Inhibitors and probes targeting endo-glycosidases

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Activity-based probes targeting glycosidases acting on plant glycans

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2.1 Introduction

Plant glycans

Plant glycans are the most abundant and structurally diverse biopolymers on the planet and are a prominent source of energy and food. Depending on the plant species and the examined tissue the molecular structure of plant glycans can contain amylose and pectins, cellulose and hemicelluloses (xylan, arabinoxylan, glucuronoxylan, xyloglucans) and mixed linkage glucans (Figure 2.1).[1,2] The abundance of these glycans in many environments has prompted many organisms to evolve enzymes to modify or metabolize these structures. These enzymes are of great interest, as discussed below, as catalysts for biomass utilization or in the context of gut microbiomes and human health.

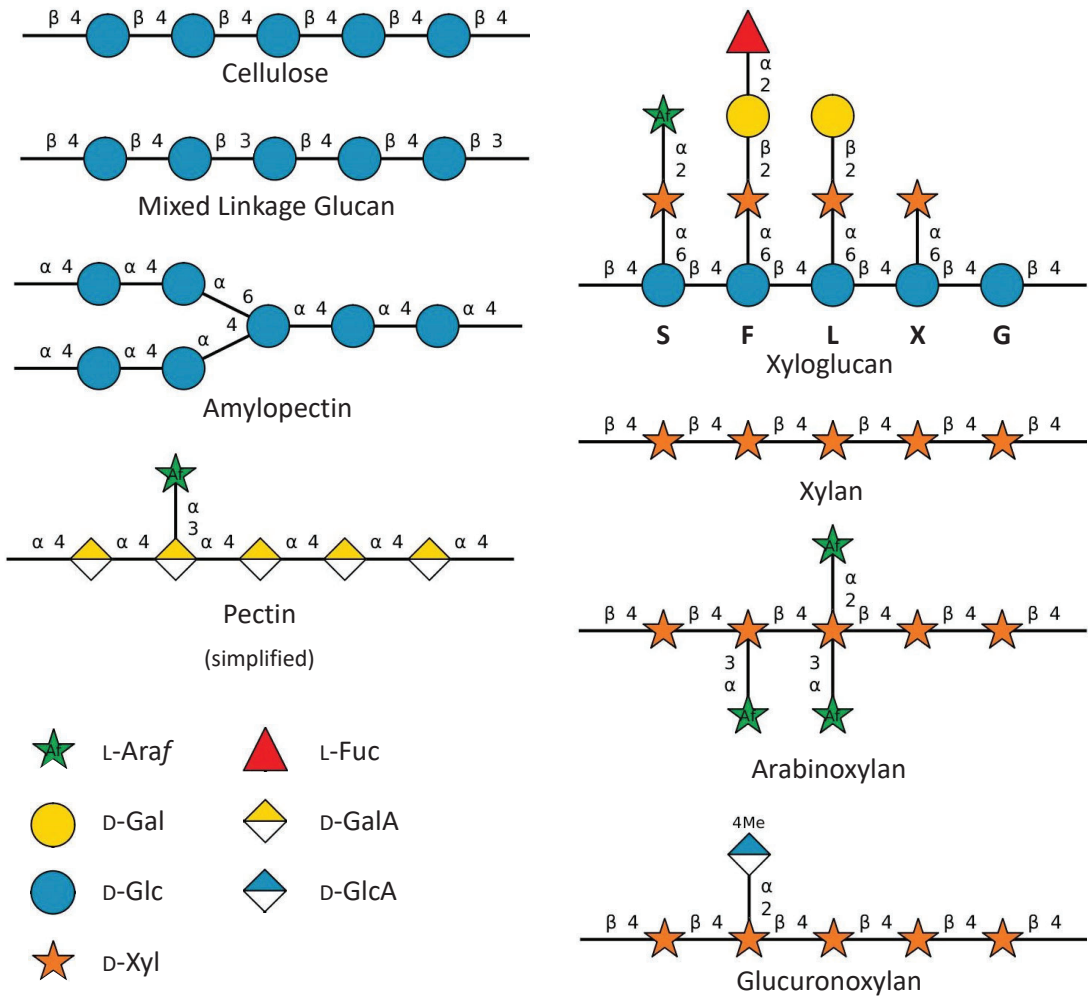


Figure 2.1| Structure of abundant plant polysaccharides. One letter abbreviation for the xyloglucan branches is shown under the structure.[3,4]

Glycoside hydrolases for biomass utilization

Efficient utilization of abundant plant biomass can provide a sustainable source of liquid fuel, platform chemicals and solvents.[5] Suitable biomass feedstocks such as wheat straw, wood and switchgrass contain between 35-48% cellulose, 22-32% hemicellulose and 12-22% polyaromatic lignin based on dry weight.[6] The recalcitrance and heterogeneity of the plant glycans is an obstacle for their efficient utilization.

Saprotrophs, organisms feeding on decaying matter, have evolved numerous glycosyl hydrolases (GHs) to degrade plant glycans.[7] Continuous efforts are made to discover highly active and stable enzymes for use in biotechnological catalysis. Many putative glycosidases have been found and documented based on genome analysis of these organisms and homology to known glycosidases (www.cazy.org).[8] The characterization of GH activity, specificity and stability based on sequence information alone, however, is complicated.

Common methodologies to properly characterize these glycosidases are laborious and require purified enzymes and therefore there is a need for new technologies to accelerate the characterization of (preferably) unpurified GHs.

Glycoside hydrolases in the human gut microbiome

An emerging field in relation to plant glycan active GHs is the study of the human gut microbiome. The human genome encodes for 97 GHs of which no more than 17 are associated with food digestion. Using our own gene products, humans are only able to enzymatically degrade the dietary glycans starch, sucrose and lactose while a healthy diet also contains many other glycans known as dietary fiber. The combined genome of the microbes living in the gut increases the digestive capability enormously, adding thousands of genes encoding GHs.[9] In this case, as well as in the previous paragraph, genetically derived primary sequence information alone does not provide sufficient information on the properties of the enzymes and, more importantly, the presence of a gene does not necessarily correlate with the expression of an active enzyme.

The importance of the constitution of an individual's microbiome is still poorly understood, but it is clear that it can be of profound influence on human health. For example, variations in microbial β-glucuronidase activity lead to variations in drug toxicity.[10] The microbial communities in the gut dynamically change their composition based on the available nutrients consisting mainly of complex glycans.[11] Several research groups have started to elucidate the metabolism of polysaccharides such as xyloglucans[12], xylans[13] and complex pectins[14] by glycosidases, secreted by gut symbionts. Monitoring these GH activities in the gut may lead to a better understanding of the significance of specific enzymatic activities in the microbiome.

Probes for plant glycan active GHs

Activity-based protein profiling (ABPP) is a powerful technique to discover and monitor glycosidases with plant polysaccharide degrading capability and can be used to increase the understanding of plant glycan degrading organisms in health an disease.[15,16] Activity-based probes (ABPs) are able to enrich low abundant enzymes in complex mixtures such as the gut microbiota or dilute secretomes, which would be difficult to detect by proteomic methods using unenriched samples.[17] Dedicated ABPs have been developed in the context of plant biomass polysaccharide processing.

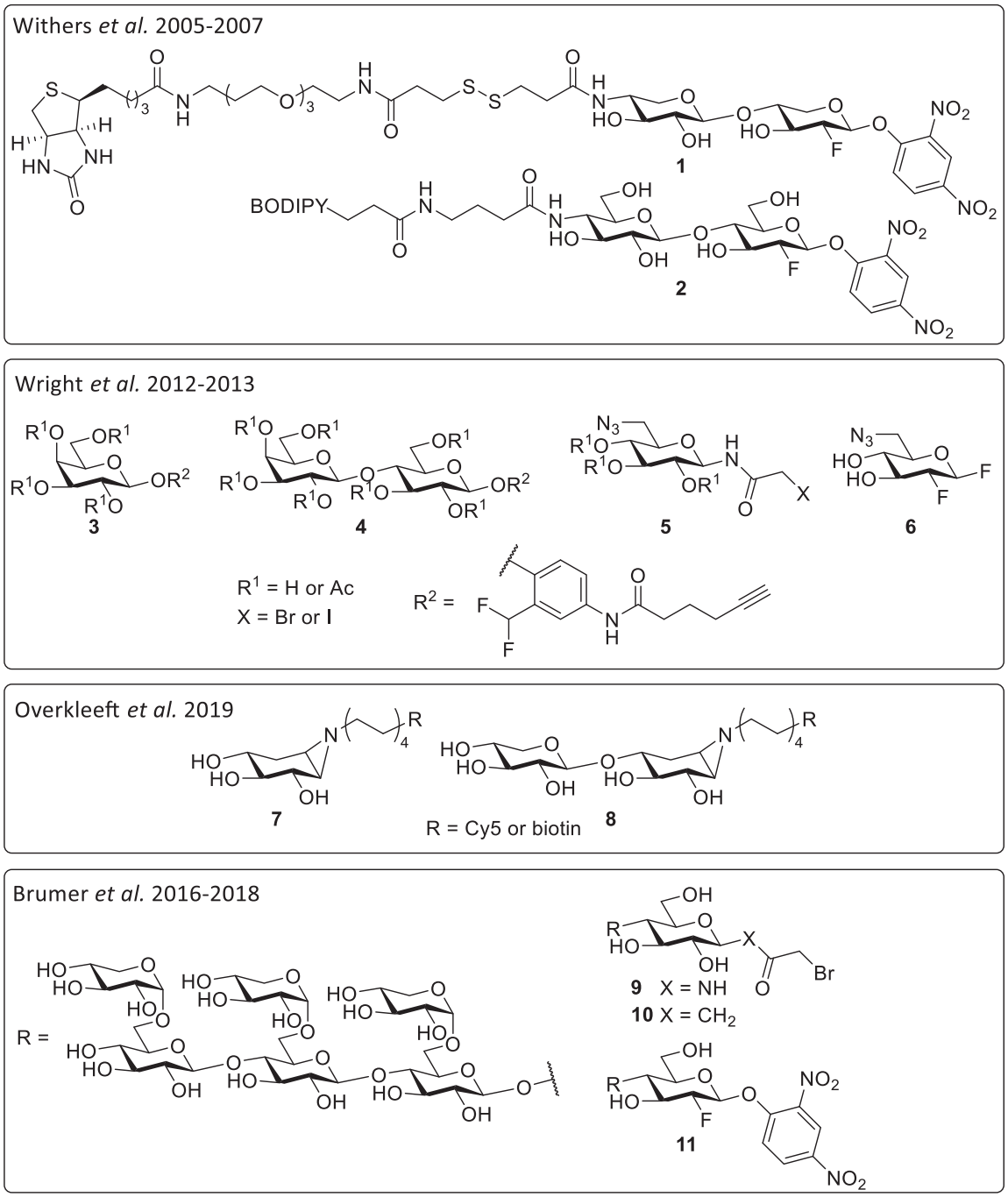


Figure 2.2| Affinity- and activity-based probes used to study biomass active enzymes.

The Withers group synthesized 2-deoxy-2-fluoro xylobiose (**1**) and cellobiose (**2**) derivatives with biotin and fluorescent reporter groups to profile endo-xylanase and cellulase activity (Figure 2.2).[18–20] This design affords selective probes for these enzymes and facilitated the discovery of a novel β-1,4-glycanase in a *Cellulomonas fimi* secretome. They also showed that the introduction of the linker with the reporter groups at the non-reducing end does not significantly change the inactivation kinetics for the two examined endo-xylanases.

The Wright group used a set of mono- and disaccharide probes with different warheads to study secretomes of *Clostridium thermocellum* and *Trichoderma reesei* (Figure 2.2).[21,22] In their protocols, the quinone methide activity-based probes (**3** and **4**) enrich carbohydrate active enzymes and other proteins associated with the cellulosome, a multi enzyme complex excreted by cellulase degrading organisms. This is probably due to diffusion of the tag away from the GH before covalent attachment. The α-halo acetamide affinity-based probes (**5**) enrich retaining and inverting GHs but do not discriminate between GHs and other carbohydrate active enzymes such as glycosyltransferases and carbohydrate kinases. The more selective 2-deoxy-2-fluoroglucose mechanism-based probe (**6**) does not show labeling in this setting, possibly due to the turnover of the covalent enzyme-probe adduct before analysis.

Overkleeft *et al.* reported the synthesis and application of β-xylosidase (**7**) and β-1,4-xylanase (**8**) probes based on the cyclitol aziridine warhead (Figure 2.2).[23] These probes react selectively with GH3 and GH10 xylan degrading enzymes in *Aspergillus niger* secretomes. The monosaccharide probe reacts selectively with exo-acting β-xylosidases. The larger recognition element of the disaccharide probe facilitates labeling of endo-acting enzymes. The exo-activity of β-xylosidases on a portion of the (excess) disaccharide probe also generates the β-xylosidase probe *in situ*. As a consequence, application of this probe shows labelling of both endo- and exo-acting retaining β-xylosidases (Figure 2.3). Curiously the retaining GH11 xylanase that is also present in the secretome did not appear to react with the probe.

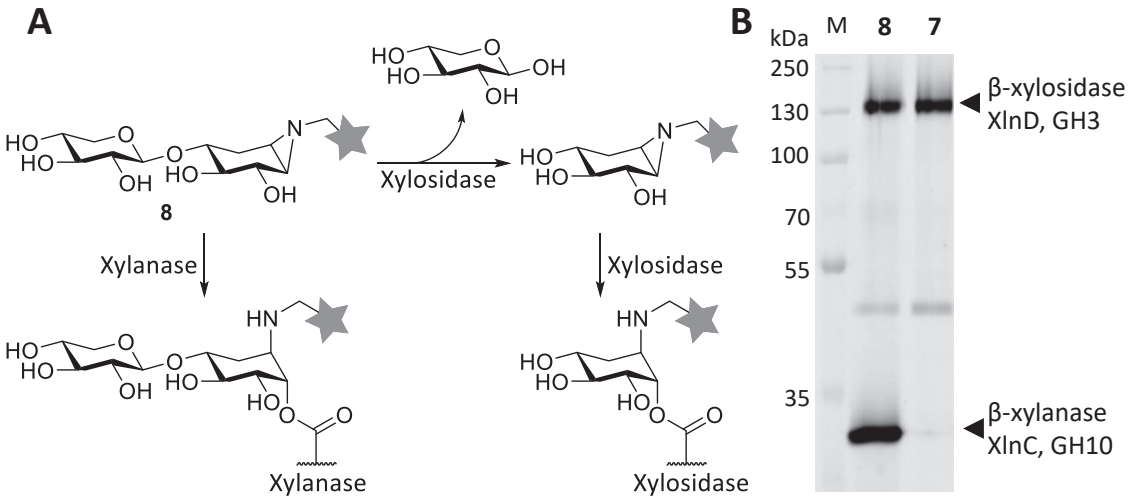


Figure 2.3|A) ABP **8** is enzymatically hydrolyzed resulting in labeling of both xylanases and xylosidases. **B**) SDS-PAGE gel showing Cy5 fluorescence of an *Aspergillus niger* secretome labeled with probe **8** or **7**.

Although mainly used as probes for crystallographic enzymology purposes, the inhibitors designed and synthesized by the Brumer group are noteworthy for containing the most extensive recognition element for xyloglucanases to date. The elaborate scaffold is accessible because the *N*-bromoacetylglycosylamine **9** and bromoketone *C*-glycoside **10** are conveniently synthesized in two steps from the oligosaccharide lactol. Xyloglucan oligosaccharides are readily available by hydrolysis of the appropriate plant material with a suitable glycosyl hydrolase.[24] The large recognition element considerably increases the affinity for xyloglucanases compared to smaller inhibitors and the inhibitors (**9** and **10**) have been used to observe enzyme substrate interactions by X-ray crystallography. As commonly observed with α-halo ketone inhibitors retaining glycosidases are covalently attached via the general acid/base residue instead of the naturally more reactive catalytic nucleophile.[25,26] To access the natural binding mode and increase selectivity a synthesis starting from the same lactol towards the mechanism-based 2-deoxy-2-fluoro inhibitor **11** was developed.[27]

Cyclitol epoxide-based ABPs for xyloglucan active glycosyl hydrolases

In this chapter a set of ABPs needed to monitor and discover cellulose and xyloglucan active retaining glycosidases is presented (Figure 2.4A). The set consists of GG, GX and XG configured cyclophellitols potentially active on retaining cellulases and xyloglucanases grouped in CAZY families 5, 7, 10, 12, 16, 44 and 51. The tag is positioned at the non-reducing end C4' position where the oligomer would naturally be elongated. This position is most likely to be large enough to accommodate the tag in the active site. Moreover, the bulky non-reducing end tag protects against cleavage by exo-glycosidases of the probe and separation of the tag and the warhead ensures labeling is only observed with the intact probe. Untagged GG and GGG configured cyclophellitols were chosen as initial targets to develop the glycosylation chemistry of this class of molecules.

α-Xylose cyclophellitol aziridine potentially targeting exo-α-xylosidases present in CAZY family 31 completes this set. Together with previously developed ABPs for β-glucosidases (**12**)[28,29], β-galactosidases (**13**)[30], α-fucosidases (**14**)[31] and α-L-arabinofuranosidases (**15**)[32] (Figure 2.4B) these probes may target most of the retaining endo- and exo-acting glycosidases active on the glycosidic linkages in xyloglucan.

For the synthesis of GX and XG configured probes **16** and **17** a strategy based on the use of three building blocks was proposed (Scheme 2.1). Selective attachment of the tag at the non-

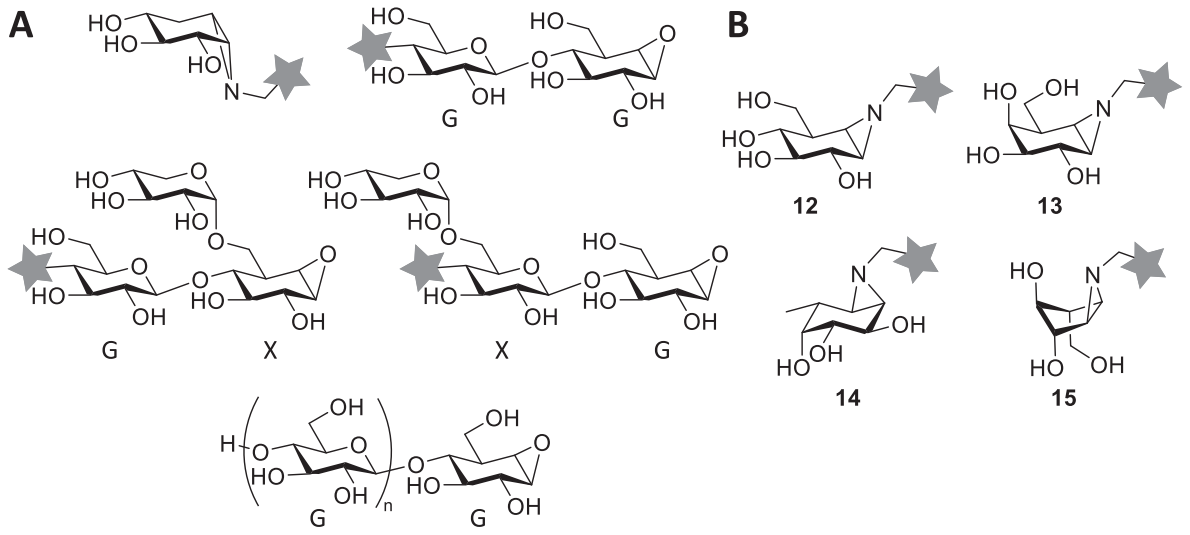
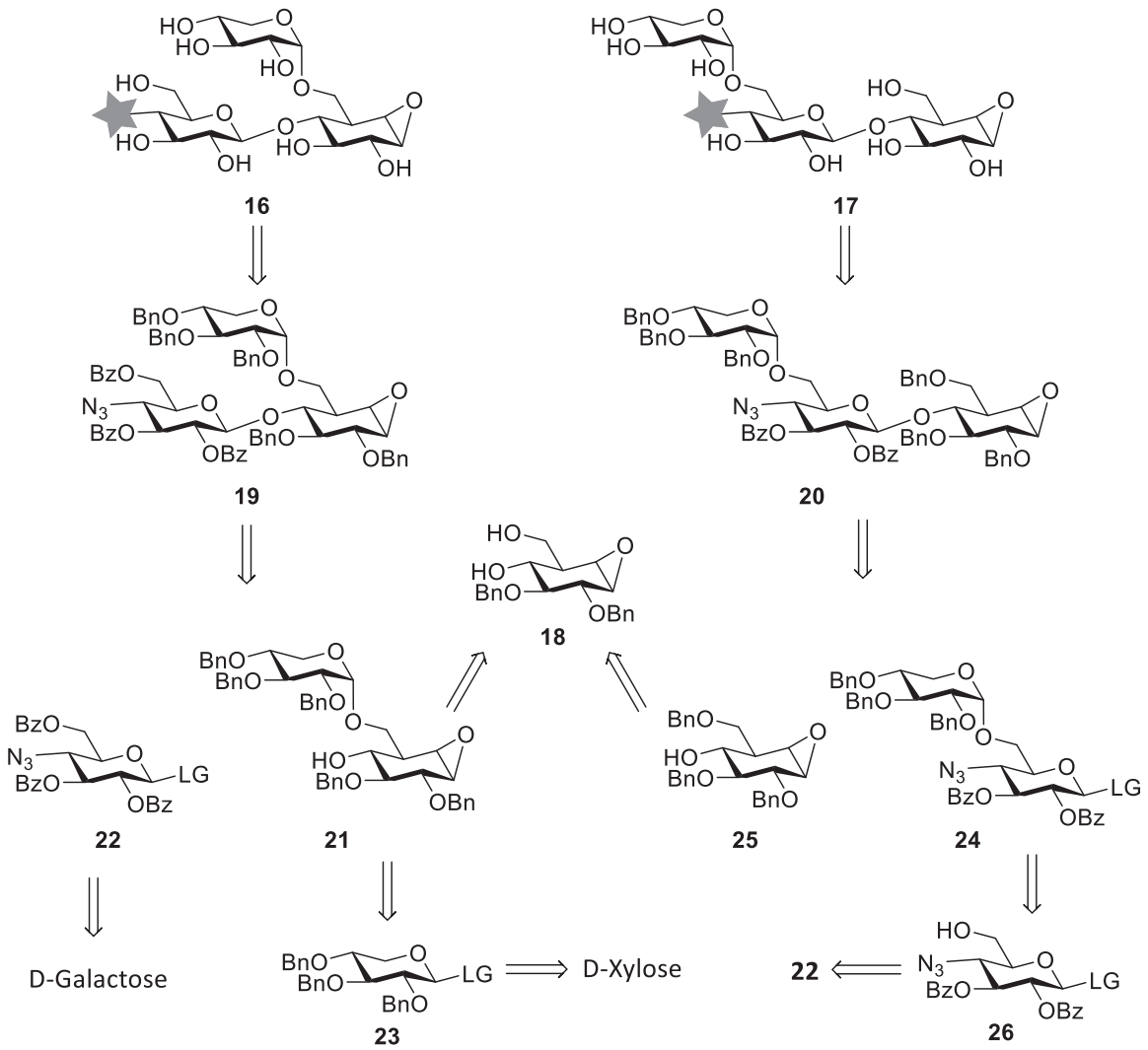


Figure 2.4| A) Structures of activity-based probes mimicking parts of the xyloglucan structure. **B**) Previously synthesized probes that target β-glucosidases (**12**), β-galactosidases (**13**), α-fucosidases (**14**) and α-L-arabinofuranosidases (**15**) relevant for xyloglucan hydrolysis. Stars denote various reporter groups.

reducing end was envisioned via amide bond formation on a C4' amine which could be masked as an azide during the synthesis. For the 1,2-trans glucosylation participating benzoyl esters were chosen. The convenient cyclophellitol synthesis first developed by Madsen *et al.*[33] yields partially benzyl ether protected building block **18**, therefore these non-participating protecting groups were selected for the 1,2-cis xylosylation. This would allow two step deprotection of the complete construct. This strategy would result in fully protected trisaccharides **19** and **20**.

