Exploring chemical space in covalent and competitive glycosidase inhibitor design

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A New Generation of Fluorescent Activity-Based Probes Targeting Starch-Degrading Glycosidases

3.1 Introduction

Starch is a main carbohydrate storage product of many plants and is an important constituent of the human diet. Starch polysaccharides are composed of two types of molecules – amylose and amylopectin (Figure 3.1).[1,2] Amylose, constituting about 20-25% of the starch content depending on the source, is a linear polymer chain consisting of up to 6,000 glucopyranose units linked through α-1,4-glucosidic bonds (Figure 3.1A). Amylopectin, constituting 75-80% of common starch, is composed of short α-1,4-linked linear chains of 10–60 glucose units and is highly branched at the α-1,6 positions (Figure 3.1B). Due to its intrinsic advantages of renewability, biodegradability, high abundance and low cost, starch has attracted significant attention from academic and industrial researchers alike and is currently used in numerous industrial applications. Besides its predominant use in food industry, starch, either in its native form or in the chemically or enzymatically modified form, has been widely applied as well in textile industries as wrap sizing agents, in paper industries as adhesives, in pharmaceutical industries as a drug carrier, and also in bioplastic producing and construction industries.[3-5]

Starch can be biodegraded by a cooperative set of enzymes, which based on their mode of actions, are divided into three groups: endoamylases (α-amylases), exoamylases (β-amylases, γ-amylases and α-glucosidases) and debranching enzymes (isoamylases and pullulanases).[6] Most of the enzymes belong to one single glycoside hydrolase (GH) family – the GH13 family, also known as the α-amylase family – based on the Carbohydrate-Active enZyme (CAZy, www.cazy.org) classification.[7] Starch-degrading enzymes are widely present in animals, plants and microorganisms. However, enzymes from microbial sources are the most preferred one for large-scale production mainly due to the advantages of cost effectiveness, easy availability, and ease of process manipulation.[8] So far, a large number of microbial starch-processing enzymes, especially retaining α-amylases which initiate the hydrolysis of starch by hydrolytic cleavage of internal α-1,4-glucosidic linkages randomly and destroy the whole starch structure quickly, are commercially available and have been used as catalysts in various industry sectors including food processing, the production of detergents, textile production, and also in paper and biofuel industries.[9-13]

The prevailing conditions in many industrial applications where the enzymes are used are rather extreme, especially with respect to temperature and pH. Considerable efforts have been made to improve the activity and stability of the enzymes in order to meet the requirements set by specific applications. One approach is to screen for novel microbial strains that have adapted to extreme environmental conditions, which has led to the identification of many extremophilic

enzymes with potential starch-degrading activities.[14-17] Another approach involves the use of protein engineering methods to create enzyme variants with increased activity/stability, altered pH optimum, or improved product specificity at given industrial process conditions.[18-20] Activity-based protein profiling (ABPP) has proven to be a powerful method for rapid detection and identification of enzyme activities of interest in their native environment. The work described in the previous chapter has revealed the efficacy of this methodology by developing maltobiose-configured activity-based probes (ABPs) (compound **1**, Figure 3.1C) to investigate retaining α-amylases in complex biological samples (Chapter 2). However, complete and efficient degradation of starch requires synergistic action of a set of enzymes, especially for amylopectin, of which the branching points containing α-1,6-glucosidic linkages are resistant to attack by α-1,4-specific amylases. In this chapter, the synthesis of two types of glucose-isomaltose (GIM) and isomaltose-glucose (IMG) configured pseudotrisaccharide ABPs is described (compound **2** and **3**, Figure 3.1C). The new probes are based on the reported pseudodisaccharide ABP **1**, which is substituted with a glucopyranose residue at the O6 position of either *epi*-cyclophellitol or non-reducing end sugar to mimic branched parts of the amylopectin structure. These probes may serve as tools for the discovery of industrially relevant starch-degrading enzymes that can accommodate α-1,6 branches in the -1 or -2 subsites.[21,22]

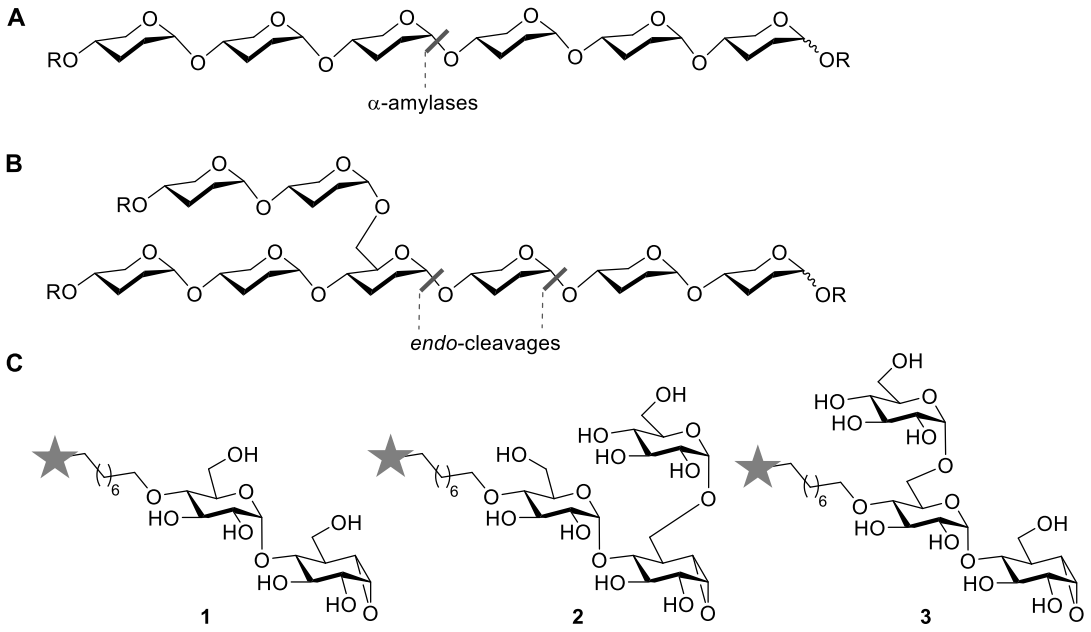
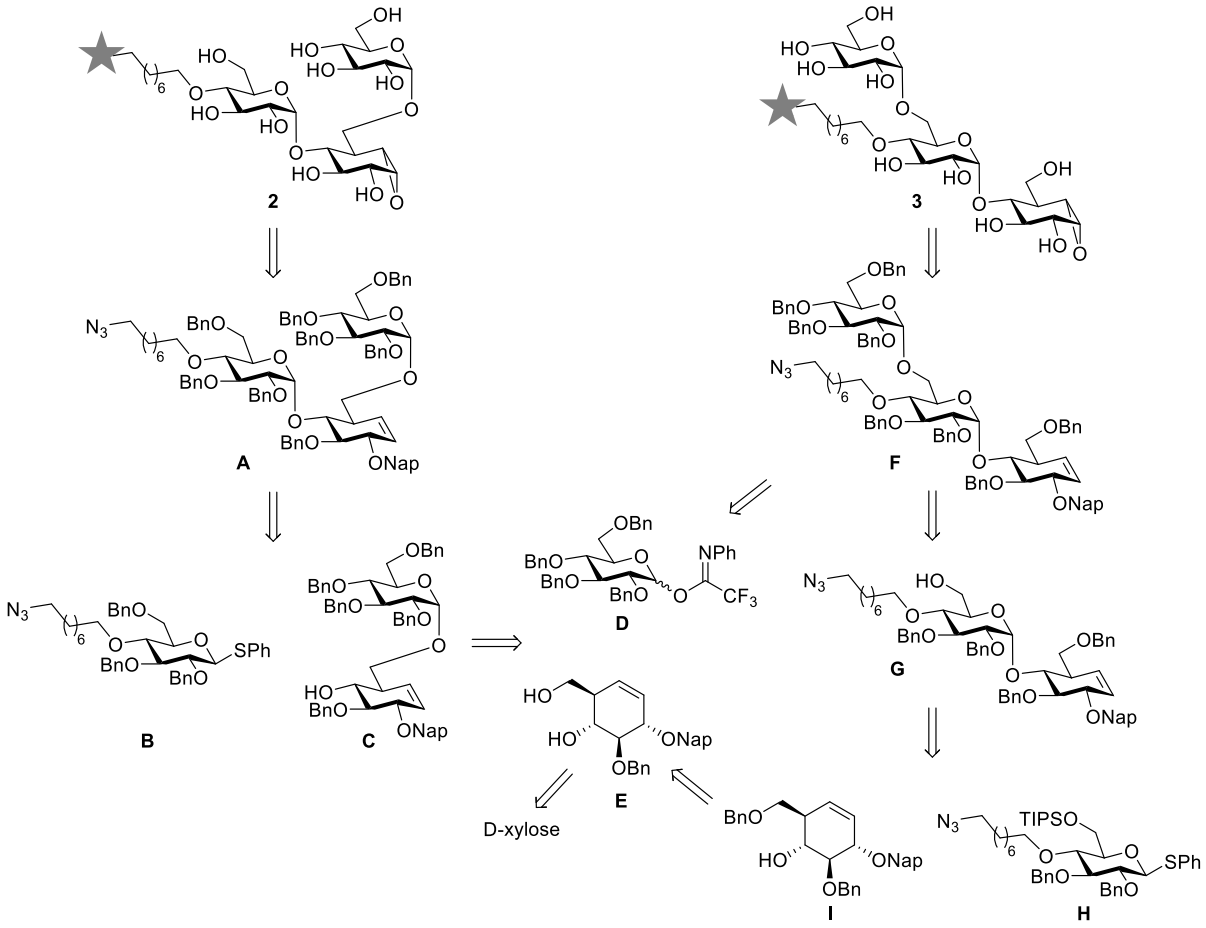


Figure 3.1. A) Chemical structure of linear amylose; B) Chemical structure of branched amylopectin; C) Structures of activity-based probes (ABPs) mimicking parts of the starch structure. Previously synthesized ABPs that target α-amylases (**1**) and new ABPs described in this chapter (**2** and **3**) that potentially target starch-degrading enzymes with potential preference for branched amylopectin-type polysaccharides.

For the preparation of the branched GIM (**2**) and IMG (**3**) configured *epi*-cyclophellitol based ABPs a synthesis strategy based on the use of three building blocks was devised (Scheme 3.1). It was envisioned that selective attachment of a reporter entity (a fluorophore or biotin) at the non-reducing end via amide coupling could be achieved by introduction of an azidooctyl group at the O4' position in an early stage. The epoxide functionality is introduced after the construction of pseudotrisaccharidic backbone to allow flexibility in the glycosylation conditions. Orthogonal protection at O2 of cyclohexene **E** with a naphthyl (Nap) ether group would allow stereoselective epoxidation after its removal following elaboration towards trisaccharide **A** or **F**. Benzyl ether protection of the remaining alcohols would allow global deprotection in one step at the end of the synthesis, and these non-participating protecting groups promote 1,2-*cis* glycosylation under the right glycosylation conditions.



Scheme 3.1. Retrosynthetic analysis of GIM (**2**) and IMG (**3**) configured probes.

Trisaccharide **A** is first disconnected into O4-alkylated thioglucoside donor **B** and pseudodisaccharide acceptor **C**. Compound **C** would be accessible from cyclohexene building block **E** by regio- and stereoselective glucosylation of the primary alcohol with per-benzylated glucosyl imidate donor **D**. Cyclohexene **E** is accessible from D-xylose based on chemistry

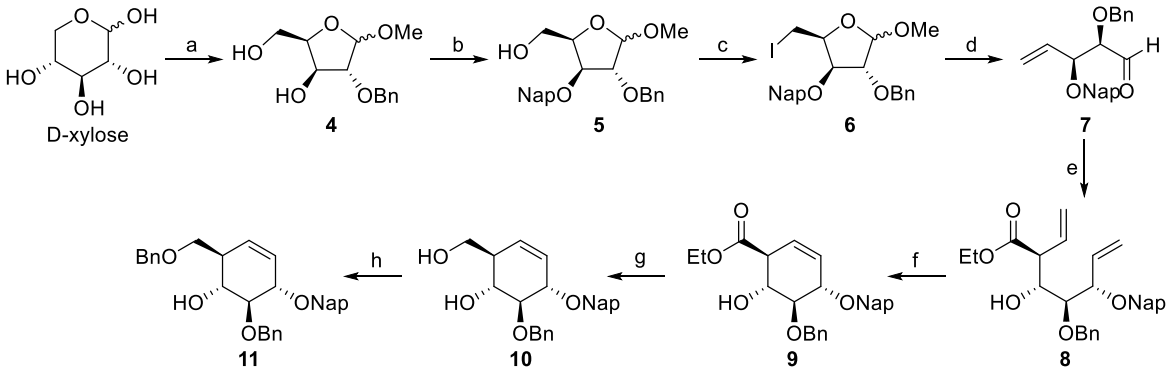
developed by Madsen and co-workers.[23,24] Similarly, trisaccharide **F** can be accessed by 1,2-*cis* glucosylation with imidate donor **D** and pseudodisaccharide acceptor **G**. Compound **G** can be synthesized by glycosylation of cyclohexene **I** with thioglucoside donor **H**, followed by desilylation. Acceptor **I** would be available from **E** by regioselective benzylation.

3.2 Results and discussion

3.2.1 Synthesis of IMG configured ABPs

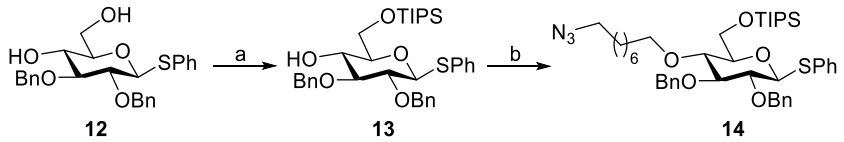
In previous work on the synthesis of maltobiose 1,6-*epi*-cyclophellitol ABPs (Chapter 2), a cyclohexene acceptor protected with a 4-methoxybenzyl (PMB) group at O6 was used for α-1,4 glycosylation reactions with different glucoside donors. Selective deprotection of the PMB ether in the resulting pseudodisaccharide followed by epoxidation with *meta*-chloroperoxybenzoic acid (*m*-CPBA) afforded an α/β mixture of epoxides (α/β as in the anomeric configuration of 'real' carbohydrates; which in terms of cyclophellitol nomenclature would translate to 'cyclophellitol/*epi*-cyclophellitol') which could be separated after silylation of O6. This key silylation step proved however not compatible with the target pseudotrisaccharidic epoxides, therefore a new cyclohexene acceptor **11** was synthesized (Scheme 3.2), in which the Nap ether can be selectively removed in a later stage to release the homoallylic alcohol for diastereoselective introduction of the desired α-epoxide.

The synthesis commenced with commercially available D-xylose, which was transformed into **4** via a four-step procedure. First, kinetic Fischer glycosylation of D-xylose afforded methyl xylofuranoside, which was then protected with a 3,5-benzylidene acetal. Benzylation of the remaining secondary alcohol followed by deprotection of the benzylidene acetal using a catalytic amount of *p*-toluenesulfonic acid (TsOH) yielded intermediate **4** in 71% over four steps. The primary alcohol in **4** was temporarily protected with a trityl group, after which the secondary alcohol was protected as a Nap ether followed by detritylation to afford intermediate **5**. Transformation of compound **5** to **10** follows procedures developed by Madsen and co-workers.[23,24] Iodination of the primary alcohol in **5** provided iodide **6**, which after Vasella fragmentation (**6** to **7**) and Barbier addition yielded diene **8**. Ring-closing metathesis of **8** was achieved by treatment with 3.5 mol-% Grubb's II catalyst, resulting in compound **9** in good yield. The ethyl ester was first reduced to the aldehyde by treatment with DIBAL-H, followed by a second reduction with $NaBH_4$ to afford diol **10**. Selective benzylation of the primary alcohol in **10** using borinate catalysis[25] yielded cylcohexene **11** in excellent yield.



Scheme 3.2. Synthesis of cyclohexene acceptor **11**. Reagents and conditions: a) *i*) acetyl chloride, MeOH, rt; *ii*) $PhCH(OMe)_2$, CSA, DMF, rt; *iii*) BnBr, NaH, TBAI, DMF, 0 °C to rt; *iv*) TsOH, DCM/MeOH, rt, 71% over 4 steps; b) *i*) TrCl, Et_3N, DMAP, DMF, rt; *ii*) NapBr, NaH, TBAI, DMF, 0 °C to rt; *iii*) TsOH, DCM/MeOH, rt, 81% over 3 steps; c) I_2, PPh_3, imidazole, THF, reflux, 95%; d) Zn, THF/H_2O, ultrasound, 40 °C, 85%; e) ethyl 4-bromocrotonate, indium powder, $La(OTf)_3$, H_2O, rt, 61%; f) Grubb's II catalyst, DCM, 40 °C, 85%; g) *i*) DIBAL-H, THF, 0 °C to rt; *ii*) $NaBH_4$, H_2O, EtOAc, rt, 87% over 2 steps; h) BnBr, 2-aminoethyl diphenylborinate, KI, K_2CO_3, MeCN, 60 °C, 95%.

