_{18} column (10 x 250 mm) was used. The applied buffers were A: 25mM NH_4OAc or 50 mM NH_4HCO_3 in H_2O, B: MeCN.

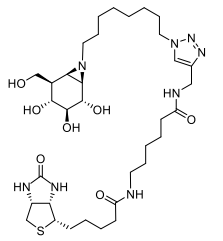


(1R,2S,3S,4R,5R,6R)-7-(8-(4-(4-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ⁴,5λ⁴-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)butyl)-1H-1,2,3-triazol-1-yl)octyl)-5-(hydroxymethyl)-7-azabicyclo[4.1.0]heptane-2,3,4-triol (3): Azide compound **1** (13 mg, 0.039 mmol, 1.0 eq.) was dissolved in DMF (0.80 mL). Green BODIPY **9** (15 mg, 0.045 mmol, 1.2 eq.), $CuSO_4$ (1.0 M in H_2O, 15 µL, 0.015 mmol, 0.38 eq.) and sodium ascorbate (1.0 M in H_2O, 16 µL, 0.016 mmol, 0.40 eq) were added to the solution under argon atmosphere. After stirring at room temperature for 12 h, the reaction volatiles were removed under reduced pressure. Then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 40%→65% B in A, 3 CV, solutions used A: 50 mM NH_4HCO_3 in H_2O, B: MeCN) and lyophilized resulting as orange product **3** JJB376 (7.7 mg, 11.7 µmol, 30%). ^{1}H-NMR (400 MHz, CD_3OD): δ ppm 7.73 (s, 1H), 6.11 (s, 2H), 4.35 (t, J = 7.0 Hz, 2H), 3.99 (dd, J = 10.1, 4.4 Hz, 1H), 3.63 – 3.58 (m, 2H), 3.13 – 3.08 (m, 1H), 3.05 – 2.98 (m, 3H), 2.78 (t, J = 7.2 Hz, 2H), 2.43 (s, 6H), 2.37 (s, 6H), 2.36 – 2.29 (m, 1H), 2.16 – 2.07 (m, 1H), 2.01 – 1.95 (m, 1H), 1.94 – 1.82 (m, 5H), 1.68 – 1.59 (m, 3H), 1.58 – 1.50 (m, 2H), 1.37 – 1.19 (m, 8H); ^{13}C-NMR (100 MHz, CD_3OD): δ ppm 154.91, 148.50, 147.87, 142.19, 132.57, 123.38, 122.61, 79.01, 73.88, 70.08,

63.73, 62.09, 51.19, 45.51, 45.45, 43.02, 32.23, 31.22, 30.81, 30.33, 30.23, 29.84, 29.06, 28.16, 27.30, 25.86, 16.49, 14.45; LC-MS: R_t 6.05 min, linear gradient 10%→90% B in 12.5 min; ESI-MS: *m/z* = 657.27 $(M+H)^+$, 637.47 $(M-F)^+$; HRMS: calculated for $C_{34}H_{51}BF_2N_6O_4$ [$M+H^+$] 657.41117, found: 657.41122.

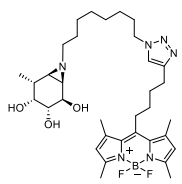


6-(3,3-dimethyl-2-((1E,3E)-5-((E)-1,3,3-trimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-3H-1l4-indol-1-yl)-N-((1-(8-((1R,2S,3S,4R,5R,6R)-2,3,4-trihydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0]heptan-7-yl)octyl)-1H-1,2,3-triazol-4-yl)methyl)hexanamide (4): Azide compound **1** (20 mg, 0.061 mmol, 1 eq.) was dissolved in DMF (1.0 mL). Blue Cy5 **10** (41 mg, 0.074 mmol, 1.2 eq.), $CuSO_4$ (1.0 M in H_2O, 25 µL, 0.025 mmol, 0.40 eq.) and sodium ascorbate (1.0 M in H_2O, 28 µL, 0.028 mmol, 0.46 eq) were added to the solution under argon atmosphere. After stirring at room temperature for 12 h, the reaction volatiles were removed under reduced pressure. Then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 41%→49% B in A, 3 CV, solutions used A: 50 mM NH_4HCO_3 in H_2O, B: MeCN) and lyophilized resulting as deep blue product **4** JJB367 (6.9 mg, 7.8 µmol, 13%). ^{1}H-NMR (600 MHz, CD_3OD): δ ppm 8.28 – 8.22 (m, 2H), 7.84 (s, 1H), 7.50 (d, *J* = 7.5 Hz, 2H), 7.44 – 7.39 (m, 2H), 7.32 – 7.25 (m, 4H), 6.62 (t, *J* = 12.4 Hz, 1H), 6.28 (dd, *J* = 13.7, 2.6 Hz, 2H), 4.45 – 4.32 (m, 4H), 4.09 (t, *J* = 7.5 Hz, 2H), 3.99 (dd, *J* = 10.1, 4.4 Hz, 1H), 3.69 – 3.54 (m, 5H), 3.12 – 3.09 (m, 1H), 3.00 (t, *J* = 9.8 Hz, 1H), 2.35 – 2.29 (m, 1H), 2.25 (t, *J* = 7.3 Hz, 2H), 2.14 – 2.09 (m, 1H), 1.99 – 1.97 (m, 1H), 1.93 (s, 6H), 1.90 – 1.79 (m, 4H), 1.73 – 1.68 (m, 10H), 1.63 (d, *J* = 6.3 Hz, 1H), 1.59 – 1.42 (m, 4H), 1.39 – 1.23 (m, 8H); ^{13}C-NMR (150 MHz, CD_3OD): δ ppm 175.74, 175.39, 174.59, 155.55, 155.47, 144.24, 143.54, 142.62, 142.51, 129.78, 129.74, 126.61, 126.28, 126.21, 124.17, 123.42, 123.28, 112.02, 111.85, 104.42, 104.23, 79.03, 73.91, 70.05, 63.72, 62.09, 51.33, 50.54, 45.54, 45.47, 44.75, 43.04, 36.46, 35.57, 31.51, 31.29, 30.37, 30.25, 29.94, 28.21, 28.13, 27.94, 27.79, 27.37, 27.31, 26.39; LC-MS: R_t 5.97 min, linear gradient 10%→90% B in 12.5 min; ESI-MS: *m/z* = 848.60 $(M)^+$; HRMS: calculated for $C_{50}H_{70}N_7O_5^+$ [M^+] 848.54329, found: 848.54304.

