Activity-based protein profiling of glucosidases, fucosidases and glucuronidases

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6

Synthesis and biological evaluation of 8-carboxy-cyclophellitol aziridine derivatives as β-glucuronidase inhibitors and activity-based probes

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6.1 Introduction

β-Glucuronidases hydrolyze β-glucuronic acid (GlcUA)-containing carbohydrates to release GlcUA and are found in all living organisms. β-Glucuronidases are classified into two glycoside hydrolase (GH) families in human, namely, GH2 and GH79, in the Carbohydrate-Active enZymes (CAZy) database, according to their amino acid sequences and tertiary structure.[1,2] β-Glucuronidases hydrolyze β-glucuronide substrates through a formal two-step double displacement mechanism, first proposed by Koshland.[3] Human GH2 lysosomal β-glucuronidase cleaves GlcUA residues from the non-reducing termini of glycosaminoglycans incuding dermatan sulphate and heparan sulfate, and its deficiency in man causes the lysosomal storage disorder, mucopolysaccharidosis type VII (MPSVII), also known as Sly syndrome.[4] The GH79 family contains a number of β-glucuronidases from mammalian, fungi and bacterial,[5] such as heparanase that is responsible for hydrolysis of heparin sulfate proteoglycans. β-glucuronidase activities are often associated with inflammation processes and have also been found responsible for the promotion of cancers.[6]

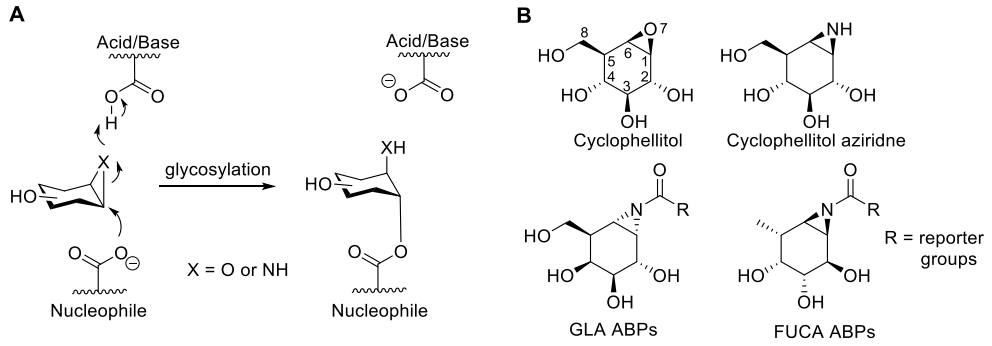


Figure 1. A) Mechanism-based inhibition of retaining glycosidases by cyclophellitol and cyclophellitol aziridine derivatives. B) Structure of the β-glucosidase inhibitors, cyclophellitol and cyclophellitol aziridine as well as activity-based probes for α-galactosidases (GLA) and α-L-fucosidases (FUCA).

The research described in the previous chapters focused on the development of configurational and functional isomers of the natural product, cyclophellitol, as an activity-based retaining *exo*-glycosidase probe scaffold. Cyclophellitol is a potent and selective mechanism-based irreversible inhibitor of retaining β-glucosidases.[7,8] Substitution of the epoxide moiety in cyclophellitol for an aziridine yields cyclophellitol aziridine,[9] which inhibits retaining β-glucosidases in a similar fashion. Structurally, cyclophellitol and its aziridine analog are close to β-glucopyranose in configuration. They fit well in the active site pocket of retaining β-glucosidases, and undergo acid-catalyzed ring-opening following nucleophilic attack on C1 by the enzyme active site nucleophilic residue (Figure 1A) to yield a stable, covalent adduct. Both cyclophellitol and cyclophellitol aziridine have been modified to yield retaining β-glucosidase-selective activity-based probes (ABPs) by grafting reporter moieties on either the cyclophellitol C8 carbon or the cyclophellitol aziridine nitrogen.[10]

The high potency and selectivity of cyclophellitol (aziridine), together with conserved catalytic mechanism of retaining GHs, opens up the potential to exploit selective inhibitors and ABPs for other class of retaining GHs. Indeed, by adapting the configuration of cyclophellitol aziridine to the natural substrate of target GHs, potent mechanism-based inhibitors of α-glucosidases, α/β-galactosidases, and α/β-mannosidases have been prepared in the past.[9,11,12] More recently, cyclophellitol aziridine-based ABPs have been developed for profiling α-galactosidases (GLA) and α-L-fucosidases (FUCA) (Figure 1B).[13-15] These ABPs were created by *N*-acylation of the aziridine nitrogen with 8-azidooctanoic acid, followed by introduction of reporter groups (biotin, fluorophores) through azide-alkyne [2+3] cycloaddition 'click' ligation. In line with these studies, this chapter investigates the potential to develop 8-carboxy-cyclophellitol aziridine inhibitors and probes aimed at inhibiting and labeling β-D-glucuronidases. Specifically, the synthesis and enzyme inhibition properties of compounds

2, **3, 12** and ABPs **4-11** (Figure 2), emulating in configuration and substitution pattern glucuronic acid (**1**) are presented. The chapter ends with describing the elucidation of the crystal structure of selected cyclophellitol aziridine derivatives in complex to the bacterial β-glucuronase from *Acidobacterium capsulatum*, AcaGH79. [5c]

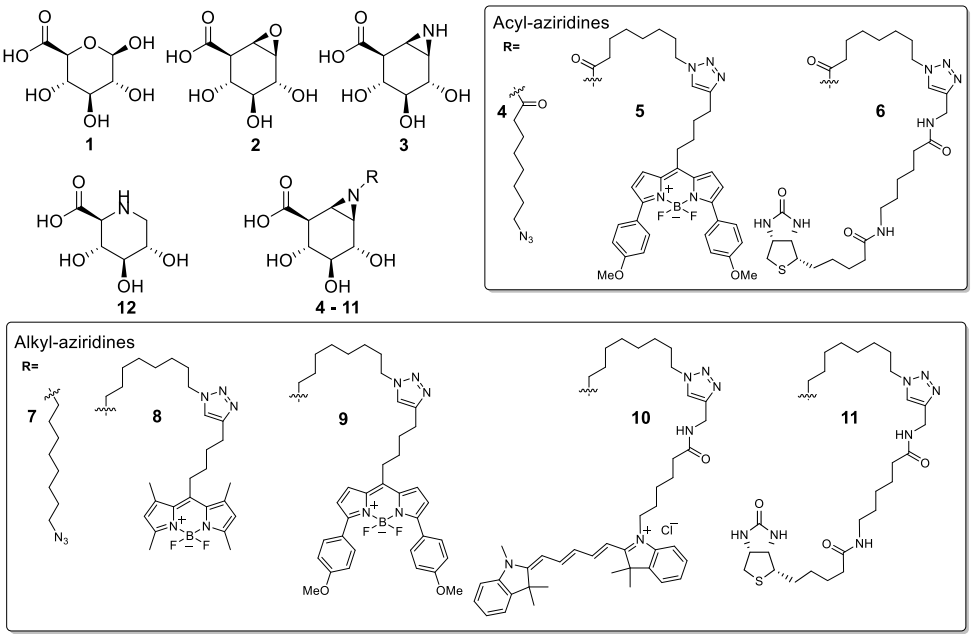


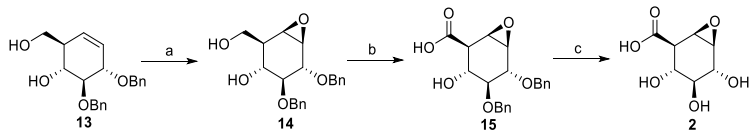
Figure 2. Structures of β-D-glucuronic acid, β-glucuronidase inhibitors and activity-based probes subject of the research described in this chapter.

6.2 Results and Discussion

Synthesis

The synthesis procedure of 8-carboxy-cyclophellitol **1** is depicted in Scheme 1. Cyclohexene diol **13**, prepared as reported before,[16, 17] was treated with *meta*-chloroperbenzoic acid in a biphasic buffer to yield epoxide **14** in a stereoselective fashion as described in the literature.[17] Next, the primary alcohol in **14** was selectively oxidized by 2,2,6,6-tetramethylpiperidine-1-oxyl and (diacetoxyiodo)benzene (TEMPO/BAIB) oxidation yielding carboxylate **15**. Addition of *t*BuOH to homogenize the biphasic (DCM/H_2O) reaction mixture enhanced the rate of hydration of the intermediate aldehyde, thus ensuring efficient oxidation of the lipophilic substrate. Purification of **15** with silica gel column chromatography caused 20-40% epoxide hydrolysis. To circumvent this, **15** was purified by HPLC using 50 mM aq. NH_4HCO_3 as the mobile phase. Hydrogenolysis with hydrogen gas and Pearlman's catalyst followed by HPLC purification yielded 8-carboxy-cyclophellitol **2**, in 4.4% overall yield based on **13**.

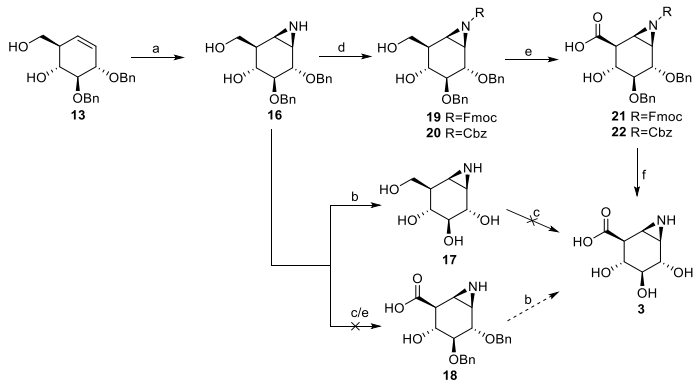
Scheme 1. Synthesis of 8-carboxy-cyclophellitol **2**.



Reagents and conditions: (a) 3-chloroperoxybenzoic acid (*m*CPBA), Na_2HPO_4 (aq., 1.0 M), 1,2-dichloroethane, 50 °C, 55%; (b) BAIB, TEMPO, DCM/*t*BuOH/H_2O (4:4:1, v/v/v), 0 °C, 35%; (c) H_2, $Pd(OH)_2$/C, MeOH, 23%.

The preparation of 8-carboxy-cyclophellitol aziridine **3** started from cyclohexane diol **13** as well (Scheme 2). Partially protected aziridine **16** was prepared following the three-step procedure for the installation described in Chapter 5 for the construction of α-*gluco*-configured cyclophellitol aziridines (first installation of the trichloroacetimidate, next iodo-imination, then hydrolysis of the formed cyclic iminal and final intramolecular iodine displacement to give the aziridine) in 45% yield. Removal of the benzyl groups was accomplished by Birch reduction, as reported previously to yield cyclophellitol aziridine (**17**).[17] In the first instance, oxidation of the primary alcohol in **17** with catalytic amounts of TEMPO and NaClO as co-oxidant at pH 10.5 was attempted, but the desired 8-carboxy-cyclophellitol aziridine (**3**) was not detected in the resulting reaction mixtures. Application of TEMPO/BAIB combination led to the formation of corresponding aldehyde (from **17**) as witnessed by LC-MS analysis. However, further oxidation of this aldehyde to **3** proceeded very sluggishly and only a small amount of the target product was isolated after silica gel chromatography. Using a biphasic solution (DCM/*t*BuOH/H_2O) and varying the concentration of the starting material in solvents also proved ineffective (Table 1). The aziridine moiety appeared to be sensitive to the oxidation conditions used. To avoid this sensitivity the aziridine nitrogen was protected as, either the fluorenylmethyloxycarbonyl (Fmoc) group (**19**) or the carboxybenzyl (Cbz) group (**20**). Subjection of **19** or **20** to the TEMPO/BAIB conditions yielded **21** or **22**, respectively, in good yields (Table 1). Birch reduction on either **21** or **22** led to the efficient and simultaneous removal of all protective groups. Cation exchange chromatography (Amberlite IR120 H^+ resin) on the crude product obtained from subjecting Fmoc aziridine **21** to the Birch conditions did not result in the isolation of pure aziridine **3**, this because of concomitant elution from the resin of Fmoc protective group remnants. However, subjecting Cbz aziridine **22** to the Birch reduction/cation exchange purification sequence gave homogeneous aziridine **3** in 79% yield (21 mg) in the final deprotection/purification sequence (16% yield over the four steps starting from **13**).

Scheme 2. Synthesis of 8-carboxy -cyclophellitol aziridine **3**.



