Cyclophellitol analogues for profiling of exo- and endo-glycosidases

Schröder, S.P.

Chapter 2

Synthesis and biochemical evaluation of D-*xylo*-cyclophellitols

Parts of this chapter have been published:
S.P. Schröder *et al.*, A Divergent Synthesis of L-*arabino*- and D-*xylo*-Configured Cyclophellitol Epoxides and Aziridines, *Eur. J. Org. Chem.* **2016**, 4787-4794

2.1 Introduction

Glycosidases are responsible for the cleavage of glycosidic linkages and are key in the turnover of glycans and glycoconjugates – a large and highly diverse class of biopolymers including polysaccharides, glycoproteins and glycolipids.[1,2] The importance of glycosidases in biological and biomedical research is reflected in the numerous studies on the discovery and development of glycosidase inhibitors. These studies are often inspired by nature, and many natural products have been identified as glycosidase inhibitors. One such natural product glycosidase inhibitor is cyclophellitol (Figure 1), a cyclitol epoxide discovered in 1990 by Atsumi and co-workers. They found that this compound, produced by the fungal strain *Phellinus sp*, inhibits retaining β-glucosidase activities by mimicking the natural substrate and

reacting with the active site nucleophile to yield a stable covalent and irreversible adduct.[3–5] Cyclophellitol is selective against retaining β-glucosidases over inverting β-glucosidases, enzymes that do not form a covalent enzyme-substrate adduct and instead directly deliver water to the activated (protonated) glycosidic linkage. This feature, which sets cyclophellitol apart from the competitive glucosidase inhibitor, deoxynojirimycin,[6] has led to a recent increased interest in the compound and its functional and configurational analogues, both in structural and chemical glycobiology studies.

In one early study on cyclophellitol analogues, substitution of the epoxide oxygen for nitrogen led to cyclophellitol aziridine, an equally strong retaining β-glucosidase inhibitor with the additional feature of a secondary amine amenable for chemical modification.[7] Another close analogue comprises conduritol B epoxide (CBE, Figure 1). CBE lacks the C5 methylene compared to cyclophellitol (cyclophellitol numbering is depicted in Figure 1) and is a synthetic retaining glucosidase inhibitor derived from the natural product, conduritol. CBE was first described by Legler and co-workers and has been the glycosidase inhibitor of choice for many years.[8,9] CBE inhibits retaining β-glucosidases considerably less effectively than cyclophellitol, and besides inhibits retaining α-glucosidases as well. Conduritol B aziridine was identified as a mechanism-based glucosidase inactivator by Withers and co-workers[10] and recently used as a starting point for the synthesis of *N*-alkylated glucosidase inhibitors.[11]

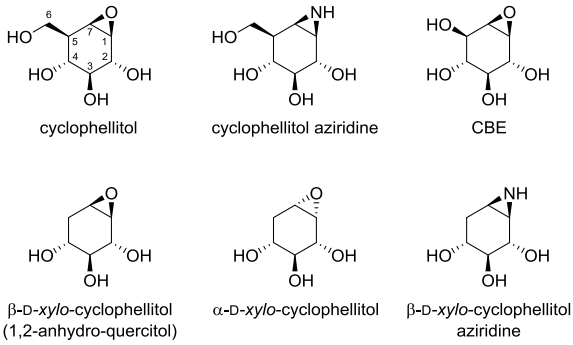
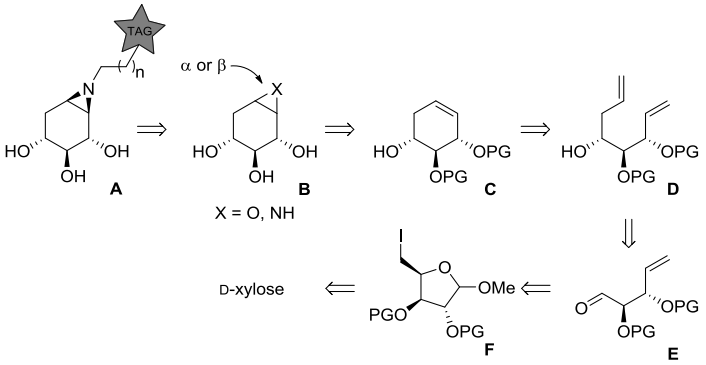


Figure 1 Chemical structures of cyclophellitol and its functional and structural analogues.

Following the discovery of cyclophellitol and CBE, quite a number of structural and configurational analogues have appeared in literature.[5,7,12,13] However, studies on cyclitol epoxides and cyclitol aziridines emulating pentopyranosides (instead of

hexopyranosides) are scarce. Ogawa and co-workers reported the synthesis of β-D-*xylo*-cyclophellitol, referred to as 1,2-anhydro-quercitol (Figure 1).[14] Configurational and functional analogues of β-D-*xylo*-cyclophellitol have not appeared in the literature. In this Chapter, a newly developed synthesis route towards β-D-*xylo*-cyclophellitol as well as the epimeric α-D-*xylo*-cyclophellitol is described. Additionally this route provides access to the aziridine analogues, and the β-aziridine is subsequently functionalized with reporter tags to afford multiple β-D-*xylo*-ABPs. Because these inhibitors and probes are structural analogues of cyclophellitol (lacking the hydroxymethylene moiety at C5), the inhibitory potency and selectivity towards human β-glucosidases GBA1 and GBA2 is investigated. Furthermore, the β-D-*xylo*-ABPs could be used as a tool to monitor β-xylosidase activity in the context of biotechnology research, for example related to food processing, paper production or biofuel development.[15,16,17] For these reasons, the efficiency of the β-D-*xylo*-ABPs to tag and visualize a β-xylosidase was studied and the results of these studies are presented as well.

It was envisioned that β-D-*xylo*-cyclophellitol ABPs **A** (Scheme 1) would be available from the set of epoxide- and aziridine inhibitors **B**. These inhibitors could be synthesized from cyclic alkene **C**, which in turn would emerge from ring-closing metathesis (RCM) of diene **D**. Several studies on asymmetric allylation of aldehyde **E** have appeared in the literature in recent years which include variations in the protective group scheme, the nature of the nucleophile and the stereochemical outcome of the newly introduced chiral center. Aldehyde **E** is readily available by Vasella fragmentation[18] of protected methyl iodofuranoside **F**, which is obtained from D-xylose in 3 steps according to literature.[19]

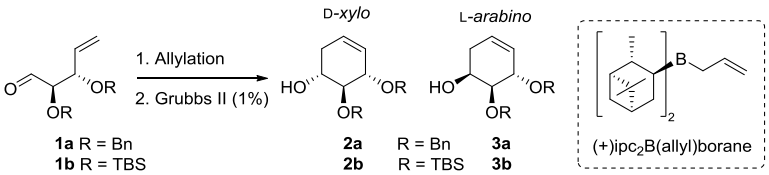


Scheme 1 Retrosynthesis of ABPs **A** and inhibitors **B**.

2.2 Results and Discussion

As the first research objective, optimal conditions were searched for the preparation enantiopure homo-allylic alcohol **C** in pure diastereomeric form and with the optimal protective group pattern for ensuing elaboration to the target epoxides and aziridines. For this, attention was focused on literature reports on nucleophilic allylation of aldehydes **E**. Madsen and co-workers thoroughly studied domino elimination-alkylation reactions of methyl iodofuranoside **F** affording products **D** directly.[20–22] The diastereomeric reaction outcome could be directed by using either zinc or indium to promote alkylation with allyl bromide, affording a diastereomeric mixture of homoallylic alcohol **D** in 1:3 and 7:2 ratios ('down'/'up'), respectively. Apart from zinc- or indium mediated Barbier additions to aldehyde **E**,[23,24] examples of Grignard-like allylations on the carbonyl group are scarce. Isopropylidene-protected aldehyde **E** has been allylated using catalytic asymmetric Keck conditions[25] or by using allyl-zinc reagents,[26] but the isopropylidene protective group would implicate acidic deprotection in the final stages of the synthesis, conditions that may be incompatible with the targeted epoxides and aziridines. It was therefore decided to investigate the efficacy of benzyl-protected alkenal **1a**[19] and *tert*-butyldimethylsilyl-protected alkenal **1b**[27], readily available according to the literature procedures, as the starting materials in the allylation studies. For practical reasons the resulting dienes were not isolated,

Table 1 Stereo-controlled allylation of aldehydes **1a** and **1b**



1a R = Bn
1b R = TBS

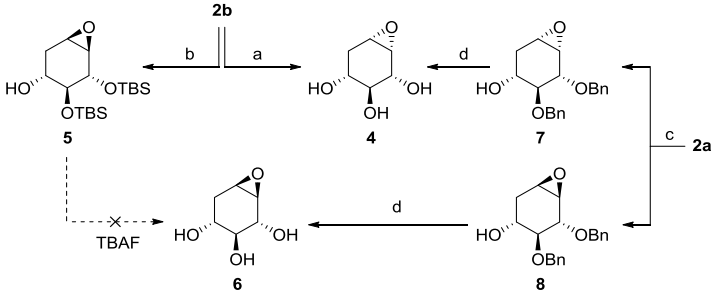
2a R = Bn **3a**
2b R = TBS **3b**

Entry	Compound	Allylation conditions	Ratio **2**:**3**[a]	Yield
1	**1a**	AllylMgBr, THF, 0 °C	1.2:1	90%[b]
2	**1a**	AllylBr, Zn, THF/H_2O, 40 °C	3:1	68%[b]
3	**1a**	(-)-ipc$_2$B(allyl)borane, THF, -90 °C	0:1	71%[c]
4	**1a**	(+)-ipc$_2$B(allyl)borane, THF, -90 °C	9:1	76%[c]
5	**1b**	(+)-ipc$_2$B(allyl)borane, THF, -90 °C	1:0	71%[c]

[a] Crude product ratio determined by ^{1}H-NMR. [b] Combined isolated yield. [c] Isolated yield of major isomer.

instead the crude dienes were directly submitted to RCM to furnish matching cyclohexenes **2** and/or **3**. Reaction of **1a** with allyl magnesium bromide resulted in a nearly 1:1 mixture of diastereomers (Table 1, entry 1). Reacting **1a** with allyl bromide and zinc under sonication (entry 2) resulted in a 3:1 preference for the formation of alkene **2a**. Ultimately it was found that with the use of the (-)-enantiomer of Brown's allylborane reagent, (-)-ipc$_2$B(allyl)borane (entry 3),[28] cyclohexene **3a** was formed in 71% yield, without the formation of observable amounts of **2a**, findings that align with the stereoselective γ-silylallylboration of aldehyde **1a** reported by Heo *et al.*[29] Similarly, by using (+)-ipc$_2$B(allyl)borane (entry 4), cyclohexene **2a** was obtained in 9:1 ratio and 76% yield. Additionally, under these same reaction conditions the target cyclic alkene **2b** could be obtained as a single isomer, when TBDMS groups were used as protecting groups (entry 5).