The glucose moiety in **19**, attached to the least reactive 4' secondary alcohol, is first disconnected leading to pseudo-disaccharide **21** and 4-deoxy-4-azido-glucoside **22**. **22** can be accessed from galactose by S_N2 displacement of the activated axial alcohol with an azide nucleophile. **21** is accessible from cyclophellitol building block **18** by regio- and stereoselective xylosylation of the primary alcohol with xylosyl donor **23**.

Trisaccharide **20** is first disconnected into disaccharide **24** and cyclophellitol building block **25** to minimize the number of steps after introduction of the valuable cyclophellitol building block. **25** can be obtained from **18** by regioselective benzylation. **24** can be obtained by 1,2-cis xylosylation with **23** and acceptor **26** which is accessible from **22** by protecting group manipulations.



Scheme 2.1| Retrosynthetic analysis of XG and GX branched probes.

2.2 Results and discussion

The synthesis of the α-xylose cyclitol aziridine probes, cellulose cyclophellitol inhibitors and probes and xyloglucan cyclophellitol probes is described in the following sections. The chapter concludes with a section in which the utility of the endo-glycosidase probes is demonstrated in preliminary labeling studies on an *Aspergillus niger* secretome.

α-Xylosidase activity-based probes

α-Xylosidase ABPs were synthesized starting from aziridine **27** (Scheme 2.2) which was prepared from D-xylose in 11 steps following literature procedures.[34] Selective alkylation of the aziridine nitrogen over the secondary alcohol was achieved using the alkyl triflate and *N,N*-diisopropylethylamine (DIPEA). Subsequently **28** was deprotected in a two-step procedure: Staudinger reduction of the azide to the amine followed by dissolving metal

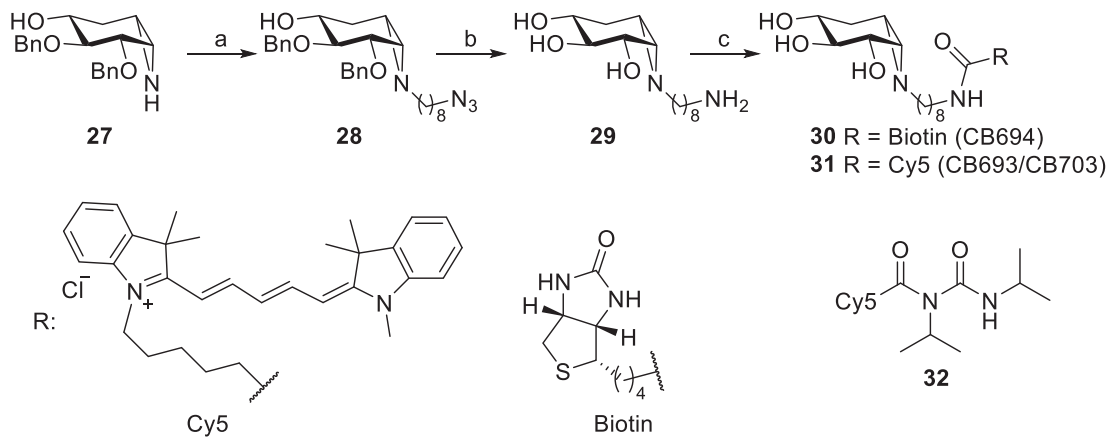
hydrogenolysis of the benzyl groups leading to **29**. The two step sequence is preferred over direct hydrogenolysis of **28** because, although sparsely reported in literature, deamination is a common side product under these conditions.[23,35]

Amide coupling of amine **29** with biotin mediated by *N,N'*-diisopropylcarbodiimide (DIC) afforded **30** after HPLC purification. Attempts to synthesize fluorescent **31** using the same conditions yielded the product contaminated with the rearranged DIC-Cy5 adduct **32**. PyBOB (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate) mediated coupling of **29** with Cy5 avoided the formation of such byproducts and the desired product was obtained after HPLC purification.

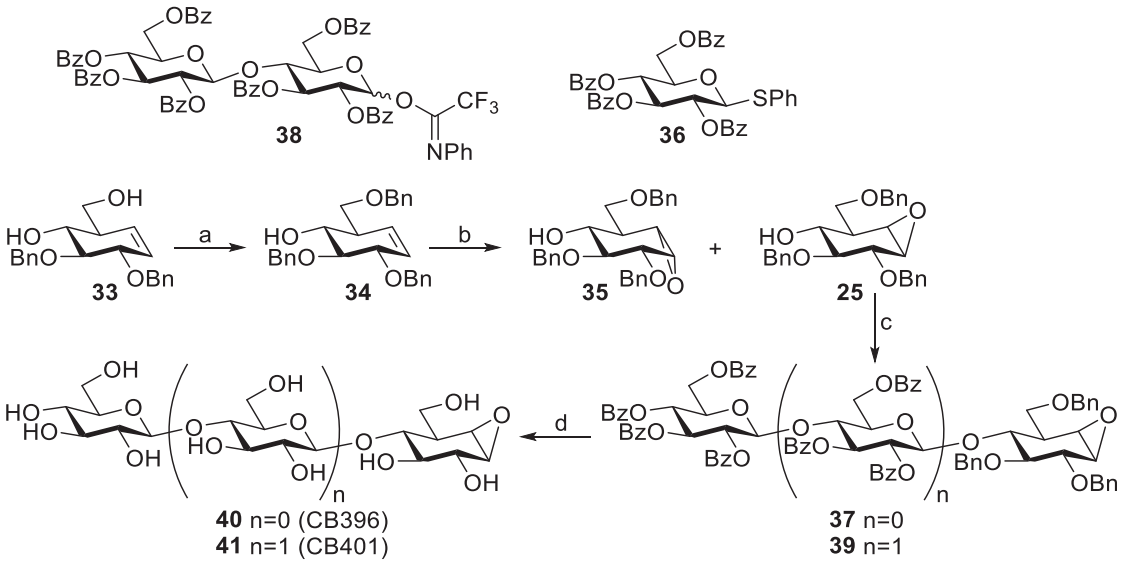
Covalent cellulase inhibitors

Alkene **33** obtained by published methods[33,36] was selectively benzylated at the primary alcohol using borinate catalysis (Scheme 2.3).[37] Epoxidation of **34** using *in situ* generated methyl(trifluoromethyl)dioxirane afforded epoxides **35** and **25** that were separated by silica column chromatography.

Glycosylation of **25** with thioglycoside donor **36**[38] afforded pseudo-disaccharide product **37**. Glycosylation with cellobiose derived *N*-phenyl trifluoroacetimidate **38** afforded pseudo-trisaccharide **39** in comparable yield. Attempts to access the trisaccharide from the thioglycoside donor employing similar conditions as for **37** were sluggish and afforded the product in low yield. This was mainly due to the poor reactivity of the fully benzoylated cellobiose donor. Attempts to increase the yield increasing the equivalents of donor were unsuccessful presumably because activated glycoside are able to react with epoxides.[39]



Scheme 2.2| Reagents and conditions: **a**) 8-azidooctyl trifluoromethanesulfonate, DIPEA, DCM, 86%. **b**) i. PPh_3 polymer bound, H_2O, MeCN, 70°C, 95%; ii. Na(s), *t*-BuOH, THF, NH_3, -60°C, quant. **c**) Biotin, DIC, DMAP, DIPEA, DMF, 18% or Cy5COOH, PyBOB, DIPEA, DMF, 19%.

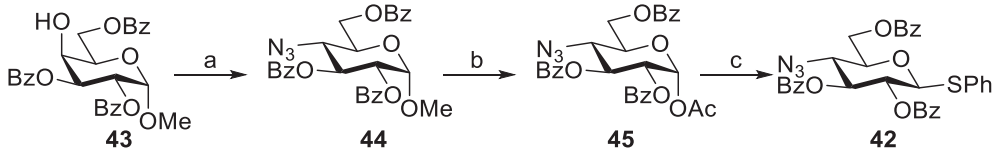


Scheme 2.3| Reagents and conditions: **a**) 2-aminoethyl diphenylborinate, KI, K_2CO_3, BnBr, MeCN, 60°C, 91%. **b**) 1,1,1 trifluoroacetone, oxone, EDTA, $NaHCO_3$, H_2O, MeCN, 0°C, 52% (**25**) and 46% (**35**). **c**) for **37**: **36**, NIS, TMSOTf, DCM, -30°C to -10°C, 53%. For **39**: **38**, TSMOTf, DCM, -15°C to 0°C, 45%. **d**) i. NaOMe, MeOH; ii. H_2, $Pd(OH)_2/C$, H_2O, MeOH, dioxane, for **40** quant., for **41** 45%.

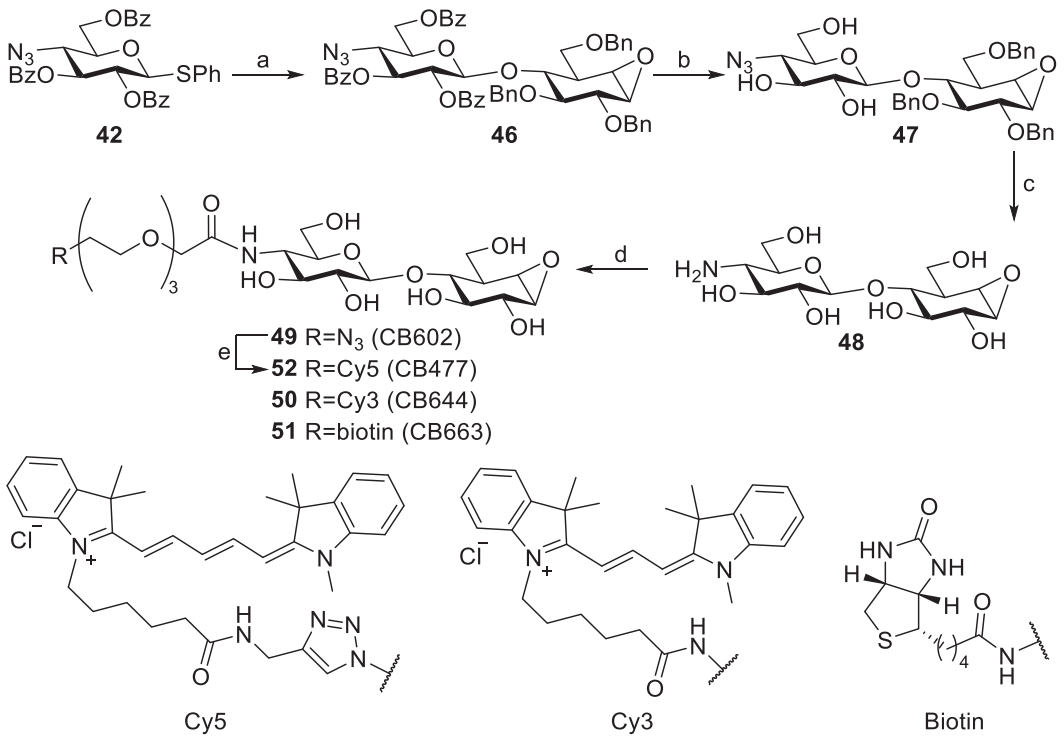
The deprotection sequence comprised of benzoyl removal by NaOMe in MeOH followed by short hydrogenation over a high loading of Pearlman's catalyst[34], provided inhibitor **40** in good yield. Trisaccharide inhibitor **41** was obtained in moderate yield mainly due to the poor solubility of the partially protected trisaccharide. To remove the methyl benzoate formed during the debenzoylation step the mixture was triturated in cold ether, lowering the yield considerably compared to the chromatography procedure used for the disaccharide.

Cellulase (GG) activity-based probes

To gain access to cellobiose configured ABPs 4-deoxy-4-azido-thioglucoside donor **42** was synthesized. The methods are similar to a published synthesis of 4-deoxy-4-fluoro-thioglucoside donors (Scheme 2.4).[40] Regioselective benzoylation of methyl α-D-galactopyranose afforded partially protected **43** of which the axial 4-OH was activated as a triflate and substituted with sodium azide leading to **44**. Acid catalyzed displacement of the



Scheme 2.4| Reagents and conditions: **a**) i. Tf_2O, pyr, DCM, -55°C to rt; ii. NaN_3, DMF, 80°C, 90%. **b**) Ac_2O, AcOH, H_2SO_4. **c**) HSPh, $BF_3{\cdot}Et_2O$, DCM, 46% over 2 steps.



Scheme 2.5| Reagents and conditions: **a**) **25**, Ph_2SO, Tf_2O, TTBP, DCM, -70°C to rt, 64%. **b**) NaOMe, MeOH, DCM, 60%. **c**) Na (s), *t*-BuOH, NH_3, -60°C, 53%. **d**) for **49**: N_3TEGCOOPFP (**S5**), DIPEA, DMF, 27%; for **50**: Cy3TEGCOOH (**S10**), DIC, PFP, DIPEA, DMF, 69%; for **51**: BiotinTEGCOOH (**S8**), DIC, PFP, DIPEA, DMF, 44%. **e**) Cy5 alkyne, THPTA, CuI, DIPEA, DMSO, 46%.

anomeric methoxy group afforded anomeric acetate **45**. Introduction of the anomeric thiophenol yielded donor **42**.

Donor **42** was reacted with acceptor **25** (Scheme 2.5). The yield of the glycosylation reaction was improved compared to the moderate yields obtained for the cellobiose and cellotriose inhibitors **37** and **39** in the previous section. This was achieved by adoption of a Tf_2O/ Ph_2SO pre-activation protocol in combination with the sterically hindered base 2,4,6-tri-*tert*-butylpyrimidine (TTBP). This way relatively high temperatures and long reaction times to activated this type of donors by N-iodosuccinimide (NIS)/triflic acid (TfOH), in the presence of the acid labile epoxide, were avoided. Disaccharide **46** is obtained in 74% yield without the use of a large excess of donor. Unreacted acceptor was recovered indicating the stability of the epoxide functionality under these conditions. Increasing the amount of donor led to diminished yield and complex mixtures presumably by reaction of the epoxide in the product with the excess activated donor.[41]

Subsequently, the benzoyl esters were removed with NaOMe (**47**) followed by reduction of the azide to avoid migration of the esters to the liberated amine. The reduction was

performed in two steps: a Staudinger reduction was performed to reduce the azide followed by benzyl removal under Birch conditions (**48**). Sodium hydroxide, formed while quenching the Birch reaction, was neutralized with NH_4Cl. Omission of this neutralization step leads to hydrolysis of the epoxide during concentration of the reaction mixture.

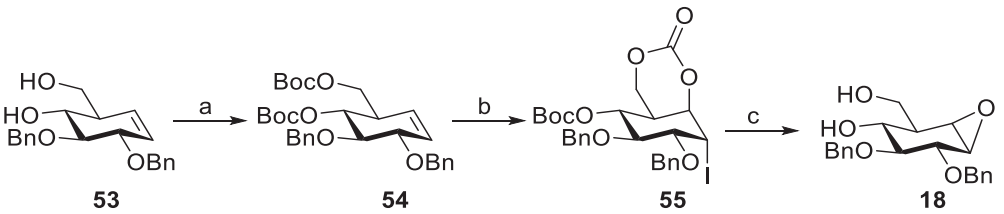
Fully deprotected disaccharide **48** was reacted with the pentafluorophenol activated ester of an azide (**S5**), Cy3 (**S10**) or biotin (**S12**) terminated triethylene glycol (TEG) spacer, yielding the azide (**49**), Cy3 (**50**) and biotin (**51**) equipped probes after semi preparative HPLC purification. Cy5 labeled probe **52** was obtained after copper catalyzed click reaction of **49** with Cy5 alkyne. Synthetic procedures towards the spacers are given in the experimental section (Scheme 2.9).

Xyloglucanase (GX) activity-based probes

To gain access to the GX motif a convenient synthesis of β-configured epoxide **18** was developed (Scheme 2.6). Epoxidation of diol **53** with *meta*-Chloroperoxybenzoic acid (mCPBA) is sluggish on this diol[33] so a diastereoselective iodocarbonylation approach to the β-epoxide was explored instead. [42–47]

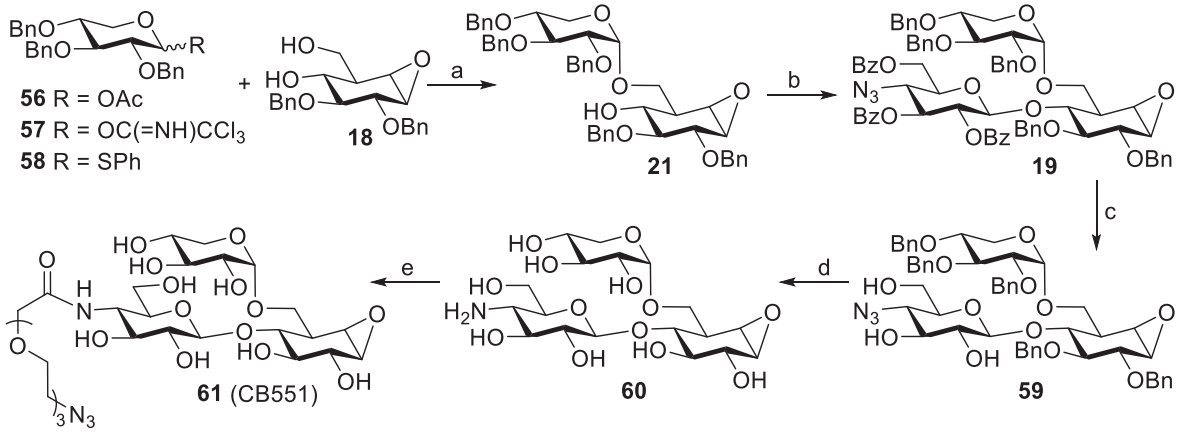
The three-step sequence started with *t*-butyloxycarbonyl (Boc) protection of diol **53** yielding fully protected **54**. Subsequent NIS induced iodocarbonylation afforded iodocarbonate **55**. The obtained iodocarbonate was treated with base in methanol generating the epoxide with concomitant solvolysis of the remaining Boc group leading to epoxide **18** in one step.

With acceptor **18** in hand access to the GX motif was gained by two subsequent glycosylations under basic or mildly acidic conditions to take the acid sensitivity of the epoxide warhead into account (Scheme 2.7). The first glycosylation on the primary alcohol was achieved by reacting xylosyl acetate donor **56**[48] with trimethylsilyl iodide (TMSI), which resulted in the formation of the xylosyl iodide and trimethylsilyl acetate (TMSOAc). The TMSOAc was removed by evaporation before addition of acceptor **18**. $OPPh_3$ was added as an



53 54 55 18

Scheme 2.6| Reagents and conditions: **a**) Boc_2O, DMAP, THF. **b**) NIS, AcOH. **c**) K_2CO_3, MeOH, 75% over three steps.



Scheme 2.7| Reagents and conditions: **a**) i. **56**, TMSI, DCM; ii. **18**, $OPPh_3$, DIPEA, DCM, 46%. **b**) **42**, Tf_2O, Ph_2SO, TTBP, DCM -70°C to rt. **c**) NaOMe, MeOH/DCM, 73% over 2 steps. **d**) i. PPh_3 polymer bound, H_2O, MeCN, 55°C; ii. Na (s), *t*-BuOH, THF, NH_3, 91%. **e**) N_3TEGCOOPFP (**S5**), Et_3N, DMF, 14%.