Reagents and conditions: (a) i) CCl_3CN, DBU, DCM, 0 ˚C; ii) I_2, $NaHCO_3$, H_2O; iii) 37% HCl aq., dioxane, 60 °C; iv) $NaHCO_3$, MeOH, 45%; (b) i) NH_3 (liq.), Li, THF, -60 °C; ii) Amberlite IR120 H^+/NH_4^+, **17**: 65%; (c) NaOCl, NaBr, TEMPO, H_2O; (d) Fmoc-OSu, pyridine, THF, **19**: 75%, or CbzCl, pyridine, THF, **20**: 75%; (e) BAIB, TEMPO, DCM/*t*BuOH/H_2O (4:4:1, v/v/v), 0˚C, **21**: 92% or **22**: 60%; (f) i) NH_3 (liq.), Li, THF, -60˚C; ii) Amberlite IR120 H^+, **3**: 79%;

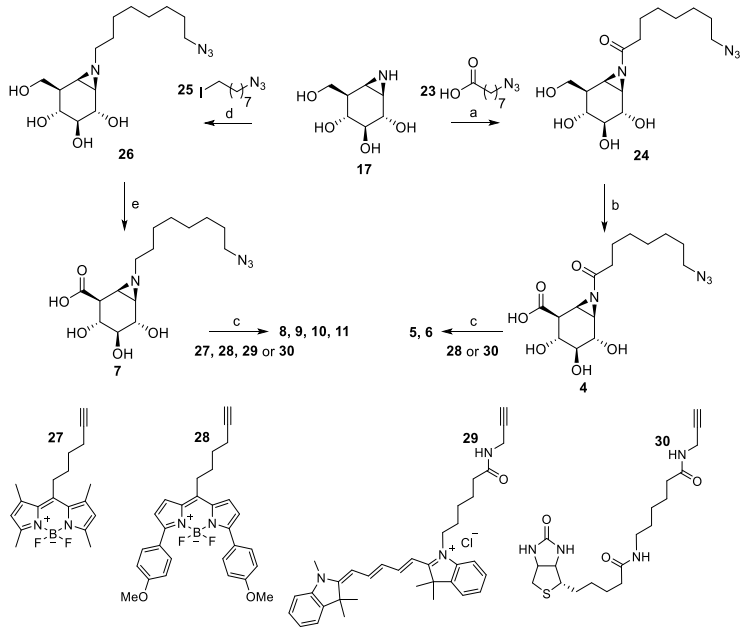
Table 1. TEMPO oxidations of cyclophellitol aziridines **16**, **17**, **19** and **20**

	Regent	Solvent	Temperature	Reaction time	Yield
16	TEMPO/NaClO NaBr, NaOH	H_2O	20 °C	6h	0
16	TEMPO/BAIB	ACN/H_2O (1/1, v/v)	0 °C	24h	0
16	TEMPO/BAIB	DCM/H_2O (2/1, v/v)	0 °C, 20 °C	5h	<1%
16	TEMPO/BAIB	DCM/*t*BuOH/H_2O (4/4/1, v/v/v)	0 °C	22h	<1%
16	TEMPO/BAIB	DCM/*t*BuOH/H_2O (8/8/1, v/v/v)	0 °C	3h	<1%
17	TEMPO/NaClO NaBr, NaOH	H_2O	20 °C	6h	0
19	TEMPO/BAIB	DCM /H_2O (2/1, v/v)	0 °C	5h	48%
19	TEMPO/BAIB	DCM/*t*BuOH/H_2O 4/4/1, v/v/v)	0 °C	5h	92%
20	TEMPO/BAIB	DCM/*t*BuOH/H_2O 4/4/1, v/v/v)	0 °C	5h	60%

With 8-carboxy-cyclophellitol (**2**) and 8-carboxy-cyclophellitol aziridine (**3**) in hand, synthetic routes of *N*-alkylated and *N*-acylated derivatives of **3** were explored. Direct acylation or alkylation of aziridine **3** proved complicated, but oxidation of the primary alcohol in partially protected, *N*-alkyl/acyl aziridine **17** proved more tractable (Scheme 3). Because compound **3** was hard to be obtained and compound **24** and **26** have already been synthesized in previous report.[14] In the first instance, *N*-acyl aziridine **24** was prepared by acylation of the aziridine

nitrogen in **17** with 8-azidooctanoic acid **23** using 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) as the activating agent. Selective oxidation of the primary alcohol in **24** proved to be challenging, as **24** appeared to be highly unstable in both acidic and (strong) basic media. Following the optimized procedure developed for the synthesis of 8-carboxy-cyclophellitol aziridine (**3**), treatment of **24** with TEMPO/BAIB at 0 ˚C under mild basic conditions yielded crude **4**. The crude reaction mixture was lyophilized and purified by HPLC using neutral (milliQ H_2O/ACN) or slightly basic (25mM aq. NH_4OAc) eluents, followed again by lyophilization.

Scheme 3. Synthesis of *N*-substituted 8-carboxy-cyclophellitol aziridine derivatives **4**-**11**.



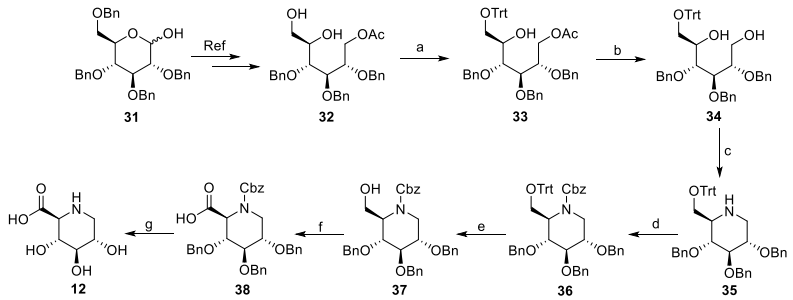
Reagents and conditions: (a) EEDQ, DMF, 57%; (b) TEMPO, BAIB, H_2O/MeCN (1/1), $NaHCO_3$, 0 **°**C, 5%; (c) $CuSO_4$ (1.0 M in H_2O), sodium ascorbate (1.0 M in H_2O), DMF, **5**: 5%, **6**: 16% , **8**: 7%, **9**: 8%, **10**: 21%, **11**: 8%; (d) K_2CO_3, DMF, 50%; (e) TEMPO, NaClO, NaBr, NaOH, H_2O, 15%.

Hydrolysis of the aziridine moiety proved to complicate the synthesis (copper (I)-catalyzed [2+3] azide-alkyne click ligation to BODIPY-alkyne **28** and biotin-alkyne **30** respectively) and purification of compounds **5** and **6** as well. Although these compounds could be obtained in crude form (as judged by LC-MS and NMR spectrum), no analytically pure material could be harvested from the crude products (the compounds proved to be active though as can be seen from the biological evaluation below).

The synthesis of *N*-alkylated 8-carboxy-cyclophellitol aziridine derivatives proved to be much easier than the synthesis of their *N*-acyl counterparts. Thus, reaction of **17** with

1-azido-8-iodooctane and K_2CO_3 in DMF afforded compound **26** in 50% yield. Selective oxidation of the primary alcohol in **26** was accomplished using TEMPO/NaClO as the oxidant at pH 10.5 to afford after HPLC purification compound **7** in 15% yield. Finally, ABPs **8-11** were prepared in moderate yields but good purity by Cu(I)-catalyzed azide alkyne click ligation with BODIPY-alkynes **27** and **28**, Cy5-alkyne **29** and biotin-alkyne **30**, respectively, followed by HPLC purification and lyophilization. The final products proved to be stable during lyophilization and HPLC purification conditions (50 mM NH_4CO_3 in H_2O), and even during LC/MS detection in the presence of 1% TFA.

Scheme 4. Synthesis of trihydroxypipecolic acid 3.



Reagents and conditions: (a) DMAP, Et_3N, TrtCl, DCM, 3 h, 75%; (b) Na, MeOH, 3 h, 91%; (c) i: $(COCl)_2$, DMSO, DCM, -65 °C, 2 h; ii: Et_3N, -65 to 5 °C, 2 h; iii: NH_4HCO_3, $NaBH_3CN$, 3 Å molecular sieves, MeOH, 0 °C, 1 h, then 0 °C to rt, 20 h, 85%; (d) CbzCl, THF, Et_3N, rt, 20 h, 72%; (e) *p*-TsOH, DCM, 0 °C to rt, 3 h, 83%; (f) TEMPO, BAIB, DCM/H_2O (2/1, v/v), 0 °C, 5 h, 71%; (g) i) BnBr, Cs_2CO_3, DMF, 3 h, 82%; ii: H_2, $Pd(OH)_2$/C, AcOH/H_2O (4/1, v/v) 16 h, 57%.

(2S,3R,4R,5S)-2,3,4-trihydroxypipecolic acid **12** was synthesized as depicted in Scheme 4. Starting from tetrabenzyl glucose **31**, intermediate diol **32** was prepared in 6 steps in 83% yield following the literature procedure.[18] Protection of the primary hydroxyl group in **32** as the trityl ether afforded **33**. After deacetylation of the C-1 hydroxyl group, Swern oxidation of the two free alcohols followed by double reductive amination gave orthogonally protected deoxynjirimycin **35** following a procedure previously developed for differently protected deoxynojirimycin derivatives. Subsequently, the amine in **35** was protected as the *tert*-butyloxycarbamate, after which the trityl ester was removed under acidic (TsOH) conditions. Subsequently the primary alcohol of **37** was oxidized (TEMPO/BAIB) to carboxylic acid **38** and hydrogenation over palladium(II) hydroxide on carbon in aq. acetic acid gave inhibitor **12**, the analytical and spectroscopical data of which matched those reported.[19]

Comparative and competitive ABPP on GH79 β-glucuronidase AcaGH79

Having the panel of inhibitors and probes **2**-**12** available, their potency (as reflected by apparent IC_{50} values) in inhibiting 4MU-β-glucuronide hydrolysis by recombinant

Acidobacterium capsulatum β-glucuronidase (AcaGH79)[15] was established firstly. All compounds proved to inhibit AcaGH79, although their potency (IC_{50} values) varied considerably (Table 1). Cy5-aziridine **10** proved to be the most potent inhibitor of the set of ABPs **5**, **6**, **8**-**11** (IC_{50} 51.8 ± 7.4 nM) with the two biotin-aziridines **6, 11** being the weakest of the series (though with an IC_{50} of around 9.5 μM and 8.1 μM, respectively, still quite potent). Of the non-tagged covalent inhibitors, acyl aziridine **4** proved to be the most potent inhibitor, slightly less outperforming than the competitive inhibitor **12**. Epoxide **2** and alkyl aziridine **7** turned out to be considerably less potent AcaGH79 inhibitors. Importantly, all ABPs excepting (at the concentrations measured) biotin aziridines **6** and **11** are able to reach subcellular β-glucuronidase at nanomolar concentrations. Additionally, the potency of alkyl aziridine **7**-**11** is roughly equal to the potency of acyl aziridines **4**-**6**. From this observation combined with the observation that alkyl aziridines are much more stable and easily accessible, one may conclude that *N*-alkylated cyclophellitol aziridine derivatives are the reagents of choice in the development of retaining β-glucuronidase activity-based probes. The cyclophellitol and cyclophellitol aziridine derivatives bind AcaGH79 covalently and irreversibly, as evident from their second order kinetic constants (measured for compounds **2**, **3**, **7**, **8**, see Figure 3A). As well, ABP **8** effectively labels AcaGH79 both at 4 °C and 37 °C (Figure 3B). Labeling of AcaGH79 with ABP **8** can be abolished by competition with either of the compounds **2**, **3, 7**, **12**, **9**-**11** or with the fluorogenic substrate, 4MU-β-glucuronide as well as by denaturing AcaGH79 prior to inclusion of the probe (Figure 3C). Competitive labeling was also obtained with acyl aziridine inhibitor **4** and ABP **5** (Figure 3D). Maximal AcaGH79 labeling with ABP **8** was observed at pH 4-5, in line with the observed maximal hydrolysis of fluorogenic substrate by AcaGH79 (Figure 3E, F).

Table 1. Potency (IC_{50}) of compounds **2**-**12** as inhibitors of recombinant AcaGH79 from *Acidobacterium capsulatum in vitro* using 4MU-β-glucuronide as the fluorogenic substrate. Data were average values of two separate experiments measured in duplicate, error ranges depict standard deviation.

Compounds	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
IC_{50}(nM)	4,520 ± 315.9	159.4 ± 19.6	95.2 ± 11.2	111.1 ± 8.4	9,532.5 ± 61.5	21,555 ± 1193.8	435.6 ± 28.9	294.9 ± 19.0	51.8 ± 7.4	8,084.4 ± 553.2	44.5 ± 3.3

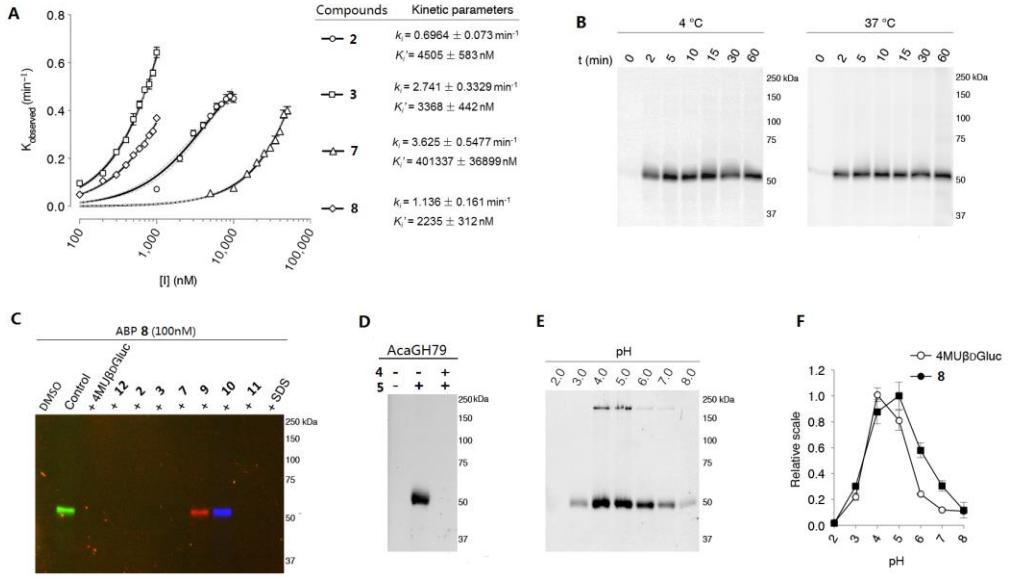


Figure 3 A) Inhibition kinetic parameters for compounds **2**, **3**, **7**, **8**. The graph depicts the obtained $K_{observed}$ and the table shows the kinetic parameters k_i and K_i' estimated with one-phase exponential association. Data shown represents average of two separate experiments in duplicate, and the error bars depict standard deviation. B) Time-dependent labeling of recombinant AcaGH79 with green-BODIPY ABP **8** at 4 °C and 37 °C, respectively. C) Competitive labeling of AcaGH79 with ABP **8** by pre-incubation with 10% SDS, substrate, inhibitors **2**, **3**, **12**, **7** or probes **9**, **10**, **11**. D) Competitive labeling of AcaGH79 with ABP **5** by pre-incubation with acyl aziridine inhibitor **4**. E) Detection of AcaGH79 labeling efficiency at various pH with ABP **8**. F) AcaGH79 labeling with ABP **8** (closed squares) compared to enzymatic activity of substrate 4-methylumbelliferyl β-D-glucuronide (4MUβDGluc) hydrolysis (open circles) at various pHs.

