Cyclophellitol analogues for profiling of exo- and endo-glycosidases

Schröder, S.P.

Chapter 8

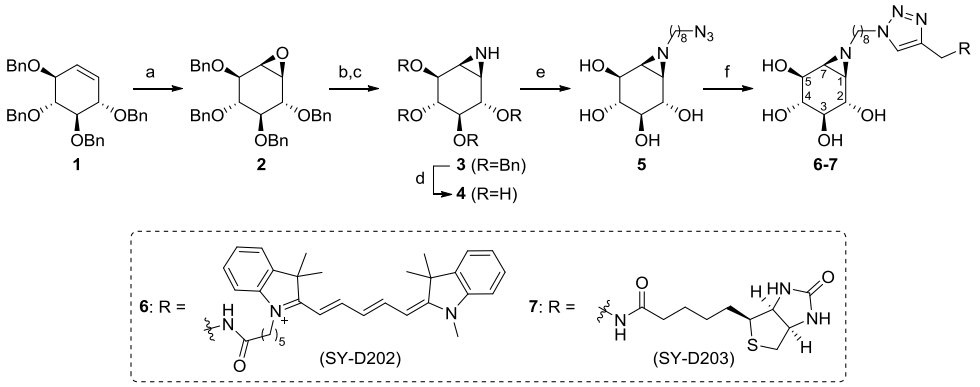
Conclusions and future prospects

Cyclophellitol and cyclophellitol aziridine are potent, selective and irreversible mechanism-based inhibitors of retaining β-glucosidases.[1,2] Cyclophellitol aziridine equipped with a reporter tag allows facile visualization and identification of active β-glucosidases in complex biological samples with high sensitivity.[3] Configurational isomers of cyclophellitol aziridine show high selectivity towards their target glycosidases, which normally processes substrates with matching configurations.[4–7] The work described in this Thesis entails modification of the cyclophellitol core in such a way that glycosidases processing different configurational sugar substrates are targeted simultaneously – thus to create broad-spectrum activity-based glycosidase probes. Additionally, the first synthetic examples of multimeric cyclophellitol aziridine activity-based probes are described. These tools enable the study of *endo*-glycosidases; a field yet unexplored by cyclophellitol-based probes.

1| **Chapter 1** provides a global overview of glycosidase functions in organisms, as well as their mechanistic aspects of catalytic glycoside hydrolysis. The basic principles of activity-based protein profiling are described, as well as the mechanism of inactivation of glycosidases by cyclitol epoxides and aziridines.

2| A new route towards D-*xylo*-cyclophellitols is described in **Chapter 2**. The key step in this synthesis route involves an asymmetric Brown allylation reaction, which following a series of chemical transformations afforded α- and β-configured D-*xylo*-cyclophellitol epoxides and aziridines. The β-aziridine was equipped with different fluorescent tags, and upon incubation with mouse liver lysates, the probe labeled β-glucosylcerebrosidase (GBA) 1 and 2. Pull-down analysis with the biotinylated probe also identified GBA1 and GBA2 in mouse liver, as well as GBA1 in mouse brain and duodenum. In addition, the D-*xylo*-cyclophellitol aziridine probes proved highly potent activity-based inactivators of GH52 β-xylosidase from *Opitutus terrae*.

It would be of interest to further investigate the influence of the substituent at C5 of cyclitols on the inhibition of glycosidases. For example, D-*xylo*-cyclophellitol aziridine equipped with a fluorescent tag was shown to label GBA1 and GBA2 in mouse liver lysate, in line with cyclophellitol aziridine probes reported previously.[3] In contrast,

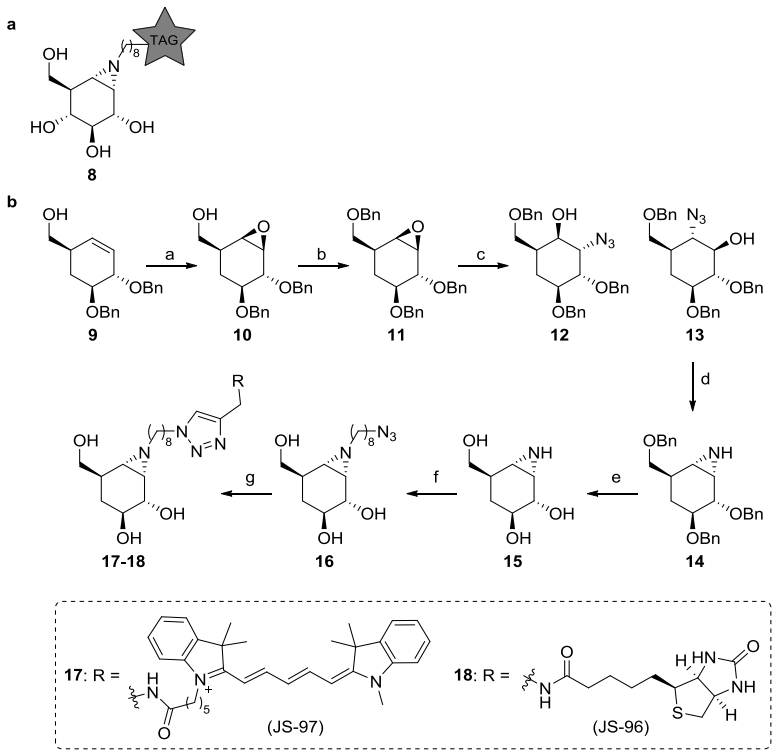


Scheme 1 Synthesis of conduritol B aziridine activity-based probes **6-7**. Reagents and conditions: a) oxone, CF_3COCH_3, $NaHCO_3$, EDTA, H_2O, MeCN, 0 °C, 87%; b) NaN_3, $LiClO_4$, DMF, 100 °C, 16h; c) polymer-bound PPh_3, MeCN, 60 °C, 33% over two steps; d) Li, NH_3, THF, -60 °C, 1h, 99%; e) 1-azido-8-iodooctane, K_2CO_3, DMF, 80 °C, 16h, 40%; f) tag-alkyne, $CuSO_4$, Na-ascorbate, DMF, yield **6**: 40%; yield **7**: 37%.

conduritol B aziridine (which possesses an equatorial hydroxyl group at C5), *N*-alkylated with various alkyl moieties, appeared highly specific for GBA1 over GBA2.[8] To further investigate the influence of the substituent at C5 in cyclitols towards the labeling of glycosidases, conduritol B aziridines **6**-**7** equipped with a reporter tag were synthesized (Scheme 1). The synthesis commenced with epoxidation of cyclohexene **1**[9] to afford **2**, which was converted into aziridine **3** by azidolysis followed by Staudinger-type ring closure. The benzyl protecting groups were removed under Birch conditions to afford conduritol B aziridine **4**, which was alkylated with an azidooctyl spacer to give **5**. Click ligation with the appropriate alkynes afforded conduritol B aziridine ABPs **6** and **7** (see the experimental section for synthetic procedures and compound characterization). Due to its C_2-symmetry axis, conduritol B epoxide is a potent inactivator of both α- and β-glucosidases.[10] Therefore, conduritol B aziridine ABPs **6** and **7** could potentially also serve as broad-spectrum α/β-glucosidase probes.

Chapter 3 describes the synthesis of two deoxygenated analogues (4-deoxy and 2,4-deoxy) of cyclophellitol aziridine. The 4-deoxygenated analogue was synthesized by deoxygenation of a common precursor in the synthesis of cyclophellitol aziridine. The synthesis of the 2,4-deoxy analogue required a different synthetic route, starting from an achiral synthon which was subjected to an iridium catalyzed asymmetric allylic alkylation followed by an asymmetric Brown allylation. It was envisioned that deoxygenation would reduce selectivity of the probe towards glycosidase active-sites, thereby enabling broad-spectrum glycosidase profiling. More specifically, the substrate configuration of OH-4 distinguishes its specificity in recognition by glucosidases and galactosidases, and OH-2 between glucosidases and mannosidases. Indeed, the 4-deoxygenated cyclophellitol aziridine probe enabled inter-class fluorescent labeling of β-glucosidases and β-galactosidases, as well as their identification by proteomics. Concomitant deoxygenation at C2 and C4 severely reduced the potency of the probe towards glycosidase labeling, however it appeared that the probe is selective for β-galactocerebrosidase.

In some initial studies aimed to establish whether a similar broad-spectrum activity could be obtained with deoxygenated analogues of *epi*-cyclophellitol aziridine **8**[4] (Scheme 2a), 4-deoxy *epi*-cyclophellitol aziridine probes were synthesized. Cyclohexene **9** (Chapter 3) was diastereoselectively epoxidized with *m*-CPBA to afford epoxide **10** (Scheme 2b). Benzylation of the primary alcohol gave **11**, which was then



Scheme 2 Synthesis of 4-deoxy *epi*-cyclophellitol aziridine probes. Reagents and conditions: a) *m*-CPBA, DCM, 0 °C, 91%; b) BnBr, NaH, TBAI, DMF, 79%; c) NaN_3, $LiClO_4$, DMF, 80 °C, 48h, ratio **12**:**13** 1:1.3, 83%; d) polymer-bound PPh_3, MeCN, 90 °C, 73%; e) Li, THF, NH_3, -60 °C, quant.; f) 8-azido-1-iodooctane, K_2CO_3, DMF, 100 °C, 55%; g) tag-alkyne, $CuSO_4$, Na-ascorbate, DMF, yield **17**: 44%; yield **18**: 56%.

reacted with sodium azide to provide a mixture of azidoalcohols **12** and **13**. Compound **13** was then subjected to Staudinger-type ring closure to afford aziridine **14**. Debenzylation under Birch conditions (**15**), followed by alkylation gave alkyl aziridine **16** which could be click-ligated with different reporter tags to afford 4-deoxy α-ABPs **17**-**18** (see the experimental section for synthetic procedures and compound characterization). While specific α-glucosidase ABP **8** positively identified the lysosomal acid α-glucosidase (GAA) in mouse kidney, liver and human fibroblast lysates,[4] deoxygenation at C4 totally abolished labeling of glycosidases (Figure 1).

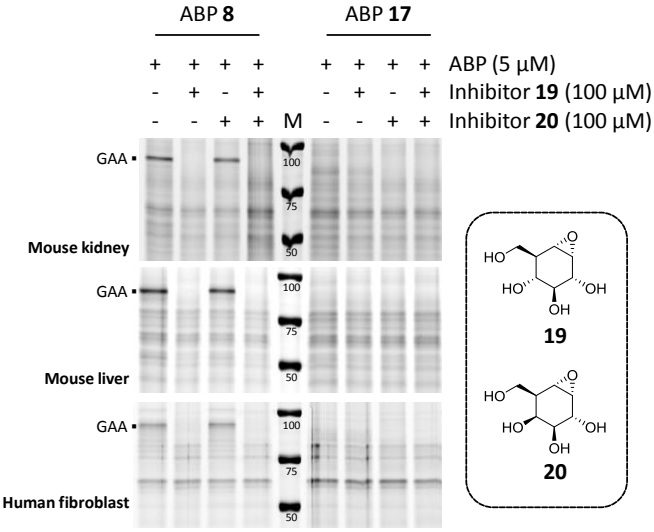
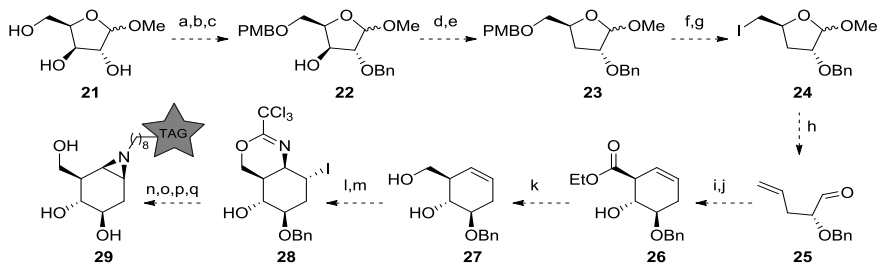


Figure 1 Labelling of mouse kidney, liver and human fibroblast lysates with *epi*-cyclophellitol aziridine probe **8** (left panel) and 4-deoxy *epi*-cyclophellitol aziridine **17** (right panel). Selective probe **8** labels GAA in all lysates and labelling can be competed with selective inhibitor **19** (and not **20**), indicating that labelling is activity-based. The 4-deoxy compound **17** does not significantly label any bands in these lysates in activity-based manner.

Alternatively, it would be of interest to develop a probe that is capable of simultaneously labeling β-glucosidases and β-mannosidases. For this purpose, C2 deoxygenated cyclophellitol aziridine probes could be synthesized (Scheme 3). Starting from methyl D-xylofuranose **21**, the 4,6-diol could be regioselectively protected as the *p*-anisylidene acetal. The remaining alcohol could be benzylated, and reductive acetal cleavage using borane and Bu_2BOTf at low temperature[11] could afford the 6-*O*-*p*-methoxybenzyl (PMB) ether **22**. The hydroxyl group could then be removed by tosylation and hydride reduction to give **23**. Acid catalyzed PMB cleavage using HCl in hexafluoroisopropanol (HFIP)[12] followed by an Appel iodination could afford compound **24**. Zinc-mediated Vasella fragmentation could give aldehyde **25**, which could be subjected to an indium catalyzed Barbier allylation[13] followed by Grubbs metathesis to afford ester **26**. Reduction of the ester could afford **27**, which is then reacted with trichloroacetonitrile and subsequently treated with *N*-iodosuccinimide to afford cyclic imidate **28**. Acidic hydrolysis followed by basification could then afford the aziridine, which is globally debenzylated under Birch conditions followed by



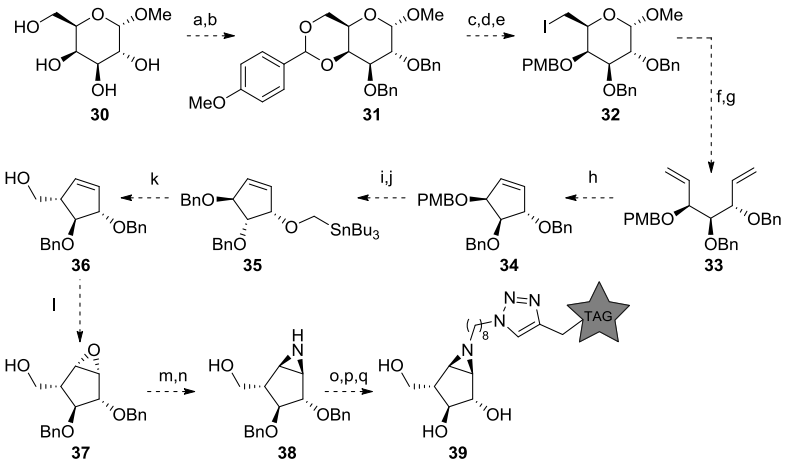
Scheme 3 Proposed synthetic scheme towards C-2 deoxy-ABPs **29**. Reagents and conditions: a) anisaldehyde dimethyl acetal, *p*-TsOH, DMF, 60 °C; b) BnBr, NaH, TBAI, DMF; c) BH_3, Bu_2BOTf, THF, DCM, -78 °C; d) TsCl, pyridine; e) $LiAlH_4$, THF, reflux; f) HCl, HFIP, DCM; g) PPh_3, I_2, THF, reflux; h) Zn, THF, H_2O, sonication; i) 4-ethylbromocrotonate, In, $La(OTf)_3$, H_2O; j) Grubbs II, DCM, reflux; k) DIBAL-H, $NaBH_4$, THF; l) CCl_3CN, DBU, DCM; m) NIS, $CHCl_3$; n) HCl, MeOH, then Et_3N; o) Li, NH_3; p) 1-azido-8-iodooctane, K_2CO_3, DMF, 100 °C; q) tag-alkyne, $CuSO_4$, Na-ascorbate, DMF.

alkylation with a click handle and click-ligation with reporter tags to finally afford 2-deoxy aziridines **29**.

Chapter 4 describes the synthesis of β-D-*lyxofuranosyl* and β-D-*arabinofuranosyl*-cyclophellitol aziridine probes. The ability of these probes to fluorescently label glycosidases in unbiased fashion was evaluated in human fibroblast and mouse liver lysates. Activity-based fluorescent labeling of glycosidases could not be detected for both probes, and pull-down analysis with the biotinylated probes did not identify glycosidases in various biological samples. It was therefore concluded that none of the biological samples (human or mouse) contained glycosidases that accepted the D-furanosyl configured probes as substrate.