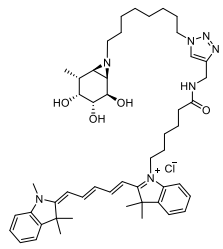


6-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)-N-((1-(8-((1R,2S,3S,4R,5R,6R)-2,3,4-trihydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0]heptan-7-yl)octyl)-1H-1,2,3-triazol-4-yl)methyl)hexanamide (5): Azide compound **1** (13 mg, 0.039 mmol, 1.0 eq.) was dissolved in DMF (0.8 mL). Biotin compound **11** (18.6 mg, 0.047 mmol, 1.2 eq.), $CuSO_4$ (1.0 M in H_2O, 15 µL, 0.015 mmol, 0.38 eq.) and sodium ascorbate (1.0 M in H_2O, 16 µL, 0.016 mmol, 0.40 eq) were added to the solution under argon atmosphere. After stirring at room temperature for 12 h, the reaction volatiles were removed under reduced pressure. Then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 15%→35% B in A, 3 CV, solutions used A: 50 mM NH_4HCO_3 in H_2O, B: MeCN) and lyophilized resulting in white powder product **5** JJB377 (9.6 mg, 13.4 µmol, 34%). ^{1}H-NMR (600 MHz, CD_3OD): δ ppm 7.84 (s, 1H), 4.49 (dd, *J* = 7.9, 4.9 Hz, 1H), 4.42 (s, 2H), 4.38 (t, *J* = 7.1 Hz, 2H), 4.30 (dd, *J* = 7.9, 4.5 Hz, 1H), 3.99 (dd, *J* = 10.1, 4.4 Hz, 1H), 3.67 – 3.55 (m, 2H), 3.23 – 3.19 (m, 1H), 3.18 – 3.14 (m, 2H), 3.13 – 3.09 (m, 1H), 3.02 (t, *J* = 9.8 Hz, 1H), 2.93 (dd, *J* = 12.7, 5.0 Hz, 1H), 2.70 (d, *J* = 12.7 Hz, 1H), 2.36 – 2.31 (m, 1H), 2.27 – 2.10 (m, 5H), 1.99 (dd, *J* = 6.3, 3.5 Hz, 1H), 1.94 (s, 1H), 1.92 – 1.86 (m, 3H), 1.79 – 1.40 (m, 13H), 1.38 – 1.28 (m, 10H); ^{13}C-NMR (150 MHz, CD_3OD): δ ppm 176.01, 175.98, 166.11, 146.26, 124.17, 79.05, 73.93, 70.11, 63.77, 63.39, 62.10, 61.63, 57.01, 51.35, 45.54, 45.46, 43.04, 41.05, 40.19, 36.83, 36.76, 35.59, 31.27, 30.37,

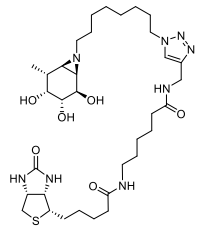
30.28, 30.12, 29.93, 29.78, 29.50, 28.22, 27.54, 27.37, 26.93, 26.51; LC-MS: R_t 3.94 min, linear gradient 10%→90% B in 12.5 min; ESI-MS: *m/z* = 723.40 $(M+H)^+$; HRMS: calculated for $C_{34}H_{58}N_8O_7S$ $[M+H^+]$ 723.42219, found: 723.42259.



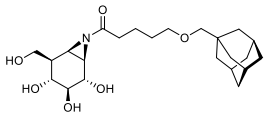
(1R,2R,3R,4R,5R,6R)-7-(8-(4-(4-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ⁴,5λ⁴-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)butyl)-1H-1,2,3-triazol-1-yl)octyl)-5-methyl-7-azabicyclo[4.1.0]heptane-2,3,4-triol (6): Azide compound **2** (14 mg, 0.045 mmol, 1.0 eq.) was dissolved in DMF (1.0 mL). Green BODIPY **9** (20 mg, 0.054 mmol, 1.2 eq.), $CuSO_4$ (1.0 M in H_2O, 18 µL, 0.018 mmol, 0.4 eq.) and sodium ascorbate (1.0 M in H_2O, 19 µL, 0.019 mmol, 0.42 eq) were added to the solution under argon atmosphere. After stirring at room temperature for 12 h, the reaction volatiles were removed under reduced pressure. Then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 47%→53% B in A, 3 CV, solutions used A: 50 mM NH_4HCO_3 in H_2O, B: MeCN) and lyophilized resulting as orange product **6** JJB350 (2.3 mg, 3.5 µmol, 8%). ^{1}H-NMR (850 MHz, CD_3OD): *δ* ppm 7.73 (s, 1H), 6.12 (s, 2H), 4.35 (t, *J* = 7.0 Hz, 2H), 3.99 (dd, *J* = 8.7, 4.3 Hz, 1H), 3.54 – 3.52 (m, 1H), 3.34 – 3.32 (m, 1H), 3.06 – 3.00 (m, 2H), 2.79 (t, *J* = 7.3 Hz, 2H), 2.44 (s, 6H), 2.39 (s, 6H), 2.31 – 2.27 (m, 1H), 2.13 – 2.10 (m, 1H), 1.96 – 1.80 (m, 6H), 1.69 – 1.62 (m, 2H), 1.56 – 1.52 (m, 2H), 1.36 – 1.21 (m, 8H), 1.14 (d, *J* = 7.5 Hz, 3H); ^{13}C-NMR (214 MHz, CD_3OD): *δ* ppm 154.93, 148.50, 147.89, 142.17, 132.59, 123.37, 122.60, 76.04, 75.14, 70.18, 62.29, 51.21, 46.47, 45.73, 36.95, 32.27, 31.22, 30.82, 30.56, 30.42, 29.89, 29.09, 28.21, 27.32, 25.90, 16.77, 16.50, 14.43; LC-MS: R_t 6.26 min, linear gradient 10%→90% B in 12.5 min; ESI-MS: *m/z* = 641.33 $(M+H)^+$; HRMS: calculated for $C_{34}H_{51}BF_2N_6O_3$ $[M+H^+]$ 641.41265, found: 641.41541.