X-ray crystallography of AcaGH79 complexed to inhibitors 3, 4 and 7

In order to provide further evidence of the covalent attachment of the β-glucuronic acid-configured ABPs to β-glucuronidase, crystal structures of AcaGH79 in complex with aziridines **4** and **7** were obtained. These structures clearly showed covalent binding of the compounds, with the aziridine ring opened by the AcaGH79 active site nucleophile, Glu287 (Figure 4A, B). Both acyl aziridine **4** and alkyl aziridine **7** adopt a $^4C_1$ conformation with the aziridine rings opened across the C1-C6 bond in trans-diaxial fashion. The structure of the AcaGH79 mutant in its catalytic nucleophile (E287Q) in complex with **7** revealed (Figure 4C) unhydrolyzed **7** present in the active site and adopting a $^4H_3$ conformation. This conformation is also seen for competitive inhibitor **12** in wild type AcaGH79 (Figure 4D), and is consistent with the expected $^1S_3$-$^4H_3$-$^4C_1$ catalytic itinerary employed by β-glucuronidases.[20]

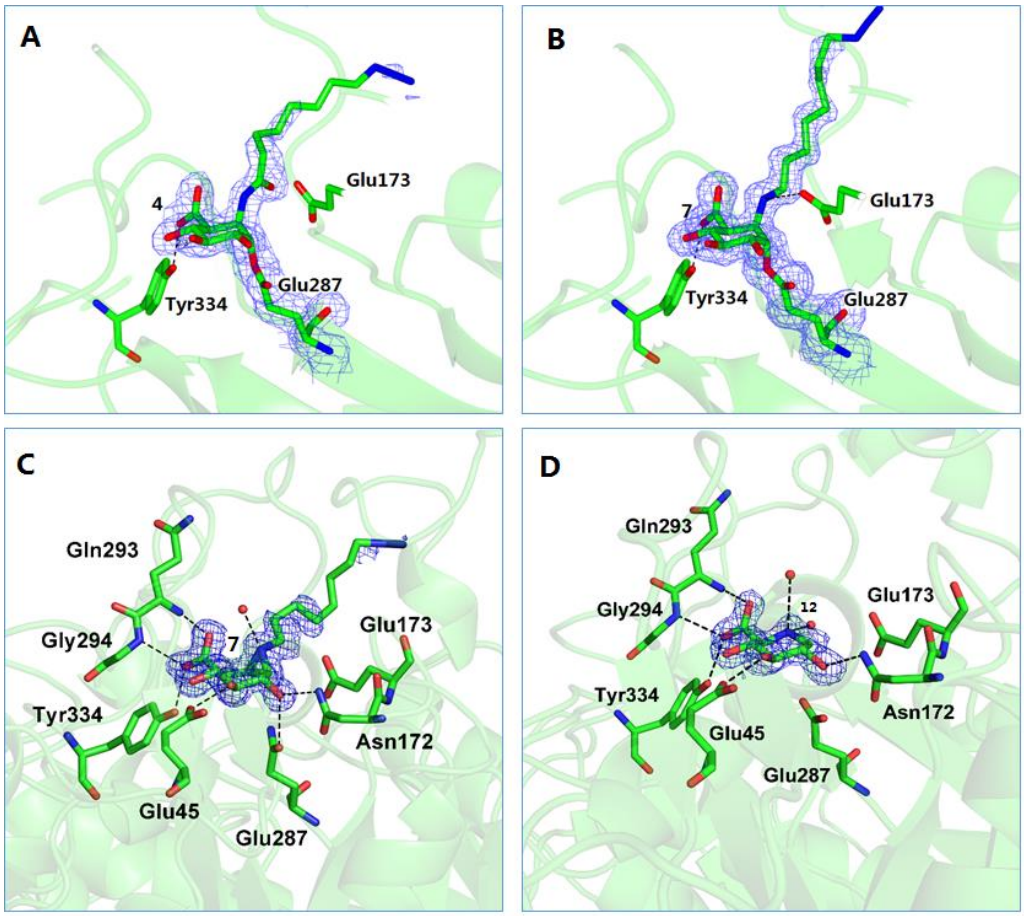


Figure 3. Crystal structures of AcaGH79 from *Acidobacterium capsulatum* in complex with **4**, **7** and **12**. A) AcaGH79-acyl aziridine **4**; B) AcaGH79-alkyl aziridine **7**; C) Mutant E287N AcaGH79--alkyl aziridine **7**; D) AcaGH79-trihydroxypipecolic acid **12**.

6.3 Conclusion

In conclusion, this chapter describes the development of potent, mechanism-based β-glucuronidase inhibitors and activity-based probes. The synthesis of *N*-acyl-8-carboxy-cyclophellitol aziridine proved challenging and no homogeneous pure material could be obtained. The corresponding *N*-alkyl derivatives however were accessible and turned out to be at least as effective in inhibiting and labeling the bacterial β-glucuronidase, AcaGH79. These results match those obtained for *N*-alkyl-cyclophellitol aziridine and *N*-acyl-cyclophellitol aziridine, the reduced counterparts of the molecules described in chapter 4 of this thesis in inhibiting and labeling retaining β-glucosidases. The crystal structure of AcaGH79 complexed to **4** and **7** together with the structure of AcaGH79 complexed to the competitive inhibitor **12** (a structure matched by that of the mutant enzyme complexed with **7**) gives a clear view on the conformational trajectory by which a substrate

glucuronide is processed by retaining β-glucuronidases. The activity-based β-glucuronidase probes described here will be further evaluated, both in activity-based protein profiling and in structural studies, in the next chapter.

6.4 Experimental sections

Gene cloning, expression and protein purification

The coding sequence of the AcGH79 gene was cloned into the pET28a (Novagen) expression vector with an *N*-terminal His6 tag. The E287N mutant was produced by polymerase chain reaction (PCR) using the primer 5'-CCTGACCCAAACGAATTC-3' (forward primer) and 5'-GAATTCGTTTGGGTCAGG-3' (reverse primer). Both the wild-type and mutant proteins were overexpressed in *Escherichia coli* strain BL21 (DE3) GOLD using LB medium. The transformed cells were grown at 37 °C in LB media containing 50 μg mL^{-1} kanamycin unitl the A600 nm reached 0.8. Expression of the recombinant proteins was induced by the addition of 1 mM isopropyl β−D-1-thiogalactopyranoside for 12 h at 25 °C. The cells were harvested by centrifugation at 8000 g for 30 min and resuspended in 50 mL lysis buffer (20 mM HEPES, NaCl 200 mM, imidazole 5 mM, pH 7.0). After 20 min of sonication and 30 min of centrifugation at 12000 ×g, the filtered supernatant containing His_6-AcGH79 was loaded onto a His Trap column (GE Healthcare), equilibrated with the lysis buffer. The column was washed with lysis buffer and the His_6-AcGH79 protein was eluted with the same buffer with supplement of 400 mM imidazole over a gradient of 100 mL. The fractions containing the His_6-AcGH79 were then loaded onto a Hiload 16/60 Superdex 75 column (GE Healthcare). The fractions containing the His_6-AcGH79 were pooled and concentrated to the final concentration of 14.5 mg mL^{-1}.

Enzyme activity assays and IC_{50} measurements for AcaGH79.

The β-D-glucuronidase activity of AcaGH79 was assayed at 37 °C by incubating with 2.5 mM 4-methylumbelliferyl-β-D-glucuronide as substrate in 150 mM McIlvaine buffer, pH 5.0, supplemented with 0.1% (w/v) BSA. To determine the apparent *in vitro* IC_{50} value, recombinant AcaGH79 was firstly pre-incubated with a range of inhibitor dilutions for 30 min at 37 °C, prior to addition of the substrate. The enzymatic reaction was quenched by adding excess NaOH-glycine (pH 10.6), after which fluorescence of liberated 4-methylumbelliferyl was measured with a fluorimeter LS55 (Perkin Elmer) using λ_{EX} 366 nm and λ_{EM} 445 nm. The *in situ* IC_{50} value was determined by incubating fibroblast cell lines expressing wild-type AcaGH79, grown to confluence, with a range of inhibitor dilutions for 2 h. Hereafter, cells were washed three times with PBS and subsequently harvested by scraping in potassium phosphate buffer (25 mM K_2HPO_4-KH_2PO_4, pH 6.5, supplemented with 0.1% (v/v) Triton X-100 and protease inhibitor cocktail (Roche)). After determination of the protein concentration (BCA kit, Pierce), lysates were aliquoted and frozen at -80 °C. Residual AcaGH79 activity was measured using the aforementioned substrate assay. Data was corrected for background fluorescence, then normalized to the untreated control condition and finally curve-fitted via one phase exponential decay function (GraphPad Prism 5.0).[9]

Determination of inhibition constants

The kinetic parameters of inhibition were determined by adding AcaGH79 to the appropriate McIlvaine buffer, containing both substrate and various concentrations of the corresponding inhibitor. The mixtures were incubated for 0 to 60 min at 37 °C and the reactions were quenched at intervals of 5 min with excess NaOH-glycine (pH 10.6).

Inhibitory constants k_i and K'_i were determined by firstly calculating the $K_{observed}$ per inhibitor concentration, followed by curve-fitting the data to a one phase exponential association function (GraphPad Prism 5.0).[9]

***In vitro* labeling and SDS-PAGE analysis and fluorescence scanning**

All the labeling samples were samples resolved on 10% SDS-PAGE. Electrophoresis in sodium dodecylsulfate containing 10% polyacrylamide gels was performed as earlier described.[21] Wet slab-gels were then scanned for ABP-emitted fluorescence using a Bio-rad ChemiDoc MP imager using green Cy2 (λ_{EX} 470 nm, bandpass 30 nm; λ_{EM} 530 nm, bandpass 28 nm) for **8**, red Cy3 (λ_{EX} 530 nm, bandpass 28 nm; λ_{EM} 605 nm, bandpass 50 nm) for **5**, **9**, and blue Cy5 (λ_{EX} 625 nm, bandpass 30 nm; λ_{EM} 695 nm, bandpass 55) for **10**. All samples were denatured with 5× Laemmli buffer (50% (v/v) 1.0 M Tris−HCl, pH 6.8, 50% (v/v) 100% glycerol, 10% (w/v) DTT, 10% (w/v) SDS, 0.01% (w/v) bromophenol blue), boiled for 4 min at 100 °C, and separated by gel electrophoresis on 10% (w/v) SDS-PAGE gels running continuously at 90 V for 30 min and 200 V for 50min.

Influence of pH on ABP labeling involved pre-incubation of AcaGH79 at pH 2−8 for 30 min on ice, prior to addition of 100 nM **8**, dissolved in Nanopure H_2O and incubating for 30 min at 37 °C. Assessment of **2**, **3**, **7**, **8** labeling kinetics involved pre-cooling of 10 ng AGH79 on ice for 15 min, followed by addition of similarly cooled 100 nM **8** solution. After mixing, **8** labeling was chased for 0−60 min at either 4 °C or 37 °C, whereafter labeling was stopped by denaturation. For alkyl aziridines competitive labeling on AcaGH79, 100 ng AcaGH79 was pre-incubated with inhibitors (25 mM 4MU-β-D-glucuronide, 100 µM **2**, **3**, 1.0 mM **12**, or 1.0 µM **7**, **9**, **10**, **11**, or boiled for 4 min in 2% (w/v) SDS, prior to labeling with 100 nM **8**, dissolved in Nanopure H_2O, for 30 min at 37 °C. For acyl aziridines competitive labeling, 100 ng wild-type AcaGH79 was pre-incubated with 1 µM inhibitor **4**, prior to labeling with 100 nM **5** in 150 mM McIlvaine buffer, pH 5.0, for 30 min at 37 °C.

X-ray crystallography and structure solution

Initial crystallization screening of native AcGH79 was conducted by sitting drop vapor diffusion method at 20 °C, by mixing 0.5 µL of the protein solution (4.5 mg mL^{-1}) and an equal volume of precipitant against 100 µL of reservoir solution composed of 0.8 M to 1.5 M sodium phosphate monobasic/postassium phosphate dibasic in the ratio of NaH_2PO_4/K_2HPO_4 = 0.5/9.5 (v/v, pH not adjusted). Some preliminary hits were obtained in several conditions and after refinement of the crystallization conditions, well diffracting crystals were obtained by mixing 0.7-0.8 µL 4.5 mg mL^{-1} protein stock with 0.5 µL precipitant composed of 0.8 M to 1.2 M NaH_2PO_4/K_2HPO_4 = 0.5/9.5 (v/v, pH not adjusted) overnight at 20 °C, using the sitting drop vapour diffusion method. The E287Q mutant of AcGH79 was crystallized by mixing 0.3-0.5 µL of the protein solution (12 mg mL^{-1}) with 0.50 µL precipitant composed of 1.2 M to 1.5 M NaH_2PO_4:K_2HPO_4 = 1.0 : 9.0 (v/v, pH not adjusted).

All four complexes, namely wt-**12**, wt-**4**, wt-**7** and E287Q-**7** were produced by soaking method. The drops containing the crystals of the corresponding wt AcGH79 or E287Q were supplemented with 1.0 µL of 5.0 mM **12**, **4** or **7** in the precipitant solution freshly prepared before soaking procedure for 1 h at 20 °C before fishing. 2 M lithium sulphate was used as cryoprotectant for all the crystals before being flash frozen in liquid nitrogen. Diffraction data for all the crystals were collected at 100 K on beamline i04-1 the Diamond Light Source and were processed using the *xia2*

implementation of XDS and programs from CCP4 suite.[22] Parts of the data processing statistics and structure refinement are listed in Table 1.