In contrast to β-D-arabinofuranosides, α-L-arabinofuranosides are common components in hemicellulosic biomass. The β-1,4-xylan backbone in hemicellulose is randomly substituted at O2, O3 or both by α-L-arabinofuranosides. These side-groups impede rapid processing of hemicellulose by *endo*-xylanases and *exo*-xylosidases, retarding the hydrolysis reaction in industrial applications (such as the production of D-xylose for the production of xylitol or ethanol). A probe that would rapidly detect α-L-arabinofuranosidase activity with high sensitivity could be of interest for the identification and development of α-L-arabinofuranosidases with high substrate turnover rate. For this purpose, an α-L-*arabino*-configured cyclophellitol aziridine probe would be of interest. The synthesis of this probe may start from commercially

available methyl α-D-galactopyranosidase **30** (Scheme 4), which is then regioselectively 4,6-*O* protected as the *p*-anisylidene acetal, followed by benzylation of the remaining alcohols to afford globally protected **31**. The acetal may then be reductively cleaved using borane and Bu_2BOTf to the 4-*O*-PMB ether,[11] followed by tosylation and nucleophilic displacement with iodine at O-6 to afford orthogonally protected iodopyranoside **32**. Zinc-mediated Vasella fragmentation followed by Wittig olefination using triphenylphosphonium methylide would afford diene **33**, which is subsequently ring-closed using Grubbs catalyst to give **34**. The PMB-group may be selectively deprotected using HCl in HFIP,[12] and the liberated alcohol condensated with tributyl-iodomethylstannane to afford **35**. Hydroxymethylation via a [2,3]-Wittig-Still rearrangement[14,15] would subsequently afford alcohol **36**. Stereoselective α-epoxidation using *m*-CPBA would then afford **37**, which is subsequently transformed into aziridine **38** by azidolysis followed by Staudinger-type ring closure. Global deprotection under Birch conditions followed by alkylation with 1-azido-8-iodooctane and subsequent click ligation with an appropriate reporter tag may finally afford α-L-*arabino*-cyclophellitol aziridine probes **39**.

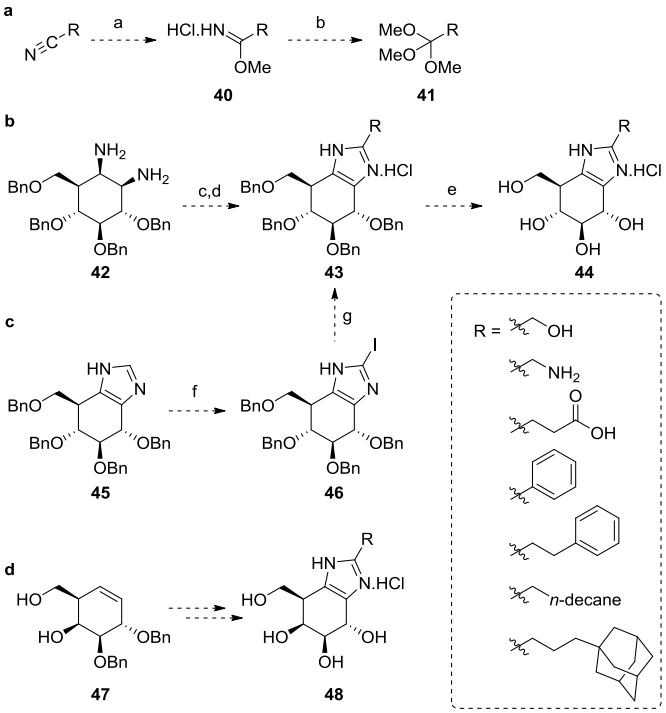


Scheme 4 Proposed synthetic scheme for the preparation of α-L-*arabino*-cyclophellitol aziridine probes **39**. Reagents and conditions: a) anisaldehyde dimethyl acetal, *p*-TsOH, DMF, 60 °C; b) BnBr, NaH, TBAI, DMF; c) BH_3, Bu_2BOTf, THF, DCM, 0 °C; d) TsCl, pyridine; e) NaI, DMF, 80 °C; f) Zn, EtOH, reflux; g) CH_3PPh_3Br, *n*-BuLi, THF, -40 °C; h) Grubbs II, DCM, reflux; i) HCl, HFIP, DCM; j) Bu_3SnCH_2I, KH, dibenzo-18-crown-6, THF, 0 °C; k) *n*-BuLi, THF, -78 °C; l) *m*-CPBA, DCM; m) NaN_3, $LiClO_4$, DMF, 100 °C; n) polymer-bound triphenylphosphine, MeCN, 80 °C; o) Li, NH_3, THF, -60 °C; p) 8-azido-1-iodooctane, K_2CO_3, DMF, 100 °C; q) alkyne-tag, $CuSO_4$, Na-ascorbate, DMF.

5| Gluco-azoles competitively inhibit glucosidases by transition-state mimicry and their ability to interact with catalytic acid residues in glucosidase active sites.[16] However, none of such azole-type inhibitors described to date possess a protic nitrogen characteristic for 1*H*-imidazoles. **Chapter 5** describes the synthesis and biochemical evaluation of gluco-1*H*-imidazole, a gluco-azole bearing a 1*H*-imidazole fused to a glucopyranose-configured cyclitol core, as well as three close analogues as novel glycosidase inhibitors. While both compounds exhibit high structural similarity and binding modes to β-glucosidases from *Thermotoga maritima* and *Thermoanaerobacterium xylanolyticum* (as shown by X-ray crystallography), it was found that unsubstituted gluco-1*H*-imidazole displayed reduced activity towards β-glucosidases, in comparison to the classical glucoimidazole. It can be postulated that the activity of gluco-1*H*-imidazole is lower due to reduced interaction of the 'glycosidic' nitrogen with the glucosidase catalytic acid, caused by prototropic tautomerization of the imidazole proton. Additionally, DFT calculations showed that protonation of glucoimidazole causes δ+ charge development at the 'anomeric' centre, ideally located for interaction with the glucosidase catalytic nucleophile. In gluco-1*H*-imidazole, protonation of the azole causes δ+ character on the 'apical' carbon of the azole ring, poorly placed for interaction with the catalytic nucleophile. In contrast, gluco-1*H*-imidazoles equipped with an *n*-butyl moiety are effective inhibitors of human GBA1, inhibiting this enzyme (deficient in Gaucher disease) in the nanomolar range. Indeed, this positive effect of a hydrophobic moiety at the 'aglycon' subsite was also observed for the classical glucoimidazoles by Vasella *et al*.[17] For example, glucoimidazole substituted with hydroxymethylene or ethylphenyl moieties at position C2 of the imidazole displayed activity in the low nanomolar range.

It would be of interest to derivatize gluco-1*H*-imidazole with different aliphatic moieties (such as hydromethyl, ethylphenyl, adamantyl or *n*-decyl) on the imidazole ring and study its effect on inhibitory activity. For this purpose, diamine **42** (Chapter 5) may be condensed with appropriate trimethyl orthoesters **41** (synthesized in two steps from the matching nitrile using methanolic HCl;[18] Scheme 5a) to afford substituted imidazoles **43**, which would afford inhibitors **44** after palladium-catalyzed debenzylation (Scheme 5b). Alternatively, 1*H*-imidazole **45** may be treated with *N*-iodosuccinimide to afford iodoazole **46**, which may be coupled with suitable organotin moieties under palladium catalysis[19,20] to afford substituted imidazoles **43**.

Moreover, the synthetic procedure described in Chapter 5 for the construction of gluco-1*H*-imidazoles could be adapted to other relevant cyclohexene scaffolds (Scheme 5 d). For example, *galacto*- configured cyclohexene **47**[21] could be utilized for the synthesis of galacto-1*H*-imidazoles **48**, which are potential inhibitors of β-galactosidases. Deficiency of active acid β-galactosidase (GLB1) causes two different lysosomal storage diseases (LSDs), namely G_{M1}-gangliosidosis[22] and Morquio B disease.[23] Chaperone therapy with small molecule inhibitors is reported to be promising treatment of these LSDs,[24] and galacto-1*H*-imidazoles **48** could be investigated for this purpose as well.



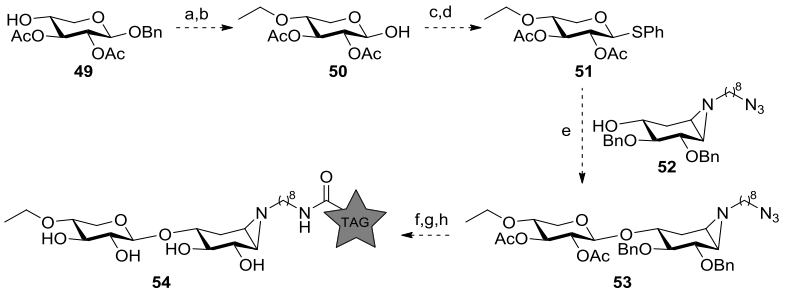
Scheme 5 (a) General synthesis of trimethyl orthoesters from terminal nitriles. (b) Proposed synthetic scheme towards substituted gluco-1*H*-imidazoles by condensation with trimethyl orthoesters. (c) Proposed synthetic scheme towards substituted gluco-1*H*-imidazoles by azole iodination and palladium catalyzed organotin coupling. Reagents and conditions: a) HCl, MeOH, (^{i}Pr)$_2$O, -20 °C; b) MeOH, Et$_2$O, reflux; c) (MeO)$_3$R, HFIP; d) (COCl)$_2$, DMSO, DCM, -60 °C; e) Pd(OH)$_2$/C, H$_2$, HCl, dioxane, MeOH, H$_2$O; f) NIS, CHCl$_3$; g) SnR$_4$, Pd(PPh$_3$)$_4$, DMF, 95 °C. (d) Galacto-1*H*-imidazoles **48** could be prepared from cyclohexene **47**[21] following the synthetic procedures reported in Chapter 5.

6| **Chapter 6** describes the synthesis of two spiro-epoxyglycosides designed to irreversibly inhibit GH99 *endo*-α-mannosidases (containing either a gluco- or mannopyranosyl residue at the non-reducing end). These enzymes are postulated to employ an unusual reaction trajectory, following through deprotonation of OH-2 to form an 1,2-anhydro epoxide transition state which is subsequently hydrolyzed by water.[25] The spiro-epoxyglycosides described contain a spiro-epoxide warhead at C-2, which was installed by a Corey-Chaykovsky spiro-epoxidation reaction. It was shown that the spiro-epoxyglycosides are able to label recombinant GH99 *endo*-α-mannosidases in concentration-, pH- and time-dependent fashion, and that labeling could be competed with the matching non-tagged inhibitors, as well as the enzyme natural substrate. Additionally, labeling of the enzyme totally abrogated the enzyme's native de-mannosylating ability. In order to unequivocally prove the postulated unusual reaction mechanism of GH99 *endo*-α-mannosidases, it would be of interest to obtain a crystal structure of the spiro-epoxyglycosides bound in the active site. Nucleophilic opening of the spiro-epoxide warhead by the putative nucleophile, together with hydrogen bonding of the putative acid/base with the aglycon would prove the suggested mechanism. Alternatively, incubation of the GH99 enzyme with the spiro-epoxyglycosides, followed by proteolysis and LC-MS/MS analysis could identify the labeled peptide fragment, and further fragmentation of this peptide could unveil the labeled nucleophile. Previously, this technique successfully identified the catalytic nucleophile in several glycosidases.[26–29]

7| **Chapter 7** describes the synthesis of *xylobiose*-cyclophellitol aziridine probes. The results described in this Chapter provide the first example that monomeric *xylo*-cyclophellitol epoxides and aziridines can readily be glycosylated with an appropriate thioglycoside donor under NIS/TMSOTf catalysis to afford 'disaccharide' cyclitol epoxides and aziridines in good yields. This finding invites the synthesis of multimeric cyclitol probes for the profiling of *endo*-glycosidases. This is exemplified by the synthesized *xylobiose*-cyclophellitol aziridine probe, which was able to visualize *endo*-xylanase activity in the secretome of industrially relevant fungus *Aspergillus niger* and profile the pH and temperature tolerance of the enzyme.

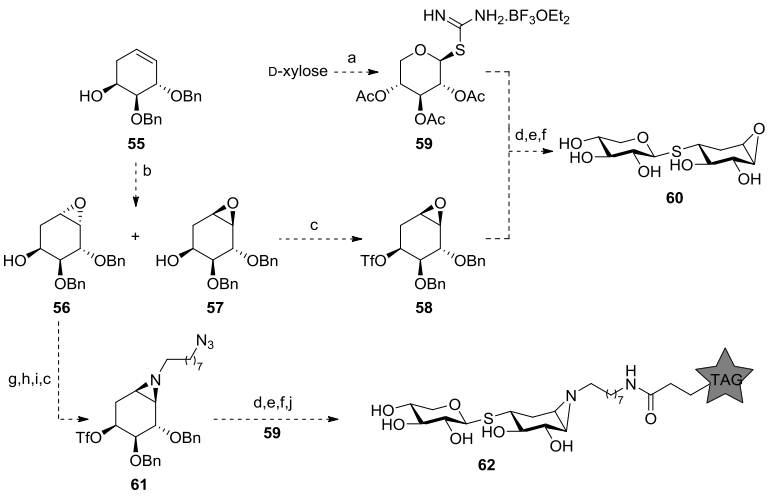
A drawback of the glycosylated *endo*-xylanase probe is that it is susceptible to hydrolysis by *exo*-xylosidases present in the complex biological sample. However, it was shown that this can be prevented by pre-incubation of the sample with a specific irreversible *exo*-xylosidase inhibitor. Alternatively, probes and covalent inhibitors that

are resistant towards *exo*-xylosidase cleavage would be of interest. Fur such purpose, 'capping' O-4 of the non-reducing pyranoside with an ether (e.g. ethoxy) group could prevent *exo*-glycosidase processing (Scheme 6). For example, compound **49**[30] could be alkylated using ethyl triflate, followed by palladium catalyzed debenzylation to afford **50**. Acetylation and reaction with thiophenol under Lewis acidic conditions affords donor **51**, which may be coupled with aziridine acceptor **52** to afford **53**. Deprotection and amide coupling then affords O-4 capped *xylobiose*-cyclophellitol aziridine **54**.



Scheme 6 Proposed synthetic scheme towards O-4 'capped' *xylobiose*-cyclophellitol aziridine **54**, which is anticipated to be resistant to hydrolysis by *exo*-glycosidases. Reagents and conditions: a) ethyl triflate, Et_3N, DCM; b) Pd/C, H_2, THF; c) Ac_2O, pyridine; d) PhSH, $BF_3{\cdot}OEt_2$, DCM; e) NIS, TMSOTf, DCM, -40 °C; f) NaOMe, MeOH, DCM; g) Na, ^tBuOH, NH_3, -60 °C; h) tag-OSu, DIPEA, DMF.