α-directing catalyst which led to the regio- and stereoselective generation of **21**.[49] Omission of the evaporation step led to significant regeneration of the acetate donor over the course of the reaction consistent with previous reports.[50]

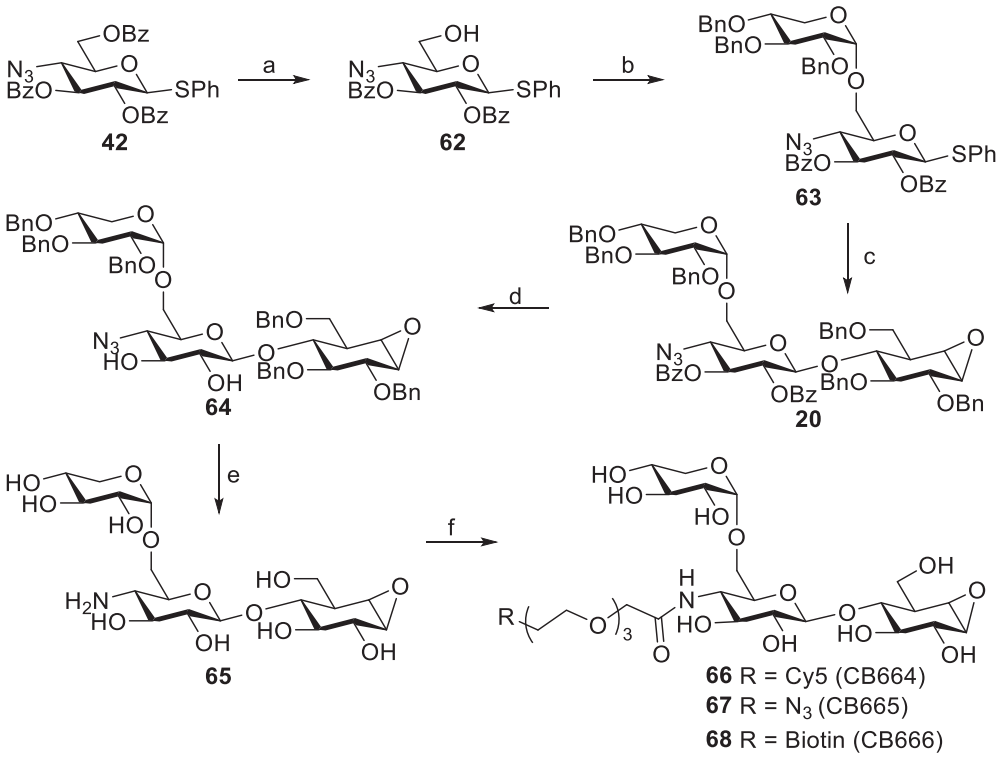
Several alternative leaving groups on the donor were examined. The reaction with trichloroacetimidate donor **57** had an equal yield to the previous method, without the need to co-evaporate the intermediate anomeric iodide. However due to the instability of the armed perbenzylated trichloroacetimidate donor the anomeric acetate was preferred. Pre-activation of thioglycoside donor **58** with tetrabutylammonium iodide (TBAI) as an α-directing additive and *N*-ethylmaleimide as a thiophenol scavenger was unsuccesfull.[51] This may be due to the reagent combination thiophenol and *in situ* generated iodo species that has been shown to react readily with aziridines and epoxides.[52]

Elaboration into the trisaccharide **19** was accomplished with the same pre-activation protocol and donor (**42**) as for the unbranched acceptor (**25**) which led to GX configured **19** in comparable yield to GG configured **46**. Deprotection of the benzoyl groups afforded **59**, which was then completely deprotected under Birch conditions to afford **60** after desalting by HW-40 size exclusion chromatography. The amine on **60** was reacted with a slight excess of the pentafluorophenol activated N_3 TEG spacer **S5** yielding azide tagged activity-based probe **61** after HPLC purification.

Xyloglucanase (XG) activity-based probes

The XG configured probes were synthesized using the conditions developed in the previous sections for the GG and GX configured probes. To this end the central 4-deoxy-4-azido-glucose building block **42** was turned into primary acceptor **62** by complete debenzoylation and subsequent silyl ether protection of the primary alcohol, benzoylation of the two secondary alcohols followed by removal of the silyl ether (Scheme 2.8).

Acceptor **62** was glycosylated α-selectively with armed donor **56** in a TMSI/$OPPh_3$ mediated reaction. Resulting disaccharide thioglycoside **63** was used as a donor in the subsequent glycosylation reaction with cyclophellitol acceptor **25** employing the Tf_2O/Ph_2SO pre-activation conditions completing the XG motif (**20**). The deprotection was accomplished by first removing the benzoyl esters under basic conditions (**64**) followed by dissolving metal hydrogenation to remove the benzyl ethers and reduce the azide to the amine, which, in this case, goes in satisfying yield to fully deprotected amine **65**. The amine was reacted with *in situ* generated pentafluorophenol activated esters of the tagged TEG spacers to yield the Cy5 (**66**), azide (**67**) and biotin (**68**) tagged XG configured ABPs after HPLC purification.



Scheme 2.8| Reagents and conditions: **a**) i. NaOMe, MeOH, DCM, 81%; ii. TBSCl, imidazole, DMF; iii. BzCl, pyr, DCM. iv) TBAF, THF, 79% over 4 steps. **b**) i. **56**, TMSI, DCM; ii. **62**, $OPPh_3$, DIPEA, DCM, rt, 70%. **c**) i. **63**, Tf_2O, Ph_2SO, TTBP, DCM, -70°C to -40°C; ii. **25**, -70°C to rt, 63%; **d**) NaOMe, MeOH, DCM, 81%. **e**) Na (s), *t*-BuOH, THF, NH_3, 76%. **f**) Cy5TEGCOOH (**S12**) or N_3TEGCOOH (**S4**) or BiotinTEGCOOH (**S8**), DIC, PFP, DIPEA, DMF, 23% **66**; 27% **67** and 26% **68**.

Profiling of an Aspergillus niger secretome

Initial assessment of the biological activity of the probes (Figure 2.5A) was made by analyzing the labeling in *Aspergillus niger* U1 mutant secretomes. The secretomes were obtained after growth in fructose containing liquid culture for four days and these secretomes are rich in many different GHs.[53,54]

Labeling with GG configured probe **52** gave, after SDS PAGE resolution of the protein content and in-gel fluorescence scanning, five mayor bands (Figure 2.5B). Comparing this band pattern with that obtained with previously developed β-glucosidase probe **12** and xylanase and xylosidase active probe **8** suggests that these bands do not correspond to exo-glucosidases or xylanases. XG branched probe **66** shows labeling of three of the five bands labeled with **52** with similar intensity indicating that these three labeled proteins may be retaining endo-xyloglucanases acting on an unbranched glucose in a xyloglucan oligomer, as do most known xyloglucanases.

GX branched probe **61** does not show labeling of any of these bands, after azide alkyne ligation of Alexa 488 to the probe, but shows labeling of a lower molecular weight protein.

Dependence of the Alexa 488 signal on probe labeling was confirmed by incubation of the secretome with various concentrations of GX probe followed by ligation to the fluorophore. This might indicate that this protein has xyloglucanase activity with activity on the branched glucose. Although this activity was historically believed to be less prevalent, enzymes have recently been characterized with dominant hydrolytic activity on the substituted glucose (Figure 2.5C).[55–57]

Labeling with the GG probe (**52**) in the secretome at different buffer pH showed different optima for the different bands (Figure 2.5D). Competition with the GGG configured inhibitor (**41**) shows inhibition of most of the bands with much higher potency for most of the bands than the disaccharide inhibitor (**40**) indicating the preference of the anticipated endo-glucanases for polysaccharide substrates (Figure 2.5E).

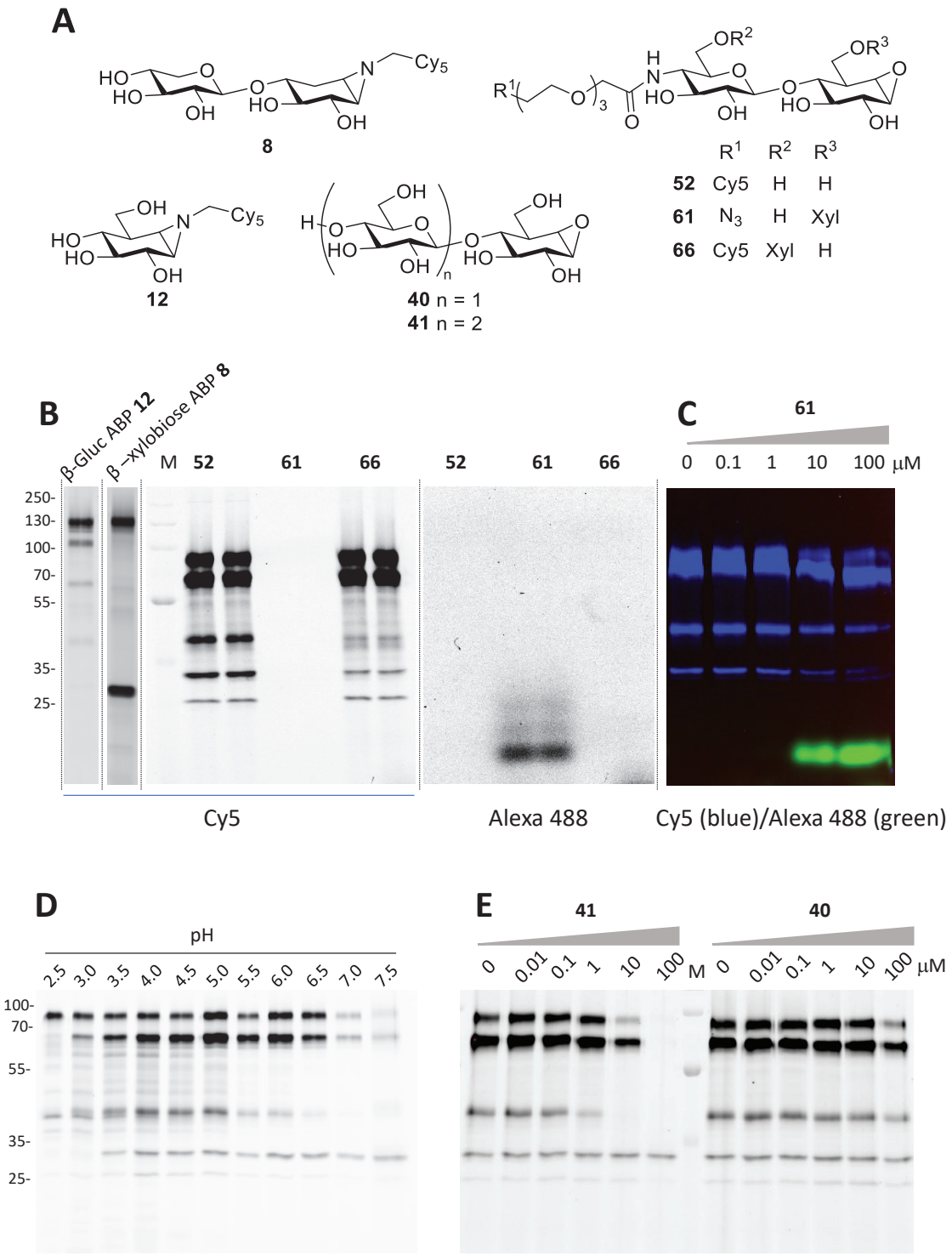


Figure 2.5| Labeling of *Aspergillus niger* secretomes in McIlvaine buffer pH 5.0 or the indicated pH. **A**) Structures of probes used in this figure. **B**) Labeling with probes with different recognition elements shows a different pattern. **C**) Incubation with various concentrations of GX **61** followed by labeling with GG **52** and ligation with Alexa 488 alkyne shows Alexa 488 labeling is dependent on concentration of **61**. **D**) Labeling with GG **52** is pH dependent. **E**) Incubation with GGG (**41**) and GG (**40**) configured inhibitors impairs labeling with GG configured probe **52**.

2.3 Conclusion

In this chapter the synthesis of a set of cellulase and xyloglucanase active ABPs is presented. Tf_2O/Ph_2SO mediated pre-activation glycosylations of thioglucoside donors and $OPPh_3$ mediated 1,2-cis xylosylations with anomeric iodide donors on cyclophellitol acceptors afforded access to the desired structural motifs.

Biological applicability of the set of endo-glucanase probes was revealed by showing distinct labeling in *Aspergillus niger* secretomes. The labeled proteins should be further characterized by use of the biotin tagged probe followed by pull-down and proteomic identification.

In the future the suite of dedicated xyloglucan ABPs can be used for the discovery of unknown enzymes from species or environments with beneficial characteristics.

2.4 Acknowledgements

Aspergillus niger secretomes were kindly provided by Jos Reijngoud, Mark Arenthorst, Gilles van Wezel and Arthur Ram from the Institute of Biology Leiden.

2.5 Experimental

ABPP procedures

Micron filtered *Aspergillus niger* secretome (2 µl) was added to McIlvaine buffer (150 mM), pH 5.0 or indicated in the figure, containing the indicated inhibitor in the indicated concentration and was shaken at 40°C for 30 minutes. The appropriate ABP (final ABP concentration 10 µM for **52**, **61** and **66**, 1 µM for **12** and 5 µM **8**) was added and the sample was shaken at 40°C for another 30 minutes (total volume 10 µl).
To samples with fluorophore containing probes loading buffer (3.5 µl) was added and the samples were boiled for 5 min and stored on ice. To the azide containing samples 2 µl 10% SDS was added followed by click mix (2 µl). The samples were left shaking for 1 hour at 40°C. 4 µl loading buffer was added and the samples were run on a 10% SDS-PAGE gel.
Fluorescently labeled bands were visualized on a ChemiDoc MP imager (BioRad) using Cy3, Cy5 and Alexa 488 multichannel settings and processed using ImageLab 6.0.1 (BioRad). PageRuler Plus Prestained protein ladder (Thermo Fisher Scientific) was used as marker.
Click mix: Alexafluor 488 (90 mM in DMSO, 0.5 µl) CuSO4 (18 mM in water, 0.5 µl), sodium ascorbate (150 mM in water, 0.5 µl), THPTA (18mM in DMSO, 0.5 µl).

General chemical synthesis procedures

All reactions were carried out in oven-dried glassware. Trace amounts of water were removed by co-evaporation with toluene. Reactions were carried out under an atmosphere of nitrogen unless stated otherwise. Tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF) dichloromethane (DCM) and toluene were of reagent grade and were stored over molecular sieves before use. Pentane, petroleum ether and diethyl ether used for workup and column chromatography were of technical grade and used as received. Ethyl acetate (EtOAc) was distilled under reduced pressure before use. Unless stated

otherwise, solvents were removed by rotary evaporation under reduced pressure at 40°C. Triflic acid anhydride (Tf_2O, Fluorochem Ltd) was distilled over P_2O_5 and stored at -20°C for no more than 3 months before use. All other chemicals (Acros, Sigma-Aldrich, TCI, Carbosynth, Merck, Boom, Honeywell & Biosolve) were used as received. Reactions were monitored by TLC analysis using Merck aluminum sheets (Silica gel 60 F254) with detection by UV absorption (254 nm) and 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. Silica gel column chromatography was performed on Screening Devices silica gel 60 (particle size of 40 – 63 μm, pore diameter of 60 Å). Gel filtration was performed on an ÄKTA explorer (GE Healthcare) using a 1.6x60 cm Toyopearl HW-40S resin eluting with a solution of NH_4HCO_3 (150mM), NH_4OAc (150mM) or AcOH (1%) in MilliQ. Fraction monitoring was performed using refractive index. 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. ^1H and ^{13}C NMR spectra were recorded on a 300/75, 400/100, 500/125, 600/150 or 800/200 MHz spectrometer. Chemical shifts (δ) are given in ppm relative to tetramethylsilane or the residual solvent. 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 mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan).

General procedure A| Cy TEG amide couplings

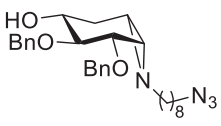
Cy-carboxylic acid was dissolved in dry DCM (0.2 M) and cooled to 0°C. Amine **S6** (1 eq), DMAP (0.05 eq) and DIC (1.2 eq) were added and the mixture was stirred overnight at rt. The mixture was loaded directly on a silica column and purification by flash chromatography.

General procedure B| Amide coupling reporter tag to warhead PFP method

The appropriate carboxylic acid (25 μmol) was dissolved in DMF (0.5 ml), 2,3,4,5,6-pentafluorophenol (23 mg, 0.13 μmol), Et_3N (10 μl, 0.13 mmol) and DIC (3.9 μl,25 μmol) were added and the mixture was stirred for 90 minutes. Part of the stock solution (1.2 eq acid compared to amine) was added to the amine and stirred overnight. LC-MS indicated full conversion and the product was purified on semi-preparative HPLC eluting with a linear gradient of solution A (MeCN) in solution B (50 mM AcOH in H_2O). The fractions were concentrated under reduced pressure, co-evaporated with water, diluted with water and lyophilized to yield the product.

α-Xylose activity-based probes

_N_-8-azidooctyl-2,3-di-_O_-benzyl-D-xylose-cyclophellitol aziridine (28)

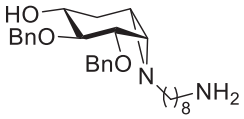


2,3-di-*O*-benzyl-D-xylose-cyclophellitol aziridine[34] **27** (0.15 g, 0.46 mmol) was dissolved in DCM (1.8 ml). The solution was cooled to 0°C and DIPEA (0.12 ml, 0.69 mmol) and freshly prepared 8-azidooctyl trifluoromethanesulfonate[29] (1M in DCM, 0.55 ml, 0.55 mmol) were added. The reaction was slowly warmed to room temperature and stirred for 21 hours. MeOH (2 ml) was added and the mixture was stirred for 2 hours. Toluene was added and the mixture was evaporated to dryness. Column chromatography (DCM/MeOH, 1/0 -> 99/1, v/v) afforded the product as an oil (0.19 g, 0.40 mmol, 86%).

^1H NMR (500 MHz, $CDCl_3$) δ 7.40 – 7.22 (m, 10H, benzyl), 4.85 (br, 1H, OH), 4.70 (d, *J* = 12.2 Hz, 1H, CH_2Bn), 4.65 (d, *J* = 12.2 Hz, 1H, CH_2Bn), 4.62 (d, *J* = 11.7 Hz, 1H, CH_2Bn), 4.50 (d, *J* = 11.7 Hz, 1H, CH_2Bn), 3.83 (dd, *J* = 4.7, 3.4 Hz, 1H, H2), 3.73 (br, 1H, H4), 3.55 (dd, *J* = 5.6, 3.4 Hz, 1H, H3), 3.21 (t, *J* = 7.0 Hz, 2H, CH_2N_3), 2.39 (dt, *J* = 11.5, 6.8 Hz, 1H, CH_2N aziridine), 2.14 – 2.05 (m, 2H, H5a/CH_2N aziridine), 1.97 (ddd, *J* = 14.3, 5.3, 1.8 Hz, 1H, H5b), 1.88 (dd, *J* = 6.3, 4.7 Hz, 1H, aziridine), 1.82 – 1.76 (m, 1H, aziridine),

1.60 – 1.50 (m, 4H, CH_2 spacer), 1.41 – 1.23 (m, 8H, CH_2 spacer). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.5, 138.3, 128.4, 128.3, 128.2, 128.1, 127.9, 127.7, 127.6, 127.6, 79.7 (C3), 76.6 (C2), 72.6 (CH_2Bn), 70.7 (CH_2Bn), 68.3 (H4), 60.4 (CH_2N aziridine), 51.4 (CH_2N_3), 39.7 (aziridine), 38.2 (aziridine), 29.5, 29.4, 29.0, 28.8, 27.1, 26.8 (C5), 26.6 (spacer). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{28}H_{39}N_4O_3$ 479.3014, found 479.3017.

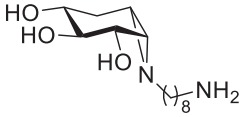
_N_-8-aminooctyl-2,3-di-_O_-benzyl-D-xylose-cyclophellitol aziridine (S1)



Azide **28** (0.189 g, 0.396 mmol) was dissolved in MeCN (7.9 ml). H_2O (71 μl, 3.96 mmol) and PPh3 polymer bound (3 mmol/g, 0.264 g, 0.792 mmol) were added and the mixture was stirred at 70°C for 13 hours. H_2O (0.5 ml) was added and the mixture was stirred for 4.5 hours at the same temperature. The solids removed by filtration, volatiles were removed under reduced pressure and the product was used and analyzed without further purification (0.171 mg, 0.378 mmol, 95%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 – 7.24 (m, 10H), 4.71 (d, *J* = 12.2 Hz, 1H, CH_2Bn), 4.66 (d, *J* = 12.2 Hz, 1H, CH_2Bn), 4.63 (d, *J* = 11.7 Hz, 1H, CH_2Bn), 4.51 (d, *J* = 11.7 Hz, 1H, CH_2Bn), 3.84 (dd, *J* = 4.7, 3.5 Hz, 1H, H2), 3.73 (q, *J* = 5.2 Hz, 1H, H4), 3.55 (dd, *J* = 5.6, 3.5 Hz, 1H, H3), 2.65 (t, *J* = 7.0 Hz, 2H, C**H$_2$**NH$_2$), 2.41 (dt, *J* = 11.5, 6.9 Hz, 1H, CH_2N aziridine), 2.13 – 2.05 (m, 2H, CH_2N aziridine/H5a), 2.02 – 1.94 (m, 1H, H5b), 1.88 (dd, J = 6.3, 4.7 Hz, 1H, aziridine), 1.83 – 1.77 (m, 1H, aziridine), 1.60 – 1.52 (m, 2H), 1.46 – 1.22 (m, 10H).^{13}C NMR (126 MHz, CDCl3) δ 138.6, 138.4, 128.5, 128.4, 127.8, 127.7, 127.6, 79.9 (C3), 76.7 (C2), 72.8 (CH2Bn), 70.9 (CH2Bn), 68.4 (C4), 60.6 (CH2N aziridine), 42.3 (CH2NH2), 39.9 (aziridine), 38.3 (aziridine), 33.8, 29.6, 29.6, 29.4, 27.3, 27.0, 26.9. HRMS (ESI) m/z: [M+H]+ calculated for C28H41N2O3 453.3112, found 453.3112.

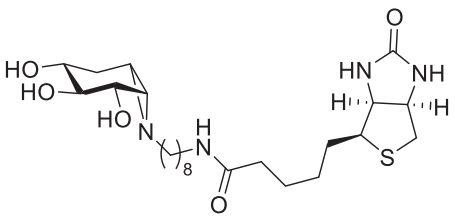
_N_-8-aminooctyl-D-xylose-cyclophellitol aziridine (29)



Ammonia (20 ml) was condensed and kept at -60°C. Sodium (0.26 g, 11.5 mmol) was added and stirred for 10 minutes. Benzyl protected **S1** (0.171 g, 0.382 mmol) dissolved in *t*-BuOH (0.35 ml, 3.68 mmol) and THF (5 ml) was slowly added to the blue solution. The color disappeared immediately so more sodium (85 mg, 3.7 mmol) was added. The blue solution was stirred for 35 minutes. Water was slowly added and the mixture was evaporated. The residue was dissolved in water and eluted over a short column of amberlite CG50 (NH_4^+) with 0.5 M NH_4OH. The combined fractions were concentrated under reduced pressure providing the product as an oil (105 mg, 0.386 mmol quant.).