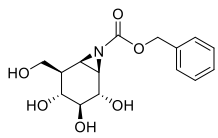
3,3-dimethyl-1-(6-oxo-6-(((1-(8-((1R,2R,3R,4R,5R,6R)-2,3,4-trihydroxy-5-methyl-7-azabicyclo[4.1.0]heptan-7-yl)octyl)-1H-1,2,3-triazol-4-yl)methyl)amino)hexyl)-2-((1E,3E)-5-((E)-1,3,3-trimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-3H-indol-1-ium (7): Azide compound **2** (8.3 mg, 0.027 mmol, 1.0 eq.) was dissolved in DMF (0.80 mL). Blue Cy5 **10** (16 mg, 0.030 mmol, 1.1 eq.), $CuSO_4$ (1.0 M in H_2O, 12 µL, 0.012 mmol, 0.44 eq.) and sodium ascorbate (1.0 M in H_2O, 13 µL, 0.013 mmol, 0.48 eq) were added to the solution under argon atmosphere. After stirring at room temperature for 12 h, the reaction volatiles were removed under reduced pressure. Then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 45%→55% B in A, 3 CV, solutions used A: 50 mM NH_4HCO_3 in H_2O, B: MeCN) and lyophilized resulting as a purple powder product **7** JJB381 (6.9 mg, 7.8 µmol, 13%). ^{1}H-NMR (850 MHz, CD_3OD): *δ* ppm 8.28 – 8.22 (m, 2H), 7.84 (s, 1H), 7.50 (d, *J* = 7.4 Hz, 2H), 7.44 – 7.38 (m, 2H), 7.34 – 7.22 (m, 4H), 6.62 (t, *J* = 12.4 Hz, 1H), 6.28 (dd, *J* = 13.7, 3.3 Hz, 2H), 4.41 (s, 2H), 4.36 (t, *J* = 7.1 Hz, 2H), 4.09 (t, *J* = 7.3 Hz, 2H), 3.99 (dd, *J* = 8.7, 4.3 Hz, 1H), 3.63 (s, 2H), 3.53 – 3.52 (m, 1H), 2.66 (s, 1H), 2.31 – 2.27 (m, 1H), 2.25 (t, *J* = 7.3 Hz, 2H), 2.15 – 2.10 (m, 1H), 1.91 – 1.79 (m, 5H), 1.73 – 1.58 (m, 16H), 1.56 – 1.50 (m, 2H), 1.50 – 1.44 (m, 2H), 1.35 – 1.27 (m, 8H), 1.14 (d, *J* = 7.5 Hz, 3H); ^{13}C-NMR (214 MHz, CD_3OD): *δ* ppm 175.72, 175.41, 174.61, 155.56, 155.48, 146.13, 144.24, 143.54, 142.63, 142.52, 129.78, 129.74, 126.61, 126.29, 126.22, 124.12, 123.42, 112.02, 111.85, 104.41, 104.23, 76.04, 75.11, 70.13, 62.29, 51.34, 50.54, 50.52, 46.47, 45.71, 44.75, 36.94, 36.46, 35.57, 31.50, 31.30, 30.56, 30.48, 29.99, 28.26, 28.12, 27.94, 27.79, 27.40, 27.31, 26.38; LC-MS: R_t 4.83 min, linear gradient 10%→90% B in 12.5 min; ESI-MS: *m/z* = 833.60 $(M)^+$; HRMS: calculated for $C_{50}H_{70}N_7O_4^+$ $[M^+]$ 833.55621, found: 833.55124.



6-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)-N-((1-(8-((1R,2R,3R,4R,5R,6R)-2,3,4-trihydroxy-5-methyl-7-azabicyclo[4.1.0]heptan-7-yl)octyl)-1H-1,2,3-triazol-4-yl)methyl) hexanamide (8): Azide compound **2** (8.3 mg, 0.027 mmol, 1.0 eq.) was dissolved in DMF (0.80 mL). Biotin compound **11** (12 mg, 0.030 mmol, 1.1 eq.), $CuSO_4$ (1.0 M in H_2O, 12 µL, 0.012 mmol, 0.44 eq.) and sodium ascorbate (1.0 M in H_2O, 13 µL, 0.013 mmol, 0.48 eq) were added to the solution under argon atmosphere. After stirring at room temperature for 12 h, the reaction volatiles were removed under reduced pressure. Then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 5%→40% B in A, 3 CV, solutions used A: 50 mM NH_4HCO_3 in H_2O, B: MeCN) and lyophilized resulting as white product **8** JJB385 (3.8 mg, 5.3 µmol, 20%). ^{1}H-NMR (850 MHz, CD_3OD): δ ppm 7.84 (s, 2H), 4.50 – 4.48 (m, 1H), 4.42 (s, 2H), 4.38 (t, *J* = 7.1 Hz, 2H), 4.30 (dd, *J* = 7.9, 4.5 Hz, 1H), 4.00 (dd, *J* = 8.7, 4.3 Hz, 1H), 3.54 – 3.52 (m, 1H), 3.36 – 3.32 (m, 1H), 3.23 – 3.18 (m, 1H), 3.17 – 3.13 (m, 2H), 2.92 (dd, *J* = 12.8, 5.0 Hz, 1H), 2.70 (d, *J* = 12.7 Hz, 1H), 2.66 (s, 1H), 2.34 – 2.30 (m, 1H), 2.23 (t, *J* = 7.5 Hz, 2H), 2.21 – 2.18 (m, 2H), 2.17 – 2.12 (m, 1H), 1.92 – 1.83 (m, 3H), 1.78 – 1.71 (m, 1H), 1.71 – 1.53 (m, 7H), 1.53 – 1.47 (m, 2H), 1.45 – 1.40 (m, 2H), 1.40 – 1.26 (m, 10H), 1.15 (d, *J* = 7.5 Hz, 3H); ^{13}C-NMR (214 MHz, CD_3OD): δ ppm 176.00, 175.96, 166.11, 146.22, 124.14, 76.06, 75.10, 70.14, 63.38, 62.30, 61.62, 57.02, 51.35, 46.48, 45.73, 41.05, 40.18, 36.94, 36.81, 36.75, 35.59, 31.29, 30.57, 30.12, 29.79, 29.50, 27.55, 27.39, 26.93, 26.52, 16.79; LC-MS: R_t 4.48 min, linear gradient 10%→90% B in 12.5 min; ESI-MS: *m/z* = 707.47 $(M+H)^+$; HRMS: calculated for $C_{34}H_{58}N_8O_6S$ $[M+H^+]$ 707.42728, found: 707.42759.

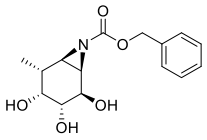
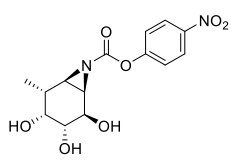


5-(((3R,5R,7R)-adamantan-1-yl)methoxy)-1-((1R,2S,3S,4R,5R,6R)-2,3,4-trihydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0]heptan-7-yl)pentan-1-one (18): *N*-Ethoxycarbonyl-2-ethoxy-1, 2-dihydroquinoline (EEDQ) (79 mg, 0.32 mmol, 1.6 eq.) and adamantane-acid **17** (85 mg, 0.32 mmol, 1.6 eq.) were dissolved in anhydrous DMF (0.32 mL) and stirred at room temperature for 2 h. Pre-activated mixed anhydride solution (160 µL, 0.80 eq.) was added to deprotected aziridine **16** (35 mg, 0.20 mmol, 1.0 eq.) in dry DMF (1.2 mL) at 0 °C and stirred for 30 min. Additional pre-activated solution (160 µL, 0.80 eq) was added. The resulting mixture was stirred at 0 °C for 2 h. The reaction was quenched by MeOH (1.0 mL) and the mixture was concentrated *in vacuo*. Then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 38%→47% B in A, 12min, solutions used A: H_2O, B: MeCN) and the fractions were lyophilized directly yielding **18** as white powder (21 mg, 0.05 mmol, 25%).^{1}H-NMR (400 MHz, CD_3OD): δ ppm 4.08 (d, *J* = 10.4, 2H), 3.72 – 3.66 (m, 2H), 3.41 (t, *J* = 6.4 Hz, 2H), 3.23 (dd, *J* = 10.0, 8.0 Hz, 1H), 3.10 – 3.02 (m, 2H), 2.74 (d, *J* = 5.6 Hz, 1H), 2.55 – 2.51 (m, 2H), 2.01-1.95 (m, 4H), 1.77 – 1.66 (m, 8H); 1.64 – 1.56 (m, 8H); ^{13}C-NMR (100 MHz, CD_3OD): δ ppm 188.5, 83.0, 79.1, 73.4, 72.2, 69.4, 63.6, 45.3, 42.4, 41.1, 40.8, 38.3, 36.6, 35.2, 30.1, 29.9, 29.8, 22.9; LC-MS: R_t 7.87min; linear gradient 10→90% B in 15 min, ESI-MS: *m/z* = 424.4 $(M+H)^+$; HRMS: calculated for $C_{14}H_{17}NO_4$ $[M+H^+]$ 424.26936, found: 424.26921.