To allow modification of trisaccharide inhibitors at the O4' position with reporter entities, a O4-alkylated donor **14** was synthesized (Scheme 3.3). Attempts to selectively protect the primary alcohol of diol **12**[26] as a PMB ether using borinate catalysis led to the formation of multiple side products. As an alternative, a silyl ether was chosen for selective protection of the primary alcohol. Reaction of **12** with triisopropylsilyl chloride (TIPSCl) proceeded smoothly, resulting in **13** in excellent yield. Subsequent alkylation of the remaining secondary alcohol was performed with an excess (4.0 eq.) of 1-azido-8-iodooctane in the presence of sodium hydride (NaH), giving product **14** in high yield. The TIPS ether was chosen over a *tert*-butyldiphenylsilyl (TBDPS) ether as the latter was found to be unstable during alkylation in the presence of strong base.



Scheme 3.3. Synthesis of thioglucoside donor **14**. Reagents and conditions: a) TIPSCl, imidazole, DCM, 0 °C to rt, 97%; b) 1-azido-8-iodooctane, NaH, 18-crown-6, DMF, 0 °C to rt, 94%.

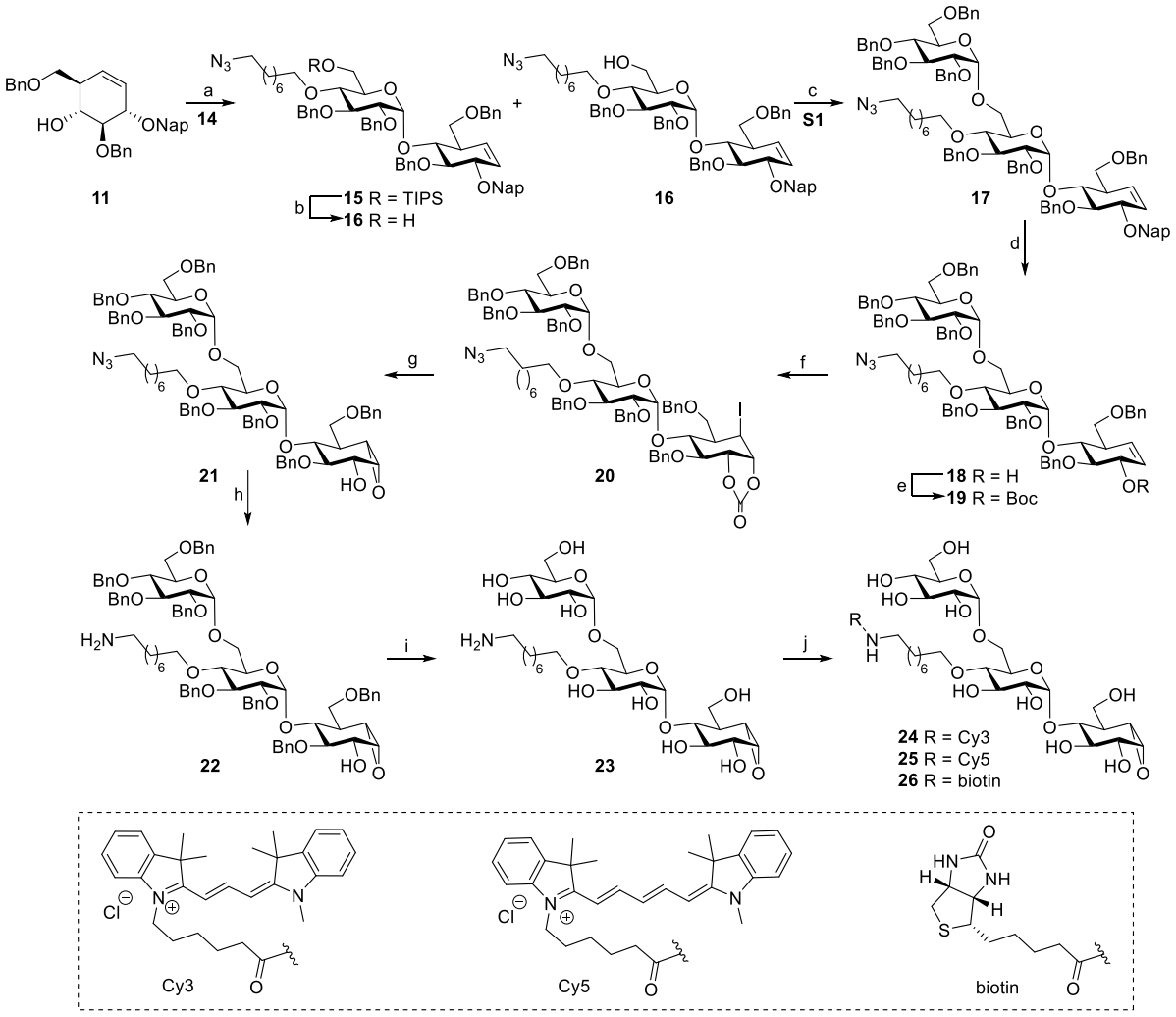
With donor **14** and acceptor **11** in hand, the synthesis of IMG probes was investigated (Scheme 3.4). Acceptor **11** was glycosylated with donor **14** under *N*-iodosuccinimide (NIS), trifluoromethanesulfonic acid (TMSOTf) and *N*,*N*-dimethyl formamide (DMF) activating conditions, affording pseudodisaccharide **15** in good α-selectivity along with the desilylated

product **16** which was formed due to prolonged reaction times (45 h) in the presence of stoichiometric amounts of Lewis acid (TMSOTf). The TIPS ether in **15** was then removed by TBAF and the so-obtained **16** was combined with that formed in the previous step. The second α-selective glycosylation on the primary alcohol of **16** was achieved by reacting with per-benzylated glucosyl imidate donor **S1** (Chapter 2) under TMSI/$OPPh_3$ conditions, yielding pseudotrisaccharide **17** in good yield. The Nap ether was removed with DDQ to provide the allylic alcohol **18**. Direct epoxidation of **18** with *m*-CPBA at 0 °C afforded an inseparable mixture of α/β epoxides in a 3/1 ratio. To improve the stereoselectivity of the reaction, an iodocarbonylation approach was employed.[27-30] *t*-Butyloxylcarbonyl (Boc) protection of the secondary alcohol afforded **19**. It was found that unreacted starting material **18** can readily react with product **19** by attacking the carbonyl group of the Boc in **19** to form a dimer side product, and an excess (15 eq.) of di-*tert*-butyl dicarbonate was needed to suppress this side reaction. Compound **19** was then reacted with NIS to yield iodocarbonate **20** with complete stereospecificity. Hydrolysis of the carbonate by sodium methoxide in methanol and subsequent intramolecular iodine displacement afforded α-epoxide **21**. The azido group was reduced by Staudinger reduction resulting in amine **22**, which was then debenzylated using Pearlman's catalyst ($Pd(OH)_2/C$) under hydrogen atmosphere, affording **23** in quantitative yield.

To ensure that the latter $Pd(OH)_2/C$ catalyzed hydrogenation reaction proceeded smoothly, several key factors need to be taken into account, including the use of a suitable solvent system, reactant concentration, catalyst poisoning by the amine, reaction time and a way to monitor the reaction process. In this case, a solvent mixture of H_2O/dioxane (1/3) was employed. The higher proportion of dioxane was needed to dissolve the lipophilic starting material and partially deprotected intermediates. At the same time, a low reactant concentration, of around 0.015 M, ensured that there was enough water to dissolve all hydrophilic products. For different substrates, the ratio of solvents or the reaction concentration may need to be adapted accordingly. To avoid catalyst poisoning by the free amine, an equivalent (relative to the reactant) amount of trifluoroacetic acid (TFA) was added to capture the amine by protonation. Of note, oxiranes are known to undergo reductive opening of the strained three-membered ring under palladium catalysis,[31] and they are prone to hydrolysis in the presence of strong acid, however the epoxide functionality remained unaffected by employing stoichiometric amounts of Pearlman's catalyst to minimize (around 3 h) reaction times. Direct transformation from azide **21** to **23** using the same hydrogenation conditions as for the conversion of **22** proved to be sluggish, and TLC-analysis indicated no further conversion of intermediates after 2 h, which

was presumably due to the emerging amine that poisoned palladium catalyst prior to being captured by TFA.

Returning to the synthesis of the target IMG probes, Cy3COOH, Cy5COOH or biotin were pre-activated as their pentafluorophenyl esters and directly coupled with the primary amine **23** to yield ABPs **24-26** after semi-preparative HPLC purification.

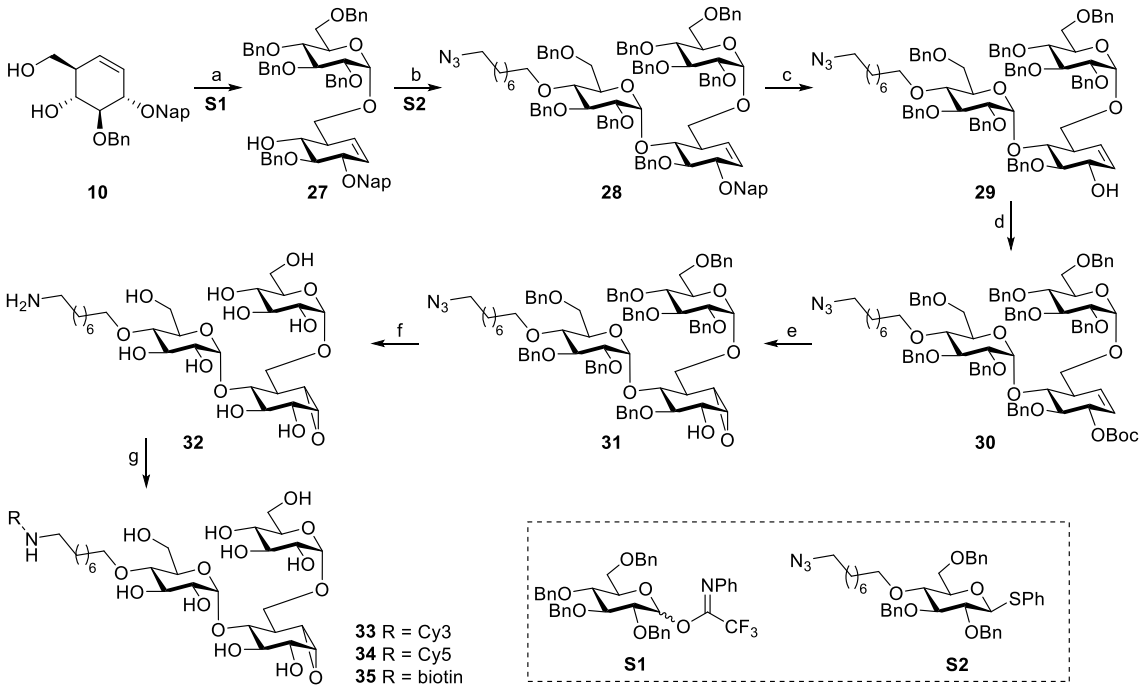


Scheme 3.4. Synthesis of ABPs **24-26**. Reagents and conditions: a) **14**, NIS, TMSOTf, DMF, DCM, 3 Å MS, 0 °C, **15** 52%, **16** 32%; b) TBAF, THF, rt, 87%; c) **S1**, TMSI, $OPPh_3$, 3 Å MS, DCM, rt, 82%; d) DDQ, DCM/H_2O (10/1), rt; e) Boc_2O, DMAP, THF, 0 °C to rt, 75% over 2 steps; f) NIS, AcOH, DCM, rt, 61%; g) NaOMe, MeOH, DCM, rt, 81%; h) polymer-bound PPh_3, MeCN, H_2O, 65 °C, 88%; i) $Pd(OH)_2/C$, TFA, H_2, dioxane, H_2O, rt, quant; j) Cy3COOH or Cy5COOH or biotin, pentafluorophenyl trifluoroacetate, DIPEA, DMF, Cy3 40%, Cy5 55%, biotin 38%.

3.2.2 Synthesis of GIM configured ABPs

The synthesis of GIM configured probes follows a strategy similar to that employed for the synthesis of the IGM configured probes as outlined in the previous section. Acceptor **10** was

glycosylated with imidate donor **S1** in a TMSI/$OPPh_3$ mediated reaction, leading to the regio- and stereoselective generation of **27** in 65% yield (Scheme 3.5). The second glycosylation of acceptor **27** with O4-alkylated thioglucoside donor **S2** (Chapter 2) was accomplished employing the same pre-activation protocol as for the synthesis of **15**, affording pseudotrisaccharide **28** in good yield and selectivity. Elaboration of **28** to epoxide **31** follows the same procedures as those developed for **21**. Deprotection of the Nap ether with DDQ (**28** to **29**) and subsequent Boc protection resulted in product **30**. Conversion of **30** into cyclic iodocarbonate with NIS was not complete after 22 h and prolonged reaction times were not tried because it was reasoned that these would lead to deprotection of the Boc group in the presence of HOAc. The carbonate intermediate and the starting material **30** were difficult to separate by standard column chromatography, therefore after work-up the resulting crude mixture was directly used for base hydrolysis, stereospecifically affording epoxide **31** in 46% over two steps, while unreacted **30** was recovered as well. Azide reduction and global debenzylation of **31** were performed under Birch conditions, affording compound **32** after desalting by size exclusion chromatography over HW40. Sodium hydroxide, formed while quenching the Birch reaction, was neutralized with HOAc. Omission of this neutralization step can lead to hydrolysis of the epoxide during concentration of the reaction mixture in the presence of aqueous base. Fully deprotected amine **32** was then coupled with the corresponding pentafluorophenyl activated esters of Cy3COOH, Cy5COOH or biotin, yielding ABPs **33**-**35** after HPLC purification.



Scheme 3.5. Synthesis of ABPs **33**-**35**. Reagents and conditions: a) **S1**, TMSI, $OPPh_3$, 3 Å MS, DCM, rt, 65%; b) **S2**, NIS, TMSOTf, DMF, DCM, 3 Å MS, 0 °C, 78%; c) DDQ, DCM/H_2O (10/1), rt, 76%; d) Boc_2O, DMAP, THF, 0 °C to rt, 85%; e) *i*) NIS, AcOH, DCM, rt; *iii*) NaOMe, MeOH, DCM, rt, 46% over 2 steps; f) Na, NH_3 (l), ^{t}BuOH, THF, -60 °C, 57%; g) Cy3COOH or Cy5COOH or biotin, pentafluorophenyl trifluoroacetate, DIPEA, DMF, Cy3 28%, Cy5 53%, biotin 52%.

3.3 Conclusion

In this chapter the synthesis of two types of branched pseudotrisacchride ABPs is presented. NIS/TMSOTf/DMF mediated α-1,4-glycosylations of thioglucoside donors and TMSI/$OPPh_3$ mediated α-1,6-glycosylations of anomeric imidate donors on cyclohexene acceptors afforded access to the desired pseudotrisacchride backbones. A key step involved orthogonal protection of the O2 position of cyclohexene with a Nap ether, which allowed selective deprotection post-glycosylation and the resulting allylic alcohol gave access to the desired α-epoxide functionality by stereoselective epoxidation.

Labeling patterns and efficiencies of the newly synthesised ABPs in complex biological samples containing starch-degrading enzymes are projected to be investigated in the near future. Once these are established, the data can be compared with that of maltobiose-configured ABPs reported previously. By mimicking parts of the branched amylopectin structure, the IMG- and GIM probes are expected to show preference or specificity towards starch-degrading enzymes that like branched substrates in -1 or -2 subsites. Furthermore, the set of branched ABPs can be used for the screening of microbial species with the aim to discover new industrially relevant enzymes with beneficial characteristics.

