Inhibitors and probes targeting endo-glycosidases

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Mechanism-based heparanase inhibitors and activity-based probes

3

3.1 Introduction

Human heparanase (HPSE), an endo-glycosidase acting on heparan sulfate polysaccharides, is implicated in various diseases and has therefore become an intensively studied potential drug target. In this chapter the chemical synthesis of covalent inhibitors and activity-based probes targeting HPSE is described.

Heparan sulfate biosynthesis and function

The main substrate of HPSE is heparan sulfate (HS, Figure 3.1), a heterogeneously decorated glycosaminoglycan synthesized by virtually all cell types in the body.[1] Its structure and biosynthesis is closely related to heparin which is only produced in mast cells. The polysaccharides are synthesized on core proteins in the Golgi apparatus by glycosyl transfer of D-glucuronic acid (GlcA) and N-acetyl-D-glucosamine (GlcNAc) residues. The growing chain

R^1 = Ac, SO$_3^-$ or H
R^2 = SO$_3^-$ or H
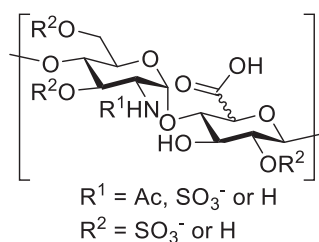
Figure 3.1│General structure of heparan sulfate.

is enzymatically modified, starting with *N*-deacetylation followed by *N*-sulfation by bifunctional *N*-deacetylase/*N*-sulfotransferases (NDST) giving rise to *N*-sulfated (NS) domains. Parts of the chain remain *N*-acetylated (NA domains) and parts become mixed (NA/NS domains). The most common modifications are 2*O*-, 3*O*- and 6*O*-sulfation of GlcNAc/GlcNS, 2*O*-sulfation of GlcA and 5*C*-epimerization of GlcA to L-iduronic acid (IdoA).[2] The complex interplay between the substrate specificities, activities and expression levels of the involved enzymes and the availability of uridine diphosphate (UDP) carbohydrate building blocks and a sulfate donor leads to variable but nonrandom modification of the constructed HS proteoglycan (PG).[3] Heparin consists for almost 90% of NS domains while for HS this is only 60%.[3] Since subsequent modifications mainly occur in NS domains heparin contains mostly IdoA and is densely sulfated while HS contains mostly GlcA and is only moderately sulfated.

The synthesized HSPGs are transported to the cell surface or released into the extracellular matrix (ECM) where they influence the activity and availability of many proteins and small molecules, either by specific sequence recognition or charge interaction.[4] HSPGs also have an important structural function in the ECM where they facilitate intercellular signaling and restrict cell mobility.[5] In their review on the physiological roles of HSPGs, Bishop *et al.* concluded: *'HSPGs interact with so many factors; one would expect few physiological systems to remain unaffected by changes in their composition.'* [6]

Heparanase

The only mammalian glycosidase known to extracellularly modify the composition of HS is heparanase (HPSE). HPSE is a retaining endo-β-D-glucuronidase belonging to GH family 79. The enzyme catalyzes the hydrolysis of the HS polysaccharide into smaller fragments. The protein is expressed as a pre-proenzyme.[7] Cleavage of the signal peptide yields proHPSE which has a 6 kDa 'exo-pocket' loop covering most of the active site cleft. This loop is also found in variable size in other GH79 glucuronidases.[8] The active site residues remain accessible in an exo-

glycosidase like pocket.[9] ProHPSE is secreted and can be endocytosed via multiple receptors on the same or on neighboring cells. After endocytosis the 'exo-pocket' loop is removed by cathepsin L in the lysosome yielding active HPSE as a heterodimer containing an 8 kDa and a 50 kDa subunit. The two catalytic residues are located in a cleft on the large subunit flanked by two HS binding domains (HBDs). The active enzyme is thus present in the lysosome but has also been found in the Golgi, the nucleus and in the extracellular matrix.

Abnormal HPSE activity has been implicated in cancer progression and other pathologies such as inflammation and diabetic nephropathy.[10–12] Pathological effects of HPSE can be a result of its increased enzymatic activity, but non-enzymatic signaling properties are also implicated in some cases.

Heparanase inhibitors

The involvement of HPSE in many physiological processes in human health and disease established HPSE as an interesting therapeutic target and stimulated the generation of different classes of inhibitors of the enzyme.[13–16] The most investigated HPSE inhibitors are densely sulfated oligo- or polysaccharides of which four preparations have made it into clinical trials to date (Figure 3.2). The sulfated oligosaccharides are mixture PI-88 (mupafostat)[17] and the more potent molecule PG545 (pixatimod)[18]. The other two preparations are glycol-split
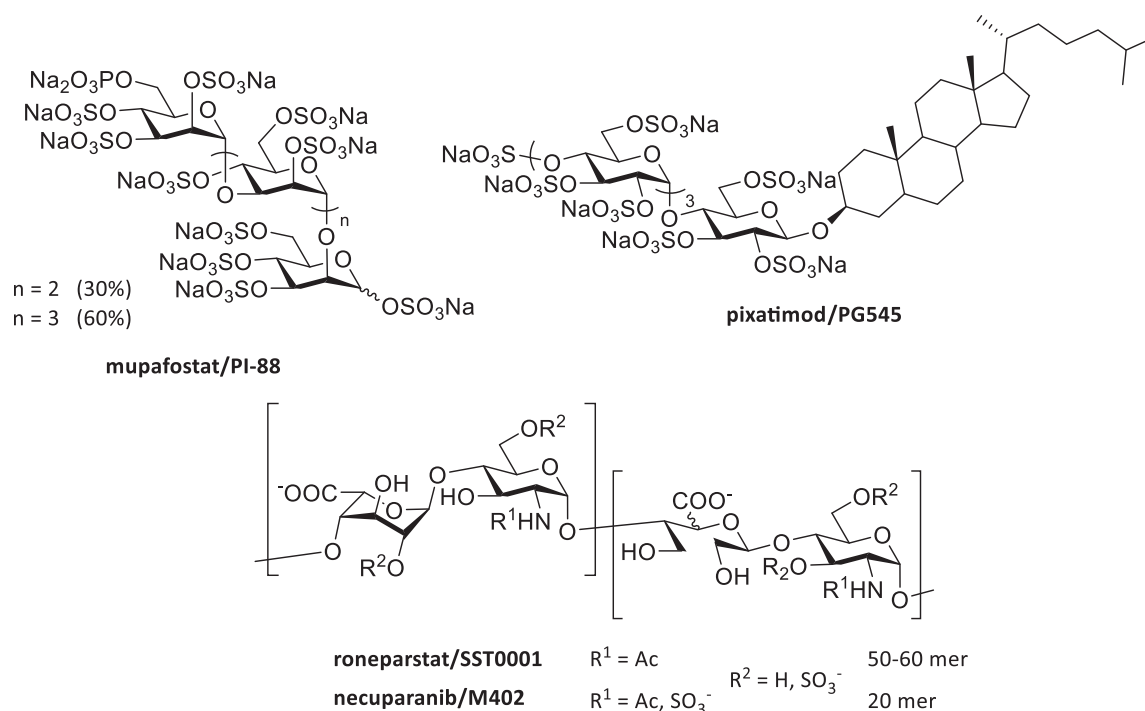
**Figure 3.2|**HPSE inhibitors examined in clinical trials.

heparin derivatives which are either N-acetylated (roneparstat/SST0001)[19] or N-sulfated (M402/necuparanib)[20]. While these compounds have all shown efficacy in pre-clinical models and an acceptable safety profile in clinical trials the efficacy in anti-cancer clinical trials has so far been moderate. A limitation in optimizing these compounds is their structural similarity to HS and heparin. This similarity makes it difficult to attribute the observed effects to inhibition of HPSE enzymatic activity, blocking of the HPSE HBDs or off-target binding to other proteins with an HBD. The high polarity of the compounds results in fast excretion necessitating high and frequent dosing.

Many small molecules with diverse structures have also been investigated for their potential as HPSE inhibitors, but the lack of a widely used, reliable and rapid inhibition assay limits the development and prohibits the quantitative comparison of the different compound classes.[15,21] Also for these compounds the mode of action, binding in the active site or elsewhere, often remains unclear. The great diversity in molecular structure suggests that the small molecules bind at various sites on the enzyme indicating multiple modes of action, a notion that is supported by molecular docking studies.[22] So far no small molecule has reached the clinic.

Mechanism-based heparanase inhibitors and ABPs

Glucuronic acid configured cyclophellitol aziridine **1** was recently published as an activity-based probe (ABP) targeting retaining β-glucuronidases (Figure 3.3).[9] LC-MS and gel-based activity-based protein profiling (ABPP) in human spleen and platelet lysates and on recombinant proteins revealed labeling of the retaining β-glucuronidases GUSB, proHPSE and HPSE. Retaining exo-β-glucosidases are reported as off-targets.[9,23]

While the labeling kinetics of **1** with exo-glucuronidase GUSB are characterized, the inhibition rate of proHPSE and HPSE is not quantified. As well, the biological significance of the
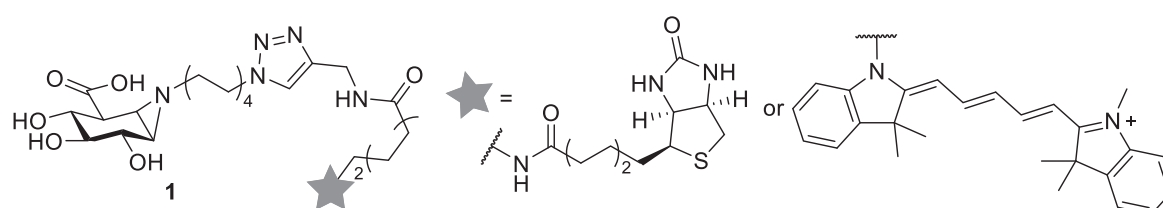

Figure 3.3| Previously reported β-D-glucuronic acid configured ABPs.[9]

reaction of proHPSE with **1** remains unclear because no enzymatic activity of proHPSE on natural substrates has been reported.

In this chapter mechanism-based inhibitors and ABPs to selectively monitor and modulate the hydrolytic activity of HPSE are described. The synthesized molecules are based on the glucuronic acid configured cyclophellitol scaffold. Selective and potent ABPs may ease the detection of low HPSE activities in various tissues and allow to better understand the role of HPSE in pathology. Activity-based protein profiling also allows the screening of HPSE inhibitors and the confirmation of HPSE inhibition *in vivo*.[24] Selective and potent mechanism-based inhibitors may allow the assessment of the therapeutic effect of the inhibition of hydrolytic activity compared to blockage of binding to HBDs. Ultimately selected inhibitors – based both on cyclophellitol-derived covalent inhibitors and compounds discovered through the application of the covalent inhibitor-based ABPs – might serve as lead compounds for the development of anti-cancer therapeutics.[25]

Extending on the results obtained with ABP, it was hypothesized that selectivity over exo-acting enzymes might be obtained by placement of the tag at the non-reducing end leading to design **2** (Scheme 3.1A). This increase in steric bulk at the non-reducing end is hypothesized to prohibit productive interaction with the pocket shaped active sites of GUSB and proHPSE,
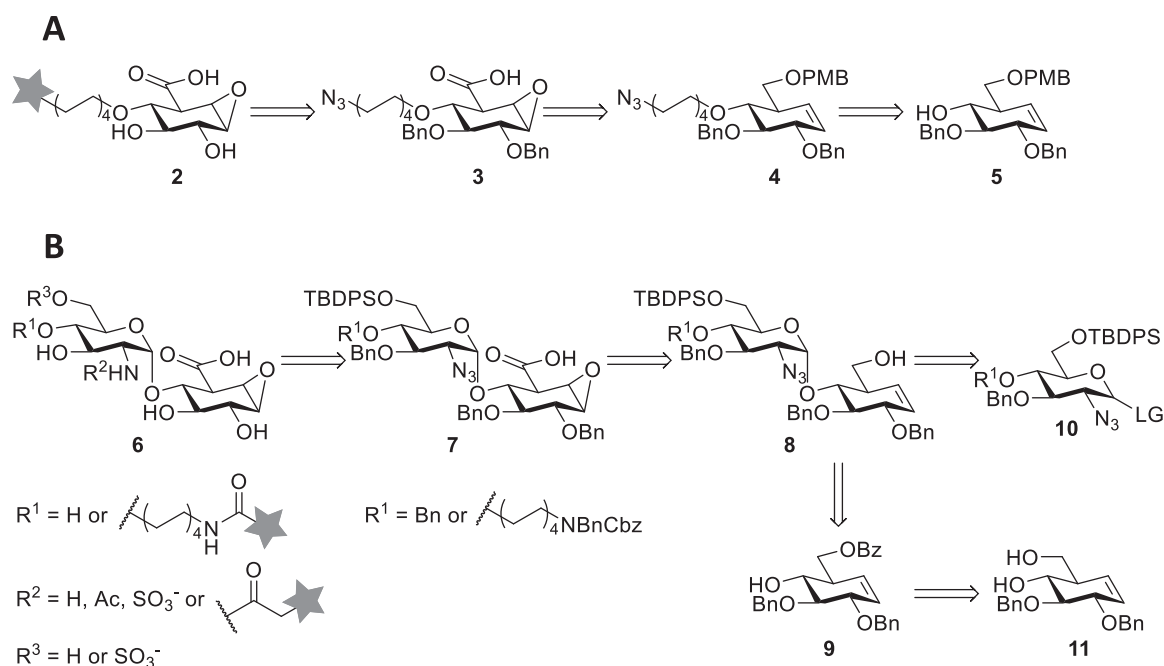
Scheme 3.1| Retrosynthetic analysis of the proposed HPSE inhibitors and probes. **A)** non-reducing end capped monosaccharide ABPs. **B)** Disaccharide mimics as inhibitors and ABPs. Stars denote various reporter groups.

while the active site cleft of mature HPSE may be able to accommodate this bulk. ABP **2** could be synthesized from protected epoxide **3** by hydrogenolysis and amide bond formation with appropriate tags. Epoxide **3** would be accessible from cyclohexene **4** by *p*-methoxybenzyl ether (PMB) removal, followed by hydrogen bond directed epoxidation and oxidation. Finally, **4** could be obtained by alkylation of solely ether protected **5**[26].

Additionally, a set of HS disaccharide cyclophellitols was designed. Analogous with other endo-glycosidase inhibitors and probes described in this thesis and elsewhere the larger recognition element is expected to increase both selectivity and potency for endo-glycosidases (**6**, Scheme 3.1B).[27,28] Orthogonal protection of specific alcohols and the 2' amine would allow the synthesis of inhibitors with a well-defined sulfation pattern. Orthogonally protected **7** may be a suitable intermediate to access selectively 6'*O*- and 2'*N*-sulfated or acylated derivatives. The 6' alcohol could be temporarily protected as a silyl ether and the 2'amine as an azide. The 2' azide, a non-participating protecting group, would also allow α-selective glycosylation. The tag was envisioned to be introduced at the end of the synthesis so the alkyl amine and the remaining alcohols were masked with benzyl ethers. The epoxide is installed post-glycosylation to allow flexibility in the glycosylation reaction conditions. The epoxide could be obtained from homoallylic alcohol **8** by stereoselective epoxidation followed by oxidation. Stereoselective glycosylation of acceptor **9** with donor **10** would afford pseudo-disaccharide **8**. Cyclohexene **9** can be obtained from previously reported diol **11**[29].

3.2   Results and discussion

In the following sections the synthesis of the monosaccharide probes and disaccharide inhibitors and probes is described. The chapter concludes with a summary of the biological evaluation of the compounds.

Monosaccharide probes

Alkylation of PMB protected cyclohexene **5**[26] with 8-azidooctyl 4-methylbenzenesulfonate[30] afforded **4** (Scheme 3.2). Removal of the temporary PMB protecting group with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) afforded homoallylic alcohol **12**, which was epoxidized in a diastereoselective reaction with meta-chloroperoxybenzoic acid (mCPBA) at 0°C (**13**). Oxidation of the primary alcohol with 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) / bis-acetoxyiodobenzene (BAIB) yielded carboxylic acid **3**. Selective hydrogenolytic benzyl removal of **3** with Pd(OH)$_2$/C proved difficult but dissolving metal reduction allowed smooth
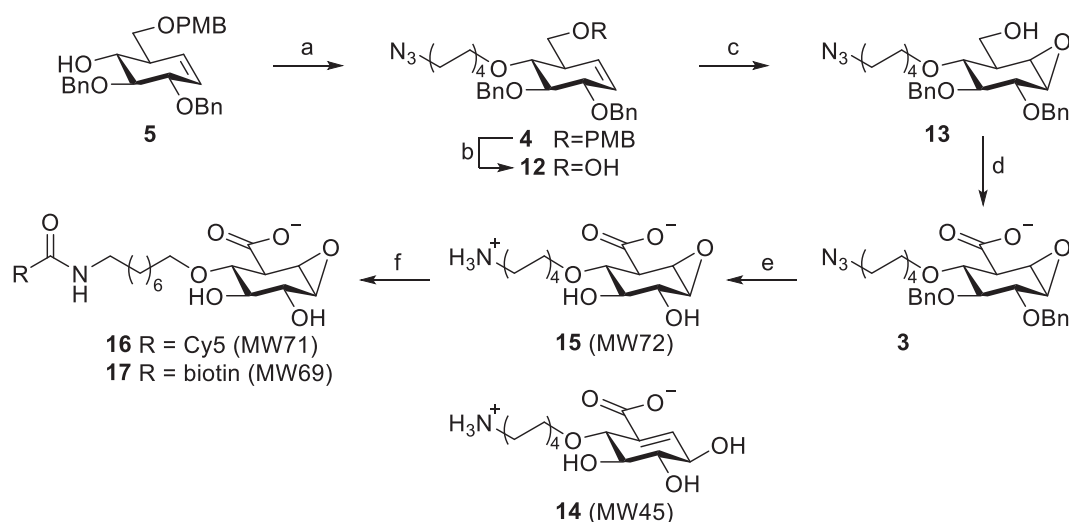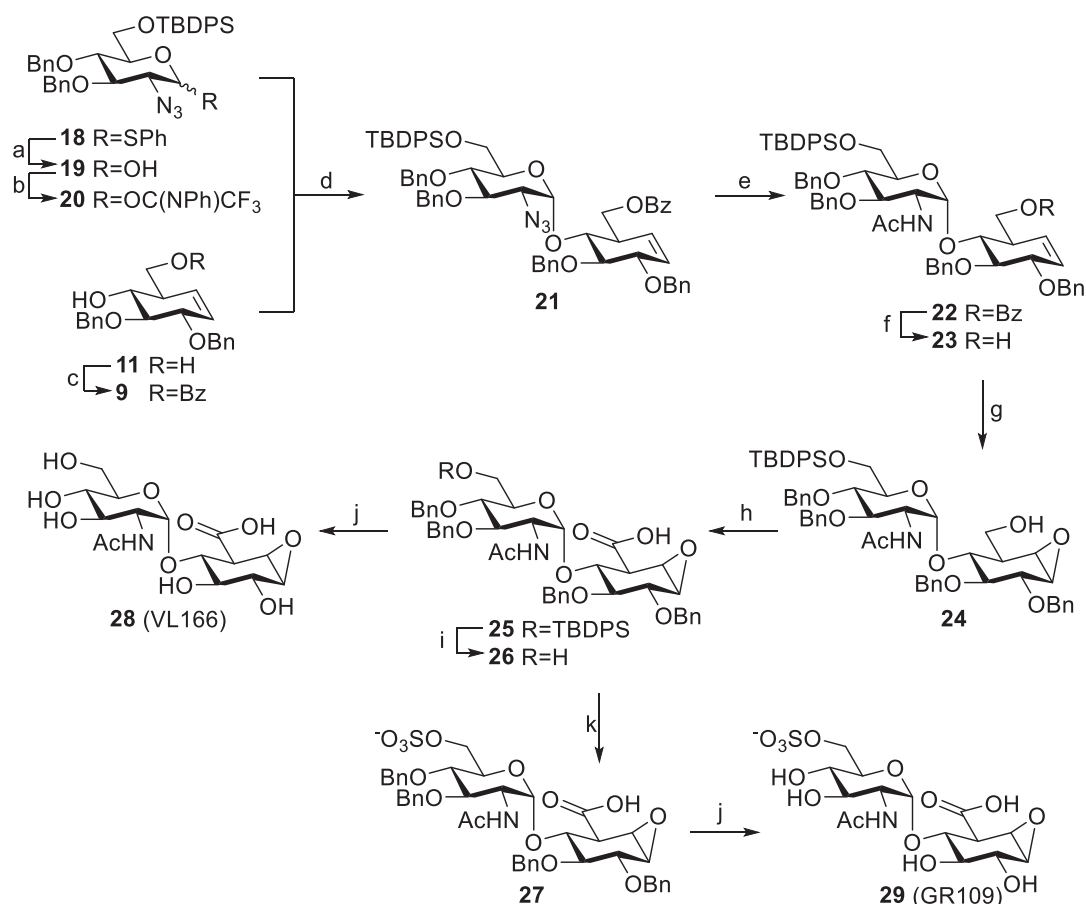
Scheme 3.2| Reagents and conditions: **a**) 8-azidooctyl 4-methylbenzenesulfonate, KHMDS, THF, 71%. **b**) DDQ, DCM, H_2O, 85%. **c**) mCPBA, DCM, 0°C, 87%. **d**) TEMPO, BAIB, *t*-BuOH, DCM, H_2O, 0°C, 90%. **e**) Na (s), NH_3, *t*-BuOH, THF, 28%. **f**) DIPEA, DMF, Cy5-NHS 14% (**16**) or biotin-NHS, 25% (**17**).

removal of the benzyl protecting groups with concomitant reduction of the azide. Quenching the reaction with NH_4Cl, followed by HPLC purification with 50 mM NH_4HCO_3 only afforded elimination product **14**. Quenching with AcOH followed HPLC purification eluting with 50 mM AcOH yielded the desired epoxide **15**. The amine was reacted with the *N*-hydroxysuccinimide activated esters of the Cy5 and biotin tags yielding probes **16** and **17** after HPLC purification.

Early stage azide reduction, sulfated and non-sulfated inhibitors

2-Azido-2-deoxy thioglucosyl donor **18**[31] was hydrolyzed to lactol **19** and converted into *N*-phenyltrifluoroacetimidate **20** (Scheme 3.3). Cyclophellitol alkene acceptor **9** was obtained by selective benzoylation of diol **11**[29,32] using mildly basic conditions. Glycosylation of these two building blocks was optimized to a protocol using a relatively high amount of triflic acid, low temperature and short reaction time. This afforded the product (**21**) in 83% up to 95% yield.

Staudinger reduction followed by acetylation of the liberated amine yielded **22**. The benzoyl ester was removed with NaOMe, setting the stage for stereoselective epoxidation of homoallylic alcohol **23**. Epoxidation with mCPBA afforded the product in a 5:1 diastereomeric mixture favoring the β-configured product (**24**). The epimeric epoxides were difficult to separate by flash column chromatography, therefore a iodocarbonylation sequence was employed to improve the stereoselectivity of the reaction.[33–38] *t*-Butyloxycarbonyl (Boc) protection of the alcohol, followed by activation of the alkene by N-iodosuccinimide (NIS) yields the iodocarbonate with complete stereospecificity. Treatment of this intermediate with
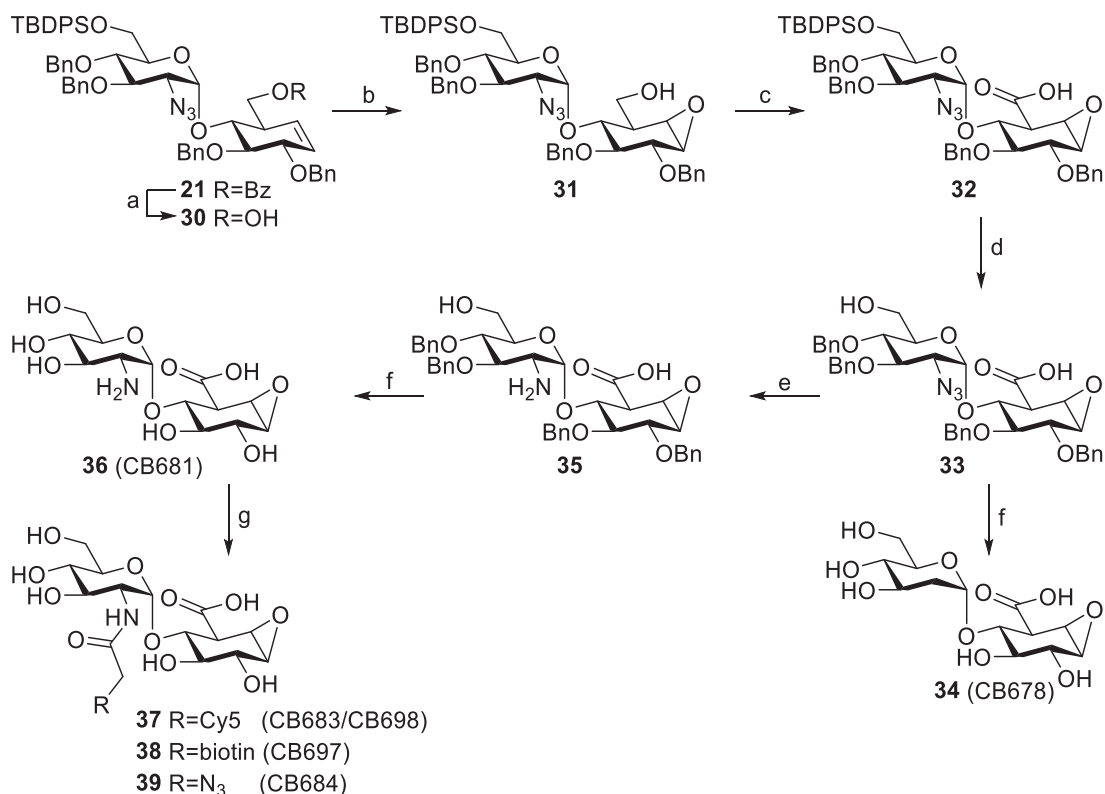
Scheme 3.3| Reagents and conditions: **a)** NIS, acetone/H_2O, 68%. **b)** *N*-phenyltrifluoroacetimidoyl chloride, Cs_2CO_3, DCM, 90%. **c)** BzCl, Et_3N, DCM, 0°C, 82%. **d)** TfOH, DCM, -78°C to -30°C, 83%. **e)** i. PPh_3, H_2O, THF; ii. Ac_2O, pyridine, 89%. **f)** NaOMe, MeOH, DCM, 97%. **g)** i. Boc_2O, DMAP, THF, 76%; ii. NIS, AcOH, DCM, 91%; iii. NaOMe, MeOH, DCM, quant. **h)** TEMPO, BAIB, DCM, *t*-BuOH, H_2O, 80%. **i)** 3HF·Et_3N, Et_3N, THF, 87%. **j)** Na(s), NH_3, *t*-BuOH, THF, 91% for **28**, 87% for **29**. **k)** SO_3·Et_3N, DMF.

base in methanol generates the epoxide with concomitant deprotection of the primary alcohol providing **24**. The primary alcohol was oxidized with TEMPO/BAIB providing carboxylic acid **25**. The silyl ether was removed with HF triethylamine complex yielding primary alcohol **26**.