^{1}H NMR (500 MHz, MeOD) δ 3.70 (dd, *J* = 7.5, 4.0 Hz, 1H, H2), 3.35 – 3.25 (m, 2H, H3/H4), 2.81 – 2.72 (m, 2H, C**H$_2$**NH$_2$), 2.35 – 2.24 (m, 2H, H5a/CH_2N aziridine), 2.15 (ddd, *J* = 11.7, 8.5, 6.4 Hz, 1H, CH_2N aziridine), 1.84 (dd, *J* = 6.5, 4.0 Hz, 1H, aziridine), 1.69 (td, *J* = 6.6, 1.2 Hz, 1H, aziridine), 1.67 – 1.53 (m, 5H, H5b/CH_2 spacer), 1.42 – 1.31 (m, 8H, CH_2 spacer). ^{13}C NMR (126 MHz, MeOD) δ 76.2 (H3), 73.6 (H2), 71.3 (H4), 61.9 (CH_2N aziridine), 45.7 (aziridine), 41.6 (CH_2NH_2), 38.3 (aziridine), 32.6 (C5), 31.0, 30.5, 30.5, 30.3, 28.3, 27.6. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{14}H_{29}N_2O_3$ 273.2172, found 273.2173.

Biotin-D-xylose-cyclophellitol aziridine (30)

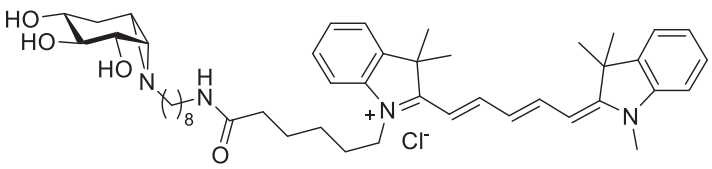


Amine **29** (6.5 mg, 24 μmol) was dissolved in DMF (0.24 ml) and to the solution was added Biotin (6.5 mg , 26 μmol). DIPEA (6.3 μl, 36 μmol), DMAP (cat.) and DIC (6.8 μl ,43 μmol) were added and the mixture was stirred overnight. LC-MS indicated conversion and the product was purified on semi-preparative HPLC eluting with a linear gradient of solution A (MeCN) in solution B (50mM NH_4HCO_3 in H_2O). The fractions were concentrated under reduced pressure, co-evaporated with water, diluted with water and lyophilized to yield the product as a white solid (2.20 mg, 4.40 μmol, 18%).

^{1}H NMR (500 MHz, MeOD) δ 4.49 (dd, *J* = 7.8, 5.0 Hz, 1H), 4.30 (dd, *J* = 7.9, 4.4 Hz, 1H), 3.70 (dd, *J* = 7.5, 4.0 Hz, 1H), 3.29 – 3.25 (m, 2H), 3.23 – 3.18 (m, 1H), 3.16 (td, *J* = 7.0, 1.9 Hz, 2H), 2.93 (dd, *J* = 12.8, 5.0

Hz, 1H), 2.71 (d, *J* = 12.7 Hz, 1H), 2.33 – 2.25 (m, 2H), 2.22 – 2.12 (m, 3H), 1.84 (dd, *J* = 6.5, 4.0 Hz, 1H), 1.65 (tddd, *J* = 26.9, 21.2, 13.2, 6.4 Hz, 8H), 1.46 (dp, *J* = 23.2, 7.8, 7.4 Hz, 4H), 1.34 (s, 8H). ^{13}C NMR (126 MHz, MeOD) δ 176.0, 76.2, 73.6, 71.3, 63.4, 62.0, 61.6, 57.0, 45.7, 41.1, 40.4, 38.3, 36.8, 32.6, 30.6, 30.5, 30.4, 30.4, 29.8, 29.5, 28.4, 28.0, 27.0. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{24}H_{43}N_4O_5S$ 499.2947, found 499.2949.

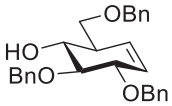
Cy5-D-xylose-cyclophellitol aziridine (31)



Amine **29** (49 mg, 0.18 mmol) was dissolved in DMF (0.25 ml). DIPEA (76 µl, 0.44 mmol), Cy5COOH (94 mg, 0.18 mmol) and PyBOB (0.10 g, 0.20 mmol) were added and the mixture was stirred overnight. LC-MS indicated conversion and the product was purified on semi-preparative HPLC eluting with a linear gradient of solution A (MeCN) in solution B (50mM NH_4HCO_3 in H_2O). The fractions were concentrated under reduced pressure, co-evaporated with water, diluted with water and lyophilized to yield the product as a blue solid (17.4 mg, 0.034 mmol, 19%).
^{1}H NMR (500 MHz, CD_3CN) δ 8.08 (t, *J* = 13.1 Hz, 2H), 7.47 (d, *J* = 7.4 Hz, 2H), 7.43 – 7.37 (m, 2H), 7.28 – 7.21 (m, 4H), 6.65 (d, *J* = 5.8 Hz, 1H), 6.55 (t, *J* = 12.4 Hz, 1H), 6.21 (dd, *J* = 21.1, 13.8 Hz, 1H), 4.00 (t, *J* = 7.5 Hz, 2H), 3.58 (dd, *J* = 7.4, 3.8 Hz, 1H), 3.54 (s, 3H), 3.24 (td, *J* = 9.4, 6.7 Hz, 1H), 3.18 – 3.13 (m, 1H), 3.07 (q, *J* = 6.6 Hz, 2H), 2.20 – 2.07 (m, 4H), 1.77 (p, *J* = 7.4 Hz, 3H), 1.67 (s, 16H), 1.56 – 1.49 (m, 2H), 1.48 – 1.37 (m, 6H), 1.26 (s, 9H).^{13}C NMR (126 MHz, CD_3CN) δ 174.9, 174.3, 173.4, 154.9, 154.8, 144.1, 143.3, 142.4, 142.3, 129.5, 129.5, 126.0, 125.9, 125.6, 123.2, 123.1, 112.0, 111.8, 104.1, 76.1, 73.3, 70.9, 61.4, 50.2, 50.1, 45.1, 44.9, 39.8, 37.8, 36.6, 32.2, 32.0, 30.4, 30.2, 30.1, 29.8, 27.9, 27.8, 27.8, 27.6, 27.5, 27.0, 26.1. HRMS (ESI) m/z: $[M]^+$ calculated for $C_{46}H_{65}N_4O_4$, 737.4996 found 737.5000.

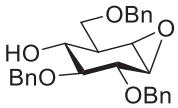
Cyclophellitol acceptor

2,3,6-tri-*O*-benzyl-cyclophellitol alkene (34)



Dibenzyl cyclohexene **33**[33,36] (2.15 g, 6.31 mmol) was co-evaporated with toluene and subsequently dissolved in acetonitrile (32 ml). K_2CO_3 (0.96 g, 6.94 mmol), KI (1.05 g, 6.31 mmol), 2-aminoethyl diphenylborinate (0.14 g, 0.63 mmol) and benzyl bromide (1.13 ml, 9.47 mmol) were added and the mixture was stirred for 20 hours at 60°C. The reaction was quenched with $NaHCO_3$ (250 ml aq. sat.) and the mixture was extracted with EtOAc (2x). The combine organic layers were washed with brine, dried with $MgSO_4$ and filtered. The solvent was evaporated under reduced pressure and the mixture was purified by column chromatography (pentane/Et_2O, 8/2 -> 7/3, v/v) to afford the product as a white solid (2.45 g, 5.74 mmol, 91%).
^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.41 – 7.30 (m, 15H), 5.78 (ddd, *J*=10.2, 2.7, 2.0, 1H, alkene), 5.67 (dt, *J*=10.3, 2.0, 1H, alkene), 5.03 (d, *J*=11.3, 1H, CH_2Bn), 4.82 (d, *J*=11.3, 1H, CH_2Bn), 4.71 (apparent q, *J*=11.5, 2H, CH_2Bn), 4.57 (d, *J*=1.3, 2H, CH_2Bn), 4.23 (ddd, *J*=7.3, 3.5, 1.8, 1H, H2), 3.75 (ddd, *J*=10.2, 8.9, 1.4, 1H, H6A), 3.71 – 3.58 (m, 3H, H3/H4/H6B), 2.58 (m, 1H, H5). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.4, 128.7, 128.6, 128.5, 128.3, 128.1, 128.0, 127.9, 127.9, 127.8, 127.7, 126.8, 84.0 (C3), 80.3 (C2), 75.1 (CH_2Bn), 73.5 (CH_2Bn), 71.7 (CH_2Bn), 71.22 (C4), 71.15 (C6), 44.1 (C5). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{28}H_{30}O_4Na$ 453.2042, found 453.2039.

2,3,6-tri-*O*-benzyl-cyclophellitol (25)

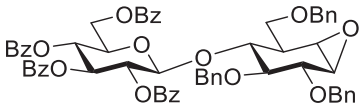


Cyclohexene **34** (1.18 g, 2.74 mmol) was dissolved in acetonitrile (18 ml). EDTA in water was added (9.0 ml, 0.4M) and the mixture was cooled to 0°C. 1,1,1 trifluoroacetone (3.7 ml, 41.13 mmol) was added followed by portion wise addition of a solid mixture of oxone (8.43 g, 13.71 mmol) and $NaHCO_3$ (1.61 g, 19.19 mmol) The reaction was stirred for 2 hours and was then diluted with water and extracted with EtOAc (3x). The combine organic layers were washed with brine and dried with $MgSO_4$. Solids were filtered of and

the solvent was evaporated under reduced pressure. Column chromatography eluting with pentane/Et_2O (8/2 -> 7/3, v/v) afforded first the product (640 mg, 1.42 mmol, 52%) and the epimeric epoxide (560 mg, 1.26 mmol, 46%) as white solids.
^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.41 – 7.25 (m, 15H), 4.93 (d, *J*=11.3, 1H, CH_2Bn), 4.81 (d, *J*=11.3, 1H, CH_2Bn), 4.69 (apparent dd, *J*=11.3, 1.5, 2H, CH_2Bn (2x)), 4.58 (d, *J*=1.4, 2H, CH_2Bn), 3.90 (dd, *J*=8.9, 5.0, 1H, H6a), 3.81 (d, J = 7.2, 1H, H2), 3.69 (t, *J*=8.7, 1H, H6b), 3.45 – 3.42 (m, 1H, H7), 3.42 – 3.30 (m, 2H, H3/H4), 3.20 (d, *J*=3.7, 1H, H1), 2.32 – 2.18 (m, 1H, H5). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.5, 138.2, 137.6, 128.7, 128.7, 128.6, 128.2, 128.1, 128.0, 127.8, 83.9 (C3), 79.5 (C2), 75.1 (CH_2Bn), 73.6 (CH_2Bn), 72.8 (CH_2Bn), 70.1 (C6), 67.5 (C4), 54.9 (C7), 54.0 (C1), 42.2 (C5). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{28}H_{30}O_5Na$ 469.1991, found 469.1985.
Epimeric epoxide **35**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.39 (m, 2H), 7.38 – 7.27 (m, 13H), 4.99 (d, *J* = 11.2 Hz, 1H, CH_2Bn), 4.86 – 4.76 (m, 2H, CH_2Bn), 4.66 (d, *J* = 11.2 Hz, 1H, CH_2Bn), 4.53 (s, 2H, CH_2Bn), 3.86 (dd, *J* = 8.0, 1.8 Hz, 1H, H2), 3.68 (dd, *J* = 4.4, 1.1 Hz, 2H, H6 (2x)), 3.59 – 3.45 (m, 2H, H3/H4), 3.36 (dd, *J* = 4.0, 1.8 Hz, 1H, epoxide), 3.18 (d, *J* = 4.0 Hz, 1H, epoxide), 2.57 (s, 1H, -OH), 2.24 – 2.16 (m, 1H, H5). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 138.5, 138.2, 138.2, 128.7, 128.6, 128.6, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 81.3 (C3), 79.7 (C2), 75.7 (CH_2Bn), 73.5 (CH_2Bn), 72.3 (CH_2Bn), 69.5 (C4), 69.1 (C6), 54.8 (epoxide), 54.7 (epoxide), 42.5 (C5).

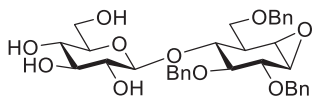
GG inhibitor

4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-benzyl-cyclophellitol (37)



Tribenzyl cyclophellitol **25** (45 mg, 0.10 mmol) and phenyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside (**36**) (0.172 mg, 0.25 mmol)[38] were co-evaporated with toluene (3x). DCM (0.5 ml) and 4Å molecular sieves were added and the mixture was stirred for 30 minutes. NIS (56 mg, 0.25 mmol) was added and the mixture was cooled to −30°C. TMSOTf (5.4 µl, 0.03mmol) was added and the mixture was warmed to −10°C during 2 hours. The reaction was quenched with triethylamine, diluted with DCM and washed with $NaHCO_3$ (aq. sat.) and brine. $MgSO_4$ was added, solids were removed by filtration and the mixture was concentrated under reduced pressure. Column chromatography eluting with pentane/EtOAc (8/2, v/v) yielded the product as a colorless oil (53 mg, 0.053 mmol, 53%).
^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.00 – 7.88 (m, 4H), 7.82 (m, 4H), 7.58 – 7.15 (m, 27H), 5.75 (t, *J*=9.7, 1H, H3'), 5.57 (t, *J*=9.7, 1H, H4'), 5.50 (dd, *J*=9.8, 8.0, 1H, H2'), 5.03 (d, *J*=11.8, 1H, CH_2Bn), 4.94 (d, *J*=8.0, 1H, H1'), 4.81 (d, *J*=11.8, 1H, CH_2Bn), 4.72 – 4.60 (m, 2H, CH_2Bn), 4.38 (d, *J*=11.9, 1H, CH_2Bn), 4.32 (dd, *J*=12.2, 3.4, 1H, H6a'), 4.23 (d, *J*=12.0, 1H, CH_2Bn), 4.17 – 4.06 (m, 1H, 6b'), 3.83 (d, *J*=7.4, 1H, H2), 3.79 – 3.69 (m, 2H, H4/H5'), 3.63 (dd, *J*=8.9, 3.4, 1H, H6a), 3.54 (dd, *J*=9.4, 7.4, 2H, H6b/H3), 3.32 (d, *J*=3.7, 1H, epoxide), 3.12 (d, *J*=3.7, 1H, epoxide), 2.21 (tt, *J*=8.9, 3.3, 1H, H5). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.1, 165.9, 165.2, 139.3, 138.2, 137.7, 133.6, 133.5, 133.3, 133.1, 129.9, 129.9, 129.8, 129.7, 129.2, 128.9, 128.9, 128.6, 128.6, 128.6, 128.5, 128.4, 128.4, 128.3, 128.0, 127.9, 127.7, 127.3, 127.1, 101.6 (C1'), 83.1 (C3), 79.5 (C2), 76.5 (C5'), 74.6 (CH_2Bn), 73.3 (C3'), 73.22 (CH_2Bn), 73.19 (CH_2Bn), 72.8 (C2'), 72.1 (C4), 69.8 (C4'), 68.3 (C6), 63.0 (C6'), 55.8 (epoxide), 53.3 (epoxide), 42.1 (C5). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{62}H_{57}O_{14}$ 1025.37428, found 1025.37453.

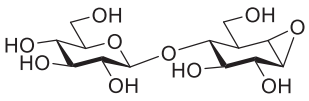
4-*O*-(β-D-glucopyranosyl)-2,3,6-tri-*O*-benzyl-cyclophellitol (S2)



Disaccharide **37** (53 mg, 0.052 mmol) was dissolved in DCM/MeOH (0.5 ml, 1/1, v/v) NaOMe (5µl, 5.4M in MeOH) was added and the mixture was stirred overnight. Et_3N·HCl (4.3 mg, 0.031 mmol) was added and the solvent was removed under reduced pressure. Column chromatography eluting with DCM/MeOH (3% -> 5% MeOH) afforded the product as a colorless oil (32 mg, 0.053 mmol, quant.).

^{1}H NMR (500 MHz, MeOD) δ = 7.35 (m, 15H), 5.00 (d, *J*=10.5, 1H, CH_2Bn), 4.82 (d, *J*=11.6, 1H, CH_2Bn), 4.72 – 4.48 (m, 4H, CH_2Bn), 4.34 (d, *J*=7.3, 1H, H1'), 4.04 – 3.87 (m, 2H, H6ab), 3.83 – 3.69 (m, 3H, H2/H4/H6a'), 3.62 – 3.52 (m, 1H, H3), 3.52 – 3.39 (m, 2H, H6b'/epoxide), 3.40 – 3.26 (m, 2H, H3'/H2'), 3.26 – 3.18 (m, 2H, epoxide/H4'), 3.18 – 3.11 (m, 1H, H5'), 2.46 – 2.35 (m, 1H, H5). ^{13}C NMR (126 MHz, MeOD) δ 139.7, 139.5, 139.3, 129.8, 129.5, 129.4, 129.2, 129.1, 128.9, 128.9, 128.7, 128.6, 104.4 (H1'), 84.7 (H3), 80.2 (H2), 78.5 (H5'), 77.9 (H3'), 77.1 (CH_2Bn), 75.9 (C2'), 75.1 (C4), 74.1 (CH_2Bn), 73.8 (CH_2Bn), 72.0 (C4'), 69.6 (C6), 63.0 (C6'), 57.2 (epoxide), 53.8 (epoxide), 44.2 (C5). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{34}H_{40}O_{10}$ 609.26942, found 609.26941.

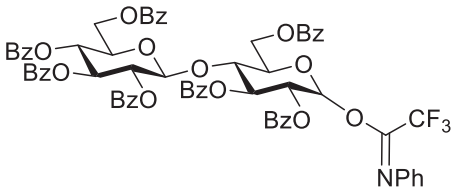
4-*O*-(β-D-glucopyranosyl)-cyclophellitol (40)



Disaccharide **S2** (18 mg, 0.030 mmol) was dissolved in H_2O/MeOH/dioxane (0.6 ml, 1/1/1 v/v) The solution was purged with nitrogen and $Pd(OH)_2$/C (18 mg) was added. The flask was purged with hydrogen and the reaction was stirred under hydrogen atmosphere 2.5 hours. The flask was purged with nitrogen solids were removed by filtration over celite and the solvent was removed in vacuo yielding the product as a white solid (10 mg, 0.030 mmol, quant.).
^{1}H NMR (400 MHz, D_2O) δ = 4.35 (d, *J*=7.9, 1H, H1'), 3.96 (dd, *J* = 11.0, 2.7 Hz, 1H, H6a), 3.88 – 3.70 (m, 3H, H6b/H6a'/H2), 3.61 (dd, *J*=12.4, 4.8, 1H, H6b'), 3.51 – 3.26 (m, 7H), 3.21 (t, *J*=8.9, 1H, H2'), 3.12 (d, *J*=2.9, 1H, epoxide), 2.25 – 2.12 (m, 1H, H5). ^{13}C NMR (101 MHz, D_2O) δ 103.0 (H1'), 77.7, 76.0 (H5'), 75.5, 74.7, 73.3 (C2'), 70.8 (C2), 69.3 (C4'), 60.4 (C6'), 59.7 (C6), 56.6 (epoxide), 55.2 (epoxide), 42.7 (C5). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{13}H_{23}O_{10}$ 339.1287, found 339.1286.

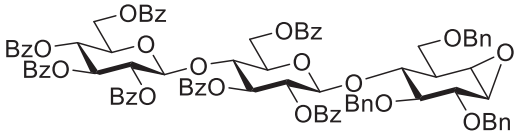
GGG inhibitor

4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl 1-(N-phenyl)-2,2,2-trifluoroacetimidate (38)



4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranose[58] (2.14 g, 2.00 mmol) was dissolved in acetone (13 ml). Cs_2CO_3 (0.977 g, 3.0 mmol) and 2,2,2-trifluoro-*N*-phenyl-acetimidoyl chloride (0.39 ml, 2.4 mmol) were added and the reaction was stirred overnight. The mixture was diluted with EtOAc and washed with $NaHCO_3$ (aq. sat.) and brine. $MgSO_4$ was added, solids were removed by filtration and the mixture was concentrated under reduced pressure. Column chromatography eluting with pentane/EtOAc (8/2, v/v) yielded the product as a white solid as an E/Z mixture (2.50 g, 1.98 mmol, 99%).
^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.17 – 7.88 (m, 18H), 7.84 – 7.71 (m, 7H), 7.67 – 7.48 (m, 6H), 7.48 – 7.12 (m, 33H), 7.12 – 7.00 (m, 3H), 6.95 (t, *J*=7.3, 1H), 6.65 (d, *J*=8.1, 1H), 6.36 (s, 2H), 6.13 (t, *J*=9.1, 1H), 5.78 (td, *J*=9.7, 4.1, 2H), 5.64 – 5.37 (m, 5H), 5.01 (m, 2H), 4.75 – 4.43 (m, 3H), 4.42 – 4.24 (m, 3H), 3.99 – 3.75 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.8, 165.5, 165.2, 165.0, 164.9, 142.9, 133.8, 133.5, 133.3, 130.0, 129.9, 129.9, 129.8, 129.8, 129.7, 129.5, 129.4, 128.8, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.3, 101.2, 76.0, 73.0, 72.7, 72.5, 72.0, 71.5, 70.5, 69.9, 69.4, 62.7, 61.9, 60.5.