3.4 Experimental methods

General experimental details

All reagents were of experimental grade and were used without further purification unless stated otherwise. Dichloromethane (DCM), acetonitrile (MeCN) and tetrahydrofuran (THF) were stored over 3 Å molecular sieves and *N*,*N*-dimethylformamide (DMF) was stored over 4 Å molecular sieves, which were dried *in vacuo* before use. All reactions were performed under an Argon or N_2 atmosphere unless stated otherwise. Reactions were monitored by analytical thin layer chromatography (TLC) using Merck aluminum sheets pre-coated with silica gel 60 with detection by UV-absorption (254 nm) and by spraying with a solution of $(NH_4)_6Mo_7O_{24}\cdot H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4\cdot H_2O$ (10 g/mL) in 10% sulfuric acid followed by charring at ~150 °C. Column chromatography was performed manually using Screening Device b.v. silica gel 60 (0.04-0.063 mm) in the indicated solvents. LC-MS analysis was performed on a LCQ Advantage Max (Thermo Finnigan) ion-trap spectrometer (ESI^+) coupled to a

Surveyor HPLC system (Thermo Finnigan) equipped with a C18 column (Gemini, 4.6 mm x 50 mm, 5 µM particle size, Phenomenex). The applied buffers were H_2O, acetonitrile (MeCN) and 1% aqueous trifluoroacetic acid (TFA). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker AV-400 (400/101 MHz), and Bruker AV-500 (500/126 MHz) spectrometers in the given solvent. Chemical shifts (δ) are given in ppm relative to tetramethylsilane (TMS) as internal standard (^{1}H NMR in $CDCl_3$) or the residual signal of the deuterated solvent. Coupling constants (J) are given in Hz. All given ^{13}C-NMR spectra are proton decoupled. The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), Ar (aromatic), C_q (quarternary carbon). 2D NMR experiments (COSY, HSQC) were carried out to assign protons and carbons of the new structures. High-resolution mass spectrometry (HRMS) analysis was performed with a LTQ Orbitrap mass spectrometer (Thermo Finnigan), equipped with an electronspray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 mL/min, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150 – 2000) and dioctyl phthalate (m/z = 391.28428) as a "lock mass". The high-resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan).

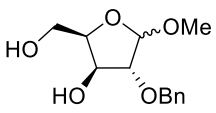
Experimental Procedures and Characterization Data of Products

Known compounds **12**[26], Cy3COOH[32], and Cy5COOH[32] were synthesized following procedures as previously described and their spectroscopic data are in agreement with those reported.

General procedure A

The appropriate carboxylic acid (25 µmol) was dissolved in DMF (200 µL). DIPEA (24 µL, 138 µmol, 5.5 eq) and pentafluorophenyl trifluoroacetate (8.6 µL, 50 µmol, 2.0 eq) were added and the mixture was stirred at rt for 2 – 3 h until LC-MS indicated almost full conversion of the free acid. Water (2.5 µL) was then added and part of the stock solution (for Cy3 and Cy5: 1.05 eq acid to amine; for biotin: 1.2 eq acid to amine) was added a solution of amine in DMF (300 µL). The reaction was stirred overnight and the product was purified on semi-preparative HPLC eluting with a linear gradient of solution A (MeCN) in solution B (50 mM NH_4OAc in H_2O). The fractions were concentrated under reduced pressure, co-evaporated with MilliQ/MeCN (1/1, at least 3 times), dissolved with MilliQ/^{t}BuOH and lyophilized to yield the product. *Note: NH_4OAc from HPLC solution is hard to remove, and repeated co-evaporation and lyophilization are necessary to remove final traces of NH_4OAc. For all final compounds, decrease of the amount is less than 0.1 – 0.3 mg after the last lyophilization.*

Compound 4



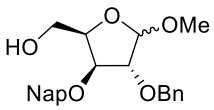
D-Xylose (15.0 g, 100 mmol) was added to a solution of acetyl chloride (3.0 mL, 42 mmol) in MeOH (300 mL). The reaction mixture was stirred at rt for 5 h, after which it was neutralized with TEA. The solution was concentrated *in vacuo* and co-evaporated with dioxane (3 x). The resulting residue was taken up in DMF (300 mL), benzaldehyde

dimethyl acetal (22.0 mL, 150 mmol) and CSA (7.0 g, 30 mmol) were added. The reaction was set up on a rotavap at rt under reduced pressure (≈16 mbar) overnight. TLC analysis indicated the presence of starting material so more benzaldehyde dimethyl acetal (7.0 mL, 50 mmol) was added and the reaction was warmed up to 40 °C under reduced pressure (≈140 mbar). After stirring for 6 h, the reaction mixture was diluted with H_2O (600 mL), extracted with EtOAc (2 x 700 mL). The combined organic layers were washed with H_2O (3 x), brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*.

The resulting residue was co-evaporated with toluene (3 x) and directly dissolved in dry DMF (300 mL). BnBr (18.0 mL, 150 mmol) and a catalytic amount of TBAI (1.85 g, 5.00 mmol) were added, after which the solution was cooled to 0 °C. Sodium hydride (60% dispersion in mineral oil, 10.0 g, 250 mmol) was added in three portions, and the reaction mixture was stirred at rt for 20 h. Then the reaction was cooled to 0 °C and quenched carefully with H_2O. The solution was further diluted with water, extracted with Et_2O (2 x). The combined organic layers were washed with H_2O (3 x), brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*.

The resulting light-yellow solid was re-dissolved in CH_2Cl_2 (100 mL) and MeOH (300 mL), and *p*-toluenesulfonic acid (1.9 g, 10 mmol) was added. The reaction mixture was stirred at rt for 48 h, after which it was quenched with Et_3N (1.4 mL, 10 mmol). The solvent was evaporated under reduced pressure and the crude was purified with silica column chromatography (Pentane/EtOAc 3:1 → 1:2) to obtain compound **4** (18.2 g, 71.7 mmol, 71%) as an anomeric mixture (α/β ratio ≈ 2:1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 – 7.15 (m, 17H), 4.92 (s, 2H, H-1α), 4.77 (d, J = 4.2 Hz, 1H, H-1β), 4.67 (d, J = 6.7 Hz, 3H), 4.64 – 4.57 (m, 4H), 4.56 – 4.51 (m, 1H), 4.40 – 4.34 (m, 2H), 4.34 – 4.29 (m, 3H), 4.17 (dt, J = 7.2, 3.3 Hz, 1H), 3.95 (d, J = 2.0 Hz, 2H), 3.91 – 3.84 (m, 6H), 3.81 (d, J = 3.3 Hz, 2H), 3.39 (s, 7H), 3.37 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 137.7, 137.4, 128.6, 128.5, 128.3, 128.1, 127.9, 107.3, 100.5, 88.2, 85.7, 82.3, 76.5, 75.8, 75.8, 72.8, 72.1, 62.2, 62.0, 55.5, 55.3 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{13}H_{18}O_5Na$ 277.1046, found 277.1043.

Compound 5



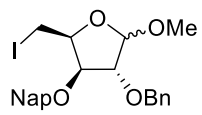
Compound **4** (18.2 g, 71.7 mmol) was dissolved in dry DMF (350 mL). Et_3N (25.0 mL, 180 mmol), trityl chloride (40.0 g, 144 mmol), and DMAP (438 mg, 3.59 mmol) were added successively and the reaction mixture was stirred at rt for 25 h. The mixture was diluted with H_2O (650 mL), extracted with EtOAc (2 x 700 mL). The combined organic layers were washed with H_2O (3 x), brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*.

The resulting oil was again taken up in dry DMF (300 mL), and NapBr (23.8 g, 108 mmol) and a catalytic amount of TBAI (1.32 g, 3.59 mmol) were added, after which the solution was cooled to 0 °C. Sodium hydride (60% dispersion in mineral oil, 7.20 g, 180 mmol) was added in 2 portions, and the reaction mixture was stirred at ambient temperature for 15 h. Then the reaction was cooled to 0 °C and quenched carefully with H_2O. The solution was further diluted with water, extracted with Et_2O (2 x).

The combined organic layers were washed with H_2O (3 x), brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*.

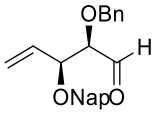
The resulting residue was redissolved in a mixture of CH_2Cl_2/MeOH (150 mL/150 mL), and *p*-toluenesulfonic acid was added until pH 2 was reached. The reaction mixture was stirred at rt for 21 h, after which it was neutralized with Et_3N. The solvent was evaporated under reduced pressure and the crude was purified with silica column chromatography (Pentane/EtOAc 5:1 → 1:1) to obtain compound **5** (23.2 g, 58.8 mmol, 81%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 – 7.75 (m, 9H), 7.71 (s, 3H), 7.51 – 7.25 (m, 23H), 4.92 – 4.83 (m, 2H), 4.83 – 4.70 (m, 3H), 4.68 – 4.61 (m, 3H), 4.58 (d, *J* = 2.0 Hz, 1H), 4.53 (d, *J* = 14.0 Hz, 3H), 4.50 – 4.44 (m, 2H), 4.32 (dt, *J* = 6.9, 4.7 Hz, 2H), 4.25 – 4.18 (m, 3H), 4.13 (dd, *J* = 3.8, 1.9 Hz, 2H), 4.09 (dd, *J* = 6.5, 4.2 Hz, 1H), 3.88 – 3.74 (m, 6H), 3.41 (s, 6H), 3.37 (s, 3H), 2.63 (t, *J* = 6.6 Hz, 2H), 2.47 (dd, *J* = 8.6, 5.0 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.6, 137.5, 135.0, 134.9, 133.3, 133.2, 133.1, 133.1, 128.5, 128.5, 128.4, 128.3, 128.1, 129.0, 128.0, 127.9, 127.8, 127.8, 126.9, 126.8, 126.3, 126.3, 126.2, 126.2, 125.7, 125.7, 108.0, 100.2, 87.3, 84.6, 82.8, 82.2, 80.7, 76.3, 72.9, 72.7, 72.6, 72.3, 62.4, 62.3, 55.7, 55.2 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{24}H_{26}O_5Na$ 417.1673, found 417.1670.

Compound 6



Alcohol **5** (23.2 g, 58.8 mmol) was co-evaporated with toluene (3 x), after which it was dissolved in anhydrous THF (250 mL). Triphenylphosphine (23.1 g, 88.2 mmol) and imidazole (8.02 g, 118 mmol) were added to the solution, which was heated to reflux. A solution of iodine (22.4 g, 88.2 mmol) in anhydrous THF (60 mL) was added, and the reaction mixture was heated at reflux until TLC-analysis revealed full conversion of the starting material (around 30 min). The reaction mixture was then cooled to rt and quenched with sat. aq. $Na_2S_2O_3$, after which the mixture was concentrated under reduced pressure to remove most of the THF. The residue was re-dissolved in EtOAc, washed with H_2O and brine, dried with $MgSO_4$, filtered and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/EtOAc 20:1 → 4:1) to obtain compound **6** (28.2 g, 56.0 mmol, 95%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 – 7.72 (m, 13H), 7.55 – 7.41 (m, 9H), 7.39 – 7.26 (m, 11H), 7.26 – 7.19 (m, 5H), 4.94 (d, *J* = 1.3 Hz, 2H), 4.88 (d, *J* = 4.2 Hz, 1H), 4.83 – 4.61 (m, 7H), 4.58 – 4.38 (m, 9H), 4.25 (dd, *J* = 6.4, 4.8 Hz, 1H), 4.10 (dd, *J* = 5.8, 2.7 Hz, 2H), 4.07 – 4.01 (m, 3H), 3.48 (d, *J* = 6.5 Hz, 1H), 3.45 (d, *J* = 6.5 Hz, 1H), 3.44 (s, 6H), 3.41 (s, 3H), 3.41 – 3.35 (m, 3H), 3.24 (dd, *J* = 10.2, 7.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 137.6, 137.4, 135.2, 135.0, 133.3, 133.3, 133.2, 133.1, 128.6, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.1, 126.8, 126.4, 126.3, 126.2, 126.1, 126.1, 125.9, 108.5, 101.0, 86.7, 84.0, 82.1, 81.9, 81.8, 77.7, 73.0, 72.8, 72.8, 72.2, 56.2, 55.7, 4.5, 3.1 ppm. HRMS (ESI) m/z: $[M+NH_4]^+$ calc for $C_{24}H_{29}IO_4N$ 522.1136, found 522.1134.

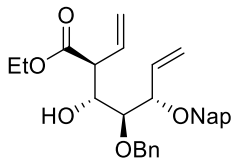
Compound 7



Zinc dust was activated and dried immediately before use: it (around 100 g) was added slowly to a vigorously stirred HCl solution (3 M, 600 mL). The mixture was stirred for 5-10 min at ambient temperature, filtered, and subsequently rinsed with water, dioxane Et_2O, after which it was dried under high vacuum at 40 °C.

Iodide **6** (28.2 g, 56.0 mmol) was divided into 3 batches (A: 9.21 g, 18.2 mmol; B: 9.44 g, 18.7 mmol; C: 9.54 g, 18.9 mmol). Each batch was dissolved in a mixture of THF/H_2O (9:1, v/v, 200 mL). Then the freshly activated zinc dust (15.0 equiv. for each) was added. The reaction mixture was flushed with argon and sonicated under argon stream at 40 °C until TLC analysis revealed full conversion of the starting material (around 1.5 h). The reaction mixture was filtrated and concentrated under reduced pressure to remove most of the THF. The combined resulting residue was diluted with Et_2O, washed with H_2O and brine, dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/Et_2O 30:1 → 10:1) to obtain compound **7** (16.4 g, 47.4 mmol, 85%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.69 (d, J = 1.5 Hz, 1H), 7.85 – 7.72 (m, 3H), 7.69 (d, J = 1.6 Hz, 1H), 7.51 – 7.43 (m, 2H), 7.39 – 7.23 (m, 6H), 5.96 (ddd, J = 17.3, 10.4, 7.7 Hz, 1H), 5.42 – 5.29 (m, 2H), 4.77 (dd, J = 15.3, 12.2 Hz, 2H), 4.62 (d, J = 12.1 Hz, 1H), 4.51 (d, J = 12.3 Hz, 1H), 4.20 (ddt, J = 7.6, 4.2, 1.0 Hz, 1H), 3.84 (dd, J = 4.1, 1.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 202.6, 137.2, 135.1, 133.9, 133.3, 133.1, 128.6, 128.3, 128.3, 128.2, 128.0, 127.8, 126.9, 126.2, 126.1, 126.0, 120.1, 85.3, 79.9, 73.6, 70.8 ppm. HRMS (ESI) m/z: $[M+NH_4]^+$ calc for $C_{23}H_{26}O_3N$ 364.1907, found 364.1903.

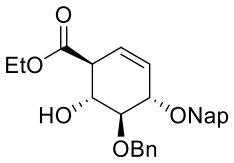
Compound 8



Ethyl 4-bromocrotonate (27.0 mL, 145 mmol), $La(OTf)_3$ (57.3 g, 96.8 mmol), and indium powder (12.2 g, 106 mmol) were added to a solution of aldehyde **7** (15.4 g, 44.5 mmol) in H_2O (220 mL). After stiring vigorously at rt for 3 days, the reaction mixture was filtered through a plug of celite, washed with EtOAc. The layers were separated and the organic layer was washed with H_2O, brine, dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/EtOAc 13:1 → 5:1) to obtain compound **8** as a clear oil (9.21 g, 20.0 mmol, 61% based on recovered starting material **7** (4.1 g, 12 mmol)). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 – 7.67 (m, 4H), 7.44 (ddd, J = 14.1, 7.4, 2.5 Hz, 3H), 7.37 – 7.23 (m, 5H), 5.92 – 5.79 (m, 1H), 5.78 – 5.65 (m, 1H), 5.48 – 5.37 (m, 2H), 5.17 (dd, J = 10.2, 1.3 Hz, 1H), 5.11 – 4.99 (m, 2H), 4.78 (d, J = 11.7 Hz, 1H), 4.57 (dd, J = 19.3, 11.5 Hz, 2H), 4.23 (t, J = 7.7 Hz, 1H), 4.10 (q, J = 7.1 Hz, 2H), 3.99 (td, J = 9.5, 8.8, 1.2 Hz, 1H), 3.57 (dd, J = 7.7, 1.1 Hz, 1H), 3.29 (t, J = 9.2 Hz, 1H), 2.71 (dd, J = 10.0, 1.9 Hz, 1H), 1.21 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6, 138.5, 135.9, 135.0, 133.4, 133.0, 133.0, 128.5, 128.2, 128.1,

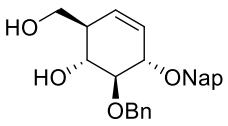
128.0, 127.8, 127.8, 126.6, 126.2, 126.1, 126.0, 120.2, 120.1, 83.0, 79.5, 74.7, 72.2, 71.0, 61.0, 55.3, 14.2 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{29}H_{32}O_5Na$ 483.2142, found 483.2139.