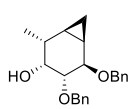


Benzyl (1R,2S,3S,4R,5R,6R)-2,3,4-trihydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0]heptane-7-carboxylate (22): Benzyl alcohol (28 µL, 0.28 mmol, 1.1 eq) and *p*-nitrophenol chloroformate (105mg, 0.32 mmol, 2.0 eq.) were dissolved in DCM (6.0 mL). Pyridine (104 µL, 1.3 mmol, 5.0 eq.) was added into the solution, the resulting mixture was stirred

at room temperature for 4 h. After quenching the reaction with brine (1.5 mL), the mixture was transferred into a separatory funnel, washed the organic layer with brine, dried over $MgSO_4$, concentrated *in vacuo*, redissolved in dry DMF 1mL, followed by addition of Et_3N (0.2 mL). Aziridine compound (45.6 mg, 0.26 mmol, 1.0 eq.) in DMF (1.0 mL) was added dropwise into active ester solution under argon atmosphere at room temperature overnight. The resulting solution was concentrated *in vacuo* and Then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 17%→23% B in A, 3 CV, solutions used A: H_2O, B: MeCN) and the fractions were lyophilized directly yielding **22** as white powder product (30 mg, 0.11 mmol, 42%). TLC: R_f 0.31 (DCM/MeOH, 5/1, v/v); ^{1}H-NMR (600 MHz, CD_3OD): δ ppm 7.39 – 7.29 (m, 5H), 5.15 – 5.09 (m, 2H), 4.04 (dd, *J* = 10.4, 4.4 Hz, 1H), 3.78 (dd, *J* = 10.4, 8.4 Hz, 1H), 3.71 (d, *J* = 8.0 Hz, 1H), 3.21 (dd, *J* = 10.0, 8.0 Hz, 1H), 3.12 – 3.02 (m, 2H), 2.75 (d, *J* = 6.0 Hz, 1H); 1.98 – 1.90 (m, 1H); ^{13}C-NMR (150 MHz, CD_3OD): δ ppm 164.6, 137.4, 129.6, 129.3, 129.1, 129.0, 79.0, 73.3, 69.3, 69.3, 63.6, 45.1.3, 43.4.7, 42.2; LC-MS: R_t 5.16 min, linear gradient 00→90% B in 15 min; ESI-MS: *m/z* = 310.3 $(M+H)^+$; HRMS: calculated for $C_{15}H_{19}NO_5$ $[M+H^+]$ 310.12851, found: 310.12876.

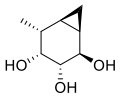



Benzyl (1R,2R,3R,4R,5R,6R)-2,3,4-trihydroxy-5- methyl-7-azabicyclo[4.1.0]heptane-7-carboxylate (24) and 4-nitrophenyl(1R,2R,3R,4R,5R,6R)-2,3,4-trihydroxy-5- methyl-7-azabicyclo[4.1.0]heptane-7-carboxylate (25): Benzyl alcohol (23 µL, 0.23 mmol, 1.1 eq) and p-nitrophenol chloroformate (80.6 mg, 0.40 mmol, 2.0 eq.) were dissolved in DCM (5 mL). Pyridine (80 µL, 1 mmol, 5.0 eq.) was added into the solution, the resulting mixture was stirred at room temperature for 4 h. After quenching the reaction with brine (1.2 mL), the mixture was transferred into a separatory funnel, washed the organic layer with brine, dried over $MgSO_4$, concentrated *in vacuo*, redissolved in dry DMF (1.0 mL), followed by addition of Et_3N (0.20 mL). Aziridine compound (32 mg, 0.20 mmol, 1.0 eq.) in DMF (1.5mL) was added dropwise under argon atmosphere at room temperature overnight. The resulting solution was concentrated *in vacuo* and Then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 19%→23% B in A, 3 CV, solutions used A: H_2O, B: MeCN) giving product **24** (10 mg, 34 µmol, 17% yield) and **25** (4.3 mg, 13 µmol, 7% yield) as white powder. **24** TLC: R_f 0.51 (DCM/MeOH, 8/1, v/v),: ^{1}H-NMR (600 MHz, CD_3OD): δ ppm 7.39 – 7.32 (m, 4H), 5.15 (dd, *J* = 21.6, 12 Hz, 2H), 4.03 (dd, *J* = 9.0, 4.2 Hz, 1H), 3.60 – 3.59 (m, 1H), 3.37 (dd, *J* = 9.0, 1.8 Hz, 1H), 2.98 (dd, *J* = 6.0, 3.6 Hz, 1H), 2.41 (d, *J* = 6.6 Hz, 1H), 2.04 – 2.02 (m, 1H); 1.89 (s, 1H), 1.19 (d, *J* = 7.8Hz, 3H); ^{13}C-NMR (150 MHz, CD_3OD): δ ppm 165.0, 137.5, 129.6, 129.3, 129.2, 129.0, 127.0, 75.7, 74.4, 69.2, 69.2, 44.6, 44.3, 36.7, 16.2; LC-MS: R_t 4.28 min, linear gradient 10→90% B in 15 min; ESI-MS: *m/z* = 294.3 $(M+H)^+$; HRMS: Calculated for $C_{15}H_{19}NO_5$ $[M+H^+]$ 294.13360. Found: 264.13364. **25** ^{1}H-NMR (400 MHz, CD_3OD): δ ppm 8.24 – 8.20 (m, 2H), 7.18 – 7.15 (m, 2H), 4.78 (t, J = 7.8 Hz, 1H), 4.66 (t, J = 6.4 Hz, 1H), 4.26 (t, *J* = 6.4 Hz, 1H), 3.98 – 3.93 (m, 2H), 2.56 – 2.52 (m, 1H), 1.04 (d, *J* = 7.6Hz, 3H); ^{13}C-NMR (100 MHz, CD_3OD): δ ppm 164.7, 161.6, 143.2, 126.9, 116.6, 81.3, 78.9, 73.4, 73.0, 55.6, 35.4, 11.3; **25** LC-MS: R_t 4.15min, linear gradient 10→90% B in 15 min; ESI-MS: *m/z* = 342.1 $(M+NH_4^+)$.