Sulfation of the primary alcohol afforded sulfate **27** for which no purification was attempted due to the amphiphilic nature. Dissolving metal hydrogenolysis of **26** and **27** provided the 6'O sulfated and non-sulfated inhibitors **28** and **29** after purification by size exclusion chromatography.

Late stage azide reduction, derivatization on the C2' position

A variation to the above described route was developed to generate diversity on the 2' position (Scheme 3.4). By delaying the azide reduction to the final stage of the synthesis, ABPs with tags on the 2'amine and inhibitors without the 2'acetyl were synthesized. Debenzoylation of **21** with NaOMe provided homoallylic alcohol **30**. Epoxide formation following the same

Scheme 3.4| Reagents and conditions: **a)** NaOMe, MeOH, DCM, quant.; **b)** i. Boc$_2$O, DMAP, THF, 82%; ii. NIS, AcOH, DCM, 71%; iii. NaOMe, MeOH, DCM, 94%. **c)** TEMPO, BAIB, DCM, *t*-BuOH, H$_2$O, quant. **d)** 3HF·Et$_3$N, THF, 86%. **e)** Zn(s), NH$_4$Cl, MeOH, toluene. **f)** Na(s), NH$_3$, *t*-BuOH, THF, quant. for **34**, 51% over 2 steps for **36**. **g)** DIC, pentafluorophenol, DIPEA, DMF, Cy5-TEG-COOH or N$_3$COOH or biotin-TEG-COOH 7% **37**; 10% **38** and 12% **39**.

procedure as executed in the previous section afforded epoxide **31**. TEMPO/BAIB oxidation to carboxylic acid **32** followed by removal of the silyl ether uneventfully afforded **33**. An attempt at dissolving metal hydrogenolysis of the benzyl ethers with concomitant reduction of the azide unexpectedly afforded the 2'deoxy product **34** in quantitative yield. Although the reductive removal of an azide group has been reported as a side reaction[39], no examples of its use as a synthetic utility were found in the literature.

Azide reduction using Zn/NH$_4$Cl granted access to amine **35**.[40] Filtration over silica was used to remove most of the zinc salts and due to the poor solubility of the product no further purification was attempted. When amine **35** was subjected to dissolving metal hydrogenolysis followed by purification by size exclusion chromatography the expected product **36** was obtained. Attempts to reduce the azide in **33** by Staudinger reduction were incompatible with the epoxide and NMR analysis revealed a product containing an alkene. This product was presumably generated by attack of PPh$_3$ on the epoxide followed by elimination of PPh$_3$O resembling Wittig type alkene formation.[41] Selective acylation of **36** with Cy5 or biotin

equipped triethylene glycol (TEG) spacers (Chapter 2) afforded putative ABPs **37** and **38** after HPLC purification. Acylation with 2-azidoacetic acid provided two step ABP **39** after size exclusion chromatography.

ABPs with a 4' tag

To gain access to disaccharide probes modified at the 4'postion with a reporter entity, a 4'-alkylated donor was synthesized (Scheme 3.5). To this end, the benzylidene acetal on **40**[42] was removed and the resulting diol was regioselectively protected as the naphthyl (Nap) ether using borinate catalysis.[43] The Nap ether was chosen over the *tert*-butyldiphenylsilyl (TBDPS) ether as a base stable protecting group to facilitate smooth alkylation of the secondary alcohol in **41**. The properly protected alkyl iodide was synthesized starting with the benzyloxycarbonyl (Cbz) protection of octanol amine (**42**) yielding **43**. The remaining alcohol was tritylated (**44**) to allow selective benzylation of the carbamate (**45**). The trityl was removed (**46**) and iodination of the resulting alcohol provided iodide **47**. The iodide was reacted with secondary alcohol **41** providing fully protected **48**. Attempts to use the non-benzylated carbamate linker in the alkylation reaction were unproductive due to intramolecular cyclization of the linker.

Application of anomeric *N*-phenyltrifluoroacetimidate derivatives of **48** as a donor in glycosylation reactions with **9** showed no stereoselectivity. Attempts to gain α-selectivity by the addition of α-directing additives such as DMF and 4-formyl morpholine[44] led to diminished yields. The good results obtained using TBDPS protected donor **20** as described in the previous section prompted the synthesis of **49**. The Nap ether in **48** was removed using DDQ (**50**)
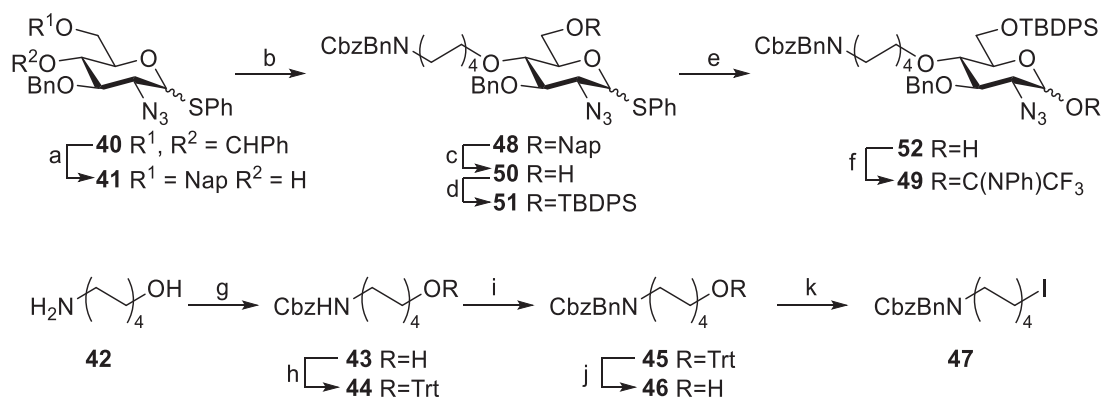
Scheme 3.5| Reagents and conditions: **a**) i. CSA, MeOH, DCE, 50°C; ii. NapBr, 2-aminoethyl diphenyl borinate, MeCN, 60°C, 93%. **b**) **47**, NaH, DMF, 88%. **c**) DDQ, DCM, MeOH, 72%. **d**) TBDPSCl, imidazole, DMF. **e**) NIS, acetone/H_2O, DCM, 79%. **f**) *N*-phenyl-trifluoroacetimidoylchloride, Cs_2CO_3, DCM, 94%. **g**) CbzCl, $NaHCO_3$, acetone/H_2O, 88%. **h**) TrtCl, Et_3N, DMF. **i**) BnBr, NaH, TBAI, DMF, 76% over 2 steps. **j**) 3% TFA/H_2O, DCM/MeOH, 84%; **k**) PPh_3, I_2, imidazole, DCM, 97%.
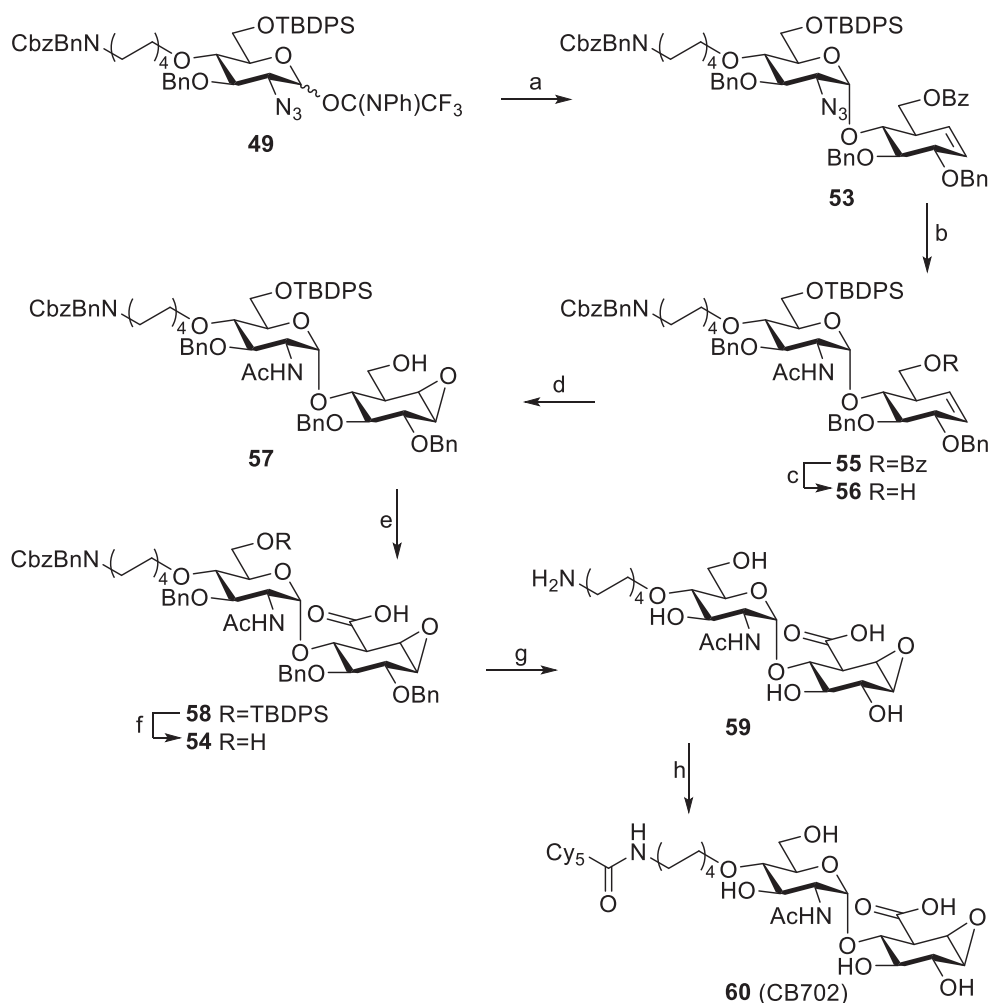
Scheme 3.6| Reagents and conditions: **a)** 9, TfOH, DCM, -78°C -> -30°C 97%. **b)** i. PPh₃, H₂O, pyridine, THF; ii. Ac₂O, pyr, DCM 98%. **c)** NaOMe, MeOH, DCM, 97%. **d)** i. Boc₂O, DMAP, THF, 82%; ii. NIS, AcOH, DCM, 68%; iii. NaOMe, MeOH, DCM, 88%. **e)** TEMPO, BAIB, *t*-BuOH, DCM, H₂O, 67%. **f)** Et₃N·3HF, THF, quant. **g)** H₂, Pd/C, H₂O, dioxane, 41%; **h)** Cy5COOH, pentafluorophenyl trifluoroacetate, DIPEA, DMF, 13%.

followed by introduction of the silyl ether (**51**). Hydrolysis of the anomeric thiophenol using NIS afforded lactol **52** which was converted to *N*-phenyltrifluoroacetimidate donor **49**.

Reaction of donor **49** with acceptor **9** provided pseudodisaccharide **53** in good yield and selectivity (Scheme 3.6). The disaccharide was elaborated into partially protected **54** using the *'Early stage azide reduction'* reaction sequence reported for the inhibitors: Staudinger reduction followed by acetylation afforded **55**. Removal of the primary benzoyl (**56**) followed by stereospecific epoxidation via a iodocarbonate intermediate afforded epoxide **57**. TEMPO/BAIB oxidation (**58**) and silyl removal delivered alcohol **54**.

Deprotection of **54** by dissolving metal hydrogenolysis did not lead to the desired product. While these conditions have been successfully applied for the deprotection of diverse cyclophellitol and cyclophellitol aziridine derivatives the contrasting result obtained in this

case is attributed to the *N*-benzyl protecting group. NMR analysis revealed the partial reduction of the *N*-benzyl group to cyclohexadiene or further reduced derivatives as indicated by the presence of non-aromatic double bonds.

Pd/C catalyzed hydrogenolysis of the CBz and benzyl groups in **54** did provide **59**. The reaction was complicated by partial reduction and nucleophilic opening of the epoxide under the reaction conditions. As well, catalyst poisoning by the emerging amine, variable activity of different batches of Pd/C catalyst, the difficulty to monitor this reaction with a multitude of different intermediates and the difficulty of dissolving starting material, intermediates and product in the same solvent made this reaction hard to optimize and reproduce. The reaction was monitored by LC-MS and the obtained mixture was purified by size exclusion (HW-40). The mixture eluted as a broad peak which was collected in three fractions. The pure fraction, based on NMR analysis, was selected for the final reaction.

Finally, Cy5COOH was activated as its pentafluorophenyl ester using pentafluorophenyl trifluoroacetate which was directly reacted with **59** to yield ABP **60** after HPLC purification.

3.3   Biological evaluation

The properties of the synthesized inhibitors and probes (Figure 3.4) were evaluated on recombinant enzymes, in cell lysates and in mice. The experiments were conducted by Liang Wu, Zachary Armstrong and coworkers in the Davies lab at the University of York in the United Kingdom and the Vlodovsky lab at the Technion Israel Institute of Technology in Haifa, Israel.

Evaluation of HPSE inhibitors

The synthesized inhibitors were screened against a panel of recombinant β-glucuronidases by gel-based competitive ABPP using ABP **1** (Figure 3.5). Heparanase was inhibited by **29** at the lowest inhibitor concentration in line with the preference of HPSE for sulfated substrates. The non-sulfated derivative **28** showed approximately ten times lower potency. Substitutions on the 2' position have a profound influence on the potency. Deacetylation of the amine (**36**) is detrimental for the potency in comparison to the GlucNAc derivative (**28**). Also the larger steric bulk of an azidoacetyl substituent (**39**) is not tolerated by HPSE. The potency of inhibitor **34** without a 2' substituent is similar to the naturally occurring NHAc substitution. HPSE is not inhibited by unsubstituted β-glucuronic acid configured cyclophellitol **61**[9] at concentrations of up to 10 µM.

|	R¹	R²	R³
28	H	NHAc	H
29	SO₃⁻	NHAc	H
34	H	H	H
36	H	NH₂	H
37	H	Cy5	H
38	H	Biotin	H
39	H	N₃	H
60	H	NHAc	Cy5

	R
16	Cy5
61	H
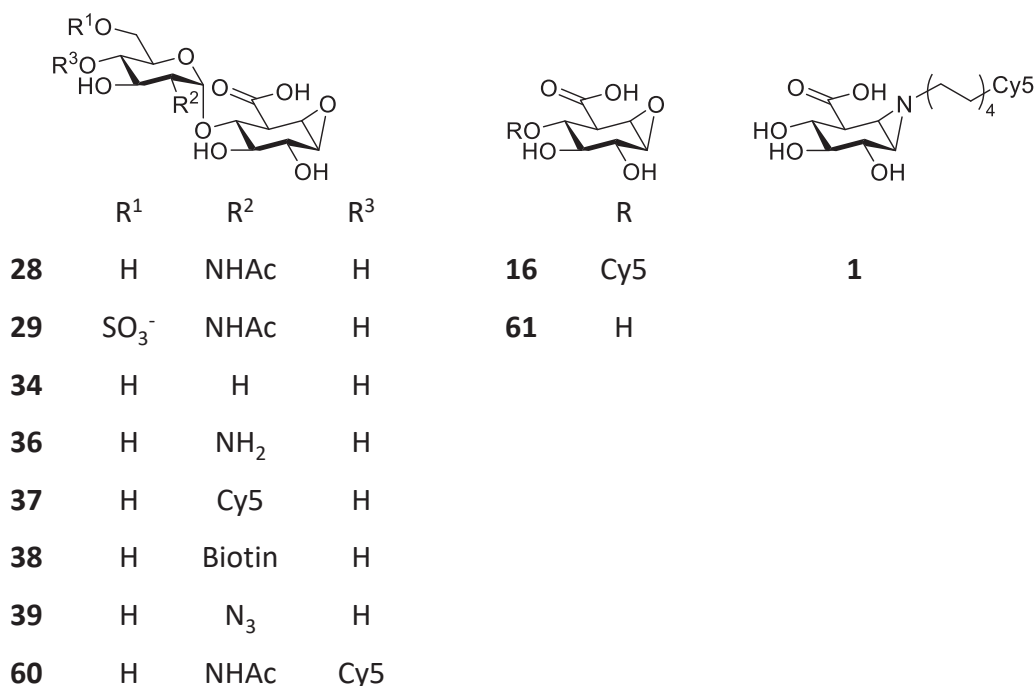
Figure 3.4| Inhibitors and ABPs used in this section.

ProHPSE has not been reported to have hydrolytic activity but is labeled by ABP **1**. No inhibition by mono- or disaccharide inhibitors (**61**, **28** or **29)** under the tested conditions (up to 10 µM) was observed. Retaining e*xo*-acting β-glucuronidase from *E. coli*[45] belonging to GH family 2 (EcGH2) is not inhibited by the disaccharide inhibitors (**28** and **29**) but is inhibited by the monosaccharide **61** although with moderate potency. The GH79 β-glucuronidase from *Acidobacterium capsulatum*[46] (AcGH79) is inhibited by mono- and disaccharide configured inhibitors (**28**, **29** and **61**) with similar potency, suggesting the enzyme may possess endo-activity in addition to the previously reported exo-activity. The GH79 heparanase from *Burkholderia pseudomallei*[47] (BpHep) is inhibited by the disaccharide inhibitors (**28** and **29**) and not by the mono saccharide **61**. Sulfation does not have a profound effect on the potency for this enzyme. X-ray crystallography of the inhibitor enzyme complexes of **61** with EcGH2 and AcGH79 and **28** and **29** with HPSE and BpHep all show selective alkylation of the catalytic nucleophile of the enzyme (data not shown).

The reactivity of inhibitor **28** towards glutathione, an abundant thiol containing antioxidant in humans, was determined with colorimetric quantitation using Ellman's reagent as a readout (Figure 3.6).[48] The assay with DMSO and iodoacetamide as baseline and positive control showed stability of **28** for at least two hours.
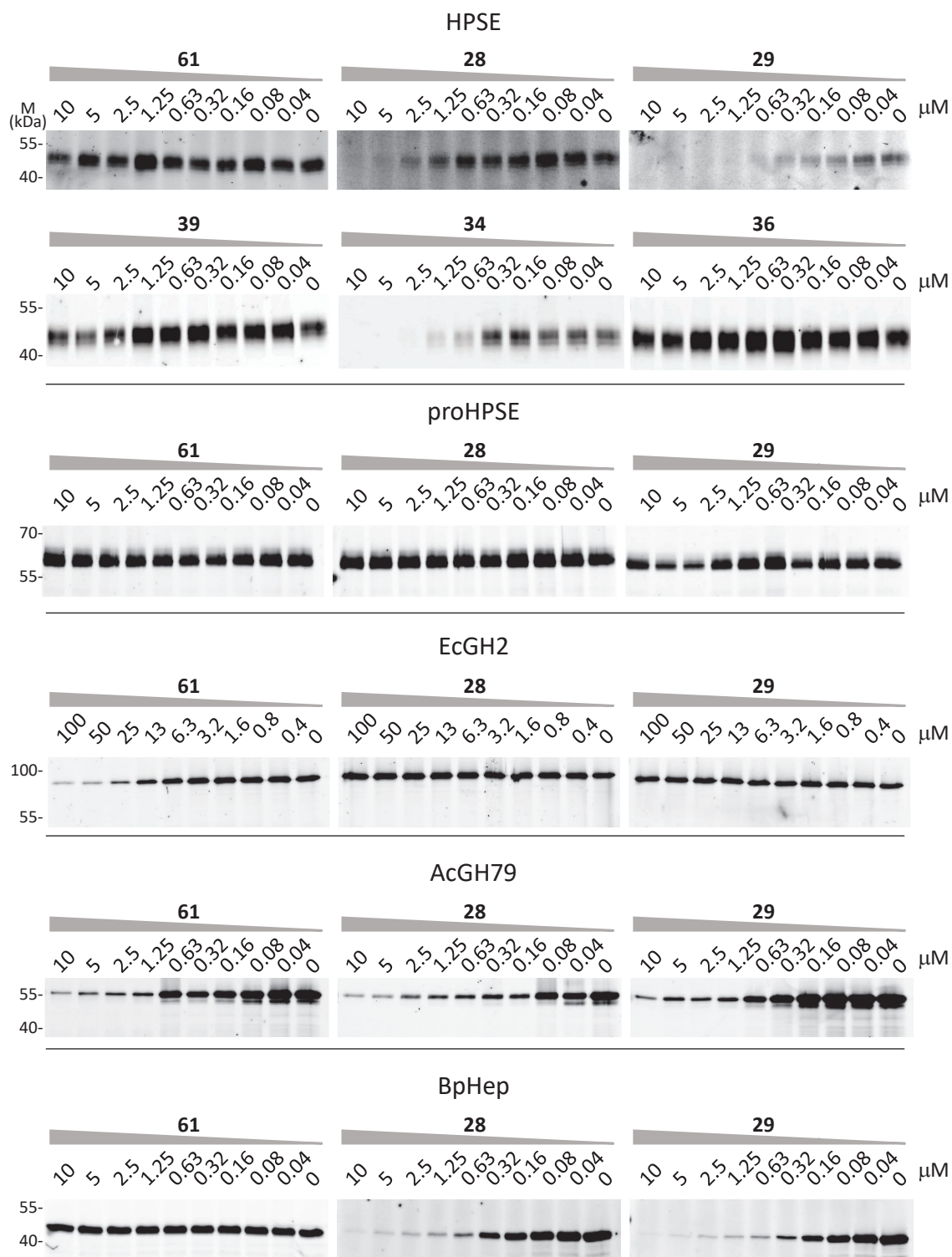
Figure 3.5| Assessment of inhibitor potency by ABP (**1**)-based screening of cyclophellitol-based inhibitors in a panel of recombinant exo- (EcGH2, AcGH79) and endo-(HPSE, BpHep) β-glucuronidases.

Inhibitors **28, 29,** and **61** were selected to be tested in several increasingly sophisticated HPSE inhibition assays. Selectivity and potency of the three inhibitors was estimated in platelet lysate by gel-based ABPP (Figure 3.7A). Monosaccharide **61** inhibited exo-acting GUSB. The
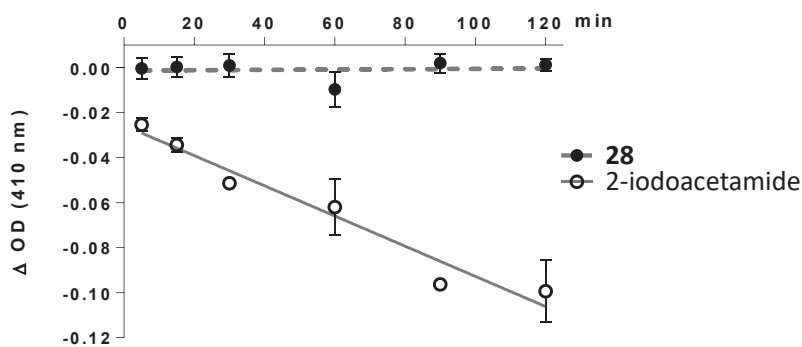
Figure 3.6| Glutathione reactivity assay. All measurements are three replicates corrected for the DMSO control at that time point. Error bars represent one standard deviation.

disaccharide mimics **28** and **29** inhibited HPSE without inhibition of GUSB in the tested concentration of up to 10 µM. The IC_{50} value for **28** (0.5 µM) is roughly ten times higher than that for **29** (0.06 µM) in accord with the results obtained with recombinant HPSE. HPSE IC_{50} values of **28** and **29** were also determined in the fondaparinux digestion assay (Figure 3.7B).[49] Hydrolysis of the synthetic anticoagulant fondaparinux by HPSE is determined by colorimetric quantitation of the hydrolysis product. In contrast to the gel-based assay where **29** was more potent then **28,** the IC_{50} of both compounds was around 0.5 µM in this assay. An undisclosed small molecule reversible inhibitor was used as a positive control.

Compounds **28** and **29** were also equally effective inhibitors of recombinant HPSE mediated hydrolysis of a extracellular matrix (ECM) generated by bovine corneal endothelial cells. [50,51] Hydrolysis of this ECM releases radiolabeled HS fragments that are quantified by size exclusion chromatography. **28** and **29** showed similar potency in this assay. Monosaccharide **61** was not active up to 5 µM (data not shown). Treatment of U87 human glioma (brain cancer) cells with inhibitors **28** or **29** followed by washing and lysis shows only modest inhibition of the naturally expressed HPSE in the above-mentioned ECM degradation assay. Treatment of the U87 cell lysates with the inhibitors does show potent inhibition (data not shown). These results indicate that **28** and **29** do not enter the cell and are thus unable to inhibit intracellular HPSE.