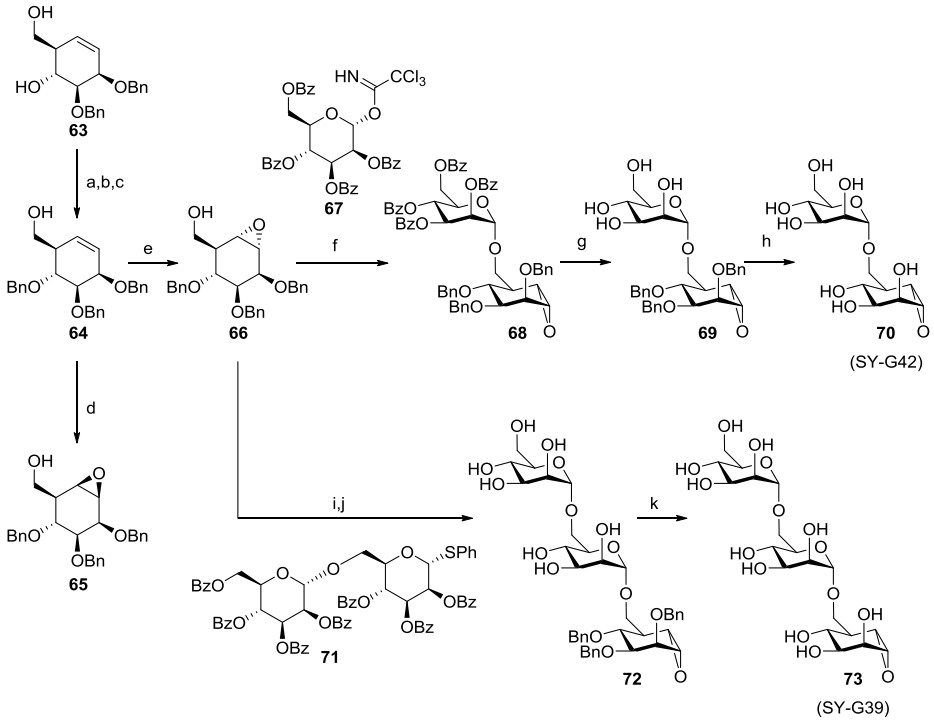
An alternative strategy would be the replacement of the hydrolysable glycosidic linkage by a thioglycosidic linkage to yield stabilized multimeric *endo*-probes. Thioglycosidic linkages are generally resistant to glycosidic processing due to the lower basicity of sulphur compared to oxygen, thereby hampering protonation of the aglycon by the enzyme acid/base residue during catalysis.[31,32] For example, 4-deoxy-4-thio-*xylobiose*-cyclophellitol **60** might be available from the coupling of triflate **58** and isothiouronium donor **59** (Scheme 7).[33,34] Donor **59** is available from D-xylose in two steps by acetylation and substitution by thiourea.[35] Triflate acceptor **58** may be obtained by epoxidation of cyclohexene **55** (Chapter 2), followed by triflation of the alcohol in β-epoxide **57**. The thioglycosidic linkage between **58** and **59** may then be installed by treatment with base, and Zemplén deacetylation followed by debenzylation under Birch conditions would afford thioglycosidic *xylobiose*-cyclophellitol **60**. The synthesis of thioglycosidic *xylobiose*-cyclophellitol aziridine **62** would follow the same strategy, by coupling triflate **61** with isothiouronium donor **59** followed by deprotection and amide coupling with appropriate succinimidyl esters.



Scheme 7 Proposed synthetic scheme towards stabilized thioglycosidic *xylobiose*-cyclophellitol **60** and fluorescent aziridine **62**. Reagents and conditions: a) 1. Ac_2O, $BF_3.OEt_2$; 2. thiourea, MeCN, 70 °C; b) Oxone, CH_3COCF_3, $NaHCO_3$, EDTA, MeCN, H_2O; c) Tf_2O, pyridine, DCM, -20 °C; d) Et_3N, MeCN; e) NaOMe, MeOH; f) Li, NH_3; g) NaN_3, $LiClO_4$, DMF, 100 °C; h) polymer-bound PPh_3, MeCN, 80 °C; i) 8-azido-1-trifluoromethanesulfonyloctane, DIPEA, $CHCl_3$; j) tag-OSu, DIPEA, DMF.

Glycoside hydrolase family GH76 *endo*-α-1,6-mannosidases are found in bacteria and fungi. In bacteria such as *Bacteroides thetaiotaomicron*, a member of the human gut microbiota, these enzymes facilitate the breakdown of large 'high mannose' glycoproteins that make up the outer cell wall of yeasts and filamentous fungi.[36,37] In contrast, GH76 *endo*-α-1,6-mannosidases in fungi display transglycosylation activity, building up the extracellular α-mannan layer.[38] GH76 *endo*-α-1,6-mannosidase gene knockouts in certain fungi impair cell growth and can induce cell death.[39,40] Therefore, the development of (irreversible) GH76 *endo*-α-1,6-mannosidase inhibitors are of interest. For this purpose, two multimeric α-1,6-*manno-epi*-cyclophellitols have been synthesized (Scheme 8). Starting from diol **63**[41], the secondary alcohol was protected by a three-step procedure involving protection of the primary alcohol with TBS, benzylation of the secondary alcohol and desilylation to afford **64**. The olefin could be stereoselectively epoxidized by choice of reagent; using *m*-CPBA β-epoxide **65** was selectively obtained, and in turn α-epoxide **66** was obtained by treatment with *in situ* generated (trifluoromethyl)methyldioxirane. Epoxide **66** was subsequently coupled with trichloroimidate donor **67**[42] to afford 'disaccharide' **68**. Zemplén deacetylation afforded **69**, which was then debenzylated using Pearlman's catalyst to afford α-1,6-

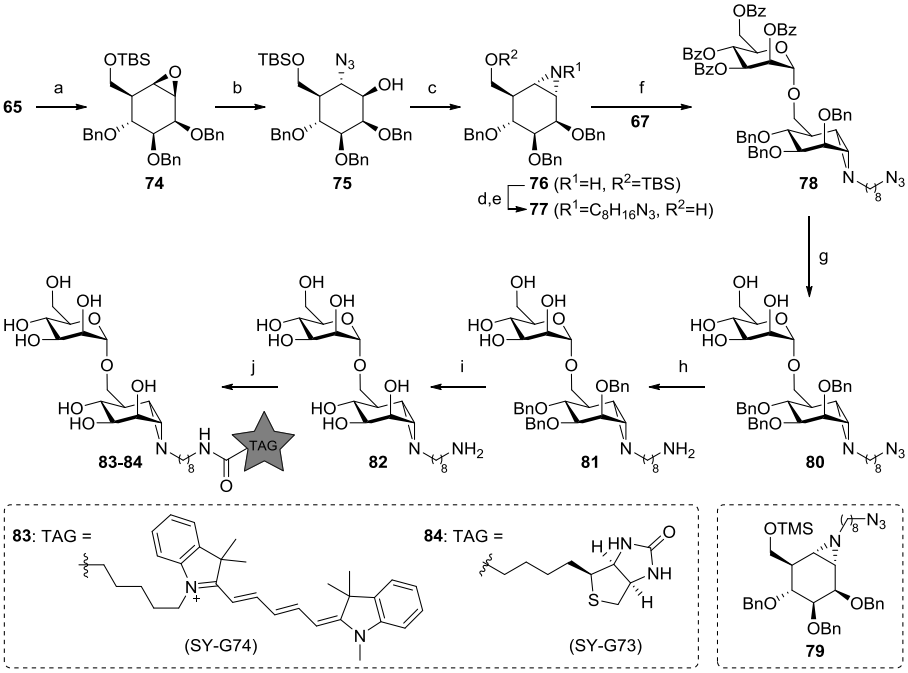
mannobiose-epi-cyclophellitol **70**. Similarly, epoxide **66** was coupled to disaccharide donor **71**[43] followed by deacetylation to afford **72** in low yield, which gave α-1,6-*mannotriose-epi*-cyclophellitol **73** after debenzylation (see the experimental section for synthetic procedures and compound characterization).



Scheme 8 Synthesis of α-1,6-*mannotriose-epi*-cyclophellitols **70** and **73**. Reagents and conditions: a) TBSCl, imidazole, DMF; b) BnBr, NaH, TBAI, DMF; c) TBAF, THF, 89% over three steps; d) *m*-CPBA, DCM , 0 °C, 84%; e) Oxone, CH_3COCF_3, $NaHCO_3$, EDTA, H_2O, MeCN, 0 °C, 89%; f) TMSOTf, DCM, -40 °C, 65%; g) NaOMe, MeOH, DCM, 81%; h) $Pd(OH)_2/C$, H_2, dioxane, MeOH, H_2O, 2h, quant; i) NIS, TMSOTf, DCM, -40 °C; j) NaOMe, MeOH, DCM, 14% over two steps; k) $Pd(OH)_2/C$, H_2, dioxane, MeOH, H_2O, 2h, quant.

In order to enable further studies on the GH76 *endo*-α-1,6-mannosidase, a set of tagged aziridines was synthesized (Scheme 9). Protection of the primary alcohol in epoxide **65** (Scheme 8) gave **74**, which was subjected to azidolysis to afford azidoalcohol **75**. Staudinger-type ring closure afforded aziridine **76**, which was alkylated and desilylated to afford acceptor **77**. Coupling with donor **67**[42] under Lewis

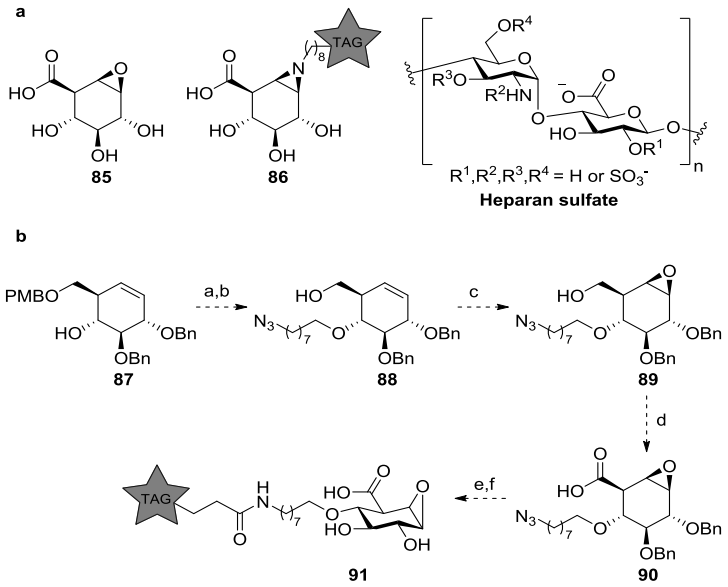
acidic conditions, using a small excess of triflic anhydride afforded 'disaccharide' **78** (of note: when TMSOTf was used as Lewis acid, only silylated acceptor **79** was formed in 78% isolated yield). Deacetylation (**80**) followed by Staudinger reduction (**81**) and debenzylation under Birch conditions afforded **82**, which was ligated with different reporter tags to afford α-*mannobiose-epi*-cyclophellitol aziridines **83** and **84** (see the experimental section for synthetic procedures and compound characterization).



Scheme 9 Synthesis of α-1,6-*mannobiose-epi*-cyclophellitol aziridines **83** and **84**. Reagents and conditions: a) TBSCl, imidazole, THF, rt, 4h, 93%; b) NaN_3, $LiClO_4$, DMF, 80 °C, 16h, 49%; c) polymer-bound PPh_3, MeCN, 90 °C, 16h, 85%; d) 8-azidooctyl trifluoromethanesulfonate, DIPEA, $CHCl_3$, 3h; e) TBAF, THF, 88% over two steps; f) imidate donor **67** (Scheme 8), TfOH, DCM, -40 °C, 74%; g) NaOMe, MeOH, DCM, 79%; h) polymer-bound PPh_3, H_2O, MeCN, 70 °C, 16h, 96%; i) Li, THF, NH_3, -60 °C, 1h, 81%; j) tag-OSu, DIPEA, DMF, 80: 29%; 81: 52%.

Recently, the development of a set of β-glucuronic acid configured cyclophellitols was reported (Scheme 10a).[6] Epoxide **85** was shown to be a potent inactivator of exo-acting β-glucuronidase (GUSB) from *Acidobacterium capsulatum* and its aziridine analogues displayed even higher potencies. Probe **86** equipped with a Cy5 fluorescent tag was shown to effectively label GUSB in human spleen lysates. Additionally, probe **86** also targets endo-acting heparanase (HPSE), as well as its inactive pro-enzyme proHPSE. HPSE is responsible for the intra- and extracellular degradation of heparan

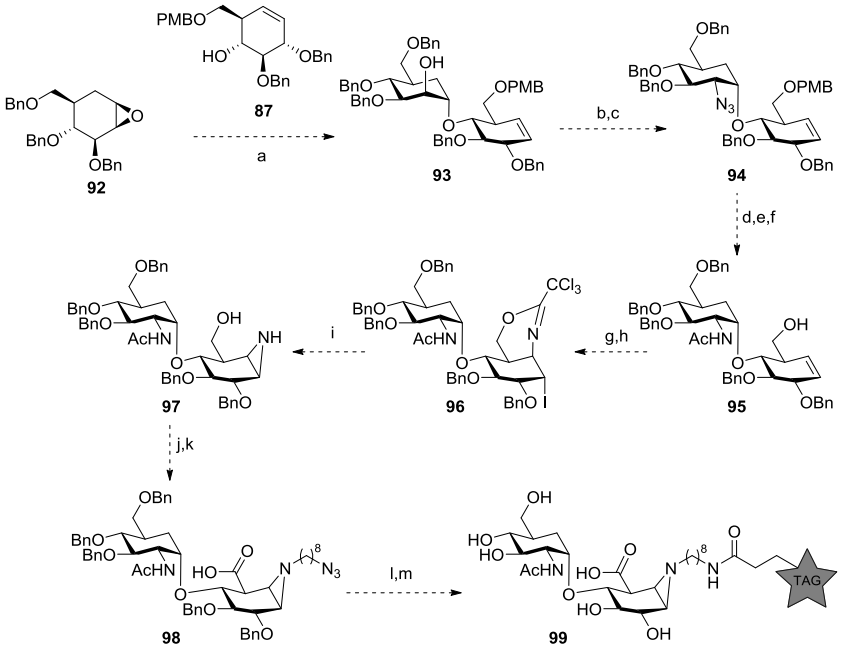
sulfate (HS), and its activity is strictly regulated. Increased extracellular HPSE activity in urine samples is a biomarker for inflammatory kidney disease, acute kidney injury and renal injury in diabetic nephropathy.[44] An activity-based probe that selectively targets HPSE could be used as a diagnostic tool for the quantification of excreted urinary HPSE levels in patients. It is envisioned that installment of a reporter tag onto C4 of epoxide **85** would block processing by exo-acting GUSB, thereby enabling selective HPSE labeling. For this purpose, cyclohexene **87** (Chapter 3) could be alkylated with an 8-azidooctyl linker, followed by PMB deprotection using DDQ to give **88** (Scheme 10b). Using *m*-CPBA, the liberated primary alcohol could then stereoselectively direct the epoxidation to β-epoxide **89**, which is then oxidized to carboxylic acid **90** using the TEMPO/BAIB system. Debenzylation under Birch conditions followed by amide coupling with different reporter tags could then afford a set of potential HPSE selective activity-based probes **91**.



Scheme 10 (a) Chemical structures of β-glucuronidase inhibitor **85**, activity-based probe **86** and the generalized structure of heparan sulfate. (b) Proposed synthetic scheme towards potential heparanase probe **91** which is resistant to processing by *exo*-glucuronidases. Reagents and conditions: a) KHMDS, 8-azido-1-iodooctane, THF; b) DDQ, THF; c) *m*-CPBA, DCM; d) TEMPO, BAIB, DCM, ^{t}BuOH, H_2O; e) Na, ^{t}BuOH, THF, NH_3; f) tag-OSu, DIPEA, DMF.

Alternatively, a stabilized heparanase 'disaccharide' probe may be constructed by introduction of a 'carbasugar' at the non-reducing end via an ether linkage (Scheme

11). For this purpose, epoxide **92** (available in multiple steps from D-glucal)[45] would be reacted with cyclohexene **87** (Chapter 3) using $Cu(OTf)_2$ as Lewis acid catalyst to regioselectively[45] afford 'disaccharide' **93** through trans-diaxial opening of the epoxide ring. The axial hydroxyl group may then be triflated and subsequently displaced by azide to afford equatorial azide **94**. Staudinger reduction, *N*-acetylation and selective cleavage of the *p*-methoxybenzyl ether using HCl in HFIP would afford cyclohexene **95**. The homoallylic alcohol may be reacted with trichloroacetonitrile under DBU catalysis, and the resulting trichloroimidate would be cyclized upon the double bond to afford **96**. Acidic hydrolysis, followed by basification could lead to aziridine **97**, which could be *N*-alkylated with 8-azidooctyl trifluoromethanesulfonate and the hydroxyl group could then be oxidized with the TEMPO/BAIB system to give **98**. Global deprotection using Birch conditions, followed by amide coupling with appropriate succinimidyl esters would finally afford stabilized 'disaccharide' heparanase ABPs **99**.