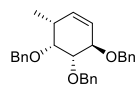


(1R,2R,3R,4R,5R,6R)-4,5-bis(benzyloxy)-2-methylbicyclo[4.1.0]heptan-3-ol (27): Diethyl zinc solution (1.0 M in Et_2O, 930 µL, 930 µmol, 10.0 eq.), $BF_3{\cdot}OEt_2$ liquid (56.7 µL, 463 µmol, 5.0 eq.) and Et_2O (97 µL, 930 µmol, 10.0 eq.) were dissolved in 0.5 mL dry DCM. Diiodomethane (149 µL, 1.8

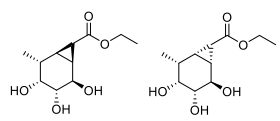
mmol, 20 eq.) was added to the solution and stirred at room temperature for 5 min. A solution of compound **26** (30 mg, 0.093 mmol 1.0 eq.) in dry DCM (0.50 mL) was added to the mixture dropwise. After stirring overnight, the reaction was quenched by saturated aqueous NH_4Cl, the mixture was poured into H_2O and extracted by EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and residue was purified by silica gel column chromatography (5%→20% EtOAc in pentane) yielding cyclopropane compound **27** (26 mg, 0.077 mmol, 83%). TLC: R_f 0.45 (EtOAc/pentane, 1/5, v/v); $[\alpha]_D^{20}$ - 101 (*c* = 0.5, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.46 – 7.21 (m, 10H), 4.82 (d, *J* = 11.8 Hz, 1H), 4.77 – 4.59 (m, 3H), 4.25 (t, *J* = 7.3 Hz, 1H), 3.75 (s, 1H), 3.17 – 3.13 (m, 1H), 2.08 (s, 1H), 1.65 – 1.59 (m, 1H), 1.51 – 1.41 (m, 1H), 1.22 (d, *J* = 12.0 Hz, 3H), 0.89 – 1.74 (m, 2H), 0.27 – 0.20 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 139.24, 138.69, 128.51, 128.41, 127.91, 127.86, 127.76, 127.52, 82.61, 77.48, 77.16, 76.84, 76.47, 73.01, 72.59, 71.00, 36.12, 18.42, 16.94, 16.18, 11.63; HRMS: calculated for $C_{22}H_{26}O_3$ [M+H$^+$] 339.19547, found: 339.19599.



(1R,2R,3R,4R,5R,6R)-5-methylbicyclo[4.1.0]heptane-2,3,4-triol (28): A mixture of product **27** (26 mg, 0.077 mmol, 1.0 eq) and $Pd(OH)_2/C$ (20 wt.% loading(dry basis), 5.0 mg) in MeOH (5.0 mL) was stirred at room temperature under hydrogen atmosphere for 24 h. The catalyst was then filtered off and washed with MeOH. The filtrate were combined and concentrated under reduced pressure. Crude product was purified by silica gel column chromatography (5%→10%, MeOH in DCM) giving the title compound **28** (12 mg, 0.076 mmol, 99%). TLC: R_f 0.60 (1/5, MeOH/DCM, v/v); $[\alpha]_D^{20}$ - 102 (*c* = 0.2, MeOH); ^{1}H-NMR (850 MHz, CD_3OD): δ ppm 4.19 (dd, *J* = 8.9, 6.5 Hz, 1H), 3.53 – 3.48 (m, 1H), 3.05 (dd, *J* = 8.9, 1.8 Hz, 1H), 1.70 – 1.66 (m, 1H), 1.35 – 1.31 (m, 1H), 1.17 (d, *J* = 7.3 Hz, 3H), 0.74 – 0.70 (m, 1H), 0.69 – 0.65 (m, 1H), 0.22 – 0.17 (m, 1H); ^{13}C-NMR (214 MHz, CD_3OD): δ ppm 76.96, 75.85, 70.40, 38.05, 19.89, 18.77, 17.94, 11.82; IR (neat, cm^{-1}): 3321, 2965, 2905, 1366, 1250, 1045, 1022, 995, 929, 812, 682; HRMS: calculated for $C_8H_{14}O_3$ [M+H$^+$] 159.10157, found: 159.10149.

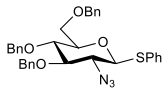


((((1R,2S,3R,6R)-6-methylcyclohex-4-ene-1,2,3-triyl)tris(oxy))tris(methylene)) tribenzene (29): To a solution of **26** (65 mg, 0.2 mmol, 1.0 eq.) in dry DMF (1 mL), benzyl bromide (47.6 µL, 0.4 mmol, 2.0 eq.) and tetrabutylammonium iodide (1.5 mg, 2.0 µmol, 0.02 eq.) were added. The mixture was cooled down to 0 °C, and NaH (60% (w/w) in mineral oil, 16 mg, 0.40 mmol, 2.0 eq.) was added slowly and keep the low temperature for 2 h. After stirring at room temperature overnight, the reaction was quenched with H_2O (0.5 mL), and the mixture was extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and residue was purified by silica gel column chromatography (0%→20% EtOAc in pentane) yielding cyclohexane **29** (80 mg, 0.19 mmol, 95%). TLC: R_f 0.62 (EtOAc/pentane, 1/8, v/v); $[\alpha]_D^{20}$ - 103 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.54 – 7.33 (m, 15H), 5.85 – 5.80 (m, 1H), 5.58 (d, *J* = 10.0 Hz, 1H), 5.03 (d, *J* = 11.8 Hz, 1H), 4.95 – 4.76 (m, 5H), 4.58 – 4.53 (m, 1H), 4.02 – 3.98 (m, 1H), 3.90 – 3.87 (m, 1H), 2.63 – 2.50 (m, 1H), 1.19 (d, *J* = 7.3 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 138.93, 132.05, 128.41, 128.37, 128.24, 128.10, 127.85, 127.58, 127.52, 127.48, 127.48, 125.52, 83.30, 77.95, 77.58, 73.82, 72.39, 72.27, 35.84, 16.69; IR (neat, cm^{-1}): 3028, 2872, 1497, 1454, 1089, 1068, 734, 696; HRMS: calculated for $C_{28}H_{30}O_3$ [M+H$^+$] 415.22677, found: 415.22683.

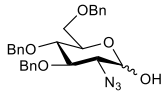


Ethyl (1S,2R,3R,4R,5R,6R,7S)-2,3,4-trihydroxy-5-methylbicyclo [4.1.0]heptane -7-carboxylate (31) and ethyl (1R,2R,3R,4R,5R,6S,7R)-2,3,4-trihydroxy-5-