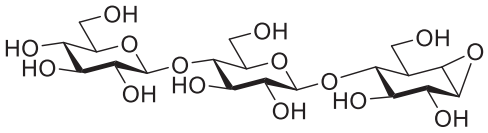
4-*O*-(4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-benzyl-cyclophellitol (39)



Donor **38** (0.311 g, 0.25 mmol) and acceptor **25** (45 mg, 0.10 mmol) were co-evaporated with toluene (3x). DCM (1.0 ml) and 4Å molecular sieves were added and the mixture was stirred for 30 minutes. The mixture was cooled to −15°C. TMSOTf (5.4 µl, 0.03 mmol) was added and the mixture was warmed to 0 °C and stirred for 4.5 hours. The reaction was quenched with triethylamine, diluted with DCM and washed with $NaHCO_3$ (aq. sat.) and brine. $MgSO_4$ was added,

solids were removed by filtration and the mixture was concentrated under reduced pressure. Column chromatography eluting with pentane/EtOAc (8/2 -> 7.5/2.5, v/v) yielded the product as a white solid. (67 mg, 0.045 mmol, 45%).
^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.01 – 7.89 (m, 10H), 7.79 – 7.69 (m, 4H), 7.60 – 7.13 (m, 36H), 7.01 (t, *J*=7.6, 2H), 6.93 – 6.77 (m, 1H), 5.62 (m, 2H, H3'/H3"), 5.47 (dd, *J*=9.8, 7.9, 1H, H2"), 5.39 (dd, *J*=9.9, 8.0, 1H, H2'), 5.31 (t, *J*=9.5, 1H, H4"), 4.90 (d, *J*=12.0, 1H, CH_2Bn), 4.82 – 4.74 (m, 3H, H1'/H1"/CH_2Bn), 4.68 – 4.53 (m, 2H, CH_2Bn), 4.33 (d, *J*=12.0, 1H, CH_2Bn), 4.25-4.12 (m, 3H, H6a'/H4'/CH_2Bn), 4.08 – 3.96 (m, 2H, H6b'/H6a"), 3.78 (d, *J*=7.4, 1H, H2), 3.73 – 3.53 (m, 4H, H6b"/H4/H5"/H6b), 3.53 – 3.40 (m, 2H, H6a/H3), 3.30 (m, 2H, H5'/epoxide), 3.09 (d, *J*=3.7, 1H, epoxide), 2.26 – 2.09 (m, 1H, H5). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.8, 165.7, 165.6, 165.5, 165.3, 165.1, 164.8, 139.2, 138.1, 137.7, 133.6, 133.5, 133.5, 133.3, 133.3, 133.2, 123.0, 129.8, 129.8, 129.7, 129.7, 129.7, 129.6, 129.6, 129.3, 128.8, 128.7, 128.7, 128.6, 128.6, 128.6, 128.5, 128.4, 128.4, 128.3, 128.1, 128.0, 127.9, 127.9, 127.7, 127.0, 126.6, 101.4 (C1'), 100.8 (C1"), 83.2 (C3), 79.5 (C2), 76.4 (C4), 76.0 (C4'), 74.3 (CH_2Bn), 73.2 (CH_2Bn), 73.2 (CH_2Bn), 73.1 (C5'), 73.0, 72.9, 72.9 (C3"/C3'/C2'), 72.4 (C5"), 71.9 (C2"), 69.6 (C4"), 68.4 (C6), 62.7 (C6"), 62.3 (C6'), 55.6 (epoxide), 53.3 (epoxide), 41.9 (C5). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{89}H_{79}O_{22}$ 1499.5058, found 1499.5058.

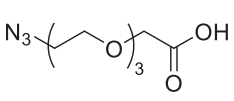
4-*O*-(4-*O*-[β-D-glucopyranosyl]-β-D-glucopyranosyl)-cyclophellitol (41)



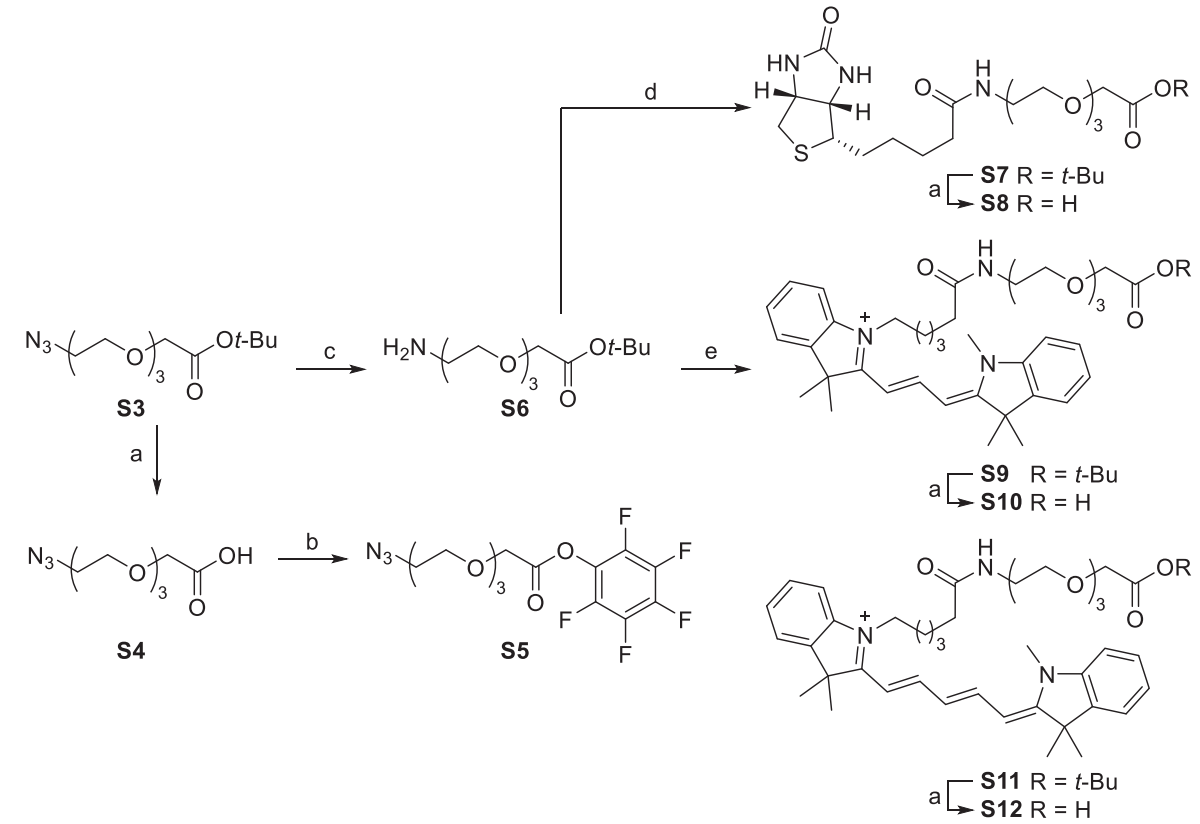
Trisaccharide **39** (64 mg, 0.043 mmol) was dissolved in DCM/MeOH (0.85 ml, 1/1, v/v) NaOMe (4 µl, 5.4M in MeOH) was added and the mixture was stirred overnight. Amberlite CG-50 (NH_4^+) was added until the mixture was no longer strongly alkaline. The resin was filtered of and the solvent was removed under reduced pressure. The residue was dissolved in MeOH (1 ml) and added to cold Et_2O (10ml). The suspension was centrifuged and the solvent was decanted. Subsequently the residue was dissolved in H_2O/MeOH/dioxane (0.4 ml, 1/1/1 v/v). The solution was purged with nitrogen and $Pd(OH)_2$/C (10 mg) was added. The flask was purged with hydrogen and the reaction was stirred under hydrogen atmosphere 2.5 hours. The flask was purged with nitrogen solids were removed by filtration over celite and the mixture was concentrated in vacuo. Water was added and the sample was lyophilized yielding the product as a white powder (8.5 mg, 0.017 mmol, 40%).
^{1}H NMR (500 MHz, D_2O) δ = 4.55 – 4.48 (m, 2H, H1 2x), 4.09 (dd, *J*=11.3, 3.6, 1H, 6a), 4.00 – 3.91 (m, 3H, 6b/6a(2x)), 3.90 – 3.81 (m, 2H, 6b), 3.79 – 3.72 (m, 1H, 6b), 3.72 – 3.64 (m, 2H, H3), 3.62 – 3.55 (m, 1H, epoxide), 3.55 – 3.48 (m, 4H, H3 (2x)/H4/H5), 3.44 (m, 1H), 3.42 – 3.36 (m, 1H, H2), 3.33 (dd, *J*=9.3, 7.9, 1H, H2), 3.25 (d, *J*=3.9, 1H, epoxide), 2.37 – 2.29 (m, 1H, H5). ^{13}C NMR (126 MHz, D_2O) δ 102.9 (C1), 102.6 (C1), 78.3, 77.7, 76.0, 75.5, 74.9, 74.8, 74.2, 73.2 (H2 2x), 70.9, 69.5, 60.6 (C6), 59.8 (C6 2x), 56.7 (epoxide), 55.3 (epoxide), 42.8 (C5). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{19}H_{33}O_{15}$ 501.1814, found 501.1818.

Tags with TEG spacers

COOH-TEG-N_3 (S4)

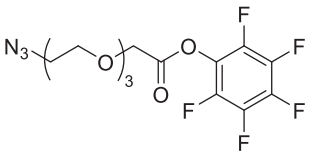


Ester **S3** (100 mg, 0.346 mmol) was dissolved in DCM/TFA (7 ml, 1/1, v/v, 0.05 M) and stirred for 30 minutes. The mixture was repeatedly co-evaporated with toluene and used and analyzed without further purification.
^{1}H NMR (400 MHz, CD_3CN) δ = 4.08 (s, 2H, OC**H_2**COOH), 3.67 – 3.57 (m, 10H), 3.37 (t, *J*=4.9, 2H, CH_2N_3). ^{13}C NMR (101 MHz, CD_3CN) δ 172.2 (COOH), 71.5, 71.1, 71.0, 71.0, 70.5, 68.8, 51.5 (CH_2N_3). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_8H_{15}N_3O_5Na$ 256.0904, found 256.0902.



Scheme 2.9| Reagents and conditions: **a**) TFA, DCM; **b**) PFPOH, DIC, DMAP, DCM, 67% **c**) PPh_3, H_2O, THF, 88%; **d**) Biotin-NHS, DIPEA, DMF, 83%; **e**) DIC, DMAP, DCM, Cy5 acid, 73% or Cy3 acid, 66%.

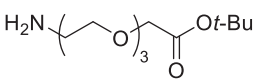
PFP-TEG-N_3 (S5)



Crude acid **S4** (0.346 mmol) was dissolved in DCM (1.9 ml, 0.2 M). 2,3,4,5,6-pentafluorophenol (70 mg, 0.381 mmol), DIC (0.059 ml, 0.381 mmol) and DMAP (cat) were added and the mixture was stirred overnight. Volatiles were evaporated under reduced pressure and the mixture was separated by column chromatography (pentane/EtOAc, 9/1 -> 8/2, v/v) providing the product as a colorless oil (93 mg, 0.23 mmol, 67 % over 2 steps).

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.56 (s, 2H, OCH_2C=O), 3.86 – 3.81 (m, 2H), 3.78 – 3.74 (m, 2H), 3.71 – 3.66 (m, 6H), 3.40 (t, *J* = 5.1 Hz, 2H, CH_2N_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.8, 71.4, 70.9, 70.8, 70.8, 70.2, 68.0 (, O**C**H_2C=O), 50.8 (**C**H_2N_3). HRMS (ESI) m/z: $[M+NH_4]^+$ calculated for $C_{14}H_{18}F_5N_4O_5$ 417.1192 found 417.1190.

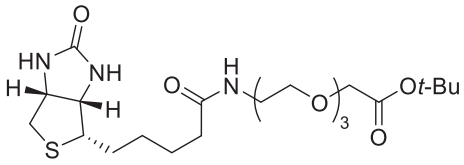
***t*-Bu-TEG-NH_2 (S6)**



Azide **S3**[59] (1.0 g, 3.46 mmol) was dissolved in THF (11.5 ml, 0.3 M), PPh_3 (1.81 g, 6.91 mmol) and H_2O (1.5 ml, 83 mmol) were added and the mixture was stirred 72 hours at rt. The mixture was diluted with H_2O (140 ml) and washed with toluene (3 x 50 ml). The combined organic layers were extracted with H_2O (4 x 20 ml), and then the water layers were combined and evaporated. The residual oil was co-evaporated with dioxane (3x) to give the title compound as an oil (803 mg, 3.04 mmol, 88%).

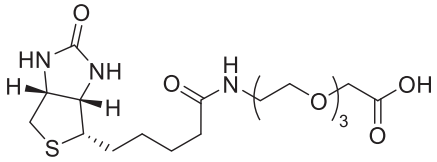
^{1}H NMR (400 MHz, $CDCl_3$) δ 4f.03 (s, 2H), 3.79 – 3.61 (m, 8H), 3.52 (t, *J* = 5.2 Hz, 2H), 2.87 (t, *J* = 5.2 Hz, 2H), 1.82 (br s, 2H, NH2), 1.48 (s, 9H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.6, 81.5, 73.3, 70.6, 70.5, 70.5, 70.2, 68.9, 41.7, 28.0 ppm HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{12}H_{26}NO_5$ 264.1806 found 264.1803.

t-Bu-TEG-biotin (S7)



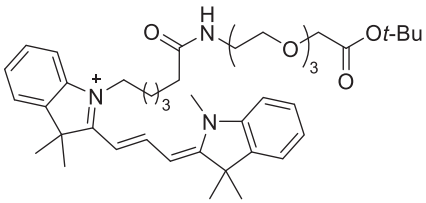
Biotin-NHS[60] (171 mg, 0.5 mmol) was dissolved in dry DMF (1.0 ml, 0.5 M), then DIPEA (105 µl, 0.6 mmol) and amine **S6** (145 mg, 0.55 mmol) were added and the mixture was stirred 16 hours at rt. The mixture was evaporated at 60°C and purification by silica column chromatography (DCM/MeOH, 49/1 -> 9/1, v/v) afforded the title compound as a white solid (202 mg, 0.42 mmol, 83%).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.02 (t, *J* = 5.2 Hz, 1H, NH), 6.94 (s, 1H, NH), 6.24 (s, 1H, NH), 4.59 – 4.43 (m, 1H), 4.36 – 4.18 (m, 1H), 4.02 (s, 2H), 3.81 – 3.61 (m, 8H), 3.57 (t, *J* = 4.8 Hz, 2H), 3.51 – 3.33 (m, 2H), 3.14 (q, *J* = 7.0 Hz, 1H), 2.90 (dd, *J* = 12.7, 4.6 Hz, 1H), 2.84 – 2.64 (m, 1H), 2.23 (t, *J* = 7.4 Hz, 2H), 1.80 – 1.60 (m, 4H), 1.48 (s, 11H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.4, 169.5, 164.4, 81.5, 70.5, 70.3, 70.3, 69.9, 69.9, 68.8, 61.7, 60.2, 55.7, 40.4, 39.0, 35.9, 28.3, 28.0, 25.6 ppm. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{22}H_{40}N_3O_7S$ 490.2582 found 490.2571.

COOH-TEG-biotin (S8)



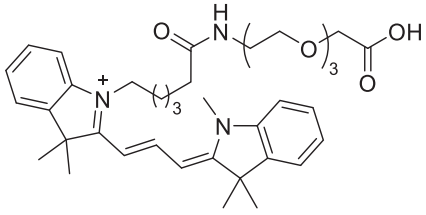
Ester **S7** (195 mg, 0.398 mmol) was dissolved in DCM/TFA (4.0 ml, 0.1 M, 20%). The mixture was stirred for 16 hours at rt, subsequently diluted with toluene (20 ml) and evaporated (3x) to furnish the title product as a white solid (173 mg, 0.399 mmol, quant.). ^{1}H NMR (400 MHz, $CDCl_3$ + 3 drops of CD_3OD) δ 7.13 (s, 1H, NH), 7.00 (s, 1H, NH), 4.55 (dd, *J* = 7.6, 4.8 Hz, 1H), 4.37 (dd, *J* = 7.6, 4.5 Hz, 1H), 4.32 – 4.05 (m, 2H), 3.83 – 3.60 (m, 8H), 3.57 (t, *J* = 4.7 Hz, 2H), 3.52 – 3.33 (m, 2H), 3.18 (q, *J* = 7.3 Hz, 1H), 2.93 (dd, *J* = 13.0, 4.8 Hz, 1H), 2.84 – 2.67 (m, 1H), 2.27 (t, *J* = 7.7 Hz, 2H), 1.80 – 1.58 (m, 4H), 1.51 – 1.39 (m, 2H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$ + CD_3OD) δ 174.7, 174.0, 165.2, 71.0, 70.6, 70.5, 70.1, 69.7, 68.8, 62.2, 60.7, 55.4, 40.5, 39.6, 35.6, 28.1, 27.9, 25.7 ppm. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{18}H_{32}N_3O_7S$ 434.19555 found 434.19565.

t-Bu-TEG-Cy3 (S9)



Reaction of **S6** (132 mg, 0.50 mmol) with Cy3-carboxylic acid[61] (229 mg, 0.5 mmol) according to general procedure A followed by flash chromatography (DCM/MeOH, 1/0 -> 94.5/5.5 v/v) afforded the title compound as a red solid (0.24 g, 0.33 mmol 66%).
^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.45 (t, *J*=13.5, 1H), 7.57 – 7.36 (m, 5H), 7.34 – 7.06 (m, 5H), 6.98 (d, *J*=13.4, 1H), 4.17 (t, *J*=7.7, 2H), 4.02 (d, *J*=1.4, 2H), 3.81 (d, *J*=1.5, 3H), 3.76 – 3.55 (m, 10H), 3.46 (dd, *J*=7.8, 3.8, 2H), 2.38 (t, *J*=7.1, 2H), 1.90 (q, *J*=9.3, 8.5, 2H), 1.75 (d, *J*=2.2, 14H), 1.71 – 1.58 (m, 2H), 1.47 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.1, 173.5, 173.3, 150.4, 142.3, 141.5, 140.3, 140.1, 128.6, 128.6, 125.2, 122.0, 121.9, 110.7, 110.5, 104.2, 103.7, 70.3, 70.1, 70.1, 69.8, 69.3, 68.6, 48.8, 48.7, 46.1, 44.4, 38.6, 35.8, 31.9, 27.8, 27.8, 26.8, 26.0, 24.9. HRMS (ESI) m/z: $[M]^+$ calculated for $C_{42}H_{60}N_3O_6$ 702.4477, found 702.4473.

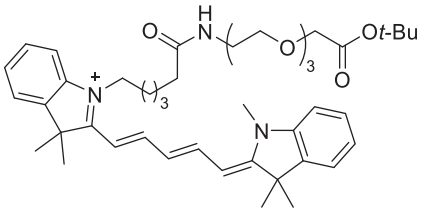
COOH-TEG-Cy3 (S10)



Tert-butyl ester **S9** (121 mg, 0.165 mmol) was dissolved in TFA/DCM (2.42 ml, 0.1 M, 17%) and stirred for 4 hours at rt. The mixture was diluted with toluene (20 ml) and evaporated (3x) to furnish the product as a red solid (112 mg, 0.164 mmol, quant.). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.41 (t, *J*=13.4, 1H), 7.83 (t, *J*=5.6, 1H), 7.48 – 7.34 (m, 4H), 7.34 – 7.23 (m, 3H), 7.16 (dd, *J*=8.0, 6.0, 2H), 6.49 (dd, *J*=19.3, 13.5, 2H), 4.20 (s, 2H), 4.06 (t, *J*=7.8, 2H), 3.80 – 3.72 (m, 2H), 3.73 – 3.55 (m, 11H), 3.52 – 3.43 (m, 2H), 2.42 (t, *J*=7.5, 2H), 1.89 – 1.64 (m, 16H), 1.60 – 1.49 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.0, 174.6, 174.2, 172.4, 150.6, 142.6, 141.8, 140.6, 140.4, 129.2, 129.0, 125.8, 125.7, 122.3, 122.2, 111.2, 110.9, 103.6, 103.4, 71.1, 70.5, 70.4, 70.0, 69.4,

68.9, 49.4, 49.2, 46.3, 44.5, 39.8, 35.5, 31.5, 28.1, 28.1, 27.1, 26.3, 25.6. HRMS (ESI) m/z: $[M]^+$ calculated for $C_{38}H_{52}N_3O_6$ 646.38506 found 646.38514.

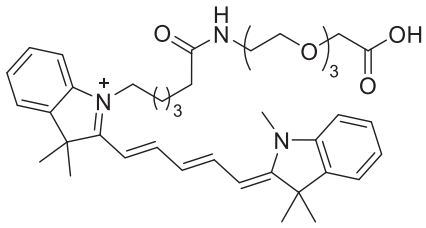
_t_-Bu-TEG-Cy5 (S11)



Reaction of **S6** (66 mg, 0.25 mmol) with Cy5-carboxylic acid[61] (130 mg, 0.25 mmol) according to general procedure A followed by flash chromatography (DCM/MeOH, 98/2 -> 95/5, v/v) afforded the title compound as a blue solid (139 mg, 0.183 mmol, 73%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (t, *J* = 13.0 Hz, 2H), 7.42 – 7.35 (m, 6H), 7.23 (dt, *J* = 10.6, 5.4 Hz, 2H), 7.14 (t, *J* = 6.8 Hz, 3H), 6.79 (t, *J* = 12.4 Hz, 1H), 6.29 (t, *J* = 14.6 Hz, 2H), 4.10 – 4.05 (m, 2H), 4.02 (s, 2H), 3.78 – 3.62 (m, 12H), 3.60 (t, *J* = 5.6 Hz, 2H), 3.51 – 3.40 (m, 2H), 2.34 (t, *J* = 7.1 Hz, 2H), 1.87 – 1.79 (m, 2H), 1.78 (s, 6H), 1.76 (s, 6H), 1.64 – 1.50 (m, 2H), 1.47 (s, 9H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.2, 173.1, 169.6, 154.0, 153.7, 142.6, 141.8, 141.2, 140.8, 128.5, 128.5, 126.2, 125.1, 124.9, 122.2, 122.1, 110.6, 110.3, 103.7, 103.5, 81.5, 77.5, 70.5, 70.4, 70.4, 70.0, 69.6, 68.9, 49.4, 49.2, 44.2, 38.9, 35.9, 31.8, 28.0, 28.0, 27.9, 27.0, 26.4, 25.1 ppm. HRMS (ESI) m/z: $[M]^+$ calculated for $C_{44}H_{62}N_3O_6$ 728.4633 found 728.4628.

COOH-TEG-Cy5 (S12)

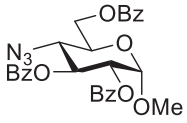


Ester **S11** (139 mg, 0.18 mmol) was dissolved in TFA/DCM (1.8 ml, 0.1 M, 50%) and stirred for 30 minutes at rt. The mixture was diluted with toluene (20 ml) and evaporated (3x) to furnish the title product as a blue solid (128 mg, 0.18 mmol, quant.).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.46 (br s, 2H, COOH), 7.93 (td, *J* = 12.9, 6.4 Hz, 2H), 7.56 (m, 1H), 7.42 – 7.31 (m, 4H), 7.31 – 7.19 (m, 3H), 7.12 (dd, *J* = 16.3, 7.9 Hz, 2H), 6.72 (t, *J* = 12.4 Hz, 1H), 6.30 (d, *J* = 13.6 Hz, 1H), 6.21 (d, *J* = 13.5 Hz, 1H), 4.22 (s, 2H), 4.04 (t, *J* = 7.3 Hz, 2H), 3.79 – 3.72 (m, 2H), 3.71 – 3.56 (m, 11H), 3.53 – 3.30 (m, 2H), 2.36 (t, *J* = 7.3 Hz, 2H), 1.87 – 1.59 (m, 16H), 1.59 – 1.44 (m, 2H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.5, 173.3, 172.9, 172.3, 153.7, 153.0, 142.8, 141.9, 141.2, 140.8, 128.9, 128.7, 126.1, 125.5, 125.1, 122.3, 122.2, 111.0, 110.4, 104.1, 103.5, 70.9, 70.5, 70.0, 69.8, 69.0, 49.5, 49.2, 44.4, 39.4, 35.9, 31.5, 31.3, 28.1, 27.1, 26.4, 25.4 ppm. HRMS (ESI) m/z: $[M]^+$ calculated for $C_{40}H_{54}N_3O_6$ 672.4007 found 672.4003.