Table 1. The statistics of the data processing and structure refinement.

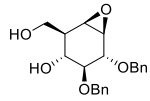
	Native	wt-**4**	wt-**7**	wt-**12**	E287Q-**7**
Data collection					
Space group	C2	C2	C2	C2	C2
Cell dimensions					
a, *b*, *c* (Å)	150.7, 44.9, 83.4	150.7, 44.8, 83.3	148.5, 44.9, 82.2	150.6, 44.7, 83.2	150.2, 45.1, 82.0
α, β, γ (°)	90°, 115.6°, 90°	90°, 115.5°, 90°	90°, 114.8°, 90°	90°, 115.5°, 90°	90°, 115.1°, 90°
Resolution (Å)	42.1 (1.19)	66.7 (1.80)	42.6 (1.60)	42.4 (1.60)	28.9 (1.24)
R_{sym} or R_{merge}	0.033 (0.443)	0.068 (0.540)	0.058 (0.664)	0.054 (0.594)	0.045 (0.489)
$I/\sigma I$	16.9 (2.2)	13.6 (2.1)	12.1 (1.8)	14.2 (1.9)	13.7 (1.9)
Completeness (%)	92.0 (55.4)	98.0 (98.0)	99.5 (99.7)	95.8 (96.3)	89.8 (49.7)
Redundancy	3.8 (2.7)	4.1 (4.1)	4.0 (3.7)	4.2 (4.3)	3.9 (2.7)
Refinement					
Resolution (Å)	1.22 (1.19)	1.85 (1.80)	1.63 (1.60)	1.63 (1.60)	1.27 (1.24)
No. reflections	147896 (6537)	45856 (3332)	64976 (3170)	63367 (3085)	126454 (5143)
R_{work}/ R_{free}	0.1595	0.1967	0.1675	0.1631	0.1699

Highest resolution shell is shown in parenthesis.

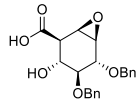
General synthesis

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 aq. 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. 1H-NMR and ^{13}C-NMR spectra were recorded on Bruker AV-850 (850/214 MHz), Bruker DMX-600 (600/150

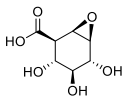
MHz) and Bruker AV-400 (400/100 MHz) spectrometer in the given solvent. Chemical shifts are given in ppm relative to the deuterated chloroform or methanol residual solvent peak or tetramethylsilane (TMS) as internal standard. Coupling constants are given in Hz. All given ^{13}C-NMR spectra are proton 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 Adventage 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: MeCN (MeCN) and C: 1% aq. 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,2R,3R,4S,5R,6R)-4,5-bis(benzyloxy)-2-(hydroxymethyl)-7-oxabicyclo[4.1.0]heptan-3-ol (14): A mixture of diol **13**[15] (3.4 g, 10 mmol, 1.0 eq.) and *m*CPBA (4.4 g, 18 mmol, 1.8 eq.) in 1, 2-dichloroethane (166 mL), NaH_2PO_4 (1.0 M in H_2O, 100 mL), and Na_2HPO_4 (1.0 M in H_2O, 100 mL) was vigorously stirred at 50 °C for 18 h. The layers were separated, and the water layer was extracted with EtOAc. The combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (88%→92%, Et_2O in pentane) to provide β-epoxide **14** (1.9 g, 5.4 mmol, 55%). ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 7.40 – 7.28 (m, 10H), 4.97 (d, *J* = 11.6 Hz, 2H), 4.83 (d, *J* = 11.2 Hz, 2H), 4.70 – 4.66 (m, 2H), 4.03 (dd, *J* = 6.4, 10.8Hz, 1H), 3.91 (dd, *J* = 5.6, 10.8Hz, 1H), 3.83(d, *J* = 7.6Hz, 1H), 3.51 – 3.46 (m, 1H), 3.42 – 3.38 (m, 1H), 3.29 – 3.28 (m, 1H), 3.17 (d, *J* = 4.0Hz, 1H), 3.01 – 2.77 (br, 1H), 2.19 – 2.13 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 138.27, 137.46, 128.70, 128.64, 128.51, 128.18, 128.09, 128.01, 127.98, 83.60, 79.45, 74.94, 72.69, 68.64, 63.99, 55.01, 53.07, 43.44; HRMS: calculated for $C_{21}H_{25}O_5$ [M+H$^+$] 357.16965, found 357.16987.

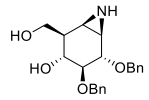


(1R,2R,3R,4S,5R,6R)-4,5-bis(benzyloxy)-3-hydroxy-7-oxabicyclo[4.1.0]heptane-2-carboxylic acid (15): 2, 3-Di-O-benzylcyclophellitol **14** (33 mg, 0.10 mmol, 1.0 eq.) was dissolved in 650 µL DCM/*t*BuOH/H_2O (4/4/1, v/v/v) mixture and was cooled down to 0˚C. TEMPO (75 mg, 19 µmol, 0.20 eq.) and BAIB (75 mg, 0.27 mmol, 2.5 eq.) were added to the solution and it was stirred at 0˚C. The reaction was monitored by TLC. The reaction was quenched after 3 h with 1.0 mL sat. NaS_2O_3 solution and extracted with EtOAc. The organic layer with crude product was dried with $MgSO_4$ and concentrated *in vacuo*. Crude product was purified via HPLC (linear gradient: 28%→38% B, 3 CV, solutions used A: 50 mM NH_4HCO_3 in H_2O, B: MeCN) and concentrated *in vacuo* resulting in **15** (12 mg, 0,032 mmol, 35%). ^{1}H-NMR (400 MHz, CD_3OD): *δ* ppm 7.36 – 7.23 (m, 10H), 4.87 – 4.73 (m, 3H), 4.66 (d, *J* = 11.5 Hz, 1H), 3.81 – 3.74 (m, 2H), 3.54 – 3.53 (m, 1H), 3.41 – 3.36 (m, 1H), 3.19 (d, *J* = 3.7 Hz, 1H), 2.68 – 2.65 (M, 1H); ^{13}C-NMR (100 MHz, CD_3OD): *δ* ppm 140.36, 139.42, 129.38, 129.15, 129.13, 129.02, 128.77, 128.41, 85.29, 80.46, 75.79, 73.62, 69.24, 56.52, 54.39, 51.36; HRMS: calculated for $C_{21}H_{22}O_6$ [M+Na$^+$] 393.13086, found 393.13077.

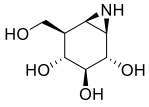


(1R,2R,3R,4S,5R,6S)-3,4,5-trihydroxy-7-oxabicyclo[4.1.0]heptane-2-carboxylic acid (2): A mixture of Product **15** (12 mg, 0.032 mmol, 1.0 eq) and $Pd(OH)_2$/C (3.0 mg, 20% wt. loading(dry basis)) in MeOH

(1.5 mL) was stirred at room temperature under hydrogen atmosphere for 24 h. The catalyst was then filtered off through celite and washed with MeOH. The filtrate was concentrated under reduced pressure and the crude product was purified by semi preparative reversed phase HPLC (linear gradient: 0%→20% B, 3 CV, solutions sed A: 50mM NH_4HCO_3 in H_2O, B: MeCN) and lyophilization gave the title compound **2** (1.4 mg, 7.4 µmol, 23 %) as white solid. ^{1}H-NMR (400 MHz, D_2O): δ ppm 3.76 (d, J = 8.5 Hz, 1H), 3.61 – 3.56 (m, 1H), 3.51 - 3.50 (m, 1H), 3.33 – 3.28 (m, 1H), 3.17 (d, J = 3.8 Hz, 1H), 2.71 – 2.68 (m, 1H); ^{13}C-NMR (100 MHz, D_2O): δ ppm 75.59, 70.74, 67.65, 56.13, 55.79, 50.77; HRMS: calculated for $C_7H_{10}O_6$ [M+Na$^+$] 213.03696, found 213.03260.

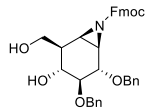


(1R,2R,3R,4S,5S,6R)-4,5-bis(benzyloxy)-2-(hydroxymethyl)-7-azabicyclo[4.1.0]heptan-3-ol (16): Building block product **13** (1.4 g, 4.11 mmol, 1 eq.) was co-evaporated thrice in toluene and was subsequently dissolved in dry DCM (100 mL). The solution was cooled down to 0 ˚C. DBU (62 µL, 0.42 mmol, 0.1 eq.) and trichloroacetonitrile (0.495 ml, 4.94 mmol, 1.2 eq.) were added to the solution. The reaction was stirred at 0 ˚C and monitored with TLC. After 4 h, extra DBU (0.1 eq.) and trichloroacetonitrile (1.2 eq.) were added to the solution. After 5 h, TLC showed that the starting material was completely consumed. Subsequently, H_2O (13 mL), $NaHCO_3$ (3.6 g, 43 mmol, 10 eq.) and iodine (3.2 g, 13 mmol, 3.1 eq.) were added to the solution. And the reaction mixture was stirred overnight at room temperature. Afterwards, it was quenched with 10% aq. NaS_2O_3, concentrated *in vacuo* and extracted with EtOAc. The organic layer with the intermediate product was dried over $MgSO_4$ and concentrated *in vacuo* again. In the following step the intermediate was dissolved in dioxane (37 mL) and 37% HCl (aq., 12 mL). The reaction mixture was refluxed at 60 ˚C for 4 h. After which it was concentrated *in vacuo* and redissolved in 100 mL MeOH. $NaHCO_3$ (14 g, 165 mmol, 40 eq.) was added until pH = 7. The reaction mixture was stirred at room temperature for 3 days. The reaction mixture was concentrated *in vacuo* 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 (4%→10%, MeOH in DCM) yielding aziridine **16** (0.66 g, 1.8 mmol, 45%) as white solid. TLC: R_f 0.5 (EtOAc/EtOH, 3/2, v/v); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.39 – 7.27 (m, 10H), 4.98 (d, J = 9.7 Hz, 1H), 4.78 (d, J = 9.3 Hz, 1H), 4.67 (dd, J = 11.4, 4.5 Hz, 2H), 3.99 (m, 2H), 3.75 (d, J = 9.4 Hz, 1H), 3.53 (t, J = 9.9 Hz, 1H), 3.39 – 3.34 (dd, J = 10.0, 8.1 Hz, 1H), 2.43 – 2.41 (m, 1H), 2.27 (d, J = 6.1 Hz, 1H), 2.08 – 2.02 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 138.54, 137.92, 128.66, 128.61, 128.05, 128.03, 128.01, 127.92, 84.38, 81.23, 74.89, 72.37, 68.36, 64.49, 42.57, 33.22, 31.72; HRMS: calculated for $C_{21}H_{26}NO_4$ [M+H$^+$] 356.1852, found 356.1856.

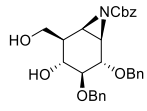


(1R,2S,3S,4R,5R,6R)-5-(hydroxymethyl)-7-azabicyclo[4.1.0]heptane-2,3,4-triol (17): Ammonia (8 mL) was condensed at -60 ˚C and lithium (75 mg, 11 mmol, 24 eq.) was added and stirred until all the lithium was dissolved. Then a solution of aziridine **16** (160mg, 0.45 mmol, 1 eq.) in dry THF (10 mL) was added to the solution above. The reaction mixture was stirred at -60 ˚C. The reaction was quenched with milliQ-H_2O (3.0 mL) after 1 h. The solution was allowed to come to room temperature and stirred until all ammonia had evaporated. Next, the solution was concentrated *in vacuo*, redissolved in milliQ-H_2O, and neutralized with Amberlite IR-120 H$^+$. Product bound to the resin was washed with water and subsequently eluted with NH_4OH solution (1.0 M) and evaporated under reduced pressure. The resulting solid was purified by Amberlite IR-120 NH_4^+ resin using milliQ-H_2O as eluens until the eluate was neutral. Concentration of the combined eluate under reduced pressure gave the fully deprotected aziridine **17** (51 mg, 0.30 mmol, 65%). ^{1}H-NMR (400 MHz, D_2O): δ ppm 3.81 – 3.74 (m, 1H), 3.54 –

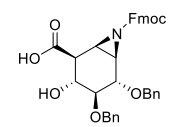
3.44 (m, 2H), 3.11 (t, *J* = 10.1 Hz, 1H), 2.88 (t, *J* = 10.1 Hz, 1H), 2.52 – 2.45 (m, 1H), 2.21 (d, *J* = 6.3 Hz, 1H), 2.01 – 1.95 (m, 1H); ^{13}C-NMR (100 MHz, D_2O): *δ* ppm 76.70, 71.67, 67.38, 61.47, 42.83, 34.78, 33.05.



(9H-fluoren-9-yl)methyl (1R,2S,3S,4R,5R,6R)-2,3-bis(benzyloxy)-4-hydroxy-5-(hydroxymethyl) -7-azabicyclo[4.1.0]heptane-7-carboxylate (19): Aziridine **16** (220 mg, 0.61 mmol, 1.0 eq.) was dissolved in THF (6.2 mL). Fmoc-OSU (160 mg, 0.62 mmol, 1.0 eq.) and pyridine (55 µL, 0.68 mmol, 1.1 eq.) were added. The reaction was stirred at room temperature for 3 h, after which TLC showed the completion of the reaction. The reaction mixture was concentrated *in vacuo*, redissolved in EtOAc. It was washed with water and brine. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo* again. The crude product was purified via silica gel column chromatography (40%→60%, EtOAc in pentane) yielding aziridine **19** (269 mg, 0.47 mmol, 75%) a white solid. TLC: R_f 0.25 (EtOAc/Pentane, 1/1, v/v); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 7.72 (t, *J* = 7.2 Hz, 2H), 7.58 – 7.53 (m, 2H), 7.43 – 7.19 (m, 14H), 4.87 (d, *J* = 11.3 Hz, 1H), 4.70 – 4.51 (m, 4H), 4.44 (d, *J* = 11.5 Hz, 1H), 4.19 (t, *J* = 5.3 Hz, 1H), 3.82 (dd, *J* = 11.1, 5.9 Hz, 1H), 3.75 – 3.61 (m, 2H), 3.35 – 3.19 (m, 2H), 2.49 – 2.41 (m, 2H), 2.00 – 1.90 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 162.64, 143.42, 143.26, 141.50, 141.44, 138.22, 137.31, 128.60, 128.56, 128.20, 128.08, 128.06, 128.02, 127.94, 127.32, 127.27, 124.78, 124.75, 120.13, 83.80, 79.45, 74.86, 71.87, 68.41, 67.44, 63.88, 47.28, 42.33, 40.28, 38.55, 29.79; HRMS: calculated for $C_{36}H_{35}NO_6$ [M+H$^+$] 578.25371, found 578.25321.