The inhibition of HPSE is expected to result in a diminished ability of the cells to migrate through the ECM. In the case of cancer cells this may reduce their tendency to metastasize. Matrigel invasion assays were performed with **28** and **29** (Figure 3.7C). At 20 µM both compounds show a significant decrease in cell mobility through a basement membrane indicating the inhibition of HPSE by these inhibitors may also have an effect on cell mobility *in vivo*. To verify this **28** was tested in a mouse metastasis model (Figure 3.7D). Balb/c mice were
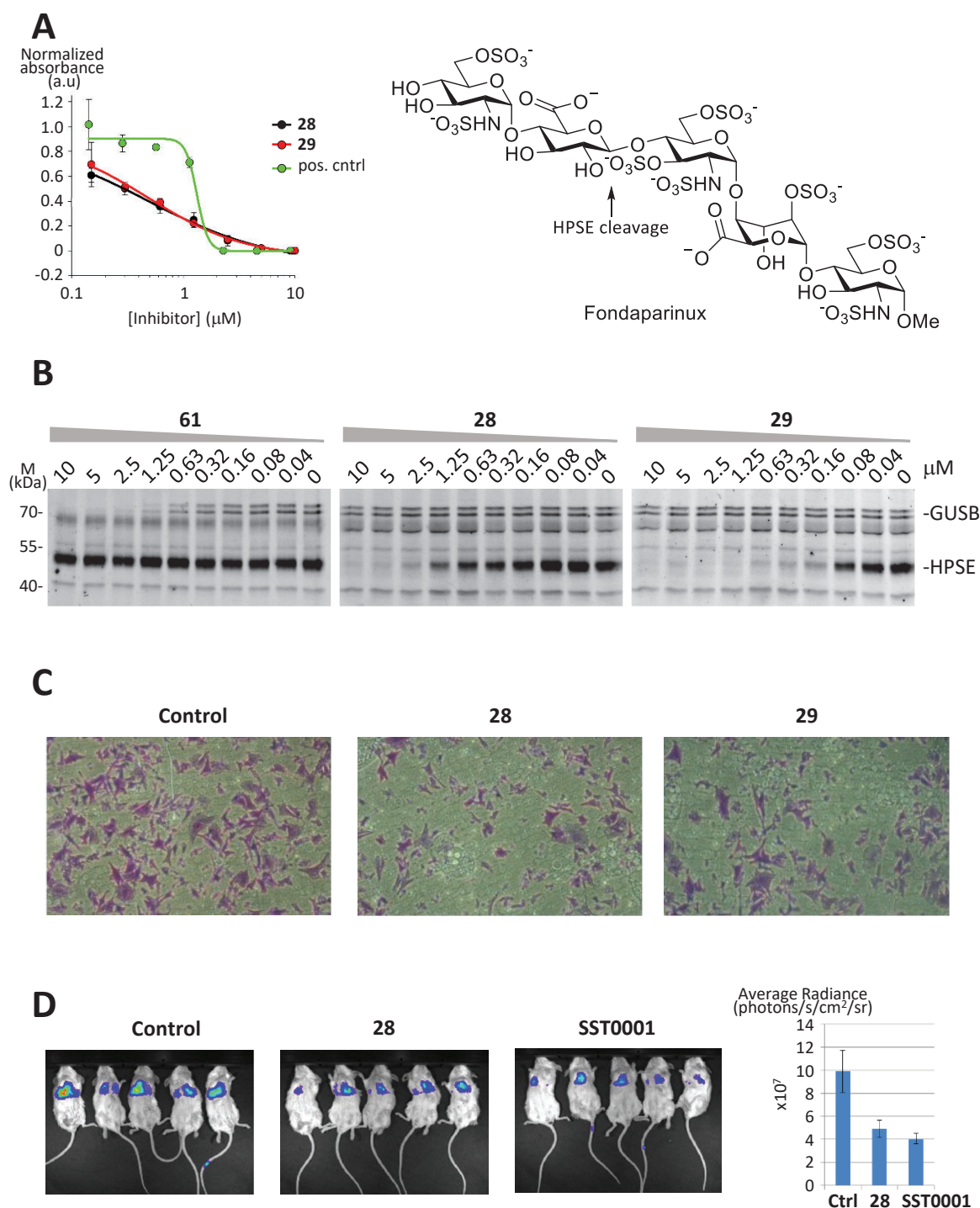
Figure 3.7| A) Inhibition of fondaparinux cleavage by recombinant heparanase. **B**) Selective HPSE and GUSB inhibition in platelet lysate. IC$_{50}$ on HPSE of **28** 534 nM, **29** 56 nM. **C**) Matrigel invasion assay: Representative pictures of the U87 glyoma cells treated with DMSO (neg control) and HPSE inhibitors after passing through a matrigel and crystal violet staining. **D**) Murine metastasis assay result after 14 days.

injected in the tail vein with 4T1 murine mammary carcinoma (breast cancer) cells expressing luciferase. Metastasis to the lungs is quantified by bioluminescent scanning after 14 days. Mice treated with **28** or roneparstat showed a significant reduction in lung metastasis compared to the untreated control.

Evaluation of HPSE ABPs

4'alkylated monosaccharide probe **16** labeled HPSE with reduced potency compared to aziridine probe **1** (Figure 3.8A). ProHPSE was labeled with similar potency so effectively the probe was more selective for proHPSE. Therefore, evaluation of these probes was not pursued further.

2'*N*-tagged disaccharide probe **37** did not show labeling of HPSE. However selective labeling of a 40 kDa protein was observed with this probe in platelets (data not shown). Labeling of a protein with this molecular weight was also observed with **1** and **60** (Figure 3.7B and Figure 3.8C).
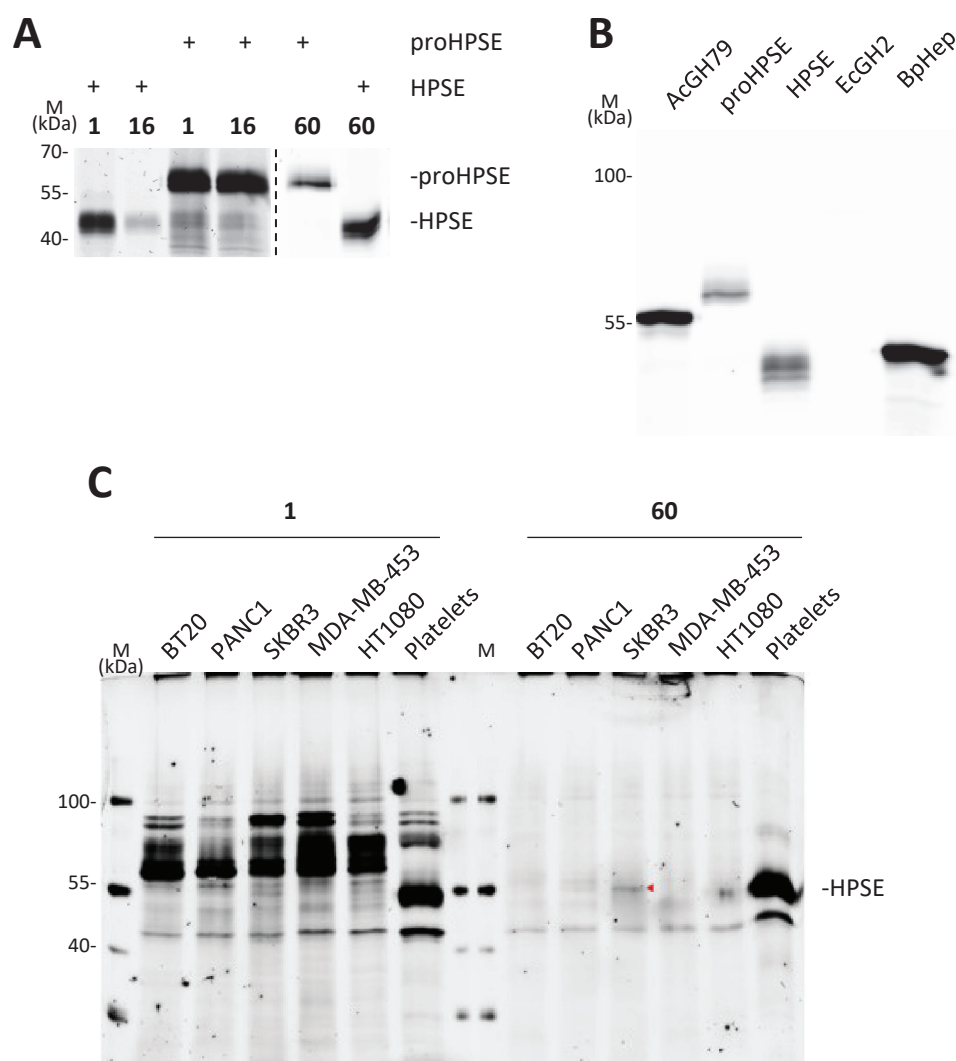
Figure 3.8|A) Labeling of recombinant proHPSE and mature HPSE (100 nM protein) with the indicated probe (100 nM for **1** and **16**, 1 μM for **60**). **B**) Labeling of recombinant human and bacterial β-glucuronidases by **60**. **C**) Comparison of labeling in human lysates with **1** and **60** showing higher selectivity of **60** (both at 1 μM).

On recombinant enzymes, 4'O-tagged disaccharide probe **60** showed decreased labeling of proHPSE while maintaining labeling efficiency for mature HPSE indicating increased selectivity for HPSE (Figure 3.8A). ABP **60** efficiently labeled recombinant endo-glucuronidases AcGH79 and BpHep without labeling exo-glucuronidase EcGH2 (Figure 3.8B). Labeling of lysates of human platelets and the cancer cell lines, BT20, SKBR3 and MDA-MB-453 (breast cancers), PANC1 (pancreatic cancer) and HT1080 (fibrosarcoma) with probes **1** and **60** clearly shows the improved HPSE selectivity of **60** (Figure 3.8C). Using both probes HPSE is clearly visible in human platelet lysate as expected. However, in the other lysates the activity of HPSE is too low to distinguish from background and off-target labeling with probe **1**. With probe **60** background and off-target labeling is reduced and HPSE activity can be observed in the SKBR3 cell lysate.

3.4 Conclusion

A set of 4'O-alkylated and glycosylated glucuronic acid configured cyclophellitol derivatives is described in this chapter. The synthesis comprises alkylation or glycosylation of a suitably protected cyclophellitol alkene, followed by generation of the glucuronic acid cyclophellitol. The glycosylated derivatives are elaborated into 2'-amino, -NHAc and -deoxy derivatives. Gel-based ABP experiments show sufficient potency for 2'-NHAc and deoxy derivatives but not for the amino derivative. 6'O-sulfation is shown to increase inhibitor potency.

Potent and selective HPSE ABPs are generated by 4'O-alkylation of disaccharide inhibitors. 4O-alkylated cyclophellitol or 2'N-acylated disaccharides do not yield potent or selective ABPs for HPSE.

Pseudodisaccharide cyclophellitol derived inhibitors **28** and **29** have shown their ability to inhibit HPSE *in vitro* and *in situ* and **28** was also active *in vivo*. In the future disaccharide inhibitors serve as a starting point for the development of selective mechanism-based HPSE inhibitors as therapeutics. The developed selective ABP may be used to assess HPSE activity in health and disease.

3.5 Acknowledgements

Lit, Gijs Ruijgrok and Merle Weizenberg are acknowledged for their synthesis work in the context of their MSc internships. Sybrin Schröder is acknowledged for valuable discussion.

3.6 Experimental

General chemical synthesis procedures are shown in the experimental section of chapter 2.

Glutatione assay

Reduced glutathione (90 µl, 50 µM in 100 mM Tris, pH = 7.5 with 10% acetonitrile) was pipetted in a 96 well plate. **28** (10 µl, 1 mM; final concentration 100 µM in DMSO) or iodoacetamide (positive control, 10 µl, 1 mM; final concentration 100 µM in DMSO) or DMSO (10 µl) was added at several time points (0, 30, 60, 90, 105, 115 minutes). After 5 more minutes Ellman's reagent (100 µl, 0.1 ml 100mM Ellman's reagent in MeOH diluted in 9.9 ml 100mM Tris pH 7.5) was added and absorbance was measured after 15 minutes at 410 nm on a Clariostar plate reader.

Monosaccharide probes

2,3-di-*O*-benzyl-4-*O*-(8-azidooctyl)-6-*O*-*p*-methoxybenzyl-cyclophellitol alkene (4)
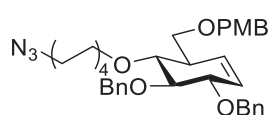
PMB-protected cyclohexene **5**[26] (230 mg, 0.50 mmol) was coevaporated with toluene thrice, after which it was dissolved in THF (1.0 ml). The solution was cooled to 0°C. KHMDS (0.5 M in toluene, 1.5 ml, 0.75 mmol) was added and the mixture was stirred for 1 hour at 0°C. 8-azidooctyl 4-methylbenzenesulfonate[30] (488 mg, 1.50 mmol) was added, the reaction mixture was warmed to rt and stirred for 18 h. The mixture was quenched by the addition of H_2O and diluted with EtOAc. The aqueous phase was extracted with EtOAc (3x) and the combined organic phases were washed with H_2O and brine. The organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by column chromatography (PE/EtOAc 15/1 -> 4/1, v/v) afforded the product as a colorless oil (219 mg, 0.355 mmol, 71%).

^{1}H NMR (400 MHz, CDCl$_3$) δ = 7.40 − 7.21 (m, 12H), 6.89 − 6.84 (m, 2H), 5.71 − 5.62 (m, 2H, Bn), 4.87 (s, 2H, Bn), 4.67 (s, 2H, Bn), 4.49 (d, J=11.9, 1H, CH$_2$PMB), 4.40 (d, J=11.9, 1H, CH$_2$PMB), 4.21 − 4.16 (m, 1H, H2), 3.84 (dt, J=8.9, 6.7, 1H, CH$_2$C**H**$_2$O), 3.79 (s, 3H, OCH$_3$), 3.70 (dd, J=10.1, 7.8, 1H, H3), 3.53 (d, J=4.0, 2H, H6), 3.44 (t, J=9.9, 1H, H4), 3.32 (dt, J=8.9, 7.0, 1H, CH$_2$C**H**$_2$O), 3.24 (t, J=7.0, 2H, CH$_2$N$_3$), 2.50 − 2.38 (m, 1H, H5), 1.66 − 1.13 (m, 12H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 159.3, 139.1, 138.7, 130.4, 129.5 (alkene), 129.4, 128.5, 128.4, 128.0, 127.9, 127.7, 127.6, 127.0 (alkene), 113.8, 85.4 (C3), 80.9 (C2), 78.7 (C4), 75.3 (Bn), 73.6 (CH$_2$**C**H$_2$O), 72.9 (Bn), 72.2 (CH$_2$PMB), 68.9 (C6), 55.3 (OCH$_3$), 51.5 (CH$_2$N$_3$), 44.5 (C5), 30.5, 29.5, 29.2, 28.9, 26.8, 26.2. HRMS (ESI) m/z: [M+Na]$^+$ calculated for C$_{37}$H$_{47}$N$_3$NaO$_5$ 636.3413, found 636.3412.

**2,3-di-*O*-benzyl-4-*O*-(8-azidooctyl)-cyclophellitol alkene (12)**
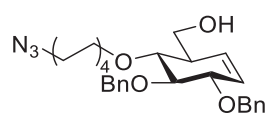
Cyclohexene **4** (91.0 mg, 0.15 mmol) was dissolved in a mixture of DCM/H_2O (3.0 ml, 19/1, v/v). DDQ (39.0 mg, 0.18 mmol) was added and the reaction mixture was stirred for 18 h at rt. The mixture was diluted with EtOAc and the organic layer was washed with NaHCO$_3$ (sat. aq. (3x)), H_2O and brine. The organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by column chromatography (PE/EtOAc, 3/1, v/v) afforded the product as colorless oil (62.0 mg, 0.128 mmol, 85%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.40 − 7.24 (m, 10H), 5.74 (dt, J = 10.1, 2.5 Hz, 1H, alkene), 5.53 (dt, J = 10.2, 1.8 Hz, 1H, alkene), 4.92 − 4.84 (m, 2H, Bn), 4.68 (s, 2H, Bn), 4.21 − 4.13 (m, 1H, H2), 3.97 (dt, J = 8.8, 6.9 Hz, 1H, Linker-CH$_2$O), 3.79 − 3.71 (m, 3H, H6 (2x)/H3), 3.55 (dt, J = 8.8, 7.4 Hz, 1H, Linker-CH$_2$O), 3.47 (t, J = 9.7 Hz, 1H, H4), 3.24 (t, J = 7.0 Hz, 2H, CH$_2$N$_3$), 2.52 − 2.45 (m, 1H, H5), 2.09 − 1.97 (m, 1H, -

OH), 1.73 – 1.49 (m, 4H), 1.41 – 1.16 (m, 8H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 138.9, 138.5, 128.5, 128.5, 128.0, 127.9, 127.9, 127.8, 127.7, 85.1 (C3), 80.8 (C2), 80.4 (C4), 75.3 (Bn), 73.6 (linker-CH$_2$O), 72.3 (Bn), 64.2 (C6), 51.6 (CH$_2$N$_3$), 45.8 (C5), 30.5, 29.5, 29.2, 28.9, 26.8, 26.1. HRMS (ESI) m/z: [M+Na]$^+$ calculated for C$_{29}$H$_{39}$N$_3$NaO$_4$ 516.2838, found 516.2834.

2,3-di-*O*-benzyl-4-*O*-(8-azidooctyl)-cyclophellitol (13)
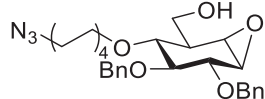
Homoallylic alcohol **12** (631 mg, 1.28 mmol) was dissolved in DCM (13 ml) and cooled to 0°C. mCPBA (442 mg, 1.97 mmol) was added and the mixture was stirred at 0°C for 18 h. A second portion of mCPBA (574 mg, 2.56 mmol) was added and the mixture was stirred for another 24 h at 0°C. The reaction was diluted with EtOAc and the organic layer was washed with a mixture of NaHCO$_3$ (sat. aq)/Na$_2$S$_2$O$_3$ (1/1, v/v, (3x)) and brine. The combined aqueous layers were extracted with EtOAc (3x). The combined organic layers were dried over MgSO$_4$, filtered and concentrated under reduced pressure. Purification by column chromatography (PE/EtOAc, 5/2, v/v) afforded the product as a colorless oil (571 mg, 1.11 mmol, 87%).
^{1}H NMR (400 MHz, CDCl$_3$) δ 7.42 – 7.26 (m, 10H), 4.81 (d, *J* = 2.8 Hz, 2H, Bn), 4.74 (dd, *J* = 28.7, 11.4 Hz, 2H, Bn), 4.00 (dd, *J* = 10.8, 5.0 Hz, 1H, H6a), 3.92 – 3.85 (m, 2H, H6b/ linker-CH$_2$O), 3.80 (dd, *J* = 8.2, 0.5 Hz, 1H, H2), 3.52 – 3.43 (m, 2H, Bn/H3), 3.32 (d, *J* = 3.6 Hz, 1H, epoxide), 3.28 – 3.21 (m, 3H, CH$_2$N$_3$/H4), 3.15 (d, *J* = 3.8 Hz, 1H, epoxide), 2.14 (m, 1H, H5), 1.55 (m, *J* = 14.3, 7.0 Hz, 4H), 1.36 – 1.21 (m, 8H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 138.7, 137.7, 128.6, 128.4, 128.1, 128.0, 127.8, 127.7, 84.9 (C3), 79.7 (C2), 76.3 (C4), 75.4 (Bn), 73.8 (linker-CH$_2$O), 73.3 (Bn), 63.2(C6), 55.9 (epoxide), 53.2 (epoxide), 51.5 (CH$_2$N$_3$), 44.0 (C5), 30.4, 29.4, 29.1, 28.9, 26.7, 26.1. HRMS (ESI) m/z: [M+Na]$^+$ calculated for C$_{29}$H$_{39}$N$_3$NaO$_5$ 532.2787, found 532.2782.

2,3-di-*O*-benzyl-4-*O*-(8-azidooctyl)-glucurono-cyclophellitol (3)
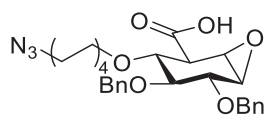
Epoxide **13** (200 mg, 393 µmol) was dissolved in DCM/*t*-BuOH/H$_2$O (12 ml, 4/4/1, v/v/v). TEMPO (12.3 mg, 78.6 µmol) and BAIB (316 mg, 982 µmol) were added and the reaction was stirred at 0°C for 22 h. The reaction was quenched with Na$_2$S$_2$O$_3$ (2 ml, aq. sat.), acidified to pH 3 and extracted with DCM (3x 20 ml). The combined organic layers were dried over MgSO$_4$, filtered and concentrated under reduced pressure. Purification by column chromatography (PE/EtOAc, 6/1, v/v, 1% AcOH) afforded the product as a colorless oil (185 mg, 0.354 mmol, 90%).
^{1}H NMR (400 MHz, CDCl$_3$) δ 7.36 – 7.28 (m, 10H), 4.81 (d, *J* = 1.1 Hz, 2H, Bn), 4.78 (d, *J* = 11.4 Hz, 1H, Bn), 4.71 (d, *J* = 11.3 Hz, 1H, Bn), 3.90 – 3.82 (m, 2H, linker-CH$_2$O/H2), 3.65 – 3.53 (m, 2H, H4/ linker-CH$_2$O), 3.52 – 3.44 (m, 2H, epoxide/H3), 3.24 (t, *J* = 7.0 Hz, 2H, CH$_2$N$_3$), 3.20 (d, *J* = 3.6 Hz, 1H, epoxide), 2.98 (dd, *J* = 10.0, 1.6 Hz, 1H, H5), 1.61 – 1.44 (m, 4H), 1.36 – 1.18 (m, 8H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 175.3 (C6), 138.6, 137.6, 128.7, 128.5, 128.2, 128.1, 127.8, 127.8, 84.2 (C3), 79.3 (C2), 75.5 (Bn), 74.9 (C4), 73.8 (linker-CH$_2$O), 73.5 (Bn), 54.4 (epoxide), 53.8 (epoxide), 51.6 (CH$_2$N$_3$), 48.6 (C5), 30.3, 29.3, 29.1, 28.9, 26.7, 25.9. HRMS (ESI) m/z: [M+Na]$^+$ calculated for C$_{29}$H$_{37}$N$_3$NaO$_6$ 546.2580, found 546.2580.

4-*O*-(8-aminooctyl)-glucurono-cyclophellitol (15) and cyclohexene 14

Carboxylic acid **3** (40.0 mg, 76.5 µmol) was dissolved in anhydrous THF, followed by the addition of *t*-BuOH (73 µl, 765 µmol) and a glass-coated stirring bar. The flask was flushed with nitrogen and ammonia (7 ml) was condensed at -60°C. Freshly cut sodium (35.0 mg, 1.53 mmol) was added portion wise at the same temperature under a flow of nitrogen. After stirring for 30 min, the reaction was quenched, warmed to rt and stirred for 1 h. The reaction mixture was concentrated under reduced pressure and coevaporated with H$_2$O (3x).
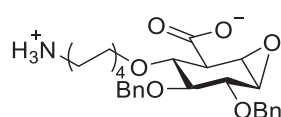
Quenching with AcOH (92 µl, 1.61 mmol) and HPLC purification with linear gradient, solutions A: 50 mM AcOH in H$_2$O and B: CH$_3$CN yielded **15** (6.7 mg, 21 µmol, 28%).

^{1}H NMR (500 MHz, D$_2$O) δ 3.80 (d, J = 8.4 Hz, 1H, H2), 3.76 – 3.70 (m, 1H, linker-CH$_2$O), 3.61 – 3.55 (m, 1H, linker-CH$_2$O), 3.49 – 3.44 (m, 2H, H4/epoxide), 3.37 (dd, J = 10.4, 8.5 Hz, 1H, H3), 3.15 (d, J = 3.7 Hz, 1H, epoxide), 2.97 (t, J = 7.6 Hz, 2H, CH$_2$NH$_2$), 2.72 (dd, J = 9.8, 1.8 Hz, 1H, H5), 1.63 (p, J = 7.4 Hz, 2H), 1.49 (d, J = 5.5 Hz, 2H), 1.39 – 1.24 (m, 8H). ^{13}C NMR (126 MHz, D$_2$O) δ 178.0 (C6), 76.9 (C4), 75.8 (C3), 73.0 (CH$_2$O), 71.2 (C2), 56.5 (epoxide), 55.5 (epoxide), 51.0 (C5), 39.5 (CH$_2$NH$_2$), 29.2, 28.0, 27.9, 26.6, 25.3, 25.0. HRMS (ESI) m/z: [M+H]$^+$ calculated for C$_{15}$H$_{28}$NO$_6$ 318.1911, found 318.1913.
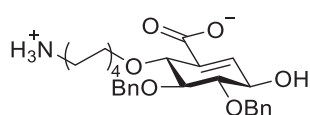
Quenching with NH$_4$HCO$_3$ (127 mg, 1.61 mmol) and HPLC purification with linear gradient, solutions A: 50 mM NH$_4$HCO$_3$ in H$_2$O and B: CH$_3$CN yielded **14** (2.20 mg, 6.9 μmol, 9%).