As the next objective, the transformation of cyclohexenes **2a** and **2b** into the corresponding epoxides was studied (Scheme 2). The α-epoxide **4** could be obtained selectively by employing a two-step procedure involving the complete deprotection of cyclohexene **2b**, followed by *m*-CPBA oxidation directed by the allylic alcohol. Next, a method to selectively afford β-epoxide **6** was investigated. Whereas reaction of **2b** with *m*-CPBA resulted in poor conversion, the cyclohexene could be easily epoxidized with dimethyldioxirane (DMDO) to afford β-epoxide **5** as a single isomer. Although the

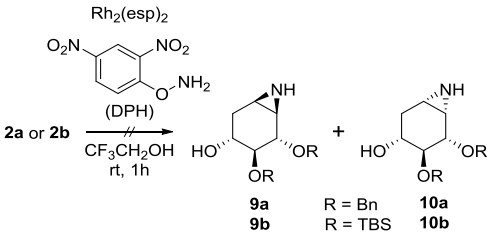


Scheme 2 Synthesis of epoxides **4** and **6**. Reagents and conditions: a) HCl, MeOH, 16h, then *m*-CPBA, $NaHCO_3$, MeOH, 16h, 64%; b) dimethyldioxirane, acetone, 2h, 77%; c) oxone, CH_3COCF_3, $NaHCO_3$, EDTA, MeCN, H_2O, 0 °C, 1h, **7**: 23%, **8**: 57%; d) $Pd(OH)_2/C$, H_2, MeOH, H_2O, dioxane, **4**: 62%, **6**: 74%.

product could be smoothly deprotected by TBAF, it proved to be troublesome to afford β-epoxide **6** in pure state due to contamination by highly polar ammonium salts. As an alternative, it was found that when benzyl protected cyclohexene **2a** was

epoxidized by methyl(trifluoromethyl)dioxirane (generated *in situ* from 1,1,1-trifluoroacetone and oxone[30]), epoxides **7** and **8** were obtained as a separable mixture. Subsequent deprotection of the benzyl groups in epoxides **7** and **8** with Pearlman's catalyst under H_2 atmosphere resulted in D-*xylo*-cyclophellitols **4** and **6**, respectively.

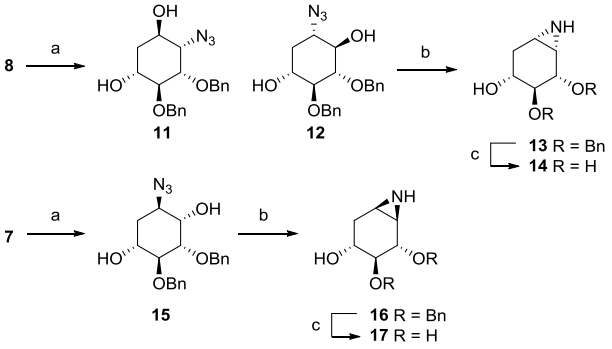
Next, the versatility of cyclohexenes **2a** and **2b** as starting materials for the synthesis of the target aziridines by means of direct aziridination was investigated. A few years ago, Jat *et al.* described the direct stereospecific synthesis of unprotected aziridines from alkenes, using $Rh_2(esp)_2$ and *O*-(2,4-dinitrophenyl)hydroxylamine (DPH) in trifluoroethanol.[31] Besides linear olefins, also cyclic alkenes were readily converted to the aziridines, and in the case of cholesterol, a directing effect of an adjacent hydroxyl group on the stereospecific delivery of the aziridine was suggested. Unfortunately, cyclohexene **2a** proved to be quite unreactive under these conditions, and it was found that high loading of catalyst (25 mol %) and aminating agent (4 equivalents) was required to effectuate full consumption of the starting material (Scheme 3). Under these conditions, aziridines **9a** and **10a** were produced as a mixture in low yield and purity. Unfortunately, similar results were observed when cyclohexene **2b** was subjected to the same reaction conditions. Therefore, further optimization of the reaction conditions was not pursued.



Scheme 3 Direct aziridination of cyclohexenes **2a** or **2b** with *O*-(2,4-dinitrophenyl)hydroxylamine (DPH) under rhodium catalysis was found unsuccessful.

In an alternative attempt to obtain the target aziridines, their synthesis from the corresponding epimeric epoxides was investigated (Scheme 4). For this purpose, the benzyl-protected epoxides were chosen as starting compounds to prevent protecting group migrations during the subsequent reactions, as well to ensure facile deprotection towards the final compounds. Epoxide **8** was reacted with sodium azide in the presence of lithium perchlorate at elevated temperature to afford a mixture of

azido-alcohols **11** and **12**. Interestingly, in contrast to the Fürst-Plattner rule, compound **12** was formed in favor of **11**. While the exact reason for this outcome is not known, it could be caused by a Lewis-acidic chelation effect of lithium due to the excess lithium perchlorate employed. A similar behavior was found with azidolysis of benzyl-protected CBE under the presence of $LiClO_4$.[32] The mixture of azido-alcohols was subsequently ring-closed under anhydrous Staudinger conditions using polymer-bound triphenylphosphine,[33] resulting in aziridine **13**, the benzyl ethers of which were removed under Birch conditions to afford D-*xylo*-cyclophellitol aziridine **14**. Similarly, epoxide **7** was treated with sodium azide and in this case the Fürst-Plattner rule was obeyed and thus only a single regioisomer **15** was formed. Ring-closure of this azido-alcohol resulted in aziridine **16**, and the benzyl groups were removed under Birch conditions resulting in D-*xylo*-cyclophellitol aziridine **17**.

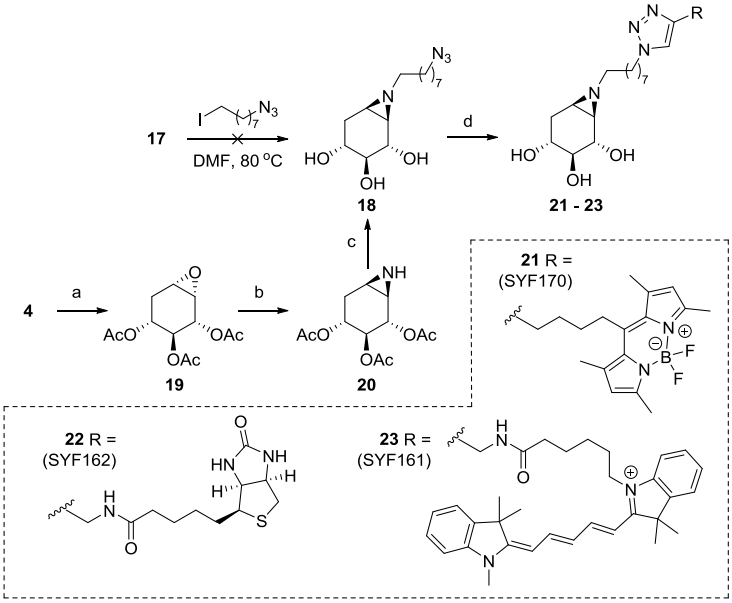


Scheme 4 Synthesis of aziridines **14** and **17** from their parent epoxides. Reagents and conditions: a) NaN_3, $LiClO_4$, DMF, 100 °C, 16h, yield **11**: 33%, yield **12**: 50%, yield **15**: 80%; b) polymer-bound triphenylphosphine, MeCN, 60 °C, 16h, yield **13**: 55%, yield **16**: 77%; c) Li, NH_3, THF, -60 °C, 1h, yield **14**: 96%, yield **17**: 73%.

Having obtained the target aziridine inhibitors, the synthesis route towards β-D-xylo-cyclophellitol aziridine ABPs was investigated (Scheme 5). Direct alkylation of the unprotected aziridine with the appropriate 1-azido-8-iodooctane linker resulted in a complex reaction mixture. An alternative synthetic route towards **18** was then demanded allowing facile alkylation of the aziridine. It was found that protected aziridines could be easily alkylated with alkyl-triflates in chloroform, in high yields without occurrence of side reactions. However, direct alkylation of aziridine **16** would necessitate the removal of the benzyl protecting groups in the presence of the azide linker in the final stage of the synthesis. As azides are prone to undergo reduction

under the standard reductive conditions of benzyl deprotections (i.e. Birch conditions, palladium catalyzed hydrogenation), other protective groups for the secondary alcohols were chosen. Therefore, α-epoxide **4** was protected with acetyl groups and the epoxide was opened with sodium azide under elevated temperatures to give a mixture of azido-alcohols, which was then subjected to Staudinger-type ring closure to give the target β-aziridine **20** in low yield. This aziridine could then be smoothly alkylated by the appropriate alkyl-triflate followed by deprotection with sodium methanolate affording alkylated aziridine **18**. The azide handle could then be functionalized by click-chemistry with different fluorogenic tags and a biotin moiety to afford β-D-xylo-cyclophellitol aziridine ABPs **21**-**23** after HPLC purification.