GG probes

Methyl 2,3,6-tri-*O*-benzoyl-4-deoxy-4-azido-α-D-glucopyranoside (44)

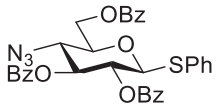


Alcohol **43**[40] (19.0 g, 37.5 mmol) was dissolved in DCM (150 ml, 0.25 M). Pyridine (16.6 ml, 206 mmol) was added and the mixture was cooled to -55°C. Tf_2O (8.84 ml, 52.5 mmol) was added and the mixture was slowly warmed to room temperature. When TLC (8/2, v/v, Pentane/EtOAc) indicated complete consumption of the starting material water and DCM were added and the organic layer was washed twice with brine, dried over $MgSO_4$ and filtered. The volatiles were removed under reduced pressure and the crude triflate was dissolved in DMF (125 ml, 0.3 M). NaN_3 (4.88 g, 75.1 mmol) was added and the mixture was stirred overnight at 80°C. The mixture was allowed to cool to room temperature and was poured over $NaHCO_3$ (aq, sat.). The water layer was extracted trice with EtOAc. The combined organic layers were washed subsequently with $NaHCO_3$ (aq, sat.) and brine, dried with $MgSO_4$ and filtered. Volatiles were removed under reduced pressure and the product was isolated after column chromatography (pentane/EtOAc, 9/1, v/v,) as a colorless oil (17.9 g, 33.8 mmol, 90%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.16 – 8.09 (m, 2H), 8.05 – 7.96 (m, 4H), 7.62 – 7.53 (m, 1H), 7.52 – 7.42 (m, 4H), 7.39 – 7.29 (m, 4H), 6.06 (t, *J* = 9.9 Hz, 1H, H3), 5.27 (dd, *J* = 10.1, 3.6 Hz, 1H, H2), 5.20 (d, *J* = 3.6 Hz, 1H, H1), 4.74 (dd, *J* = 12.2, 2.4 Hz, 1H, H6a), 4.66 (dd, *J* = 12.2, 4.7 Hz, 1H, H6b), 4.15 – 4.04 (m,

1H, H5), 3.95 (t, *J* = 10.1 Hz, 1H, H4), 3.42 (s, 3H, OMe). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 166.1, 165.8, 165.5, 133.4, 133.4, 133.3, 129.9, 129.7, 129.7, 129.0, 128.8, 128.5, 128.4, 97.1 (C1), 71.9 (C2), 71.1 (C3), 68.0 (5), 63.3 (C6), 61.0 (C4), 55.6 (OMe). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{28}H_{25}N_3O_8Na$ 554.1539, found 554.1542.

Phenyl 2,3,6-tri-*O*-benzoyl-4-deoxy-4-azido-1-thio-β-D-glucopyranoside (42)



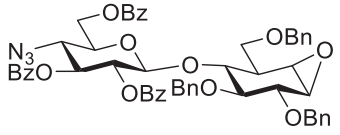
44 (17.88 g, 33.64 mmol) was dissolved in Ac_2O (63.5 ml, 673 mmol). The mixture was cooled to 0°C and AcOH (9.24 ml, 161 mmol) and H_2SO_4 (1.79 ml, 33.6 mmol) were added slowly. The mixture was allowed to warm to rt overnight. TLC (9/1, v/v, pentane/EtOAc) showed full conversion to a lower running spot. $NaHCO_3$ (aq. sat.) was added slowly and the water layer was extracted with toluene three times. The combined organic layers were washed with $NaHCO_3$ (aq. sat.) and brine, dried with $MgSO_4$, filtered and the volatiles were removed under reduced pressure.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 – 8.08 (m, 2H), 8.04 – 7.98 (m, 2H), 7.94 – 7.87 (m, 2H), 7.64 – 7.46 (m, 5H), 7.43 – 7.33 (m, 4H), 6.57 (d, *J* = 3.7 Hz, 1H, H1), 6.00 (dd, *J* = 10.8, 9.2 Hz, 1H, H3), 5.43 (dd, *J* = 10.2, 3.7 Hz, 1H, H2), 4.67 (d, *J* = 3.0 Hz, 2H, H6ab), 4.16 (dt, *J* = 10.5, 3.0 Hz, 1H, H5), 4.00 (t, *J* = 10.1 Hz, 1H, H4), 2.18 (s, 3H, OAc). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 168.7, 166.2, 165.7, 165.5, 133.7, 133.7, 133.5, 129.9, 129.9, 129.6, 128.8, 128.7, 128.6, 128.6, 89.4 (C1), 70.9 (C3), 70.7 (C5), 70.3 (C2), 62.9 (C6), 60.5 (C4), 20.9 (OAc).

The crude product **45** was dissolved in DCM (85ml, 0.4 M) and thiophenol (4.12 ml, 40.4 mmol) and $BF_3{\cdot}Et_2O$ (4.98 ml, 40.4 mmol) were added and the reaction was stirred for 40 hours. Thiophenol (2.05 ml, 20 mmol) and $BF_3.Et_2O$ (4.98 ml, 40.4 mmol) were added and the reaction was stirred for another 2 hours. The reaction was quenched with $NaHCO_3$ (aq. sat.) and the organic layer was washed with $NaHCO_3$ (aq. sat.) and brine and was subsequently dried over $MgSO_4$ and filtered. The volatiles were removed under reduced pressure the product was crystalized out of Et_2O and pentane as a white solid. (9.43 g, 15.47 mmol, 46%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.11 – 8.07 (m, 2H), 7.95 (m, 4H), 7.68 – 7.59 (m, 1H), 7.57 – 7.33 (m, 10H), 7.29 – 7.22 (m, 1H), 7.19 – 7.10 (m, 2H), 5.70 (t, *J* = 9.5 Hz, 1H, H3), 5.36 (dd, *J* = 10.0, 9.4 Hz, 1H, H2), 4.95 (d, *J* = 10.0 Hz, 1H, H1), 4.81 (dd, *J* = 12.1, 1.9 Hz, 1H, H6a), 4.59 (dd, *J* = 12.1, 4.8 Hz, 1H, H6b), 3.92 – 3.76 (m, 2H, H4/H5). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 166.2, 165.7, 165.3, 133.7, 133.5, 133.5, 131.5, 130.0, 130.0, 130.0, 129.7, 129.1, 129.0, 128.8, 128.7, 128.6, 128.6, 128.5, 86.2 (C1), 76.6 (C5), 75.1 (C3), 70.4 (C2), 63.6 (C6), 60.8 (C4). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{33}H_{27}N_3O_7SNa$ 632.1467, found 632.1467.

4-*O*-(2,3,6-tri-*O*-benzoyl-4-deoxy-4-azido-β-D-glucopyranosyl)-2,3,6-tri-*O*-benzyl-cyclophellitol (46)

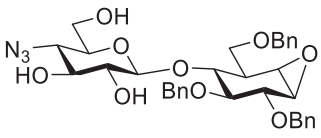


A mixture of **42** (150 mg, 0.246 mmol), Ph_2SO (68 mg, 0.336 mmol) and TTBP (303 mg, 1.22 mmol) was co-evaporated twice with dry toluene and dissolved in dry DCM (2.5 ml). Crushed 3Å molecular sieves were added and the mixture was stirred for 45 min at room temperature. The mixture was cooled to -60°C and freshly distilled Tf_2O (49 µl, 0.291 mmol) was added. The mixture was allowed to warm to -40°C within 30 minutes and was subsequently cooled back to -70°C. **25** (100 mg, 0.224 mmol, co-evaporated trice with dry toluene) was added in DCM (1.0 ml). The mixture was slowly warmed to room temperature overnight. Pyridine (0.05 ml) was added and the mixture was poured over brine. DCM was added and the layers were separated. The organic layer was washed with brine, dried with $MgSO_4$ and filtered. The volatiles were evaporated under reduced pressure and the product was isolated column chromatography (pentane/EtOAc, 9/1 -> 8/2, v/v) provided the product (136 mg, 0.143 mmol, 64%).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.04 – 8.00 (m, 2H), 7.92 (m, 4H), 7.61 – 7.12 (m, 24H), 5.50 (t, J=9.8, 1H, H3'), 5.35 (dd, J=9.8, 8.0, 1H, H2'), 4.97 (d, J=11.9, 1H, CH_2Bn), 4.86 – 4.79 (m, 2H, CH_2Bn/H1'), 4.66 (d, J=11.5, 1H, CH_2Bn), 4.61 (d, J=11.5, 1H, CH_2Bn), 4.40 – 4.27 (m, 2H, CH_2Bn/H6a'), 4.24 (dd, J=12.2, 4.2, 1H, H6b'), 4.17 (d, J=11.9, 1H, CH_2Bn), 3.82 (d, J=7.4, 1H, H2), 3.78 (t, J=10.0, 1H, H4'), 3.68 (t, J=9.8, 1H, H4), 3.60 (dd, J=8.8, 3.4, 1H, H6a), 3.52 (dd, J=9.6, 7.4, 1H, H3), 3.46 (t, J=8.5, 1H, H6b), 3.34 – 3.29

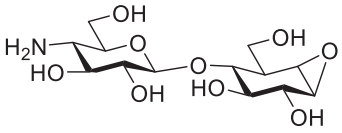
(m, 2H, H5'/epoxide), 3.11 (d, J=3.7, 1H/epoxide), 2.22 – 2.14 (m, 1H, H5). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.1, 165.7, 165.3, 139.3, 138.2, 137.7, 133.6, 133.3, 129.9, 129.9, 129.7, 129.1, 128.9, 128.7, 128.6, 128.3, 128.0, 127.9, 127.7, 127.3, 126.8, 101.5 (C1'), 83.2 (C3), 79.5 (C2), 76.5 (C4), 74.4 (CH_2Bn), 74.0 (C3'), 73.2 (CH_2Bn), 73.1 (CH_2Bn), 72.9 (C2'), 72.6 (C5'), 68.4 (C6), 63.2 (C6'), 60.9 (C4'), 55.6 (epoxide), 53.3 (epoxide), 41.9 (C5). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{55}H_{51}N_3O_{12}Na$ 968.3365, found 968.3387.

4-*O*-(4-deoxy-4-azido-β-D-glucopyranosyl)-2,3,6-tri-*O*-benzyl-cyclophellitol (47)



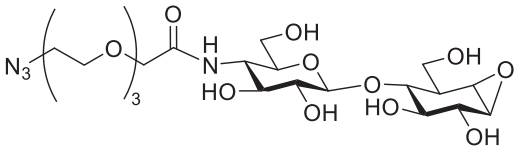
46 (62 mg, 0.098 mmol) was dissolved in a mixture of DCM and methanol (1.3 ml, 1/1, v/v). NaOMe (12 µl of a 5.4 M solution, 0.065 mmol) was added and the mixture was stirred overnight. The reaction was quenched by adding solid CO_2. Volatiles were removed under reduced pressure and column chromatography (EtOAc/DCM, 1/4, v/v) provided the product (25 mg, 0.058 mmol, 60%).
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 – 7.24 (m, 15H), 4.87 – 4.79 (m, 2H, CH_2Bn (2x)), 4.77 (d, *J* = 11.4 Hz, 1H, CH_2Bn), 4.69 (d, *J* = 11.3 Hz, 1H, CH_2Bn), 4.65 – 4.56 (m, 2H, CH_2Bn), 4.37 (d, *J* = 7.8 Hz, 1H, H1'), 3.90 – 3.80 (m, 3H, H2/H6ab), 3.78 – 3.71 (m, 2H,H4/OH), 3.56 – 3.43 (m, 3H, H3/H3'/H6a'), 3.37 – 3.29 (m, 3H, H4'/H6b'/epoxide), 3.26 (m, 1H, H2'), 3.17 (d, *J* = 3.7 Hz, 1H, epoxide), 2.91 (m, 1H, H5'), 2.79 (d, *J* = 2.9 Hz, 1H, OH), 2.38 – 2.27 (m, 1H, H5), 1.87 (s, 1H, OH). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.7, 137.5, 137.4, 128.7, 128.2, 128.2, 128.2, 128.1, 127.8, 127.0, 102.4 (C1'), 82.9 (C3), 79.5 (C2), 75.8 (C3'), 75.1 (C4), 74.9 (C5'), 74.6 (C2'), 74.6 (CH_2Bn), 73.6 (CH_2Bn), 73.2 (CH_2Bn), 69.1 (C6), 61.9 (C6'), 61.1 (C4'), 56.2 (epoxide), 53.2 (epoxide), 42.3 (C5). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{34}H_{39}N_3O_9Na$ 656.2579, found 656.2594.

4-*O*-(4-deoxy-4-amino-β-D-glucopyranosyl)-cyclophellitol (48)



Ammonia (3 ml) was condensed at -50°C. Sodium (36 mg, 1.57 mmol) was added. **47** (33 mg, 0.052 mmol) was dissolved in THF (3 ml) and *t*-BuOH (0.15 ml, 1.57 mmol) and the solution was added dropwise at -60°C. After 45 minutes the reaction was quenched with NH_4Cl (97 mg, 1.82 mmol). The mixture was warmed to room temperature and the ammonia was evaporated.
The crude product was purified by size exclusion chromatography over HW-40 eluting with 150 mM NH_4HCO_3 in H_2O yielding the product as a white solid (9.3 mg, 0.028 mmol, 53%).
^{1}H NMR (400 MHz, D_2O) δ 4.42 (d, *J* = 7.8 Hz, 1H, H1'), 4.04 (dd, *J* = 11.3, 3.6 Hz, 1H, H6a), 3.94 – 3.78 (m, 3H, H6b/H6a'/H2), 3.70 (t, *J* = 12.5, 5.3 Hz, 1H, H6b'), 3.52 (m, 1H, epoxide), 3.49 – 3.45 (m, 2H, H4/H3), 3.44 – 3.34 (m, 2H, H5'/H3'), 3.29 (dd, *J* = 9.3, 7.7 Hz, 1H, H2'), 3.20 (d, *J* = 3.8 Hz, 1H, epoxide), 2.76 (t, *J* = 9.8 Hz, 1H, H4'), 2.28 (m, 1H, H5). ^{13}C NMR (101 MHz, D_2O) δ 103.1 (C1'), 77.7 (C4), 76.0 (C5'), 75.0 (C3'), 74.7 (C3), 73.7 (C2'), 70.8 (C2), 60.6 (C6'), 59.7 (C6), 56.5 (epoxide), 55.2 (epoxide), 52.1 (C4'), 42.6 (C5). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{13}H_{24}NO_9$ 338.1446, found 338.1453.

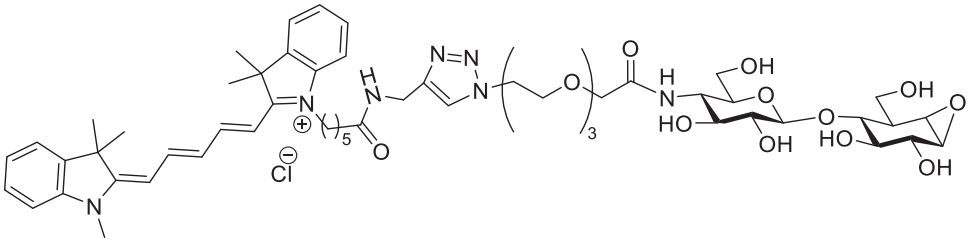
GG azide probe (49)



48 (38 mg, 0.113 mmol) was dissolved in DMF (0.4 ml, 0.3 M) and added to PFP ester **S5** (59 mg, 0.147 mmol). DIPEA (20 µl, 0.136 mmol) was added and the reaction was stirred overnight. LCMS indicated full conversion and the product was purified on semi-preparative HPLC eluting with a linear gradient of solution A (MeCN) in solution B (50 mM AcOH in H_2O). The fractions were concentrated under reduced pressure, diluted with water and lyophilized to yield the product as a white solid (16.6 mg, 0.031 mmol, 27%).
^{1}H NMR (500 MHz, D_2O) δ 4.45 (d, *J* = 7.9 Hz, 1H, H1'), 4.11 (s, 2H, OCH_2C=O), 4.06 (dd, *J* = 11.3, 3.5 Hz, 1H, H6a), 3.91 (dd, *J* = 11.3, 6.7 Hz, 1H, H6b), 3.87 – 3.81 (m, 2H, H2/H4'), 3.75 – 3.67 (m, 11H, TEG (10H)/H6a'), 3.65 (dd, *J* = 10.1, 9.3 Hz, 1H, H3'), 3.60 – 3.56 (m, 2H, H3/H6b'), 3.55 – 3.53 (m, 1H, epoxide), 3.52 – 3.46 (m, 4H, H4/CH_2N_3/H5'), 3.38 (dd, *J* = 9.3, 7.9 Hz, 1H, H2'), 3.21 (d, *J* = 3.9 Hz, 1H, epoxide), 2.33 – 2.27 (m, 1H, H5). ^{13}C NMR (126 MHz, D_2O) δ = 173.2, 103.0 (C1'), 77.7 (C3), 74.8, 74.8

(C5'/C3), 73.8 (C2'), 73.2 (C3'), 70.9 (C2), 70.3, 69.6, 69.5, 69.5, 69.5, 69.3 (OCH_2CH_2O/ O**C**H_2C=O), 60.6 (C6'), 59.8 (C6), 56.6 (epoxide), 55.3 (epoxide), 51.1 (C4'), 50.2 (CH_2N_3), 42.8 (C5). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{21}H_{37}N_4O_{13}$ 553.2352 found 553.2349.

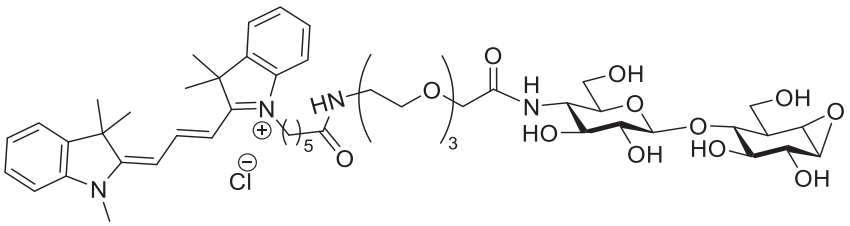
GG Cy5 probe (52)



To **49** (3.1 mg, 5.6 µmol) was added a stock solution of DMSO (0.2 ml) containing THPTA (1.68 µmol), CuI (0.56 µmol) and DIPEA (0.67 µmol). To this solution was added Cy5 alkyne (3.3 mg, 5.9 µmol). The mixture was stirred overnight after which LC-MS analysis indicated full consumption of the starting azide. The product was purified on semi-preparative HPLC eluting with a linear gradient of solution A (MeCN) in solution B (50mM NH_4HCO_3 in H_2O). The fractions were concentrated under reduced pressure, diluted with water and lyophilized yielding the compound as a blue solid. (2.8 mg, 2.6 µmol, 46%).

^{1}H NMR (850 MHz, MeOD) δ 8.24 (t, *J* = 12.9 Hz, 2H, alkene), 7.92 (s, 1H, triazole), 7.49 (d, *J* = 7.4 Hz, 2H, phenyl), 7.42 (td, *J* = 7.7, 3.6 Hz, 2H, phenyl), 7.32 – 7.28 (m, 2H, phenyl), 7.28 – 7.25 (m, 2H, phenyl), 6.63 (t, *J* = 12.4 Hz, 1H, alkene), 6.28 (d, *J* = 13.7 Hz, 2H, alkene), 4.58 – 4.56 (m, 2H, OCH_2C**H_2**N), 4.42 (s, 2H, TriazoleC**H_2**NH), 4.36 (d, *J* = 7.9 Hz, 1H, H1'), 4.10 (m, 3H, H6b/CH_2N=C), 4.01 (s, 2H, O**C**H_2C=O), 3.90 (t, *J* = 5.1 Hz, 2H, OC**H_2**CH_2N), 3.83 (dd, *J* = 10.9, 7.1 Hz, 1H, H6a), 3.79 – 3.70 (m, 2H, H4'/H2), 3.69 – 3.59 (m, 15H, TEG10H /H6a'/H3'/CH_3N), 3.56 – 3.49 (m, 2H, H6b'/H5), 3.43 – 3.34 (m, 3H, epoxide/H3/H4), 3.30 – 3.27 (m, 1H, H2'), 3.04 (d, *J* = 3.7 Hz, 1H, epoxide), 2.25 (t, *J* = 7.4 Hz, 2H, HNC=OC**H_2**), 2.20 – 2.16 (m, 1H, H5), 1.82 (q, *J* = 7.7 Hz, 2H, HNC=$OCH_2CH_2CH_2C$**H_2**), 1.75 – 1.67 (m, 14H, HNC=OCH_2C**H_2**/CH_3 4x), 1.48 (q, *J* = 7.9 Hz, 2H, HNC=OCH_2CH_2C**H_2**). ^{13}C NMR (214 MHz, MeOD) δ 175.7, 175.4, 174.6, 173.7, 155.5, 155.5, 146.1, 144.3, 143.6, 142.6, 142.5, 129.8, 129.7, 126.6, 126.3, 126.2, 125.0, 123.4, 123.3, 112.1, 111.8, 104.7 (C1'), 104.4 (Cy), 104.3 (Cy), 80.5 (C4), 77.0 (C5'), 76.9 (C3), 75.8 (C2'), 74.9 (C3'), 72.9 (C2), 71.9 (TEG), 71.4 (TEG 2x), 71.3 (TEG), 71.2 (O**C**H_2C=O), 70.3 (O**C**H_2CH_2N), 62.8 (C6'), 62.1 (C6), 56.9 (epoxide), 56.5 (epoxide), 52.9 (C4'), 51.4 (OCH_2**C**H_2N), 44.9 (C5), 44.8 (**C**H_2N=C), 36.5 (HNC=OC**H_2**), 35.6 (CH_2NH), 31.5 (CH_3N), 28.2 (HNC=$OCH_2CH_2CH_2$**C**H_2), 28.0 (2x **C**H_3Cq), 27.8 (2x **C**H_3Cq), 27.4 (HNC=OCH_2CH_2**C**H_2), 26.4 (HNC=OCH_2**C**H_2CH_2). HRMS (ESI) m/z: $[M]^+$ calculated for $C_{56}H_{78}N_7O_{14}$ 1072.5601, found 1072.5618.