^{1}H NMR (500 MHz, D$_2$O) δ 5.97 (t, J = 1.8 Hz, 1H, alkene), 4.34 – 4.30 (m, 1H, H4), 4.23 – 4.18 (m, 1H, H1), 3.68 – 3.63 (m, 3H, linker-CH$_2$O/H3), 3.46 – 3.39 (m, 1H, H2), 2.96 (t, J = 7.5 Hz, 2H, CH$_2$NH$_2$), 1.66 – 1.57 (m, 2H), 1.54 – 1.46 (m, 2H), 1.38 – 1.24 (m, 8H). ^{13}C NMR (126 MHz, D$_2$O) δ 174.7 (C6), 137.9 (C5), 131.5 (CH alkene), 80.2 (C4), 75.2 (C2), 73.9 (C3), 71.1 (C1), 70.6 (CH$_2$O), 39.5 (CH$_2$NH$_2$), 29.3, 28.1, 27.9, 26.6, 25.3, 25.0. HRMS (ESI) m/z: [M+H]$^+$ calculated for C$_{15}$H$_{28}$NO$_6$ 318.19111 found 318.19109.

Cy5 probe 16
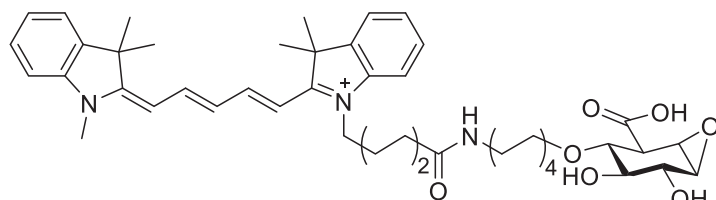
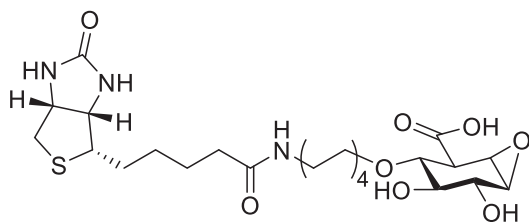Amine **15** (10.3 mg, 32.5 μmol) was dissolved in anhydrous DMF (0.5 ml), Cy5-NHS ester (30.0 mg, 48.8 μmol) and DIPEA (10.6 μl, 65.0 μmol) were added. The reaction mixture was stirred for 18 h followed by purification by preparative RP-HPLC (linear gradient, solutions used: A: 50 mM AcOH in H$_2$O, B: CH$_3$CN) and lyophilization. The product was obtained as a blue solid (3.50 mg, 4.48 μmol, 14%).

^{1}H NMR (500 MHz, MeOD) δ 8.25 (t, J = 13.0 Hz, 2H), 7.49 (d, J = 7.5 Hz, 2H), 7.44 – 7.38 (m, 2H), 7.32 – 7.24 (m, 4H), 6.64 (t, J = 12.4 Hz, 1H), 6.29 (dd, J = 13.7, 6.5 Hz, 2H), 4.11 (t, J = 7.4 Hz, 2H), 3.79 – 3.71 (m, 1H), 3.69 (d, J = 8.2 Hz, 1H, H2), 3.67 – 3.59 (m, 4H), 3.48 (t, J = 9.8 Hz, 1H, H4), 3.39 (s, 1H, epoxide), 3.27 (dd, J = 10.1, 8.3 Hz, 1H, H3), 3.12 (t, J = 7.0 Hz, 2H), 2.99 (d, J = 3.6 Hz, 1H, epoxide), 2.69 (dd, J = 8.1, 1.5 Hz, 1H, H5), 2.20 (t, J = 7.2 Hz, 2H), 1.87 – 1.79 (m, 2H), 1.73 (s, 11H), 1.71 – 1.66 (m, 2H), 1.55 – 1.37 (m, 9H), 1.29 (s, 13H). ^{13}C NMR (126 MHz, MeOD) δ 155.5, 144.3, 143.6, 142.7, 142.5, 129.8, 129.7, 126.7, 126.2, 123.4, 123.3, 112.1, 111.9, 104.3, 78.2 (C3), 77.9 (C4), 73.9, 73.0 (C2), 56.9 (epoxide), 56.7 (epoxide), 52,1 (C5 from 2D), 50.6, 50.5, 44.8, 40.4, 36.7, 31.5, 31.3, 30.5, 30.3, 28.2, 28.0, 27.9, 27.8, 27.3, 27.0, 26.6. HRMS (ESI) m/z: [M]$^+$ calculated for C$_{47}$H$_{64}$N$_3$O$_7$ 782.4739, found 782.4772.

Biotin probe 17

Amine **15** (6.00 mg, 18.9 μmol) was dissolved in anhydrous DMF (0.5 ml). Biotin-NHS ester (6.6 mg, 28 μmol) and DIPEA (6.5 μl, 39 μmol) were added and the reaction mixture was stirred for 18 h. The mixtures was purified by semi-preparative HPLC (linear gradient, 50 mM AcOH in H$_2$O and CH$_3$CN) and lyophilized. This afforded the product as a white solid (2.60 mg, 4.79 μmol, 25%).

^{1}H NMR (500 MHz, D$_2$O) δ 4.62 (dd, J = 7.9, 4.9 Hz, 1H), 4.43 (dd, J = 7.9, 4.5 Hz, 1H), 3.84 (d, J = 8.4 Hz, 1H, H2), 3.79 – 3.74 (m, 1H), 3.62 – 3.56 (m, 1H), 3.56 – 3.53 (m, 1H, epoxide), 3.49 (t, J = 10.1 Hz, 1H, H4), 3.44 – 3.39 (m, 1H, H3), 3.36 – 3.31 (m, 1H), 3.23 – 3.13 (m, 3H, epoxide), 3.00 (dd, J = 13.1, 5.0 Hz, 1H), 2.87 (d, J = 9.8 Hz, 1H, H5), 2.79 (d, J = 13.0 Hz, 1H), 2.25 (t, J = 7.1 Hz, 2H), 1.78 – 1.55 (m, 4H), 1.55 – 1.46 (m, 4H), 1.46 – 1.35 (m, 2H), 1.35 – 1.23 (m, 8H). ^{13}C NMR (126 MHz, D$_2$O) δ 176.7 (C6),

76.5 (C4), 75.7 (C3), 73.3, 71.1 (C2), 62.1, 60.3, 56.0 (epoxide), 55.6 (epoxide), 55.5, 49.8 (C5), 39.8, 39.4, 35.6, 29.3, 28.5, 28.4, 28.3, 27.8, 27.7, 26.1, 25.3, 25.2. HRMS (ESI) m/z: [M+H]$^+$ calculated for $C_{25}H_{42}N_3O_8S$ 544.2693, found 544.2689.

Disaccharide inhibitors

### 2-Azido-2-deoxy-3,4-di-*O*-benzyl-6-*O*-tert-butyldiphenylsilyl-ᴅ-glucopyranose (19)
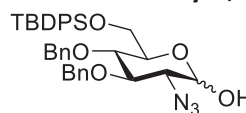
Thioglycoside **18**[31] (3.58 g, 5.00 mmol) was dissolved in acetone/H_2O (50 ml, 9/1, v/v). The mixture was cooled to 0°C and NIS (2.25 g, 10.0 mmol) was added. The reaction was stirred for 4 hours. Upon completion $Na_2S_2O_3$ (aq. sat) was added and the reaction turned colorless. The mixture was concentrated under reduced pressure, the residue was dissolved in EtOAc, washed with $NaHCO_3$ (aq. sat.) and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The product was obtained after chromatography (Et$_2$O/pentane, 1/19 -> 1/5, v/v) as a colorless oil (2.13 g, 3.41 mmol, 68%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.79 − 7.59 (m, 7H), 7.42 − 7.24 (m, 23H), 7.23 − 7.12 (m, 3H), 5.23 (t, *J* = 3.4 Hz, 1H), 4.99 − 4.78 (m, 5H), 4.74 (d, *J* = 10.9 Hz, 1H), 4.66 (d, *J* = 10.8 Hz, 1H), 4.43 (dd, *J* = 7.8, 5.1 Hz, 1H), 4.15 − 3.78 (m, 7H), 3.78 − 3.66 (m, 1H), 3.50 − 3.26 (m, 4H), 1.06 (d, *J* = 1.6 Hz, 14H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 138.1, 137.9, 137.9, 137.8, 136.0, 135.9, 135.7, 135.7, 133.6, 133.6, 133.1, 133.0, 129.8, 129.8, 129.8, 128.6, 128.6, 128.5, 128.4, 128.3, 128.1, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.7, 96.1, 92.1, 83.1, 80.2, 78.3, 77.5, 77.2, 76.8, 76.1, 75.8, 75.8, 75.2, 71.9, 67.6, 64.2, 62.8, 62.5, 60.6, 26.9, 19.4, 19.4. HRMS (ESI) m/z: [M+Na]$^+$ calculated for $C_{36}H_{41}N_3O_5Na$ 646.2708, found 646.2702.

2-Azido-2-deoxy-3,4-di-*O*-benzyl-6-*O*-tert-butyldiphenylsilyl-ᴅ-glucopyranosyl *N*-phenyltrifluoroacetimidate(20)
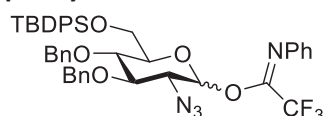
Lactol **19** (2.10 g, 3.37 mmol) was dissolved in DCM (16.8 ml). 2,2,2-trifluoro-*N*-phenyltrifluoroacetimidoyl chloride (0.15 ml, 0.92 mmol) and Cs_2CO_3 (1.32 g, 4.04 mmol) were added and the reaction was stirred overnight at room temperature. The reaction was filtered over celite and concentrated in vacuo. Purification by flash silica column chromatography (Et$_2$O/pentane, 1/38 -> 1/19, v/v) yielded the product as a colorless oil (2.41 g, 3.03 mmol, 90%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.80 − 7.58 (m, 4H), 7.51 − 7.18 (m, 19H), 7.16 − 7.00 (m, 1H), 6.81 (d, *J* = 7.8 Hz, 1H), 6.76 − 6.71 (m, 1H), 5.00 − 4.82 (m, 3H), 4.76 (dd, *J* = 10.7, 3.9 Hz, 1H), 4.08 − 3.79 (m, 4H), 3.65 (d, *J* = 10.2 Hz, 1H), 1.07 (d, *J* = 1.0 Hz, 9H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 143.5, 143.3, 137.9, 137.8, 137.7, 137.7, 136.1, 136.0, 135.7, 135.6, 133.6, 133.5, 132.9, 132.8, 130.0, 129.9, 129.9, 129.5, 128.9, 128.8, 128.7, 128.7, 128.7, 128.4, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 126.5, 124.6, 124.5, 120.6, 119.5, 82.9, 80.4, 77.6, 77.5, 77.2, 77.1, 76.8, 76.5, 76.0, 75.5, 75.5, 74.6, 65.6, 63.2, 62.1, 27.0, 26.9, 19.5. HRMS (ESI) m/z: [M+Na]$^+$ calculated for $C_{44}H_{45}F_3N_4O_5SiNa$ 817.3004 found 817.3005.

2,3-di-*O*-benzyl-6-*O*-benzoyl-cyclophellitol alkene (9)
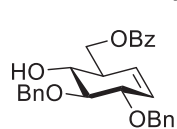
Diol **11** (1.36 g, 4.00 mmol) was dissolved in DCM (20 ml). Et$_3$N (2.79 ml, 20.0 mmol) and benzoyl chloride (0.56 ml, 4.80 mmol) were added at -50°C and the reaction was slowly warmed to room temperature overnight. H_2O and DCM were added and the layers were separated. The organic phase was washed with $NaHCO_3$ (aq. sat.) and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Column chromatography (EtOAc/pentane, 1/19 -> 1/9, v/v) afforded the product as a colorless oil (1.45 g, 3.27 mmol, 82%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 8.00 (dd, *J* = 8.3, 1.4 Hz, 2H), 7.60 − 7.49 (m, 1H), 7.49 − 7.27 (m, 12H), 5.80 (dt, *J* = 10.3, 2.2 Hz, 1H, alkene), 5.70 (dt, *J* = 10.3, 2.0 Hz, 1H, alkene), 5.04 (d, *J* = 11.2 Hz, 1H, Bn), 4.80 − 4.61 (m, 3H, Bn), 4.58 (dd, *J* = 10.9, 3.7 Hz, 1H, H6a), 4.44 (dd, *J* = 11.0, 5.5 Hz, 1H, H6b), 4.27 − 4.20 (m, 1H, H2), 3.78 − 3.64 (m, 2H, H4/H3), 2.75 − 2.65 (m, 1H, H5). ^{13}C NMR (101 MHz, CDCl$_3$) δ 166.7 (Bz), 138.9, 138.5, 138.2, 133.2, 129.7, 128.7, 128.6, 128.5, 128.1, 128.1, 128.0, 127.9, 127.8 (alkene),

127.4 (alkene), 83.8 (C3), 80.4 (C2), 75.2 (Bn), 71.6 (Bn), 69.9 (C4), 64.4 (C6), 43.4 (C5). HRMS (ESI) m/z: [M+Na]$^+$ calculated for $C_{28}H_{28}NaO_5$ 467.1829, found 467.1832.

2,3-di-*O*-benzyl-4-*O*-(2-Azido-2-deoxy-3,4-di-*O*-benzyl-6-*O*-tert-butyldiphenylsilyl-ᴅ-glucopyranosyl)-6-*O*-benzoyl-cyclophellitol alkene (21)
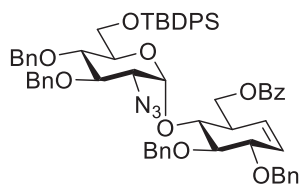
Donor **20** (2.41 g, 3.03 mmol) and acceptor **9** (1.04 g, 2.33 mmol) were co-evaporated with anhydrous toluene (3x). the mixture was dissolved in DCM (15 ml) and activated MS 3 Å were added. The mixture was stirred at room temperature overnight. The mixture was cooled to -78°C. TfOH (0.04 ml, 0.45 mmol) was added and the reaction was warmed to -30°C over 70 minutes and kept at this temperature for 60 minutes. The reaction was quenched with NaHCO₃ (aq. sat.), diluted with Et₂O and washed with H₂O, NaHCO₃ (aq. sat.), and brine, dried over MgSO₄ and filtered. Volatiles were removed under educed pressure and column chromatography (EtOAc/pentane, 1/19->1/9, v/v) afforded the product (2.02 g, 1.92 mmol, 83%).

^{1}H NMR (400 MHz, CDCl₃) δ 7.84 – 7.80 (m, 2H), 7.61 – 7.51 (m, 4H), 7.45 – 7.13 (m, 29H), 5.83 – 5.77 (m, 1H, alkene), 5.71 (d, *J* = 3.9 Hz, 1H, H1'), 5.64 – 5.57 (m, 1H, alkene), 5.08 (d, *J* = 10.7 Hz, 1H, Bn), 4.98 (d, *J* = 10.7 Hz, 1H, Bn), 4.89 – 4.81 (m, 3H, Bn (3x)), 4.73 – 4.62 (m, 3H, Bn (3x)), 4.47 (dd, *J* = 11.1, 3.2 Hz, 1H, H6a), 4.33 – 4.24 (m, 2H, H6b/H2), 4.05 – 3.91 (m, 3H, H3'/H4'/H4), 3.91 – 3.81 (m, 2H, H6a'/H3'), 3.80 – 3.70 (m, 2H, H6b'/H5'), 3.29 (dd, *J* = 10.4, 3.9 Hz, 1H, H2'), 2.78 (br, 1H, H5), 0.98 (s, 9H, *t*-Bu). ^{13}C NMR (101 MHz, CDCl₃) δ 166.2 (OBz), 138.9, 138.3, 138.2, 138.1, 135.9, 135.7, 133.6, 133.2, 133.1, 129.7, 129.7, 129.6, 128.7, 128.6, 128.6, 128.6, 128.4, 128.3, 128.0, 128.0, 127.9, 127.8, 127.8, 127.8, 127.6, 127.6, 98.2 (C1'), 84.4, 81.0 (C2), 80.2 (C4'), 78.1 (C3'), 75.7 (Bn), 75.2 (Bn), 74.8 (Bn), 74.4 (C4), 72.8 (C5'), 71.8 (Bn), 64.5 (C6), 63.6 (C2'), 62.2 (C6'), 43.3 (C5), 27.0 (*t*-Bu)., 19.4 (*t*-Bu). HRMS (ESI) m/z: [M+Na]$^+$ calculated for $C_{64}H_{67}N_3O_9SiNa$ 1072.4539, found 1072.4573.

2,3-di-*O*-benzyl-4-*O*-(2-*N*-acetyl-2-deoxy-3,4-di-*O*-benzyl-6-*O*-tert-butyldiphenylsilyl-ᴅ-glucopyranosyl)-6-*O*-benzoyl-cyclophellitol alkene (22)
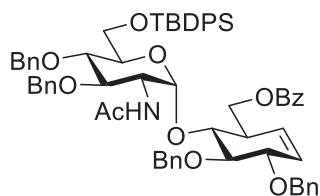
Azide **21** (0.22 g, 0.21 mmol) was dissolved in THF/H₂O (2.6 ml, 17/3, v/v) and PPh₃ (0.14 g, 0.50 mmol) and pyridine (5 μl, 0.06 mmol) were added. The solution was stirred at 50°C for 3 hours. The reaction was concentrated under reduced pressure, dissolved in DCM and dried over MgSO₄. The solids were removed by filtration and the filtrate was concentrated under reduced pressure. The residue was coevaporated with toluene and dissolved in DCM (2.0 ml). Acetic anhydride (0.30 ml, 3.1 mmol) and pyridine (0.25 ml, 3.1 mmol) were added and the reaction was stirred overnight. The reaction was cooled to 0°C and quenched with H₂O. The layers were separate and the organic phase was washed with CuSO₄ (aq. sat), NaHCO₃ (aq. sat) and brine, dried over MgSO₄, filtered and concentrated under reduced pressure. Column chromatography (EtOAc/pentane, 9/1 -> 8/2, v/v) afforded the product (0.20 g, 0.19 mmol, 89%).

^{1}H NMR (400 MHz, CDCl₃) δ 7.97 – 7.92 (m, 2H), 7.72 – 7.63 (m, 4H), 7.42 – 7.13 (m, 29H), 6.62 (d, *J* = 9.7 Hz, 1H, NH), 5.86 – 5.81 (m, 1H, alkene), 5.73 – 5.66 (m, 1H, alkene), 4.98 – 4.77 (m, 5H, H1', Bn (4x)), 4.72 – 4.55 (m, 5H, H6a, Bn (4x)), 4.52 – 4.47 (m, 1H, H6b), 4.38 (td, *J* = 10.0, 3.5 Hz, 1H, H2'), 4.23 – 4.16 (m, 1H, H2), 3.99 – 3.71 (m, 7H), 2.70 – 2.63 (m, 1H, H5), 1.46 (s, 3H, Ac), 1.03 (s, 9H, TBDPS). ^{13}C NMR (101 MHz, CDCl₃) δ 170.4 (Ac), 166.2 (Bz), 138.6, 138.3, 137.9, 137.7, 136.0, 135.7, 133.7, 133.2, 133.0, 130.0, 129.8, 129.7, 129.7, 128.8, 128.7, 128.6, 128.6, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 126.8, 100.5 (C1'), 81.8, 81.4, 79.0 (C2), 77.8, 77.4, 75.4 (Bn), 75.3 (Bn), 74.9 (Bn), 73.5, 71.6 (Bn), 64.1 (C6), 62.5 (C6'), 53.5 (C2'), 44.0 (C5), 26.9 (TBDPS), 22.9 (NHAc), 19.4 (TBDPS). HRMS (ESI) m/z: [M+Na]$^+$ calculated for $C_{66}H_{71}NO_{10}SiNa$ 1088.4739, found 1088.4741.

2,3-di-*O*-benzyl-4-*O*-(2-*N*-acetyl-2-deoxy-3,4-di-*O*-benzyl-6-*O*-tert-butyldiphenylsilyl-D-glucopyranosyl)-cyclophellitol alkene (23)
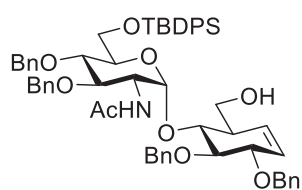
Benzoyl ester **22** (0.52 g, 0.49 mmol) was dissolved in MeOH/DCM (13.5 ml, 4.4/1, v/v). NaOMe (5.4 M in MeOH, 0.03 ml,0.15 mmol) was added and the reaction was stirred overnight. The reaction was quenched with NH$_4$Cl. Volatiles were removed under reduced pressure. The residue was dissolved in EtOAc and washed with H$_2$O and brine, dried over MgSO$_4$, filtered and concentrated under reduced pressure. Column chromatography (EtOAc/pentane, 9/1->3/2, v/v) yielded the product (0.46 g, 0.47 mmol, 97%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.70 (dd, *J* = 16.1, 7.0 Hz, 4H), 7.46 – 7.11 (m, 26H), 6.70 (d, *J* = 9.6 Hz, 1H, NH), 5.90 – 5.82 (m, 1H, alkene), 5.67 – 5.60 (m, 1H, alkene), 4.95 – 4.89 (m, 2H, H1', Bn), 4.83 (d, *J* = 10.6 Hz, 1H, Bn), 4.75 – 4.50 (m, 6H, Bn (6x)), 4.39 – 4.30 (m, 1H, H2'), 4.21 – 4.15 (m, 1H, H2), 3.99 – 3.56 (m, 9H), 2.41 – 2.34 (m, 1H, H5), 1.47 (s, 3H, NAc), 1.05 (s, 9H, TBDPS). ^{13}C NMR (101 MHz, CDCl$_3$) δ 170.4 (NAc), 138.5, 138.1, 137.9, 137.5, 136.0, 135.7, 133.6, 133.1, 129.8, 129.4, 128.7, 128.6, 128.6, 128.4, 128.3, 128.3, 128.2, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 126.7, 100.2 (C1'), 81.6, 81.2, 78.8 (C2), 78.0, 77.8, 75.2 (Bn), 74.8 (Bn), 73.5, 71.5 (Bn), 62.8 (C6'), 62.5 (C6), 53.3 (C2'), 46.1 (C5), 26.9 (TBDPS), 22.8 (Ac), 19.3 (TBDSP). HRMS (ESI) m/z: [M+Na]$^+$ calculated for C$_{59}$H$_{67}$NO$_9$SiNa 984.4477, found 984.4506.

2,3-di-*O*-benzyl-4-*O*-(2-*N*-acetyl-2-deoxy-3,4-di-*O*-benzyl-6-*O*-tert-butyldiphenylsilyl-D-glucopyranosyl)-6-*O*-tert-butyloxycarbonyl-cyclophellitol alkene (S1)
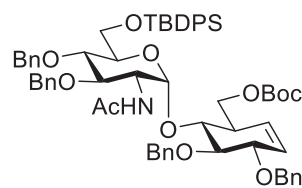
Alcohol **23** (0.72 g, 0.75 mmol) was coevaporated three times with toluene and dissolved in dry THF (5.4 ml, 0.14 M). Boc$_2$O (0.19 ml, 0.81 mmol) and DMAP (9 mg, 75 µmol) were added and the reaction was stirred at room temperature. After 1h the reaction was cooled to 0 C and quenched with H$_2$O. The reaction mixture was further diluted with H$_2$O and extracted with Et$_2$O. The organic layer was washed with NH$_4$Cl (aq. sat.), NaHCO$_3$ (aq. sat.) and brine, dried over MgSO$_4$, filtered and concentrated in vacuo. Flash column chromatography (PE/EtOAc, 9/1 -> 2/3, v/v) yielded the product (0.60 g, 0.57 mmol, 76%).

^{1}H NMR (300 MHz, CDCl$_3$) δ 7.78 – 7.63 (m, 4H), 7.47 – 7.11 (m, 26H), 6.75 (d, *J* = 9.7 Hz, 1H, NH), 5.82 (dt, *J* = 10.2, 2.5 Hz, 1H, alkene), 5.62 (ddd, *J* = 10.3, 2.6, 1.6 Hz, 1H, alkene), 4.99 – 4.84 (m, 3H, H1', Bn (2x)), 4.80 (d, *J* = 11.1 Hz, 1H, Bn), 4.75 – 4.51 (m, 5H, Bn (5x)), 4.45 – 4.27 (m, 3H, H2', H6ab), 4.23 (dt, *J* = 6.3, 2.3 Hz, 1H, H2), 4.04 – 3.68 (m, 7H), 2.52 (h, *J* = 3.1 Hz, 1H, H5), 1.45 (s, 3H, Ac), 1.42 (s, 9H, Boc), 1.06 (s, 9H, TBDPS). ^{13}C NMR (75 MHz, CDCl$_3$) δ 170.3 (NAc), 153.4 (Boc), 138.6, 138.3, 137.8, 137.6, 135.9, 135.6, 133.7, 133.0, 129.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.2, 128.2, 128.0, 128.0, 128.0, 127.9, 127.8, 127.7, 127.7, 127.7, 127.6, 127.5, 126.8, 100.5 (C1'), 82.1, 81.8, 81.7, 79.3 (C2), 77.7, 75.2 (Bn), 75.1 (Bn), 74.9 (Bn), 73.4, 71.5 (Bn), 66.1 (C6), 62.5 (C6'), 53.5 (C2'), 43.9 (C5), 27.7 (Boc), 26.9 (TBDPS), 22.7 (Ac), 19.3 (TBDPS). HRMS (ESI) m/z: [M+H]$^+$ calculated for C$_{64}$H$_{76}$NO$_{11}$Si 1062.5182, found 1062.5182.