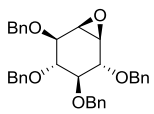


Scheme 11 Proposed synthetic scheme towards stabilized 'disaccharide' heparanase probes **99**. Reagents and conditions: a) $Cu(OTf)_2$, DCM , rt; b) Tf_2O, pyridine, DCM, -20 °C; c) NaN_3, DMF; d) PPh_3, H_2O, MeCN; e) Ac_2O, pyridine; f) HCl, HFIP, DCM; g) CCl_3CN, DBU, DCM; h) NIS, $CHCl_3$; i) HCl, MeOH, then Et_3N; j) 8-azidooctyl trifluoromethanesulfonate, DIPEA, DCM; k) TEMPO, BAIB, H_2O, ^tBuOH, DCM; l) Na, ^tBuOH, THF, NH_3; m) tag-OSu, DIPEA, DMF.

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).

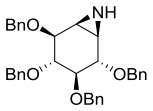
Compound 2



Cyclohexene **1**[9] (1.84 g, 3.63 mmol) was dissolved in a mixture of MeCN (24 mL), dioxane (12 mL) and aq. EDTA buffer (0.4 mM, 12 mL) and the mixture was cooled to 0 °C. 1,1,1-trifluoroacetone (4.9 mL, 54.4 mmol) was added via a pre-cooled needle, and subsequently a mixture of Oxone (11.1 g, 18.1 mmol) and $NaHCO_3$ (2.1 g, 25.4 mmol) was added in 6 portions over 1 h. The mixture was stirred for an additional 2.5 h, then diluted with H_2O (200 mL), extracted with EtOAc (3 x 100 mL) and the combined organic fractions were washed with brine, dried over $MgSO_4$, filtrated and concentrated. Flash purification by silica column chromatography (pentane/EtOAc, 25:1 → 10:1) afforded the title compound as a white solid (1.65 g, 87%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.20 (m, 20H), 4.88 – 4.65 (m, 8H), 3.97 – 3.84 (m, 3H), 3.71 – 3.57 (m, 1H), 3.47 (dd, *J* = 10.4, 7.9 Hz, 1H), 3.31 (d, *J* = 3.2 Hz,

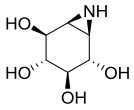
1H), 3.19 (d, *J* = 3.8 Hz, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.5, 138.1, 137.5, 128.4, 128.4, 128.2, 128.0, 127.9, 127.8, 127.7, 127.5, 127.50, 83.3, 79.2, 79.1, 78.9, 75.8, 75.4, 73.2, 73.0, 55.2, 53.9 ppm.

Compound 3



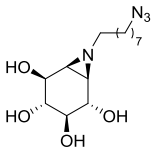
Epoxide **2** (1.55 g, 2.97 mmol) was dissolved in DMF (30 mL), sodium azide (1.93 g, 29.7 mmol) and $LiClO_4$ (6.32 g, 59.4 mmol) were added and the mixture was stirred overnight at 100 °C. The mixture was diluted with H_2O (300 mL) and extracted with Et_2O (3 x 150 mL). The combined organic fractions were washed with H_2O (200 mL) and brine, dried over $MgSO_4$, filtrated and concentrated. Flash purification by silica column chromatography (pentane/EtOAc, 5:1) gave the azido-alcohols (1.36 g) as an inseparable mixture. This mixture was co-evaporated with toluene (3x) and dissolved in dry MeCN (12 mL) under argon. Polymer-bound triphenylphosphine (~3 mmol/g, 1.6 g, 4.8 mmol) was added and the mixture was stirred overnight at 60 °C. The reaction mixture was filtered and evaporated. Flash purification by silica column chromatography (pentane/EtOAc, 3:1) afforded the title compound as a white solid (508 mg, 33% over 2 steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 – 7.26 (m, 20H), 4.91 – 4.69 (m, 8H), 3.87 (d, *J* = 7.7 Hz, 2H), 3.66 (t, *J* = 9.4 Hz, 1H), 3.46 (dd, *J* = 10.3, 7.8 Hz, 1H), 2.53 (dd, *J* = 5.1, 2.8 Hz, 1H), 2.37 (d, *J* = 5.8 Hz, 1H), 0.75 (brs, NH) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.9, 138.2, 128.5, 128.4, 128.4, 128.3, 128.2, 128.0, 127.9, 127.9, 127.9, 127.7, 127.6, 127.5, 84.4 (broad, assigned by HSQC), 79.9, 79.7, 75.9, 75.4, 73.0, 34.4, 29.8 (broad, assigned by HSQC) ppm.

Compound 4



Ammonia (10 mL) was condensed in a flask at -60 °C, and lithium wire (56 mg, 8.0 mmol) was added. The resulting deep-blue solution was stirred for 30 minutes to dissolve all lithium. Aziridine **3** (104 mg, 0.2 mmol) was taken up in dry THF (1 mL) and added to the reaction mixture. After stirring for 1 h, the mixture was quenched with H_2O. The mixture was slowly warmed to rt and evaporated. The crude was dissolved in H_2O and eluted over a column packed with Amberlite CG-50 (NH_4^+) with 0.5M NH_4OH as eluent, affording the title compound as an oil (32 mg, 99%). ^{1}H NMR (400 MHz, D_2O) δ 3.87 (dt, *J* = 6.0, 3.3 Hz, 1H), 3.68 (dd, *J* = 5.6, 2.5 Hz, 1H), 3.20 (dd, *J* = 5.8, 2.6 Hz, 2H), 2.61 (dd, *J* = 6.1, 3.6 Hz, 1H), 2.34 (d, *J* = 6.2 Hz, 1H) ppm. ^{13}C NMR (101 MHz, D_2O) δ 75.6, 72.0, 71.5, 70.9, 35.4, 34.9 ppm. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_6H_{12}O_9$ 162.07608, found 162.07613. This analytical data is in accordance with the literature.[2]

Compound 5



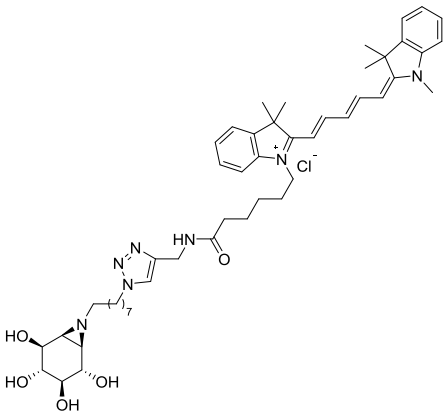
Aziridine **4** (32 mg, 0.2 mmol) was dissolved in DMF (1 mL). Potassium carbonate (38 mg, 0.28 mmol) and 1-azido-8-iodooctane (83 mg, 0.3 mmol) were added and the mixture was stirred overnight at 80 °C. H-NMR analysis of the crude reaction mixture showed mainly starting material. Therefore, additional potassium carbonate (27 mg, 0.2 mmol) and 1-azido-8-iodooctane (110 mg, 0.4 mmol) were

added and the mixture was stirred 4 h at 120 °C and subsequently concentrated. Flash purification by silica column chromatography (DCM/MeOH, 9:1) afforded the title product as an oil (25 mg, 40%). ^{1}H NMR (400 MHz, D_2O) δ 3.61 (dd, *J* = 8.4, 3.6 Hz, 1H), 3.54 (d, *J* = 8.1 Hz, 1H), 3.19 (t, *J* = 6.8 Hz, 2H), 3.14 (dd, *J* = 10.4, 8.5 Hz, 1H), 2.97 (dd, *J* = 10.4, 8.2 Hz, 1H), 2.28 (dt, *J* = 11.6, 7.3 Hz, 1H), 2.07 (dt, *J* = 11.6, 7.4 Hz, 1H), 1.84 (dd, *J* = 6.2, 3.7 Hz, 1H), 1.54 (d, *J* = 6.2 Hz, 1H), 1.53 - 1.43 (m, 4H), 1.35 - 1.22 (d, *J* = 19.0 Hz, 8H) ppm. ^{13}C NMR (101 MHz, D_2O) δ 77.8, 74.0, 73.4, 73.1, 62.0, 52.4, 45.8, 45.4, 30.6, 30.5, 30.2, 29.9, 28.3, 27.8 ppm.

General procedure for click reactions

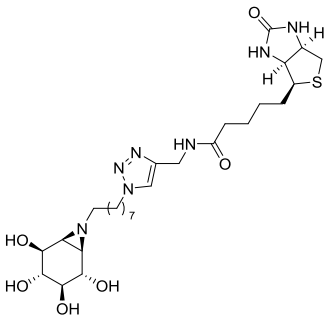
The azido compound (3-5 mg) was dissolved in DMF (0.5 mL), then the alkyne-tag (1.1 eq), $CuSO_4$ (0.2 eq) and sodium ascorbate (0.4 eq) were added and the mixture was stirred for 72 h at rt. The reaction mixture was concentrated and purified by semi-preparative reversed phase HPLC (linear gradient. Solutions used: A: 50 mM NH_4HCO_3 in H_2O, B: acetonitrile).

Compound 6 (SY-D202)



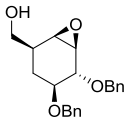
Following the general procedure starting from compound **5** (5.7 mg, 18.1 µmol), the product was obtained as a blue powder (6.3 mg, 40%). ^{1}H NMR (500 MHz, MeOD) δ 8.33 – 8.18 (m, 2H), 7.86 (s, 1H), 7.51 (d, *J* = 7.4 Hz, 2H), 7.47 – 7.38 (m, 2H), 7.36 – 7.25 (m, 4H), 6.64 (t, *J* = 12.4 Hz, 1H), 6.30 (dd, *J* = 13.8, 1.8 Hz, 2H), 4.43 (s, 2H), 4.39 (t, *J* = 7.1 Hz, 2H), 4.11 (t, *J* = 7.5 Hz, 2H), 3.71 (dd, *J* = 8.4, 3.6 Hz, 1H), 3.65 (s, 3H), 3.64 (d, *J* = 8.2 Hz, 1H), 3.24 (dd, *J* = 10.4, 8.4 Hz, 1H), 3.07 (dd, *J* = 10.4, 8.1 Hz, 1H), 2.36 (dt, *J* = 11.6, 7.3 Hz, 1H), 2.27 (t, *J* = 7.3 Hz, 2H), 2.19 – 2.11 (m, 1H), 1.93 – 1.80 (m, 5H), 1.75 (s, 12H), 1.72 (dd, *J* = 8.8, 6.4 Hz, 2H), 1.63 (d, *J* = 6.3 Hz, 1H), 1.56 (q, *J* = 7.4, 7.0 Hz, 2H), 1.49 (p, *J* = 7.7, 7.2 Hz, 2H), 1.40 – 1.28 (m, 8H) ppm. ^{13}C NMR (125 MHz, MeOD) δ 174.3, 174.0, 173.2, 154.1, 142.8, 142.1, 141.2, 141.1, 128.4, 128.3, 125.2, 124.9, 124.8, 122.7, 122.0, 121.9, 110.6, 110.4, 103.0, 102.8, 76.5, 72.7, 72.1, 71.8, 60.6, 49.9, 49.1, 44.4, 44.0, 43.4, 35.1, 34.2, 30.1, 29.9, 29.1, 29.0, 28.5, 26.8, 26.7, 26.5, 26.4, 26.0, 25.9, 25.0 ppm.

Compound 7 (SY-D203)



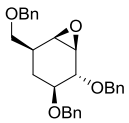
Following the general procedure starting from compound **5** (6.4 mg, 20.3 µmol), the product was obtained as a white powder (4.51 mg, 37%). ^{1}H NMR (500 MHz, MeOD) δ 7.86 (s, 1H), 4.52 (dd, *J* = 7.9, 4.2 Hz, 1H), 4.45 (s, 2H), 4.40 (t, *J* = 7.1 Hz, 2H), 4.31 (dd, *J* = 7.9, 4.5 Hz, 1H), 3.71 (dd, *J* = 8.4, 3.6 Hz, 1H), 3.65 (d, *J* = 8.1 Hz, 1H), 3.27 – 3.18 (m, 2H), 3.08 (dd, *J* = 10.4, 8.1 Hz, 1H), 2.95 (dd, *J* = 12.9, 5.0 Hz, 1H), 2.73 (d, *J* = 12.7 Hz, 1H), 2.38 (dt, *J* = 11.6, 7.2 Hz, 1H), 2.26 (t, *J* = 7.3 Hz, 2H), 2.17 (dt, *J* = 11.6, 7.3 Hz, 1H), 1.95 – 1.87 (m, 3H), 1.79 – 1.66 (m, 3H), 1.65 (d, *J* = 6.2 Hz, 1H), 1.63 – 1.55 (m, 3H), 1.48 – 1.41 (m, 2H), 1.41 – 1.27 (m, 8H) ppm. ^{13}C NMR (125 MHz, MeOD) δ 174.6, 144.9, 122.7, 76.5, 72.7, 72.1, 71.8, 61.9, 60.6, 60.2, 55.6, 49.9, 44.4, 44.0, 39.7, 35.1, 34.2, 29.9, 29.1, 29.0, 28.5, 28.3, 28.0, 26.7, 25.9, 25.3 ppm.

Compound 10



Cyclohexene **9** (3.11 mmol, 1.0 g) was dissolved in DCM. At 0 °C, *m*-CPBA (1.74 g, 7.79 mmol) was added. After stirring overnight, the reaction mixture was diluted with DCM before washing with sat. aq. $NaHCO_3$/10% $Na_2S_2O_3$ (1:1 v/v) and brine. The organic layer was dried, concentrated and purified by column chromatography (pentane/EtOAc, 2:1) to yield the title compound (970 mg, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.23 (m, 10H), 4.79 (dd, *J* = 2.5 Hz, 2H), 4.71 – 4.59 (m, 2H), 3.78 – 3.62 (m, 3H), 3.44 (ddd, *J* = 12.1, 8.0, 3.8 Hz, 1H), 3.27 (d, *J* = 3.4 Hz, 1H), 3.19 (d, *J* = 3.7 Hz, 1H), 2.15 (dt, *J* = 10.8, 5.5 Hz, 1H), 1.72 (dt, *J* = 12.9, 4.3 Hz, 1H), 1.16 (q, *J* = 12.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.7, 138.1, 128.6, 128.5, 128.0, 128.0, 127.8, 127.7, 79.3, 78.9, 73.2, 71.8, 64.9, 54.4, 54,0, 37.5, 23.8. IR: (neat) υ 2928, 2872, 1454, 1092, 1059, 735 cm^{-1}. HRMS (ESI): *m/z* = $[M+Na]^+$ calcd. for $C_{21}H_{24}O_4$ 363.15668, found 363.15666.

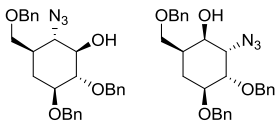
Compound 11



Epoxide **9** (2.8 mmol, 0.95 g) was dissolved in DMF under argon. At 0 °C NaH (60 wt%, 146 mg, 3.64 mmol, 1.3 equiv.) and TBAI (104 mg, 0.28 mmol) were added. After 10 minutes BnBr (466 µL, 3.92 mmol, 1.4 equiv.) was added. The reaction mixture stirred at room temperature. After reaction completion, it was quenched with water. The mixture was extracted with ether, washed with water (3x), brine and dried over $MgSO_4$. The concentrated crude product was then purified by column chromatography (pentane/EtOAc, 94:6) to obtain the title compound (0.95 g, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.22 (m, 15H), 4.84 – 4.73 (m, 2H), 4.69 – 4.50 (m, 4H), 3.72 (d, *J* = 8.0 Hz, 1H), 3.61 – 3.53 (m, 1H), 3.47 – 3.38 (m, 2H), 3.29 (d, *J* = 3.5 Hz, 1H), 3.18 (d, *J* = 3.7 Hz, 1H), 2.30 (dq, *J* = 12.3, 6.3 Hz, 1H), 1.76 (dt, *J* = 12.9, 4.2 Hz, 1H), 1.11 (q, *J* = 12.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.7, 138.3, 138.2, 128.6, 128.6, 128.5, 128.0, 127.9, 127.8, 127.8, 127.8, 127.7, 79.3, 79.0, 73.5, 73.2, 72.2, 71.8, 54.6, 54.1, 35.8, 24.3. IR:

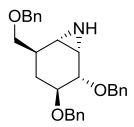
(neat) υ 2860, 1452, 1092, 1028, 733, 696 cm^{-1}. HRMS (ESI): *m/z* = $[M+Na]^+$ calcd. for $C_{28}H_{30}O_4$ 453.20363, found 453.20294.