Compound 9



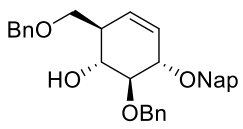
Compound **8** (9.21 g, 20.0 mmol) was dissolved in DCM (100 mL), after which the solution was sonicated under argon stream for 30 min. The second-generation Grubbs catalyst (600 mg, 0.70 mmol, 3.5 mol-%) was added and the reaction mixture was heated at reflux in the dark for 17 h under argon atmosphere. The reaction mixture was concentrated and purified with silica column chromatography (Pentane/EtOAc 9:1 → 5:1) to obtain compound **9** (7.38 g, 17.0 mmol, 85%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 – 7.67 (m, 4H), 7.52 – 7.42 (m, 3H), 7.39 – 7.25 (m, 5H), 5.82 (ddd, J = 10.2, 2.9, 2.2 Hz, 1H), 5.67 (dt, J = 10.2, 2.2 Hz, 1H), 4.98 (d, J = 11.4 Hz, 1H), 4.88 – 4.77 (m, 3H), 4.26 – 4.10 (m, 4H), 3.68 (dd, J = 9.8, 7.5 Hz, 1H), 3.26 (dq, J = 8.8, 2.9 Hz, 1H), 2.99 (d, J = 2.4 Hz, 1H), 1.26 (t, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.0, 138.5, 135.6, 133.4, 133.1, 128.6, 128.4, 128.4, 128.0, 128.0, 127.9, 127.8, 126.8, 126.3, 126.1, 126.0, 124.2, 82.6, 79.3, 75.0, 72.1, 70.5, 61.4, 50.2, 14.3 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{27}H_{28}O_5Na$ 455.1829, found 455.1824.

Compound 10



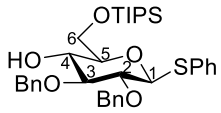
Ester **9** (4.0 g, 9.2 mmol) was co-evaporated with toluene (3 x), after which it was dissolved in dry THF (45 mL). DIBAL-H (1 M in toluene, 46 mL, 46 mmol) was added to the solution at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and TLC analysis revealed full conversion of the starting material. It was then quenched with EtOAc (18 mL) at 0 °C, followed by slow addition of H_2O (9.2 mL) and sodium borohydride (2.3 g, 60 mmol). The reaction mixture was stirred for 18 h at ambient temperature, after which it was concentrated *in vacuo*. The crude gelatinous product was dispersed with EtOAc (250 mL), to which 1 M HCl solution was added at 0 °C under stirring until the mixture became clear solution (200 mL HCl in total). The layers were separated and the water layer was extracted with EtOAc (150 mL). The combined organic layers were washed with H_2O and brine, dried with $MgSO_4$, and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/EtOAc 2:1 → 1:2) to obtain compound **10** (3.14 g, 8.05 mmol, 87%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 – 7.76 (m, 4H), 7.50 – 7.44 (m, 3H), 7.37 – 7.27 (m, 5H), 5.80 (ddd, J = 10.2, 2.9, 2.1 Hz, 1H), 5.51 (dt, J = 10.2, 2.0 Hz, 1H), 5.05 (d, J = 11.4 Hz, 1H), 4.86 (d, J = 11.7 Hz, 1H), 4.77 (dd, J = 15.1, 11.5 Hz, 2H), 4.30 – 4.19 (m, 1H), 3.77 (ddd, J = 11.5, 7.4, 4.2 Hz, 1H), 3.73 – 3.64 (m, 3H), 2.95 (d, J = 1.3 Hz, 1H), 2.52 (tq, J = 7.2, 2.7 Hz, 1H), 2.48 – 2.38 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.5, 135.7, 133.4, 133.2, 128.7, 128.4, 128.1, 128.0, 127.8, 127.6, 127.5, 126.8, 126.3, 126.1, 126.0, 83.5, 80.3, 75.0, 72.9, 71.7, 65.6, 45.4 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{25}H_{26}O_4Na$ 413.1723, found 413.1719.

Compound 11



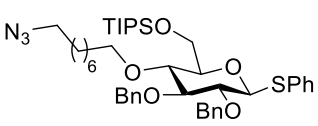
Compound **10** (781 mg, 2.00 mmol) was dissolved in dry MeCN (10 mL), K_2CO_3 (304 mg, 2.20 mmol), KI (332 mg, 2.00 mmol), 2-aminoethyl diphenylborinate (45 mg, 0.20 mmol) and BnBr (0.36 mL, 3.0 mmol) were added and the mixture was stirred for 21 hours at 60 °C. The reaction was quenched with sat. aq. $NaHCO_3$ (150 mL) and the mixture was extracted with EtOAc (2 x). The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/EtOAc 11:1 → 7:1) to obtain compound **11** (913 mg, 1.90 mmol, 95%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 – 7.72 (m, 4H), 7.44 (ddt, J = 8.4, 6.8, 3.2 Hz, 3H), 7.38 – 7.19 (m, 10H), 5.75 (dt, J = 10.2, 2.4 Hz, 1H), 5.62 (dt, J = 10.2, 2.0 Hz, 1H), 5.01 (d, J = 11.3 Hz, 1H), 4.87 – 4.73 (m, 3H), 4.50 (d, J = 1.1 Hz, 2H), 4.27 – 4.19 (m, 1H), 3.76 – 3.62 (m, 2H), 3.57 (qd, J = 9.0, 5.4 Hz, 2H), 2.99 (d, J = 1.4 Hz, 1H), 2.57 – 2.50 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.7, 138.2, 135.8, 133.3, 133.0 ($5C_q$ Ar), 129.0, 128.5, 128.5, 128.5, 128.5, 128.3, 128.3, 128.0, 128.0, 127.8, 127.7, 127.7, 127.6, 127.3, 126.8, 126.648, 126.2, 126.0, 126.0, 83.9, 80.2, 75.0, 73.4, 71.7, 71.2, 71.1, 44.1 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{32}H_{32}O_4Na$ 503.2193, found 503.2189.

Compound 13



Compound **12** (2.32 g, 5.13 mmol) was dissolved in dry DCM (50 mL), imidazole (874 mg, 12.8 mmol) was added and the mixture was stirred at rt until imidazole fully dissolved. After which, the mixture was cooled to 0 °C and stirred for 1 hour. TIPSCl (1.43 mL, 6.67 mmol) was added at 0 °C and the reaction was warmed to rt slowly and stirred overnight. The mixture was diluted with DCM, washed with sat. aq. NH_4Cl, sat. aq. $NaHCO_3$, H_2O and brine, dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/EtOAc 50:1 → 20:1) to obtain compound **13** (3.03 g, 4.98 mmol, 97%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 – 7.49 (m, 2H, CH Ar), 7.43 – 7.21 (m, 13H, CH Ar), 4.89 – 4.82 (m, 3H, 3CH*H* Bn), 4.71 (dd, J = 17.0, 10.0 Hz, 2H, 1CH*H* Bn and H1), 4.02 (dd, J = 10.3, 4.9 Hz, 1H, H6a), 3.94 (dd, J = 10.4, 5.5 Hz, 1H, H6b), 3.73 (td, J = 9.2, 1.7 Hz, 1H, H4), 3.58 (t, J = 8.8 Hz, 1H, H4), 3.49 – 3.44 (m, 1H, H2), 3.44 – 3.36 (m, 1H, H5), 3.05 (d, J = 1.7 Hz, 1H, OH), 1.19 – 1.02 (m, 21H, TIPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.7, 138.2, 134.0 ($3C_q$ Ar), 131.9, 129.0, 128.7, 128.5, 128.4, 128.1, 128.0, 128.0, 127.6 (15CH Ar), 87.7 (C1), 86.4 (C3), 80.2 (C2), 78.5 (C5), 75.7, 75.5 ($2CH_2$ Bn), 73.2 (C4), 65.1 (C6), 18.1 ($6CH_3$ TIPS), 11.9 (3CH TIPS) ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{35}H_{48}O_5SSiNa$ 631.2884, found 631.2879.

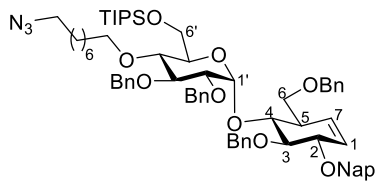
Compound 14



Compound **13** (3.03 mg, 4.98 mmol) was co-evaporated with toluene (2 x) and dissolved in dry DMF (25 mL). After cooling to 0 °C, NaH (300 mg, 7.47 mmol), 18-crown-6 (263 mg, 0.996 mmol) and 1-azido-8-iodooctane

(2.80 g, 9.96 mmol) were added successively. The mixture was stirred at 0 °C for 10 min, then warmed to rt and stirred for 7 h. TLC-analysis indicated the presence of starting material so another portion of NaH (300 mg, 7.47 mmol) and 1-azido-8-iodooctane (2.80 g, 9.96 mmol) were added at 0 °C and the mixture was stirred at rt overnight. The reaction was quenched with H_2O at 0 °C, diluted with Et_2O, washed with H_2O (3 x) and brine, dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/EtOAc 100:1 → 30:1) to obtain compound **14** (3.56 g, 4.67 mmol, 94%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 – 7.52 (m, 2H, CH Ar), 7.42 – 7.20 (m, 13H, CH Ar), 4.89 – 4.77 (m, 3H, 3CH*H* Bn), 4.66 (dd, *J* = 18.9, 10.0 Hz, 2H, 1CH*H* Bn and H1), 4.05 – 3.96 (m, 1H, H6a), 3.91 (dd, *J* = 11.1, 3.9 Hz, 1H, H6b), 3.82 (dt, *J* = 8.7, 6.5 Hz, 1H, 1CH*H*O linker), 3.69 – 3.57 (m, 2H, 1CH*H*O linker and H3), 3.46 (dt, *J* = 12.8, 9.2 Hz, 2H, H2/H4 and H5), 3.31 – 3.19 (m, 3H, H2/H4 and CH_2N_3), 1.63 – 1.49 (m, 4H, $2CH_2$ linker), 1.40 – 1.21 (m, 8H, $4CH_2$ linker), 1.21 – 1.03 (m, 21H, TIPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.6, 138.3, 134.2 ($3C_q$ Ar), 131.9, 128.9, 128.6, 128.5, 128.3, 128.0, 127.9, 127.8, 127.4 (15CH Ar), 87.5 (H1), 87.0 (H3), 80.7, 80.5 (H2 and H4), 77.5 (H5), 76.0, 75.5 ($2CH_2$ Bn), 73.2 (CH_2O linker), 62.5 (C6), 51.6 (CH_2N_3), 30.6, 29.5, 29.2, 28.9, 26.8, 26.3 ($6CH_2$ linker), 18.2 ($6CH_3$ TIPS), 12.1 (3CH TIPS). HRMS (ESI) m/z: $[M+NH_4]^+$ calc for $C_{43}H_{67}N_4O_5SSi$ 779.4596, found 779.4589.

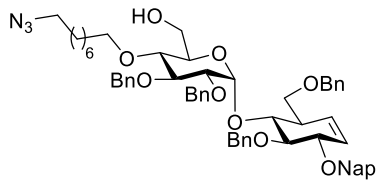
Compound 15



Donor **14** (2.08 g, 2.73 mmol) was co-evaporated with toluene (3 x) and dissolved in dry DCM (33 mL) under nitrogen and stirred over fresh flame-dried 3 Å molecular sieves, after which DMF (3.36 mL, 43.7 mmol, 16 equiv. of donor) was added to the solution. The solution was cooled to 0 °C, NIS (615 mg, 2.73 mmol) and TMSOTf (494 µL, 2.73 mmol) were added successively. The mixture was pre-activated at 0 °C for 1 h. Then acceptor **11** (875 mg, 1.82 mmol) was dissolved with dry DCM (3 mL in total) and added to the solution and the reaction was stirred at 0 °C for 45 h. The reaction was then quenched with a mixture of sat. aq. $Na_2S_2O_3$ and sat. aq. $NaHCO_3$, stirred vigorously at rt until the brown color faded. The mixture was filtered and diluted with DCM. The layers were separated and the organic layer was washed with H_2O (2 x), brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (Pentane/EtOAc 20:1 → 11:1) to afford product **15** (*dr* > 20/1, 1.08 g, 0.953 mmol, 52%) as a clean oil and with eluent Pantane/EtOAc = 3:1, a TIPS fall-off side product (here after compound **16**, 576 mg, 0.590 mmol, 32%) was also obtained as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (dd, *J* = 6.1, 3.4 Hz, 1H, CH Ar), 7.75 – 7.68 (m, 3H, CH Ar), 7.48 – 7.18 (m, 19H, CH Ar), 7.15 (s, 4H, CH Ar), 5.79 (dt, *J* = 10.2, 2.5 Hz, 1H, H1/H7), 5.67 (dt, *J* = 10.1, 2.2 Hz, 1H, H1/H7), 5.57 (d, *J* = 3.6 Hz, 1H, anomeric), 5.01 – 4.92 (m, 2H), 4.86 (d, *J* = 10.8 Hz, 1H), 4.80 – 4.69 (m, 3H), 4.55 – 4.44 (m, 3H), 4.42 (d, *J* = 12.2 Hz, 1H), 4.30 (dq, *J* = 7.4, 2.4 Hz, 1H), 4.11 (t, *J* = 9.1 Hz, 1H), 3.91 (q, *J* = 9.3, 8.3 Hz, 2H), 3.85 – 3.69 (m, 4H), 3.59 (td, *J* = 9.9, 8.8, 4.9 Hz, 3H), 3.44 (q, *J* = 9.5, 8.1 Hz, 1H), 3.36 (dd, *J* = 9.8, 3.5 Hz, 1H),

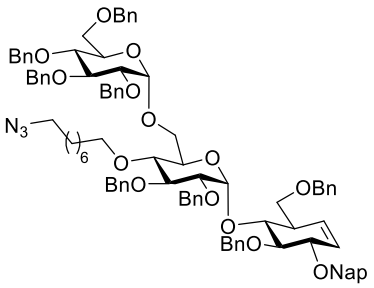
3.20 (t, *J* = 7.0 Hz, 2H, CH_2N_3), 2.70 (ddp, *J* = 8.4, 5.8, 2.9 Hz, 1H, H5), 1.55 (h, *J* = 7.2 Hz, 4H, $2CH_2$ linker), 1.36 – 1.20 (m, 8H, $4CH_2$ linker), 1.09 – 0.94 (m, 21H, TIPS). ^{13}C NMR (126 MHz, $CDCl_3$) *δ* 139.5, 139.1, 138.5, 138.3, 136.0, 133.4, 133.0 ($7C_q$ Ar), 129.9, 128.4, 128.4, 128.3, 128.3, 128.2, 128.0, 127.8, 127.7, 127.6, 127.5, 127.5, 127.4, 127.0, 126.8, 126.7, 126.6, 126.1, 125.9, 96.7 (anomeric), 85.1, 81.9, 80.5, 80.1, 77.6, 75.6, 74.0, 73.5, 73.1, 72.9, 72.5, 71.9, 70.0, 62.4, 51.5 (CH_2N_3), 44.0 (C5), 30.6, 29.5, 29.3, 28.9, 26.8, 26.3 ($6CH_2$ linker), 18.1 ($3CH_3$ TIPS), 18.1 ($3CH_3$ TIPS), 12.1 (3CH TIPS) ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{69}H_{89}N_3O_9SiNa$ 1154.6260, found 1154.6259.