1-iodo-2,3-di-*O*-benzyl-4-*O*-(2-*N*-acetyl-2-deoxy-3,4-di-*O*-benzyl-6-*O*-tert-butyldiphenylsilyl-D-glucopyranosyl)-6,7-*O*-carbonyl-cyclophellitol alkane (S2)
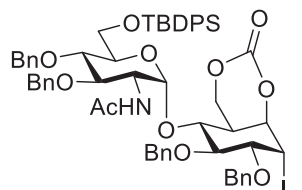
To a solution of Boc protected **S1** (0.22 g, 0.21 mmol) in DCM/AcOH (1.1 ml, 0.2 M, 2/1, v/v), NIS (0.09 g, 0.41 mmol) was added. After 22h the reaction mixture was diluted with Et$_2$O and quenched with Et$_3$N. The organic layer was washed with NH$_4$Cl (aq. sat.), NaHCO$_3$ (aq. sat.), Na$_2$S$_2$O$_3$ (aq. sat.) and brine, dried over MgSO$_4$, filtered and concentrated in vacuo. Flash column chromatography (PE/EtOAc, 9/1 -> 3/2, v/v) yielded the product (0.21 g, 0.19 mmol, 91%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.74 – 7.62 (m, 4H), 7.48 – 7.11 (m, 26H), 6.43 (d, *J* = 9.7 Hz, 1H, NH), 5.12 – 4.99 (m, 2H, Bn, H6a), 4.91 – 4.83 (m, 4H, H1', H0, Bn (2x)), 4.72 – 4.63 (m, 4H, H1, Bn (3x)), 4.60 (d, *J* = 10.5 Hz, 1H, Bn), 4.55 (d, *J* = 11.0 Hz, 1H, Bn), 4.41 (td, *J* = 10.0, 3.3 Hz, 1H, H2'), 4.06 (t, *J* = 6.7 Hz,

1H, H6b), 4.01 – 3.82 (m, 5H), 3.82 – 3.72 (m, 2H), 3.13 (dd, J = 9.3, 4.0 Hz, 1H, H2), 2.62 (dd, J = 10.4, 2.6 Hz, 1H, H5), 1.28 (s, 3H, Ac), 1.06 (s, 9H, TBDPS). ^{13}C NMR (101 MHz, CDCl$_3$) δ 170.4, 146.8, 138.4, 138.0, 137.0, 136.8, 135.8, 135.5, 133.6, 132.8, 129.9, 129.8, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 101.4, 82.1, 81.6, 81.4, 77.6, 77.3, 76.7, 76.2, 75.3, 75.0, 74.0, 72.2, 68.3, 62.6, 60.4, 53.3, 35.1, 30.4, 26.9, 22.5, 19.4. HRMS (ESI) m/z: [M+H]$^+$ calculated for C$_{60}$H$_{67}$INO$_{11}$Si 1132.3523, found 1132.3525.

2,3-di-*O*-benzyl-4-*O*-(2-*N*-acetyl-2-deoxy-3,4-di-*O*-benzyl-6-*O*-tert-butyldiphenylsilyl-D-glucopyranosyl)-cyclophellitol (24)
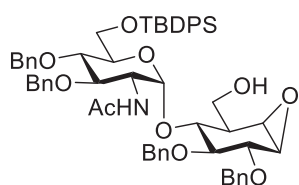
Iodide **S2** (0.12 g, 0.11 mmol) was dissolved in MeOH/DCM (1.9 ml, 0.06 M, 1.6/1, v/v), NaOMe (4.37 M in MeOH, 0.09 ml, 0.39 mmol) was added and the reaction was stirred for 20 hours. Et$_3$N·HCl was added and the solution was concentrated in vacuo. EtOAc was added and the solution was washed with H$_2$O and brine, dried over MgSO$_4$, filtered and concentrated in vacuo. The product was used and analyzed without further purification (0.12 g, 0.12 mmol, quantitative).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.76 – 7.66 (m, 4H), 7.47 – 7.15 (m, 26H), 6.83 (d, J = 9.7 Hz, 1H, NH), 4.95 (d, J = 11.0 Hz, 1H, Bn), 4.88 (d, J = 3.3 Hz, 1H, H1'), 4.85 – 4.77 (m, 2H), 4.73 – 4.63 (m, 4H), 4.47 (d, J = 11.2 Hz, 1H), 4.35 (td, J = 9.8, 3.3 Hz, 1H, H2'), 4.12 – 4.05 (m, 1H, H6a), 4.01 – 3.93 (m, 2H, H6b/H6a'), 3.93 – 3.78 (m, 4H, H6b'), 3.72 – 3.52 (m, 3H, H3'/H4), 3.34 – 3.31 (m, 1H, epoxide), 3.15 (d, J = 3.7 Hz, 1H, epoxide), 2.03 – 1.97 (m, 1H, H5), 1.44 (s, 3H, NAc), 1.06 (s, 9H, TBDPS). ^{13}C NMR (101 MHz, CDCl$_3$) δ 170.3 (NAc), 138.6, 138.2, 137.5, 137.2, 136.1, 135.7, 133.7, 133.1, 129.8, 128.8, 128.7, 128.6, 128.4, 128.3, 128.2, 128.2, 127.9, 127.9, 127.7, 127.7, 127.6, 100.8 (C1'), 83.1, 81.5, 80.1, 77.6, 75.9, 75.6 (Bn), 75.1 (Bn), 74.9 (Bn), 73.7, 72.8 (Bn), 62.7 (C6'), 61.8 (C6), 56.6 (epoxide), 53.3 (C2'), 52.2 (epoxide), 44.5 (C5), 26.9 (TBDPS), 22.8 (Ac), 19.4 (TBDPS). HRMS (ESI) m/z: [M+Na]$^+$ calculated for C$_{59}$H$_{67}$NO$_{10}$SiNa 1000.4426, found 1000.4452.

2,3-di-*O*-benzyl-4-*O*-(2-*N*-acetyl-2-deoxy-3,4-di-*O*-benzyl-6-*O*-tert-butyldiphenylsilyl-D-glucopyranosyl)-glucurono-cyclophellitol (25)
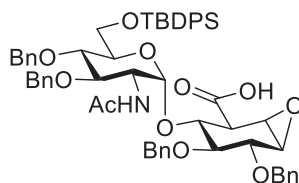
Alcohol **24** (0.13 g, 0.13 mmol) was dissolved in *t*-BuOH/DCM/H$_2$O (4.9 ml, 5/4/1, v/v). and cooled down to 0°C. TEMPO (4 mg, 0.03 mmol) and BAIB (0.10 g, 0.32 mmol) were added and the reaction was stirred 23 hours. The reaction was diluted with DCM and quenched with Na$_2$S$_2$O$_3$ (aq. sat.). The layers were separated and the water layer was acidified with AcOH. The water layer was extracted with DCM (4x). The combined organic layers were dried over MgSO$_4$, filtered and concentrated under reduced pressure. Column chromatography (EtOAc/pentane, 4/1 -> 1/1, v/v, 1% AcOH) afforded the product (0.10 mg, 0.10 mmol, 80%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.76 (d, J = 3.4 Hz, 2H), 7.68 (d, J = 6.8 Hz, 2H), 7.50 – 7.13 (m, 26H), 6.04 (d, J = 9.6 Hz, 1H, NH), 4.97 – 4.74 (m, 6H, Bn(5x)/H1'), 4.69 – 4.53 (m, 3H, Bn (3x)), 4.30 – 4.22 (m, 1H, H2'), 4.08 (d, J = 9.7 Hz, 1H), 3.99 (t, J = 9.5 Hz, 1H), 3.95 – 3.87 (m, 2H), 3.80 – 3.64 (m, 3H), 3.48 – 3.42 (m, 1H, H2), 3.29 (s, 1H, epoxide), 3.17 (d, J = 3.5 Hz, 1H, epoxide), 2.79 – 2.73 (m, 1H, H5), 1.25 (s, 3H, Ac), 1.05 (s, 9H, TBDPS). ^{13}C NMR (101 MHz, CDCl$_3$) δ 170.6, 170.1 (NHAc/COOH), 138.6, 138.4, 137.6, 137.1, 136.6, 135.8, 133.7, 133.6, 130.0, 129.8, 128.8, 128.8, 128.7, 128.5, 128.4, 128.4, 128.2, 128.2, 128.0, 127.9, 127.8, 127.7, 100.5 (C1'), 81.8, 81.4, 79.1, 77.7, 75.9, 75.6 (Bn), 75.4 (Bn), 75.4 (Bn), 73.2, 72.9 (Bn), 62.2 (C6'), 54.1 (epoxide), 53.7 (epoxide), 53.4 (C2'), 48.7 (C5), 27.0 (TBDPS), 22.5 (Ac), 19.3 (TBDPS). HRMS (ESI) m/z: [M+Na]$^+$ calculated for C$_{59}$H$_{65}$NO$_{11}$SiNa 1014.4219, found 1014.4223.

### 2,3-di-*O*-benzyl-4-*O*-(2-*N*-acetyl-2-deoxy-3,4-di-*O*-benzyl-D-glucopyranosyl)-glucurono-cyclophellitol (26)
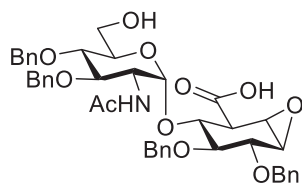
Silyl protected **25** (89 mg, 0.090 mmol) was dissolved in anhydrous THF (1.9 ml). $Et_3N \cdot 3HF$ (0.046 ml ,0.28 mmol) was added and the reaction was stirred for 20 hours. More $Et_3N \cdot 3HF$ (0.030 ml, 0.18 mmol) was added and the reaction was stirred 23 hours. The reaction was diluted with DCM and washed with water. The water layer was extracted with DCM (4x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. Column chromatography (EtOAc/pentane, 4/1 -> 9/1, v/v, 1% AcOH) yielded the product (62 mg, 0.082 mmol, 87%).

^1H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.15 (m, 20H), 6.35 (d, *J* = 9.4 Hz, 1H, NH), 4.96 (d, *J* = 10.7 Hz, 1H, Bn), 4.88 – 4.74 (m, 4H, H1'/Bn (3x)), 4.74 – 4.63 (m, 2H, Bn), 4.63 – 4.49 (m, 2H, Bn), 4.39 – 4.28 (m, 1H, H2'), 4.02 – 3.89 (m, 3H, H4/H2/H6a'), 3.87 – 3.77 (m, 3H, H4'/H5'/H6b'), 3.68 – 3.61 (m, 1H, H3'), 3.49 (t, *J* = 9.3 Hz, 1H, H3), 3.44 – 3.39 (m, 1H, epoxide), 3.22 – 3.16 (m, 1H, epoxide), 2.88 (d, *J* = 8.3 Hz, 1H, H5), 1.19 (s, 3H, Ac). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.2, 172.3 (NAc/COOH), 138.6, 138.3, 137.7, 137.1, 129.1, 128.8, 128.8, 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 128.3, 128.2, 127.9, 127.6, 127.6, 125.4, 100.5 (C1'), 81.8 (C3'), 81.5 (C3), 79.3, 77.6, 76.7, 75.8 (Bn), 75.2 (Bn), 75.1 (Bn), 73.3, 72.8 (Bn), 61.0 (C6'), 54.4 (epoxide), 53.6 (epoxide/C2'), 49.4 (C5), 22.7 (Ac). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{43}H_{47}NO_{11}Na$ 776.3041 found 776.3058.

2,3-di-*O*-benzyl-4-*O*-(2-*N*-acetyl-2-deoxy-3,4-di-*O*-benzyl-6-*O*-sulfo-D-glucopyranosyl)-glucurono-cyclophellitol (27)
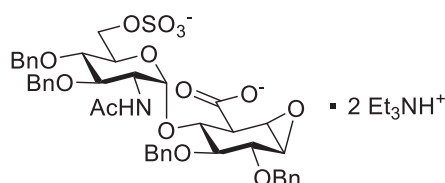
Alcohol **26** (0.062 g, 0.082 mmol) was dissolved in dry DMF (1.1 ml, 0.08 M). $SO_3 \cdot Et_3N$ (0.031 g, 0.17 mmol) was added and the mixture was stirred for 4h. Et_3N (0.07 ml, 0.5 mmol) was added, the reaction mixture was diluted with MeOH (2 ml) and concentrated under reduced pressure. The crude mixture was analyzed and used without purification.

^1H NMR (400 MHz, MeOD) δ 7.45 – 7.17 (m, 20H), 5.42 (d, *J* = 3.9 Hz, 1H, H1'), 4.90 – 4.59 (m, 8H, Bn), 4.41 (d, *J* = 10.6 Hz, 1H, H6a'), 4.24 (d, *J* = 10.6 Hz, 1H, H6b'), 4.17 (dd, *J* = 10.0, 3.9 Hz, 1H, H2'), 4.08 (t, *J* = 9.7 Hz, 1H, H4), 3.91 – 3.83 (m, 2H, H5'/H4'), 3.78 – 3.63 (m, 3H, H3'/H3/H2), 3.52 – 3.47 (m, 1H, epoxide), 3,33 (under solvent peak, 1H, epoxide) 3.20 (dt, *J* = 8.3, 6.7 Hz, 19H, H5/Et_3N), 1.74 (s, 3H), 1.31 (td, *J* = 7.3, 1.4 Hz, 29H, Et_3N). ^{13}C NMR (101 MHz, MeOD) δ 174.2, 172.9 (NHAc/COOH), 140.1, 139.8, 139.6, 139.0, 129.5, 129.5, 129.4, 129.3, 129.2, 129.0, 128.7, 128.6, 128.5, 128.5, 99.3 (C1'), 84.8 (C3), 82.0, 81.4, 79.0 (C2), 76.2 (Bn), 75.9 (Bn), 75.2 (Bn), 73.3 (Bn), 72.8 (C4), 71.5, 66.8 (C6'), 55.6 (epoxide), 54.2 (C2'), 53.8 (epoxide), 49.4 (C5), 47.9 (Et_3N), 22.9 (Ac), 9.3 (Et_3N). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{43}H_{47}NO_{14}S$ 834.2790, found 834.2785.

4-*O*-(2-*N*-acetyl-2-deoxy-D-glucopyranosyl)-glucurono-cyclophellitol (28)
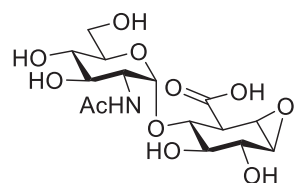
Ammonia (10 ml) was condensed at -70°C. Sodium (17 mg, 0.72 mmol) was added and the solution turned blue. Benzyl protected **26** (14 mg, 0.018 mmol), dissolved in anhydrous THF (2 ml) and *t*-BuOH (0.068 ml, 0.72 mmol) was added. After stirring at -60°C for 15 minutes the blue color faded and more sodium (12 mg, 0.51 mmol) was added. After another 30 minutes the bl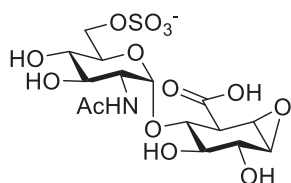ue color faded again the reaction was quenched with NH_4Cl (66 mg, 1.23 mmol) The ammonia was evaporated, water was added and the compound was desalted by size exclusion over HW-40 (1% AcOH in water).Lyophilization afforded the compound as a white solid (6.42 mg, 0.016 mmol, 91%).

^1H NMR (500 MHz, D_2O) δ 5.30 (d, *J* = 3.7 Hz, 1H, H1'), 3.97 – 3.93 (m, 1H, H2'), 3.93 – 3.88 (m, 4H, H4/H6' (2x)), 3.85 – 3.81 (m, 2H), 3.64 – 3.56 (m, 3H, epoxide), 3.32 – 3.29 (m, 1H, epoxide), 2.99 (d, *J* = 9.6 Hz, 1H, H5), 2.13 (s, 3H, Ac). ^{13}C NMR (126 MHz, D_2O) δ 177.6, 174.4 (NAc/COOH), 97.7 (C1'), 76.2,

75.0, 72.0, 71.5, 71.0, 69.7, 60.1 (C6'), 56.2 (epoxide), 55.5 (epoxide), 54.0 (C2'), 50.8 (C5), 22.0 (Ac). HRMS (ESI) m/z: [M+Na]$^+$ calculated for $C_{15}H_{23}NO_{11}Na$ 416.1163, found 416.1164.

4-O-(2-N-acetyl-2-deoxy-6-O-sulfo-D-glucopyranosyl)-glucurono-cyclophellitol (29)

Ammonia was condensed at -60°C under an inert atmosphere and Na (37 mg, 1.6 mmol) was added. The solution turned dark blue. Benzyl protected **27** (35 μmol, 33 mg) was dissolved in THF/t-BuOH (0.6 ml, 2/3, v/v) and added to the solution. After stirring for 45 minutes the reaction was quenched with NH_4Cl (0.10 g, 1.92 mmol). Ammonia was evaporated at room temperature, diluted in water and concentrated under reduced pressure. The compound was desalted by size exclusion over HW-40 (1% AcOH in water). Lyophilization afforded the compound as a white solid (14.9 mg, 0.03 mmol, 87% over 2 steps).

^{1}H NMR (400 MHz, D_2O) δ 5.22 (d, J = 3.7 Hz, 1H, H1'), 4.33 (dd, J = 11.0, 2.2 Hz, 1H, H6a'), 4.15 (dd, J = 11.1, 2.1 Hz, 1H, H6b'), 3.88 – 3.75 (m, 4H, H2'/H5'), 3.70 (dd, J = 10.7, 9.1 Hz, 1H), 3.56 (dd, J = 10.1, 9.1 Hz, 1H), 3.50 – 3.44 (m, 2H, epoxide), 3.15 (d, J = 3.7 Hz, 1H, epoxide), 2.83 (dd, J = 9.7, 1.9 Hz, 1H, H5), 1.99 (s, 3H, Ac). ^{13}C NMR (101 MHz, D_2O) δ 180.6, 177.7, 174.3 (NAc/COOH/AcOH), 97.4 (C1'), 76.5, 74.4, 71.6, 70.8, 69.9, 69.0, 66.3 (C6'), 56.1 (epoxide), 55.3 (epoxide), 53.7 (C2'), 50.9 (C5), 22.7 (AcOH), 21.9 (Ac). HRMS (ESI) m/z: [M+2H]$^+$ calculated for $C_{15}H_{24}NO_{14}S$ 474.09120 found 474.09129.

Late stage azide reduction

### 2,3-di-O-benzyl-4-O-(2-Azido-2-deoxy-3,4-di-O-benzyl-6-O-tert-butyldiphenylsilyl-D-glucopyranosyl)-cyclophellitol alkene (30)
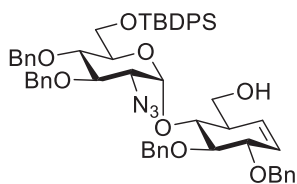
Ester **21** (0.98 g, 0.93 mmol) was dissolved in MeOH/DCM (1/1, v/v, 18 ml). NaOMe (25 wt%, 0.1 ml, 0.44 mmol) was added and the reaction mixture was stirred overnight. NH_4Cl was added and volatiles were removed under reduced pressure. The crude product was purified using column chromatography (pentane/EtOAc, 1/19 -> 3/17, v/v) yielding the product as a colorless oil (0.91 g, 0.96 mmol, quant.).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 – 7.61 (m, 4H), 7.44 – 7.21 (m, 24H), 7.17 – 7.10 (m, 2H), 5.80 (dt, J = 10.2, 2.4 Hz, 1H, alkene), 5.65 (d, J = 4.0 Hz, 1H H1'), 5.59 (dt, J = 10.2, 2.2 Hz, 1H, alkene), 5.06 (d, J = 10.9 Hz, 1H, Bn), 4.95 (d, J = 10.9 Hz, 1H, Bn), 4.89 – 4.79 (m, 3H, Bn), 4.71 – 4.57 (m, 3H, Bn), 4.23 (m, 1H, H2), 4.02 – 3.89 (m, 3H, H4/ H3'/H4), 3.88 – 3.80 (m, 3H, H6'ab/H5'), 3.76 – 3.57 (m, 3H, H6ab/H3), 3.29 (dd, J = 10.3, 4.0 Hz, 1H, H2'), 2.54 – 2.44 (m, 1H, H5), 1.04 (s, 9H, TBDPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.0, 138.3, 138.0, 136.0, 135.8, 133.4, 133.1, 129.9, 128.9, 128.6, 128.6, 128.6, 128.5, 128.5, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.5, 98.2 (C1'), 84.2 (C3), 80.9 (C2), 80.3 (C4'), 78.4 (C3'), 75.7 (Bn), 75.3 (Bn), 74.6 (Bn), 74.4 (C4), 72.9 (C5'), 71.7 (Bn), 63.6 (C2'), 63.0 (C6'), 62.6 (C6), 45.5 (C5), 27.0 (TBDPS), 19.4 (TBDPS). HRMS (ESI) m/z: [M+Na]$^+$ calculated for $C_{57}H_{63}N_3O_8SiNa$ 968.4277, found 968.4274.

2,3-di-O-benzyl-4-O-(2-Azido-2-deoxy-3,4-di-O-benzyl-6-O-tert-butyldiphenylsilyl-D-glucopyranosyl)-6-O-tert-butyloxycarbonyl-cyclophellitol alkene (S3)
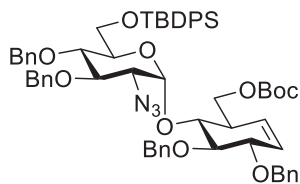
Alcohol **30** (0.92 g, 0.93 mmol) was co-evaporated with toluene and subsequently dissolved in THF (9.3 ml, 0.1 M). DMAP (91 mg, 0.74 mmol) and Boc_2O (0.41 g, 1.87 mmol) were added. The reaction was stirred at room temperature for 3 hours. Water was added and the mixture was stirred for 15 minutes. The mixture was extracted with Et_2O (3x), the combined organic layers were washed with NH_4Cl (aq. sat.), $NaHCO_3$ (aq. sat.) and brine , dried over $MgSO_4$, filtered and concentrated under reduced pressure. The product was obtained after column chromatography (Et_2O/pentane, v/v/, 0/1 -> 1/9) as a colorless oil (0.80 g, 0.76 mmol, 82%).

^{1}H NMR (500 MHz, CDCl$_3$) δ 7.73 – 7.59 (m, 4H), 7.44 – 7.12 (m, 26H), 5.76 (dt, J = 10.2, 2.4 Hz, 1H, alkene), 5.66 (d, J = 3.9 Hz, 1H, H1'), 5.57 (dt, J = 10.2, 2.3 Hz, 1H, alkene), 5.05 (d, J = 10.9 Hz, 1H, Bn), 4.94 (d, J = 10.9 Hz, 1H, Bn), 4.91 – 4.83 (m, 3H, Bn), 4.74 (d, J = 10.9 Hz, 1H, Bn), 4.70 – 4.60 (m, 2H, Bn), 4.26 – 4.16 (m, 2H, H6a/H2), 4.03 – 3.94 (m, 3H, H3'/H6b/H6a'), 3.93 – 3.81 (m, 4H, H4/H3/H6b'/H4'), 3.75 – 3.69 (m, 1H, H5'), 3.28 (dd, J = 10.4, 3.9 Hz, 1H, H2'), 2.69 – 2.61 (m, 1H, H5), 1.25 (s, 9H, Boc), 1.03 (s, 9H, TBDPS). ^{13}C NMR (126 MHz, CDCl$_3$) δ 153.5 (Boc), 139.0, 138.3, 138.1, 136.0, 135.7, 133.8, 133.2, 129.7, 129.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.5, 98.0 (C1'), 84.3, 82.1 (Boc), 80.7 (C2), 80.3 (C3'), 78.2, 75.7 (Bn), 75.1 (Bn), 74.6 (Bn), 74.1, 72.7 (C5'), 71.8 (Bn), 66.6 (C6), 63.7 (C2'), 62.3 (C6'), 43.0 (C5), 27.7, 27.0 (TBDPS/Boc), 19.4(TBDPS). HRMS (ESI) m/z: [M+Na]$^+$ calculated for C$_{62}$H$_{71}$N$_3$O$_{10}$SiNa 1068.48009, found 1068.48007.

1-iodo-2,3-di-*O*-benzyl-4-*O*-(2-azido-2-deoxy-3,4-di-*O*-benzyl-6-*O*-tert-butyldiphenylsilyl-ᴅ-glucopyranosyl)-6,7-*O*-carbonyl-cyclophellitol alkane (S4)
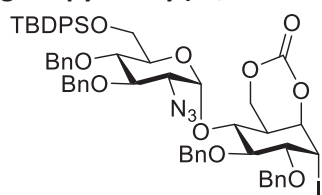
Alkene **S3** (0.80 g, 0.77 mmol) was dissolved in AcOH/DCM (1/2, v/v, 5.1 ml, 0.15 M). NIS (0.35 g , 1.54 mmol) was added and the reaction was stirred for 18 hours in the dark. The mixture was diluted with Et$_2$O, washed with Na$_2$S$_2$O$_3$ (aq.), NaHCO$_3$ (aq. sat. 2x) and brine, dried over MgSO$_4$, filtered and concentrated under reduced pressure. The product was obtained after column chromatography (Et$_2$O/pentane, 1/4 -> 1/1, v/v) as a foam (0,61 g, 0.55 mmol, 71%).

^{1}H NMR (500 MHz, CDCl$_3$) δ = 7.64 (ddt, J=6.6, 2.8, 1.4, 4H), 7.45 – 7.22 (m, 24H), 7.21 – 7.11 (m, 2H), 5.50 (d, J=3.9, 1H, H1'), 5.03 (d, J=10.7, 1H, Bn), 4.93 – 4.83 (m, 5H, H7/Bn (4x)), 4.72 – 4.63 (m, 2H, Bn (2x)), 4.63 – 4.54 (m, 3H, H1/Bn/H6a), 4.27 (dd, J=11.6, 3.3, 1H, H6b), 4.00 – 3.93 (m, 2H, H6a'/H3), 3.94 – 3.82 (m, 4H, H3'/H6b'/H4/H4'), 3.63 (dt, J=9.7, 2.3, 1H, H5'), 3.36 (dd, J=10.2, 3.9, 1H, H2'), 3.13 (dd, J=8.8, 4.0, 1H, H2), 2.79 – 2.73 (m, 1H, H5), 1.06 (s, 9H, TBDPS).