GG Cy3 probe (50)

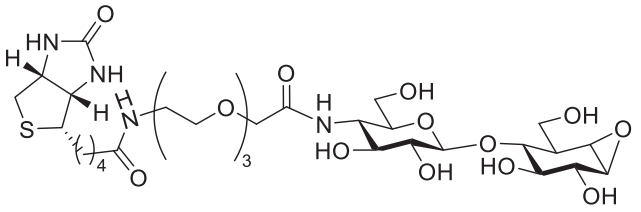


Amine **48** (4.7 mg, 14 µmol) was reacted with stock solution TEG Cy3 **S10** (0.34 ml) according to general procedure B. Providing the product as a red solid contaminated with a small amount of an unknown byproduct (9.7 mg, 9.7 µmol, 69%).

^{1}H NMR (600 MHz, D_2O) δ = 8.48 (t, *J*=13.4, 1H), 7.59 – 7.53 (m, 2H), 7.50 – 7.43 (m, 2H), 7.37 – 7.30 (m, 4H), 6.36 – 6.26 (m, 2H), 4.46 (d, *J*=8.0, 1H), 4.13 – 4.05 (m, 5H), 3.95 – 3.88 (m, 1H), 3.87 – 3.80 (m, 2H), 3.73 – 3.65 (m, 6H), 3.66 – 3.54 (m, 10H), 3.54 – 3.47 (m, 4H), 3.42 – 3.37 (m, 1H), 3.31 – 3.26 (m, 2H), 3.23 (d, *J*=3.8, 1H), 2.33 – 2.27 (m, 1H), 2.28 – 2.22 (m, 2H), 1.90 – 1.82 (m, 2H), 1.73 – 1.70 (m, 12H), 1.69 – 1.62 (m, 2H), 1.41 – 1.32 (m, 2H). ^{13}C NMR (151 MHz, D_2O) δ 177.2, 176.0, 175.5, 173.6, 151.3, 143.3, 142.7, 141.6, 141.5, 129.3, 126.0, 123.0, 122.9, 112.0, 111.7, 103.6, 102.8, 102.7, 78.5,

75.4, 74.4, 73.7, 71.5, 70.8, 70.2, 70.1, 70.0, 69.4, 61.3, 60.5, 57.2, 55.9, 51.7, 49.8, 49.7, 44.3, 43.3, 31.5, 27.9, 27.7, 27.2, 26.0, 25.6. HRMS (ESI) m/z: $[M]^+$ calculated for $C_{51}H_{73}N_4O_{14}$ 965.5118 found 965.5116.

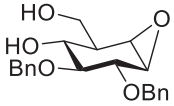
GG biotin probe (51)



Amine **48** (4.7 mg, 14 μmol) was reacted with stock solution TEG biotin **S8** (0.34 ml) according to general procedure B. Providing the product as a white solid. (4.68 mg, 6.2 μmol, 44%).
^{1}H NMR (850 MHz, MeOD) δ = 4.50 (dd, *J*=7.9, 4.0, 1H, biotin), 4.36 (d, *J*=7.9, 1H, H1'), 4.31 (dd, *J*=7.9, 4.5, 1H, biotin), 4.12 (dd, *J*=10.9, 4.1, 1H, 6A), 4.05 (d, *J*=2.4, 2H, O**CH$_2$**C=O), 3.85 (dd, *J*=10.9, 7.1, 1H, 6B), 3.77 – 3.71 (m, 3H), 3.71 – 3.67 (m, 5H), 3.67 – 3.61 (m, 4H), 3.57 – 3.54 (m, 3H), 3.52 (ddd, *J*=10.4, 6.1, 2.1, 1H), 3.42 (dt, *J*=3.7, 1.1, 1H, epox), 3.42 – 3.36 (m, 4H), 3.30 – 3.28 (m, 1H, H2'), 3.22 (ddd, *J*=9.0, 5.9, 4.5, 1H, biotin), 3.05 (d, *J*=3.7, 1H, epoxide), 2.93 (dd, *J*=12.8, 5.0, 1H, biotin), 2.71 (d, *J*=12.7, 1H, biotin), 2.23 (t, *J*=7.4, 1.4, 2H, biotin), 2.21 – 2.17 (m, 1H, H5), 1.77 – 1.71 (m, 1H, biotin), 1.71 – 1.57 (m, 3H, biotin), 1.48 – 1.42 (m, 2H, biotin). ^{13}C NMR (214 MHz, MeOD) δ 176.2, 173.8, 104.7 (C1'), 80.5, 77.0, 76.9, 75.8 (C2'), 74.9, 72.9, 71.8, 71.4, 71.4, 71.2, 71.1, 70.6, 63.4 (biotin), 62.8 (C6'), 62.2 (C6), 61.6 (biotin), 57.0 (biotin), 56.8 (epoxide), 56.5 (epoxide), 52.9, 45.0 (C5), 41.0, 40.3, 36.8, 29.8, 29.5, 26.8. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{31}H_{53}N_4O_{15}S$ 753.3223 found 753.3219.

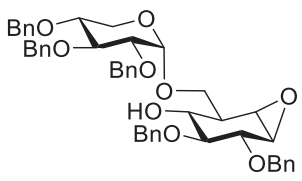
GX probes

2,3-di-*O*-benzyl-cyclophellitol (18)



Diol **53** (102 mg, 0.30 mmol) was dissolved in THF (3 ml, 0.1 M). DMAP (30 mg, 0.24 mmol) and Boc_2O (262 mg, 1.2 mmol) were added and the mixture was stirred overnight. The mixture was diluted with Et_2O and washed with NH4Cl (aq. sat.) $NaHCO_3$ (aq. sat.) and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure.
The residue was dissolved in AcOH (1.3 ml, 0.2 M) and NIS (118 mg, 0.523 mmol) was added and the mixture was stirred overnight. The mixture was diluted with Et_2O and washed with $NaHCO_3$ (aq. sat.), $Na_2S_2O_3$ (aq. sat.) and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The iodide was dissolved in MeOH (3 ml, 0.05 M), K_2CO_3 (96 mg, 0.696 mmol) was added and the mixture was stirred overnight. The mixture was filtered, the solvent evaporated and the residue was dissolved in EtOAc. The solution was washed with H_2O and brine, dried over $MgSO_4$, filtered and concentrated. The product was obtained after chromatography (Et_2O/pentane, 9/1, v/v) as a white solid (80 mg, 0.23 mmol, 75%) Spectra matched previously recorded data.[33,36]

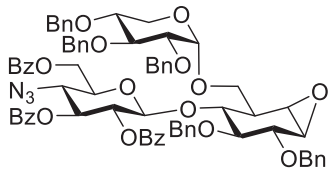
6-*O*-(2,3,4-tri-*O*-benzyl-α-D-xylopyranosyl)-2,3-di-*O*-benzyl-cyclophellitol (21)



Diol **18** (47 mg, 0.132 mmol) was co-evaporated with toluene (3x). DCM (1 ml), DIPEA (30 μl, 0.173 mmol), $OPPh_3$ (160 mg, 0.575 mmol) and 3Å molecular sieves were added and the mixture was stirred for 30 minutes at room temperature.
Acetate donor **56** (80 mg, 0.173 mmol) was co-evaporated with toluene (3x). The dry residue was dissolved in DCM (0.5 ml) followed by the addition of TMSI (25 μl, 0.173 mmol) at room temperature. The mixture was stirred for 30 minutes and turned deep red. The mixture was co-evaporated with toluene (2x) and dissolved in DCM (1 ml) and

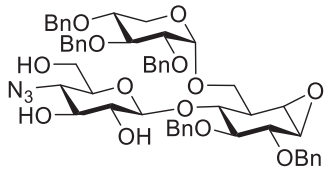
0.8 ml was added to the reaction flask containing **18**. Upon addition the color faded immediately and the reaction was stirred for 23 hours.
The reaction was quenched by the addition of $NaHCO_3$ (aq. sat.). The solution was separated from the solids and diluted with DCM. The layers were separated and the organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The product was isolated after column chromatography (pentane/EtOAc, 85/15, v/v) as a white solid (47 mg, 0.061 mmol, 46%).
^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.42 – 7.18 (m, 25H), 4.95 – 4.60 (m, 11H, CH_2Bn (5x)/H1'), 3.92 – 3.84 (m, 2H, H3'/H6a), 3.81 (d, *J*=8.1, 1H, H2), 3.73 (dd, *J*=9.4, 5.7, 1H, H6b), 3.63 (q, *J*=3.2, 1H,H5a'), 3.59 – 3.51 (m, 2H, H5b'/H4'), 3.47 (dd, *J*=9.6, 3.6, 1H, H2'), 3.43 – 3.38 (m, 2H, epoxide/H3), 3.33 (t, *J*=9.6, 1H, H4), 3.20 (d, *J*=3.7, 1H, epoxide), 2.88 (s, 1H, OH), 2.39 – 2.31 (m, 1H, H5). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 139.0, 138.6, 138.4, 138.3, 137.6, 128.7, 128.6, 128.6, 128.6, 128.5, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 127.7, 97.5 (C1), 83.7 (C3), 81.5 (C3'), 79.8 (C2'), 79.3 (C2), 78.1 (C4'), 75.9 (CH_2Bn), 75.1 (CH_2Bn), 73.7 (CH_2Bn), 73.6 (CH_2Bn), 72.9 (CH_2Bn), 68.1 (C4), 68.0 (C6), 60.2 (C5'), 54.6 (epoxide), 54.2 (epoxide), 41.7 (C5). $^1J_{H,C}$ 4.70 ppm, 97.5 ppm = 168 Hz. HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{47}H_{50}O_9Na$ 781.3353, found 781.3362.

6-*O*-(2,3,4-tri-*O*-benzyl-α-D-xylopyranosyl)-4-*O*-(2,3,6-tri-*O*-benzoyl-4-deoxy-4-azido-β-D-glucopyranosyl)-2,3-di-*O*-benzyl-cyclophellitol (19)



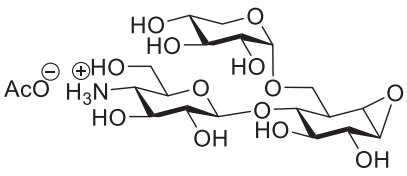
A mixture of **42** (74 mg, 0.121 mmol), Ph_2SO (31 mg, 0.151 mmol) and TTBP (75 mg, 0.303 mmol) was co-evaporated twice with dry toluene and dissolved in dry DCM (1.0 ml). Crushed 3Å molecular sieves were added and the mixture was stirred for 2 hours at room temperature. The mixture was cooled to -60°C and freshly distilled Tf_2O (23 µl, 0.139 mmol) was added. The mixture was allowed to warm to -40°C and was subsequently cooled back to -70°C. **21** (46 mg, 0.061 mmol, co-evaporated trice with dry toluene) was added in DCM (0.5 ml). The mixture was slowly warmed to room temperature overnight. Pyridine (0.05 ml) was added and the mixture was poured over brine. DCM was added and the layers were separated. The organic layer was washed with brine, dried with $MgSO_4$ and filtered. The volatiles were evaporated under reduced pressure and the product was isolated after size exclusion over sephadex LH-20 eluting with DCM/MeOH (1/1, v/v) (79 mg). The product was not completely pure and a yield over two steps is provided after the next step. HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{74}H_{71}N_3O_{16}Na$ 1280.4727, found 1280.4735.

6-*O*-(2,3,4-tri-*O*-benzyl-α-D-xylopyranosyl)-4-*O*-(4-deoxy-4-azido-β-D-glucopyranosyl)-2,3-di-*O*-benzyl-cyclophellitol (59)



19 (79 mg) was dissolved in MeOH/DCM (2 ml, 1/1, v/v). NaOMe (10 µl, 5.4M, 0.042 mmol) was added and the mixture was stirred overnight. Solid CO_2 was added the mixture was stirred for a few minutes and the solvent was evaporated under reduced pressure. Column chromatography (DCM/EtOAc, 1/0 -> 4/1, v/v) provided the product (42 mg, 0.045 mmol, 73% over 2 steps).
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 – 7.26 (m, 25H), 4.89 (s, 2H, CH_2Bn), 4.83 (d, *J* = 3.5 Hz, 1H, H1 xyl), 4.80 (s, 2H, CH_2Bn), 4.79 – 4.66 (m, 5H, CH_2Bn), 4.61 (d, *J* = 11.6 Hz, 1H, CH_2Bn), 4.27 (d, *J* = 7.7 Hz, 1H, H1 glu), 3.97 (dd, *J* = 9.8, 3.8 Hz, 1H, H6a), 3.94 – 3.84 (m, 2H H3 xyl/H6b), 3.83 (m, 1H, H2), 3.75 – 3.69 (br, 1H, OH), 3.69 – 3.65 (m, 1H, H5a xyl), 3.61 – 3.45 (m, 7H, H4/H3/H5b xyl/H2 xyl/H4 xyl/H6a glu/epoxide), 3.36 – 3.23 (m, 4H, H3 gluc /H2 glu/H6b glu/H4 glu), 3.20 (d, *J* = 3.8 Hz, 1H, epoxide), 3.13 (br, 1H, OH), 2.96 – 2.88 (m, 1H, H5 gluc), 2.47 – 2.41 (m, 1H, H5).^{13}C NMR (126 MHz, $CDCl_3$) δ = 138.9, 138.4, 138.2, 138.2, 137.5, 128.7, 128.6, 128.6, 128.5, 128.5, 128.2, 128.2, 128.1, 128.1, 128.0, 127.9, 127.7, 127.4, 102.2 (C1 glu), 97.1 (C1 xyl), 82.3 (C3) , 81.2 (C3 xyl), 79.7 (C2 xyl), 79.5 (C2), 78.1 (C4 xyl), 75.8 (C3 gluc), 75.8 (CH_2Bn), 75.0 (C5 gluc), 74.7 (C4), 74.7 (CH_2Bn), 74.1 (C2 gluc), 73.9 (CH_2Bn), 73.6 (CH_2Bn), 73.2 (CH_2Bn), 66.1 (C6), 61.9 (C6 glu), 61.1 (C4 gluc), 60.5 (C5 xyl), 55.6 (epoxide), 54.1 (epoxide), 41.9 (C5). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{53}H_{59}N_3O_{13}Na$ 968.3940, found 968.3959.

6-*O*-(α-D-xylopyranosyl)-4-*O*-(4-deoxy-4-amino-β-D-glucopyranosyl)-cyclophellitol (60)

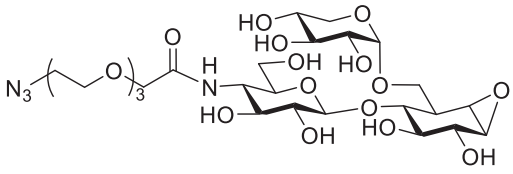


59 (42 mg, 44 µmol) was dissolved in MeCN (1.5 ml). H_2O (150 µl) and polymer bound PPh_3 (84 mg, 3 mmol/g, 250 µmol) were added and the mixture was stirred at 55°C overnight. The reaction was cooled to room temperature, filtered and the solvent was removed under reduced pressure.

Ammonia (5 ml) was condensed at -50°C. Sodium (53 mg, 2.2 mmol) was added. The crude starting material was dissolved in THF (1.5 ml) and *t*-BuOH (0.25 ml, 3.5 mmol) and was added dropwise at -60°C. After 60 minutes the reaction was quenched with AcOH (0.2 ml, 3.52 mmol). The mixture was warmed to room temperature and the ammonia was evaporated. The crude product was purified by size exclusion chromatography over HW-40 eluting with 150 mM NH_4HCO_3 in H_2O yielding the product as a white solid (19 mg, 40 µmol, 91%).

^{1}H NMR (400 MHz, D_2O) δ 4.70 (1H, obscured by HOD peak, H1 xyl), 4.23 (d, *J* = 7.9 Hz, 1H, H1 glu), 3.74 (dd, *J* = 9.5, 3.8 Hz, 1H, H6a), 3.71 – 3.12 (m, 15H), 3.05 (d, *J* = 3.8 Hz, 1H, epoxide), 3.00 (t, *J* =f 10.0 Hz, 1H, H4 glu), 2.36 – 2.26 (m, 1H, H5). ^{13}C NMR (101 MHz, D_2O) δ = 102.7 (C1 glu), 98.3 (C1 xyl), 77.8, 74.2, 73.4, 73.0, 72.3, 71.7, 71.2, 70.6, 69.2, 65.6 (C6), 61.3, 60.2, 56.2 (epoxide), 56.2 (epoxide), 52.1 (C4 glu), 40.7 (C5). $^1J_{H,C}$ 4.23 ppm, 98.3 ppm = 171 Hz, $^1J_{H,C}$ 4.70 ppm, 102.7 ppm = 162 Hz. HRMS (ESI) m/z: $[M+H_3O]^+$ calculated for $C_{18}H_{34}NO_{14}$ 488.1974 found 488.1978.

GX azide probe (61)

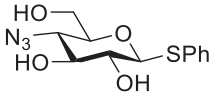


60 (19 mg, 40 µmol) was dissolved in DMF (0.5 ml, 0.1 M) and added to PFP ester **S5** (24 mg, 61 µmol). Et_3N (1 drop) was added and the reaction was stirred overnight. LC-MS indicated full conversion and the product was purified on semi-preparative HPLC eluting with a linear gradient of solution A (MeCN) in solution B (50mM AcOH in H_2O). The fractions were concentrated under reduced pressure, diluted with water and lyophilized to yield the product as a white solid (4.3 mg, 5.6 µmol 14%).

^{1}H NMR (850 MHz, D_2O/MeOD) δ 4.93 (d, *J* = 3.7 Hz, 1H, H1 xyl), 4.41 (d, *J* = 7.9 Hz, 1H, H1 glu), 4.11 (s, 2H, OC**H$_2$**C=ON), 4.02 – 3.99 (m, 1H, H6a), 3.92 (t, *J* = 9.3 Hz, 1H, H6b), 3.86 – 3.82 (m, 2H, H2/H4 glu), 3.77 – 3.71 (m, 10H, TEG), 3.71 – 3.63 (m, 5H, H6a glu/H5a xyl/epoxide), 3.62 – 3.55 (m, 3H), 3.54 – 3.49 (m, 5H, H3/H2 xyl/CH_2N_3), 3.42 (t, *J* = 9.9 Hz, 1H, H4), 3.39 – 3.36 (m, 1H, H2 glu), 3.28 (d, *J* = 3.9 Hz, 1H, epoxide), 2.57 – 2.54 (m, 1H, H5). ^{13}C NMR (214 MHz, D_2O/MeOD) δ 174.0 (amide), 104.1 (C1 glu), 99.6 (C1 xyl), 79.1 (C4), 75.9, 75.6, 74.8 (C2 glu), 74.3, 74.1, 72.5 (C2 xyl), 71.8 (C2), 71.3 (TEG), 70.7 (TEG), 70.6 (TEG), 70.5 (TEG), 70.5, 70.3 (TEG), 66.8, 62.4, 61.7, 57.3 (epoxide), 57.2 (epoxide), 52.2 (C4 glu), 51.2 (CH_2N_3), 42.0 (C5). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{26}H_{45}N_4O_{17}$ 685.2774 found 685.2771.

XG probes

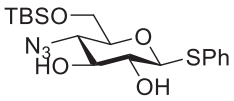
Phenyl 4-deoxy-4-azido-1-thio-β-D-glucopyranoside (S13)



Benzoyl protected building block **42** (1.98 g, 3.25 mmol) was dissolved in MeOH (32 ml) and DCM (7.5 ml). NaOMe (0.12 ml, 5.4 M, 0.65 mmol) was added and the mixture was stirred for 20 hours. The reaction was neutralized with AcOH and dry loaded in silica. Silica with the adsorbed material was loaded on a silica column and eluted (pentane/EtOAc, 7/3 → 6/4, v/v) to provide the product as a white solid (790 mg, 2.63 mmol, 81%).

^{1}H NMR (400 MHz, MeOD) δ 7.56 – 7.52 (m, 2H), 7.33 – 7.22 (m, 3H), 4.59 (d, *J* = 9.8 Hz, 1H, H1), 3.80 (dd, *J* = 12.3, 2.1 Hz, 1H, H6a), 3.69 (dd, *J* = 12.3, 4.5 Hz, 1H, H6b), 3.53 (dd, *J* = 9.6, 8.6 Hz, 1H, H3), 3.41 (t, *J* = 9.8 Hz, 1H, H4), 3.29 – 3.20 (m, 2H, H2/H5). ^{13}C NMR (101 MHz, MeOD) δ 135.0, 132.8, 129.9, 128.4, 89.4 (C1), 80.1 (C5), 78.9 (C3), 73.8 (C2), 63.1 (C4), 62.6 (C6).

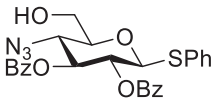
Phenyl 6-*O*-tert-butyldimethylsilyl-4-deoxy-4-azido-1-thio-β-D-glucopyranoside (S14)



Triol **S13** (780 mg, 2.62 mmol) was dissolved in DMF (13 ml, 0.2 M). Imidazole (267 mg, 3.93 mmol) and TBSCl (475 mg, 3.15 mmol) were added and the mixture was stirred overnight. The reaction was diluted with water and extracted with EtOAc (3x) the combine organic layers were washed with water (3x) and brine, dried over $MgSO_4$ and concentrated under reduced pressure yielding a viscous oil (1.17 g) that was used and analyzed without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 – 7.57 (m, 2H, SPh), 7.35 – 7.30 (m, 3H, SPh), 4.50 (d, *J* = 9.7 Hz, 1H, H1), 3.96 (dd, *J* = 11.6, 1.7 Hz, 1H, H6a), 3.88 (dd, *J* = 11.6, 3.9 Hz, 1H, H6b), 3.68 – 3.53 (m, 2H, H3/H4), 3.40 (dd, *J* = 9.7, 8.5 Hz, 1H, H2), 3.29 – 3.24 (m, 1H, H5), 0.98 (s, 9H, *t*-Bu), 0.16 (s, 3H, CH_3Si), 0.15 (s, 3H, CH_3Si). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 132.9, 131.6, 129.0, 128.2, 87.4 (C1), 79.4 (C5), 76.7 (C3), 72.0 (C2), 62.7 (C6), 61.3 (C4), 25.9 (*t*-Bu), 18.4 (*t*-Bu), -5.2 (Me), -5.4 (Me). HRMS (ESI) m/z: $[M+NH_4]^+$ calculated for $C_{18}H_{33}N_4O_4SSi$ 429.19863, found 429.19861.