Compound 12 and 13



Epoxide **11** (933 mg, 2.2 mmol) was dissolved in DMF under argon. NaN_3 (21.7 mmol, 1.4 g, 10 equiv.) and $LiClO_4$ (43.4 mmol, 4.6 g, 20 equiv.) were added. The reaction mixture was stirred at 80 °C for two days. The completed reaction was quenched with water, extracted with ether and washed with water and brine. The crude product, after drying over $MgSO_4$ and concentrating, was purified by column chromatography (pentane/EtOAc, 1:19 → 1:9) resulting in a mixture of the two *trans*-azido alcohols **12**-**13** in a ratio of 1:1.3 (853 mg, 83%). **12:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.27 (m, 15H), 5.04 (d, *J* = 11.3 Hz, 1H), 4.70 (dd, *J* = 11.4, 5.2 Hz, 2H), 4.60 (d, *J* = 11.5 Hz, 1H), 4.52 (s, 2H), 3.57 (dd, *J* = 9.1, 4.5 Hz, 1H), 3.53 (d, *J* = 2.2 Hz, 1H), 3.50 (dd, *J* = 4.6, 1.8 Hz, 1H), 3.47 (s, 1H), 3.45 (d, *J* = 2.0 Hz, 1H), 3.35 (q, *J* = 9.3 Hz, 2H), 2.62 (d, *J* = 2.0 Hz, 1H), 2.20 (dd, *J* = 9.9, 4.6 Hz, 1H), 1.51 (s, 1H), 1.49 (d, *J* = 3.3 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.6, 138.4, 138.3, 128.8, 128.6, 128.6, 128.2, 128.1, 127.8, 127.8, 127.7, 85.1, 79.7, 76.4, 75.6, 73.4, 72.0, 70.4, 64.3, 37.8, 31.2. IR: (neat) υ 2924, 2859, 2099, 1452, 1086, 1070, 733, 696 cm^{-1}. HRMS (ESI): *m/z* = $[M+H]^+$ calcd. for $C_{28}H_{31}N_3O_4$ 474.23873, found 474.23836. **13:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.26 (m, 14H), 4.81 (d, *J* = 11.7 Hz, 1H), 4.77 – 4.62 (m, 3H), 4.56 – 4.43 (m, 2H), 4.02 (s, 1H), 3.98 – 3.94 (m, 2H), 3.82 – 3.73 (m, 1H), 3.70 (dd, *J* = 9.3, 2.5 Hz, 1H), 3.58 (dd, *J* = 9.3, 3.5 Hz, 1H), 3.45 (d, *J* = 1.3 Hz, 1H), 1.94 (s, 1H), 1.90 (d, *J* = 22.9 Hz, 1H), 1.78 (dd, *J* = 8.9, 4.9 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.1, 138.6, 137.5, 128.7, 128.5, 128.5, 128.1, 127.9, 127.7, 127.7, 127.6, 80.5, 77.6, 73.8, 73.5, 73.4, 72.7, 72.2, 63.9, 35.0, 28.3. IR: (neat) υ 2924, 2864, 2102, 1454, 1090, 1076, 731, 696 cm^{-1}. HRMS (ESI): *m/z* = $[M+H]^+$ calcd. for $C_{28}H_{31}N_3O_4$ 474.23873, found 474.23849.

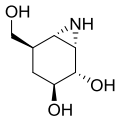
Compound 14



Trans-azido alcohol **13** (0.54 mmol, 254 mg) was co-evaporated with toluene (3x) and dissolved in dry acetonitrile under argon. Polymer-bound triphenyl phosphine (717 mg, 2.2 mmol, 4 equiv.) was added to a dried closed tube. The resin was rinsed with anhydrous acetonitrile (3x) before the starting material was transferred to the closed tube. The reaction mixture was stirred at 90 °C overnight. The mixture was then allowed to cool to room temperature, filtrated and concentrated at room temperature. The crude product was purified by column chromatography (DCM/MeOH, 200:1 → 50:1) to afford the title product (168 mg, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.23 (m, 16H), 4.82 (p, *J* = 12.4 Hz, 2H), 4.71 – 4.59 (m, 2H), 4.59 – 4.44 (m, 2H), 3.73 (dd, *J* = 8.0, 3.5 Hz, 1H), 3.65 (ddd, *J* = 11.4, 8.0, 3.3 Hz, 1H), 3.50 – 3.37 (m, 2H), 2.48 (dd, *J* = 6.0, 3.5 Hz, 1H), 2.30 (d, *J* = 6.1 Hz, 1H), 2.21 (dt, *J* = 12.2, 6.1 Hz, 1H), 1.84 (ddd, *J* = 12.8, 5.3, 3.4 Hz, 1H), 1.14 (q, *J* = 12.0 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.3, 139.1, 138.4, 128.6, 128.4, 128.4, 127.9, 127.8, 127.8, 127.6, 127.5, 80.4, 77.6, 73.4, 73.3, 72.6, 72.4, 36.3, 35.0, 34.7, 31.8.

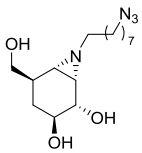
IR: (neat) υ 2857, 1450, 1094, 1074, 735, 696 cm^{-1}. HRMS (ESI): *m/z* = $[M+H]^+$ calcd. for $C_{28}H_{31}NO_3$ 430.23767, found 430.23746.

Compound 15



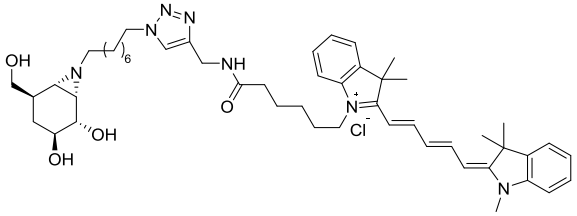
Ammonia was condensed at -60 °C and lithium (1.5 mmol, 11 mg, 30 equiv.) was added. The mixture was stirred for 30 minutes. Then, the aziridine **14** (22 mg, 50 µmol) in THF (1 mL) was added and stirred at -60 °C for 1 hour. The reaction was quenched with D_2O and concentrated. The crude product was purified by column chromatography using Amberlite CG-50 (NH_4^+) with 0.5*N* NH_4OH, affording the title compound as an oil (9 mg, quant.). ^1H NMR (500 MHz, D_2O) δ 3.76 (dd, *J* = 8.7, 3.6 Hz, 1H), 3.64 (dd, *J* = 11.0, 5.7 Hz, 1H), 3.58 (dd, *J* = 11.0, 7.0 Hz, 1H), 3.52 (ddd, *J* = 12.2, 8.7, 3.5 Hz, 1H), 2.58 (dd, *J* = 6.4, 3.7 Hz, 1H), 2.35 (d, *J* = 6.4 Hz, 1H), 2.05 (dq, *J* = 12.2, 6.1 Hz, 1H), 1.67 (dt, *J* = 12.4, 4.4 Hz, 1H), 1.14 (q, *J* = 12.4 Hz, 1H). ^{13}C NMR (126 MHz, D_2O) δ 73.2, 69.5, 64.5, 37.1, 36.5, 34.4, 33.2. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_7H_{14}NO_3$ 160.09682, found 160.09662.

Compound 16



Aziridine **14** (71 mg, 0.166 mmol) was debenzylated and purified as described above, and subsequently dissolved in DMF-d_7 (1.6 mL). K_2CO_3 (0.2 mmol, 27 mg, 1.2 equiv.) and 1-azido-8-iodooctane (0.33 mmol, 93 mg, 2 equiv.) were added. The reaction mixture was stirred overnight at 100 °C. Then, the mixture was concentrated and purified by column chromatography (DCM/MeOH, 20:1 → 9:1) affording the title compound as an oil (29 mg, 55% over 2 steps). ^1H NMR (400 MHz, MeOD) δ 3.60 – 3.57 (m, 1H), 3.57 – 3.50 (m, 2H), 3.47 (dt, *J* = 10.8, 5.5 Hz, 1H), 3.28 (t, *J* = 6.8 Hz, 2H), 2.33 (dt, *J* = 11.7, 7.5 Hz, 1H), 2.16 (dt, *J* = 11.6, 7.5 Hz, 1H), 2.00 (dt, *J* = 12.5, 5.7 Hz, 1H), 1.87 (dd, *J* = 6.4, 3.6 Hz, 1H), 1.70 (d, *J* = 6.4 Hz, 1H), 1.59 (dq, *J* = 13.9, 6.9, 6.1 Hz, 5H), 1.35 (s, 8H), 1.00 (q, *J* = 12.2 Hz, 1H). ^{13}C NMR (101 MHz, MeOD) δ 75.1, 71.3, 66.1, 62.3, 52.4, 46.9, 44.8, 39.6, 35.3, 30.6, 30.4, 30.2, 29.9, 28.3, 27.8. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{15}H_{29}N_4O_3$ 313.22342, found 313.22342.

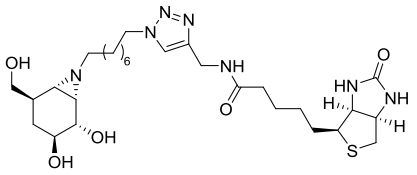
Compound 17 (JS-97)



Following the general procedure starting from compound **16** (4.8 mg, 15 µmol) the title compound was obtained as a blue solid (6.7 mg, 44%). ^1H NMR (500 MHz, D_2O) δ 7.79 (s, 2H), 7.34 (t, *J* = 8.1 Hz, 2H), 7.23 (dt, *J* = 25.5, 8.0 Hz, 3H), 7.09 (dt, *J* = 19.1, 7.5 Hz, 3H), 6.32 (t, *J* = 12.2 Hz, 1H), 6.00 (dd, *J* = 32.4, 13.7 Hz, 2H), 4.35 (s, 2H), 4.23 (t, *J* = 6.9 Hz, 2H), 3.92 (s, 1H), 3.68 (dd, *J* = 8.7, 3.7 Hz, 1H), 3.56 (dd, *J* = 10.9, 5.5 Hz, 1H), 3.52 (s, 2H), 3.51 – 3.43 (m, 3H), 2.22 (t, *J* = 7.0 Hz, 2H), 2.15 (t, *J* = 7.4 Hz, 2H), 2.05 (s, 8H), 2.02 – 1.93 (m, 2H), 1.92 (s, 5H), 1.77 (d, *J* = 6.8 Hz, 1H), 1.67 (s, 3H), 1.63 – 1.55 (m, 3H), 1.41 (d, *J* = 10.3 Hz, 12H),

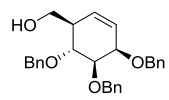
1.06 (d, *J* = 12.7 Hz, 3H), 1.03 (s, 5H). ^{13}C NMR (126 MHz, D_2O) δ 176.0, 173.8, 172.7, 153.2, 142.6, 141.9, 141.1, 128.5, 125.3, 125.0, 124.3, 122.2, 110.7, 103.2, 102.6, 72.7, 70.0, 64.3, 59.7, 50.3, 49.0, 48.9, 45.8, 44.1, 43.5, 36.9, 35.4, 34.3, 33.1, 30.9, 29.5, 28.5, 28.4, 28.1, 27.0, 26.8, 26.6, 26.4, 25.6, 25.5, 25.1, 0.9. HRMS (ESI) m/z: $[M]^+$ calc for $C_{50}H_{70}N_7O_4^+$ 832.54838, found 832.54852.

Compound 18 (JS-96)



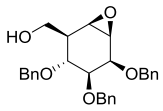
Following the general procedure starting from compound **16** (4.8 mg, 15 µmol), the title compound was obtained as a white solid (5.1 mg, 56%). ^{1}H NMR (400 MHz, MeOD) δ 7.86 (s, 1H), 4.54 (dd, *J* = 7.9, 4.5 Hz, 2H), 4.43 (s, 2H), 4.38 (t, *J* = 7.0 Hz, 2H), 4.33 (dd, *J* = 7.9, 4.5 Hz, 1H), 3.63 (dd, *J* = 8.5, 3.8 Hz, 1H), 3.58 (dd, *J* = 10.9, 5.6 Hz, 1H), 3.55 – 3.45 (m, 2H), 3.22 (dt, *J* = 9.8, 5.4 Hz, 1H), 2.95 (dd, *J* = 12.9, 5.0 Hz, 1H), 2.72 (d, *J* = 12.9 Hz, 1H), 2.24 (dt, *J* = 14.2, 7.4 Hz, 4H), 2.01 (dq, *J* = 12.4, 5.8 Hz, 1H), 1.93 (dd, *J* = 6.5, 3.9 Hz, 1H), 1.88 (d, *J* = 8.2 Hz, 4H), 1.73 (d, *J* = 6.5 Hz, 1H), 1.65 (td, *J* = 13.8, 6.3 Hz, 5H), 1.55 (dt, *J* = 15.5, 8.1 Hz, 5H), 1.39 (p, *J* = 8.5, 7.9 Hz, 3H), 1.29 (s, 5H), 1.03 (q, *J* = 12.4 Hz, 1H). ^{13}C NMR (101 MHz, MeOD) δ 176.6, 166.1, 146.1, 124.4, 74.8, 71.2, 65.7, 63.3, 61.9, 61.5, 56.8, 51.4, 46.8, 44.8, 41.0, 39.0, 36.5, 35.4, 34.9, 31.0, 30.2, 30.1, 29.7, 29.4, 29.2, 28.0, 27.1, 26.5. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{28}H_{48}N_7O_5S$ 594.34321, found 594.34332.

Compound 64



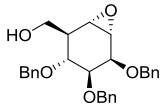
Diol **63**[13] (400 mg, 1.18 mmol) was dissolved in dry DMF (6 mL), imidazole (200 mg, 2.94 mmol) was added and the mixture was cooled to -15 °C (ice/EtOH). Then, TBSCl (186 mg, 1.23 mmol) was added and the mixture was stirred for 2 h while the cooling bath was allowed to warm to rt. The mixture was quenched and diluted with H_2O (120 mL) and extracted with Et_2O (3 x 50 mL). The combined organic layers were washed with H_2O (100 mL) and brine, dried over $MgSO_4$, filtrated and concentrated. The crude product was co-evaporated with toluene, dissolved in dry DMF (6 mL) and cooled to 0 °C. Then, TBAI (43 mg, 0.12 mmol), BnBr (280 µL, 2.35 mmol) and NaH (60 wt%, 89 mg, 2.23 mmol) were added and the mixture was stirred overnight at rt. The reaction was quenched and diluted with H_2O (60 mL) at 0 °C, and extracted with Et_2O (3 x 30 mL). The combined organic layers were washed with H_2O (30 mL) and brine, dried over $MgSO_4$, filtrated and concentrated. The crude product was co-evaporated with toluene, dissolved in THF (6 mL) and TBAF (1M in THF, 3.5 mL, 3.5 mmol) was added. After 2 h, the mixture was concentrated, and flash purification by silica column chromatography (pentane/EtOAc, 3:1 → 2:1) afforded the title compound as an oil (451 mg, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.22 (m, 15H), 5.85 (ddd, *J* = 9.9, 4.6, 2.7 Hz, 1H), 5.66 (dd, *J* = 10.0, 2.5 Hz, 1H), 4.96 (d, *J* = 11.2 Hz, 1H), 4.80 – 4.62 (m, 5H), 4.13 (t, *J* = 4.0 Hz, 1H), 3.97 (dd, *J* = 8.9, 7.3 Hz, 1H), 3.76 – 3.63 (m, 3H), 2.46 – 2.36 (m, 1H), 2.09 (brs, OH) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.9, 138.6, 138.5, 130.5, 128.6, 128.5, 128.5, 128.3, 128.0, 127.9, 127.8, 127.7, 126.9, 80.7, 77.0, 74.5, 72.7, 72.0, 71.8, 64.5, 46.5 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{28}H_{30}O_4$ 453.2036, found 453.2049.