With β-D-*xylo*-cyclophellitol ABPs **21**-**23** in hand, their labeling efficiency towards recombinant lysosomal β-glucocerebrosidase (GBA1) was investigated, and compared to known *gluco*-configured cyclophellitol aziridine probes. The enzyme was incubated



Scheme 5 Synthesis of β-D-*xylo*-cyclophellitol aziridine ABPs **21**-**23**. Reagents and conditions: a) Ac_2O, DMAP, pyridine, rt, 16h, 88%; b) 1. NaN_3, $Et_3N.HCl$, 80 °C, 16h; 2. polymer-bound triphenylphosphine, 60 °C, 24h, 13% over 2 steps; c) 1. 8-azidooctyl trifluoromethanesulfonate, DIPEA, DCM, rt, 24h; 2. NaOMe, MeOH, rt, 16h, 73% over 2 steps; d) tag-alkyne, $CuSO_4$, sodium ascorbate, DMF/H_2O, rt, 16h, **21**: 58%; **22**: 82%, **23**: 58%.

in McIlvaine buffer pH 5.2 with different concentrations of **21** or **23** (Figure 2a) for 30 minutes at 37 °C and subsequently the remaining unreacted enzyme was post-labeled by 10 nM β-D-*gluco*-cyclophellitol ABPs JJB367[34] or JJB70[35] (Figure 2d), respectively. From these experiments, it is apparent that **21** and **23** label GBA1 in a concentration dependent manner, and that the labeling is activity-based. Indeed, it is known that GBA1 displays activity towards 4-methylumbelliferyl β-D-xylopyranoside,[36] and the reduced potency (compared to the β-D-*gluco*-cyclophellitol ABPs) presumably arises from the absence of the C5-hydroxymethylene group which may be important for the stabilization of the enzyme-inhibitor transition state complex. Interestingly, while the optimal probe concentration for labeling GBA1 with BODIPY-green tagged **21** is approximately 1.0 μM, the Cy5 analogue **23** does not accomplish full enzyme labeling at this concentration, and the optimum lies between 1 and 10 μM.

In addition to these studies, a competitive assay was executed with the complementary D-*xylo*-cyclophellitol inhibitors **4, 6**, **14, 17**, **18**, **21** and **22** (Figure 2b). Thus, recombinant GBA1 was pre-incubated with different concentrations of inhibitor, followed by labeling of the residual active enzyme with 2 μM of **23**. Nearly complete competition was achieved by pre-incubation with 100 μM β-epoxide **6**. The β-aziridine **17** was approximately 10 times more potent, as full inhibition was achieved around 10 μM. Alkylation of the aziridine (**18**) further increased its potency, however it was found that click ligation of the azidoalkyl linker with a BODIPY-green (**21**) or biotin (**22**) reporter tag reduced its inhibitory activity. Finally, in line with the enzymatic catalytic mechanism, the α-epoxide **4** and -aziridine **14** did not react with the enzyme. The IC_{50} values for inhibition of GBA1 were also determined and are in line with the competition experiments (Figure 2c).

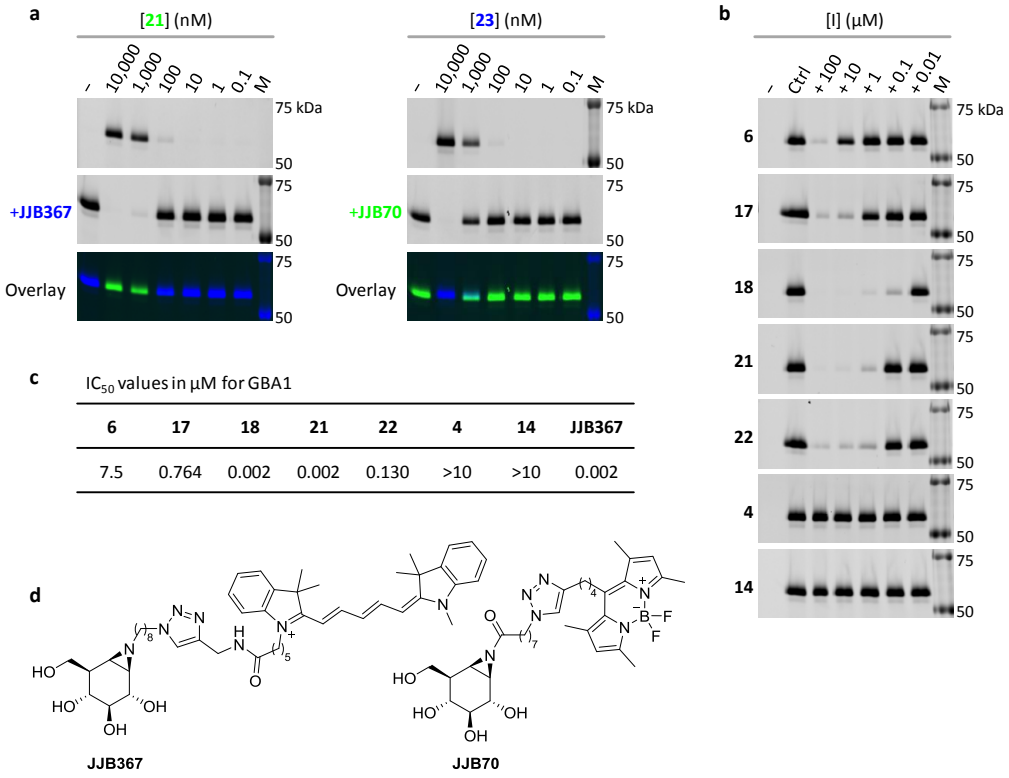


Figure 2 a) determination of the concentration optimum for labeling recombinant GBA1 with ABP **21** (left) and **23** (right). rGBA (1 pmol) was incubated with different concentrations of *xylo*-ABP at pH 5.2 for 30 min at 37 °C and subsequently incubated with 10 nM JJB367 or JJB70. b) Competition assay with various α/β-D-xylo-cyclophellitol epoxides and aziridines. c) IC_{50} values of these inhibitors for GBA1. Values reported are averages from two measurement using β-4-MU-glucopyranoside as substrate at pH 5.2. d) Chemical structures of β-glucosidase selective ABPs JJB367 and JJB70.

Next, the labeling of **23** in mouse liver lysate (C57bl/6j, Jackson's laboratories) was investigated (Figure 3). In this experiment, lysate was pre-incubated with different concentrations of β-epoxide (Figure 3a), β-aziridine (Figure 3b), β-alkyl aziridine (Figure 3c) or BODIPY-aziridine (Figure 3d). These competitors contained either the D-*gluco* (top row) or D-*xylo* (bottom row) configuration. After pre-incubation, the remainder of active enzymes was post-labeled by incubation with either 2 μM JJB367

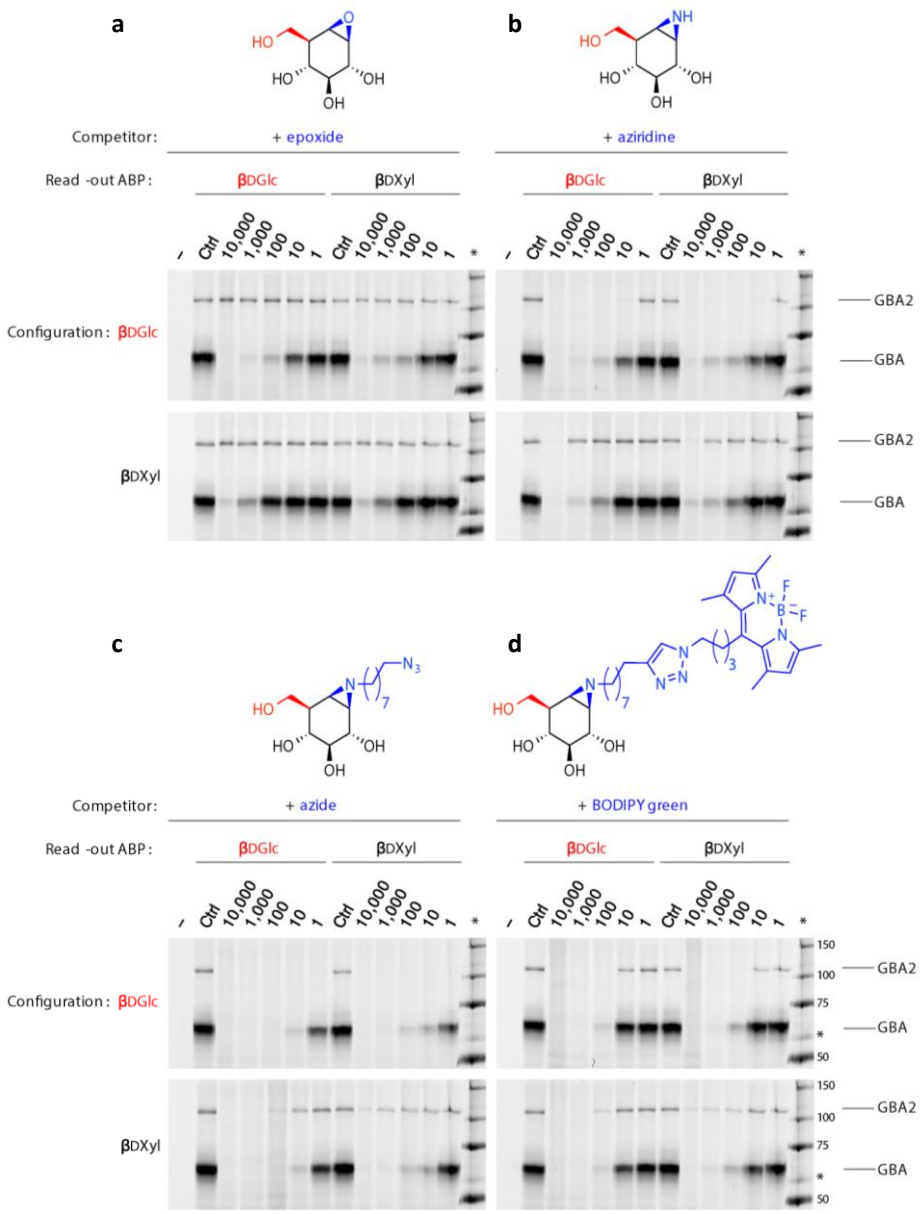


Figure 3 Competition assay of covalent D-*xylo* or D-*gluco* configured cyclophellitols in mouse liver lysate. The lysate was pre-incubated with the cyclitol inhibitor depicted above the gel (top gel: D-*gluco* configuration, bottom gel: D-*xylo* configuration) with different concentrations, and subsequently labeled with D-*gluco* (left) or D-*xylo* configured cyclophellitol aziridine ABP (right).