Compound 16



Compound **15** (1.04 g, 0.918 mmol) was co-evaporated with toluene (3 x) and dissolved in dry THF (9.2 mL). TBAF (1.0 M in THF, 2.3 mL, 2.3 mmol) was added and the reaction was stirred at rt for 3 h. Then the mixture was quenched with sat. aq. NH_4Cl at 0 °C, diluted with EtOAc, washed with H_2O and brine, dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (Pentane/EtOAc 7:1 → 3:1) to afford product **16** (787 mg, 0.806 mmol, 87%) as a clean oil. ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.84 – 7.64 (m, 4H, CH Ar), 7.52 – 7.04 (m, 23H, CH Ar), 5.79 (dt, *J* = 10.1, 2.7 Hz, 1H, H1/H7), 5.66 (dd, *J* = 12.4, 3.1 Hz, 2H, H1/H7 and anomeric H1'), 5.03 (d, *J* = 11.8 Hz, 1H), 4.98 – 4.84 (m, 2H), 4.84 – 4.65 (m, 3H), 4.62 – 4.39 (m, 4H), 4.37 – 4.28 (m, 1H), 4.10 (t, *J* = 9.1 Hz, 1H), 4.00 – 3.86 (m, 2H), 3.81 (dt, *J* = 8.9, 6.4 Hz, 1H), 3.77 – 3.48 (m, 6H), 3.43 – 3.17 (m, 4H), 2.70 (dp, *J* = 10.8, 3.6 Hz, 1H, H5), 1.93 – 1.78 (m, 1H, OH), 1.55 (p, *J* = 6.7 Hz, 4H, $2CH_2$ linker), 1.30 (dd, *J* = 15.5, 7.8 Hz, 8H, $4CH_2$ linker). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 139.3, 138.9, 138.2, 138.1, 135.8, 133.3, 133.0 ($7C_q$ Ar), 129.7, 128.5, 128.4, 128.4, 128.3, 128.2, 128.0, 127.8, 127.8, 127.8, 127.7, 127.6, 127.6, 127.2, 126.8, 126.8, 126.7, 126.1, 125.9 (27CH Ar, C1 and C7), 96.9 (anomeric), 85.1, 81.6, 80.7, 79.5, 78.2, 75.5, 74.2, 73.4, 73.1, 73.0, 72.0, 71.7, 69.6, 61.9, 51.5 (CH_2N_3), 44.0 (C5), 30.5, 29.5, 29.2, 28.9, 26.8, 26.2 ($6CH_2$ linker) ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{60}H_{69}N_3O_9Na$ 998.4926, found 998.4923.

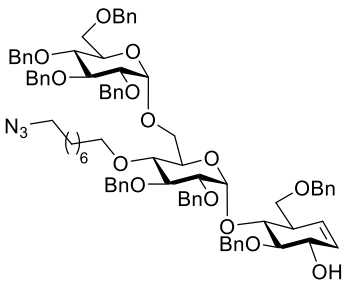
Compound 17



Imidate donor **S1** (1.49 g, 2.09 mmol) and acceptor **16** (1.36 g, 1.39 mmol) were co-evaporated with toluene (3 x), then $OPPh_3$ (2.32 g, 8.34 mmol) and activated 3 Å molecular sieves were added under nitrogen. The mixture was dissolved with dry DCM (28 mL) and stirred at rt for 30 min. Then TMSI (297 µL, 2.09 mmol) was added slowly and the reaction was stirred at rt for 43 h. The reaction was then quenched with a mixture of sat. aq. $Na_2S_2O_3$ and sat. aq. $NaHCO_3$, stirred vigorously at rt until the deep red color faded. The mixture was filtered over celite and diluted with DCM. The layers were separated and the organic layer was washed with H_2O (2 x), brine, dried over Na_2SO_4, filtered and

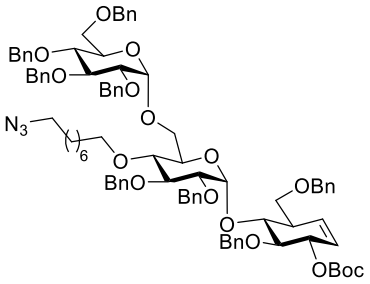
concentrated *in vacuo*. The crude was purified by silica gel column chromatography (Pentane/EtOAc 11:1 → 6:1) to afford product **17** (*dr* > 20/1, 1.74 g, 1.16 mmol, 82%) as a light-yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 – 7.75 (m, 1H, CH Ar), 7.74 – 7.66 (m, 3H, CH Ar), 7.49 – 6.96 (m, 43H, CH Ar), 5.76 (dt, *J* = 10.2, 2.4 Hz, 1H, H1/H7), 5.66 (dt, *J* = 10.2, 2.0 Hz, 1H, H1/H7), 5.64 (d, *J* = 3.7 Hz, 1H, anomeric α-1,4), 5.09 (d, *J* = 3.5 Hz, 1H, anomeric α-1,6), 5.02 – 4.88 (m, 3H, 3CH*H* Bn), 4.84 (dd, *J* = 10.9, 6.0 Hz, 2H, 2CH*H* Bn), 4.78 – 4.70 (m, 3H, 3CH*H* Bn), 4.69 – 4.43 (m, 7H, 7CH*H* Bn), 4.40 – 4.34 (m, 3H, 3CH*H* Bn), 4.33 – 4.29 (m, 1H), 4.12 (t, *J* = 9.2 Hz, 1H), 3.97 – 3.90 (m, 2H), 3.90 – 3.85 (m, 1H), 3.82 (dt, *J* = 9.2, 6.7 Hz, 1H), 3.79 – 3.46 (m, 12H), 3.25 (dd, *J* = 9.8, 3.7 Hz, 1H), 3.15 (t, *J* = 7.0 Hz, 2H, CH_2N_3), 2.71 (ddp, *J* = 8.5, 5.8, 2.9 Hz, 1H, H5), 1.60 – 1.45 (m, 4H, $2CH_2$ linker), 1.26 (td, *J* = 16.6, 13.7, 5.0 Hz, 8H, $4CH_2$ linker). ^{13}C NMR (126 MHz, $CDCl_3$) δ 139.4, 139.1, 139.0, 138.6, 138.5, 138.4, 138.2, 138.1, 136.0, 133.4, 133.0 ($11C_q$ Ar), 129.8, 128.5, 128.5, 128.5, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 127.5, 127.5, 127.1, 126.7, 126.7, 126.1, 126.1, 125.9, 97.3 (anomeric α-1,6), 96.8 (anomeric α-1,4), 85.4, 81.9, 80.9, 80.1, 79.9, 77.8, 77.7, 75.7, 75.5, 75.2, 74.1, 73.6, 73.2, 73.1, 73.0, 72.9, 72.0, 71.7, 70.4, 70.0, 68.6, 65.6, 51.5 (CH_2N_3), 43.9 (C5), 30.6, 29.6, 29.2, 28.9, 26.8, 26.2 ($6CH_2$ linker) ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{94}H_{103}N_3O_{14}Na$ 1520.7332, found 1520.7339.

Compound 18



Compound **17** (1.74 g, 1.16 mmol) was dissolved in a mixture of DCM/H_2O (10/1, 22 mL). Then DDQ (277 mg, 1.22 mmol) was added and the reaction mixture was stirred for 2 h at rt. The mixture was diluted with DCM, washed with sat. aq. $NaHCO_3$ (3 x), H_2O and brine, dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/EtOAc 9:1 → 4:1) to obtain compound **18** (1.25 g) as a colourless oil contaminated with a small amount of unknown impurities which may come from dirty tubes, thus a yield over two steps is provided after the next step.

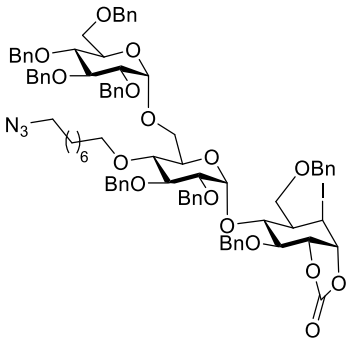
Compound 19



Compound **18** (1.25 g, 0.920 mmol) was co-evaporated with toluene (3 x) and dissolved in dry THF (20 mL). Boc_2O (3.01 g, 13.8 mmol) and DMAP (11 mg, 0.092 mmol) were added at 0 °C. Then the reaction mixture was stirred at rt for 5 h. The reaction was quenched with sat. aq. NH_4Cl at 0 °C and concentrated *in vacuo* to remove most of the THF. Then the residue was diluted with Et_2O, washed with sat. aq. NH_4Cl, sat. aq. $NaHCO_3$, H_2O and brine, dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/EtOAc 15:1 → 7:1) and size

exclusion (eluent MeOH/DCM: 1/1) to obtain pure compound **19** (1.27 g, 0.871 mmol, 75% over two steps) as a colourless oil. *Note: size exclusion was used to separate the unknow contaminations from the previous step.* ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 – 6.98 (m, 40H, CH Ar), 5.69 (dt, J = 10.1, 2.0 Hz, 1H, H7), 5.63 (dt, J = 10.1, 2.4 Hz, 1H, H1), 5.45 (d, J = 3.6 Hz, 1H, anomeric α-1,4), 5.43 – 5.38 (m, 1H, H2), 5.05 (d, J = 3.5 Hz, 1H, anomeric α-1,6), 4.96 – 4.81 (m, 4H, 4CH*H* Bn), 4.79 – 4.71 (m, 3H, 3CH*H* Bn), 4.65 – 4.52 (m, 3H, 3CH*H* Bn), 4.51 – 4.43 (m, 3H, 3CH*H* Bn), 4.41 – 4.34 (m, 3H, 3CH*H* Bn), 4.12 (dd, J = 9.5, 8.4 Hz, 1H, H4), 3.96 – 3.78 (m, 4H), 3.77 – 3.45 (m, 12H), 3.25 (dd, J = 9.8, 3.7 Hz, 1H), 3.16 (t, J = 7.0 Hz, 2H, CH_2N_3), 2.74 – 2.65 (m, 1H, H5), 1.58 – 1.46 (m, 4H, $2CH_2$ linker), 1.41 (s, 9H, $3CH_3$ Boc), 1.32 – 1.11 (m, 8H, $4CH_2$ linker). ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.2 (C=O Boc), 139.1, 139.0, 139.0, 138.6, 138.6, 138.4, 138.3, 138.2 ($8C_q$ Ar), 131.1 (C1), 128.5, 128.5, 128.4, 128.4, 128.4, 128.1, 128.3, 128.0, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 127.6, 127.6, 127.6, 127.5, 127.5, 127.2, 127.0 (40CH Ar), 125.3 (C7), 97.4 (anomeric α-1,6), 97.2 (anomeric α-1,4), 83.4, 82.4 (C_q Boc), 81.9, 81.9, 80.1, 79.9, 77.7, 77.7, 77.6, 75.7, 75.5, 75.2, 74.1, 73.9, 73.6, 73.3, 73.1, 72.9, 72.0, 71.8, 70.4, 69.9, 68.6, 65.7, 51.5 (CH_2N_3), 43.8 (C5), 30.6, 29.6, 29.3, 28.9 ($4CH_2$ linker), 27.8 ($3CH_3$ Boc), 26.8, 26.2 ($2CH_2$ linker) ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{88}H_{103}N_3O_{16}Na$ 1480.7231, found 1480.7232.

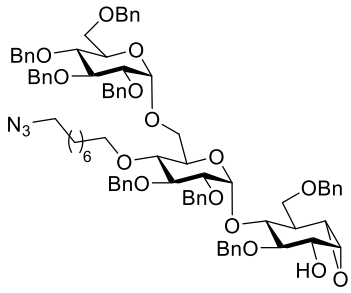
Compound 20



Compound **19** (635 mg, 0.436 mmol) was dissolved in a mixture of DCM/HOAc (0.15 M, 1/1 (v/v)), NIS (196 mg, 0.872 mmol) was added and the reaction mixture was stirred at rt for 22 h. The mixture was diluted with Et_2O and quenched with Et_3N (4.0 mL). Then the Et_2O layer was washed with sat. aq. NH_4Cl, sat. aq. $NaHCO_3$, sat. aq. $Na_2S_2O_3$, H_2O and brine, dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/EtOAc 11:1 → 6:1) to obtain compound **20** (402 mg, 0.263 mmol, 61%) as a colourless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 – 7.01 (m, 40H, CH Ar), 5.32 (d, *J* = 3.7 Hz, 1H, anomeric α-1,4), 5.20 – 5.12 (m, 1H), 5.01 (d, *J* = 3.6 Hz, 1H, anomeric α-1,6), 4.93 (d, *J* = 10.9 Hz, 1H), 4.88 – 4.68 (m, 7H), 4.67 – 4.57 (m, 4H), 4.54 – 4.40 (m, 5H), 4.34 (d, *J* = 11.8 Hz, 1H), 4.08 – 3.94 (m, 3H), 3.89 (t, *J* = 8.0 Hz, 1H), 3.87 – 3.72 (m, 5H), 3.69 – 3.52 (m, 6H), 3.48 (dd, *J* = 9.3, 6.0 Hz, 1H), 3.42 (t, *J* = 9.4 Hz, 1H), 3.26 (dd, *J* = 9.9, 3.8 Hz, 1H), 3.17 (t, *J* = 7.0 Hz, 2H, CH_2N_3), 2.03 (s, 1H, H5), 1.54 (ddt, *J* = 32.1, 14.4, 7.1 Hz, 4H, $2CH_2$ linker), 1.32 – 1.18 (m, 8H, $4CH_2$ linker). ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.0 (C=O), 138.9, 138.8, 138.5, 138.4, 138.0, 137.9, 137.8, 137.3 ($8C_q$ Ar), 128.7, 128.5, 128.5, 128.5, 128.4, 128.2, 128.2, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.6, 127.1 (40CH Ar), 98.1 (anomeric α-1,4), 97.8 (anomeric α-1,6), 81.9, 81.6, 81.3, 80.3, 80.2, 79.4, 77.9, 77.7, 76.2, 75.6, 75.6, 75.3, 74.0, 73.6, 73.6, 73.4, 73.3, 72.4, 72.3, 71.5, 70.4, 68.7, 66.7, 51.5 (CH_2N_3), 43.9 (C5, assign by HSQC), 30.6, 29.6, 29.2, 28.9, 26.8,

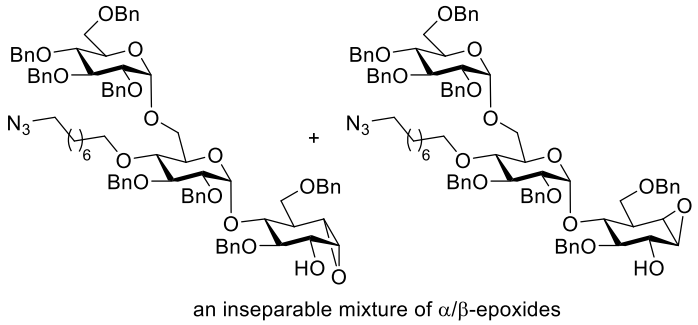
26.2 (6CH_2 linker), 25.0 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{84}H_{94}IN_3O_{16}Na$ 1550.5571, found 1550.5573.

Compound 21



Compound **20** (402 mg, 0.263 mmol) was dissolved in a mixture of DCM/MeOH (1.9 mL/3.3 mL), NaOMe (25 wt% in MeOH, 178 µL, 0.789 mmol) was added and the mixture was stirred at rt overnight. The reaction was quenched with $Et_3N{\cdot}HCl$ (131 mg, 0.95 mmol) and concentrated *in vacuo*. The residue was diluted with EtOAc, washed with H_2O and brine, dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/EtOAc 7:1 → 3:1) to obtain compound **21** (0.29 g, 0.21 mmol, 81%) as a clean oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 – 7.00 (m, 40H, CH Ar), 4.99 – 4.91 (m, 4H, anomeric α-1,4 and α-1,6 and 2CH*H* Bn), 4.84 (dd, *J* = 12.1, 10.8 Hz, 2H, 2CH*H* Bn), 4.76 (dd, *J* = 10.9, 6.0 Hz, 2H, 2CH*H* Bn), 4.67 – 4.54 (m, 4H, 4CH*H* Bn), 4.49 – 4.34 (m, 6H, 6CH*H* Bn), 4.02 – 3.71 (m, 10H), 3.69 – 3.60 (m, 5H), 3.59 – 3.52 (m, 2H), 3.44 – 3.37 (m, 1H), 3.33 (dd, *J* = 4.0, 2.0 Hz, 1H, epoxide), 3.28 (dd, *J* = 9.8, 3.6 Hz, 1H), 3.18 (t, *J* = 7.0 Hz, 2H, CH_2N_3), 3.13 (dd, *J* = 4.1, 0.8 Hz, 1H, epoxide), 2.52 (dt, *J* = 7.9, 4.1 Hz, 1H, H5), 2.40 (d, *J* = 5.0 Hz, 1H, OH), 1.53 (dq, *J* = 14.4, 7.7, 7.2 Hz, 4H, 2CH_2 linker), 1.36 – 1.21 (m, 8H, 4CH_2 linker). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.9, 138.9, 138.9, 138.5, 138.5, 138.3, 138.2, 138.1 (8C_q Ar), 128.6, 128.5, 128.5, 128.5, 128.4, 128.4, 128.1, 128.1, 128.0, 128.0, 128.0, 127.8, 127.8, 127.8, 127.7, 127.7, 127.7, 127.7, 127.6, 127.6 (40CH Ar), 98.0 (anomeric), 97.5 (anomeric), 82.0, 81.7, 80.9, 80.1, 80.1, 78.1, 77.7, 77.4, 75.7, 75.6, 75.3, 74.8, 73.6, 73.4, 73.4, 73.1, 72.3, 71.9, 70.5, 70.4, 69.6, 68.6, 66.3, 55.5 (epoxide), 55.4 (epoxide), 51.5 (CH_2N_3), 42.4 (C5), 30.6, 29.6, 29.2, 28.9, 26.8, 26.2 (6CH_2 linker) ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{83}H_{95}N_3O_{15}Na$ 1396.6655, found 1396.6656.