^{13}C NMR (126 MHz, CDCl$_3$) δ 146.7 (carbonate), 138.5, 137.9, 137.8, 137.2, 136.0, 135.8, 133.6, 132.9, 129.9, 129.9, 128.7, 128.5, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 127.9, 127.8, 127.7, 127.4, 98.6 (C1'), 83.4 (C3), 81.1 (C7), 80.2 (C3'), 78.0, 77.2 (C2), 75.8 (Bn), 75.4 (Bn), 75.1 (Bn), 74.0, 73.4 (C5'), 72.5 (Bn), 68.6 (C6), 63.6 (C2'), 62.0 (C6'), 34.0 (C5), 29.9 (C1), 27.1 (TBDPS), 19.5 (TBDPS). HRMS (ESI) m/z: [M+NH$_4$]$^+$ calculated for C$_{58}$H$_{66}$IN$_4$O$_{10}$Si 1133.3587, found 1133.3593.

### 2,3-di-*O*-benzyl-4-*O*-(2-azido-2-deoxy-3,4-di-*O*-benzyl-6-*O*-tert-butyldiphenylsilyl-ᴅ-glucopyranosyl)-cyclophellitol (31)
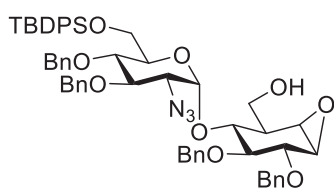
Iodide **S4** (0.606 g 0.543 mmol) was dissolved in DCM/MeOH (5.4 ml, 1/1, v/v). NaOMe (4.4M, 0.04 ml, 1.63 mmol) was added and the reaction was left to stir overnight. More NaOMe (4.4M, 0.04 ml, 1.63 mmol) was added and the reaction was stirred for 7 hours. Upon completion the reaction was quenched with NH$_4$Cl (35 mg, 0.65 mmol). The solvent was removed under reduced pressure and column chromatography (pentane/EtOAc, 85/15 -> 65/35, v/v) afforded the product as an oil (0.492 g, 0.511 mmol, 94%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.71 – 7.62 (m, 4H), 7.44 – 7.24 (m, 24H), 7.18 – 7.12 (m, 2H), 5.61 (d, J = 4.0 Hz, 1H, H1'), 4.99 (d, J = 10.9 Hz, 1H, Bn), 4.91 – 4.76 (m, 5H, Bn), 4.69 – 4.61 (m, 2H, Bn), 3.97 – 3.78 (m, 6H, H3'/H6ab/H6ab'), 3.78 – 3.65 (m, 4H), 3.41 – 3.36 (m, 1H, epoxide), 3.24 (dd, J = 10.3, 4.0 Hz, 1H, H2'), 3.17 (d, J = 3.8 Hz, 1H, epoxide), 2.23 – 2.16 (m, 1H, H5), 1.05 (s, 9H, TBDPS). ^{13}C NMR (101 MHz, CDCl$_3$) δ 138.9, 138.0, 137.5, 136.0, 135.8, 133.6, 133.2, 129.8, 128.7, 128.6, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 128.0, 127.8, 127.7, 127.6, 127.5, 97.9 (C1'), 84.8, 80.5, 80.3 (C3'), 78.3, 75.7 (Bn), 75.2 (Bn), 74.6 (Bn), 72.9 (Bn and CH), 70.4, 63.4(C2'), 62.7, 62.1 (C6/C6'), 56.0 (epoxide), 52.8 (epoxide), 43.2 (C5), 27.0 (TBDPS), 19.4 (TBDPS). HRMS (ESI) m/z: [M+NH$_4$]$^+$ calculated for C$_{57}$H$_{67}$N$_4$O$_9$Si 979.4672, found 979.4669.

2,3-di-*O*-benzyl-4-*O*-(2-azido-2-deoxy-3,4-di-*O*-benzyl-6-*O*-tert-butyldiphenylsilyl-ᴅ-glucopyranosyl)-glucurono-cyclophellitol (32)
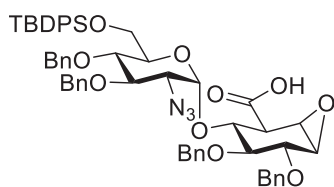
Alcohol **31** (0.492 g, 0.511 mmol) was dissolved in *t*-BuOH/ DCM/H_2O (3.5 ml, 6/4/1, v/v). TEMPO (0.016 g, 0.10 mmol) and BAIB (0.412 g, 1.28 mmol) were added and the reaction was stirred overnight. After TLC analysis (EtOAc/pentane, 1/3 with 0.5% Et_3N, v/v) showed full conversion of the starting material the reaction was diluted with DCM and water. $Na_2S_2O_3$ was added the mixture was shaken and the layers were separated. The water layer was extracted with DCM (4x). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The product was obtained after column chromatography (pentane/EtOAc, 3/17 -> 1/4 v/v, 0.5% AcOH,) as an oil (0.538 g, 0.525 mmol, quant.).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 – 7.63 (m, 4H), 7.43 – 7.25 (m, 24H), 7.21 – 7.16 (m, 2H), 5.38 (d, *J* = 3.7 Hz, 1H, H1'), 4.94 – 4.80 (m, 5H, Bn), 4.76 – 4.62 (m, 3H, Bn), 4.04 (t, *J* = 9.6 Hz, 1H, H4), 4.00 – 3.89 (m, 2H, H3', H6a'), 3.86 – 3.79 (m, 3H, H2/H6b'/H4'), 3.64 (m, 1H, H5'), 3.54 (dd, *J* = 9.6, 8.2 Hz, 1H, H3), 3.29 (dd, *J* = 10.2, 3.8 Hz, 1H, H2'), 3.21 – 3.18 (m, 1H, epoxide), 3.09 (d, *J* = 3.6 Hz, 1H, epoxide), 2.89 (dd, *J* = 9.4, 1.5 Hz, 1H, H5), 1.04 (s, 9H, TBDPS). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.2 (COOH), 138.7, 138.2, 137.9, 137.4, 136.2, 135.8, 133.6, 133.3, 129.9, 129.8, 128.7, 128.6, 128.6, 128.5, 128.4, 128.2, 128.1, 128.1, 127.9, 127.9, 127.9, 127.8, 127.7, 98.4 (C1'), 83.0, 80.3 (C3'), 79.4, 78.1 (C2/C4'), 75.8 (Bn), 75.2 (Bn), 75.0 (Bn), 73.6 (C4), 73.2 (Bn), 72.5 (C5'), 63.8 (C2'), 62.3 (C6'), 53.9 (epoxide), 53.5 (epoxide), 48.1 (C5), 27.0 (TBDPS), 19.4 (TBDPS). HRMS (ESI) m/z: [M+NH4]+ calculated for $C_{57}H_{65}N_4O_{10}Si$ 993.44645 found 993.44650.

2,3-di-*O*-benzyl-4-*O*-(2-azido-2-deoxy-3,4-di-*O*-benzyl-ᴅ-glucopyranosyl)-glucurono-cyclophellitol (33)
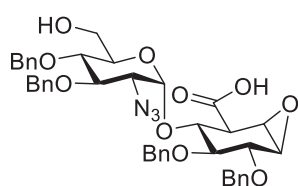
Silyl protected **32** (0.525 g, 0.538 mmol) was dissolved in THF (2.7 ml), 3HF·Et_3N (0.44 ml, 2.69 mmol (8.07 mmol HF)) was added and the mixture was stirred for 45 hours. The reaction was poured over water and extracted with DCM (4x). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Column chromatography (EtOAc/pentane, 35/65 -> 45/55, v/v, 0.5% AcOH) afforded the product as a white solid (0.340 g, 0.461 mmol, 86%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 – 7.23 (m, 20H), 5.41 (d, *J* = 4.0 Hz, 1H, H1'), 4.90 (s, 2H, Bn), 4.87 – 4.79 (m, 3H, Bn), 4.74 (d, *J* = 11.3 Hz, 1H, Bn), 4.63 (dd, *J* = 14.7, 11.2 Hz, 2H, Bn), 4.12 (t, *J* = 9.7 Hz, 1H, H4), 3.93 – 3.85 (m, 2H, H3'/H2), 3.81 (dd, *J* = 11.8, 2.5 Hz, 1H, H6a'), 3.72 (ddd, *J* = 10.1, 4.8, 2.5 Hz, 1H, H5'), 3.64 – 3.58 (m, 2H, H6b'/H3), 3.46 – 3.41 (m, 2H, H4'/epoxide), 3.26 (dd, *J* = 10.3, 3.9 Hz, 1H, H2'), 3.20 (d, *J* = 3.7 Hz, 1H, epoxide), 3.04 (dd, *J* = 9.5, 2.1 Hz, 1H, H5). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.8 (C6), 138.8, 137.8, 137.7, 137.4, 128.7, 128.7, 128.6, 128.4, 128.2, 128.2, 128.1, 128.1, 127.5, 127.4, 98.7 (C1'), 83.0 (C3), 80.3 (C3'), 79.6 (C2), 78.4 (C4'), 75.6 (Bn), 75.2 (Bn), 74.9 (Bn), 74.3 (C4), 73.2 (Bn), 72.1 (C5), 63.6 (C2'), 61.9 (C6'), 54.3 (epoxide), 53.7 (epoxide), 48.4 (C5). HRMS (ESI) m/z: [M+NH4]+ calculated for $C_{41}H_{47}N_4O_{10}$ 755.3287 found 755.3284.

4-*O*-(2-deoxy-ᴅ-glucopyranosyl)-glucurono-cyclophellitol (34)
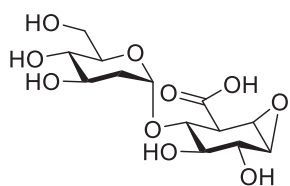
Ammonia (8 ml) was condensed at -70°C. Sodium (31 mg, 1.35 mmol) was added and the solution turned blue. Benzyl protected **33** (50 mg, 0.068 mmol), dissolved in anhydrous THF (2ml). and *t*-BuOH (0.16 ml, 1.69 mmol) was added After stirring at -60°C for 45 minutes the reaction was quenched with NH_4Cl (109 mg, 2.04 mmol) The ammonia was evaporated, water was added and the compound was desalted by size exclusion over HW-40 (1% AcOH in water).Lyophilization afforded the compound as a white solid. (22.7 mg, 0.068 mmol, quant.)

^{1}H NMR (500 MHz, D_2O) δ 5.31 (d, *J* = 3.7 Hz, 1H, H1'), 3.88 (ddd, *J* = 11.9, 9.1, 4.9 Hz, 1H, H3'), 3.84 – 3.76 (m, 4H, H6ab'/H4/H2), 3.67 (dt, *J* = 10.0, 3.1 Hz, 1H, H5'), 3.48 – 3.45 (m, 1H, epoxide), 3.43 (dd, *J*

= 10.2, 8.7 Hz, 1H/H3), 3.35 (dd, *J* = 10.0, 9.2 Hz, 1H, H4'), 3.18 (d, *J* = 3.7 Hz, 1H, epoxide), 2.80 (dd, *J* = 9.8, 1.9 Hz, 1H, H5), 2.19 (ddd, *J* = 13.3, 5.0, 1.3 Hz, 1H, H2a'), 1.64 (ddd, *J* = 13.2, 11.9, 3.9 Hz, 1H, H2'). ^{13}C NMR (126 MHz, D_2O) δ 179.8 (COOH), 97.7 (C1'), 75.7 (C3), 73.7, 71.6 (C5'), 70.4, 70.3 (C4'), 67.6 (C3'), 59.7 (C6'), 55.6 (epoxide), 54.7 (epoxide), 50.4 (C5), 36.4 (C2'). HRMS (ESI) m/z: [M+H]$^+$ calculated for $C_{13}H_{24}O_{10}$ 354.13947 found 354.13937.

2,3-di-*O*-benzyl-4-*O*-(2-amino-2-deoxy-3,4-di-*O*-benzyl-D-glucopyranosyl)-glucurono-cyclophellitol (35)
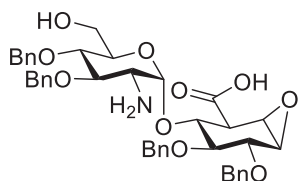
Azide **33** (50 mg, 0.068 mmol) was dissolved in MeOH/toluene (2.5 ml, 4/1,v/v). Zn dust (132 mg, 2.02 mmol) and NH_4Cl (144 mg, 2.69 mmol) were added portion wise over the 2 hours. The reaction was stirred for another hour followed by filtration over silica with MeOH/toluene (1/1, v/v). This afforded the poorly soluble product which was analyzed and used without further purification.

^{1}H NMR (400 MHz, MeOD) δ 7.39 – 7.22 (m, 20H), 5.52 (d, *J* = 3.6 Hz, 1H, H1'), 4.79 (d, *J* = 11.3 Hz, 1H, Bn), 4.75 (d, *J* = 11.9 Hz, 1H, Bn), 4.69 (d, *J* = 9.6 Hz, 2H, Bn), 4.54 (d, *J* = 11.3 Hz, 1H, Bn), 4.21 (t, *J* = 9.7 Hz, 1H, H4), 4.00 (d, *J* = 7.1 Hz, 1H, H2), 3.95 – 3.84 (m, 3H, H3'/H6a'/H5'), 3.84 – 3.74 (m, 2H, H3/H6b'), 3.64 (t, *J* = 9.0 Hz, 1H, H4'), 3.50 (dd, *J* = 4.1, 1.6 Hz, 1H, epoxide), 3.28 (d, *J* = 3.7 Hz, 1H,epoxide), 3.23 (dd, *J* = 10.1, 3.6 Hz, 1H, H2'), 3.10 (d, *J* = 9.7 Hz, 1H, H5). HRMS (ESI) m/z: [M+H]$^+$ calculated for $C_{41}H_{46}NO_{10}$ 712.3116 found 712.3112.

4-*O*-(2-amino-2-deoxy-D-glucopyranosyl)-glucurono-cyclophellitol (36)
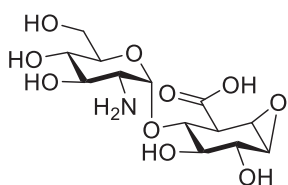
Anhydrous THF (1 ml). and *t*-BuOH (0.32 ml, 3.4 mmol) were added to benzyl protected **35** (crude, 0.068 mmol). The flask was cooled to -70°C and ammonia (5 ml) was condensed directly in the flask. Sodium (63 mg, 2.72 mmol) was added and the solution turned blue. After stirring at -60°C for 45 minutes the reaction was quenched with NH_4Cl (0.18 g, 3.4 mmol) The ammonia was evaporated, water was added and the compound was desalted by size exclusion over HW-40 (1% AcOH in water).Lyophilization afforded the compound as a white solid (12.1 mg, 0.035 mmol, 51% over 2 steps).

^{1}H NMR (500 MHz, D_2O) δ 5.49 (d, *J* = 3.8 Hz, 1H, H1'), 3.93 – 3.85 (m, 3H, H3'/H4/H2), 3.83 (d, *J* = 2.9 Hz, 2H, H6ab'), 3.75 (dt, *J* = 10.1, 2.9 Hz, 1H, H5'), 3.58 – 3.50 (m, 3H, H3/H4'/epoxide), 3.30 (dd, *J* = 10.7, 3.8 Hz, 1H, H2'), 3.21 (d, *J* = 3.7 Hz, 1H, epoxide), 2.90 (dd, *J* = 9.8, 1.8 Hz, 1H, H5). ^{13}C NMR (126 MHz, D_2O) δ 177.7 (C6), 96.1 (C1'), 76.4 (C3), 75.2, 72.1 (C5'), 71.4, 69.7, 69.2 (C4'), 59.7 (C6'), 56.2 (epoxide), 55.4 (epoxide), 54.4 (C2'), 50.9 (C5). HRMS (ESI) m/z: [M+H]$^+$ calculated for $C_{13}H_{22}NO_{10}$ 352.1235, found 352.1238.

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

The appropriate carboxylic acid (50 µmol) was dissolved in DMF (1 ml), 2,3,4,5,6-pentafluorophenol (46 mg, 0.26 mmol), Et$_3$N (20 µl, 0.26 mmol) and DIC (7.8 µl, 50 µmol) were added and the mixture was stirred for 90 minutes. Part of the stock solution (1.5 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 (50mM AcOH in H_2O). The fractions were concentrated under reduced pressure, coevaporated with water, diluted with water and lyophilized to yield the product.
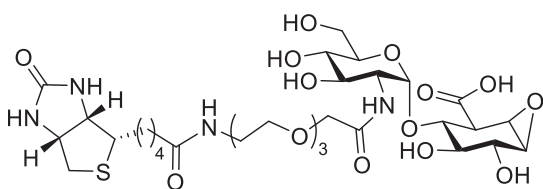
2'-N Cy5 conjugated probe 37
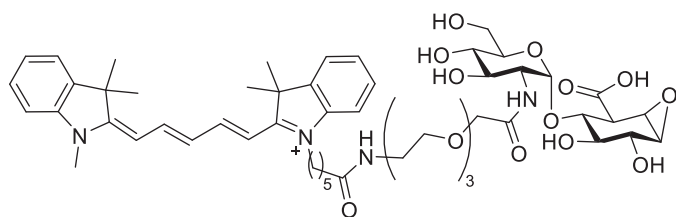
Amine **36** (11.6 mg, 33 µmol) was reacted with Peg Cy5 (Chapter 2) according to general procedure A. This afforded the product as a blue solid (2.35 mg, 2.25 µmol, 7%).

^{1}H NMR (600 MHz, MeOD) δ 8.24 (t, J = 13.1 Hz, 2H), 7.49 (dt, J = 7.6, 1.5 Hz, 2H), 7.44 − 7.39 (m, 2H), 7.32 − 7.29 (m, 2H), 7.26 (td, J = 7.4, 0.9 Hz, 2H), 6.65 (t, J = 12.4 Hz, 1H), 6.30 (dd, J = 13.7, 3.5 Hz, 2H), 5.23 (d, J = 3.8 Hz, 1H), 4.11 (t, J = 7.5 Hz, 2H), 4.05 (d, J = 15.2 Hz, 1H), 4.00 (d, J = 15.2 Hz, 1H), 3.94 − 3.87 (m, 3H), 3.84 − 3.80 (m, 1H), 3.75 − 3.62 (m, 14H), 3.55 (t, J = 5.5 Hz, 2H), 3.44 − 3.35 (m, 4H), 3.30 − 3.26 (m, 1H), 2.99 (d, J = 3.5 Hz, 1H), 2.76 (dd, J = 9.1, 2.2 Hz, 1H), 2.25 (t, J = 7.4 Hz, 2H), 1.86 − 1.80 (m, 2H), 1.75 − 1.66 (m, 14H), 1.51 − 1.44 (m, 2H). ^{13}C NMR (151 MHz, MeOD) δ 178.2, 176.1, 175.3, 174.6, 173.0, 155.5, 144.3, 143.6, 142.7, 142.5, 129.8, 129.7, 126.7, 126.2, 123.4, 123.3, 112.1, 111.8, 104.5, 104.4, 99.4, 77.9, 77.6, 74.1, 73.3, 73.1, 72.6, 71.7, 71.5, 71.4, 71.3, 71.1, 70.6, 63.0, 57.0, 56.6, 55.4, 52.9, 50.5, 50.5, 44.8, 40.3, 36.7, 31.6, 28.2, 28.0, 27.8, 27.4, 26.6. HRMS (ESI) m/z: [M]$^+$ calculated for C$_{53}$H$_{73}$N$_4$O$_{15}$ 1005.5058, found 1005.5067.

2'-N biotin conjugated probe 38

Amine **36** (14.2 mg, 40.4 µmol) was reacted with Peg biotin (Chapter 2) according to general procedure A. This afforded the product as a white solid (3.00 mg, 3.90 µmol, 10%).

^{1}H NMR (500 MHz, MeOD) δ 5.20 (s, 1H), 4.52 (dd, J = 7.9, 4.8 Hz, 1H), 4.33 (dd, J = 7.9, 4.4 Hz, 1H), 4.12 − 3.99 (m, 2H), 3.95 − 3.84 (m, 3H), 3.84 − 3.63 (m, 12H), 3.58 (t, J = 5.4 Hz, 2H), 3.46 − 3.37 (m, 4H), 3.23 (dt, J = 9.8, 5.3 Hz, 1H), 3.04 (d, J = 3.4 Hz, 1H), 2.94 (dd, J = 12.8, 5.0 Hz, 1H), 2.81 − 2.75 (m, 1H), 2.71 (d, J = 12.8 Hz, 1H), 2.24 (t, J = 7.4 Hz, 2H), 1.80 − 1.53 (m, 4H), 1.49 − 1.40 (m, 2H). ^{13}C NMR (126 MHz, MeOD) δ 176.5, 173.1, 99.4, 77.7, 77.7, 74.1, 73.0, 72.9, 72.3, 71.6, 71.3, 71.2, 71.1, 70.6, 63.4, 61.6, 57.1, 57.0, 56.6, 55.3, 49.5, 49.3, 49.2, 49.0, 48.8, 48.7, 48.5, 41.0, 40.3, 36.7, 29.7, 29.4, 26.8, 23.5. HRMS (ESI) m/z: [M+H]$^+$ calculated for C$_{31}$H$_{51}$N$_4$O$_{16}$S 767.3009, found 767.3015.

4-*O*-(2-*N*-azidoacetyl-2-deoxy-D-glucopyranosyl)-glucurono-cyclophellitol (39)
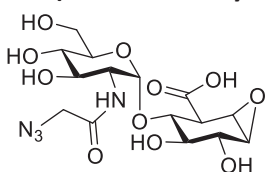
Amine **36** (8.4 µmol) was reacted with N$_3$AcOH according to general procedure A. The product was purified over HW-40 eluting with AcOH in water (1%, v/v). The fractions were concentrated under reduced pressure, coevaporated with water, diluted with water and lyophilized to yield the product as a white solid (0.45 mg, 1.04 µmol, 12%).

^{1}H NMR (600 MHz, D$_2$O) δ 5.17 (d, J = 3.8 Hz, 1H, H1'), 4.03 (d, J = 1.1 Hz, 2H,CH$_2$N$_3$), 3.89 (dd, J = 10.7, 3.8 Hz, 1H, H2'), 3.79 − 3.71 (m, 5H, H4/H6'ab/H2/H3'), 3.69 (dt, J = 10.1, 3.1 Hz, 1H, H5'), 3.50 − 3.45 (m, 3H, H3/H4'/epoxide), 3.19 − 3.13 (m, 1H, epoxide/Et$_3$N), 2.87 (dd, J = 9.5, 1.9 Hz, 1H, H5). ^{13}C NMR (151 MHz, D$_2$O) δ 178.3, 171.6, 98.5 (C1'), 77.0, 76.0, 72.9 (C5'), 72.2, 71.7, 70.4, 60.9 (C6'), 57.0 (epoxide), 56.4 (epoxide), 54.9 (C2'), 52.7 (CH$_2$N$_3$), 51.5 (C5). HRMS (ESI) m/z: [M+H]$^+$ calculated for C$_{15}$H$_{23}$N$_4$O$_{11}$ 435.1358, found 435.1358.

Activity-based probes with 4' linker

8-Benzyloxycarbonylamino-octan-1-ol (43)
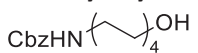
8-Amino-octan-1-ol (**42**) (6.65 g, 45.8 mmol) was dissolved in acetone/water (700 ml, 2/1, v/v). NaHCO$_3$ (11.53 g, 137.3 mmol) was added followed by dropwise

addition of carboxybenzyl chloride (9.78 ml, 68.7 mmol). After TLC showed full conversion the acetone was removed in vacuo. The remaining water layer was extracted with EtOAc (3x). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The product was obtained by column chromatography (EtOAc/pentane, 3/7, v/v) as an oil. (11.26 g, 40.30 mmol, 88%) Spectral data is in accordance with literature.[52]

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.39 − 7.28 (m, 5H), 5.09 (s, 2H), 3.63 (t, J = 6.6 Hz, 2H), 3.18 (q, J = 6.7 Hz, 2H), 1.62 − 1.40 (m, 5H), 1.41 − 1.23 (m, 9H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 156.5, 136.8, 128.6, 128.3, 128.2, 66.7, 63.1, 41.2, 32.8, 30.1, 29.4, 29.3, 26.7, 25.8. HRMS (ESI) m/z: [M+Na]$^+$ calculated for $C_{16}H_{25}NO_3Na$ 302.1727, found 302.1734.

8-Benzyloxycarbonylamino-1-trityloxy octane(44)

CbzHN⌒⌒OTrt (4)

Alcohol **43** (9.8 g, 35 mmol) was dissolved in DMF (58 ml, 0.6 M). Trityl chloride (13.7 g, 175 mmol) and Et$_3$N (24.5 ml, 175 mmol) were added and the reaction was stirred for 24h. Trityl chloride (4.4 g, 17.5 mmol) and Et$_3$N (5.0 ml, 36 mmol) were added and the reaction was stirred for 48h. Upon completion the mixture was diluted with H$_2$O and extracted with Et$_2$O (3x). The combine organic layers were washed with H$_2$O (5x) and brine, dried over MgSO$_4$, filtered and concentrated in vacuo. The product was used without further purification.