(left panel) or **23** (right panel). Incubation with **23** resulted in the labeling of GBA1, as well as GBA2 (control lanes). Moreover, the labeling intensity of both enzymes appeared comparable to that of JJB367 at this concentration. Both D-*gluco* and D-*xylo* derivatives selectively inhibit GBA1 over GBA2 (Figure 3a), with the D-*gluco* compound being the most potent of the two. The β-aziridines display similar potency towards GBA1 (Figure 3b). However, the D-*gluco* configured aziridine simultaneously inhibits GBA2, whereas the D-*xylo* aziridine only inhibits this enzyme at the highest concentration tested (10 μM). Subsequently, functionalization of the aziridines with an azidoalkyl spacer results in an increased potency towards GBA1 and GBA2 for both configurations (Figure 3c). This trend is retained for the aziridines functionalized with a BODIPY fluorophore, while the inhibitory potency appears to be slightly reduced for both compounds (Figure 3d).

Lastly, mouse liver, brain and duodenum tissue lysates (C57bl/6j, Jackson's laboratories) were labeled with the biotinylated D-*xylo* ABP and the hits were analyzed by proteomics. For this purpose, the lysates were incubated with **22** with or without pre-incubation with **23** (competition), the resulting biotinylated proteins were enriched with magnetic streptavidin beads, and following tryptic digestion of the

Table 2 Pull-down analysis by LC-MS/MS proteomics of mouse brain, duodenum and liver tissue lysates labeled by biotinylated ABP **22**.

	Probe	Protein	Accession code	Mass (kDa)	Protein score	Unique peptides	Sequence coverage
Brain	DMSO	-	-	-	-	-	-
	23 → 22	-	-	-	-	-	-
	22	GBA1	P17439	58	1.536	6	12 %
Duodenum	DMSO	-	-	-	-	-	-
	23 → 22	-	-	-	-	-	-
	22	GBA1	P17439	58	25.884	9	24 %
Liver	DMSO	GANAB	Q8BHN3	107	1.697	12	15 %
		Glucosidase II	O08795	60	1.206	4	5 %
	23 → 22	-	-	-	-	-	-
	22	GBA1	P17439	58	75.141	21	66 %
		GBA2	Q69ZF3	105	2.409	9	15 %

proteins the peptide fragments were analyzed by nano LC-MS/MS (Table 2). Pull-down analysis of **22** in mouse brain lysates clearly identified the labeling of GBA1, which was positively absent in the competition experiment and negative control. Likewise, labeling with **22** in mouse duodenum identified GBA1 as single glycosidase hit. Finally, a pull-down was performed on mouse liver lysate. This resulted in the identification of GBA1 as well as GBA2, corroborating with the bands labeled by **23** (Figure 3).

The ability of D-*xylo* ABP **23** to label recombinant GH52 β-xylosidase from *Opitutus terrae* (PB90-1, ~81 kDa) was investigated next by incubation of the enzyme with the probe at pH 6.8, 37 °C for 30 minutes with increasing concentrations of **23** (Figure 4a). Optimal labeling was achieved at 500 nM. Furthermore the optimal pH for labeling was determined by incubating the enzyme with the probe at different pH values (Figure 4b), and the most profound labeling was observed at pH 6. A similar experiment was performed, varying the incubation temperature, and optimal labeling occurred at 50 °C (Figure 4c). Interestingly, in contrast with this temperature optimum, *O. terrae* strains are unable to grow at temperatures above 37 °C.[37] Lastly, a competition assay was performed. Pre-incubation of the enzyme with different concentrations of covalent inhibitor followed by labeling with 500 nM **23** shows that β-epoxide **6** is unable to fully inactivate the enzyme at the highest concentration (100 μM) employed (Figure 4d). The β-aziridine **17** proved to be a superior competitor, and alkylation of the aziridine (**18**) further improved its potency. Similarly to the labeling of GBA1 (Figure 2b), the potency was slightly reduced when the aziridine was equipped with a biotin scaffold (**22**). Minor competition was observed by α-epoxide **4** at the highest concentration applied, while cyclophellitol was totally inactive, in line with the enzymatic activity.

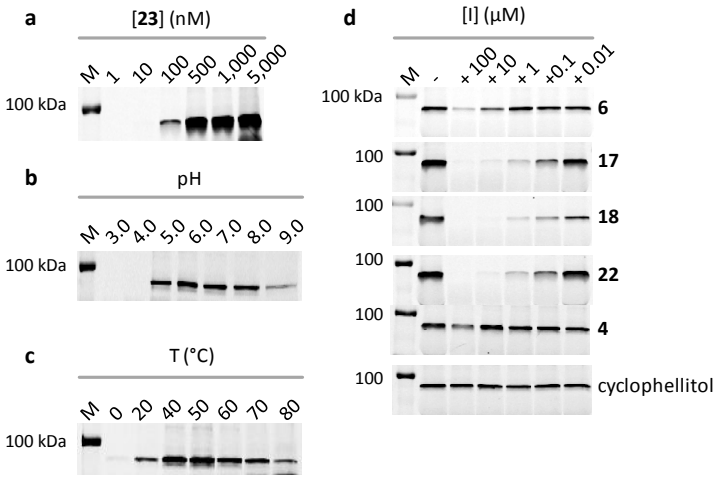


Figure 4 Activity-based labeling of β-xylosidase from *Opitutus terrae* (PB90-1, 50 ng). a) Concentration optimum (50 mM phosphate buffer pH 6.8 at 37 °C, 30 minutes). Optimal probe concentration of 500 nM was used for further screening. b) pH optimum. c) Temperature screening. d) Competitive ABPP assay.

2.3 Conclusion

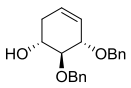
In summary, this Chapter describes the synthesis of D-*xylo*-cyclophellitol, a pentopyranoside-configured analogue of cyclophellitol. The key synthetic step in the synthesis involves the asymmetric allylation of alkenal **1a** towards D-*xylo*-configured cyclohexene **2a**, which is under full diastereomeric control by choice of allylating agent. Additionally, the synthesis of structural analogues of D-*xylo*-cyclophellitol is described bearing an aziridine as electrophilic warhead, and the aziridine is further equipped with several reporter tags to afford D-*xylo*-cyclophellitol activity-based probes. While these probes only partially resemble the natural substrate (lacking the C5 hydroxymethylene group), they are activity-based inactivators of GBA1, albeit with reduced potency in comparison with cyclophellitol and its analogues. Probe **23** selectively labels GBA1 and GBA2 in mouse liver lysate, and through a competition assay it was found that the D-*xylo*-cyclophellitols display similar selectivity towards GBA1 and GBA2 compared to the cyclophellitols, except for D-*xylo*-cyclophellitol aziridine **17** which, in contrast to cyclophellitol aziridine, shows higher activity towards GBA1 over GBA2. Pull-down analysis with biotinylated ABP **22** positively identified GBA1 and GBA2 as unique glycosidase hits in this lysate. Lastly, it was shown that D-*xylo*-cyclophellitols are activity-based covalent inhibitors of β-xylosidase from *O. terrae*, and using ABP **23** the temperature and pH dependence of labeling by the enzyme was visualized, and the potency of these D-*xylo*-cyclophellitols was determined by a competition assay.

The D-*xylo*-cyclophellitol aziridine ABPs could be used for labeling of GBA1 and GBA2 in biological samples, however the configurationally matching D-*gluco*-cyclophellitol aziridine ABP JJB367 proved to be superior for this purpose. Instead, D-*xylo* ABPs **21**-**23** may be used for identification of retaining *exo*-β-xylosidases in biologically relevant samples. Additionally, these ABPs could be used to screen for β-xylosidases with extreme pH and/or temperature tolerance, thereby providing a potential useful tool for the food, paper and biofuel industries.[15,16,17]

Experimental procedures

General: Chemicals were purchased from Acros, Sigma Aldrich, Biosolve, VWR, Fluka, Merck and Fisher Scientific and used as received unless stated otherwise. Tetrahydrofuran (THF), N,N-dimethylformamide (DMF) and toluene were stored over molecular sieves before use. Traces of water from reagents were removed by co-evaporation with toluene in reactions that required anhydrous conditions. All reactions were performed under an argon atmosphere unless stated otherwise. TLC analysis was conducted using Merck aluminum sheets (Silica gel 60 F_{254}) with detection by UV absorption (254 nm), by spraying with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ (10 g/L) in 10% sulfuric acid or a solution of $KMnO_4$ (20 g/L) and K_2CO_3 (10 g/L) in water, followed by charring at ~150 ˚C. Column chromatography was performed using Screening Device b.v. silica gel (particle size of 40 – 63 μm, pore diameter of 60 Å) with the indicated eluents. For reversed-phase HPLC purifications an Agilent Technologies 1200 series instrument equipped with a semi-preparative column (Gemini C18, 250 x 10 mm, 5 μm particle size, Phenomenex) was used. LC/MS analysis was performed on a Surveyor HPLC system (Thermo Finnigan) equipped with a C_{18} column (Gemini, 4.6 mm x 50 mm, 5 μm particle size, Phenomenex), coupled to a LCQ Adventage Max (Thermo Finnigan) ion-trap spectrometer (ESI^+). The applied buffers were H_2O, MeCN and 1% aqueous TFA. ^1H NMR and ^{13}C NMR spectra were recorded on a Brüker AV-400 (400 and 101 MHz respectively) or a Brüker DMX-600 (600 and 151 MHz respectively) spectrometer in the given solvent. Chemical shifts are given in ppm (δ) relative to the residual solvent peak or tetramethylsilane (0 ppm) as internal standard. Coupling constants are given in Hz. 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).