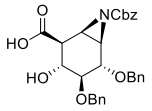


Benzyl(1R,2S,3S,4R,5R,6R)-2,3-bis(benzyloxy)-4-hydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0] heptane-7-carboxylate (20): Aziridine **16** (82 mg, 0.23 mmol, 1 eq.) was dissolved in THF (2.3 mL). CbzCl (39 mg, 0.23 mmol, 1.0 eq.) and pyridine (22.3 µL, 0.28 mmol, 1.2 eq.) were added to the solution. The reaction was stirred at room temperature for 1.5 h and was monitored by TLC. It was shown that all the starting material was converted to a higher running product. The reaction mixture was extracted with EtOAc and concentrated *in vacuo*. The crude product was purified via silica gel column chromatography (1%→9% MeOH in DCM) yielding aziridine **20** (85 mg, 0.17 mmol, 75%). TLC: R_f 0.6(MeOH/DCM, 1/19, v/v); $[\alpha]_D^{20}$ +49.2 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 7.40 – 7.26 (m, 15H), 5.14 – 5.13 (m, 2H), 4.96 (d, *J* = 11.3 Hz, 1H), 4.83 (d, *J* = 11.5 Hz, 1H), 4.66 – 4.61 (m, 2H), 4.02 – 3.98 (m, 1H), 3.90 (br, 1H), 3.81 (d, 1H), 3.45 (t, *J* = 9.7 Hz, 1H), 3.35 (m, 1H), 2.86 – 2.79 (m, 2H), 2.12 – 2.07 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 162.55, 138.26, 137.31, 135.45, 128.82, 128.67, 128.63, 128.22, 128.13, 128.00, 127.97, 83.84, 79.53, 74.93, 72.07, 68.66, 68.64, 64.20, 42.40, 40.35, 38.83; HRMS: calculated for $C_{29}H_{31}NO_6$ [M+H$^+$] 490,22241, found 490,22215.

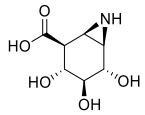


(1R,2S,3R,4S,5S,6R)-7-(((9H-fluoren-9-yl)methoxy)carbonyl)-4,5-bis(benzyloxy)-3-hydroxy-7-az abicyclo[4.1.0]heptane-2-carboxylic acid (21): Fmoc-protected aziridine **19** (172 mg, 0.30 mmol, 1 eq.) was dissolved in 1.5 mL DCM/*t*BuOH/H_2O (v/v/v, 4:4:1) mixture and cooled down to 0 ˚C. TEMPO (9.3 mg, 60 µmol, 0.20 eq.) and BAIB (240 mg, 0.74 mmol, 2.5 eq.) were added to the solution and it was stirred at 0 ˚C. The reaction was monitored by TLC. Extra TEMPO (0.10 eq.) and BAIB (1.2 eq.) were added after 3.5 h. The reaction was quenched with sat. aq. NaS_2O_3 solution (5.0 mL), extracted with EtOAc. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (40%→90% EtOAc with 0.15% AcOH in Pentane) yielding aziridine **21** (162 mg, 0.27 mmol, 92%) as white solid. TLC: R_f 0.45 (EtOH/EtOAc/AcOH, 0.98/9/0.02, v/v); $[\alpha]_D^{20}$ +18 (*c* = 0.5, $CHCl_3$); IR (neat, cm^{-1}): 2922, 2852,

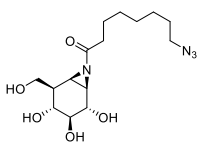
1724, 1450, 1433, 1381, 1278, 1251, 1215, 1111, 1074, 1055, 1082, 987, 756, 698; ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.65 (dd, *J* = 7.2, 3.2 Hz, 2H), 7.55 – 7.49 (m, 2H), 7.41 – 7.24 (m, 14H), 4.82 (d, *J* = 11.3 Hz, 1H), 4.64 (d, *J* = 10.9 Hz, 2H), 4.52 – 4.40 (m, 2H), 4.33 (dd, *J* = 10.7, 6.2 Hz, 1H), 4.13 (t, *J* = 6.1 Hz, 1H), 3.81 (t, *J* = 10.0 Hz, 1H), 3.74 (d, *J* = 8.1 Hz, 1H), 3.30 (dd, *J* = 9.8, 8.4 Hz, 1H), 2.96 (dd, *J* = 5.7, 3.0 Hz, 1H), 2.80 (dd, *J* = 9.9, 2.8 Hz, 1H), 2.52 (d, *J* = 6.0 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 174.24, 162.21, 143.63, 143.39, 141.38, 141.35, 138.23, 137.28, 128.62, 128.54, 128.24, 128.12, 127.99, 127.92, 127.32, 127.28, 125.17, 125.02, 120.09, 120.04, 83.00, 79.12, 74.98, 72.22, 68.16, 66.93, 47.22, 47.00, 39.40, 39.13; HRMS: calculated for $C_{36}H_{33}NO_7$ [M+H$^+$], 592.23298; found 592.23273.



(1R,2S,3R,4S,5S,6R)-4,5-bis(benzyloxy)-7-((benzyloxy)carbonyl)-3-hydroxy-7-azabicyclo[4.1.0]heptane-2-carboxylic acid (22): Aziridine **20** (85 mg, 0.17 mmol, 1.0 eq.) was dissolved in DCM/*t*BuOH/H_2O mixture (1.2 mL , 4:4:1, v/v/v) and cooled down to 0 ˚C. TEMPO (5.3 mg, 34 µmol, 0.20 eq.) and BAIB (137 mg, 42 mmol, 2.5 eq.) were added to the solution and it was stirred at 0˚C for 3 h. The reaction was monitored with TLC. The reaction was quenched by sat. aq. NaS_2O_3 solution (3.0 mL), extracted with EtOAc. The organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (0.5%→10% MeOH in DCM) yielding aziridine **22** (50 mg, 0.10 mmol, 60%). TLC: R_f 0.3 (MeOH/DCM, 1/19, v/v); $[\alpha]_D^{20}$ +56 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.46 – 6.26 (m, 15H), 5.11 – 5.02 (m, 2H), 4.89 (d, *J* = 10.7 Hz, 1H), 4.79 – 4.62 (m, 3H), 3.86 – 3.80 (m, 2H), 3.42 – 3.34 (m, 1H), 3.15 (br, 1H), 2.87 – 2.80 (m, 2H; ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 162.24, 138.30, 137.30, 135.55, 128.70, 128.63, 128.54, 128.42, 128.19, 128.10, 127.98, 127.94, 127.89, 83.13, 79.11, 74.99, 72.32, 68.55, 67.05, 39.58, 39.40; HRMS: calculated for $C_{29}H_{29}NO_7$ [M+H$^+$] 504.20168, found 504.20148.

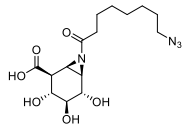


(1R,2S,3R,4S,5S,6R)-3,4,5-trihydroxy-7-azabicyclo[4.1.0]heptane-2-carboxylic acid (3): Ammonia 10 mL was condensed at -60 ˚C and lithium (23 mg, 3.4 mmol, 24 eq.) was dissolved in the liquid. After 30 min, aziridine **22** (50 mg, 0.14 mmol, 1.0 eq.) was dissolved in dry THF (6.0 mL) and added to the solution. The reaction mixture was stirred at -60 ˚C for 0.5 h, then 15 mg extra lithium was added to the solution for stirring one more hour. The reaction was quenched with 4.0 mL milliQ-H_2O. The solution was allowed to come to room temperature and stirred until all ammonia had extracted. Next, the solution was concentrated *in vacuo*, redissolved in milliQ-H_2O, and neutralized with Amberlite IR-120 H$^+$. Product bound to the resin was washed with water and subsequently eluted with NH_4OH solution (1.0 M) and evaporated under reduced pressure. Concentration of the combined eluate fractions under reduced pressure gave the fully deprotected aziridine **3** (21 mg, 0.11 mmol, 79%). ^{1}H-NMR (400 MHz, D_2O): δ ppm 3.61 (d, *J* = 8.5 Hz, 1H), 3.51 – 3.46 (m, 1H), 3.23 – 3.19 (m, 1H), 2.62 – 2.50 (m, 2H), 2.20 – 2.18 (m, 1H); ^{13}C-NMR (100 MHz, D_2O): δ ppm 179.42, 76.65, 72.21, 67.95, 49.86, 34.26, 33.41; HRMS: calculated for $C_7H_{11}NO_5$ [M+H$^+$] 190.07100, found 190.07105.

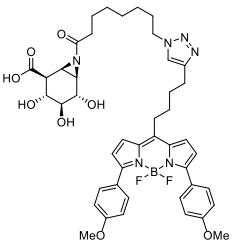


8-Azido-1-((1R,2S,3S,4R,5R,6R)-2,3,4-trihydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0]heptan-7-yl)octan-1-one (24): 8-azidooctanoic acid **23** (93 mg, 0.50 mmol, 1.2 eq.) and EEDQ (123 mg, 0.50 mmol, 1.2 eq.) were dissolved in DMF (0.5 mL) and stirred at room temperature for 2 h. Pre-activated mixed anhydride solution (300 µL, 0.60 eq.) was added to the deprotected aziridine **17** solution at 0 °C and stirred for 30 min. Additional pre-activated

solution (200 µL, 0.40 eq) was added. The resulting mixture was stirred at 0 °C for 3 h. The reaction was quenched by MeOH (2.0 mL) and the mixture was concentrated *in vacuo*. Then the crude product was purified by HPLC (linear gradient: 25%→31% B in A, 3 CV, solutions used: A: H_2O, B: MeCN). After lyophilization, pure product **24** was obtained as colorless oil (27 mg, 0.079 mmol, 18.9%). ^{1}H-NMR (400 MHz, CD_3OD): δ ppm 4.05 (dd, J = 10.4, 4.4 Hz, 1H), 3.69 - 3.65 (m, 2H), 3.30 - 3.25 (m, 2H, and MeOD solvent signals), 3.19 (dd, J = 10.0, 8.4,1H), 3.06 (t, J = 9.6 Hz, 1H), 3.01 (dd, J = 6.0, 2.8Hz, 1H), 2.72 (d, J = 6.0 Hz, 1H), 2.48 (t, J = 7.2Hz, 2H), 1.99 - 1.93 (m, 1H), 1.63–1.54 (m, 4H), 1.36 (s, 6H); ^{13}C-NMR (101MHz, CD_3OD): δ ppm 188.54, 79.02, 73.37, 69.34, 63.69, 52.38, 45.2, 42.40, 41.00, 36.83, 30.09, 29.80 27.61 25.93; IR (neat, cm^{-1}): 3341.9, 2934.6, 2860.2, 2363.2, 2343.2, 2095.8, 1671.7, 1559.8, 1418.4, 1371.6, 1278.3, 1180.9, 1088.7, 1027.3, 976.6, 668.2; HRMS: calculated for $C_{15}H_{27}N_4O_5$ [M+H$^+$] 343.19760, found 343.19761.

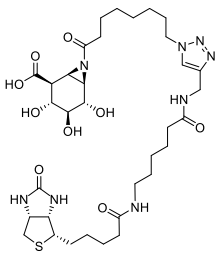


(1R,2S,3R,4S,5S,6R)-7-(8-azidooctanoyl)-3,4,5-trihydroxy-7-azabicyclo[4.1.0]heptane-2-carboxylic acid (4): The primary alcohol compound **24** (89 mg, 0.26 mmol, 1.0 eq.) was dissolved in MeCN/H_2O (3.0 mL, 1/1, v/v). The solution was cooled to 0 °C, then BAIB (183 mg, 0.57 mmol, 2.2 eq), the solution of TEMPO (8.1 mg, 0.052 mmol, 0.20 eq) in MeCN/H_2O (200 µL, 1/1, v/v) and $NaHCO_3$ (88 mg, 1.0 mmol, 4.0 eq.) was added, the resulting mixture was stirred for 2 h at 0 °C. More addition of BAIB (183 mg, 0.57 mmol, 2.2 eq), the solution of TEMPO (8.1 mg, 0.052 mmol, 0.20 eq.) in MeCN/H_2O (100 µL, 1/1, v/v) and $NaHCO_3$ (50 mg, 0.60 mmol, 2.4 eq) was added and the reaction mixture stirred overnight from 0 °C to room temperature, then the reaction was quenched with 10% $Na_2S_2O_3$. After lyophilization, the crude product was purified by semi-preparative reversed HPLC (linear gradient: 16%→19% B in A, 3 CV, solutions used A: 25 mM NH_4OAc in H_2O, B: MeCN) and the fraction were freeze-dried to afford the desired product as a white powder **4** (4.2 mg, 0.012 mmol, 5%). ^{1}H-NMR (400 MHz, CD_3OD): δ ppm 3.67 (dd, J = 13.1, 9.0 Hz, 2H), 3.27 (t, J = 7.0 Hz, 2H), 3.13 (dd, J = 5.8, 2.5 Hz, 1H), 2.66 (d, J = 5.8 Hz, 1H), 2.61 – 2.49 (m, 2H), 2.49 – 2.38 (m, 1H), 2.28 – 2.17 (m, 1H), 1.68 – 1.52 (m, 4H), 1.43 – 1.28 (m, 6H); ^{13}C-NMR (100 MHz, CD_3OD): δ ppm 78.88, 73.55, 69.40, 52.47, 50.68, 41.71, 41.54, 36.76, 30.10, 30.02, 29.85, 27.68, 26.03; ^{1}H-NMR (400 MHz, D_2O): δ ppm 3.78 (d, J = 8.4 Hz, 1H), 3.64 (t, J = 10.2 Hz, 1H), 3.32 – 3.25 (m, 3H), 3.17 (dd, J = 5.7, 3.3 Hz, 1H), 2.75 (d, J = 5.8 Hz, 1H), 2.64 (dd, J = 9.9, 3.3 Hz, 1H), 2.52 – 2.32 (m, 2H), 2.24 (d, J = 7.0 Hz, 1H), 1.61 – 1.48 (m, 4H), 1.29 - 1.32 (m, 6H); ^{13}C-NMR (100 MHz, D_2O): δ ppm 190.68, 77.04, 72.20, 68.74, 51.93, 51.22, 41.13, 41.00, 36.32, 28.75, 28.64, 28.60, 26.45, 25.01; LC-MS: R_t 3.12 min, linear gradient 10%→90% B with 10% NH_4OAc in 15 min; ESI-MS: m/z = 357.2 (M+H)$^+$; HRMS: calculated for $C_{15}H_{24}N_4O_6$ [M+H$^+$] 357.17686, found: 357.17687.