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.47 − 7.41 (m, 6H), 7.37 − 7.25 (m, 11H), 7.25 − 7.19 (m, 3H), 5.09 (s, 2H), 4.71 (s, 1H), 3.17 (q, J = 6.7 Hz, 2H), 3.03 (t, J = 6.6 Hz, 2H), 1.65 − 1.55 (m, 2H), 1.51 − 1.40 (m, 2H), 1.39 − 1.19 (m, 8H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 156.5, 144.6, 136.8, 128.8, 128.6, 128.3, 128.2, 127.8, 126.9, 86.4, 66.7, 63.7, 41.2, 30.1, 30.1, 29.5, 29.3, 26.8, 26.3. HRMS (ESI) m/z: [M+Na]$^+$ calculated for $C_{35}H_{39}NO_3Na$ 544.2822, found 544.2830.

8-*N*-benzyl(benzyloxycarbonyl)-1-trityloxy octane(46)

CbzBnN⌒⌒OH (4)

Carbamate **44** (18.3 g, 35 mmol) was dissolved in DMF (96 ml, 0.36 M) and the solution was cooled to 0°C. NaH (60 wt%, 2.9 g, 74 mmol) was added and the reaction was stirred at 0°C for 10 minutes. Benzyl bromide (5.9 ml, 49 mmol) and TBAI (1.3 g, 3.5 mmol) were added and the solution was stirred overnight at room temperature. The reaction mixture was cooled down to 0°C and quenched with H$_2$O. It was then further diluted with H$_2$O and extracted with Et$_3$O (3x). The combined organic layers were washed with H$_2$O (5x) and brine, dried over MgSO$_4$, filtered and concentrated under reduced pressure. Column chromatography (PE/Et$_2$O, 98/2 -> 4/1, v/v) yielded the product (16.3 g, 26.7 mmol, 76%, over 2 steps).

Trityl ether **45** (16.3 g, 26 mmol) was dissolved in DCM/MeOH (104 ml, 1/1, 0.25 M). TFA (3% in H$_2$O, 7.5 ml, 3.0 mmol) was added and the reaction was stirred for 27 hours. More TFA (0.23 ml, 3.0 mmol) was added and the reaction was stirred for another 24 hours. TLC showed complete conversion and the reaction was quenched with NaHCO$_3$, concentrated in vacuo, dissolved in EtOAc and extracted with H$_2$O (3x) and brine, dried over MgSO$_4$, filtrated and concentrated in vacuo. Column chromatography (PE/EtOAc, 9/1 -> 7/3, v/v) afforded the product (8.1 g, 21.9 mmol, 84%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.40 − 7.12 (m, 10H), 5.16 (d, J = 13.1 Hz, 2H), 4.48 (d, 2H), 3.57 (t, J = 6.7 Hz, 2H), 3.29 − 3.14 (m, 2H), 2.32 (s, 1H), 1.57 − 1.43 (m, 4H), 1.38 − 1.14 (m, 8H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 156.8, 156.2, 137.9, 136.8, 136.7, 128.5, 128.4, 127.9, 127.8, 127.3, 67.1, 62.7, 50.4, 50.1, 47.2, 46.2, 32.6, 29.2, 29.2, 28.0, 27.6, 26.6, 25.6. HRMS (ESI) m/z: [M+Na]$^+$ calculated for $C_{23}H_{31}NO_3Na$ 392.2196, found 392.2200.

8-*N*-benzyl(benzyloxycarbonyl)-1-iodo-octane(47)

CbzBnN⌒⌒I (4)

Alcohol **46** (8.1 g, 21.3 mmol) was dissolved in DCM (250 ml, 0.085 M) and cooled to 0°C. PPh$_3$ (6.7 g, 25.6 mmol), imidazole (1.7 g, 25.6 mmol) and I$_2$ (6.5 g, 26 mmol) were added to the solution. After stirring for 1 hour at 0 °C the reaction was warmed to rt and stirred for an additional 4 hours. The reaction was quenched with NaHCO$_3$ (aq. sat.) and the water layer was extracted three times with DCM. The combined organic layers were washed with NH$_4$Cl (aq. sat.), NaHCO$_3$ (aq. sat.), Na$_2$S$_2$O$_3$ (aq. sat.) and brine, dried over MgSO$_4$, filtrated and concentrated

in vacuo. Column chromatography (PE/EtOAc, 24/1 -> 4/1, v/v) yielded the product (10.4 g, 21.6 mmol, 97%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.42 – 7.13 (m, 10H), 5.17 (d, J = 11.8 Hz, 2H), 4.49 (d, J = 8.0 Hz, 2H), 3.30 – 3.11 (m, 4H), 1.83 – 1.70 (m, 2H), 1.58 – 1.41 (m, 2H), 1.41 – 1.15 (m, 8H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 156.8, 156.2, 138.0, 128.6, 128.5, 128.0, 127.9, 127.3, 67.2, 50.5, 50.2, 47.2, 46.3, 33.5, 30.4, 29.1, 28.4, 28.1, 27.7, 26.7, 7.4. HRMS (ESI) m/z: [M+Na]$^+$ calculated for C$_{23}$H$_{30}$INO$_2$Na 502.1213, found 502.1216.

### Phenyl 2-azido-2-deoxy-3-O-benzyl-6-O-(2-Naphthylmethyl)-1-thio-α-D-glucopyranoside (41)
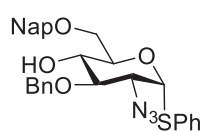
Phenyl 2-azido-2-deoxy-3-O-benzyl-4,6-benzylidene-1-thio-α-D-glucopyranoside[42] (**40**, 1.70 g, 3.6 mmol) was suspended in MeOH (30 mL). DCE (5 ml) was added to obtain a clear solution. The solution was heated to 50°C and camphorsulfonic acid (0.42 g, 1.8 mmol) was added. When TLC analysis showed full conversion of the starting material the reaction was cooled down to 0°C and quenched with Et$_3$N. Solvents were removed in vacuo and the crude residue was dissolved in EtOAc, washed with HCl (aq. 1M), NaHCO$_3$ (aq. sat.), brine and dried over MgSO$_4$.

The solvent was evaporated and the crude diol was dissolved in anhydrous MeCN (15 ml). 2-aminoethyl diphenyl borinate (0.081 g, 0.36 mmol), 2-bromomethyl-naphtalene (1.2 g, 5.4 mmol), KI (0.60 g, 3.6 mmol) and K$_2$CO$_3$ (0.55 g, 4.0 mmol) were added. The mixture was heated to 60°C and stirred overnight. The reaction mixture was transferred to a separatory funnel containing EtOAc and H$_2$O, layers were separated and the water layer was re-extracted three times with EtOAc. The organic layers were combined, washed with brine, dried over MgSO$_4$, filtered and concentrated in vacuo. Column chromatography (pentane/EtOAc, 9/1 -> 3/1, v/v) afforded the product (1.77 g, 3.35 mmol, 93%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.83 – 7.75 (m, 3H), 7.73 – 7.69 (m, 1H), 7.52 – 7.26 (m, 10H), 7.24 – 7.17 (m, 3H), 5.54 (d, J = 5.4 Hz, 1H, H1), 4.91 (d, J = 11.1 Hz, 1H), 4.80 (d, J = 11.1 Hz, 1H), 4.69 (d, J = 12.1 Hz, 1H), 4.61 (d, J = 12.1 Hz, 1H), 4.34 (dt, J = 9.6, 4.2 Hz, 1H, H5), 3.85 (dd, J = 10.0, 5.4 Hz, 1H, H2), 3.78 – 3.60 (m, 4H, C6ab/H4/H3), 2.66 (d, J = 3.1 Hz, 1H, OH). ^{13}C NMR (101 MHz, CDCl$_3$) δ 137.9, 135.2, 133.4, 133.2, 133.0, 132.2, 129.1, 128.7, 128.3, 128.2, 128.2, 127.9, 127.8, 127.8, 126.6, 126.2, 126.0, 125.6, 87.3 (C1), 81.4 (C3), 75.4, 73.7 (Bn/Nap), 72.1 (C4), 71.2 (C5), 69.6 (C6), 63.6 (C2). HRMS (ESI) m/z: [M+Na]$^+$ calculated for C$_{30}$H$_{29}$N$_3$O$_4$SNa 550.1771, found 550.1771.

Phenyl 2-azido-2-deoxy-3-O-benzyl-4-O-(8-N-benzyl(benzyloxycarbonyl)-1-octyl)-6-O-(2-Naphthylmethyl)-1-thio-D-glucopyranoside (48)
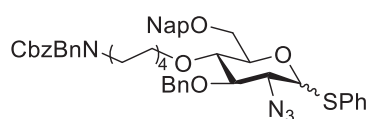
Alcohol **41** (α/β mixture) (2.3 g, 6.2 mmol) and iodide **47** (7.7 g, 16 mmol) were dissolved in dry DMF (13 ml, 0.5 M) and the solution was cooled to 0°C. NaH (60% dispersion in mineral oil, 0.43 g, 10.7 mmol) was added and the reaction was stirred for 18h at rt. The reaction mixture was cooled to 0°C and quenched with water. The water layer was extracted with Et$_2$O (3x), the combined organic layers were washed with water (5x) and brine, dried over MgSO$_4$, filtrated and concentrated in vacuo. Column chromatography (PE/Et$_2$O, 49/1 -> 4/1, v/v) yielded the product (4.4 g, 5.4 mmol, 88%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.79 (d, J = 7.9 Hz, 3H), 7.74 (s, 1H), 7.54 – 7.12 (m, 23H), 5.60 (d, J = 5.4 Hz, 1H), 5.17 (d, J = 10.2 Hz, 2H), 4.88 (d, J = 10.5 Hz, 1H), 4.84 (d, J = 10.5 Hz, 1H), 4.78 (d, J = 12.1 Hz, 1H), 4.60 (d, J = 12.2 Hz, 1H), 4.48 (d, J = 9.4 Hz, 2H), 4.33 – 4.27 (m, 1H), 3.91 (dd, J = 10.3, 5.4 Hz, 1H), 3.79 (dd, J = 10.8, 3.7 Hz, 1H), 3.76 – 3.64 (m, 3H), 3.54 (t, J = 9.4 Hz, 1H), 3.39 (q, J = 7.0 Hz, 1H), 3.27 – 3.12 (m, 2H), 1.52 – 1.32 (m, 4H), 1.20 – 1.00 (m, 8H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 156.2, 138.0, 137.8, 135.3, 133.6, 133.2, 133.0, 132.1, 129.1, 128.6, 128.5, 128.5, 128.2, 128.0, 128.0, 127.9, 127.8, 127.7, 127.7, 126.7, 126.2, 126.0, 126.0, 87.3, 81.8, 78.4, 75.7, 75.7, 73.7, 73.6, 73.6, 73.4, 72.0, 68.3, 67.2, 64.0, 50.5, 50.2, 47.3, 46.3, 30.4, 29.8, 29.5, 29.3, 28.1, 27.7, 26.8, 26.1. HRMS (ESI) m/z: [M+Na]$^+$ calculated for C$_{53}$H$_{58}$N$_4$O$_6$SNa 901.3969, found 901.3969.

Phenyl 2-azido-2-deoxy-3-*O*-benzyl-4-*O*-(8-*N*-benzyl(benzyloxycarbonyl)-1-octyl)-1-thio-D-glucopyranoside (50)
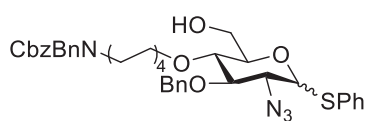
Naphthyl ether **48** (4.8 g, 5.5 mmol) was dissolved in DCM/MeOH (38 ml, 0.15 M, 4/1, v/v) and flushed with N_2 for 15 min. DDQ (3.7 g, 16 mmol) was added and the reaction was stirred for 2.5 hours in the dark. The reaction mixture was concentrated in vacuo and the residue was dissolved in EtOAc. The organic layer was washed with $NaHCO_3$ (aq. sat. 3x) and brine, dried over $MgSO_4$, filtrated and concentrated in vacuo. Column chromatography (PE/Et_2O, 47/3 -> 2/3. v/v) yielded the product (2.6 g, 3.9 mmol, 72%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.49 (dd, *J* = 7.8, 1.7 Hz, 2H), 7.43 − 7.22 (m, 17H), 7.17 (d, *J* = 6.6 Hz, 1H), 5.54 (d, *J* = 5.3 Hz, 1H), 5.17 (d, *J* = 13.2 Hz, 2H), 4.88 (s, 2H), 4.49 (d, *J* = 8.3 Hz, 2H), 4.18 (dt, *J* = 9.9, 3.2 Hz, 1H), 3.84 (dd, *J* = 10.3, 5.3 Hz, 1H), 3.82 − 3.73 (m, 4H), 3.62 − 3.54 (m, 1H), 3.43 (t, *J* = 9.3 Hz, 1H), 3.31 − 3.15 (m, 2H), 1.60 − 1.43 (m, 4H), 1.32 − 1.14 (m, 8H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 137.8, 132.6, 129.3, 128.6, 128.6, 128.6, 128.2, 128.1, 128.0, 127.9, 87.2, 81.6, 78.5, 75.8, 73.6, 72.7, 67.3, 64.1, 61.6, 30.5, 29.5, 29.3, 26.8, 26.2. HRMS (ESI) m/z: [M+Na]$^+$ calculated for $C_{42}H_{50}N_4O_6SNa$ 761.3343, found 761.3365.

Phenyl 2-azido-2-deoxy-3-*O*-benzyl-4-*O*-(8-*N*-benzyl(benzyloxycarbonyl)-1-octyl)-6-*O*-tert-butyldiphenylsilyl-1-thio-α-D-glucopyranoside (51)
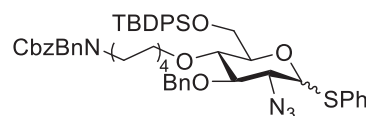
Alcohol **50** (3.4 g, 4.5 mmol) was dissolved in DMF (22.8 ml, 0.2 M). TBDPSCl (2.5 g, 9.1 mmol) and imidazole (1.6 g, 22.8 mmol) were added. After stirring for 17h TLC showed complete conversion and the reaction was quenched with H_2O. The reaction mixture was then diluted further with H_2O and extracted with Et_2O (5x). The combined organic layers were washed with H_2O (5x) and brine, dried over $MgSO_4$, filtrated and concentrated in vacuo. Column chromatography (Pe/Et_2O, 47/3, v/v) yielded the product (4.3 g, 4.4 mmol, 95% contaminated with excess silyl reagent).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.75 − 7.64 (m, 6H), 7.48 − 7.19 (m, 23H), 7.19 − 7.13 (m, 1H), 5.59 (d, *J* = 5.4 Hz, 1H), 5.16 (d, *J* = 11.2 Hz, 2H), 4.88 (s, 2H), 4.48 (d, *J* = 6.8 Hz, 2H), 4.15 (d, *J* = 9.6 Hz, 1H), 3.94 (dd, *J* = 11.5, 3.1 Hz, 1H), 3.91 − 3.78 (m, 3H), 3.74 (t, *J* = 9.6 Hz, 1H), 3.68 − 3.60 (m, 2H), 3.27 − 3.14 (m, 2H), 1.57 − 1.44 (m, 4H), 1.30 − 1.12 (m, 8H), 1.03 (s, 9H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 137.8, 136.0, 135.6, 134.9, 134.3, 133.7, 133.1, 131.5, 129.8, 129.1, 128.6, 128.5, 128.2, 127.8, 127.7, 127.4, 87.2, 81.9, 78.2, 76.0, 73.6, 73.2, 67.3, 64.2, 62.3, 50.6, 50.2, 47.4, 46.4, 30.6, 29.7, 29.5, 26.9, 26.3, 19.5. HRMS (ESI) m/z: [M+Na]$^+$ calculated for $C_{58}H_{68}N_4O_6SSiNa$ 999.4521, found 999.4539.

2-azido-2-deoxy-3-*O*-benzyl-4-*O*-(8-*N*-benzyl(benzyloxycarbonyl)-1-octyl)-6-*O*-tert-butyldiphenylsilyl-D-glucopyranose (52)
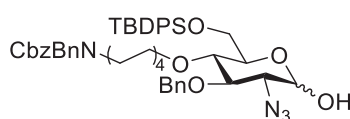
Thioglycoside **51** (6.2 g, 6.3 mmol) was dissolved in acetone/H_2O/DCM (49.5 ml, 0.13 M, 9/1/1, v/v). The reaction mixture was flushed with N_2. NIS (3.1 g, 13.5 mmol) was added and the reaction was stirred in the dark for 7 hours. The reaction was quenched with solid $Na_2S_2O_3$. The reaction mixture was concentrated in vacuo and dissolved in EtOAc. The organic layer was washed with $Na_2S_2O_3$ (aq. sat.) and brine, dried over $MgSO_4$, filtrated and concentrated in vacuo. Column chromatography (PE/Et_2O, 20/1 -> 4/1, v/v) yielded the product. (4.4 g, 5.0 mmol, 79%, α/β = 1/5).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.75 − 7.63 (m, 10H), 7.46 − 7.12 (m, 54H), 5.25 (t, *J* = 3.2 Hz, 1H), 5.17 (d, *J* = 11.4 Hz, 5H), 4.88 − 4.77 (m, 5H), 4.52 − 4.41 (m, 6H), 3.97 − 3.75 (m, 9H), 3.67 − 3.48 (m, 5H), 3.41 − 3.13 (m, 12H), 3.00 (s, 1H), 1.54 − 1.41 (m, 9H), 1.27 − 1.11 (m, 21H), 1.08 − 1.00 (m, 23H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 138.0, 136.1, 135.7, 129.8, 128.6, 128.3, 128.0, 127.8, 127.7, 96.1, 92.1, 83.1, 80.1, 78.2, 77.6, 76.3, 75.7, 73.4, 72.2, 67.6, 67.3, 64.2, 62.8, 62.6, 30.5, 29.6, 29.4, 27.0, 26.9, 26.2, 19.4. HRMS (ESI) m/z: [M+Na]$^+$ calculated for $C_{52}H_{64}N_4O_7SiNa$ 907.4436, found 907.4436.

2-azido-2-deoxy-3-*O*-benzyl-4-*O*-(8-*N*-benzyl(benzyloxycarbonyl)-1-octyl)-6-*O*-tert-butyldiphenylsilyl-D-glucopyranosyl N-phenyltrifluoroacetimidate (49)
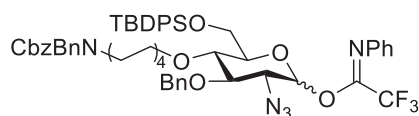
Lactol **52** (3.8 g, 4.3 mmol) was dissolved in DCM (3.5 ml, 0.2 M). 2,2,2-trifluoro-*N*-phenylacetimidoyl chloride (1.0 ml, 6.4 mmol) and Cs_2CO_3 (2.1 g, 6.4 mmol) were added. And the mixture was stirred for 6.5 hours. Upon completion the suspension was filtrated over celite and the filtrate was concentrated under reduced pressure. Column chromatography (PE/Et_2O, 20/1 -> 9/10, v/v) yielded the product (4.2 g, 4.0 mmol, 94%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.74 – 7.64 (m, 4H), 7.46 – 7.02 (m, 26H), 6.80 (d, *J* = 7.7 Hz, 1H), 6.72 (d, *J* = 7.4 Hz, 1H), 5.17 (d, *J* = 10.6 Hz, 2H), 4.94 – 4.79 (m, 2H), 4.49 (d, *J* = 7.4 Hz, 2H), 3.98 – 3.78 (m, 3H), 3.78 – 3.53 (m, 3H), 3.31 – 3.14 (m, 2H), 1.52 (s, 4H), 1.21 (d, *J* = 16.1 Hz, 8H), 1.05 (s, 9H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 138.0, 137.8, 135.9, 135.6, 133.7, 133.6, 132.9, 129.9, 129.8, 129.4, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.4, 127.4, 127.3, 126.3, 124.5, 124.4, 120.6, 119.4, 82.9, 80.3, 77.6, 76.6, 75.9, 74.7, 73.7, 73.5, 67.2, 65.4, 63.0, 62.0, 50.7, 50.6, 50.2, 47.3, 46.4, 30.5, 30.5, 29.7, 29.6, 29.4, 28.2, 27.8, 26.9, 26.9, 26.3, 19.5.

2,3-di-*O*-benzyl-4-*O*-(2-Azido-2-deoxy-3-*O*-benzyl-4-*O*-(8-*N*-benzyl(benzyloxycarbonyl)-1-octyl)6-*O*-tert-butyldiphenylsilyl-α-D-glucopyranosyl)-6-*O*-benzoyl-cyclophellitol alkene (53)
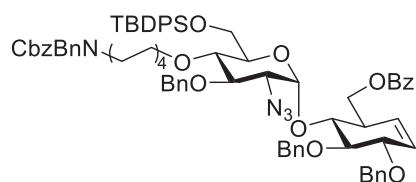
Donor **49** (1.3 g, 1.3 mmol) and acceptor **9** (0.39 g, 0.9 mmol) were co-evaporated with toluene (3x) and dissolved in dry DCM (10.6 ml, 0.068 M). 3Å molecular sieves were added and the mixture was stirred for 1 hour. The mixture was cooled to -78°C and TfOH (0.1 M in DCM, 2.8 ml, 0.28 mmol) was added. The reaction mixture was slowly warmed to -30°C within 1.5 hours and stirred for an additional hour at this temperature. The reaction was quenched with Et$_3$N at -30°C and diluted with DCM. The organic layer was washed with water and the water layer was extracted with DCM (2x). The combined organic layers were washed with brine, dried over MgSO$_4$, filtrated and concentrated in vacuo. Column chromatography (Pentane/EtOAc, 20/1 -> 3/1, v/v) yielded the product (1.2 g, 0.88 mmol, 97%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.83 (d, *J* = 7.3 Hz, 2H), 7.60 – 7.52 (m, 4H), 7.44 – 7.13 (m, 34H), 5.80 (dt, *J* = 10.2, 2.1 Hz, 1H), 5.68 (d, *J* = 3.9 Hz, 1H), 5.63 – 5.57 (m, 1H), 5.17 (d, *J* = 10.3 Hz, 2H), 5.07 (d, *J* = 10.7 Hz, 1H), 4.98 (d, *J* = 10.7 Hz, 1H), 4.88 (d, *J* = 10.5 Hz, 1H), 4.83 (d, *J* = 10.6 Hz, 1H), 4.71 (d, *J* = 11.5 Hz, 1H), 4.64 (d, *J* = 11.5 Hz, 1H), 4.53 – 4.44 (m, 3H), 4.32 – 4.24 (m, 2H), 4.02 – 3.89 (m, 3H), 3.83 – 3.73 (m, 2H), 3.71 – 3.54 (m, 5H), 3.29 – 3.14 (m, 3H), 2.82 – 2.75 (m, 1H), 1.55 – 1.42 (m, 4H), 1.25 – 1.11 (m, 8H), 0.97 (s, 9H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 166.2, 138.9, 138.2, 138.1, 135.9, 135.6, 133.7, 133.3, 133.0, 129.8, 129.6, 129.5, 128.6, 128.6, 128.5, 128.4, 128.3, 127.9, 127.8, 127.7, 127.6, 127.6, 127.5, 98.1, 84.4, 81.0, 80.0, 77.9, 75.6, 74.8, 74.2, 73.3, 72.9, 71.8, 67.2, 64.4, 63.4, 62.1, 50.5, 43.2, 30.5, 29.6, 29.4, 27.0, 26.9, 26.2, 19.4. HRMS (ESI) m/z: [M+Na]$^+$ calculated for $C_{80}H_{90}N_4O_{11}SiNa$ 1333.6268, found 1333.6309.

2,3-di-*O*-benzyl-4-*O*-(2-*N*-acetyl-2-deoxy-3-*O*-benzyl-4-*O*-(8-*N*-benzyl(benzyloxycarbonyl)-1-octyl)6-*O*-tert-butyldiphenylsilyl-α-D-glucopyranosyl)-6-*O*-benzoyl-cyclophellitol alkene (55)
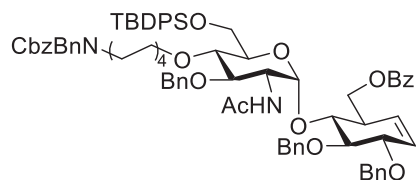
Azide **53** (1.2 g, 0.88 mmol) was dissolved in THF/H_2O (10 ml, 0.085 M, 17/3, v/v). PPh$_3$ (0.58 g, 2.2 mmol) and pyridine (0.080 ml, 1.0 mmol) were added. The reaction mixture was heated to 50°C and stirred for 3 hours. The reaction mixture was concentrated in vacuo. The residue was dissolved in DCM, dried with MgSO$_4$, and concentrated in vacuo.

The crude product was co-evaporated with toluene and dissolved in DCM (8.8 ml, 0.1 M). Acetic anhydride (1.40 ml, 13.4 mmol) and pyridine (1.2 ml, 13.4 mmol) were added and the reaction mixture was stirred for 16.5 hours. The mixture was cooled to 0°C and quenched with water. The organic layer was washed with CuSO$_4$ (aq. sat.), NaHCO$_3$ (aq. sat.) and brine, dried over MgSO$_4$, filtrated and

concentrated in vacuo. Column chromatography (PE/EtOAc, 9/1 -> 3/2, v/v) yielded the product (1.1 g, 0.86 mmol, 98%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.98 − 7.91 (m, 2H), 7.74 − 7.64 (m, 4H), 7.48 − 7.42 (m, 1H), 7.39 − 7.19 (m, 33H), 6.64 (d, J = 9.6 Hz, 1H), 5.83 (dt, J = 10.3, 2.5 Hz, 1H), 5.68 (dt, J = 10.3, 2.0 Hz, 1H), 5.18 (d, J = 12.6 Hz, 2H), 5.01 − 4.90 (m, 2H), 4.82 (d, J = 11.3 Hz, 1H), 4.74 − 4.44 (m, 8H), 4.38 (td, J = 9.6, 3.4 Hz, 1H), 4.23 − 4.17 (m, 1H), 3.97 − 3.78 (m, 6H), 3.78 − 3.58 (m, 4H), 3.23 (dt, J = 32.6, 7.5 Hz, 2H), 2.68 (t, J = 6.2 Hz, 1H), 1.58 − 1.44 (m, 9H), 1.29 − 1.13 (m, 8H), 1.05 (s, 10H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 170.0, 165.8, 156.6, 155.9, 138.5, 137.8, 137.6, 137.4, 136.7, 135.6, 135.4, 133.5, 132.9, 132.8, 129.7, 129.5, 129.3, 129.2, 128.4, 128.4, 128.3, 128.1, 128.0, 127.8, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.1, 126.6, 100.1, 81.4, 81.2, 78.7, 77.5, 76.9, 74.9, 74.6, 73.4, 72.9, 71.2, 66.9, 63.8, 62.3, 53.0, 50.3, 50.0, 47.1, 46.1, 43.7, 30.3, 29.3, 29.1, 27.9, 27.6, 26.7, 26.6, 26.0, 22.6, 19.1. HRMS (ESI) m/z: [M+Na]$^+$ calculated for C$_{82}$H$_{94}$N$_2$O$_{12}$SiNa 1349.6468, found 1349.6487.