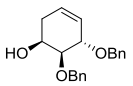
Compound 2a



To a stirred solution of aldehyde **1a**[19] (960 mg, 3.25 mmol) in dry THF (33 mL) was added (+)-ipc$_2$B(allyl)borane (1M in pentane, 4.9 mL) slowly at -90 °C under inert atmosphere. The reaction mixture was stirred for 1 h at -90 °C. After allowing the solution to reach rt, MeOH (6 mL) was added followed by the slow addition of 30% H_2O_2 (20 mL) and sodium-phosphate buffer (0.1 M, pH 7, 20 mL). The biphasic solution was separated, and the aqueous layer was extracted with EtOAc (3 x 75 mL). The combined organic phases were then washed with aq. 10% $Na_2S_2O_3$, water and brine, dried over $MgSO_4$, filtered and evaporated. The crude product was dissolved in dry degassed DCM (13 mL) under argon, and Grubbs 2nd generation catalyst (1% mol) was added. The reaction was refluxed (40 °C) in the dark for 15 h. The solvent was evaporated and flash purification by silica column chromatography (pentane/EtOAc, 9:1) gave product **2a** as colorless oil (760 mg, 76% over 2 steps). ^1H NMR (400 MHz, $CDCl_3$): δ 7.61 – 7.11 (m, 10H), 5.70 (s,

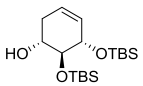
2H), 4.99 (d, *J* = 11.4 Hz, 1H), 4.80 – 4.52 (m, 3H), 4.17 (d, *J* = 6.6 Hz, 1H), 3.80 (dd, *J* = 15.3, 9.0 Hz, 1H), 3.65 – 3.50 (t, *J* = 8.2 Hz, 1H), 2.85 (s, 1H), 2.47 (dd, *J* = 16.2, 4.8 Hz, 1H), 2.23 – 2.04 (m, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 138.6, 138.3, 128.6, 128.5, 128.4, 128.0, 127.9, 127.9, 127.8, 127.7, 126.4, 126.0, 83.5, 80.2, 74.6, 71.4, 68.8, 32.5 ppm. IR: (neat) ν 3350, 3030, 1453, 1352, 1060, 734 cm^{-1}. $[\alpha]D_{20}$ (c = 0.4, DCM): +50. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{20}H_{22}O_3$ 311.16417, found 311.16429.

Compound 3a



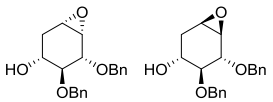
The reaction was carried out following the same procedure described for **2a**, employing aldehyde **1a**[19] (600 mg, 2.02 mmol) and (-)-ipc$_2$B(allyl)borane to give the product as colorless oil (444 mg, 71% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 – 7.26 (m, 10H), 5.73 (s, 2H), 4.80 – 4.53 (m, 4H), 4.24 – 4.13 (m, 2H), 3.71 (dd, *J* = 5.8, 2.5 Hz, 1H), 2.48 – 2.20 (m, 2H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 138.6, 138.4, 128.6, 128.5, 127.9, 127.9, 127.9, 127.8, 126.9, 125.3, 80.4, 75.6, 72.3, 71.9, 66.8, 31.40 ppm. IR: (neat) υ 3439, 3030, 2870, 1496, 1452, 1066, 1026, 732, 694 cm^{-1}. $[\alpha]D_{20}$ (c = 0.1, $CHCl_3$): +84. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{20}H_{22}O_3$ 311.16417, found 311.16435.

Compound 2b



The reaction was carried out following the same procedure described for **2a**, employing aldehyde **1b**[27] (2.22 g, 6.44 mmol) and (+)-ipc$_2$B(allyl)borane to give the product as colorless oil (1.62 g, 71% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.72 (dt, *J* = 10.1, 3.5 Hz, 1H), 5.67 – 5.60 (m, 1H), 3.98 (s, 1H), 3.83 (s, 2H), 3.52 (d, *J* = 7.1 Hz, 1H), 2.45 – 2.36 (m, 1H), 2.18 – 2.10 (m, 1H), 0.90 (s, 9H), 0.89 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H), 0.11 (s, 3H), 0.10 (s, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 126.4, 126.2, 72.8, 70.4, 68.9, 30.5, 25.8, 25.7, 18.0, 17.9, -4.4, -4.6, -4.7 ppm. IR: (neat) υ 3512, 2927, 1471, 1251, 1076, 833 cm^{-1}. $[\alpha]D_{20}$ (c = 0.3, DCM): +71. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{18}H_{38}O_3Si_2$ 381.2252, found 381.2260.

Compound 7 and 8

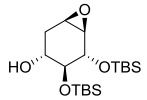


7 8

To a stirred solution of cyclohexene **2a** (1.0 g, 3.22 mmol) in MeCN/0.4 mM aq. EDTA (2:1 v/v, 32 mL), 1,1,1-trifluoroacetone (3.2 mL, 35.4 mmol) was added at 0 °C. Subsequently a solid mixture of $NaHCO_3$ (2.2 g, 25.8 mmol) and oxone (10.9 g, 35.44 mmol) was added in 6 portions over 60 min and the reaction was left to stir at 0 °C for 1 h. Then, water (300 mL) was added and the crude products were extracted with EtOAc (3 x 150 mL). The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and concentrated. Flash purification by silica column chromatography (pentane/EtOAc, 4:1) afforded pure **7** and **8** as colorless oils (**7**: 244 mg, 23%; **8**: 601 mg, 57%). <u>For α-isomer **7**:</u> ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 – 7.15 (m, 10H), 4.91 (d, *J* = 11.3 Hz, 1H), 4.83 (d, *J* = 11.9 Hz, 1H), 4.77 (d, *J* = 11.9 Hz, 1H), 4.61 (d, *J* = 11.3 Hz, 1H), 3.88 (dd, *J* = 6.7, 2.3 Hz, 1H), 3.67 – 3.47 (m, 2H), 3.36 (dd, *J* = 3.7, 2.3 Hz, 1H), 3.26 (t, *J* = 4.0 Hz, 1H), 2.68 (br s, OH), 2.35 (ddd, *J* = 15.4, 6.2, 4.6 Hz, 1H), 2.01 (dd, *J* = 15.3, 8.6 Hz, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 138.3,

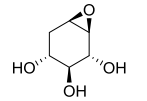
138.1, 128.7, 128.6, 128.1, 128.0, 80.7, 78.8, 75.0, 72.0, 68.4, 54.1, 51.6, 29.9 ppm. IR: (neat) υ 3300, 1103, 1094, 1073 cm^{-1}. $[\alpha]D_{20}$ (c = 0.5, DCM): +24. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{20}H_{22}O_4$ 349.14103, found 349.14087. For β-isomer **8**: ^1H NMR (400 MHz, $CDCl_3$): δ 7.53 – 7.01 (m, 10H), 4.96 (d, *J* = 11.3 Hz, 1H), 4.82 (d, *J* = 11.3 Hz, 1H), 4.73 – 4.60 (m, 2H), 3.83 (d, *J* = 8.0 Hz, 1H), 3.58 (td, *J* = 10.3, 5.4 Hz, 1H), 3.35 – 3.24 (m, 2H), 3.16 (d, *J* = 3.6 Hz, 1H), 2.60 (ddd, *J* = 14.6, 5.3, 1.8 Hz, 1H), 2.50 (br s, OH), 1.81 (ddd, *J* = 14.6, 10.6, 1.6 Hz, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 138.4, 137.6, 128.8, 128.7, 128.2, 128.1, 128.1, 128.0, 84.3, 80.0, 75.0, 72.7, 65.2, 54.0, 53.6, 31.3 ppm. IR: (neat) υ 3350, 3031, 1110, 1070, 1048 cm^{-1}. $[\alpha]D_{20}$ (c = 0.4, DCM): +23. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{20}H_{22}O_4$ 349.14103, found 349.14111.

Compound 5



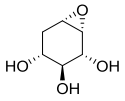
Dimethyldioxirane (DMDO) was freshly prepared according to the procedure by Murray and Singh.[38] Cyclohexene **2b** (72 mg, 0.2 mmol) was dissolved in acetone (3.8 mL) and cooled to 0 °C. Then, a solution of dimethyldioxirane (0.08 M in acetone, 3.8 mL) was added dropwise, and the mixture was stirred for 3 h at rt. Then the mixture was concentrated, and flash purification by silica column chromatography (pentane/EtOAc 15:1) afforded the product as a colorless oil (58 mg, 77%). ^1H NMR (400 MHz, $CDCl_3$): δ 3.81 (d, *J* = 6.7 Hz, 1H), 3.50 – 3.43 (m, 1H), 3.39 (dd, *J* = 8.4, 6.7 Hz, 1H), 3.21 (s, 1H), 3.00 (d, *J* = 3.6 Hz, 1H), 2.47 (dd, *J* = 14.9, 4.1 Hz, 1H), 2.43 (d, *J* = 3.4 Hz, 1H), 1.86 (ddd, *J* = 14.9, 8.8, 2.8 Hz, 1H), 0.94 (s, 9H), 0.90 (s, 9H), 0.18 (s, 3H), 0.14 (s, 3H), 0.11 (s, 3H), 0.09 (s, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 77.0, 72.5, 66.7, 56.5, 52.2, 30.9, 26.0, 25.9, 18.1, 18.1, -3.5, -4.1, -4.4, -4.6 ppm. IR: (neat) υ 3439, 2929, 1766, 1249, 1093, 835 cm^{-1}. $[\alpha]D_{20}$ (c = 1.0, DCM): +11. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{18}H_{38}O_4Si_2$ 397.2201, found 397.2205.

Compound 6[14]



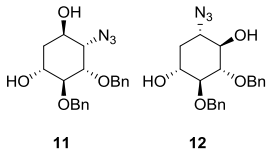
Compound **8** (20 mg, 0.06 mmol) was dissolved in a mixture of MeOH:H_2O:1,4-dioxane (1:1:1, 1.2 mL) and $Pd(OH)_2/C$ (10 mol%) was added under argon. The solution was flushed with H_2 and stirred under H_2 (1 atm) for 5 h at RT. The mixture was filtered over a Celite pad and the solvent was evaporated. The crude deprotected epoxide was absorbed on silica gel and purified by silica column chromatography (DCM/MeOH, 9:1), affording product **6** as a colorless oil (6.6 mg, 74%). ^1H NMR (400 MHz, $CDCl_3$): δ 3.78 (d, *J* = 8.3 Hz, 1H), 3.53 – 3.40 (m, 2H), 3.28 (dd, *J* = 10.2, 8.3 Hz, 1H), 3.21 (d, *J* = 3.6 Hz, 1H), 2.56 (ddd, *J* = 14.9, 5.3, 1.9 Hz, 1H), 1.85 (ddd, *J* = 14.9, 10.6, 1.7 Hz, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 76.6, 71.2, 65.5, 56.6, 54.9, 31.2 ppm. IR: (neat) υ 3378, 3320 cm^{-1}. $[\alpha]D_{20}$ (c = 0.1, MeOH): -20. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_6H_{10}O_4$ 147.06519, found 147.06521.