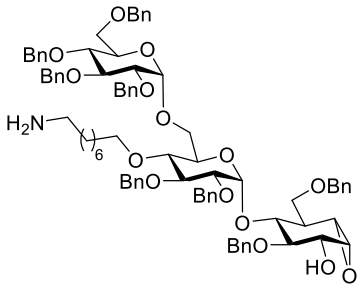
Direct epoxidation of compound 18 with *m*-CPBA



Compound **18** (84 mg, 62 µmol) was co-evaporated with toluene (3x) and dissolved in dry DCM (1.2 mL). After cooling to 0 ^{0}C, *m*-CPBA (71%, 28 mg, 0.12 mmol) was added and the mixture was stirred at 0 ^{0}C for 22 h. TLC-analysis indicated the presence of starting material so another portion of *m*-CPBA (28 mg, 0.12 mmol) was added and the mixture was stirred at 0 ^{0}C for 24 h. The reaction mixture was then diluted with EtOAc, washed with a mixture of sat. aq. $NaHCO_3$ and sat. aq. $Na_2S_2O_3$ (3x), H_2O and brine, dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The crude

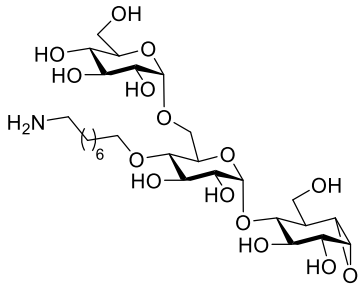
was purified with silica column chromatography (Pentane/EtOAc 10:1 → 3:1) to obtain product (66 mg, 48 µmol, 77%) as an inseparable mixture of α/β epoxides (3/1, determined by ^{1}H NMR).

Compound 22



Compound **21** (107 mg, 78.0 µmol) was dissolved in MeCN (1.6 mL). H_2O (100 µL) and polymer-bound PPh_3 (3 mmol/g loading, 130 mg, 390 µmol) were added and the mixture was stirred at 65 ^{0}C for 23 h. After which, another 150 µL H_2O was added and the mixture was stirred at 65 ^{0}C for another 2 h. The reaction was then cooled to rt, filtered and the solvent was concentrated *in vacuo*. The crude was purified with silica column chromatography (DCM/MeOH 50:1 → 19:1, with 0.1% Et_3N) to obtain product **22** (92 mg, 68 µmol, 88%) which was co-evaporated with toluene (3x) to remove Et_3N residue. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 – 7.08 (m, 40H, CH Ar), 4.99 – 4.91 (m, 4H), 4.84 (t, *J* = 10.7 Hz, 2H), 4.76 (dd, *J* = 10.9, 4.8 Hz, 2H), 4.64 – 4.56 (m, 4H), 4.51 – 4.35 (m, 6H), 4.02 – 3.92 (m, 2H), 3.92 – 3.71 (m, 8H), 3.69 – 3.59 (m, 5H), 3.55 (dt, *J* = 9.7, 2.7 Hz, 2H), 3.40 (dd, *J* = 10.0, 9.0 Hz, 1H), 3.33 (dd, *J* = 4.1, 1.9 Hz, 1H), 3.30 – 3.16 (br s, 2H), 3.28 (dd, *J* = 9.8, 3.6 Hz, 1H), 3.12 (d, *J* = 4.1 Hz, 1H), 2.70 – 2.63 (m, 2H, CH_2NH_2), 2.52 (dt, *J* = 7.4, 4.0 Hz, 1H, H5), 2.34 (s, CH_3 toluene residue), 1.59 – 1.51 (m, 2H, CH_2 linker), 1.45 (q, *J* = 7.1 Hz, 2H, CH_2 linker), 1.33 – 1.20 (m, 8H, 4CH_2 linker). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.9, 138.9, 138.9, 138.5, 138.5, 138.3, 138.2, 138.0 (8C_q Ar), 129.1, 128.6, 128.5, 128.4, 128.4, 128.4, 128.3, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 125.4 (40CH Ar), 98.0 (anomeric), 97.5 (anomeric), 82.0, 81.6, 81.0, 80.1, 80.0, 78.1, 77.7, 77.4, 75.7, 75.6, 75.2, 74.8, 73.6, 73.4, 73.1, 72.3, 71.9, 70.5, 70.3, 69.6, 68.6, 66.4, 55.6 (epoxide), 55.4 (epoxide), 42.3 (C5), 41.5 (CH_2NH_2), 32.0, 30.6, 29.6, 29.4, 26.8, 26.3 (6CH_2 linker) ppm. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{83}H_{98}NO_{15}$ 1348.6931, found 1348.6934.

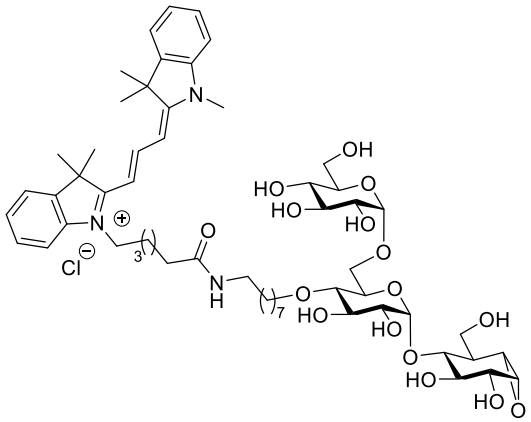
Compound 23



Compound **22** (43 mg, 32 µmol) was dissolved in dioxane (0.75 mL), then H_2O (0.5mL) was added under stirring. Upon addition of H_2O, the clear solution turned into white emulsion, so more dioxane (in total 0.75 mL) was added until the solution became clear again. The solution was purged with nitrogen for 2 minutes under vigorous stirring. TFA (2.5 µL, 32 µmol) and $Pd(OH)_2$/C (20 wt%, 22 mg, 32 µmol) were added under nitrogen atmosphere. While stirring vigorously, the N_2 balloon was replaced with a H_2 balloon and flushed with H_2 for 2 minutes. After stirring for 3 h under H_2 atmosphere, the mixture was filtered over a small celite pad and concentrated under reduced pressure. Product **23** including minor impurities was obtained as a white powder (quant) after lyophilization which can be directly used for

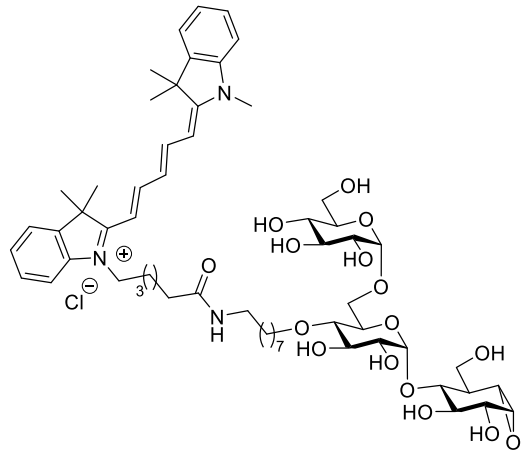
coupling with fluorescent tags. *Note: the hydrogenation reaction was always performed on small scales (25.0 – 33.0 µmol) and it usually took around 3 h for full deprotection of the benzyl groups. It's better to monitor the reaction hourly by TLC-analysis to avoid further hydrolysis or reductive opening of the epoxide moiety.* ^{1}H NMR (500 MHz, D_2O) δ 5.14 (d, J = 4.1 Hz, 1H, anomeric α-1,4), 4.95 (d, J = 3.7 Hz, 1H, anomeric α-1,6), 3.95 (tq, J = 8.6, 3.0, 2.5 Hz, 4H), 3.91 – 3.51 (m, 12H), 3.50 – 3.42 (m, 3H), 3.38 – 3.30 (m, 2H), 3.01 – 2.94 (m, 2H, CH_2NH_2), 2.28 – 2.19 (m, 1H, H5), 1.69 – 1.53 (m, 4H, $2CH_2$ linker), 1.34 (d, J = 7.3 Hz, 8H, $4CH_2$ linker). ^{13}C NMR (126 MHz, D_2O) δ 100.7 (anomeric α-1,4), 98.9 (anomeric α-1,6), 80.4, 77.7, 73.4, 73.2, 73.1, 72.9, 72.0, 71.8, 71.6, 70.7, 70.4, 69.4, 66.8, 60.4, 57.3 (epoxide), 55.2 (epoxide), 42.8 (C5), 39.6 (CH_2NH_2), 29.3, 28.4, 28.1, 26.7, 25.5, 25.2 ($6CH_2$ linker) ppm. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{27}H_{50}NO_{15}$ 628.3175, found 628.3172.

Compound 24



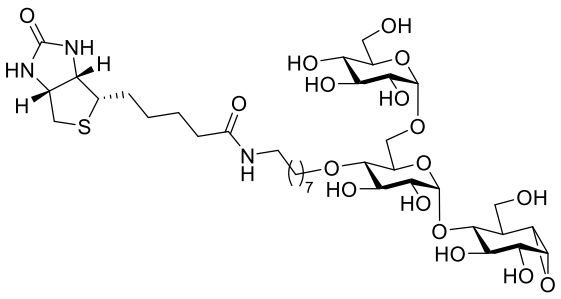
Amine **23** (14.0 mg, 22.3 µmol) was reacted with Cy3 acid (1.05 eq) for 22 h according to general procedure A. This afforded product **24** (9.9 mg, 9.0 µmol, 40%) as a red powder after lyophilization (3x). ^{1}H NMR (500 MHz, MeOD) δ 8.55 (t, J = 13.5 Hz, 1H), 7.55 (ddd, J = 7.5, 1.2, 0.6 Hz, 2H), 7.46 (dddd, J = 8.0, 7.5, 3.9, 1.2 Hz, 2H), 7.40 – 7.28 (m, 4H), 6.44 (dd, J = 13.5, 6.3 Hz, 2H), 4.94 (1H, anomeric α-1,4, obscured by H_2O peak), 4.82 (d, J = 3.7 Hz, 1H, anomeric α-1,6), 4.16 (t, J = 7.5 Hz, 2H, CH_2N^+), 4.06 – 4.02 (m, 1H), 3.96 – 3.63 (m, 14H), 3.59 (dd, J = 9.8, 8.7 Hz, 1H), 3.52 (dt, J = 9.0, 6.6 Hz, 1H), 3.43 (ddd, J = 16.3, 9.7, 3.8 Hz, 2H), 3.34 (dd, J = 9.7, 8.9 Hz, 1H), 3.28 – 3.18 (m, 3H), 3.12 (t, J = 7.1 Hz, 2H, $NHCH_2$), 3.09 – 3.02 (m, 1H), 2.21 (t, J = 7.3 Hz, 2H, CH_2C=O), 2.10 – 2.04 (m, 1H, H5), 1.86 (p, J = 7.7 Hz, 2H), 1.77 (s, 12H), 1.71 (p, J = 7.4 Hz, 2H), 1.59 – 1.40 (m, 6H), 1.40 – 1.21 (m, 8H). ^{13}C NMR (126 MHz, MeOD) δ 176.7, 176.0, 175.7, 152.1, 144.1, 143.4, 142.2, 142.1, 130.0, 130.0, 126.8, 126.8, 123.6, 123.4, 112.5, 112.3, 103.8, 103.7, 103.1 (anomeric α-1,4), 100.5 (anomeric α-1,6), 83.7, 80.0, 75.3, 75.0, 74.8, 74.0, 74.0, 73.7, 73.5, 72.6, 72.3, 71.7, 68.8, 62.6, 61.8, 58.0 (epoxide), 55.3 (epoxide), 50.7 (C_q), 50.6 (C_q), 45.2 (C5), 45.1 (CH_2N^+), 40.4 ($NHCH_2$), 36.7 (CH_2C=O), 31.8 (NCH_3), 31.4, 30.5, 30.4, 30.3 ($4CH_2$ linker), 28.3 ($2CH_3$), 28.2 (CH_2 linker), 28.2 ($2CH_3$), 27.9, 27.3, 27.1, 26.6 ($4CH_2$ linker) ppm. *Note: peaks at 23.3 and 179.0 ppm in ^{13}C NMR are belong to NH_4OAc residue which can be removed after repeated lyophilization.* HRMS (ESI) m/z: $[M]^+$ calc for $C_{57}H_{84}N_3O_{16}$ 1066.5846, found 1066.5844.

Compound 25



Amine **23** (20.0 mg, 31.8 µmol) was reacted with Cy5 acid (1.05 eq) for 28 h according to general procedure A. This afforded product **25** (19.8 mg, 17.5 µmol, 55%) as a blue powder after lyophilization (3x). ^{1}H NMR (500 MHz, MeOD) δ 8.28 – 8.19 (m, 2H), 7.49 (dd, J = 7.5, 1.3 Hz, 2H), 7.41 (tdd, J = 7.7, 3.3, 1.2 Hz, 2H), 7.33 – 7.23 (m, 4H), 6.64 (t, J = 12.4 Hz, 1H), 6.28 (dd, J = 13.7, 4.0 Hz, 2H), 4.94 (1H, anomeric α-1,4, obscured by H_2O peak), 4.82 (d, J = 3.7 Hz, 1H, anomeric α-1,6), 4.10 (d, J = 7.4 Hz, 2H, CH_2N^+), 4.04 (dd, J = 10.9, 3.0 Hz, 1H), 3.99 – 3.55 (m, 15H), 3.52 (dt, J = 9.0, 6.7 Hz, 1H), 3.48 – 3.39 (m, 2H), 3.38 – 3.32 (m, 1H), 3.30 – 3.19 (m, 3H), 3.12 (t, J = 7.2 Hz, 2H, $NHCH_2$), 3.06 (t, J = 9.5 Hz, 1H), 2.20 (t, J = 7.3 Hz, 2H, CH_2C=O), 2.08 (ddd, J = 9.7, 6.9, 3.1 Hz, 1H, H5), 1.86 – 1.78 (m, 2H), 1.72 (s, 14H), 1.59 – 1.50 (m, 2H), 1.50 – 1.40 (m, 4H), 1.40 – 1.24 (m, 8H). ^{13}C NMR (126 MHz, MeOD) δ 175.7, 175.3, 174.6, 155.5, 155.4, 144.2, 143.5, 142.6, 142.5, 129.8, 129.7, 126.6, 126.3, 126.2, 123.4, 123.3, 112.0, 111.9, 104.4, 104.3, 103.1 (anomeric α-1,4), 100.5 (anomeric α-1,6), 83.6, 80.0, 75.3, 74.9, 74.9, 74.8, 74.0, 73.9, 73.7, 73.6, 73.6, 73.5, 72.6, 72.2, 71.6, 68.7, 62.5, 61.8, 58.0 (epoxide), 55.3 (epoxide), 50.5 (C_q), 50.5 (C_q), 45.1 (C5), 44.8 (CH_2N^+), 40.4 ($NHCH_2$), 36.7 (CH_2C=O), 31.6 (NCH_3), 31.4, 30.5, 30.4, 30.3 (4CH_2 linker), 28.2 (CH_2 linker), 28.0 (2CH_3), 27.9 (CH_2 linker), 27.8 (2CH_3), 27.4, 27.1, 26.6 (3CH_2 linker) ppm. HRMS (ESI) m/z: $[M]^+$ calc for $C_{59}H_{86}N_3O_{16}$ 1092.6003, found 1092.6000.