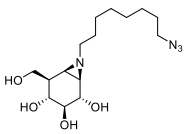


(1R,2S,3R,4S,5S,6R)-7-(8-(4-(4-(5,5-difluoro-3,7-bis(4-methoxyphenyl)-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)butyl)-1H-1,2,3-triazol-1-yl)octanoyl)-3,4,5-trihydroxy-7-azabicyclo[4.1.0]heptane-2-carboxylic acid (5): Acyl Azido-aziridine compound **4** (5.2 mg, 0.015 mmol, 1.0 eq.) was dissolved in DMF (0.60 mL), red BODIPY-alkyne (8.5 mg, 0.017 mmol, 1.2 eq.), $CuSO_4$ (1.0 M in H_2O, 10 µL, 0.010 mmol, 0.60 eq.) and sodium ascorbate (1.0 M in H_2O, 11 µL, 0.011 mmol, 0.70 eq.) were added to the solution under argon atmosphere, and the mixture was stirred at room temperature for 2 h, the reaction was checked with LC-MS within the elution system of 10% NH_4OAc. The reaction mixture was lyophilization and then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 42%→48% B in A, 3 CV, solutions used

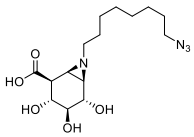
A: 25 mM NH_4OAc in H_2O, B: MeCN) and the fractions were freeze-dried to afford the desired product **5** as a purple powder (1.2 mg, 0.0014 mmol, 9.5%); ^{1}H-NMR (500 MHz, CD_3OD): δ ppm 7.87 – 7.81 (m, 4H), 7.74 – 7.70 (m, 2H), 7.67 – 7.61 (m, 2H), 7.45 (d, *J* = 4.4 Hz, 2H), 6.99 – 6.95 (m, 4H), 6.69 (d, *J* = 4.4 Hz, 2H), 4.33 (t, *J* = 7.1 Hz, 2H), 3.85 (s, 7H), 3.81 (d, *J* = 13.3 Hz, 4H), 3.08 (t, *J* = 5.1 Hz, 2H), 2.82 – 2.73 (m, 3H), 2.24 – 2.09 (m, 5H), 2.04 (s, 4H), 1.91 – 1.83 (m, 8H), 1.66 – 1.50 (m, 6H), 1.39 – 1.22 (m, 20H); LC-MS: R_t 7.51 min, linear gradient 0%→90% B with 10% NH_4OAc in 15 min; ESI-MS: *m*/*z* = 841.6 ($M+H^+$); HRMS: calculated for $C_{44}H_{51}BF_2N_6O_{48}$ $[M+H]^+$ 841.39023, found: 841.38982.



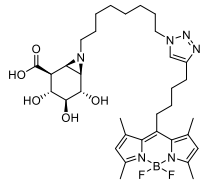
(1R,2S,3R,4S,5S,6R)-3,4,5-trihydroxy-7-(8-(4-((6-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)hexanamido)methyl)-1H-1,2,3-triazol-1-yl)octanoyl)-7-azabicyclo[4.1.0]heptane-2-carboxylic acid (6): Acyl azido-aziridine compound **4** (9.5 mg, 0.027 mmol, 1 eq.) was dissolved in DMF (1.0 mL) in eppi tube, Biotin-Ahx-alkyne (11 mg, 0.029 mmol, 1.1 eq.), $CuSO_4$ (1.0 M in H_2O) (5.3 µL, 0.0053 mmol, 0.20 eq.) and sodium ascorbate (1.0 M in H_2O) (5.6 µL, 0.0056 mmol, 0.21 eq.) was added to the solution under argon atmosphere, and the mixture was stirred at room temperature overnight. The reaction was checked by LC-MS within the elution system of 10% aq. NH_4OAc. The volatiles were removed by lyophilisation and then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 12%→27% B in A, 3 CV, solutions used A: 25mM NH_4OAc in H_2O, B: MeCN), the fractions were lyophilized directly to afford product **6** as a white powder (3.3 mg, 0.0044 mmol, 16.4%). ^{1}H-NMR (600 MHz, D_2O): δ ppm 7.84 (s, 1H), 4.58 (dd, *J* = 7.8, 4.8 Hz, 1H), 4.42 (s, 2H), 4.39 – 4.37 (m, 3H), 3.78 (d, *J*=8.4 Hz, 1H), 3.66 (t, *J* = 10.2 Hz, 1H), 3.34- 3.28 (m, 2H), 3.17 – 3.16 (m, 1H), 3.13-3.10 (m, 2H), 2.96 (dd, *J* = 12.6, 4.8Hz, 1H), 2.76-2.72(m, 2H), 2.70 (s, 2H), 2.66 (dd, *J* = 9.6, 3 Hz, 1H), 2.27 (t, *J* = 7.2 Hz, 3H), 2.22 (t, *J* = 7.2 Hz, 3H), 1.87 – 1.84 (m, 2H), 1.63-1.42 (m, 13H), 1.38-1.34 (m, 2H), 1.38 - 1.30 (m, 8H); ^{13}C-NMR (100 MHz, D_2O): δ ppm 190.7, 182.4, 177.7, 177.5, 166.2, 124.7, 77.3, 72.4, 68.9, 63.0, 61.2, 56.3, 56.2, 51.4, 51.3, 41.3, 41.2, 40.6, 40.0, 39.6, 36.5, 36.4, 36.3, 35.2, 30.1, 28.9, 28.9, 28.8, 28.6, 28.6, 28.5, 26.3, 26.2, 26.1, 25.8, 25.1, 24.2; LC-MS: R_t 5.06 min, linear gradient 0%→90% B with 10% NH_4OAc in 15 min; ESI-MS: *m*/*z* = 751.8 ($M+H^+$); HRMS: calculated for $C_{34}H_{54}N_8O_9S$ $[M+H^+]$ 751.38027, found: 751.38028.



(1R,2S,3S,4R,5R,6R)-7-(8-azidooctyl)-5-(hydroxymethyl)-7-azabicyclo[4.1.0]heptane-2,3,4-triol (26): Unprotected aziridine **17**[14] (163 mg, 0.93 mmol, 1.0 eq.) was dissolved in DMF (4.0 mL). 1-Azido-8-iodooctane **25** (379 mg, 1.3 mmol, 1.5 eq.) and K_2CO_3 (448 mg, 4.0 mmol, 4.3 eq.) were added to the solution and the reaction mixture was stirred at 80 °C under refluxing conditions for 24 h, the volatiles were filtered via a pad of celite and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (MeOH in DCM, 2%→20%) yielding **26** as light yellow oil (150 mg, 0.46 mmol, 49%). TLC: R_f 0.39 (DCM/MeOH, 5/1, v/v); $[\alpha]_D^{20}$ +50 (*c* = 1, MeOH); ^{1}H-NMR (400 MHz, CD_3OD): δ ppm 4.00 (dd, *J* = 10.1, 4.6 Hz, 1H), 3.66 – 3.59 (m, 2H), 3.28 (t, *J* = 6.8 Hz, 2H), 3.15 – 3.02 (m, 2 H), 2.40 – 2.33 (m, 1H), 2.18 – 2.11 (m, 1H), 2.01 – 1.98 (m, 1H), 1.93 – 1.86 (m, 1H), 1.66 (d, *J* = 6.3 Hz, 1H), 1.62 – 1.54 (m, 4H), 1.42 – 1.29 (m, 8H); ^{13}C-NMR (100 MHz, CD_3OD): δ ppm 78.92, 73.79, 70.15, 63.76, 62.05, 52.40, 45.36, 45.34, 42.98, 30.45, 30.24, 30.14, 29.85, 28.25, 27.72; IR (neat, cm^{-1}): 3315, 2926, 2095, 1464, 1348, 1256, 1096, 1020, 818; LC-MS: R_t 4.52 min, linear gradient 10%→90% B in 12.5 min; ESI-MS: *m*/*z* = 329.2 $(M+H)^+$; HRMS: calculated for $C_{15}H_{28}N_4O_4$ $[M+H^+]$ 329.21833, found: 329.21809.

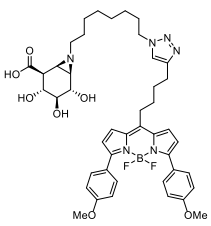


(1R,2S,3R,4S,5S,6R)-7-(8-azidooctyl)-3,4,5-trihydroxy-7-azabicyclo[4.1.0]heptane-2-carboxylic acid (7): Compound **26** (77 mg, 0.23 mmol, 1.0 eq.), TEMPO (0.23 mg, 0.0015 mmol, 0.0065 eq.), and NaBr (9.5 mg, 0.092 mmol, 0.40 eq.) were dissolved in water (5.0 mL) at 2 °C. A 13% sodium hypochlorite solution (0.24 mL, 0.51 mmol, 2.2 eq.) was added dropwise to the mixture, which was adjusted to pH 10.5 by adding aq. NaOH (0.50 M). The reaction was conducted at 2 °C and checked with LC-MS with an elution system of 10% aq. NH_4OAc. Upon completion, the reaction was quenched by adding 96% EtOH (1.0 mL) and the pH was adjusted to 7 by adding aq. 0.5 M HCl. The volatiles were removed by lyophilization and then the crude product was purified by semi-preparative reverse phase HPLC (linear gradient: 19%→26% B in A, 3 CV, solutions used A: 50 mM NH_4HCO_3 in H_2O, B: MeCN), the fractions were concentrated and lyophilized to afford product **7** as a white powder (12.0 mg, 0.035 mmol, 15%). ^{1}H-NMR (850 MHz, CD_3OD): δ ppm 3.63 (d, J = 8.2 Hz, 1H), 3.62 – 3.57 (m, 1H), 3.27 (t, J = 6.9 Hz, 2H), 3.18 – 3.16 (m, 1H), 2.41 – 2.38 (m, 1H), 2.35 – 2.30 (m, 1H), 2.18 – 2.10 (m, 2H), 1.62 – 1.55 (m, 4H), 1.53 (d, J = 6.2 Hz, 1H), 1.43 – 1.27 (m, 8H); ^{13}C-NMR (214 MHz, CD_3OD): δ ppm 78.89, 74.25, 70.09, 62.24, 52.47, 50.87, 44.64, 43.67, 30.53, 30.28, 30.21, 29.91, 28.39, 27.80; ^{1}H-NMR (400 MHz, D_2O): δ 3.62 (d, J = 8.7 Hz, 1H), 3.50 (t, J = 10.2 Hz, 1H), 3.24 (t, J = 6.9 Hz, 2H), 3.20 – 3.15 (m, 1H), 2.52 (dd, J = 9.9, 3.7 Hz, 1H), 2.32 - 2.24 (m, 1H), 2.15 - 2.04 (m, 2H), 1.68 (d, J = 6.3 Hz, 1H), 1.56 – 1.49 (m, 2H), 1.46 - 1.35 (m, 2H), 1.34 - 1.13 (m, 8H); ^{13}C-NMR (100 MHz, D_2O): δ ppm 179.81, 76.44, 71.91, 68.53, 59.87, 51.23, 50.17, 42.90, 42.35, 28.42, 28.34, 28.11, 27.91, 26.31, 25.86; LC-MS: R_t 4.41 min, linear gradient 10%→90% B in 12.5 min; ESI-MS: m/z = 343.2 $(M+H)^+$; HRMS: calculated for $C_{15}H_{26}N_4O_5$ $[M+H^+]$ 343.19760, found: 343.19777.