Compound 4[14]



Starting from epoxide **7** (40 mg, 0.12 mmol), the reaction was carried out following the procedure described for **6** to afford the product as a colorless oil (11 mg, 62%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.89 (dd, *J* = 8.6, 1.9 Hz, 1H), 3.53 (td, *J* = 10.3, 7.4 Hz, 1H), 3.41 – 3.37 (m, 1H), 3.37 – 3.27 (m, 2H), 2.43 (ddd, *J* = 15.4, 7.2, 5.4 Hz, 1H), 1.78 (dd, *J* = 15.5, 10.4 Hz, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 73.7, 71.6, 68.5, 57.5, 52.2, 30.8 ppm. IR: (neat) υ 3469, 3362, 3209 cm^{-1}. [α]D_{20} (c = 0.2, MeOH): +12. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_6H_{10}O_4$ 147.06519, found 147.06519.

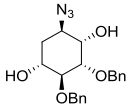
Compound 11 and 12



11 **12**

To a solution of epoxide **8** (200 mg, 0.62 mmol) in dry DMF (6.2 mL), NaN_3 (200 mg, 3 mmol) and $LiClO_4$ (124 mg, 1.2 mmol) were added and the reaction mixture was heated at 100 °C for 18 h under inert atmosphere. H_2O (60 mL) was added, the crude product was extracted with EtOAc (3 x 30 mL) and then the combined organic fractions were washed with H_2O and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated. Flash purification by silica column chromatography (pentane/EtOAc 8:2) afforded the desired azido-alcohols (**11**: 79 mg, 33%; **12**: 111 mg, 50%) as yellow oils. For isomer **11**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 – 7.21 (m, 10H), 4.80 – 4.51 (m, 4H), 4.06 (td, *J* = 7.2, 4.0 Hz, 1H), 3.98 – 3.86 (m, 2H), 3.67 (m, 2H), 3.05 (br s, OH), 2.40 (br s, OH), 1.96 (ddd, *J* = 10.9, 6.9, 3.9 Hz, 1H), 1.87 (ddd, *J* = 13.7, 7.4, 4.1 Hz, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 138.1, 137.2, 128.7, 128.7, 128.3, 128.3, 128.1, 127.9, 79.8, 78.9 (broad signal, assigned by HSQC), 73.9, 73.8, 68.9, 66.4, 64.4, 35.4 ppm. IR: (neat) υ 3380, 2102 cm^{-1}. [α]D_{20} (c = 0.5, DCM): -4. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{20}H_{23}N_3O_4$ 329.15808, found 392.15802. For isomer **12**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42 – 7.26 (m, 10H), 4.97 (d, *J* = 11.3 Hz, 1H), 4.93 (d, *J* = 11.2 Hz, 1H), 4.78 (d, *J* = 11.2 Hz, 1H), 4.70 (d, *J* = 11.3 Hz, 1H), 3.56 (ddd, *J* = 11.9, 8.7, 4.6 Hz, 1H), 3.49 (t, *J* = 9.1 Hz, 1H), 3.41 – 3.20 (m, 3H), 2.63 (br s, OH), 2.36 (br s, OH), 2.17 (dt, *J* = 12.9, 4.6 Hz, 1H), 1.39 (dd, *J* = 24.8, 12.4 Hz, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 138.3, 138.2, 128.9, 128.8, 128.2, 128.2, 128.0, 85.5, 83.6, 76.8, 75.7, 75.6, 69.2, 60.0, 33.7 ppm. IR: (neat) υ 3380, 2109 cm^{-1}. [α]D_{20} (c = 0.5, DCM): -51. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{20}H_{23}N_3O_4$ 392.15808, found 392.15797.

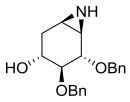
Compound 15



Starting from epoxide **7** (244 mg, 0.75 mmol), the reaction was carried out following the same procedure described for **8** to afford the desired azido-alcohol as a single isomer (222 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41 – 7.23 (m, 10H), 4.77 – 4.56 (m, 4H), 3.88 (m, 3H), 3.81 (dd, *J* = 6.4, 1.5 Hz, 1H), 3.67 (t, *J* = 6.2 Hz, 1H), 2.91 (br s, OH), 2.47 (br s, OH), 2.14 – 1.99 (m, 1H), 1.92 (ddd, *J* = 13.8, 8.0, 3.9 Hz, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 138.1, 137.2, 128.9, 128.7, 128.5, 128.3, 128.1, 127.9, 80.2, 78.9 (broad signal, assigned by HSQC), 74.0, 73.8,

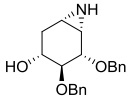
70.6, 68.5, 58.8, 31.7 ppm. IR: (neat) υ 3437, 2916, 2100, 1257, 1074 cm^{-1}. [α]D_{20} (c = 0.4, DCM): -18. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{20}H_{23}N_3O_4$ 392.15808, found 392.15802.

Compound 16



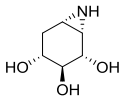
Compound **15** (506 mg, 1.37 mmol) was dissolved in dry CH_3CN (14 mL) and polymer-bound PPh_3 (3 mmol/g loading, 913 mg, 2.74 mmol) was subsequently added to the solution. The reaction was left to stir for 15 h at 60 °C under inert atmosphere. Then the beads were removed by filtration, the organic solvent was evaporated and flash purification by silica column chromatography (DCM/MeOH 49:1) afforded the product as a colorless oil (361 mg, 81%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43 – 7.22 (m, 10H), 4.94 (d, *J* = 11.4 Hz, 1H), 4.78 (d, *J* = 11.4 Hz, 1H), 4.66 (d, *J* = 5.5 Hz, 1H), 4.64 (d, *J* = 5.4 Hz, 1H), 3.79 (d, *J* = 7.7 Hz, 1H), 3.59 (td, *J* = 10.1, 5.3 Hz, 1H), 3.29 (dd, *J* = 9.7, 7.7 Hz, 1H), 2.47 – 2.32 (m, 2H), 2.27 (d, *J* = 5.9 Hz, 1H), 1.73 (ddd, *J* = 13.4, 10.5, 3.0 Hz, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 138.6, 138.0, 128.6, 128.6, 128.0, 128.0, 127.9, 127.9, 84.9 (broad), 81.6 (broad), 74.7, 72.3, 65.6, 33.0, 31.5, 30.9 ppm. IR: (neat) υ 3300, 2862, 1454, 1062 cm^{-1}. [α]D_{20} (c = 0.15, DCM): +15. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{20}H_{23}NO_3$ 326.17507, found 326.17493.

Compound 13



Starting from a mixture of azido-alcohols **11** and **12** (40 mg, 0.11 mmol), the reaction was carried out following the procedure described for **15** to afford the desired product as a colorless oil (20 mg, 55%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56 – 7.02 (m, 10H), 4.77 – 4.71 (m, 3H), 4.57 (d, *J* = 11.5 Hz, 1H), 3.89 (t, *J* = 4.5 Hz, 1H), 3.70 (dd, *J* = 12.0, 6.3 Hz, 1H), 3.56 (dd, *J* = 6.4, 4.9 Hz, 1H), 2.54 (dd, *J* = 6.0, 4.4 Hz, 1H), 2.44 (t, *J* = 4.6 Hz, 1H), 2.18 (dt, *J* = 14.4, 4.8 Hz, 1H), 1.94 (ddd, *J* = 14.4, 6.5, 1.2 Hz, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 138.6, 138.5, 128.6, 128.5, 128.0, 127.9, 127.8, 80.5, 77.8, 73.5, 71.2, 68.8, 32.2, 29.5, 28.2 ppm. IR: (neat) υ 3300, 1111, 1070, 1057, 697 cm^{-1}. [α]D_{20} (c = 0.3, DCM): -45. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{20}H_{23}NO_3$ 326.17507, found 326.17494.

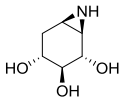
Compound 14



Ammonia (3 mL) was condensed at -60°C under inert atmosphere. Lithium (8 mg, 1.1 mmol) was added and the mixture was stirred until all lithium was completely dissolved (30 min). To the resulting dark-blue mixture, a solution of aziridine **13** (18 mg, 0.06 mmol) dissolved in dry THF (1 mL) was subsequently added. The reaction mixture was stirred for 1 h at -60 °C before being quenched with MilliQ-H_2O. The solution was allowed to come to RT and stirred in a warm water bath (40 °C) until all ammonia had evolved. After solvent evaporation under reduced pressure the crude was re-dissolved in 0.5N NH_4OH and purified over Amberlite CG-50 (NH_4^+). The product was eluted with 0.5N NH_4OH solution and obtained as a colorless oil (5.9 mg, 73%). ^{1}H NMR (400 MHz, D_2O): δ 3.83 (dd, J = 8.6, 3.7 Hz, 1H), 3.44 (m, 1H), 3.21 (dd, J = 10.2, 8.7 Hz, 1H), 2.49 (dd, J = 5.7, 3.8 Hz, 1H), 2.42 – 2.27 (m, 2H), 1.56 (m, 1H) ppm. ^{13}C NMR (101 MHz, D_2O): δ

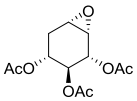
74.3, 71.9, 69.4, 35.4, 31.0, 27.8 ppm. IR: (neat) υ 3306, 1670, 1199, 1136 cm^{-1}. $[\alpha]D_{20}$ (c = 0.1, MeOH): -26. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_6H_{11}NO_3$ 146.08117, found 146.08132.

Compound 17



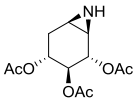
Starting from aziridine **16** (40 mg, 0.12 mmol), the reaction was carried out following the same procedure described for **13** to afford the product as a colorless oil (17 mg, 96%). ^{1}H NMR (400 MHz, D_2O): δ 3.64 (d, *J* = 8.3 Hz, 1H), 3.37 (td, *J* = 10.5, 5.4 Hz, 1H), 3.18 (dd, *J* = 8.6, 1.0 Hz, 1H), 2.46 (br s, 1H), 2.34 (dd, *J* = 13.8, 5.2 Hz, 1H), 2.20 (d, *J* = 6.0 Hz, 1H), 1.79 – 1.58 (m, 1H) ppm. ^{13}C NMR (101 MHz, D_2O): δ 77.5, 72.7, 66.2, 34.57, 31.3, 30.9 ppm. IR: (neat) υ 3365, 1653, 1463 cm^{-1}. $[\alpha]D_{20}$ (c = 0.3, MeOH): -24. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_6H_{11}NO_3$ 146.08117, found 146.08115.