methylbicyclo[4.1.0]heptane-7-carboxylate (32): A 2-necked pear flask was charged with **29** (80 mg, 0.19 mmol, 1.0 eq.), Copper(II) acetylacetonate ($Cu(AcAc)_2$) (5.0 mg, 0.019 mmol, 0.10 eq.) and dry EtOAc (0.2 mL). The mixture was refluxed at 90 °C and ethyl diazoacetate (13% wt. in DCM, 46 µL, 0.38 mmol, 2.0 eq.) was added in dry EtOAc (0.80 mL) by syringe pump over 6 h. After full conversion of starting material, the mixture was concentrated under reduced pressure, and silica gel column chromatography (1%→10%, EtOAc in pentane) produced the mixture of α and β cyclopropane ester mixture **30**, which was treated by 20 mg $Pd(OH)_2/C$ (20% wt. loading (dry basis)) in MeOH (2.0 mL) at room temperature under hydrogen atmosphere overnight. The reaction mixture was filtered with celite and washed with MeOH. The solvent was removed under reduced pressure and residue was purified by silica gel column chromatography (0%→10% MeOH in DCM) giving target α-cyclopropane **31** (11 mg, 0.049 mmol, 26%) and byproduct β-cyclopropane **32** (4.6 mg, 0.020 mmol, 11%). **31** TLC: R_f 0.34 (MeOH/DCM, 1/9, v/v); ^{1}H-NMR (400 MHz, CD_3OD): δ ppm 4.20 (dd, J = 8.9, 6.2 Hz, 1H), 4.10 (q, J = 7.3 Hz, 2H), 3.55 – 3.51 (m, 1H), 3.08 – 3.03 (m, 1H), 1.98 – 1.92 (m, 1H), 1.84 – 1.76 (m, 1H), 1.58 (t, J = 4.6 Hz, 1H), 1.29 – 1.22 (m, 4H), 1.19 (d, J = 7.3 Hz, 3H); ^{13}C-NMR (100 MHz, CD_3OD): δ ppm 175.52, 76.17, 75.59, 68.90, 61.70, 36.92, 30.95, 29.08, 26.80, 18.47, 14.53; HRMS: calculated for $C_{11}H_{18}O_5$ [M+H$^+$] 231.11261, found: 231.11039. **32** TLC: R_f 0.29 (1/9, MeOH/DCM, v/v); ^{1}H-NMR (400 MHz, CD_3OD): δ ppm 4.15 – 4.06 (m, 2H), 3.82 (d, J = 8.7 Hz, 1H), 3.68 – 3.65 (m, 1H), 3.28 – 3.24 (m, 1H), 2.20 – 2.10 (m, 1H), 2.08 (t, J = 4.6 Hz, 1H), 1.57 – 1.51 (m, 2H), 1.27 – 1.21 (m, 3H), 1.12 (d, J = 6.9 Hz, 3H); ^{13}C-NMR (100 MHz, CD_3OD): δ ppm 78.07, 74.08, 70.34, 61.58, 33.45, 30.32, 29.37, 23.83, 16.82, 14.55; HRMS: calculated for $C_{11}H_{18}O_5$ [M+H$^+$] 231.11261, found: 231.11058.

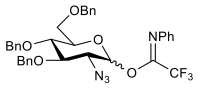


(2S,3R,4R,5S,6R)-3-azido-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-2-(phenylthio)tetrahydro-2H-pyran (47): A mixture of compound **46** (5.7 g, 21 mmol, 1.0 eq.), 'Stick' reagent, imidazole-1-sulfonyl azide (5.5 g, 26 mmol, 1.2 eq.) and $CuSO_4{\cdot}5H_2O$ (52 mg, 0.21 mmol, 0.010 eq.) in 100 mL EtOH was stirred at room temperature overnight. The resulting solution was concentrated to remove most of EtOH *in vacuo*, diluted by 1.0 M aqueous HCl (100 mL), extracted by EtOAc. The organic layer washed by sat. aq. $NaHCO_3$ and brine and dried over $MgSO_4$ and concentrated *in vacuo*. After three times co-evaporation with toluene, the residue was dissolved in dry DMF (60 mL), followed by addition of BnBr (6.5 mL, 55 mmol, 2.6 eq.), tetrabutylammonium iodide (77 mg, 0.21 mmol, 0.010 eq) and NaH (60%(w/w) in mineral oil, 1.9 g, 55 mmol, 2.6 eq.). The reaction mixture was stirred at 0 °C to room temperature overnight, quenched with MeOH (20 mL) and extracted by Et_2O. The crude product was purified by silica gel column chromatography (0%→15%, EtOAc in pentane) to afford product **47** as a white powder (5.2 g, 8.9 mmol, 43%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.60 (d, J = 6.9 Hz, 2H), 7.38 – 7.15 (m, 18H), 4.84 (d, J = 3.6 Hz, 2H), 4.82 – 4.76 (m, 1H), 4.65 – 4.58 (m, 2H), 4.58 – 4.51 (m, 1H), 4.41 (d, J = 10.1 Hz, 1H), 3.81 – 3.70 (m, 2H), 3.61 (t, J = 9.3 Hz, 1H), 3.48 – 3.52 (m, 2H), 3.34 (t, J = 9.7 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 149.09, 138.32, 137.96, 137.72, 133.77, 131.27, 129.12, 128.66, 128.61, 128.51, 128.35, 128.16, 128.04, 127.98, 127.75, 86.06, 85.20, 79.45, 77.65, 76.04, 75.19, 73.56, 68.85, 65.16; HRMS: calculated for $C_{33}H_{33}N_3O_4S$ [M+Na$^+$] 590.20840, found: 590.20796.

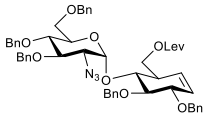


(3R,4R,5S,6R)-3-azido-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-ol (48): Compound **47** (568 mg, 1.0 mmol, 1.0 eq.) was dissolved in DCM/H_2O (11 mL, 10/1, v/v) and cooled to 0°C. *N*-iodosuccinimide (225 mg, 1.0 mmol, 1.0 eq.) and TFA (0.74 mL, 1.0 mmol, 1.0 eq.) were added into the solution and stirred for 3 h, and quenched by sat. aq. $Na_2S_2O_3$ (25 mL). The resulting mixture

was diluted by EtOAc, washed by sat. aq. $NaHCO_3$ twice and dried over $MgSO_4$. After concentration *in vacuo*, the residue was purified by silica gel column chromatography (10%→40%, EtOAc in pentane) to afford mixture product **48** as white crystals (449 mg, 0.94 mmol, 94%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.43 – 7.08 (m, 22H), 5.32 (d, J = 3.5 Hz, 1H), 4.88 (s, 2H), 4.84 – 4.76 (m, 2H), 4.61 – 4.47 (m, 5H), 4.11 – 4.05 (m, 1H), 4.04 – 3.96 (m, 1H), 3.71 – 3.53 (m, 4H), 3.52 – 3.34 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 137.94, 137.92, 128.62, 128.59, 128.27, 128.22, 128.14, 128.12, 128.05, 127.99, 127.96, 127.93, 96.30, 92.21, 83.19, 80.25, 78.58, 77.79, 75.73, 75.69, 75.18, 75.15, 75.00, 73.68, 73.63, 70.76, 68.70, 68.64, 67.56, 64.13.

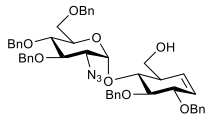


(3R,4R,5S,6R)-3-azido-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl (E)-2,2,2-trifluoro-N-phenylacetimidate (49): Compound **48** (316 mg, 0.66 mmol, 1.0 eq.) was dissolved in 7 mL actone/H_2O (20/1, v/v) and cooled to 0 °C. *N*-phenyl trifluoroacetimidoyl chloride (280 mg, 1.3 mmol, 2.0 eq.) and Cs_2CO_3 (261 mg, 0.80 mmol, 1.2 eq.) were added into the solution and stirred for 24 h, quenched by adding Et_3N dropwise. The resulting mixture was diluted by EtOAc, washed by H_2O and brine aqueous and dried over $MgSO_4$. After concentration *in vacuo*, the residue was purified by silica gel column chromatography (0%→10%, EtOAc in pentane) yielding product **49** as light yellow oil (293 mg, 0.45 mmol, 68%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.42 – 7.23 (m, 18H), 7.17 (d, J = 6.2 Hz, 3H), 7.10 (t, J = 7.4 Hz, 1H), 6.82 (d, J = 7.7 Hz, 2H), 4.92 (s, 2H), 4.88 – 4.76 (m, 1H), 4.67 – 4.45 (m, 4H), 4.07 – 3.87 (m, 2H), 3.86 – 3.77 (m, 2H), 3.76 – 3.60 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 177.75, 143.39, 137.85, 137.78, 137.76, 137.72, 128.87, 128.63, 128.56, 128.51, 128.17, 128.11, 128.02, 128.00, 127.95, 124.51, 93.89, 93.83, 80.37, 77.58, 75.79, 75.39, 75.18, 73.65, 73.51, 67.92, 63.03.