Compound 65



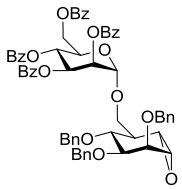
Cyclohexene **64** (451 mg, 1.05 mmol) was dissolved in DCM (10 mL) and cooled to 0 °C on a large ice-bath. Then, mCPBA (<77% wt, 470 mg, 2.1 mmol) was added and the mixture was stirred overnight while the cooling bath was slowly allowed to reach rt. The mixture was diluted with DCM (100 mL) and washed with a mixture of sat. aq. $NaHCO_3$ and aq. 10% $Na_2S_2O_3$ (1:1 v/v, 3 x 50 mL) and brine, dried over $MgSO_4$, filtrated and concentrated. Flash purification by silica column chromatography (pentane/EtOAc, 2:1 → 1:1) afforded the title compound as a white solid (393 mg, 84%). Additionally, α-epoxide **66** was obtained as an oil (36 mg, 8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 – 7.23 (m, 15H), 4.88 (d, *J* = 11.0 Hz, 1H), 4.83 (d, *J* = 12.4 Hz, 1H), 4.72 (d, *J* = 12.4 Hz, 1H), 4.66 – 4.58 (m, 2H), 4.52 (d, *J* = 11.0 Hz, 1H), 4.03 (t, *J* = 4.6 Hz, 1H), 3.94 (dd, *J* = 10.7, 5.2 Hz, 1H), 3.88 (dd, *J* = 10.7, 6.2 Hz, 1H), 3.75 (t, *J* = 8.5 Hz, 1H), 3.45 (dd, *J* = 9.1, 4.9 Hz, 1H), 3.32 (dd, *J* = 3.7, 2.5 Hz, 1H), 3.20 (t, *J* = 4.1 Hz, 1H), 2.58 – 2.29 (brs, OH), 2.14 (ddt, *J* = 8.5, 5.4, 3.1 Hz, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.3, 138.2, 138.1, 128.5, 128.5, 128.4, 128.2, 128.2, 128.0, 127.9, 127.8, 80.0, 74.7, 74.2, 72.6, 71.5, 70.5, 63.0, 54.9, 51.0, 44.3 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{28}H_{30}O_5$ 469.1985, found 469.1990.

Compound 66



Cyclohexene **64** (326 mg, 0.76 mmol) was dissolved in a mixture of MeCN (7.6 mL) and aq. EDTA (0.4 mM, 3.8 mL) and cooled to 0 °C. 1,1,1-trifluoroacetone (1.0 mL, 11.4 mmol) was added via a pre-cooled needle and subsequently a mixture of Oxone (2.33 g, 3.79 mmol) and $NaHCO_3$ (445 mg, 5.3 mmol) was added in 6 portions over 1 h. After stirring an additional hour, the mixture was diluted with H_2O (100 mL) and extracted with EtOAc (3 x 50 mL). The combined organic fractions were washed with brine, dried over $MgSO_4$, filtrated and concentrated. Flash purification by silica column chromatography (pentane/EtOAc, 4:1 → 2:1) afforded the title compound as an oil (302 mg, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 16 (m, 15H), 4.90 (dd, *J* = 18.0, 11.7 Hz, 2H), 4.73 – 4.53 (m, 4H), 4.22 (t, *J* = 2.6 Hz, 1H), 3.81 – 3.75 (m, 1H), 3.75 – 3.66 (m, 2H), 3.61 (dd, *J* = 10.8, 5.9 Hz, 1H), 3.19 (t, *J* = 3.1 Hz, 1H), 3.11 (d, *J* = 3.7 Hz, 1H), 2.27 (brs, OH), 2.19 (dt, *J* = 7.8, 5.7 Hz, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.5, 138.4, 138.3, 128.5, 128.5, 128.4, 128.2, 127.9, 127.9, 127.8, 127.7, 127.7, 80.2, 74.8, 74.7, 74.0, 73.5, 73.1, 62.6, 54.3, 54.2, 44.3 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{28}H_{30}O_5$ 469.1985, found 469.1996.

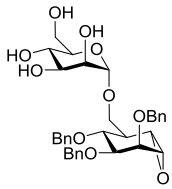
Compound 68



Acceptor **66** (45 mg, 0.1 mmol) and trichloroimidate donor **67**[42] (89 mg, 0.12 mmol) were combined in a flask, co-evaporated with toluene (3x) and dissolved in dry DCM (1 mL). The mixture was cooled to -40 °C and TMSOTf (5.4 µL, 30 µmol) was added. After stirring for 1 h, the reaction was quenched with Et_3N (50 µL) and warmed to rt. The mixture was diluted with H_2O (30 mL) and extracted with DCM (3 x 15 mL). The combined organic fractions were washed with brine,

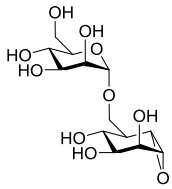
dried over $MgSO_4$, filtrated and concentrated. Flash purification by silica column chromatography (pentane/EtOAc, 5:1) afforded the title compound as an oil (67 mg, 65%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (ddd, *J* = 12.9, 8.3, 1.2 Hz, 4H), 7.92 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.82 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.62 – 7.57 (m, 1H), 7.56 – 7.51 (m, 1H), 7.50 – 7.46 (m, 1H), 7.45 – 7.22 (m, 26H), 6.13 (t, *J* = 10.0 Hz, 1H), 5.90 (dd, *J* = 10.2, 3.3 Hz, 1H), 5.73 (dd, *J* = 3.2, 1.8 Hz, 1H), 5.08 (d, *J* = 1.7 Hz, 1H), 4.97 (dd, *J* = 11.9, 4.1 Hz, 2H), 4.77 (d, *J* = 12.3 Hz, 1H), 4.72 (d, *J* = 11.7 Hz, 1H), 4.63 (dd, *J* = 21.5, 11.6 Hz, 3H), 4.44 – 4.36 (m, 2H), 4.28 (t, *J* = 2.6 Hz, 1H), 3.91 (dd, *J* = 9.9, 7.5 Hz, 1H), 3.85 – 3.80 (m, 1H), 3.77 (dd, *J* = 9.6, 3.0 Hz, 2H), 3.29 (t, *J* = 3.1 Hz, 1H), 3.27 (d, *J* = 3.5 Hz, 1H), 2.44 (td, *J* = 7.8, 3.9 Hz, 1H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.2, 165.6, 165.5, 165.5, 138.7, 138.6, 133.6, 133.5, 133.3, 133.2, 130.0, 129.9, 129.9, 129.4, 129.2, 129.1, 128.7, 128.6, 128.6, 128.4, 128.1, 128.0, 127.9, 127.9, 127.8, 98.0, 80.4, 75.0, 74.6, 74.0, 73.3, 73.2, 70.5, 70.1, 69.4, 68.3, 66.9, 62.8, 54.5, 54.2, 42.4 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{62}H_{56}O_{14}$ 1047.3562, found 1047.3627.

Compound 69



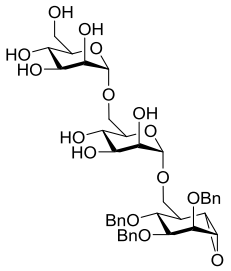
Compound **68** (56 mg, 55 µmol) was dissolved in a mixture of MeOH (0.5 mL) and DCM (0.5 mL), NaOMe (5.4 M in MeOH, 5.1 µL, 27 µmol) was added and the mixture was stirred overnight at rt. The mixture was concentrated and flash purification by silica column chromatography (DCM/MeOH, 19:1) afforded the title compound as an oil (27 mg, 81%). ^{1}H NMR (500 MHz, $CDCl_3$ + MeOD) δ 7.41 – 7.24 (m, 15H), 4.89 (d, *J* = 11.5 Hz, 2H), 4.80 (m, under solvent peak, assigned by HSQC, 1H), 4.72 (d, *J* = 12.0 Hz, 1H), 4.63 (d, *J* = 2.8 Hz, 2H), 4.61 – 4.56 (m, 1H), 4.28 (t, *J* = 2.7 Hz, 1H), 3.88 – 3.81 (m, 2H), 3.81 – 3.72 (m, 4H), 3.69 (ddd, *J* = 9.5, 6.0, 3.1 Hz, 2H), 3.59 (dd, *J* = 9.8, 3.9 Hz, 1H), 3.54 (ddd, *J* = 9.5, 4.5, 2.7 Hz, 1H), 3.23 (t, *J* = 3.1 Hz, 1H), 3.16 (d, *J* = 3.6 Hz, 1H), 2.27 (td, *J* = 7.8, 3.9 Hz, 1H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$ + MeOD) δ 137.9, 137.8, 137.8, 127.7, 127.6, 127.6, 127.3, 127.3, 127.1, 127.1, 126.9, 126.9, 99.8, 79.5, 74.2, 74.0, 73.1, 72.8, 72.5, 72.1, 70.8, 70.2, 66.5, 66.1, 60.8, 53.5 (2 C), 41.8 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{34}H_{40}O_{10}$ 631.2514, found 631.2531.

Compound 70 (SY-G42)



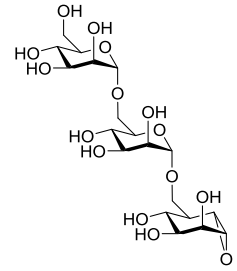
Compound **69** (20 mg, 33 µmol) was dissolved in a mixture of dioxane/MeOH/H_2O (1:1:1, 1.5 mL) under N_2. $Pd(OH)_2$/C (20 wt%, 12 mg, 16 µmol) was added and the mixture was purged with H_2 gas with a balloon. After stirring vigorously for 2 h, the mixture was filtrated over Celite and evaporated, affording the title compound as an oil (12 mg, quant.). ^{1}H NMR (400 MHz, D_2O) δ 4.89 (d, *J* = 1.6 Hz, 1H), 4.41 (t, *J* = 2.9 Hz, 1H), 3.97 – 3.90 (m, 2H), 3.89 – 3.78 (m, 2H), 3.76 – 3.68 (m, 2H), 3.65 – 3.57 (m, 3H), 3.51 (dd, *J* = 10.4, 3.5 Hz, 1H), 3.40 – 3.36 (m, 1H), 3.31 (d, *J* = 3.5 Hz, 1H), 2.14 (ddd, *J* = 9.4, 6.3, 3.3 Hz, 1H) ppm. ^{13}C NMR (101 MHz, D_2O) δ 99.7, 72.9, 70.5, 70.3, 70.0, 67.1, 66.7, 66.1, 65.6, 60.9, 55.8, 55.2, 42.3 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{13}H_{22}O_{10}$ 361.1105, found 361.1118.

Compound 72



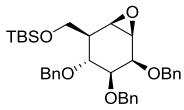
Acceptor **66** (62 mg, 0.14 mmol) and thioglycoside donor **71**[43] (194 mg, 0.17 mmol) were combined in a flask, co-evaporated with toluene (3x) and dissolved in dry DCM (1.4 mL). Molecular sieves (4 Å) were added and the mixture was stirred for 1 h at rt, and subsequently cooled to -40 °C. Then, NIS (36 mg, 0.17 mmol) and TMSOTf (7.5 µL, 42 µmol) were added. After stirring for 1 h, the reaction was quenched with Et_3N (50 µL) and warmed to rt. The mixture was diluted DCM (50 mL), washed with 10% aq. $Na_2S_2O_3$ (2 x 20 mL) and brine, dried over $MgSO_4$, filtrated and concentrated. Flash purification by silica column chromatography (pentane/EtOAc, 2:1) afforded the target trisaccharide which was highly contaminated with inseparable byproducts. The product was taken up in a mixture of MeOH (0.5 mL) and DCM (0.5 mL) and 5 drops of NaOMe (5.4 M in MeOH) were added. After stirring the reaction overnight, the reaction was neutralized by addition of Amberlite CG-50 (H^+), filtrated and concentrated. Flash purification by silica column chromatography (DCM/MeOH, 19:1 → 15:85) afforded the title compound as an oil (15 mg, 14%). ^{1}H NMR (400 MHz, MeOD) δ 7.44 – 7.18 (m, 15H), 4.87 (d, *J* = 2.4 Hz, 1H), 4.79 (d, *J* = 1.5 Hz, 1H), 4.78 – 4.70 (m, 2H), 4.70 – 4.55 (m, 4H), 4.30 (t, *J* = 2.8 Hz, 1H), 3.90 (dd, *J* = 11.2, 5.4 Hz, 1H), 3.88 – 3.79 (m, 4H), 3.78 (d, *J* = 8.5 Hz, 1H), 3.75 – 3.68 (m, 4H), 3.68 – 3.61 (m, 5H), 3.57 (dd, *J* = 9.9, 3.7 Hz, 1H), 3.24 (t, *J* = 3.1 Hz, 1H), 3.15 (d, *J* = 3.6 Hz, 1H), 2.22 (td, *J* = 7.6, 3.8 Hz, 1H) ppm. ^{13}C NMR (101 MHz, MeOD) δ 140.0, 139.9, 139.8, 129.5, 129.4, 129.4, 129.2, 129.1, 129.0, 128.8, 128.7, 128.7, 101.9, 101.4, 81.4, 76.0, 75.8, 74.7, 74.4, 74.3, 73.7, 73.5, 72.8, 72.6, 72.0, 72.0, 68.6, 68.3, 67.9, 67.0, 62.8, 55.4, 55.2, 43.9 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{40}H_{50}O_{15}$ 793.3042, found 793.3082.

Compound 73 (SY-G39)



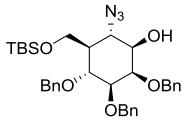
Compound **72** (10 mg, 13 µmol) was dissolved in a mixture of dioxane/MeOH/H_2O (1:1:1, 1.0 mL) under N_2. $Pd(OH)_2$/C (20 wt%, 10 mg, 14 µmol) was added and the mixture was purged with H_2 gas with a balloon. After stirring vigorously for 2 h, the mixture was filtrated over celite and evaporated, affording the title compound as an oil (6.9 mg, quant.). ^{1}H NMR (500 MHz, D_2O) δ 4.91 (s, 2H), 4.44 (t, *J* = 2.9 Hz, 1H), 4.02 – 3.91 (m, 4H), 3.89 (d, *J* = 10.8 Hz, 1H), 3.85 (t, *J* = 3.4 Hz, 1H), 3.83 (t, *J* = 3.1 Hz, 1H), 3.80 – 3.64 (m, 6H), 3.68 – 3.60 (m, 2H), 3.53 (dd, *J* = 10.4, 3.4 Hz, 1H), 3.41 (t, *J* = 3.0 Hz, 1H), 3.34 (d, *J* = 3.6 Hz, 1H), 2.17 (ddd, *J* = 9.2, 6.2, 3.2 Hz, 1H) ppm. ^{13}C NMR (125 MHz, D_2O) δ 99.9, 99.5, 72.8, 71.1, 70.9, 70.6, 70.4, 70.0, 67.2, 66.8, 66.6, 66.2, 65.7, 65.6, 61.0, 55.9, 55.3, 42.3 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{19}H_{32}O_{15}$ 523.1633, found 523.1649.

Compound 74



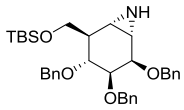
Epoxide **65** (393 mg, 0.88 mmol) was dissolved in dry THF (9 mL), cooled to 0 °C and then imidazole (120 mg, 1.76 mmol) and TBSCl (160 mg, 1.06 mmol) were added and the mixture was stirred for 4 h at rt. The reaction was quenched by addition of H_2O (100 mL) and extracted with Et_2O (3 x 50 mL). The combined organic phases were washed with brine, dried over $MgSO_4$, filtrated and concentrated. Flash purification by silica column chromatography (pentane/EtOAc, 10:1) afforded the title compound as a colorless oil (459 mg, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 – 7.21 (m, 15H), 4.84 (dd, *J* = 11.8, 9.4 Hz, 2H), 4.73 (d, *J* = 12.5 Hz, 1H), 4.63 (s, 2H), 4.46 (d, *J* = 11.3 Hz, 1H), 4.03 (t, *J* = 4.6 Hz, 1H), 3.90 (dd, *J* = 9.5, 4.9 Hz, 1H), 3.73 (t, *J* = 9.4 Hz, 1H), 3.57 (t, *J* = 8.4 Hz, 1H), 3.47 – 3.40 (m, 2H), 3.26 (t, *J* = 4.0 Hz, 1H), 2.14 (dddd, *J* = 9.4, 7.7, 4.9, 2.5 Hz, 1H), 0.88 (s, 9H), 0.04 (s, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.6, 138.5, 138.3, 128.5, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 80.0, 74.5, 73.4, 72.6, 71.5, 70.8, 62.2, 54.5, 51.9, 44.9, 26.1, 18.4, -5.3 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{34}H_{44}O_5Si$ 583.2850, found 583.2861.