Compound 19



Epoxide **4** (226 mg, 1.55 mmol) was dissolved in dry pyridine (5 mL) and cooled to 0 °C. Then, acetic anhydride (2.2 mL, 23.2 mmol) and a catalytic amount of DMAP were added and the mixture was stirred overnight at rt. The mixture was diluted with EtOAc (50 mL), quenched with sat. aq. $NaHCO_3$ (30 mL) and stirred vigorously for 3 h. The water layer was discarded, the organic phase was diluted with EtOAc (200 mL), washed with sat. aq. $NaHCO_3$, H_2O and brine, dried over $MgSO_4$, filtrated and concentrated. Flash purification by silica column chromatography (pentane/EtOAc 2:1) afforded the product as a yellow solid (371 mg, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.34 – 5.22 (m, 2H), 4.93 – 4.80 (m, 1H), 3.44 (d, *J* = 3.9 Hz, 1H), 3.29 – 3.23 (dd, *J* = 4.9, 4.2 Hz, 1H), 2.60 (ddd, *J* = 15.3, 7.7, 5.0 Hz, 1H), 2.12 (s, 3H), 2.04 – 2.01 (m, 1H), 2.02 (s, 3H), 2.02 (s, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.6, 170.1, 169.8, 71.9, 70.2, 68.8, 54.3, 50.5, 29.0, 20.9, 20.9, 20.8 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{12}H_{16}O_7$ 295.0788, found 295.0795.

Compound 20



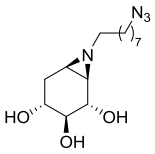
Epoxide **19** (204 mg, 0.75 mmol) was co-evaporated with toluene (3x) and dissolved in DMF under argon. Sodium azide (487 mg, 7.49 mmol) and triethylamine hydrochloride (113 mg, 0.82 mmol) were added and the mixture was stirred overnight at 80 °C. The mixture was diluted with 1N HCl (300 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with H_2O, brine, dried over $MgSO_4$, filtrated and concentrated. Flash purification by silica column chromatography (pentane/EtOAc 2:1) afforded the azido-alcohols as a mixture (169 mg, 72%) which was used directly in the next step without further characterization. The mixture of azido-alcohols (160 mg, 0.507 mmol) was co-evaporated with toluene, dissolved in dry THF, and polymer-bound triphenylphosphine (~3 mmol/g loading, 338 mg, 1.02 mmol) was added. After stirring for 24 h at 60 °C, the mixture was filtrated and concentrated. Flash purification by silica column chromatography (pentane/EtOAc 1:2) afforded the product as an oil (18 mg, 13%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.15 – 4.94 (m, 3H), 2.56 – 2.47 (m, 2H), 2.21 (d, *J* = 5.8 Hz, 1H), 2.09 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.83 (dddd, *J* = 10.5, 8.1, 6.1, 3.4 Hz, 1H) ppm. ^{13}C NMR

(101 MHz, $CDCl_3$): δ 170.4, 170.2, 169.9, 73.6, 73.5, 67.3, 32.4, 31.0, 29.6, 21.1, 21.0, 20.9 ppm. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{12}H_{17}NO_6$ 272.1129, found 272.1129.

1-azido-8-trifluoromethylsulfonyloctane

To dry DCM (5.8 mL) was added 8-azidooctan-1-ol (100 mg, 0.58 mmol) and pyridine (57 µL, 0.70 mmol) and the mixture was cooled to -20 °C. Triflic anhydride (118 µL, 0.70 mmol) was added and the mixture was stirred for 15 minutes. Then the mixture was diluted with DCM, and washed with cold water (3 x 10 mL). The organic layer was dried over $MgSO_4$, filtrated and concentrated at rt. The crude product was used directly for the alkylation of the aziridine. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.55 (t, *J* = 6.5 Hz, 2H), 3.27 (t, *J* = 6.9 Hz, 2H), 1.90 – 1.75 (m, 2H), 1.65 – 1.55 (m, 2H), 1.49 – 1.30 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 77.8, 51.6, 29.4, 29.0, 28.9, 28.9, 26.7, 25.2 ppm.

Compound 18

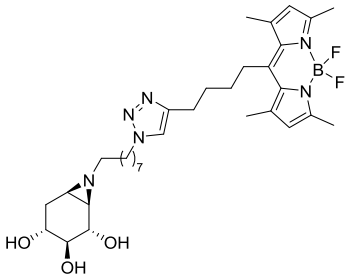


Aziridine **20** (18 mg, 66 µmol) was dissolved in dry DCM (700 µL) and cooled to 0 °C. Then, DIPEA (13 µL, 132 µmol) and 8-azidooctyl trifluoromethanesulfonate (0.5 M, 264 µL) were added and the mixture was stirred 24 h at rt. The reaction was quenched by adding sat. aq. $NaHCO_3$ and the mixture was extracted with EtOAc (3x). The combined organics were washed with brine, dried over $MgSO_4$, filtrated and concentrated. Flash purification by silica column chromatography (pentane/EtOAc 5:1) afforded the product as an oil (21 mg), which was taken up in methanol (500 µL) and subsequently NaOMe (0.1 M, 98 µL) was added. After stirring 16 h at rt, the mixture was quenched by adding triethylamine hydrochloride until the pH of the mixture was neutral. The mixture was concentrated, and flash purification by silica column chromatography (DCM/MeOH 93:7) afforded the product as an oil (14 mg, 73% over two steps). ^{1}H NMR (400 MHz, CD_3OD): δ 3.60 (d, *J* = 8.0 Hz, 1H), 3.39 (m, 1H), 3.28 (t, *J* = 6.8 Hz, 2H), 3.05 (dd, *J* = 9.8, 8.1 Hz, 1H), 2.32 (dd, *J* = 13.8, 5.4 Hz, 1H), 2.24 (m, 1H), 1.85 – 1.80 (m, 1H), 1.67 – 1.62 (m, 1H), 1.62 – 1.34 (m, 12H) ppm. ^{13}C NMR (101 MHz, CD_3OD): δ 79.1, 74.0, 68.1, 61.7, 52.4, 45.3, 41.1, 33.4, 30.6, 30.5, 30.2, 29.9, 28.3, 27.8 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{14}H_{26}N_4O_3$ 321.1897, found 321.1899.

General procedure for click reactions

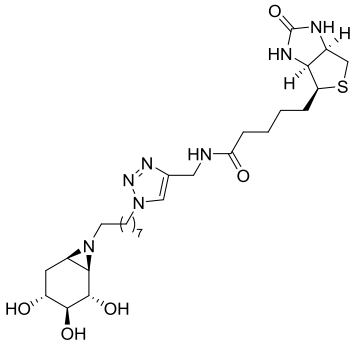
The azido compound (3-5 mg) was dissolved in degassed DMF (0.2 mL), then the alkyne-tag (1.1 eq), $CuSO_4$ (0.2 eq) and sodium ascorbate (0.4 eq) were added and the mixture was stirred for 16 h at rt. The reaction mixture was concentrated and purified by semi-preparative reversed phase HPLC (linear gradient. Solutions used: A: 50 mM NH_4HCO_3 in H_2O, B: acetonitrile).

Compound 21 (SYF170)



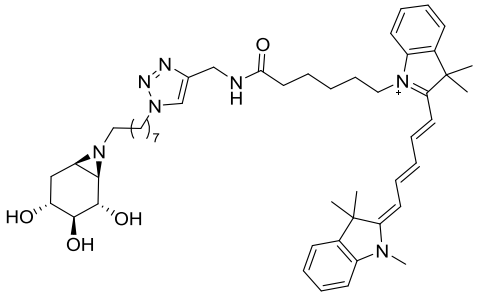
Following the general procedure starting from compound 18 (4.4 mg, 14.7 μmol), the product was obtained as an orange powder (5.4 mg, 58%). ^{1}H NMR (500 MHz, CD_3OD): δ 7.73 (s, 1H), 6.11 (s, 2H), 4.35 (t, *J* = 6.9 Hz, 2H), 3.60 (d, *J* = 8.0 Hz, 1H), 3.38 (m, 1H), 3.05 (dd, *J* = 9.8, 8.0 Hz, 1H), 3.03 – 2.98 (m, 2H), 2.78 (t, *J* = 7.2 Hz, 2H), 2.44 (s, 6H), 2.38 (s, 6H), 2.30 (dd, *J* = 13.1, 5.5 Hz, 1H), 2.20 (t, *J* = 7.3 Hz, 2H), 1.92 – 1.82 (m, 5H), 1.81 – 1.77 (m, 1H), 1.68 – 1.58 (m, 3H), 1.56 (d, *J* = 6.3 Hz, 1H), 1.54 – 1.48 (m, 2H), 1.35 – 1.20 (m, 7H) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ 153.6, 147.2, 146.6, 140.8, 131.2, 122.0, 121.3, 77.8, 72.7, 66.8, 60.3, 49.9, 48.5, 44.0, 39.7, 32.1, 30.9, 29.9, 29.5, 29.1, 29.0, 28.5, 27.8, 26.8, 25.9, 24.6, 15.2, 13.1 ppm. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{33}H_{49}BF_2N_6O_3$ 627.4000, found 627.4029.

Compound 22 (SYF162)



Following the general procedure starting from compound **18** (4.3 mg, 14.5 μmol), the product was obtained as a white powder (6.9 mg, 82%). ^{1}H NMR (500 MHz, CD_3OD): δ 7.87 (s, 1H), 4.53 – 4.49 (m, 1H), 4.44 (s, 2H), 4.40 (m, 2H), 4.31 (dd, *J* = 7.9, 4.5 Hz, 1H), 3.61 (d, *J* = 8.0 Hz, 1H), 3.40 (td, *J* = 10.1, 5.5 Hz, 1H), 3.25 – 3.18 (m, 1H), 3.07 (dd, *J* = 9.9, 8.1 Hz, 1H), 2.95 (dd, *J* = 12.7, 5.0 Hz, 1H), 2.72 (d, *J* = 12.7 Hz, 1H), 2.33 (dd, *J* = 13.8, 5.5 Hz, 1H), 2.26 (m, 4H), 1.91 (m, 2H), 1.86 (dd, *J* = 9.6, 3.5 Hz, 1H), 1.79 – 1.51 (m, 7H), 1.48 – 1.27 (m, 11H) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ 174.6, 164.8, 144.9, 122.8, 77.8, 72.7, 66.8, 62.0, 60.3, 60.3, 55.7, 50.0, 44.1, 39.8, 39.7, 35.2, 34.2, 32.1, 29.9, 29.1, 29.1, 28.6, 28.4, 28.1, 26.9, 26.0, 25.4 ppm. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{27}H_{45}N_7O_5S$ 580.3276, found 580.3279.