Phenyl 2,3-*O*-di-benzoyl-4-deoxy-4-azido-1-thio-β-D-glucopyranoside (62)

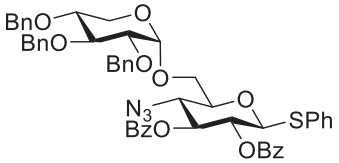


Crude diol **S14** (2.62 mmol) was dissolved in DCM (26 ml, 0.1 M). Pyridine (1.06 ml, 13.1 mmol) and BzCl (0.76 ml, 6.55 mmol) were added and the mixture was stirred overnight. The reaction was quenched with water, poured over $NaHCO_3$ (aq. sat.) and extracted with DCM. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure.

The crude product was dissolved in THF (21 ml, 0.1M), TBAF (5.24 ml, 1 M in THF, 5.24 mmol) was added and the mixture was stirred for 22 hours. The reaction was poured over $NaHCO_3$ (aq. sat.). The mixture was extracted with EtOAc (2x). The combined organic layers were washed with brine (2x), dried over $MgSO_4$ and the volatiles were removed under reduced pressure. The pure product was obtained after chromatography (pentane/Et_2O, 8/2 → 65/35, v/v) as a colorless oil (1.05 g, 2.07 mmol, 79% over 3 steps).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 – 7.89 (m, 4H), 7.56 – 7.48 (m, 2H), 7.48 – 7.43 (m, 2H), 7.42 – 7.34 (m, 4H), 7.34 – 7.28 (m, 3H), 5.66 (t, *J* = 9.7 Hz, 1H, H3), 5.36 (t, *J* = 9.7 Hz, 1H, H2), 4.97 (d, *J* = 10.0 Hz, 1H, H1), 4.03 (dd, *J* = 12.4, 2.3 Hz, 1H, H6a), 3.95 (t, *J* = 10.1 Hz, 1H, H4), 3.86 (dd, *J* = 12.4, 4.1 Hz, 1H, H6b), 3.53 (ddd, *J* = 10.3, 4.1, 2.4 Hz, 1H, H5). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 165.8, 165.3, 133.6, 133.5, 133.0, 131.9, 130.0, 130.0, 129.2, 129.1, 128.8, 128.6, 128.6, 86.3 (C1), 78.8 (C5), 75.0 (C3), 70.6 (C2), 62.0 (C6), 59.9 (C4). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{26}H_{23}N_3O_6SNa$ 528.11998, found 528.12004.

Phenyl 6-*O*-(2,3,4-tri-*O*-benzyl-α-D-xylopyranosyl)-2,3-*O*-di-benzoyl-4-deoxy-4-azido-1-thio-β-D-glucopyranoside (63)



Acceptor **62** (100 mg, 0.197 mmol) was co-evaporated with toluene (3x). DCM (2 ml), DIPEA (86 µl, 0.493 mmol), $OPPh_3$ (274 mg, 0.985 mmol) and 3Å molecular sieves were added and the mixture was stirred for 1 hour at room temperature.

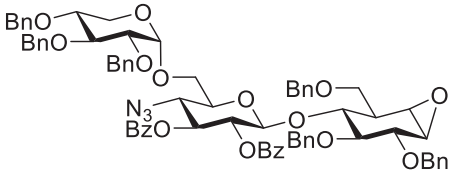
Acetate donor **56**[48] (182 mg, 0.394 mmol) was co-evaporated with toluene (3x). The dry residue was dissolved in DCM (1 ml) followed by the addition of TMSI (59 µl, 0.414 mmol) at room temperature. The mixture was stirred for 30 minutes and turned deep red. The mixture was co-evaporated with toluene (2x), dissolved in DCM (0.5 ml) and added to the reaction flask containing the acceptor. Upon addition the color faded immediately and the reaction was stirred overnight.

The reaction was quenched by the addition of water. The mixture was filtered over celite and diluted with EtOAc. The layers were separated and the organic layer was washed with , $NaHCO_3$ (aq. sat.) and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The product was isolated after column chromatography (pentane/Et_2O, 8/2, v/v) (125 mg, 0.138 mmol, 70%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 – 7.92 (m, 2H), 7.88 – 7.83 (m, 2H), 7.56 – 7.48 (m, 4H), 7.44 – 7.21 (m, 22H), 5.59 (t, *J* = 9.7 Hz, 1H, H3), 5.32 (t, *J* = 9.7 Hz, 1H, H2), 4.96 – 4.87 (m, 3H, H1', CH_2Bn), 4.85

(d, *J* = 10.0 Hz, 1H, H1), 4.75 (m, 2H, CH_2Bn), 4.66 – 4.61 (m, 2H, CH_2Bn), 3.99 – 3.85 (m, 4H, H4/H6ab/H3'), 3.71 – 3.55 (m, 4H, H5ab'/H4'/H5), 3.49 (dd, *J* = 9.6, 3.5 Hz, 1H, H2'). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 165.8, 165.3, 139.1, 138.5, 138.3, 133.9, 133.6, 133.5, 131.7, 130.1, 130.0, 129.3, 129.2, 128.9, 128.7, 128.6, 128.5, 128.5, 128.2, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.7, 97.5 (C1'), 86.5 (C1), 81.2 (C3'), 80.1 (C2'), 78.8, 78.2(C4'/C5), 75.9 (CH_2Bn), 75.0 (C3), 73.6 (CH_2Bn), 73.4 (CH_2Bn), 70.7 (C2), 66.0 (C6), 60.4 (C5' and C4 weak). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{52}H_{49}N_3O_{10}SNa$ 930.3063, found 930.3023.

4-*O*-(6-*O*-(2,3,4-tri-*O*-benzyl-α-D-xylopyranosyl)-2,3-*O*-di-benzoyl-4-deoxy-4-azido-β-D-glucopyranosyl)-2,3,6-tri-*O*-benzyl-cyclophellitol (20)

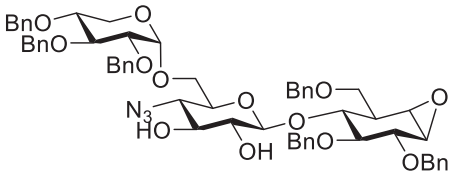


Donor **63** (108 mg, 0.119 mmol), Ph_2SO (33 mg, 0.162 mmol) and TTBP (134 mg, 0.54 mmol) were co-evaporated with toluene (2x). DCM (1.2 ml) and 3 Å molecular sieves were added and the mixture was stirred for 30 minutes at room temperature. The mixture was cooled to -70°C and Tf_2O (0.25 ml, 0.59 M in DCM, 0.148 mmol) was added and the mixture was allowed to warm to -40°C within 30 minutes. The mixture was cooled again to -70°C and acceptor **25** (48 mg, 0.108 mmol) dissolved in DCM (0.5 ml) was added.

The mixture was slowly warm to room temperature overnight, diluted with DCM and poured over brine. The layers were separated and the organic layer was dried over $MgSO_4$. The product was obtained after chromatography (Pentane/EtOAc, 9/1 -> 8/2, v/v) (85 mg, 0.068 mmol, 63%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 – 7.89 (m, 2H), 7.88 – 7.84 (m, 2H), 7.52 – 7.46 (m, 2H), 7.44 – 7.17 (m, 36H), 5.43 (t, *J* = 9.9 Hz, 1H, H3'), 5.30 (dd, *J* = 9.8, 7.9 Hz, 1H, H2'), 4.97 (d, *J* = 12.3 Hz, 1H, CH_2Bn), 4.91 (d, *J* = 3.5 Hz, 1H, H1"), 4.89 – 4.81 (m, 3H, CH_2Bn), 4.80 (d, *J* = 8.0 Hz, 1H, H1'), 4.73 (d, *J* = 11.8 Hz, 1H, CH_2Bn)), 4.65 – 4.57 (m, 4H, CH_2Bn)), 4.55 (d, *J* = 11.4 Hz, 1H, CH_2Bn), 4.32 (d, *J* = 11.8 Hz, 1H, CH_2Bn), 4.15 (d, *J* = 11.8 Hz, 1H, CH_2Bn), 3.94 (t, *J* = 10.1 Hz, 1H, H4'), 3.92 – 3.87 (m, 1H, H3"), 3.86 (d, *J* = 7.4 Hz, 1H, H2), 3.69 (t, *J* = 9.8 Hz, 1H, H4), 3.62 (m, 2H, H6a/H6a'), 3.59 – 3.47 (m, 5H, H5ab"/H6b'/H4"/H3), 3.43 (t, *J* = 8.5 Hz, 1H, H6b), 3.37 – 3.31 (m, 2H, H2"/epoxide), 3.17 (ddd, *J* = 10.2, 3.9, 1.6 Hz, 1H, H5'), 3.09 (d, *J* = 3.7 Hz, 1H, epoxide), 2.18 (dddd, *J* = 9.8, 8.2, 3.5, 1.4 Hz, 1H, H5). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 165.8, 165.2, 139.4, 139.0, 138.6, 138.5, 138.2, 137.7, 133.5, 133.4, 130.0, 129.9, 129.3, 129.0, 128.6, 128.6, 128.5, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.1, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.6, 127.3, 126.7, 101.6 (C1'), 97.7 (C1"), 83.3 (C3), 81.3 (C3"), 79.9 (C2"), 79.6 (C2), 78.1 (C4"), 75.9 (C4), 75.7 (CH_2Bn), 74.9 (C5'), 74.2 (CH_2Bn), 74.0 (C3'), 73.4 (CH_2Bn), 73.2 (CH_2Bn), 73.1 (C2'), 73.1 (CH_2Bn), 73.0 (CH_2Bn), 68.6 (C6), 65.5 (C6'), 60.4 (C4'/C5" weak), 55.5 (epoxide), 53.3 (epoxide), 42.0 (C5). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{74}H_{73}N_3O_{15}Na$ 1266.4934, found 1266.4942.

4-*O*-(6-*O*-(2,3,4-tri-*O*-benzyl-α-D-xylopyranosyl)-4-deoxy-4-azido-β-D-glucopyranosyl)-2,3,6-tri-*O*-benzyl-cyclophellitol (64)

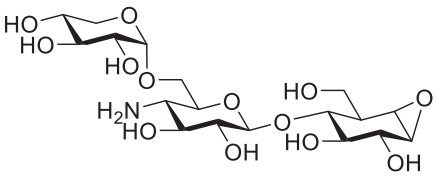


20 (57 mg, 68 µmol) was dissolved in MeOH/DCM (1 ml, 1/1, v/v). NaOMe (10 µl, 5.4M, 42 µmol) was added and the mixture was stirred overnight. NH_4Cl was added and the solvent was evaporated under reduced pressure. Column chromatography (DCM/EtOAc, 1/0 -> 9/1, v/v) provided the product (57 mg, 55 µmol, 81%).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.36 – 7.21 (m, 30H), 4.86 (d, *J*=11.7, 1H, CH_2Bn), 4.84 – 4.78 (m, 3H, CH_2Bn/H1"), 4.76 (d, *J*=10.9, 1H, CH_2Bn), 4.70 (d, *J*=11.8, 1H, CH_2Bn), 4.68 – 4.55 (m, 7H, CH_2Bn), 4.41 (d, *J*=7.8, 1H, H1'), 3.90 – 3.77 (m, 5H, H4/H3"/H6ab/), 3.61 – 3.46 (m, 6H), 3.44 – 3.37 (m, 2H), 3.35 (dd, *J*=9.6, 3.5, 1H, H2"), 3.30 – 3.28 (m, 1H, epoxide), 3.15 – 3.09 (m, 2H, H2', epoxide), 3.00 (ddd, *J*=10.1, 4.2, 1.5, 1H), 2.33 – 2.26 (m, 1H, H5). ^{13}C NMR (126 MHz, $CDCl_3$) δ 139.0, 138.8, 138.7, 138.6, 137.5, 128.6, 128.6, 128.5, 128.5, 128.5, 128.5, 128.4, 128.1, 128.1, 128.0, 128.0, 127.8, 127.7, 127.7, 127.6, 127.5, 127.1, 102.9 (H1'), 97.7 (H1"), 83.5, 81.3, 79.8 (H2"), 79.7, 78.1, 75.7, 75.5, 75.0, 74.7,

74.3, 74.2, 73.4, 73.0, 72.7, 69.3, 66.2, 61.2, 60.3, 56.1 (epoxide), 53.1 (epoxide), 41.9 (C5). HRMS (ESI) m/z: $[M+NH_4]^+$ calculated for $C_{60}H_{69}N_4O_{13}$ 1053.4856, found 1053.4848.

4-*O*-(6-*O*-(α-D-xylopyranosyl)-4-deoxy-4-amino-β-D-glucopyranosyl)-cyclophellitol (65)

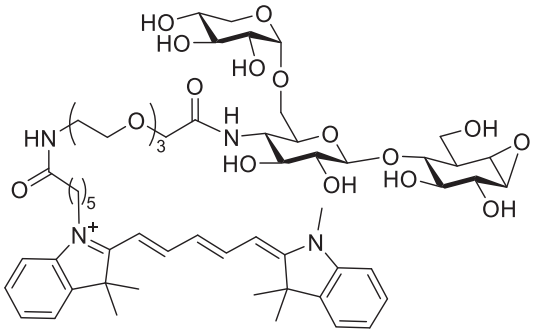


Ammonia (8 ml) was condensed at -50°C. Sodium (77 mg, 3.4 mmol) was added. Starting material **64** (58 mg, 0.056 mmol) was dissolved in THF (2 ml) and *t*-BuOH (0.37 ml, 3.9 mmol) and the mixture was added dropwise at – 65°C. After 40 minutes the reaction was quenched with NH_4Cl (195 mg, 3.6 mmol). The mixture was warmed to room temperature and the ammonia was evaporated.

The crude product was purified by size exclusion chromatography over HW-40 eluting with 1% AcOH in H_2O yielding the product as a white solid (20 mg, 0.043 mmol, 76%).

^{1}H NMR (400 MHz, MeOD) δ 4.84 (d, *J* = 3.7 Hz, 1Hn H1xyl), 4.38 (d, *J* = 7.9 Hz, 1H, H1gluc), 4.11 (dd, *J* = 10.9, 4.1 Hz, 1H, H6a), 3.94 – 3.62 (m, 5H), 3.61 – 3.35 (m, 9H), 3.30 – 3.26 (m, 1H, H2gluc), 3.06 (d, *J* = 3.6 Hz, 1H, epoxide), 2.89 (t, *J* = 9.8 Hz, 1H, H4gluc), 2.23 – 2.16 (m, 1H, H5). ^{13}C NMR (101 MHz, MeOD) δ 105.2 (C1xyl), 100.9 (C1gluc), 81.5, 76.8, 75.6, 75.3, 75.0, 73.7, 73.5, 72.9, 71.4, 69.3, 63.4, 62.0 (C6), 56.7 (epoxide), 56.5 (epoxide), 56.0 (C4gluc), 44.9 (C5). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{18}H_{32}NO_{13}$ 470.1868, found 470.1871.

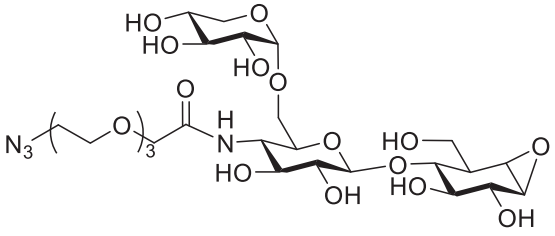
XG Cy5 probe (66)



Amine **65** (4.4 mg, 9.4 μmol) was reacted with stock solution TEG Cy5 **S12** (0.24 ml) according to general procedure B (2.64 mg, 2.2 μmol, 23%).

^{1}H NMR (600 MHz, MeOD) δ = 8.30 – 8.21 (m, 2H), 7.50 (dt, *J*=7.5, 1.5, 2H), 7.44 – 7.39 (m, 2H), 7.32 – 7.23 (m, 4H), 6.64 (t, *J*=12.4, 1H), 6.29 (dd, *J*=13.7, 4.9, 2H), 4.73 (d, *J*=3.6, 1H, H1xyl), 4.32 (d, *J*=8.0, 1H, H1gluc), 4.14 – 4.08 (m, 3H), 4.03 (d, *J*=1.7, 2H), 3.82 (dd, *J*=10.9, 7.2, 1H), 3.79 – 3.58 (m, 16H), 3.57 – 3.46 (m, 5H), 3.45 – 3.39 (m, 2H), 3.39 – 3.32 (m, 7H), 3.05 (d, *J*=3.7, 1H, epoxide), 2.24 (t, *J*=7.4, 2H), 2.22 – 2.17 (m, 1H, H5), 1.86 – 1.80 (m, 2H), 1.76 – 1.66 (m, 14H), 1.51 – 1.44 (m, 2H). ^{13}C NMR (151 MHz, MeOD) δ 176.0, 175.4, 174.7, 173.5, 144.3, 143.6, 142.7, 142.5, 129.8, 129.7, 126.6, 126.3, 123.4, 123.3, 112.1, 111.8, 105.6, 104.4 (C1gluc), 104.3, 100.0 (C1xyl), 82.7, 77.1, 75.7, 75.1, 74.9, 74.8, 73.9, 72.8, 71.8, 71.7, 71.4, 71.4, 71.2, 71.1, 71.1, 70.5, 68.0, 63.2, 62.4, 56.6 (epoxide), 56.5 (epoxide), 53.3, 50.6, 50.5, 49.8, 49.6, 44.9 (C5), 44.8, 40.3, 36.7, 31.5, 28.2, 28.0, 27.8, 27.4, 26.5. HRMS (ESI) m/z: $[M]^+$ calculated for $C_{58}H_{83}N_4O_{18}$ 1123.5697 found 1123.5690.

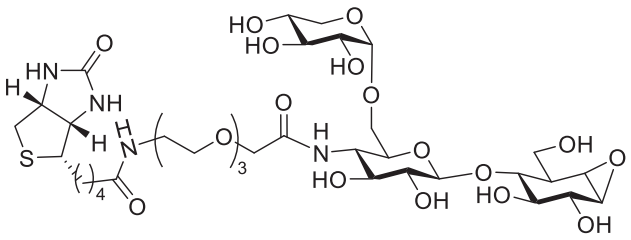
XG azide probe (67)



Amine **65** (4.4 mg, 9.4 μmol) was reacted with stock solution TEG azide **S4** (0.24 ml) according to general procedure B (1.74 mg, 2.54 μmol, 27%).

^{1}H NMR (850 MHz, MeOD) δ 4.74 (d, *J* = 3.6 Hz, 1H, H1xyl), 4.32 (d, *J* = 8.0 Hz, 1H, H1gluc), 4.12 (dd, *J* = 10.9, 4.5 Hz, 1H), 4.03 (d, *J* = 1.9 Hz, 2H), 3.83 (dd, *J* = 10.9, 7.2 Hz, 1H), 3.81 – 3.77 (m, 1H), 3.77 – 3.74 (m, 2H), 3.74 – 3.67 (m, 11H), 3.59 (t, *J* = 9.4 Hz, 1H), 3.57 – 3.52 (m, 2H), 3.49 (t, *J* = 10.7 Hz, 1H), 3.45 – 3.40 (m, 4H), 3.40 – 3.37 (m, 1H), 3.36 – 3.32 (m, 2H), 3.06 (d, *J* = 3.6 Hz, 1H, epoxide), 2.23 – 2.19 (m, 1H, H5). ^{13}C NMR (214 MHz, MeOD) δ 173.5, 105.5 (C1gluc), 100.0 (C1xyl), 82.7, 77.1, 75.6, 75.1, 74.9, 74.8, 73.9, 72.8, 71.9, 71.7, 71.5, 71.4, 71.4, 71.2, 71.0, 68.0, 63.2, 62.4, 56.6 (epoxide), 56.5 (epoxide), 53.3, 51.8, 44.9 (C5). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{26}H_{45}N_4O_{17}$ 685.2774 found 685.2768.

XG biotin probe (68)



Amine **65** (4.4 mg, 9.4 μmol) was reacted with stock solution TEG biotin **S8** (0.24 ml) according to general procedure B (2.14 mg, 2,44 μmol, 26%).

^{1}H NMR (850 MHz, MeOD) δ 4.74 (d, *J* = 3.6 Hz, 1H, H1xyl), 4.50 (ddd, *J* = 7.9, 5.0, 0.9 Hz, 1H), 4.34 – 4.29 (m, 2H, H1gluc), 4.12 (dd, *J* = 10.9, 4.5 Hz, 1H, H6a), 4.05 (d, *J* = 2.7 Hz, 2H), 3.84 (dd, *J* = 10.9, 7.1 Hz, 1H, H6b), 3.81 – 3.78 (m, 1H), 3.77 – 3.71 (m, 4H), 3.71 – 3.67 (m, 6H), 3.67 – 3.64 (m, 2H), 3.61 (t, *J* = 9.4 Hz, 1H), 3.58 – 3.52 (m, 4H), 3.50 (t, *J* = 10.7 Hz, 1H), 3.46 – 3.41 (m, 2H, epoxide), 3.41 – 3.37 (m, 3H), 3.36 – 3.34 (m, 2H), 3.34 – 3.32 (m, 2H), 3.24 – 3.20 (m, 1H), 3.06 (d, *J* = 3.6 Hz, 1H, epoxide), 2.93 (dd, *J* = 12.8, 5.0 Hz, 1H), 2.71 (d, *J* = 12.7 Hz, 1H), 2.27 – 2.19 (m, 3H, H5), 1.78 – 1.57 (m, 4H), 1.48 – 1.42 (m, 2H). ^{13}C NMR (214 MHz, MeOD) δ 176.2, 173.5, 105.5 (C1gluc), 100.0 (C1xyl), 82.7, 77.1, 75.7, 75.1, 74.9, 74.8, 73.9, 72.8, 71.8, 71.7, 71.4, 71.4, 71.2, 71.1, 70.6, 68.0, 63.4, 63.2, 62.4 (C6), 61.6, 57.0, 56.7, 56.5 (epoxide), 53.3 (epoxide), 44.9 (C5), 41.0, 40.3, 36.8, 29.8, 29.5, 26.9. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{31}H_{53}N_4O_{15}S$ 753.3223 found 753.3219.

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