Compound 75



Epoxide **74** (441 mg, 0.79 mmol) was dissolved in DMF (8 mL). Sodium azide (510 mg, 7.9 mmol) and $LiClO_4$ (1.67 g, 15.7 mmol) were added and the mixture was stirred overnight at 80 °C. The mixture was diluted with H_2O (100 mL) and extracted with Et_2O (3 x 50 mL). The combined organic phases were washed with brine, dried over $MgSO_4$, filtrated and concentrated. Flash purification by silica column chromatography (pentane/EtOAc, 15:1) afforded the title compound as a colorless oil (234 mg, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.12 (m, 15H), 4.69 – 4.41 (m, 6H), 4.15 – 4.04 (m, 1H), 3.96 – 3.87 (m, 2H), 3.77 – 3.67 (m, 4H), 3.66 (dd, *J* = 8.5, 2.6 Hz, 1H), 0.87 (s, 9H), 0.04 (s, 3 H), 0.03 (s, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.0, 137.8, 128.5, 128.4, 128.1, 127.9, 127.8, 127.6, 78.6, 76.7 (under solvent peak, assigned by HSQC), 74.8, 73.2, 73.1, 72.5 (broad), 70.8, 63.9 (broad), 61.2, 44.6, 25.9, 18.1, -5.5, -5.5 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{34}H_{45}N_3O_5Si$ 626.3021, found 626.3023.

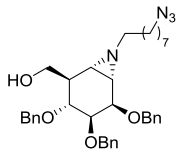
Compound 76



Polymer-bound triphenylphosphine (~3 mmol/g loading, 242 mg, 0.73 mmol) was added to a flame dried microwave tube and rinsed with dry MeCN (3x). Azido-alcohol **75** (219 mg, 0.36 mmol) was co-evaporated with toluene (3x), dissolved in dry MeCN (3.6 mL) and transferred to the microwave tube under nitrogen atmosphere. The tube was fitted with a cap and the reaction was stirred overnight at 90 °C. The mixture was then filtrated and concentrated. Flash purification by silica column chromatography (pentane/EtOAc, 3:1) afforded the title compound as a colorless oil (172 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.20 (m, 15H), 4.93 (d, *J* = 12.3 Hz, 1H), 4.87 (d, *J* = 11.4 Hz, 1H), 4.71 (d, *J* = 13.0 Hz, 2H), 4.64 (d, *J* = 11.8 Hz, 1H), 4.55 (d, *J* = 11.4 Hz, 1H), 4.20 (t, *J* = 2.3 Hz, 1H), 3.78 – 3.73 (m, 2H), 3.72 – 3.66 (m, 1H), 3.61 (dd, *J* = 9.7, 8.3 Hz, 1H), 2.42 (dd, *J* = 5.7, 2.2 Hz, 1H), 2.32 (d, *J* = 5.8 Hz, 1H),

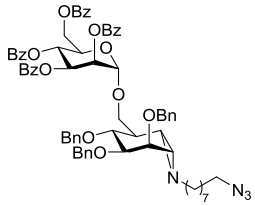
2.11 (td, *J* = 8.0, 4.1 Hz, 1H), 0.88 (s, 9H), 0.04 (s, 3H), 0.03 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.3, 139.2, 128.4, 128.4, 128.1, 127.8, 127.7, 127.5, 127.5, 75.6, 75.2, 74.4, 73.3, 73.0, 63.7, 45.6, 34.3, 31.7, 26.0, 18.4, -5.2, -5.2 ppm. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{34}H_{45}NO_4Si$ 560.3191, found 560.3203.

Compound 77



Freshly prepared 8-azidooctyl trifluoromethanesulfonate (0.5 M in $CHCl_3$, 1.1 mL, 0.57 mmol) was added to a solution of aziridine **76** (159 mg, 0.28 mmol) and DIPEA (99 µL, 0.57 mmol) in $CHCl_3$ (2.8 mL) and the mixture was stirred overnight at rt. The reaction was quenched by addition of MeOH (3 mL) and the mixture was stirred for 3 h at rt. The mixture was concentrated, dissolved in EtOAc (150 mL) and washed with H_2O (3 x 75 mL) and brine, dried over $MgSO_4$, filtrated and concentrated. The crude was dissolved in dry THF (2.8 mL), TBAF (1M in THF, 0.85 mL, 0.85 mmol) was added and the mixture was stirred overnight at rt. The mixture was diluted with H_2O (200 mL) and extracted with EtOAc (3 x 50 mL). The combined organic phases were washed with H_2O (50 mL) and brine, dried over $MgSO_4$, filtrated and concentrated. Flash purification by silica column chromatography (pentane/EtOAc, 4:1) afforded the title compound as a colorless oil (146 mg, 88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 – 7.20 (m, 15H), 4.89 (dd, *J* = 11.7, 9.3 Hz, 2H), 4.73 (d, *J* = 12.2 Hz, 1H), 4.70 (d, *J* = 11.7 Hz, 1H), 4.64 (d, *J* = 11.7 Hz, 1H), 4.57 (d, *J* = 11.3 Hz, 1H), 4.18 (t, *J* = 2.5 Hz, 1H), 3.79 – 3.75 (m, 2H), 3.74 – 3.70 (m, 1H), 3.63 (dt, *J* = 10.3, 5.0 Hz, 1H), 3.23 (t, *J* = 6.9 Hz, 2H), 2.24 – 2.10 (m, 4H), 1.76 (dd, *J* = 6.0, 2.4 Hz, 1H), 1.61 – 1.54 (m, 2H), 1.50 (d, *J* = 6.0 Hz, 1H), 1.48 – 1.40 (m, 2H), 1.39 – 1.22 (m, 8H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.9, 138.8, 128.4, 128.4, 128.2, 127.8, 127.6, 127.6, 127.6, 127.5, 81.7, 76.4, 74.7, 74.5, 73.2, 72.9, 64.7, 60.7, 51.5, 45.3, 42.7, 40.3, 29.7, 29.5, 29.1, 28.9, 27.3, 26.7 ppm. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{36}H_{46}N_4O_4$ 599.3592, found 599.3605.

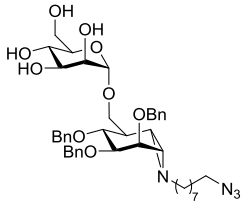
Compound 78



Donor **67**[42] (207 mg, 0.28 mmol) and acceptor **77** (139 mg, 0.23 mmol) were combined in a flask, co-evaporated with toluene (3x) and dissolved in dry DCM (2.3 mL). 4 Å molecular sieves were added, the mixture was stirred for 2 h and then cooled to -40 °C. TfOH (20.5 µL, 0.23 mmol) was added in three portions over 1 h and after stirring for additional 15 minutes the mixture was quenched by addition of Et_3N (100 µL). The mixture was diluted with DCM (50 mL), washed with sat. aq. $NaHCO_3$ (2 x 20 mL) and brine. The organic layer was dried over $MgSO_4$, filtrated and concentrated. Flash purification by silica column chromatography (pentane/EtOAc, 5:1) afforded the title compound as a colorless oil (201 mg, 74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (t, *J* = 8.6 Hz, 4H), 7.91 (d, *J* = 7.5 Hz, 2H), 7.83 (d, *J* = 7.5 Hz, 2H), 7.63 – 7.15 (m, 27H), 6.14 (t, *J* = 9.8 Hz, 1H), 5.92 (dd, *J* = 10.1, 3.2 Hz, 1H), 5.75 (d, *J* = 1.7 Hz, 1H), 5.17 – 5.07 (m, 1H), 4.95 (dd, *J* = 11.9, 2.6 Hz, 2H), 4.79 (d, *J* = 12.4 Hz, 1H), 4.75 – 4.60 (m, 3H), 4.57 (d, *J* = 11.5 Hz, 1H), 4.48 – 4.37 (m, 2H), 4.23 (s, 1H), 3.92 – 3.65 (m, 4H), 3.16 (t, *J* = 6.9 Hz, 2H), 2.42 – 2.28

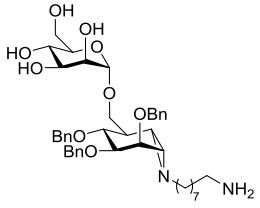
(m, 2H), 2.19 (dt, *J* = 11.7, 6.9 Hz, 1H), 1.90 – 1.80 (m, 1H), 1.68 (d, *J* = 5.9 Hz, 1H), 1.52 (d, *J* = 6.3 Hz, 4H), 1.40 – 1.20 (m, 8H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.2, 165.6, 165.4, 139.1, 139.0, 138.9, 133.6, 133.5, 133.3, 133.2, 130.0, 129.9, 129.8, 129.8, 129.4, 129.2, 129.1, 128.7, 128.6, 128.5, 128.5, 127.9, 127.9, 127.7, 127.6, 127.6, 97.6, 81.7, 75.4, 74.8, 74.5, 73.1, 73.0, 70.6, 70.3, 69.3, 69.2, 67.0, 62.8, 60.8, 51.5, 43.1, 42.9, 40.2, 29.8, 29.6, 29.2, 28.9, 27.4, 26.8 ppm. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{70}H_{72}N_4O_{13}$ 1177.5169, found 1177.5215.

Compound 80



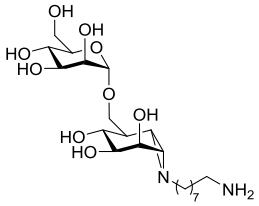
Compound **78** (198 mg, 0.17 mmol) was dissolved in a mixture of DCM/MeOH (1:1 v/v, 1.6 mL), NaOMe (5.4 M in MeOH, 16 µL, 84 µmol) was added and the mixture was stirred overnight at rt. The mixture was concentrated and flash purification by silica column chromatography (DCM/MeOH, 40:1 → 40:3) afforded the title compound as a colorless oil (101 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.07 (m, 15H), 4.91 – 4.84 (m, 2H), 4.82 (s, 1H), 4.70 (d, *J* = 12.3 Hz, 1H), 4.66 (d, *J* = 11.7 Hz, 1H), 4.60 (d, *J* = 11.7 Hz, 1H), 4.46 (d, *J* = 11.4 Hz, 1H), 4.14 (s, 1H), 3.96 (t, *J* = 9.6 Hz, 1H), 3.93 – 3.83 (m, 2H), 3.80 (d, *J* = 9.4 Hz, 1H), 3.73 (dd, *J* = 9.8, 2.6 Hz, 1H), 3.71 – 3.63 (m, 2H), 3.58 (t, *J* = 9.2 Hz, 1H), 3.52 (dd, *J* = 9.5, 3.5 Hz, 1H), 3.47 (d, *J* = 9.7 Hz, 1H), 3.22 (t, *J* = 7.0 Hz, 2H), 2.31 – 2.14 (m, 2H), 2.14 – 2.04 (m, 1H), 1.71 (dd, *J* = 5.7, 1.9 Hz, 1H), 1.57 (p, *J* = 6.9 Hz, 2H), 1.47 (d, *J* = 5.9 Hz, 1H), 1.45 – 1.35 (m, 2H), 1.36 – 1.17 (m, 8H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.0, 138.9, 128.5, 128.4, 127.9, 127.7, 127.6, 127.6, 100.2, 81.6, 74.8, 74.5, 73.1, 72.9, 72.6, 71.9, 71.2, 68.6, 66.2, 61.0, 60.6, 51.6, 43.1, 42.8, 40.3, 29.7, 29.6, 29.2, 28.9, 27.3, 26.8 ppm. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{42}H_{56}N_4O_9$ 761.4120, found 761.4150.

Compound 81



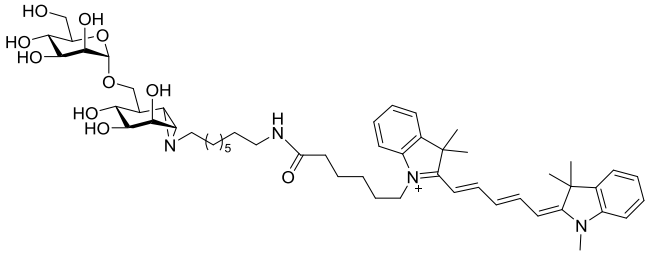
Compound **80** (98 mg, 0.13 mmol) was dissolved in MeCN (2.6 mL), polymer-bound triphenylphosphine (~3 mmol/g loading, 86 mg, 0.26 mmol) and H_2O (23 µL, 1.3 mmol) were added and the mixture was stirred overnight at 70 °C. Then, more H_2O (500 µL) was added and the mixture was stirred for 4 h at 70 °C. The mixture was filtrated and concentrated to afford the product in high purity as an oil (91 mg, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.16 (m, 15H), 4.89 (d, *J* = 11.8 Hz, 2H), 4.84 (s, 1H), 4.72 (d, *J* = 12.3 Hz, 1H), 4.65 (q, *J* = 11.8 Hz, 2H), 4.59 – 4.41 (m, 1H + NH2), 4.20 (s, 1H), 3.97 (dd, *J* = 22.0, 11.5 Hz, 2H), 3.87 (s, 1H), 3.81 – 3.66 (m, 3H), 3.63 – 3.43 (m, 4H), 2.64 (t, *J* = 7.1 Hz, 2H), 2.57 (dd, *J* = 11.6, 5.9 Hz, 1H), 2.21 (td, *J* = 9.3, 4.3 Hz, 1H), 1.76 (d, *J* = 5.5 Hz, 2H), 1.49 (d, *J* = 5.8 Hz, 1H), 1.48 – 1.37 (m, 4H), 1.35 – 1.20 (m, 8H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.0, 139.0, 138.8, 128.4, 128.4, 128.4, 128.0, 127.8, 127.6, 127.5, 127.5, 100.4, 81.9, 75.6, 74.9, 74.5, 73.2, 72.9, 72.8, 71.9, 71.2, 68.5, 66.2, 60.9, 60.8, 43.3, 42.7, 41.4, 40.4, 32.1, 29.8, 29.4, 28.9, 27.2, 26.6 ppm. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{42}H_{58}N_2O_9$ 735.4215, found 735.4224.

Compound 82



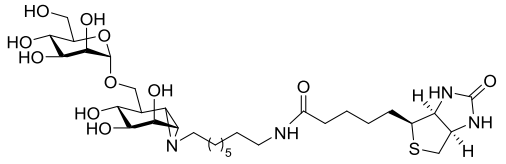
Ammonia (5 mL) was condensed at -60 °C, lithium (21 mg, 3.06 mmol) was added and the mixture was stirred for 30 minutes resulting in a deep blue solution. Compound **81** (45 mg, 61 µmol) dissolved in THF (1 mL) was added dropwise to this solution, the mixture was stirred for 1 hour at -60 °C and subsequently quenched by addition of water (1 mL). The mixture was evaporated, redissolved in H_2O (1 mL) and purified by elution over Amberlite CG-50 (NH_4^+) with NH_4OH (0.5 M) as eluent. The product fractions were combined and evaporated, then the product was redissolved in MeOH, filtrated over Celite and evaporated, affording the product as an oil (23 mg, 81%). ^{1}H NMR (400 MHz, D_2O) δ 4.88 (s, 1H), 4.31 (s, 1H), 3.94 (s, 1H), 3.89 – 3.76 (m, 3H), 3.76 – 3.66 (m, 2H), 3.66 – 3.56 (m, 2H), 3.47 – 3.31 (m, 2H), 2.96 – 2.85 (m, 2H), 2.48 – 2.30 (m, 1H), 2.26 – 2.09 (m, 1H), 1.99 – 1.85 (m, 2H), 1.77 (d, *J* = 5.9 Hz, 1H), 1.63 – 1.55 (m, 2H), 1.55 –1.45 (m, 2H), 1.38 –1.18 (m, 8H) ppm. ^{13}C NMR (101 MHz, D_2O) δ 99.6, 72.8, 71.1, 70.6, 70.0, 67.6, 67.5, 66.7, 66.0, 60.8, 59.7, 43.9, 42.5, 40.4, 39.5, 28.5, 28.0, 27.1, 26.4, 25.5 ppm. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{21}H_{40}N_2O_9$ 465.2807, found 465.2810.