Compound 23 (SYF161)



Following the general procedure starting from compound 18 (4.6 mg, 15.3 μmol), the product was obtained as a blue powder (7.6 mg, 58%). ^{1}H NMR (500 MHz, CD_3OD): δ 8.28 (t, *J* = 13.1 Hz, 2H), 7.88 (s, 1H), 7.51 (d, *J* = 7.4 Hz, 2H), 7.45 – 7.39 (m, 2H), 7.33 – 7.30 (m, 2H), 7.30 – 7.25 (m, 2H), 6.65 (t, *J* = 12.4 Hz, 1H), 6.31 (d, *J* = 13.7 Hz, 2H), 4.42 (s, 2H), 4.40 – 4.36 (m, 2H), 4.13 – 4.09 (m, 2H), 3.65 (s, 3H), 3.60 (d, *J* = 8.0 Hz, 1H), 3.40 (m, 1H), 3.07 (dd, *J* = 9.8, 8.0 Hz, 1H), 2.32 (dd, *J* = 13.9, 5.4 Hz, 1H), 2.26 (m, 4H), 1.93 – 1.79 (m, 5H), 1.76 – 1.67 (m, 2H), 1.74 (s, 12H), 1.68 – 1.61 (m,

1H), 1.59 (d, *J* = 6.3 Hz, 1H), 1.50 (m, 3H), 1.43 – 1.23 (m, 9H) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ 174.4, 174.0, 173.3, 154.2, 154.2, 142.9, 142.2, 141.3, 141.2, 128.4, 128.4, 125.4, 124.9, 124.9, 122.9, 122.1, 122.0, 110.7, 110.5, 103.1, 103.0, 77.8, 72.7, 66.8, 60.3, 50.0, 44.0, 43.4, 39.8, 35.2, 34.3, 32.1, 30.2, 30.0, 29.1, 29.1, 28.6, 26.9, 26.8, 26.6, 26.5, 26.0, 26.0, 25.1 ppm. HRMS (ESI) m/z: $[M]^+$ calc for $C_{49}H_{68}N_7O_4$ 819.5406, found 819.5336.

General procedure for SDS-PAGE experiments

Recombinant enzyme (rGBA: 1 pmol, PB90-1 β-xylosidase: 50 ng) or mouse liver lysate (Jackson's laboratories, C57Bl6/J, 40 µg total protein per sample) was diluted in 150 mM phosphate buffer with appropriate pH. A solution of the ABP with appropriate concentration was added and the mixture was incubated for 30 minutes at 37 °C. In the case of competition experiments, the enzyme solution was pre-incubated with appropriate inhibitor concentrations for 30 minutes at 37 °C. Then, laemmli (4X) was added and the mixture was heated at 100 °C for 5 minutes. The samples were loaded on 10% acrylamide SDS-PAGE gels and the gels were ran at a constant 90V. Wet slab gels were scanned on fluorescence using a Typhoon FLA9500 Imager (GE Healthcare) using λ_{EX} 635 nm; λ_{EM} > 665 nm. Images were acquired, processed and quantified with Image Quant (GE Healthcare).

Determination of IC_{50} values

The activity of recombinant GBA (Cerezyme, Genzyme) was measured at 37 °C with 4-methylumbelliferyl β-D-glucopyranoside as substrate as reported previously.[39] To determine the half-maximal inhibitory concentration (IC_{50}) value, the inhibitors were pre-incubated for 30 min with the enzyme before addition of the substrate mixture. The incubation mixture contained 3 mM β-4-MU-glucopyranoside, 0.2% (w/v) sodium taurocholate, 0.1% (v/v) Triton X-100 and 0.1% (w/v) BSA in 150 mM McIlvaine buffer, pH 5.2. After stopping the incubation with excess NaOH-glycine (pH 10.6), fluorescence of liberated 4-methylumbelliferone was measured with a fluorimeter LS 55 (Perkin Elmer) using λex 366 nm and λem 445 nm. The IC50 values were estimated by non-linear regression analysis of blank-corrected datapoints, using a one-phase exponential decay function (GraphPad Prism 5.0).

References

1 V. Gieselmann, *Biochim. Biophys. Acta*, 1995, **1270**, 103–136.
2 K. A. Stubbs, *Carbohydr. Res.*, 2014, **390**, 9–19.
3 S. Atsumi, H. Iinuma, C. Nosaka and K. Umezawa, *J. Antibiot.*, 1990, **43**, 49–53.
4 J. Marco-Contelles, *Eur. J. Org. Chem.*, 2001, 1607–1618.
5 B. P. Rempel and S. G. Withers, *Glycobiology*, 2008, **18**, 570–586.
6 H. Paulsen, I. Sangster and K. Heyns, *Chem. Ber.*, 1967, **100**, 802–815.
7 K. Tatsuta, *Pure Appl. Chem.*, 1996, **68**, 1341–1346.
8 G. Legler, *Hoppe. Seylers. Z. Physiol. Chem.*, 1966, **345**, 197–214.
9 G. Legler, *Hoppe. Seylers. Z. Physiol. Chem.*, 1968, **349**, 767–774.
10 G. Caron and S. G. Withers, *Biochem. Biophys. Res. Commun.*, 1989, **163**, 495–499.

11 B. T. Adams, S. Niccoli, M. A. Chowdhury, A. N. K. Esarik, S. J. Lees, B. P. Rempel and C. P. Phenix, *Chem. Commun.*, 2015, **51**, 11390–11393.

12 E. Borges de Melo, A. da Silveira Gomes and I. Carvalho, *Tetrahedron*, 2006, **62**, 10277–10302.

13 V. W. Tai, P. Fung, Y. Wong and T. K. M. Shing, *Tetrahedron: Asymm.*, 1994, **5**, 1353–1362.

14 S. Ogawa, S. Uetsuki, Y. Tezuka, T. Morikawa, A. Takahashi and K. Sato, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 1493–1498.

15 P. Biely, *Trends Biotechnol.*, 1985, **3**, 286–290.

16 A. Sunna and G. Antranikian, *Crit. Rev. Biotechnol.*, 1997, **17**, 39–67.

17 D. B. Jordan and K. Wagschal, *Appl. Microbiol. Biotechnol.*, 2010, **86**, 1647–1658.

18 B. Bernet and A. Vasella, *Helv. Chim. Acta*, 1979, **62**, 1990–2016.

19 F. G. Hansen, E. Bundgaard and R. Madsen, *J. Org. Chem.*, 2005, **70**, 10139–10142.

20 C. S. Poulsen and R. Madsen, *J. Org. Chem.*, 2002, **67**, 4441–4449.

21 L. Hyldtoft, C. S. Poulsen and R. Madsen, *Chem. Commun.*, 1999, 2101–2102.

22 L. Hyldtoft and R. Madsen, *J. Am. Chem. Soc.*, 2000, **122**, 8444–8452.

23 L. Keinicke and R. Madsen, *Org. Biomol. Chem.*, 2005, **3**, 4124–4128.

24 J. C. Grim, K. C. A. Garber and L. L. Kiessling, *Org. Lett.*, 2011, **13**, 3790–3793.

25 G. Sabitha, K. Shankaraiah and J. S. Yadav, *Eur. J. Org. Chem.*, 2013, 4870–4878.

26 V. R. Doddi, A. Kumar and Y. D. Vankar, *Tetrahedron*, 2008, **64**, 9117–9122.

27 J. Uenishi and H. Ohmiya, *Tetrahedron*, 2003, **59**, 7011–7022.

28 P. K. Brown, H C; Jadhav, *J. Am. Chem. Soc.*, 1983, **105**, 2092–2093.

29 J. N. Heo, E. B. Holson and W. R. Roush, *Org. Lett.*, 2003, **5**, 1697–1700.

30 D. Yang, M.-K. Wong and Y.-C. Yip, *J. Org. Chem.*, 1996, **60**, 3887–3889.

31 J. L. Jat, M. P. Paudyal, H. Gao, Q.-L. Xu, M. Yousufuddin, D. Devarajan, D. H. Ess, L. Kurti and J. R. Falck, *Science*, 2014, **343**, 61–65.

32 P. Serrano, A. Llebaria and A. Delgado, *J. Org. Chem.*, 2005, **70**, 7829–7840.

33 P. Pöchlauer, E. P. Müller and P. Peringer, *Helv. Chim. Acta*, 1984, **67**, 1238–1247.

34 J. Jiang, Thesis: Activity-based protein profiling of glucosidases, fucosidases and glucoronidases; Leiden University, 2016.

35 K. Y. Li, J. Jiang, M. D. Witte, W. W. Kallemeijn, H. van den Elst, C. S. Wong, S. D. Chander, S. Hoogendoorn, T. J. M. Beenakker, J. D. C. Codée, J. M. F. G. Aerts, G. A. van der Marel and H. S. Overkleeft, *Eur. J. Org. Chem.*, 2014, **2014**, 6030–6043.

36 S. van Weely, M. Brandsma, A. Strijland, J. M. Tager and J. M. F. G. Aerts, *BBA - Mol. Basis Dis.*, 1993, **1181**, 55–62.

37 K. J. Chin, W. Liesack and P. H. Janssen, *Int. J. Syst. Evol. Microbiol.*, 2001, **51**, 1965–1968.

38 R. W. Murray and M. Singh, *Org. Synth.*, 1997, **74**, 91–97.

39 M. D. Witte, W. W. Kallemeijn, J. Aten, K.-Y. Li, A. Strijland, W. E. Donker-Koopman, A. M. C. H. van den Nieuwendijk, B. Bleijlevens, G. Kramer, B. I. Florea, B. Hooibrink, C. E. M. Hollak, R. Ottenhoff, R. G. Boot, G. A. van der Marel, H. S. Overkleeft and J. M. F. G. Aerts, *Nat. Chem. Biol.*, 2010, **6**, 907–913.