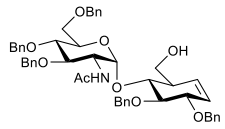


((1R,4S,5R,6R)-6-(((2S,3R,4R,5S,6R)-3-azido-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-4,5-bis(benzyloxy) cyclohex-2-en-1-yl)methyl 4-oxopentanoate (50): Mixture of thioglycoside donor **47** (108 mg, 0.19 mmol, 1.2 eq.) and flame-dried molecular sieve (3Å) was suspended in dry DCM (1.0 mL), the final concentration of the thioglycoside donor was 60 mM. Then, *N*-formylmorpholine (306 µL, 3.0 mmol, 16 eq.) was added to the mixture. The resulting mixture was stirred at room temperature for 10 min and at reaction temperature specified for particular reaction for additional 10 min. Subsequently, NIS (43 mg, 0.19 mmol, 1.2 eq.) and trimethylsilyltriflate (62 µL, 0.34 mmol, 1.8 eq.) were added, the reaction progress was monitored by TLC (pentane/EtOAc, 5/2, v/v). Upon completion activation of the glycosyl donor, acceptor **45** (70 mg, 0.16 mmol, 1.0 eq.) was added to the reaction mixture. The progress of glycosylation was monitored by TLC. Upon completion of reaction sat. aq. $NaHCO_3$, and $Na_2S_2O_3$ were added to the mixture, followed by vigorous stirring until the brown color of the reaction mixture faded away. The resulting mixture was filtered and extracted by DCM, dried over $MgSO_4$, filtered and concentrated before silica gel column chromatography (EtOAc in pentane, 10%→30%) yielding pure product **50** as light yellow oil (40 mg, 0.045 mmol, 28%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.39 – 7.18 (m, 23H), 7.19 – 7.10 (m, 2H), 5.82 – 5.73 (m, 2H), 5.59 – 5.51 (m, 1H), 5.08 (d, J = 10.9 Hz, 1H), 4.95 – 4.80 (m, 3H), 4.76 (d, J = 10.8 Hz, 1H), 4.71 – 4.57 (m, 3H), 4.55 – 4.44 (m, 2H), 4.30 (dd, J = 11.0, 3.4 Hz, 1H), 4.24 – 4.18 (m, 1H), 4.06 (dd, J = 11.0, 5.3 Hz, 1H), 3.98 – 3.86 (m, 3H), 3.85 – 3.67 (m, 3H), 3.61 – 3.55 (m, 1H), 3.28 (dd, J = 10.4, 4.0 Hz, 1H), 2.69 – 2.60 (m, 3H), 2.52 – 2.47 (m, 2H), 2.15 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 206.35, 172.63, 138.99, 138.19, 138.03, 138.00, 137.98, 128.56, 128.56, 128.54,

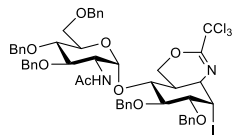
128.49, 128.40, 128.12, 128.04, 128.02, 127.99, 127.94, 127.93, 127.85, 127.80, 127.63, 127.40, 127.36, 98.35, 84.17, 80.90, 80.25, 78.19, 75.50, 75.14, 74.76, 74.52, 73.64, 71.77, 71.59, 68.05, 64.52, 63.30, 42.90, 37.91, 29.96, 27.89; HRMS: calculated for $C_{53}H_{57}N_3O_{10}$ [M+Na$^+$] 918.39362, found: 918.39371.



((1R,4S,5R,6R)-6-(((2S,3R,4R,5S,6R)-3-azido-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-4,5-bis(benzyloxy) cyclohex-2-en-1-yl)methanol (51): Compound **50** (40 mg, 0.045 mmol, 1.0 eq.) was dissolved in pyridine/AcOH mixture (0.5 mL, 4/1, v/v) at room temperature, followed by addition of hydrazine acetate, NH_2NH_2·AcOH (20.5 mg, 2.23 mmol, 5.0 eq.). The resulting mixture was stirred at room temperature for 1 h and quenched by acetone. The reaction mixture was diluted by EtOAc, washed by H_2O and brine aqueous and dried over $MgSO_4$. After concentration *in vacuo*, the residue was purified by silica gel column chromatography (EtOAc in pentane, 20%→40%) yielding colorless oil product **51** (30 mg, 0.038 mmol, 84%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.38 – 7.24 (m, 23H), 7.11 – 7.06 (m, 2H), 5.84 – 5.75 (m, 2H), 5.66 – 5.59 (m, 1H), 5.10 (d, *J* = 10.9 Hz, 1H), 4.95 – 4.81 (m, 3H), 4.75 (d, *J* = 10.8 Hz, 1H), 4.70 – 4.63 (m, 1H), 4.64 – 4.55 (m, 2H), 4.52 – 4.46 (m, 1H), 4.41 (d, *J* = 10.9 Hz, 1H), 4.30 – 4.25 (m, 1H), 4.13 (t, *J* = 9.6 Hz, 1H), 3.99 – 3.89 (m, 4H), 3.67 – 3.61 (d, *J* = 10.3 Hz, 2H), 3.41 – 3.20 (m, 3H), 2.50 – 2.44 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 139.16, 138.29, 137.84, 137.42, 137.10, 129.66, 128.66, 128.64, 128.61, 128.54, 128.39, 128.27, 128.23, 128.19, 128.16, 128.05, 127.81, 127.76, 127.33, 97.94, 84.79, 81.54, 80.64, 78.79, 75.62, 75.31, 74.64, 73.65, 73.01, 71.75, 71.70, 68.73, 63.27, 61.42, 45.60; HRMS: calculated for $C_{48}H_{51}N_3O_8$ [M+Na$^+$] 820.35684, found: 820.35694.