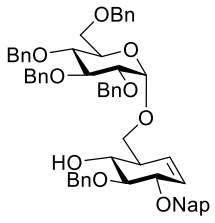
Compound 26



Amine **23** (13.4 mg, 21.4 µmol) was reacted with biotin (1.2 eq) for 25 h according to general procedure A. This afforded product **26** (7.1 mg, 8.3 µmol, 38%) as a white powder after lyophilization (3x). ^{1}H NMR (500 MHz, MeOD) δ 4.95 (d, J = 3.9 Hz, 1H, anomeric α-1,4), 4.83 (d, J = 3.7 Hz, 1H, anomeric α-1,6), 4.50 (ddd, J = 7.9, 5.0, 0.9 Hz, 1H, NHC*H* biotin), 4.31 (dd, J = 7.9, 4.5 Hz, 1H, NHC*H* biotin), 4.04 (dd, J = 10.9, 3.0 Hz, 1H), 3.96 – 3.83 (m, 3H), 3.83 – 3.63 (m, 8H), 3.59 (dd, J = 9.8, 8.7 Hz, 1H), 3.56 – 3.50 (m, 1H), 3.43 (ddd, J = 17.0, 9.7, 3.8 Hz, 2H), 3.35 (dd, J = 9.7, 8.8 Hz, 1H), 3.29 – 3.25 (m, 2H), 3.24 – 3.12 (m, 4H), 3.07 (dd, J = 10.0, 8.9 Hz, 1H), 2.93 (dd, J = 12.8, 5.0 Hz, 1H, SC*H*H), 2.71 (d, J = 12.7 Hz, 1H, SC*H*H), 2.20 (t, J = 7.3 Hz, 2H, CH_2C=O), 2.13 – 2.04 (m, 1H, H5), 1.77 – 1.41 (m, 10H), 1.38 – 1.28 (m, 8H), 1.22 (s, CH_3, ^{t}BuOH residue). ^{13}C NMR (126 MHz, MeOD) δ 176.0 (NHCONH), 166.1 (CONH), 103.1 (anomeric α-1,4), 100.5 (anomeric α-1,6), 83.6, 80.0, 75.3, 75.0, 74.8, 74.0, 73.7, 73.5, 72.6, 72.3, 71.7, 68.8, 63.4,

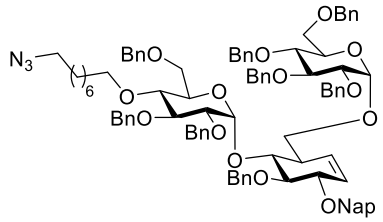
62.5, 61.8, 61.6, 58.0 (epoxide), 57.0 (SCH), 55.3 (epoxide), 45.1 (C5), 41.1 (SCH_2), 40.4 ($NHCH_2$), 36.8 ($CH_2C=O$), 31.4 (CH_2 linker), 31.1 (CH_3, ^{t}BuOH residue), 30.5, 30.4, 30.4, 29.8, 29.5, 27.9, 27.1, 27.0 ($8CH_2$ linker) ppm. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{37}H_{64}N_3O_{17}S$ 854.3951, found 854.3950.

Compound 27



The imidate donor **S1** (1.5 g, 2.1 mmol) and cyclohexane acceptor **10** (0.78 g, 2.0 mmol) were co-evaporated with toluene (3 x), then $OPPh_3$ (3.34 g, 12.0 mmol) and activated 3 Å molecular sieves were added under nitrogen. The mixture was dissolved with dry DCM (40 mL) and stirred at rt for 30 min. Then TMSI (342 µL, 2.4 mmol) was added slowly and the reaction was stirred at rt for 24 h. The reaction was then quenched with a mixture of sat. aq. $Na_2S_2O_3$ and sat. aq. $NaHCO_3$, stirred vigorously at rt until the deep red color faded. The mixture was filtered over celite and diluted with DCM. The layers were separated and the organic layer was washed with H_2O (2 x), brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude was first purified by silica gel column chromatography (Pentane/EtOAc 9:1 → 3:1) to give an inseparable mixture of product and hydrolyzed donor. Then the mixture was further purified with size exclusion (eluent MeOH/DCM: 1/1) to afford product **27** as a mixture of two isomers (α-1,6 linkage/β-1,6 linkage ≈ 14/1, 1.2 g, 1.3 mmol, 65%) as a clean oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 – 7.74 (m, 4H, CH Ar), 7.50 – 7.41 (m, 3H, CH Ar), 7.41 – 7.22 (m, 23H, CH Ar), 7.19 – 7.09 (m, 2H, CH Ar), 5.77 – 5.71 (m, 1H, H7), 5.59 (dt, *J* = 10.2, 2.0 Hz, 1H, H1), 5.01 – 4.71 (m, 9H, 8CH*H* Bn and H1'), 4.64 (d, *J* = 12.0 Hz, 1H, 1CH*H* Bn), 4.58 (d, *J* = 12.1 Hz, 1H, 1CH*H* Bn), 4.46 (dd, *J* = 11.5, 5.9 Hz, 2H, 2CH*H* Bn), 4.27 – 4.20 (m, 1H, H2), 3.94 (t, *J* = 9.3 Hz, 1H, H3'), 3.80 (dd, *J* = 9.5, 6.1 Hz, 1H, H6a), 3.76 – 3.66 (m, 4H, H3, H4, H5' and H6'a), 3.65 – 3.59 (m, 2H, H6'b and H4'), 3.56 (dd, *J* = 9.6, 3.7 Hz, 1H, H2'), 3.45 (dd, *J* = 9.5, 6.4 Hz, 1H, H6b), 3.28 (d, *J* = 1.2 Hz, 1H, OH), 2.66 – 2.58 (m, 1H, H5). ^{13}C NMR (126 MHz, $CDCl_3$) δ 139.0, 138.9, 138.3, 138.3, 138.0, 136.0, 133.4, 133.1 ($8C_q$ Ar), 128.6, 128.6, 128.5, 128.3, 128.1, 128.1, 128.0, 128.0, 128.0, 127.8, 127.7, 127.7, 127.5, 127.4, 126.7, 126.2, 126.0, 126.0 (32CH Ar, C1 and C7), 97.6 (C1'), 83.9 (C3), 82.2 (C3'), 80.1, 80.0 (C2 and C2'), 77.7 (C4'), 75.8, 75.2, 75.2, 73.6, 73.3 ($5CH_2$ Bn/Nap), 72.6 (C4), 72.0 (CH_2 Bn/Nap), 70.5 (C5'), 70.3 (C6), 68.5 (C6'), 43.9 (C5) ppm. HRMS (ESI) m/z: $[M+NH_4]^+$ calc for $C_{59}H_{64}O_9N$ 930.4576, found 930.4569.

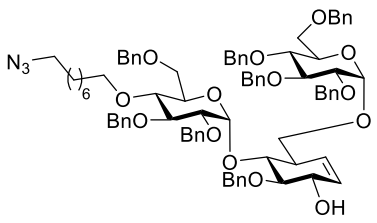
Compound 28



Donor **S2** (1.37 g, 1.97 mmol) was co-evaporated with toluene (3 x) and dissolved in dry DCM (22.0 mL) under nitrogen and stirred over fresh flame-dried 3 Å molecular sieves, after which DMF (2.4 mL, 31.5 mmol, 16.0 eq. of donor) was added to the solution. The solution was cooled to 0 °C, NIS (442 mg, 1.97 mmol) and TMSOTf (380 µL, 1.97 mmol) were added successively. The mixture was pre-activated at 0 °C for 1 h. Then

acceptor **27** (1.20 g, 1.31 mmol) was dissolved with dry DCM (4 mL in total) and added to the solution and the reaction was stirred at 0 °C for 61 h. The reaction was then quenched with a mixture of sat. aq. $Na_2S_2O_3$ and sat. aq. $NaHCO_3$, stirred vigorously at rt until the brown color faded. The mixture was filtered over celite and diluted with DCM. The layers were separated and the organic layer was washed with H_2O (2 x), brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude was first purified by silica gel column chromatography (Pentane/EtOAc 11:1 → 5:1), then the impure fractions were collected and further purified with size exclusion (eluent MeOH/DCM: 1/1) to afford product **28** (*dr* > 20/1, 1.55 g, 1.03 mmol, 78%) as a clean oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 – 7.73 (m, 1H, CH Ar), 7.70 (d, *J* = 8.4 Hz, 1H, CH Ar), 7.68 – 7.63 (m, 2H, CH Ar), 7.46 – 7.38 (m, 2H, CH Ar), 7.36 – 7.06 (m, 41H, CH Ar), 5.80 (ddd, *J* = 10.2, 2.7, 1.8 Hz, 1H), 5.75 (dt, *J* = 10.2, 2.1 Hz, 1H), 5.61 (d, *J* = 3.7 Hz, 1H, anomeric α-1,4), 4.98 – 4.62 (m, 12H, 11CH*H* Bn and anomeric α-1,6), 4.57 (dd, *J* = 15.4, 12.1 Hz, 2H), 4.51 – 4.45 (m, 3H), 4.38 (dd, *J* = 20.7, 12.1 Hz, 2H), 4.20 (ddt, *J* = 6.7, 4.4, 2.0 Hz, 1H), 4.02 – 3.85 (m, 4H), 3.82 – 3.63 (m, 9H), 3.62 – 3.52 (m, 2H), 3.49 – 3.33 (m, 3H), 3.19 (t, *J* = 7.0 Hz, 2H, CH_2N_3), 2.83 (tdq, *J* = 7.4, 4.6, 2.5 Hz, 1H, H5), 1.51 (p, *J* = 7.1 Hz, 2H, CH_2 linker), 1.40 (p, *J* = 6.8 Hz, 2H, CH_2 linker), 1.30 – 1.10 (m, 8H, 4CH_2 linker). ^{13}C NMR (126 MHz, $CDCl_3$) δ 139.4, 139.0, 138.9, 138.5, 138.4, 138.4, 138.2, 138.1, 135.9, 133.4, 133.0 (11C_q Ar), 128.6, 128.5, 128.5, 128.5, 128.5, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.7, 127.7, 127.6, 127.5, 127.4, 127.1, 126.8, 126.6, 126.1, 126.0, 125.9 (47CH Ar and C1 and C7), 97.7 (anomeric α-1,4), 97.1 (anomeric α-1,6), 84.0, 82.1, 81.9, 80.1, 80.0, 79.5, 77.9, 77.7, 75.8, 75.6, 75.5, 75.2, 74.0, 73.6, 73.5, 73.3, 72.9, 72.8, 71.9, 71.5, 70.6, 68.8, 68.6, 68.5, 51.5 (CH_2N_3), 43.5 (C5), 30.5, 29.5, 29.2, 28.9, 26.8, 26.1 (6CH_2 linker) ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{94}H_{103}N_3O_{14}Na$ 1520.7332, found 1520.7339.

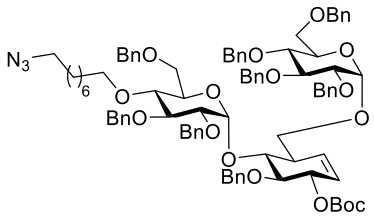
Compound 29



Compound **28** (1.52 g, 1.01 mmol) was dissolved in a mixture of DCM/H_2O (10/1, 20 mL). Then DDQ (252 mg, 1.11 mmol) was added and the reaction mixture was stirred for 1.5 h at rt. The reaction was diluted with DCM, washed with sat. aq. $NaHCO_3$ (3 x), H_2O and brine, dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/EtOAc 9:1 → 4:1) to obtain compound **29** (1.05 g, 0.773 mmol, 76%) as a clean oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 – 7.09 (m, 40H, CH Ar), 5.98 (ddt, *J* = 10.3, 4.6, 1.5 Hz, 1H), 5.81 (ddt, *J* = 10.3, 4.6, 1.1 Hz, 1H), 4.96 (d, *J* = 10.7 Hz, 1H), 4.87 – 4.74 (m, 6H), 4.66 – 4.52 (m, 7H), 4.49 – 4.39 (m, 3H), 4.37 – 4.32 (m, 1H), 4.20 (d, *J* = 12.0 Hz, 1H), 4.07 (dd, *J* = 11.6, 4.5 Hz, 1H), 3.95 (t, *J* = 9.2 Hz, 1H), 3.88 – 3.77 (m, 2H), 3.76 – 3.61 (m, 9H), 3.59 – 3.42 (m, 5H), 3.37 (dt, *J* = 9.0, 6.8 Hz, 1H), 3.19 (t, *J* = 7.0 Hz, 2H, CH_2N_3), 2.67 (q, *J* = 7.1, 6.7 Hz, 1H, H5), 1.51 (p, *J* = 6.9 Hz, 2H, CH_2 linker), 1.46 – 1.37 (m, 2H, CH_2 linker), 1.29 – 1.09 (m, 8H, 4CH_2 linker). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.9, 138.8, 138.5, 138.4, 138.1, 138.1, 138.0,

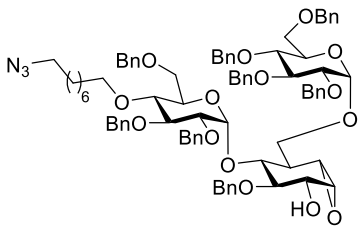
137.7 ($8C_q$ Ar), 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.1, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.4, 126.5 (40CH Ar and C1 and C7), 97.1 (anomeric), 95.9 (anomeric), 82.4, 82.1, 79.9, 78.6, 77.8, 77.5, 76.3, 75.9, 75.6, 75.2, 73.9, 73.6, 73.4, 73.3, 72.6, 72.1, 71.4, 70.3, 70.2, 69.2, 68.5, 68.4, 65.6, 51.5 (CH_2N_3), 41.7 (C5), 30.5, 29.5, 29.2, 28.9, 26.8, 26.2 ($6CH_2$ linker) ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{83}H_{95}N_3O_{14}Na$ 1380.6706, found 1380.6706.

Compound 30



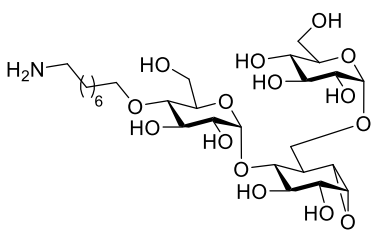
Compound **29** (1.04 g, 0.766 mmol) was co-evaporated with toluene (3 x) and dissolved in dry THF (15.3 mL) and cooled to 0 °C. Boc_2O (2.51 g, 11.5 mmol) and DMAP (9.3 mg, 76.6 µmol) were added at 0 °C. Then the reaction mixture was stirred at rt for 4 h. The reaction was quenched with sat. aq. NH_4Cl at 0 °C and concentrated *in vacuo* to remove most of the THF. Then the residue was diluted with Et_2O, washed with sat. aq. NH_4Cl, sat. aq. $NaHCO_3$, H_2O and brine, dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/EtOAc 15:1 → 6:1) to obtain compound **30** (944 mg, 0.648 mmol, 85%) as a clean oil. *Note: the starting material can react with the product by attacking the carbonyl group of Boc to form a dimer side product, thus far more excessive Boc_2O was needed to compete the side reaction.* ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 – 7.08 (m, 40H, CH Ar), 5.86 (ddd, J = 10.2, 3.1, 2.0 Hz, 1H), 5.65 (dt, J = 10.3, 2.4 Hz, 1H), 5.44 (d, J = 3.7 Hz, 1H, anomeric α-1,4), 5.34 (dq, J = 6.9, 2.4 Hz, 1H, H2), 4.95 (d, J = 10.8 Hz, 1H, 1CH*H* Bn), 4.91 – 4.86 (m, 2H, 2CH*H* Bn), 4.85 – 4.71 (m, 5H, anomeric α-1,6 and 4CH*H* Bn), 4.64 (d, J = 2.1 Hz, 2H, 2CH*H* Bn), 4.60 – 4.50 (m, 4H, 4CH*H* Bn), 4.47 (d, J = 10.8 Hz, 1H, 1CH*H* Bn), 4.38 (dd, J = 25.5, 12.1 Hz, 2H, 2CH*H* Bn), 4.01 – 3.85 (m, 4H), 3.81 – 3.61 (m, 9H), 3.57 (ddd, J = 19.4, 10.0, 2.3 Hz, 2H), 3.49 – 3.34 (m, 3H), 3.19 (t, J = 7.0 Hz, 2H, CH_2N_3), 2.83 (dq, J = 7.8, 2.7 Hz, 1H, H5), 1.56 – 1.47 (m, 2H, CH_2 linker), 1.40 (s, 11H, CH_2 linker and $3CH_3$ Boc), 1.31 – 1.09 (m, 8H, $4CH_2$ linker). ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.2 (C=O, Boc), 139.0, 138.9, 138.9, 138.4, 138.3, 138.3, 138.2, 138.0 ($8C_q$ Ar), 129.8 (C1/C7), 128.5, 128.4, 128.4, 128.4, 128.3, 128.2, 128.0, 128.0, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.2, 127.0 (40CH Ar), 125.3 (C1/C7), 97.8 (anomeric α-1,4), 97.1 (anomeric α-1,6), 82.3 (C_q, Boc), 82.1, 81.9, 81.8, 79.9, 79.4, 77.8, 77.6, 76.3, 75.8, 75.7, 75.5, 75.2, 74.0, 73.6, 73.4, 73.2, 72.8, 71.5, 70.6, 68.7, 68.6, 68.4, 51.5 (CH_2N_3), 43.1 (C5), 30.5, 29.5, 29.2, 28.9 ($4CH_2$ linker), 27.8 ($3CH_3$ Boc), 26.8, 26.1 ($2CH_2$ linker) ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{88}H_{103}N_3O_{16}Na$ 1480.7231, found 1480.7229.