2,3-di-*O*-benzyl-4-*O*-(2-*N*-acetyl-2-deoxy-3-*O*-benzyl-4-*O*-(8-*N*-benzyl(benzyloxycarbonyl)-1-octyl)6-*O*-tert-butyldiphenylsilyl-α-D-glucopyranosyl)-cyclophellitol alkene (56)
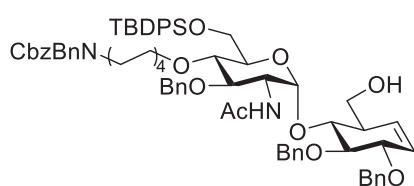
Benzoyl ester **55** (1.1 g, 0.86 mmol) was dissolved in MeOH/DCM (21 ml, 0.04 M, 9.5/1, v/v), NaOMe (4.37 M in MeOH, 0.12 ml, 0.57 mmol) was added and the mixture was stirred for 21.5 hours. NH$_4$Cl was added and the reaction was concentrated in vacuo. The residue was dissolved in in EtOAc, washed with water and brine, dried over MgSO$_4$, filtrated and concentrated in vacuo. Column chromatography (PE/EtOAc, 4/1 -> 3/2, v/v) yielded the product (1.0 g, 0.83 mmol, 97%).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.75 − 7.66 (m, 4H), 7.43 − 7.13 (m, 31H), 6.70 (d, J = 9.7 Hz, 1H), 5.88 − 5.81 (m, 1H), 5.66 − 5.60 (m, 1H), 5.17 (d, J = 10.8 Hz, 2H), 4.96 − 4.89 (m, 2H), 4.71 (dd, J = 11.2, 5.7 Hz, 2H), 4.65 (d, J = 11.6 Hz, 1H), 4.57 (d, J = 11.6 Hz, 1H), 4.54 − 4.46 (m, 3H), 4.34 − 4.25 (m, 1H), 4.21 − 4.16 (m, 1H), 3.96 − 3.75 (m, 7H), 3.67 − 3.51 (m, 4H), 3.31 − 3.14 (m, 2H), 2.42 − 2.34 (m, 1H), 1.55 − 1.46 (m, 4H), 1.20 (d, J = 20.5 Hz, 8H), 1.05 (s, 9H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 170.3, 138.6, 138.0, 137.9, 137.5, 135.9, 135.8, 135.6, 133.7, 133.1, 129.7, 129.6, 129.5, 128.6, 128.5, 128.4, 128.3, 128.2, 128.2, 128.1, 127.9, 127.9, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 126.6, 100.1, 81.4, 78.9, 77.9, 77.7, 74.9, 74.8, 73.6, 73.1, 71.4, 67.1, 62.7, 62.3, 60.4, 53.1, 50.5, 50.1, 47.2, 46.2, 46.1, 30.4, 29.5, 29.3, 28.1, 27.7, 26.8, 26.8, 26.2, 22.7, 19.3. HRMS (ESI) m/z: [M+Na]$^+$ calculated for C$_{75}$H$_{90}$N$_2$O$_{11}$SiNa 1245.6206, found 1245.6245.

2,3-di-*O*-benzyl-4-*O*-(2-*N*-acetyl-2-deoxy-3-*O*-benzyl-4-*O*-(8-*N*-benzyl(benzyloxycarbonyl)-1-octyl)6-*O*-tert-butyldiphenylsilyl-α-D-glucopyranosyl)-6-*O*-tert-butyloxycarbonyl-cyclophellitol alkene (S5)
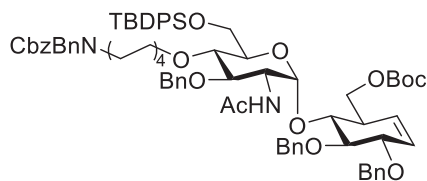
Alcohol **56** (0.53 g, 0.43 mmol) was co-evaporated with toluene (3x) and dissolved in THF (3.2 ml, 0.14 M). Boc$_2$O (0.38 g, 1.8 mmol) and DMAP (0.16 mM in THF, 0.32 ml, 0.05 mmol) were added. The reaction was stirred for 1 hour and quenched with H$_2$O. The reaction mixture was diluted with Et$_2$O and washed with NH$_4$Cl (aq. sat.), NaHCO$_3$ (aq. sat.) and brine, dried over MgSO$_4$, filtrated and concentrated in vacuo. Column chromatography (PE/EtOAc, 9/1 -> 7/3, v/v) yielded the product (0.47 g, 0.35 mmol, 82%).

^{1}H NMR (300 MHz, CDCl$_3$) δ 7.76 − 7.65 (m, 4H), 7.43 − 7.14 (m, 31H), 6.73 (d, J = 9.7 Hz, 1H), 5.87 − 5.78 (m, 1H), 5.67 − 5.57 (m, 1H), 5.18 (d, J = 7.0 Hz, 2H), 4.94 (d, J = 11.1 Hz, 1H), 4.86 (d, J = 3.5 Hz, 1H), 4.78 (d, J = 11.3 Hz, 1H), 4.73 − 4.44 (m, 7H), 4.42 − 4.17 (m, 4H), 3.96 − 3.56 (m, 10H), 3.31 − 3.06 (m, 2H), 2.52 (s, 1H), 1.58 − 1.37 (m, 16H), 1.28 − 1.12 (m, 8H), 1.05 (s, 9H). ^{13}C NMR (75 MHz, CDCl$_3$) δ 170.4, 153.4, 138.8, 138.0, 137.9, 137.6, 135.9, 135.7, 135.6, 133.9, 133.1, 129.6, 128.6, 128.6, 128.6, 128.5, 128.3, 128.3, 128.2, 128.0, 128.0, 127.9, 127.7, 127.6, 127.5, 127.3, 126.8, 100.5, 82.1, 81.7, 79.3, 77.7, 77.6, 77.5, 77.2, 76.8, 75.0, 74.9, 73.5, 73.2, 71.5, 67.2, 66.1, 62.5, 53.3, 50.5, 50.2, 47.3, 46.3, 44.0, 30.5, 29.6, 29.4, 27.8, 26.9, 26.8, 26.2, 22.7, 19.4. HRMS (ESI) m/z: [M+H]$^+$ calculated for C$_{80}$H$_{99}$N$_2$O$_{13}$Si 1323.6911, found 1323.6910.

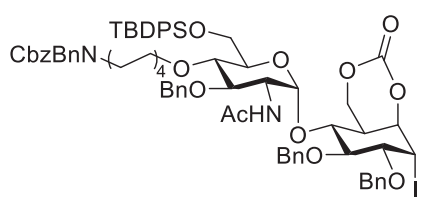**1-iodo-2,3-di-*O*-benzyl-4-*O*-(2-*N*-acetyl-2-deoxy-3-*O*-benzyl-4-*O*-(8-*N*-benzyl(benzyloxycarbonyl)-1-octyl)-6-*O*-tert-butyldiphenylsilyl-α-D-glucopyranosyl)-6,7-*O*-carbonyl-cyclophellitol alkane (S6)**

Alkene **S5** (0.47 g, 0.35 mmol) was dissolved in DCM/AcOH (1.8 ml, 0.2 M, 2/1, v/v). NIS (0.16 g, 0.71 mmol) was added and the reaction was stirred for 20 hours. Additional NIS (0.039 g, 0.18 mmol) was added and the reaction was stirred for 2 hours. The solution was diluted with Et_2O and quenched with Et_3N. The organic layer was washed with NH_4Cl (aq. sat.), $NaHCO_3$ (aq. sat.), $Na_2S_2O_3$ (aq. sat.) and brine, dried over $MgSO_4$, filtrated and concentrated in vacuo. Column chromatography (PE/EtOAc, 9/1 -> 3/2, v/v) yielded the product (0.33 g, 0.24 mmol, 68%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 – 7.62 (m, 4H), 7.46 – 7.11 (m, 31H), 6.37 (d, *J* = 9.7 Hz, 1H), 5.17 (d, *J* = 11.3 Hz, 2H), 5.08 (d, *J* = 10.5 Hz, 1H), 5.01 (d, *J* = 12.1 Hz, 1H), 4.87 – 4.77 (m, 3H), 4.71 – 4.44 (m, 7H), 4.34 (td, *J* = 9.9, 3.4 Hz, 1H), 4.04 (dd, *J* = 12.2, 2.7 Hz, 1H), 3.96 – 3.71 (m, 7H), 3.71 – 3.54 (m, 3H), 3.32 – 3.14 (m, 3H), 3.12 (dd, *J* = 9.3, 4.0 Hz, 1H), 2.66 – 2.57 (m, 1H), 1.56 – 1.44 (m, 4H), 1.24 (d, *J* = 4.8 Hz, 11H), 1.04 (d, *J* = 5.9 Hz, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.5, 146.8, 138.6, 138.1, 137.1, 136.8, 135.9, 135.6, 133.9, 132.9, 130.0, 129.8, 128.8, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.3, 128.0, 127.9, 127.7, 127.6, 101.5, 82.2, 81.7, 81.4, 77.8, 77.4, 76.7, 76.3, 75.0, 74.2, 73.4, 72.3, 68.4, 67.2, 62.7, 53.2, 50.6, 50.2, 47.3, 46.3, 35.2, 30.6, 30.5, 29.7, 29.5, 28.2, 27.8, 27.0, 26.9, 26.4, 22.5, 19.5. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{76}H_{90}N_2O_{13}SiI$ 1393.5251, found 1393.5247.

2,3-di-*O*-benzyl-4-*O*-(2-*N*-acetyl-2-deoxy-3-*O*-benzyl-4-*O*-(8-*N*-benzyl(benzyloxycarbonyl)-1-octyl)6-*O*-tert-butyldiphenylsilyl-α-D-glucopyranosyl)-cyclophellitol (57)
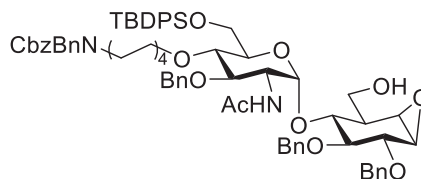
Iodocarbonate **S6** (0.28 g, 0.24 mmol) was dissolved in MeOH/DCM (3.4 ml, 0.7 M, 12:5, v/v). NaOMe (4.37 M in MeOH, 0.13 ml, 0.57 mmol) was added and the reaction was stirred for 15.5 hours. The reaction was quenched with Et_3N·HCl and concentrated in vacuo. The residue was dissolved in EtOAc, washed with H_2O and brine, dried over $MgSO_4$, filtrated and concentrated in vacuo. The product was used without further purification (0.26 g, 0.21 mmol, 88%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 – 7.66 (m, 4H), 7.45 – 7.14 (m, 31H), 6.80 (d, *J* = 9.7 Hz, 1H), 5.20 – 5.14 (m, 2H), 4.94 (d, *J* = 11.0 Hz, 1H), 4.86 (d, *J* = 3.5 Hz, 1H), 4.78 (d, *J* = 11.2 Hz, 1H), 4.72 – 4.62 (m, 3H), 4.52 – 4.43 (m, 3H), 4.32 – 4.25 (m, 1H), 4.09 – 4.01 (m, 1H), 3.99 – 3.86 (m, 3H), 3.85 – 3.74 (m, 3H), 3.58 (tdt, *J* = 11.9, 9.1, 5.8 Hz, 5H), 3.35 – 3.31 (m, 1H), 3.30 – 3.13 (m, 3H), 2.03 – 1.96 (m, 1H), 1.56 – 1.44 (m, 5H), 1.43 (s, 3H), 1.30 – 1.14 (m, 8H), 1.05 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.2, 138.6, 138.0, 137.4, 137.2, 136.0, 135.6, 133.7, 133.1, 129.7, 128.7, 128.6, 128.6, 128.5, 128.3, 128.2, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 127.5, 127.3, 127.2, 100.6, 83.1, 81.3, 80.0, 77.5, 77.4, 77.1, 76.8, 75.8, 75.5, 74.7, 73.8, 73.0, 72.7, 67.2, 62.6, 61.7, 56.5, 53.5, 53.1, 52.2, 50.5, 50.2, 47.3, 46.3, 45.9, 44.5, 30.5, 29.7, 29.6, 29.4, 28.2, 26.9, 26.8, 26.2, 22.7, 19.3. HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{75}H_{90}N_2O_{12}SiNa$ 1261.6155, found 1261.6184.

2,3-di-*O*-benzyl-4-*O*-(2-*N*-acetyl-2-deoxy-3-*O*-benzyl-4-*O*-(8-*N*-benzyl(benzyloxycarbonyl)-1-octyl)6-*O*-tert-butyldiphenylsilyl-α-D-glucopyranosyl)-glucurono-cyclophellitol (58)
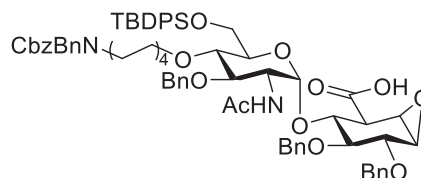
Alcohol **54** (0.25 g, 0.21 mmol) was dissolved in *t*-BuOH/DCM/H_2O (7.7 ml, 26 mM, 5/4/1, v/v). TEMPO (7 mg, 0.04 mmol) and BAIB (0.161 g, 0.50 mmol) were added and the solution was stirred for 24 hours. The reaction was diluted with DCM and H_2O and quenched with $Na_2S_2O_3$ (aq. sat.). The water layer was acidified with AcOH and extracted with DCM (4x). The combined organic layers were dried over $MgSO_4$, filtrated and concentrated in vacuo. Column chromatography (PE/EtOAc, 9/1 -> 1/1, v/v, 1% AcOH) yielded the product (0.17 g, 0.13 mmol, 67%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 – 7.65 (m, 4H), 7.46 – 7.14 (m, 31H), 6.07 (d, *J* = 9.7 Hz, 1H), 5.17 (d, *J* = 13.7 Hz, 2H), 4.93 (dd, *J* = 7.3, 3.7 Hz, 2H), 4.78 (t, *J* = 11.5 Hz, 2H), 4.64 (d, *J* = 11.3 Hz, 1H), 4.57 (dd,

J = 11.1, 5.0 Hz, 2H), 4.49 (d, J = 7.4 Hz, 2H), 4.19 (td, J = 10.0, 3.6 Hz, 1H), 3.98 – 3.61 (m, 9H), 3.58 (dd, J = 10.4, 8.4 Hz, 1H), 3.47 (dd, J = 10.1, 8.1 Hz, 1H), 3.32 (t, J = 2.9 Hz, 1H), 3.29 – 3.14 (m, 3H), 2.80 (dd, J = 8.8, 2.2 Hz, 1H), 1.55 – 1.43 (m, 4H), 1.31 – 1.14 (m, 11H), 1.03 (s, 9H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 170.8, 170.6, 138.7, 138.0, 137.6, 137.1, 136.3, 135.8, 133.8, 133.6, 129.8, 129.7, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 127.4, 127.2, 100.0, 81.6, 81.5, 79.1, 77.5, 75.4, 75.2, 73.2, 73.2, 72.8, 67.2, 62.2, 54.2, 53.6, 53.2, 50.5, 50.2, 48.8, 47.3, 46.3, 30.6, 29.6, 29.4, 28.2, 27.8, 27.1, 27.0, 26.9, 26.8, 26.3, 22.5, 19.3. HRMS (ESI) m/z: [M+Na]$^+$ calculated for C$_{75}$H$_{88}$N$_2$O$_{13}$SiNa 1275.5948, found 1275.5986.

2,3-di-*O*-benzyl-4-*O*-(2-*N*-acetyl-2-deoxy-3-*O*-benzyl-4-*O*-(8-*N*-benzyl(benzyloxycarbonyl)-1-octyl)-α-D-glucopyranosyl)-glucurono-cyclophellitol (54)
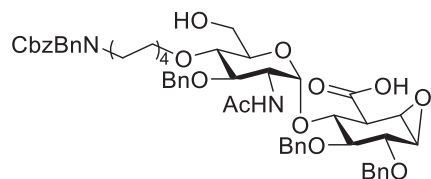
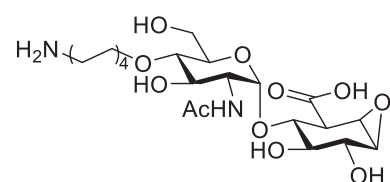Silyl protected **58** (0.60 g, 0.48 mmol) was dissolved in THF (7.2 ml, 0.05 M). Et$_3$N·3HF (0.29 ml, 1.8 mmol) was added and the reaction was stirred for 42 hours. The reaction mixture was diluted with DCM and water and the layers were separated. The water layer was extracted with DCM (4x) and the combined organic layers were dried over MgSO$_4$, filtrated and concentrated in vacuo. Column chromatography (PE/EtOAc, 2/3 -> 0/1, v/v, 1 % AcOH) yielded the product (0.49 g, 0.48 mmol, quant.).

^{1}H NMR (400 MHz, CDCl$_3$) δ 7.40 – 7.11 (m, 25H), 6.27 (d, J = 9.7 Hz, 1H), 5.17 (d, J = 11.6 Hz, 2H), 4.96 (d, J = 10.8 Hz, 1H), 4.87 – 4.75 (m, 3H), 4.67 (d, J = 11.3 Hz, 1H), 4.56 (t, J = 10.8 Hz, 2H), 4.48 (d, J = 9.5 Hz, 2H), 4.22 (td, J = 10.0, 3.6 Hz, 1H), 3.99 – 3.87 (m, 3H), 3.79 – 3.66 (m, 4H), 3.60 – 3.39 (m, 6H), 3.28 – 3.14 (m, 4H), 2.89 (dd, J = 9.0, 2.1 Hz, 1H), 1.50 (d, J = 6.7 Hz, 4H), 1.20 (d, J = 19.5 Hz, 11H). ^{13}C NMR (101 MHz, CDCl$_3$) δ 172.9, 172.6, 156.9, 156.3, 138.7, 138.0, 137.9, 137.7, 137.1, 137.0, 129.1, 128.7, 128.7, 128.7, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.2, 128.0, 127.9, 127.6, 127.5, 127.5, 127.3, 125.4, 100.3, 81.6, 81.5, 79.3, 77.9, 77.5, 77.4, 77.2, 76.8, 76.8, 75.7, 75.0, 73.4, 73.2, 72.8, 67.2, 66.2, 64.3, 61.1, 54.5, 53.6, 53.4, 50.5, 50.2, 49.6, 47.3, 46.3, 43.4, 30.6, 29.8, 29.6, 29.4, 28.2, 27.8, 26.8, 26.2, 22.6. HRMS (ESI) m/z: [M+H]$^+$ calc for C$_{59}$H$_{71}$N$_2$O$_{13}$ 1015.4951, found 1015.4954.

4-*O*-(2-*N*-acetyl-2-deoxy-4-*O*-(8-amino-1-octyl)-α-D-glucopyranosyl)-glucurono-cyclophellitol (59)

Benzyl protected **54** (0.10 g, 0.10 mmol) was dissolved in dioxane/water (10 ml, 0.01 M, 8/5) and flushed with N$_2$ for 5 min. 10% Pd/C (0.21 g, 0.2 mmol) was added and the suspension was flushed with N$_2$ for another 5 min. The N$_2$ balloon was replaced with an H$_2$ balloon and the solution was flushed with H$_2$ for 10 min and stirred at rt for 7 hours and 40 minutes. The H$_2$ balloon was replaced by an N$_2$ balloon, the reaction mixture was flushed for 5 min, filtrated over celite and concentrated under reduced pressure. Size exclusion chromatography over HW-40 eluting with H$_2$O 1% AcOH yielded a broad peak that was collected in three fractions. Based on NMR the pure product fraction was selected (0.021 g, 0.041 mmol, 41%).

^{1}H NMR (500 MHz, MeOD) δ 5.11 (d, J = 3.8 Hz, 1H), 3.90 – 3.83 (m, 4H), 3.81 – 3.75 (m, 1H), 3.74 – 3.66 (m, 2H), 3.62 (dd, J = 11.6, 5.3 Hz, 1H), 3.58 – 3.50 (m, 1H), 3.42 (dd, J = 10.0, 8.2 Hz, 1H), 3.39 – 3.36 (m, 1H), 3.10 (dd, J = 10.0, 8.8 Hz, 1H), 3.01 (d, J = 3.6 Hz, 1H), 2.90 (t, J = 7.7 Hz, 2H), 2.74 (dd, J = 9.2, 2.1 Hz, 1H), 1.99 (s, 3H), 1.69 – 1.49 (m, 4H), 1.45 – 1.33 (m, 8H). ^{13}C NMR (126 MHz, MeOD) δ 178.3 (weak), 174.0, 99.8, 80.2, 78.2, 77.8, 73.9, 73.7, 73.3, 73.2, 62.7, 57.0, 56.6, 56.1, 52.9, 40.8, 31.1, 29.9, 29.9, 28.7, 27.2, 27.2, 22.8. HRMS (ESI) m/z: [M+H]$^+$ calc for C$_{23}$H$_{41}$N$_2$O$_{11}$ 521.2705, found 521.2703.

4'O Cy5 conjugated probe 60 (CB702)
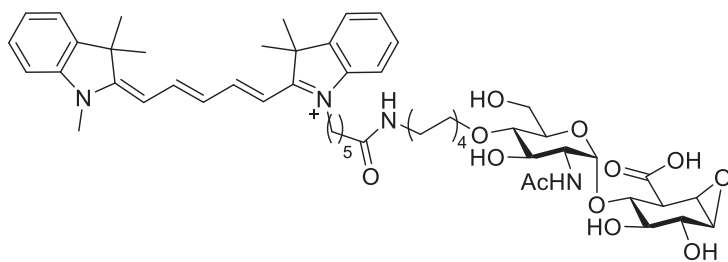
Cy5 carboxylic acid (11.6 mg, 22.3 µmol) was dissolved in DMF (0.25 ml). DIPEA (12 µl, 70 µmol), and pentafluorophenyl trifluoroacetate (3.8 µl, 22.3 µmol) were added and the mixture was stirred for one hour. LC-MS indicated the presence of starting material so more DIPEA (6 µl, 35 µmol) and pentaflurophenyl trifluoroacetate (3.8 µl, 22.3 µmol) were added. After stirring for 30 minutes water (2 µl) and DMF (0.25 ml) were added and the solution was added to amine **59** (12.2 mg, 23µmol).

The reaction was stirred overnight and the product was purified on semi-preparative HPLC eluting with a linear gradient of solution A (MeCN) in solution B (50mM AcOH in H_2O). The fractions were concentrated under reduced pressure, coevaporated with water, diluted with water and lyophilized to yield the product as a blue solid (2,88 mg, 2,82 µmol, 13%).

^{1}H NMR (850 MHz, CD_3CN) δ 8.02 (t, *J* = 13.1 Hz, 2H), 7.47 – 7.42 (m, 2H), 7.40 – 7.34 (m, 2H), 7.26 – 7.19 (m, 4H), 6.50 (t, *J* = 12.4 Hz, 1H), 6.16 (t, *J* = 13.1 Hz, 2H), 5.01 (d, *J* = 3.9 Hz, 1H), 3.96 (t, *J* = 7.5 Hz, 2H), 3.74 – 3.65 (m, 7H), 3.64 – 3.58 (m, 2H), 3.53 (dd, *J* = 12.0, 5.2 Hz, 1H), 3.49 (s, 5H), 3.38 (dd, *J* = 10.0, 8.4 Hz, 1H), 3.35 (dd, *J* = 3.8, 2.1 Hz, 1H), 3.10 (t, *J* = 9.6 Hz, 1H), 3.06 – 2.99 (m, 3H), 2.69 (dd, *J* = 9.3, 2.1 Hz, 1H), 2.08 (t, *J* = 7.3 Hz, 3H), 1.90 (s, 3H), 1.74 – 1.69 (m, 2H), 1.62 (s, 12H), 1.58 – 1.52 (m, 2H), 1.50 – 1.42 (m, 2H), 1.37 – 1.30 (m, 4H), 1.20 (s, 8H). ^{13}C NMR (214 MHz, CD_3CN) δ 175.5, 174.8, 174.4, 174.2, 154.6, 143.9, 143.2, 142.2, 142.1, 129.6, 126.0, 125.4, 123.2, 123.1, 112.0, 111.8, 103.9, 103.8, 98.3, 98.3, 79.5, 76.8, 76.7, 74.1, 72.5, 72.2, 72.1, 61.6, 57.1, 56.3, 56.3, 55.1, 51.7, 50.0, 50.0, 44.7, 40.0, 36.5, 31.8, 30.5, 29.9, 29.7, 27.7, 27.6, 27.5, 27.3, 26.7, 26.3, 26.1, 23.0. HRMS (ESI) m/z: $[M]^+$ calculated for $C_{55}H_{77}N_4O_{12}$ 985.5523, found 985.5533.

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