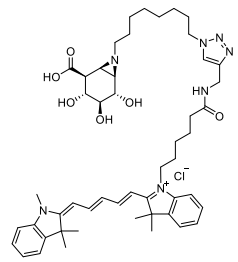


(1R,2S,3R,4S,5S,6R)-7-(8-(4-(4-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)butyl)-1H-1,2,3-triazol-1-yl)octyl)-3,4,5-trihydroxy-7-azabicyclo[4.1.0]heptane-2-carboxylic acid (8): Azide compound **7** (5.0 mg, 0.015 mmol, 1.0 eq.) was dissolved in DMF (0.5 mL) in an 1 mL Eppendorf tube, green BODIPY-alkyne **27** (6.9 mg, 0.017 mmol, 1.2 eq.), $CuSO_4$ (1.0 M in H_2O, 8.8 µL, 0.0088 mmol, 0.60 eq) and sodium ascorbate (1.0 M in H_2O, 9.5 µL, 0.0095 mmol, 0.65 eq.) were added to the solution under argon atmosphere, and the mixture was stirred at room temperature overnight. The reaction was checked by LC-MS with an elution system of 10% aq. NH_4OAc. The volatiles were concentrated *in vacuo* and then the crude product was purified by semi-preparative reverse phase HPLC (linear gradient: 35%→44% B in A, 3 CV, solutions used A: 25 mM NH_4HCO_3 in H_2O, B: MeCN) and the fractions were concentrated and lyophilized giving **8** as an orange powder (2.2 mg, 0.0032 mmol, 22%). ^{1}H-NMR (850 MHz, CD_3OD): δ ppm 7.90 (s, 2H), 7.75 (s, 1H), 6.11 (s, 2H), 4.34 (t, J = 7.0 Hz, 2H), 3.63 – 3.58 (m, 2H), 3.18 – 3.12 (m, 1H), 3.05 – 3.01 (m, 2H), 2.78 (t, J = 7.3 Hz, 2H), 2.49 - 2.45 (m, 1H), 2.43 (s, 6H), 2.38 (s, 6H), 2.22 – 2.18 (m, 2H), 2.11 – 2.08 m, 1H), 1.92 – 1.88 (m, 5H), 1.88 – 1.83 (m, 2H), 1.69 – 1.63 (m, 2H), 1.57 – 1.48 (m, 2H), 1.36 – 1.21 (m, 8H); ^{13}C-NMR (214 MHz, CD_3OD): δ ppm 154.92, 148.51, 147.88, 142.17, 132.57, 123.41, 122.59, 78.66, 74.02, 69.83, 62.02, 51.23, 44.79, 43.39, 32.31, 31.24, 30.87, 30.36, 29.87, 29.09, 28.19, 27.34, 25.91, 16.50, 14.43; LC-MS: R_t 6.24 min, linear gradient 10%→90% B, 12.5 min; ESI-MS: m/z = 671.2 $(M+H)^+$; HRMS: calculated for $C_{34}H_{49}BF_2N_6O_5$ $[M+H^+]$ 671.39043, found: 671.39001.

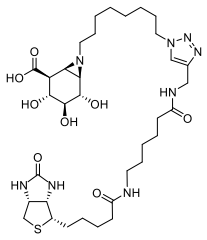
(1R,2S,3R,4S,5S,6R)-7-(8-(4-(4-(5,5-difluoro-3,7-bis(4-methoxyphenyl)-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)butyl)-1H-1,2,3-triazol-1-yl)octyl)-3,4,5-trihydroxy-7-azabicyclo[4.1.0]heptane-2-carboxylic acid (9):



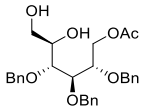
Azide compound **7** (6.0 mg,0.018 mmol, 1.0 eq.) was dissolved in DMF (0.6 mL), red BODIPY-alkyne **28** (10 mg, 0.021 mmol, 1.2 eq.), $CuSO_4$ (1.0 M in H_2O, 10 µL, 0.011 mmol, 0.60 eq) and sodium ascorbate (1.0 M in H_2O, 11 µL, 0.012 mmol, 0.65 eq.) were added to the solution under argon atmosphere, and the mixture was stirred at room temperature overnight, the reaction was checked by LC-MS with an elution system of 10% aq. NH_4OAc. The volatiles were concentrated *in vacuo* and then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 43%→50% B in A, 12 min, solutions used A: 25 mM NH_4HCO_3 in H_2O, B: MeCN) and the fractions were concentrated and lyophilized to afford product **9** as a purple powder (1.9 mg, 0.0023 mmol, 13%).^{1}H-NMR (850 MHz, CD_3OD): δ ppm 7.87 – 7.83 (m, 4H), 7.71 (s, 1H), 7.44 (d, J = 4.3 Hz, 2H), 6.99 – 6.95 (m, 4H), 6.69 (d, J = 4.3 Hz, 2H), 4.33 (t, J = 7.1 Hz, 2H), 3.85 (s, 6H), 3.65 – 3.58 (m, 2H), 3.18 – 3.16 (t, J = 9.2 Hz, 1H), 3.08 – 3.06 (m, 2H), 2.80 – 2.78 (m, 2H), 2.39 – 2.38 (m, 1H), 2.30 – 2.25 (m, 1H), 2.13 – 2.11 (m, 1H), 1.89 – 1.82 (m, 6H), 1.51 (d, J = 6.2 Hz, 1H), 1.34 – 1.20 (m, 11H); ^{13}C-NMR (214 MHz, CD_3OD): δ ppm 162.18, 158.78, 148.59, 146.79, 137.49, 132.16, 128.44, 126.52, 123.26, 121.03, 114.61, 78.88, 74.21, 70.08, 62.19, 55.82, 51.27, 50.83, 44.62, 43.65, 34.22, 31.28, 30.99, 30.42, 30.38, 30.22, 29.91, 28.26, 27.40, 25.82; LC-MS: R_t 7.00 min; linear gradient 10%→90% B in 12.5 min; ESI-MS: m/z = 827.3 $(M+H)^+$; HRMS: calculated for $C_{44}H_{53}BF_2N_6O_7$ $[M+H^+]$ 827.41172, found: 827.41098.



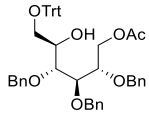
1-(6-(((1-(8-((1R,2S,3R,4S,5S,6R)-2-carboxy-3,4,5-trihydroxy-7-azabicyclo[4.1.0]heptan-7-yl)octyl)-1H-1,2,3-triazol-4-yl)methyl)amino)-6-oxohexyl)-3,3-dimethyl-2-((1E,3E)-5-((E)-1,3,3-trimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-3H-indol-1-ium chloride (10): Azide compound **7** (6.0 mg, 0.017 mmol, 1.0 eq.) was dissolved in DMF (0.6 mL), Cy-5 allkyne **29** (12 mg, 0.021 mmol, 1.2 eq.), $CuSO_4$ (1.0 M in H_2O, 10 µL, 0.011 mmol, 0.6 eq) and sodium ascorbate (1.0 M in H_2O, 11 µL, 0.012 mmol, 0.65 eq.) were added to the solution under argon atmosphere, and the mixture was stirred at room temperature overnight, the reaction was checked by LC-MS with an elution system of 10% aq. NH_4OAc. The volatiles were concentrated *in vacuo* and then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 40%→50% B in A, 12 min, solutions used A: 25 mM NH_4HCO_3 in H_2O, B: MeCN) and the fractions were concentrated and lyophilizedto afford product **10** as a dark blue powder (2.5 mg, 0.0029 mmol, 17%). ^{1}H-NMR (850 MHz, CD_3OD): δ ppm 8.26 – 8.22 (m, 2H), 7.85 (s, 1H), 7.49 – 7.48 (m, 1.3 Hz, 2H), 7.42 – 7.39 (m, 2H), 7.31 – 7.28 (m, 1H), 7.27 – 7.25 (m, 1H), 6.62 (t, J = 12.4 Hz, 1H), 6.29 – 6.27 (m, 2H), 4.41 (s, 2H), 4.36 (t, J = 7.0 Hz, 2H), 4.08 (t, J = 7.6 Hz, 2H), 3.63 – 3.58 (m, 3H), 3.16 (t, J = 9.2 Hz, 1H), 2.40 – 2.35 (m, 1H), 2.25 (t, J = 7.3 Hz, 2H), 2.16 – 2.09 (m, 2H), 1.89 – 1.83 (m, 8H), 1.84 – 1.78 (m, 2H), 1.72 – 1.68 (m, 10H), 1.56 – 1.42 (m, 7H), 1.34 – 1.22 (m, 10H); ^{13}C-NMR (214 MHz, CD_3OD): δ ppm 175.75, 175.36, 174.62, 155.53, 155.48, 146.14, 144.26, 143.53, 142.63, 142.52, 129.79, 129.73, 126.65, 126.25, 126.22, 124.18, 123.41, 123.27, 112.05, 111.85, 104.43, 104.28, 78.91, 74.23, 70.11, 62.14, 51.35, 50.81, 50.54, 50.50, 44.77, 44.61, 43.66, 36.47, 35.59, 31.53, 31.25, 30.30, 30.19, 29.86, 28.20, 28.12, 27.95, 27.81, 27.33, 27.30, 26.40, 24.18; LC-MS: R_t 6.29 min, linear gradient 10%→90% B in 12.5 min; ESI-MS: m/z = 862.5 $(M+H)^+$; HRMS: calculated for $C_{50}H_{68}BF_2N_7O_6$ $[M+H^+]$ 863.53038, found: 863.52618.



(1R,2S,3R,4S,5S,6R)-3,4,5-trihydroxy-7-(8-(4-((6-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)hexanamido)methyl)-1H-1,2,3-triazol-1-yl)octyl)-7-azabicyclo[4.1.0]heptane-2-carboxylic acid (11): Azide compound **7** (9.2 mg, 0.027 mmol, 1.0 eq.) was dissolved in DMF (0.8 mL) in eppi tube, Biotin-alkyne **30** (12.6 mg, 0.032 mmol, 1.2 eq.), $CuSO_4$ (1.0 M in H_2O, 10 µL, 0.011 mmol, 0.60 eq.) and sodium ascorbate (1.0 M in H_2O, 11 µL, 0.012 mmol, 0.65 eq.) were added to the solution under argon atmosphere, and the mixture was stirred at room temperature overnight, the reaction was checked with LC-MS with an elution system of 10% aq. NH_4OAc. The volatiles were concentrated *in vacuo* and then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 40%→50% B in A, 12 min, solutions used A: 25 mM NH_4HCO_3 in H_2O, B: MeCN) and the fractions were concentrated and lyophilized to afford product **11** as a white powder (2.6 mg, 0.0035 mmol, 13%). ^{1}H-NMR (600 MHz, CD_3OD): δ ppm 7.87 (s, 1H), 4.49 (dd, *J* = 7.9, 4.9 Hz, 1H), 4.46 – 4.34 (m, 4H), 4.31 (dd, *J* = 7.9, 4.4 Hz, 1H), 3.70 - 3.54 (m, 2H), 3.24 – 3.12 (m, 4H), 2.96 – 2.89 (m, 1H), 2.70 (d, *J* = 12.7 Hz, 1H), 2.43 (br, 1H), 2.34 - 21 (m, 6H), 1.98 – 1.81 (m, 3H), 1.78 – 1.47 (m, 11H), 1.44 (q, *J* = 7.5 Hz, 2H), 1.39 – 1.24 (m, 10H); ^{13}C-NMR (150 MHz, CD_3OD): δ ppm 176.00, 175.98, 166.14, 124.20, 79.21, 78.79, 74.15, 70.06, 69.31, 63.68, 63.38, 62.13, 61.62, 57.03, 51.39, 44.75, 43.60, 41.06, 40.20, 36.82, 36.75, 35.62, 31.30, 30.40, 30.25, 30.12, 29.96, 29.79, 29.50, 28.25, 27.56, 27.40, 26.94, 26.70, 26.54; LC-MS: R_t 4.06 min, linear gradient 10-90% B in 12.5 min; ESI-MS: *m*/*z* = 737.3 $(M+H)^+$; HRMS: calculated for $C_{34}H_{56}N_8O_8S$ $[M+H^+]$ 737.40146, found: 737.40167.

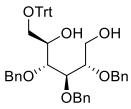


(2S,3R,4R,5R)-2,3,4-tris(benzyloxy)-5,6-dihydroxyhexyl acetate (32): The Intermediate **32** (20.6 g, 41.7 mmol) was prepared via previous reported synthesis,[17] in 6 steps from compound **31** in 83% total yield. $[\alpha]_D^{20}$ +11.8 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm 7.35 – 7.24 (m, 15H), 4.70 – 4.55 (m, 6H), 4.31 – 4.26 (m, 1H), 4.23 – 4.18 (m, 1H), 3.96 – 3.92 (m, 1H), 3.84 – 3.81 (m, 1H), 3.76 – 3.71 (m, 3H), 3.67 – 3.63 (m, 1H), 1.97 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm 170.75, 137.80, 137.66, 137.49, 128.80, 128.60, 128.58, 128.56, 128.22, 128.20, 128.07, 128.06, 78.04, 77.08, 76.73, 76.42, 73.94, 73.77, 73.36, 71.81, 63.63, 63.53, 20.96; IR (neat, cm^{-1}): 3447, 2876, 1738, 1497, 1454, 1368, 1231, 1026, 733, 696; HRMS: calculated for $C_{29}H_{34}NO_7$ $[M+Na^+]$ 517.21967, found: 517.21919.

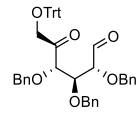


(2S,3R,4R,5R)-2,3,4-tris(benzyloxy)-5-hydroxy-6-(trityloxy)hexyl acetate (33): Product **32** (20 g, 41 mmol, 1.0 eq) was dissolved in DCM (500 mL). Then DMAP (0.25 g, 2.1 mmol, 0.05 eq.), Et_3N (10 mL, 72 mmol, 1.7 eq) and triphenylmethyl chloride (15 g, 46 mmol, 1.3 eq.) were added. The reaction was then stirred for 3 h at room temperature. Afterwards aq. sat. $NaHCO_3$ solution (600 mL) was added to quench the reaction. The product was extracted from the water layer with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude mixture was purified by silica gel column chromatography (10%→20%, EtOAc in pentane) providing product **33** (23 g, 31 mmol, 75 %). $[\alpha]_D^{20}$ +6 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.45 – 7.41 (m, 6H), 7.29 – 7.19 (m, 22H), 7.13 – 7.10 (m, 2H), 4.65 – 4.56 (m, 3H), 4.48 – 4.44 (m, 3H), 4.38 (dd, *J* = 12.0, 3.6 Hz, 1H), 4.15 – 4.04 (m, 2H), 3.94 – 3.85 (m, 2H), 3.77 – 3.74 (m, 1H), 3.36 – 3.29 (m, 2H), 3.01 (d, *J* = 5.6 Hz, 1H), 1.90 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 170.77, 143.86, 138.09, 137.86, 137.82, 128.78, 128.51, 128.46, 128.36, 128.25, 128.13, 127.95, 127.83, 127.74, 127.18, 86.75,

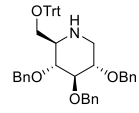
78.35, 77.11, 76.81, 74.31, 73.15, 72.86, 70.69, 64.74, 64.12, 20.95; IR (neat, cm^{-1}): 3030, 2876, 2347, 1738, 1449, 1231, 1067, 737, 696, 633; HRMS: calculated for $C_{48}H_{48}O_7$ [M+Na^+] 759.32922, found: 759.32905.