Compound 31



Compound **30** (936 mg, 0.642 mmol) was dissolved in a mixture of DCM/HOAc (0.2 M, 2/1 (v/v)), NIS (288 mg, 1.28 mmol) was added and the reaction was stirred at rt for 22 h. The mixture was diluted with Et_2O and quenched with Et_3N (2.6 mL). Then the Et_2O layer was washed with sat. aq. NH_4Cl, sat. aq. $NaHCO_3$, sat. aq. $Na_2S_2O_3$, H_2O and brine, dried over Na_2SO_4, filtrated and concentrated *in vacuo*. As the conversion was not complete, the crude (896 mg, yellow oil) was obtained as a mixture of an iodocarbonate intermediate and starting material **30** (ratio ≈ 1.5:1, determined by ^{1}H NMR) and it's difficult to separate them by standard column chromatography. Therefore the crude was directly dissolved in a mixture of DCM/MeOH (2.7 mL/4.3 mL), NaOMe (25 wt% in MeOH, 0.24 mL, 1.05 mmol) was added and the mixture was stirred at rt overnight. The reaction was quenched with $Et_3N{\cdot}HCl$ (173 mg, 1.26 mmol) and concentrated *in vacuo*. The residue was diluted with EtOAc, washed with H_2O and brine, dried over Na_2SO_4, filtrated and concentrated *in vacuo*. The crude was purified with silica column chromatography (Pentane/EtOAc 12:1 → 3:1) to obtain compound **31** (404 mg, 0.294 mmol, 46% over two steps) as a clean oil, and unreacted starting material **30** (292 mg, 0.20 mmol) from the first step was recovered as well. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52 – 6.95 (m, 40H, CH Ar), 4.97 – 4.74 (m, 8H), 4.68 (d, J = 11.9 Hz, 1H), 4.62 – 4.52 (m, 6H), 4.49 (d, J = 10.8 Hz, 1H), 4.39 (dd, J = 20.7, 12.0 Hz, 2H), 3.99 (ddd, J = 10.0, 5.7, 2.4 Hz, 2H), 3.90 (td, J = 9.3, 7.5 Hz, 2H), 3.82 (ddd, J = 10.1, 3.7, 2.3 Hz, 1H), 3.79 – 3.52 (m, 10H), 3.48 – 3.34 (m, 4H), 3.29 (dd, J = 4.1, 2.3 Hz, 1H, epoxide), 3.20 (t, J = 7.0 Hz, 2H, CH_2N_3), 3.17 (dd, J = 4.1, 1.0 Hz, 1H, epoxide), 2.68 (d, J = 5.8 Hz, 1H, OH), 2.62 (q, J = 4.8 Hz, 1H, H5), 1.54 (p, J = 7.0 Hz, 2H, CH_2 linker), 1.44 (p, J = 6.7 Hz, 2H, CH_2 linker), 1.36 – 1.10 (m, 8H, 4CH_2 linker). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.8, 138.7, 138.7, 138.3, 138.3, 138.1, 138.1, 138.0 (8C_q Ar), 128.6, 128.5, 128.4, 128.4, 128.4, 128.3, 128.0, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6 (40CH Ar), 98.6 (anomeric α-1,4), 97.7 (anomeric α-1,6), 82.0, 81.6, 80.0, 79.5, 79.3, 79.0, 78.0, 77.6, 75.7, 75.6, 75.2, 74.4, 73.6, 73.4, 73.3, 73.3, 73.0, 71.5, 70.9, 69.1, 68.8, 68.5, 68.4, 54.9 (epoxide), 54.8 (epoxide), 51.5 (CH_2N_3), 41.6 (C5), 30.4, 29.4, 29.1, 28.9, 26.7, 26.1 (6CH_2 linker) ppm. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{83}H_{95}N_3O_{15}Na$ 1396.6655, found 1396.6655.

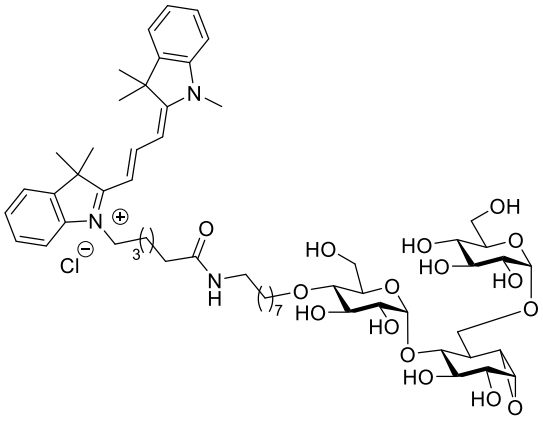
Compound 32



Ammonium (6.0 mL) was condensed in a dry flask at -60 ^{0}C. Sodium (81 mg, 3.5 mmol) was added and the resulting deep-blue solution was stirred for 15 min to dissolve all sodium. Compound **31** (60 mg, 44 μmol) and ^{t}BuOH (340 μL, 3.5 mmol) were taken up in anhydrous THF (1.5 mL) and slowly added into the reaction mixture under a flow of argon. After stirring for 1 h, the reaction was carefully quenched with aq. HOAc (225 μL HOAc in 0.5 mL H_2O, 3.92 mmol), slowly warmed to rt and stirred for 1 h. The reaction mixture was concentrated

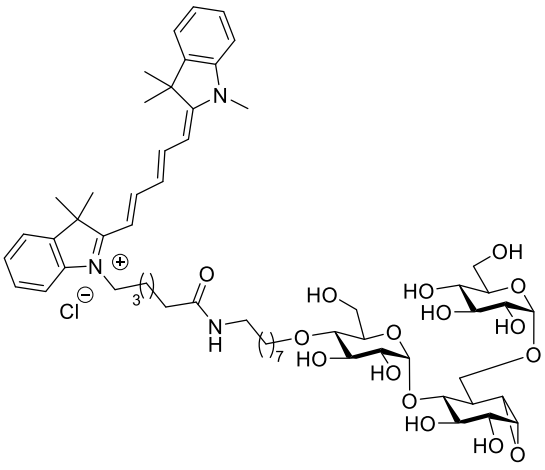
and desalted by size exclusion over HW-40 (1% HOAc in water). The compound **32** was obtained as a white powder (16.1 mg, 25.5 µmol, 57%) after lyophilization. ^{1}H NMR (500 MHz, D_2O) δ 5.10 (d, J = 4.0 Hz, 1H, anomeric α-1,4), 4.95 (d, J = 3.7 Hz, 1H, anomeric α-1,6), 3.98 (ddd, J = 16.1, 9.4, 3.9 Hz, 2H), 3.89 – 3.62 (m, 12H), 3.60 – 3.52 (m, 2H), 3.51 – 3.40 (m, 4H), 3.28 (t, J = 9.5 Hz, 1H), 2.98 (td, J = 7.5, 1.2 Hz, 2H, CH_2NH$_2$), 2.41 (ddd, J = 9.4, 6.2, 2.9 Hz, 1H, H5), 1.62 (dp, J = 29.4, 6.9 Hz, 4H, 2CH$_2$ linker), 1.35 (dt, J = 10.3, 5.6 Hz, 8H, 4CH$_2$ linker). ^{13}C NMR (126 MHz, D_2O) δ 100.9 (anomeric α-1,4), 98.8 (anomeric α-1,6), 81.1, 77.6, 73.3 (CH$_2$O linker), 73.3, 72.9, 72.2, 71.9, 71.6, 71.4, 70.6, 69.5, 67.2 (C6), 60.4, 60.3 (C6' and C6''), 57.4 (epoxide), 55.2 (epoxide), 41.1 (C5), 39.6 (CH$_2$NH$_2$), 29.2, 28.3, 28.1, 26.8, 25.5, 25.1 (6CH$_2$ linker) ppm. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{27}H_{50}NO_{15}$ 628.3175, found 628.3178.

Compound 33



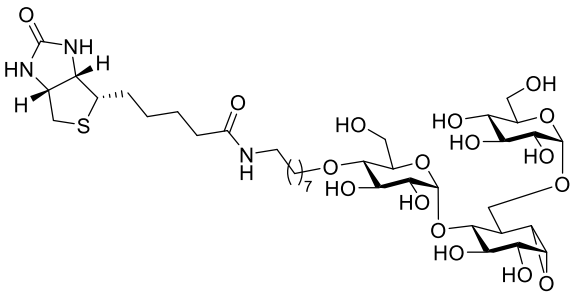
Amine **32** (12.3 mg, 19.6 µmol) was reacted with Cy3 acid (1.05 eq) for 24 h according to general procedure A. This afforded product **33** (6.1 mg, 5.5 µmol, 28%) as a red powder after lyophilization (2x). ^{1}H NMR (500 MHz, MeOD) δ 8.55 (t, J = 13.5 Hz, 1H), 7.60 – 7.52 (m, 2H), 7.46 (tdd, J = 7.6, 4.1, 1.2 Hz, 2H), 7.39 – 7.28 (m, 4H), 6.45 (d, J = 6.1 Hz, 1H), 6.43 (d, J = 6.0 Hz, 1H), 4.99 (d, J = 3.9 Hz, 1H, anomeric α-1,4), 4.83 (1H, anomeric α-1,6, obscured by H_2O peak), 4.16 (t, J = 7.5 Hz, 2H, CH$_2$N$^+$), 4.01 (dd, J = 9.9, 6.4 Hz, 1H), 3.88 – 3.74 (m, 5H), 3.73 – 3.52 (m, 11H), 3.42 (ddd, J = 12.7, 9.8, 3.8 Hz, 2H), 3.36 – 3.32 (m, 1H), 3.29 – 3.26 (m, 3H), 3.15 – 3.07 (m, 3H), 2.28 – 2.16 (m, 3H, H5 and CH$_2$C=O), 1.92 – 1.80 (m, 2H), 1.77 (s, 12H, 4CH$_3$), 1.71 (p, J = 7.4 Hz, 2H), 1.60 – 1.41 (m, 6H), 1.39 – 1.26 (m, 8H). ^{13}C NMR (126 MHz, MeOD) δ 176.7, 176.0, 175.7, 152.1, 144.1, 143.4, 142.2, 142.1, 130.0, 130.0, 126.8, 126.8, 123.6, 123.4, 112.5, 112.3, 103.8, 103.7, 103.3 (anomeric α-1,4), 100.8 (anomeric α-1,6), 84.0, 79.4, 75.3, 74.8, 74.8, 74.2, 74.0, 73.8, 73.5, 72.6, 71.9, 68.7, 62.8, 62.4, 58.1 (epoxide), 55.4 (epoxide), 50.7 (C$_q$), 50.6 (C$_q$), 45.1 (CH$_2$N$^+$), 43.3 (C5), 40.4 (NHCH$_2$), 36.7 (*C*H$_2$C=O), 31.8 (NCH$_3$), 31.4, 30.5, 30.4, 30.3 (4CH$_2$ linker), 28.3 (2CH$_3$), 28.2 (CH$_2$ linker), 28.2 (2CH$_3$), 27.9, 27.3, 27.1, 26.6 (4CH$_2$ linker) ppm. HRMS (ESI) m/z: $[M]^+$ calc for $C_{57}H_{84}N_3O_{16}$ 1066.5846, found 1066.5849.

Compound 34



Amine **32** (13.8 mg, 22.0 µmol) was reacted with Cy5 acid (1.05 eq) for 17 h according to general procedure A. This afforded the product **34** (13.2 mg, 11.7 µmol, 53%) as a blue powder after lyophilization (3x). ^{1}H NMR (500 MHz, MeOD) δ 8.29 – 8.20 (m, 2H), 7.52 – 7.47 (m, 2H), 7.42 (tdd, J = 7.7, 3.6, 1.2 Hz, 2H), 7.33 – 7.23 (m, 4H), 6.63 (t, J = 12.4 Hz, 1H), 6.28 (dd, J = 13.7, 4.6 Hz, 2H), 4.99 (d, J = 3.9 Hz, 1H, anomeric α-1,4), 4.83 (1H, anomeric α-1,6, obscured by H_2O peak), 4.11 (t, J = 7.4 Hz, 2H, CH_2N^+), 4.01 (dd, J = 9.9, 6.4 Hz, 1H), 3.89 – 3.74 (m, 5H), 3.72 – 3.50 (m, 11H), 3.42 (ddd, J = 12.3, 9.7, 3.8 Hz, 2H), 3.34 (d, J = 8.9 Hz, 1H), 3.31 – 3.25 (m, 3H), 3.15 – 3.07 (m, 3H), 2.27 – 2.17 (m, 3H, H5 and CH_2C=O), 1.87 – 1.79 (m, 2H), 1.73 (s, 14H), 1.59 – 1.50 (m, 2H), 1.50 – 1.39 (m, 4H), 1.39 – 1.25 (m, 8H). ^{13}C NMR (126 MHz, MeOD) δ 175.7, 175.4, 174.7, 155.5, 144.2, 143.6, 142.6, 142.5, 129.8, 129.8, 126.6, 126.3, 126.3, 123.4, 123.3, 112.1, 111.9, 104.4, 104.3, 103.3 (anomeric α-1,4), 100.8 (anomeric α-1,6), 84.0, 79.4, 75.3, 74.8, 74.8, 74.1, 74.0, 73.8, 73.5, 72.6, 71.9, 68.7, 62.8, 62.4, 58.1 (epoxide), 55.4 (epoxide), 50.5 (C_q), 50.5 (C_q), 44.8 (CH_2N^+), 43.3 (C5), 40.4 ($NHCH_2$), 36.7 (CH_2C=O), 31.5 (NCH_3), 31.4, 30.5, 30.4, 30.3 (4CH_2 linker), 28.2 (CH_2 linker), 28.0 (2CH_3), 27.9 (CH_2 linker), 27.8 (2CH_3), 27.4, 27.1, 26.6 (3CH_2 linker) ppm. HRMS (ESI) m/z: $[M]^+$ calc for $C_{59}H_{86}N_3O_{16}$ 1092.6003, found 1092.6005.

Compound 35



Amine **32** (10.1 mg, 16.1 µmol) was reacted with biotin (1.2 eq) for 19 h according to general procedure A. This afforded the product **35** (7.1 mg, 8.3 µmol, 52%) as a white powder after lyophilization (2x). ^{1}H NMR (500 MHz, D_2O) δ 5.10 (d, J = 4.0 Hz, 1H, anomeric α-1,4), 4.96 (d, J = 3.7 Hz, 1H, anomeric α-1,6), 4.64 – 4.58 (m, 1H, NHC*H*), 4.42 (dd, J = 8.0, 4.5 Hz, 1H, NHC*H*), 4.03 – 3.94 (m, 2H), 3.89 – 3.62 (m, 12H), 3.57 (td, J = 9.6, 3.8 Hz, 2H), 3.52 – 3.39 (m, 4H), 3.33 (ddd, J = 9.1, 5.8, 4.5 Hz, 1H, SCH), 3.28 (t, J = 9.4 Hz, 1H), 3.17 (hept, J = 6.7 Hz, 2H, NHCH_2), 3.00 (dd, J = 13.0, 5.0 Hz, 1H, SC*H*H), 2.79 (d, J = 13.1 Hz, 1H, SCH*H*), 2.41 (ddd, J = 9.2, 6.1, 3.1 Hz, 1H, H5), 2.25 (t, J = 7.1 Hz, 2H, CH_2C=O), 1.78 – 1.54 (m, 6H), 1.50 (t, J = 6.6 Hz, 2H), 1.45 – 1.26 (m, 10H), 1.24 (s, CH_3, ^{t}BuOH residue). ^{13}C NMR (126 MHz, D_2O) δ 176.6 (NHCONH), 165.4 (CONH), 101.0 (anomeric α-1,4), 98.8 (anomeric α-1,6), 81.1, 77.5, 73.3, 73.3, 72.9, 72.9, 72.2, 71.9, 71.7, 71.4, 70.6, 69.5, 67.2, 62.1, 60.4, 60.3, 60.3, 57.4 (epoxide), 55.5

(SCH), 55.2 (epoxide), 41.1 (C5), 39.8 (SCH_2), 39.3 ($NHCH_2$), 35.6 ($CH_2C=O$), 29.7 (CH_3, a small peak, ^{t}BuOH residue), 29.3, 28.5, 28.3, 28.3, 27.9, 27.7, 26.0, 25.3, 25.2 ($9CH_2$ linker), 22.6 (CH_3COONH_4 residue *which was removed after repeated lyophilization*) ppm. HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{37}H_{64}N_3O_{17}S$ 854.3951, found 854.3948.

3.5 References

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