Compound 83 (SY-G74)



Compound **82** (8.5 mg, 18.2 µmol) was dissolved in DMF (0.5 mL), then DIPEA (6.3 µL, 36.4 µmol) and Cy5-OSu[46] (12.3 mg, 20.0 µmol) was added and the mixture was stirred overnight. The mixture was diluted with H_2O (0.5 mL) and the resulting crude was purified by HPLC (NH_4HCO_3) affording the product as a blue solid (5.2 mg, 29%). ^{1}H NMR (500 MHz, D_2O) δ 7.81 – 7.72 (m, 2H), 7.37 (t, *J* = 6.4 Hz, 2H), 7.26 (q, *J* = 7.1 Hz, 2H), 7.19 (dd, *J* = 10.8, 8.2 Hz, 2H), 7.11 (q, *J* = 7.3 Hz, 2H), 6.34 (t, *J* = 12.5 Hz, 1H), 6.03 (dd, *J* = 18.9, 13.5 Hz, 2H), 4.83 (d, *J* = 1.4 Hz, 1H), 4.25 (dd, *J* = 3.2, 1.7 Hz, 1H), 4.01 (br s, 2H), 3.89 (dd, *J* = 3.3, 1.7 Hz, 1H), 3.84 (dd, *J* = 12.2, 2.1 Hz, 1H), 3.82 – 3.70 (m, 3H), 3.67 – 3.61 (m, 2H), 3.61 – 3.56 (m, 1H), 3.52 (s, 3H), 3.43 (t, *J* = 10.0 Hz, 1H), 3.36 (dd, *J* = 10.5, 3.5 Hz, 1H), 2.97 (t, *J* = 7.0 Hz, 2H), 2.22 – 2.10 (m, 3H), 2.01 – 1.94 (m, 1H), 1.88 – 1.84 (m, 1H), 1.82 (d, *J* = 4.6 Hz, 1H), 1.80 – 1.75 (m, 2H), 1.65 (d, *J* = 6.1 Hz, 1H), 1.63 – 1.54 (m, 2H), 1.44 (s, 6H), 1.41 (s, 6H), 1.38 – 1.21 (m, 8H), 1.15 – 1.02 (m, 8H) ppm. ^{13}C NMR (125 MHz, D_2O) δ 175.9, 173.5, 173.1, 153.1, 142.7, 142.0, 141.1, 141.0, 128.5, 125.1, 124.3, 122.3, 110.9, 110.7, 103.1, 102.9, 99.6, 72.9, 71.1, 70.8, 70.2, 67.6, 67.4, 66.8, 66.1, 60.9, 59.7, 49.0, 48.9, 44.1, 43.6, 42.5, 40.6, 39.4, 35.5, 30.8, 29.0, 28.6, 28.5, 28.4, 27.0, 26.9, 26.8, 26.6, 26.3, 25.5, 25.1, 22.9 ppm. HRMS (ESI) m/z: $[M]^+$ calc for $C_{53}H_{77}N_4O_{10}$ 930.5712, found 930.5668.

Compound 84 (SY-G73)



Compound **82** (6.6 mg, 14.3 μmol) was dissolved in DMF (0.5 mL), then DIPEA (5 μL, 28.5 μmol) and biotin-OSu[47] (5.4 mg, 15.7 μmol) was added and the mixture was stirred overnight. The mixture was diluted with H_2O (0.5 mL) and the resulting crude was purified by HPLC (NH_4HCO_3) affording the product as a white solid (5.1 mg, 52%). ^{1}H NMR (500 MHz, D_2O) δ 4.90 (d, *J* = 1.4 Hz, 1H), 4.58 (dd, *J* = 7.9, 4.8 Hz, 1H), 4.39 (dd, *J* = 7.9, 4.5 Hz, 1H), 4.35 – 4.28 (m, 1H), 3.96 (dd, *J* = 3.3, 1.7 Hz, 1H), 3.89 – 3.78 (m, 3H), 3.76 – 3.69 (m, 2H), 3.67 – 3.58 (m, 2H), 3.44 (dd, *J* = 19.3, 9.7 Hz, 1H), 3.41 – 3.37 (m, 1H), 3.30 (dt, *J* = 9.8, 5.4 Hz, 1H), 3.21 – 3.10 (m, 2H), 2.97 (dd, *J* = 13.0, 5.0 Hz, 1H), 2.75 (d, *J* = 13.0 Hz, 1H), 2.41 (dt, *J* = 13.4, 6.8 Hz, 1H), 2.25 – 2.18 (m, 3H), 2.01 – 1.93 (m, 2H), 1.81 (d, *J* = 6.1 Hz, 1H), 1.74 – 1.55 (m, 4H), 1.55 – 1.43 (m, 4H), 1.42 – 1.33 (m, 2H), 1.33 – 1.23 (m, 8H) ppm. ^{13}C NMR (125 MHz, D_2O) δ 176.6, 99.7, 73.0, 71.2, 70.7, 70.1, 67.7, 67.6, 66.8, 66.2, 62.1, 61.0, 60.3, 59.8, 55.5, 44.1, 42.6, 40.6, 39.8, 39.3, 35.6, 28.8, 28.7, 28.3, 28.3, 27.9, 27.7, 26.5, 26.0, 25.3 ppm. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{31}H_{54}N_4O_{11}S$ 691.3583, found 691.3608.

References

1 S. Atsumi, K. Umezawa, H. Iinuma, H. Naganawa, H. Nakamura, Y. Iitaka and T. Takeuchi, *J. Antibiot.*, 1989, **43**, 49–53.

2 G. Caron and S. G. Withers, *Biochem. Biophys. Res. Commun.*, 1989, **163**, 495–499.

3 W. W. Kallemeijn, K. Y. Li, M. D. Witte, A. R. A. Marques, J. Aten, S. Scheij, J. Jiang, L. I. Willems, T. M. Voorn-Brouwer, C. P. A. A. van Roomen, R. Ottenhoff, R. G. Boot, H. van den Elst, M. T. C. Walvoort, B. I. Florea, J. D. C. Codée, G. A. van der Marel, J. M. F. G. Aerts and H. S. Overkleeft, *Angew. Chem. Int. Ed.*, 2012, **51**, 12529–12533.

4 J. Jiang, C. L. Kuo, L. Wu, C. Franke, W. W. Kallemeijn, B. I. Florea, E. van Meel, G. A. van der Marel, J. D. C. Codée, R. G. Boot, G. J. Davies, H. S. Overkleeft and J. M. F. G. Aerts, *ACS Cent. Sci.*, 2016, **2**, 351–358.

5 J. Jiang, W. W. Kallemeijn, D. W. Wright, A. M. C. H. van den Nieuwendijk, V. C. Rohde, E. C. Folch, H. van den Elst, B. I. Florea, S. Scheij, W. E. Donker-Koopman, M. Verhoek, N. Li, M. Schürmann, D. Mink, R. G. Boot, J. D. C. Codée, G. A. van der Marel, G. J. Davies, J. M. F. G. Aerts and H. S. Overkleeft, *Chem. Sci.*, 2015, **6**, 2782–2789.

6 L. Wu, J. Jiang, Y. Jin, W. W. Kallemeijn, C.-L. Kuo, M. Artola, W. Dai, C. van Elk, M. van Eijk, G. A. van der Marel, J. D. C. Codée, B. I. Florea, J. M. F. G. Aerts, H. S. Overkleeft and G. J. Davies, *Nat. Chem. Biol.*, 2017, **13**, 867–873.

7 L. I. Willems, T. J. M. Beenakker, B. Murray, S. Scheij, W. W. Kallemeijn, R. G. Boot, M. Verhoek, W. E. Donker-Koopman, M. J. Ferraz, E. R. Van Rijssel, B. I. Florea, J. D. C. Codée, G. A. Van Der Marel, J. M. F. G. Aerts and H. S. Overkleeft, *J. Am. Chem. Soc.*, 2014, **136**, 11622–11625.

8 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.

9 G. Luchetti, K. Ding, M. D'Alarcao and A. Kornienko, *Synthesis*, 2008, 3142–3147.

10 B. P. Rempel and S. G. Withers, *Glycobiology*, 2008, **18**, 570–586.

11 J. M. Hernández-Torres, J. Achkar and A. Wei, *J. Org. Chem.*, 2004, **69**, 7206–7211.

12 A. G. Volbeda, H. A. V. Kistemaker, H. S. Overkleeft, G. A. van der Marel, D. V. Filippov and J. D. C. Codée, *J. Org. Chem.*, 2015, **80**, 8796–8806.

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

14 Z. You, *Tetrahedron Lett.*, 1993, **34**, 2597–2600.

15 H. Ovaa, B. Lastdrager, J. D. C. Codeé, G. A. van der Marel, H. S. Overkleeft and J. H. van Boom, *J. Chem. Soc. Perkin Trans. 1*, 2002, 2370–2377.

16 T. M. Gloster, P. Meloncelli, R. V. Stick, D. Zechel, A. Vasella and G. J. Davies, *J. Am. Chem. Soc.*, 2007, **129**, 2345–2354.

17 N. Panday, Y. Canac and A. Vasella, *Helv. Chim. Acta*, 2000, **83**, 58–79.

18 S. M. McElvain, R. E. Kent and C. L. Stevens, *J. Am. Chem. Soc.*, 1946, **68**, 1922–1925.

19 P. Lang, G. Magnin, G. Mathis, A. Burger and J. F. Biellmann, *J. Org. Chem.*, 2000, **65**, 7825–7832.

20 A. A. Van Aerschot, P. Mamos, N. J. Weyns, S. Ikeda, E. De Clercq and P. A. Herdewijn, *J. Med. Chem.*, 1993, **36**, 2938–2942.

21 Y. Harrak, C. M. Barra, A. Delgado, A. R. Castaño and A. Llebaria, *J. Am. Chem. Soc.*, 2011, **133**, 12079–12084.

22 N. Brunetti-Pierri and F. Scaglia, *Mol. Genet. Metab.*, 2008, **94**, 391–396.

23 A. Caciotti, S. C. Garman, Y. Rivera-Colón, E. Procopio, S. Catarzi, L. Ferri, C. Guido, P. Martelli, R. Parini, D. Antuzzi, R. Battini, M. Sibilio, A. Simonati, E. Fontana, A. Salviati, G. Akinci, C. Cereda, C. Dionisi-Vici, F. Deodato, A. d'Amico, A. d'Azzo, E. Bertini, M. Filocamo, M. Scarpa, M. di Rocco, C. J. Tifft, F. Ciani, S. Gasperini, E. Pasquini, R. Guerrini, M. A. Donati and A. Morrone, *Biochim. Biophys. Acta - Mol. Basis Dis.*, 2011, **1812**, 782–790.

24 K. Higaki, L. Li, U. Bahrudin, S. Okuzawa, A. Takamuram, K. Yamamoto, K. Adachi, R. C. Paraguison, T. Takai, H. Ikehata, L. Tominaga, I. Hisatome, M. Iida, S. Ogawa, J. Matsuda, H. Ninomiya, Y. Sakakibara, K. Ohno, Y. Suzuki and E. Nanba, *Hum. Mutat.*, 2011, **32**, 843–852.

25 A. J. Thompson, R. J. Williams, Z. Hakki, D. S. Alonzi, T. Wennekes, T. M. Gloster, K. Songsrirote, J. E. Thomas-Oates, T. M. Wrodnigg, J. Spreitz, A. E. Stutz, T. D. Butters, S. J. Williams and G. J. Davies, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 781–786.

26 C. Braun, T. Lindhorst, N. B. Madsen and S. G. Withers, *Biochemistry*, 1996, **35**, 5458–5463.

27 H. D. Ly, S. Howard, K. Shum, S. He, A. Zhu and S. G. Withers, *Carbohydr. Res.*, 2000, **329**, 539–547.

28 J. C. Díaz Arribas, A. G. Herrero, M. Martín-Lomas, F. Javier Cañada, S. He and S. G. Withers, *Eur. J. Biochem.*, 2000, **267**, 6996–7005.

29 S. S. Lee, S. He and S. G. Withers, *Biochem. J.*, 2001, **359**, 381–386.

30 L. Ziser and S. G. Withers, *Carbohydr. Res.*, 1994, **265**, 9–17.

31 T. H. Fife and T. J. Przystas, *J. Am. Chem. Soc.*, 1980, **102**, 292–299.

32 V. L. Y. Yip and S. G. Withers, *Angew. Chem. Int. Ed.*, 2006, **45**, 6179–6182.

33 F. M. Ibatullin, S. I. Selivanov and A. G. Shavva, *Synthesis*, 2001, **2001**, 419–422.

34 T. Belz, Y. Jin, J. Coines, C. Rovira, G. J. Davies and S. J. Williams, *Chem. Commun.*, 2017, **53**, 9238–9241.

35 S. Ghosh, P. Tiwari, S. Pandey, A. K. Misra, V. Chaturvedi, A. Gaikwad, S. Bhatnagar and S. Sinha, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 4002–4005.

36 A. J. Thompson, F. Cuskin, R. J. Spears, J. Dabin, J. P. Turkenburg, H. J. Gilbert and G. J. Davies, *Acta Crystallogr. Sect. D Biol. Crystallogr.*, 2015, **71**, 408–415.

37 F. Cuskin, E. C. Lowe, M. J. Temple, Y. Zhu, E. A. Cameron, N. A. Pudlo, N. T. Porter, K. Urs, A. J. Thompson, A. Cartmell, A. Rogowski, B. S. Hamilton, R. Chen, T. J. Tolbert, K. Piens, D. Bracke, W.

Vervecken, Z. Hakki, G. Speciale, J. L. Munōz-Munōz, A. Day, M. J. Peña, R. McLean, M. D. Suits, A. B. Boraston, T. Atherly, C. J. Ziemer, S. J. Williams, G. J. Davies, D. W. Abbott, E. C. Martens and H. J. Gilbert, *Nature*, 2015, **520**, 388–388.

38 J. Ao, J. L. Chinnici, A. Maddi and S. J. Free, *Eukaryot. Cell*, 2015, **14**, 792–803.

39 A. Maddi, C. Fu and S. J. Free, *PLoS One*, 2012, **7**, e38872.

40 H. Kitagaki, H. Wu, H. Shimoi and K. Ito, *Mol. Microbiol.*, 2002, **46**, 1011–1022.

41 C. S. Wong, Thesis: The Synthesis of Mannose-derived Bioconjugates and Enzyme Inhibitors; Leiden University, 2015.

42 B. Becker, R. H. Furneaux, F. Reck and O. a. Zubkov, *Carbohydr. Res.*, 1999, **315**, 148–158.

43 H. Franzyk, M. Meldal, H. Paulsen and K. Bock, *J. Chem. Soc. Perkin Trans. 1*, 1995, 2883–2898.

44 T. J. Rabelink, B. M. van den Berg, M. Garsen, G. Wang, M. Elkin and J. van der Vlag, *Nat. Rev. Nephrol.*, 2017, **13**, 201–212.

45 V. Bordoni, V. Porkolab, S. Sattin, M. Thepaut, I. Frau, L. Favero, P. Crotti, A. Bernardi, F. Fieschi and V. Di Bussolo, *RSC Adv.*, 2016, **6**, 89578–89584.

46 M. V. Kvach, A. V. Ustinov, I. A. Stepanova, A. D. Malakhov, M. V. Skorobogatyi, V. V. Shmanai and V. A. Korshun, *Eur. J. Org. Chem.*, 2008, 2107–2117.

47 K. Susumu, H. T. Uyeda, I. L. Medintz, T. Pons, J. B. Delehanty and H. Mattoussi, *J. Am. Chem. Soc.*, 2007, **129**, 13987–13996.