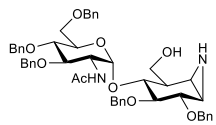


N-((2S,3R,4R,5S,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-2-(((1R,2R,5S,6R)-5,6-bis(benzyloxy)-2-(hydroxymethyl)cyclohex-3-en-1-yl)oxy)tetrahydro-2H-pyran-3-yl)acetamide (52): Compound **51** (22 mg, 0.027 mmol, 1.0 eq.) was dissolved in $CHCl_3$ (350 µL), followed by addition of pyridine (300 µL) and thioacetic acid (300µL). The resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted by EtOAc, washed by H_2O and brine aqueous and dried over $MgSO_4$. After concentration *in vacuo*, the residue was purified by silica gel column chromatography (EtOAc in pentane, 50%→90%) yielding product **52** as colorless oil (15.4 mg, 0.019 mmol, 70%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.37 – 7.24 (m, 23H), 7.16 – 7.11 (m, 2H), 6.33 (d, *J* = 9.7 Hz, 1H), 5.88 (dt, *J* = 10.3, 2.7 Hz, 1H), 5.72 – 5.67 (m, 1H), 5.05 (d, *J* = 3.7 Hz, 1H), 4.90 (d, *J* = 11.3 Hz, 1H), 4.79 – 4.62 (m, 4H), 4.60 – 4.50 (m, 4H), 4.44 (d, *J* = 10.8 Hz, 1H), 4.34 (td, *J* = 9.9, 3.6 Hz, 1H), 4.19 – 4.14 (m, 1H), 4.03 – 3.86 (m, 3H), 3.77 (dd, *J* = 8.3, 5.9 Hz, 1H), 3.73 – 3.65 (m, 3H), 3.64 – 3.51 (m, 3H), 2.47 – 2.39 (m, 1H), 1.50 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 170.10, 138.46, 137.97, 137.88, 137.69, 129.62, 128.73, 128.67, 128.61, 128.60, 128.49, 128.28, 128.16, 128.10, 128.06, 128.03, 127.98, 127.88, 127.85, 127.70, 126.83, 126.45, 99.55, 81.53, 81.41, 78.67, 78.12, 76.51, 75.11, 75.06, 74.36, 73.57, 72.39, 71.55, 69.03, 62.56, 52.70, 46.10, 23.01; HRMS: calculated for $C_{50}H_{55}NO_9$ [M+Na$^+$] 837.37690, found: 837.37663.



***N*-((2S,3R,4R,5S,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-2-(((4aR,5R,6S,7R,8S,8aR)-6,7-bis(benzyloxy)-8-iodo-2-(trichloromethyl)-4a,5,6,7,8,8a-hexahydro-4H-benzo[d][1,3]oxazin-5-yl)oxy)tetrahydro-2H-pyran-3-yl)acetamide (53):** Product **52** (11 mg,

0.013 mmol, 1.0 eq.) was thrice co-evaporated with toluene and was subsequently dissolved in dry DCM (100 mL). The solution was cooled down to 0 ˚C. DBU (2.7 µL, 0.027 mmol, 2.0 eq.) and trichloroacetonitrile (0.95 µL, 1.4 µmol, 0.10 eq.) were added to the solution. The reaction was stirred at 0˚C for 2 h, TLC showed that the starting material was completely converted into a higher running product. Subsequently, H_2O (42 µL), $NaHCO_3$ (15 mg, 0.18 mmol, 13 eq.) and iodine (11 mg, 0.044 mmol, 3.1 eq.) were added to the solution. It was stirred overnight at room temperature. The reaction mixture was quenched with NaS_2O_3 (10% aqueous), concentrated *in vacuo* and extracted with EtOAc. The organic layer with the intermediate product was dried over $MgSO_4$ and concentrated for purification by silica gel column chromatography (EtOAc in pentane, 10%→40%) yielding product **53** as colorless oil (9.0 mg, 8.3 µmol, 62%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.42 – 7.04 (m, 25H), 6.46 (d, *J* = 9.8 Hz, 1H), 5.14 (d, *J* = 11.6 Hz, 1H), 5.04 (d, *J* = 10.3 Hz, 1H), 4.94 (d, *J* = 3.5 Hz, 1H), 4.85 (t, *J* = 3.5 Hz, 1H), 4.82 – 4.69 (m, 3H), 4.59 (d, *J* = 11.0 Hz, 3H), 4.56 – 4.47 (m, 3H), 4.43 – 4.34 (m, 1H), 4.21 – 4.13 (m, 1H), 4.09 – 4.04 (m, 1H), 4.03 – 3.99 (m, 1H), 3.90 (t, *J* = 9.3 Hz, 1H), 3.77 – 3.70 (m, 4H), 3.53 (t, *J* = 10.0 Hz, 1H), 2.75 (dd, *J* = 9.3, 3.8 Hz, 1H), 2.69 – 2.62 (m, 1H), 1.36 (s, 3H); HRMS: calculated for $C_{52}H_{54}Cl_3N_2O_9$ [M+H$^+$] 1083.20123, found: 1083.20164.



***N*-((2S,3R,4R,5S,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-2-(((1R,2R,3R,4S,5S,6R)-4,5-bis(benzyloxy)-2-(hydroxymethyl)-7-azabicyclo[4.1.0]heptan-3-yl)oxy)tetrahydro-2H-pyran-3-yl)acetamide (54):** The intermediate **53** (9.0 mg, 8.3 µmol, 1.0 eq.) was dissolved in dioxane (0.5 mL) and 2 drops of HCl 37% in water. The reaction mixture was refluxed at 60 ˚C for 4 h. The reaction progress was monitored by TLC with pentane/EtOAc mixture (2/1, v/v). After which it was concentrated *in vacuo* and redissolved in MeOH (0.50 mL), and $NaHCO_3$ (14 mg, 0.17 mmol, 20 eq.) was added making the pH > 7. The reaction mixture was stirred at room temperature for three days. The reaction mixture was concentrated *in vacuo*, redissolved in H_2O (5.0 mL) and extracted with EtOAc. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. The crude product was purified via silica gel column chromatography (2%→6%, MeOH in DCM) yielding aziridine **54** as white solid (4.0 mg, 4.8 µmol, 58%). TLC: R_f 0.43 (MeOH/DCM, 1/9, v/v); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.37 – 7.24 (m, 20H), 7.24 – 7.13 (m, 5H), 4.96 (d, *J* = 11.1 Hz, 1H), 4.90 (d, *J* = 3.6 Hz, 1H), 4.77 (d, *J* = 11.2 Hz, 2H), 4.66 (d, *J* = 10.2 Hz, 3H), 4.63 – 4.52 (m, 3H), 4.52 – 4.44 (m, 3H), 4.32 (td, *J* = 9.8, 3.6 Hz, 1H), 4.26 – 4.19 (m, 1H), 4.09 – 4.00 (m, 3H), 3.82 (d, *J* = 7.7 Hz, 1H), 3.73 – 3.59 (m, 6H), 3.58 – 3.50 (m, 1H), 2.59 – 2.55 (m, 1H), 2.35 (d, *J* = 6.2 Hz, 1H), 2.07 – 1.98 (m, 1H), 1.42 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 170.25, 138.56, 138.11, 138.05, 137.70, 128.94, 128.73, 128.68, 128.58, 128.55, 128.44, 128.23, 128.17, 128.12, 127.98, 127.96, 127.85, 127.75, 127.61, 100.62, 81.63, 78.03, 75.25, 75.10, 74.85, 73.57, 72.47, 72.41, 69.18, 68.30, 62.80, 53.28, 44.01, 31.52, 31.50, 30.49, 29.85, 29.07, 23.87, 23.14, 22.82; HRMS: calculated for $C_{50}H_{56}N_2O_9$ [M+H$^+$] 829.40586, found: 829.40593.

8.4 References

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