(2S,3R,4R,5R)-2,3,4-tris(benzyloxy)-6-(trityloxy)hexane-1,5-diol (34): Product **33** (21 g, 29 mmol, 1.0 eq.) was co-evaporated with toluene and then dissolved in dry MeOH (500 mL). Thereafter catalytic amount of Na (0.60 g) was added. The mixture was stirred for 3 h at room temperature and then concentrated at reduced pressure. The residue was dissolved in H_2O (300 mL). The product was extracted from the water layer with EtOAc. The combined organic layers were washed with brine (300 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. Finally the reaction mixture was purified by silica gel column chromatography (12%→50%, EtOAc in pentane) giving product **34** (18.56 g, 26.71 mmol, 91 %). $[\alpha]_D^{20}$ -4 (*c*=1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.72 – 7.67 (m, 6H), 7.51 – 7.35 (m, 22H), 7.32 – 7.27 (m, 2H), 4.95 – 4.71 (m, 4H), 4.69 – 4.63 (m, 2H), 4.33 (br, 1H), 4.17 – 4.07 (m, 2H), 4.06 – 3.99 (m, 1H), 3.98 – 3.89 (m, 1H), 3.85 – 3.75 (m, 1H), 3.69 – 3.62 (m, 1H), 3.61 – 3.45 (m, 2H), 2.76 (br, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 143.71, 138.09, 137.75, 137.60, 129.06, 128.75, 128.61, 128.31, 128.22, 128.17, 128.04, 127.80, 127.74, 127.62, 127.59, 126.97, 125.97, 86.53, 79.39, 79.01, 76.91, 74.30, 72.81, 72.75, 70.63, 64.58, 61.64; IR (neat, cm^{-1}): 2932, 1736, 1449, 1371, 1240, 1043, 745, 696; HRMS: calculated for $C_{46}H_{46}NO_6$ [M+Na^+] 717.31866, found: 717.31864.

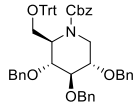


(2R,3R,4S)-2,3,4-tris(benzyloxy)-5-oxo-6-(trityloxy)hexanal: $COCl_2$ (8.7 mL, 101 mmol, 4.0 eq.) was dissolved in DCM (92 mL) and cooled to -78 °C. Then DMSO (9.0 mL, 126 mmol, 5.0 eq.) in DCM (54 mL) was added dropwise over 10 min. The mixture was stirred for 40 min while kept below -70 °C. Then a solution of **34** (17 g, 25 mmol, 1.0 eq.) in DCM (50 mL) was added dropwise over 15 min. After this the mixture was kept below -65 °C for 2 h while stirring, followed by dropwise addition of Et_3N (42 mL, 302 mmol, 12 eq.) over 10 min. The mixture was then warmed to -5 °C over 2 h and concentrated under reduced pressure.

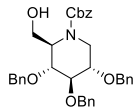


(2R,3R,4R,5S)-3,4,5-tris(benzyloxy)-2-((trityloxy)methyl)piperidine (35): Crude product aldehyde (1.0 eq.) was co-evaporated with toluene thrice after which it was dissolved in MeOH (500 mL). Subsequently NH_4HCO_2 (32 g, 504 mmol, 20 eq.) was added. The solution was then cooled to 0 °C and stirred for 20 min. Activated molecular sieves (250 g, 4 Å) were added and the mixture was stirred for another 20 min. Then $NaBH_3CN$ (6.3 g, 101 mmol, 4.0 eq.) was added. The mixture was cooled to 0 °C and stirred for 1 hour, after which the cooling was removed and the mixture was stirred for another 20 h. It was then filtered and concentrated under reduced pressure. The residue was dissolved in EtOAc (300 mL). The solution was then washed with aq., sat. $NaHCO_3$ (300 mL). The product was extracted from the water layer with EtOAc. Thereafter the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. At last the mixture was purified by silica gel column chromatography (11%→25%, EtOAc in pentane) giving product **35** (14 g, 21 mmol, 85 % over 2 steps). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.51 – 7.20 (m, 28H), 6.90 – 6.88 (m, 2H), 4.97 (d, *J* = 10.8 Hz, 1H), 4.81 – 4.67 (m, 4H), 4.22 (d, *J* = 10.8 Hz, 1H), 3.58 – 3.50 (m, 3H), 3.41 – 3.28 (m, 2H), 3.24 – 3.15 (m, 1H), 2.79 – 2.71 (m, 1H), 2.62 – 2.50 (m, 1H), 1.98 (br, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 143.79, 138.93, 138.67, 138.21, 128.83, 128.51, 128.47, 128.26, 128.11, 128.05, 127.92, 127.85, 127.76, 127.66, 127.58, 127.16, 87.51, 86.65, 75.89, 80.93, 80.38, 75.10, 72.90,

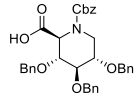
63.55, 60.41, 48.45; IR (neat, cm^{-1}): 3030, 2874, 1736, 1495, 1449, 1086, 1047, 731, 694; HRMS: calculated for $C_{46}H_{45}NO_4$ [M+H$^+$] 676.34214, found: 676.34168.



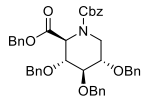
Benzyl (2R,3R,4R,5S)-3,4,5-tris(benzyloxy)-2-((trityloxy)methyl)piperidine-1-carboxylate (36): Product **35** (160 mg, 0.24 mmol, 1.0 eq.) was dissolved in 3mL THF, Et_3N (33 μL, 0.24 mmol, 1.0 eq.) and CbzCl (82 mg, 0.48 mmol, 2.0 eq.) were added into the solution, then the mixture was stirred at room temperature for 20 h. The reaction was diluted by EtOAc, washed by sat. aq. $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (5%→30%, EtOAc in pentane) giving in product **36** (140 mg, 0.17 mmol, 72%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.65 – 7.13(m, 35H), 5.20 (br, 2H), 4.87 (d, J = 11.1 Hz, 2H), 4.78 – 4.67 (m, 2H), 4.63 – 4.42 (m, 3H), 4.42 – 3.99 (m, 2H), 3.91 – 3.80 (m, 2H), 3.72 – 3.40 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 143.90, 141.13, 138.17, 138.05, 136.56, 128.96, 128.88, 128.68, 128.42, 128.31, 127.97, 127.92, 127.88, 127.82, 127.72, 127.67, 127.64, 127.51, 127.08, 126.94, 126.11, 86.70, 82.09, 78.31, 74.77, 73.90, 73.07, 70.53, 67.35, 65.18, 61.89, 56.42, 29.75; HRMS: calculated for $C_{54}H_{51}NO_6$ [M+H$^+$] 810.37891, found: 810.37956.



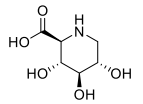
Benzyl (2R,3R,4R,5S)-3,4,5-tris(benzyloxy)-2-(hydroxymethyl)piperidine-1-carboxylate (37): Product **36** (140 mg, 0.17 mmol, 1.0 eq.) was dissolved in DCM/MeOH (2.0 mL, 1/1, v/v), followed by addition of *p*-toluenesulfonic acid monohydrate (6.6 mg, 0.035mmol, 0.20 eq.). After stirring overnight at room temperature, the reaction was quenched with Et_3N. The reaction was diluted by EtOAc, washed by brine. The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The mixture was then purified by silica gel column chromatography (10%→50%, EtOAc in pentane) to afford product **37** (80 mg, 0.14 mmol, 83%). $[\alpha]_D^{20}$ + 2.2 (c = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.37 – 7.24 (m, 20H), 5.14 (s, 2H), 4.86 – 4.58 (m, 5H), 4.53 (d, J = 11.8 Hz, 1H), 4.02 – 3.85 (m, 3H), 3.78 – 3.67 (m, 5H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 156.37, 138.13, 138.03, 136.38, 128.63, 128.56, 128.52, 128.49, 128.20, 128.06, 128.03, 127.92, 127.88, 127.80, 77.53, 75.52, 73.37, 71.35, 67.63, 61.48; HRMS: calculated for $C_{35}H_{37}NO_6$ [M+H$^+$] 568.26936, found: 568.26952.



(2S,3R,4R,5S)-3,4,5-tris(benzyloxy)-1-((benzyloxy)carbonyl)piperidine-2-carboxylic acid (38): The primary alcohol compound **37** (80 mg, 0.14 mmol, 1.0 eq.) was dissolved in DCM/H_2O (1.5 mL, 2/1, v/v), then BAIB (112.7 mg, 0.35 mmol, 2.5 eq.) and TEMPO (4.4 mg, 0.028 mmol, 0.20 eq.) were added in the solution, the resulting mixture was stirred for 5 h at room temperature. The reaction was quenched with 10% $Na_2S_2O_3$, was extracted by EtOAc and washed by brine. The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The mixture was then purified by silica gel column chromatography (25%→50%, EtOAc in pentane with 1% AcOH) to afford product **38** (58 mg, 0.10 mmol, 71%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 10.85 (br, 1H), 7.44 – 7.21 (m, 20H), 5.47 – 5.07 (m, 3H), 4.93 – 4.51 (m, 5H), 4.46 – 4.14 (m, 3H), 3.90 – 3.56 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 174.90, 174.74, 156.91, 156.17, 138.06, 137.58, 137.38, 136.29, 128.91, 128.67, 128.41, 128.38, 128.26, 128.00, 127.90, 127.77, 127.71, 127.39, 126.09, 77.48, 77.16, 76.84, 74.17, 73.75, 73.38, 71.64, 71.51, 70.72, 67.71, 55.95, 55.05, 40.74, 39.97; HRMS: calculated for $C_{35}H_{35}NO_7$ [M+H$^+$] 582.24863, found: 582.24883.



Dibenzyl (2S,3R,4R,5S)-3,4,5-tris(benzyloxy)piperidine-1,2-dicarboxylate: Product **38** (180 mg, 0.32 mmol, 1.0 eq.) was dissolved in DCM/H_2O (3.0 mL, 2/1, v/v) and cooled to 0 °C. TEMPO (10 mg, 0.064 mmol, 0.20 eq.) and BAIB (255 mg, 0.79 mmol, 2.5 eq.) were added at 0 °C. After 6 h stirring, the reaction mixture was quenched by 10% aq. $Na_2S_2O_3$ solution and extracted by DCM, being successively washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was co-evaporated twice with toluene. To a stirred solution of the residual syrup and benzyl bromide (76 µL, 0.63 mmol, 2.0 eq.) in dry DMF (5.0 mL) was added cesium carbonate (103 mg, 0.32 mmol, 1.0 eq.) at room temperature. The mixture was stirred for 3 h, and poured into water, before the mixture was extracted with Et_2O. The extract was washed by water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10 % → 30 %, EtOAc in pentane) to give Bn protected **38** (160 mg, 0.24 mmol, 75% over two steps). $[\alpha]_D^{20}$ +19 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, DMSO-d_6): δ ppm 7.34 – 7.22 (m, 25H), 5.14 – 5.07 (m, 3H), 4.99 – 4.95 (m, 2H), 4.62 – 4.47 (m, 6H), 4.25 – 4.24 (m, 1H), 4.03 – 3.99 (m, 1H), 3.87 – 3.85 (m, 1H), 3.76 – 3.72 (m, 1H), 3.64 – 3.60 (m, 1H), 3.09 (s, 1H); ^{13}C-NMR (100 MHz, DMSO-d_6): δ ppm 168.46, 155.21, 138.01, 137.44, 136.24, 135.07, 128.20, 128.04, 127.87, 127.81, 127.71, 127.63, 127.57, 127.54, 127.28, 127.20, 127.12, 127.06, 126.94, 126.90, 126.73, 74.51, 73.45, 70.89, 70.56, 69.90, 66.21, 66.03, 55.94, 40.26.



(2S,3R,4R,5S)-3,4,5-trihydroxypiperidine-2-carboxylic acid (12): A mixture of Product Bn-protected **38** (160 mg, 0.24 mmol, 1.0 eq.) and $Pd(OH)_2$/C (50 mg, 20% wt. loading(dry basis)) in 80% AcOH in H_2O (5.0 mL) was stirred at room temperature under a hydrogen atmosphere for 24 h. The catalyst was then filtered off and washed with MeOH. The filtrate and washings were combined and concentrated under reduced pressure to give semi-crystalline solid. Recrystallization from water ethanol gave final product **12** (24 mg, 0.14 mmol, 57%) as crystalline solid.^{1}H-NMR (400 MHz, D_2O): δ ppm 3.75 – 3.67 (m, 1H), 3.62 (t, *J* = 9.6 Hz, 1H), 3.47 (t, *J* = 9.0 Hz, 1H), 3.41 – 3.34 (m, 2H), 2.81 (t, *J* = 11.8 Hz, 1H), 1.88 (s, 1H); ^{13}C-NMR (100 MHz, D_2O): δ ppm 173.10, 76.05, 70.77, 67.73, 61.71, 45.70; HRMS: calculated for $C_6H_{11}NO_5$ [M+H$^+$] 178.07100, found: 178.07105.

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