Chapter 3

Synthesis of mannosylated cyclophellitols[1]

Introduction

Glucocerebrosidase (GBA) catalyses the hydrolysis of β-glucosylceramide into glucose and ceramide.[2] Impaired functioning of GBA leads to the accumulation of glucosylceramide causing Gaucher disease, the most prevalent lysosomal storage disorder.[3] The severity of Gaucher disease is related to the degree of activity of GBA in lysosomes. Monitoring GBA levels would give insight in the extent of Gaucher disease, allow for tailor-made personalized treatment of patients and be useful for the development of new therapies. The development of activity-based probes (ABPs) is a new and effective approach to detect, identify and monitor enzymes. The most sensitive ABPs for GBA to date have been developed by Witte *et al.*[4] These probes are characterized by the covalent attachment of a fluorescent BODIPY label at the C7 (cyclophellitol numbering) of cyclophellitol, an efficient mechanism-based β-glucosidase inhibitor (**1** and **2**, see Figure 1a).[5,6] GBA is a retaining glycosidase and processes β-glucosylceramide with retention of the anomeric configuration.[7] Hydrolysis proceeds via a double displacement mechanism as depicted in Figure 1b. As the glycosidic bond of the enzyme bound α-

glucoside can be hydrolysed, cyclophellitol lacks the pyranose ring oxygen. Upon binding of cyclophellitol to the enzyme a double replacement event is blocked (Figure 1c).

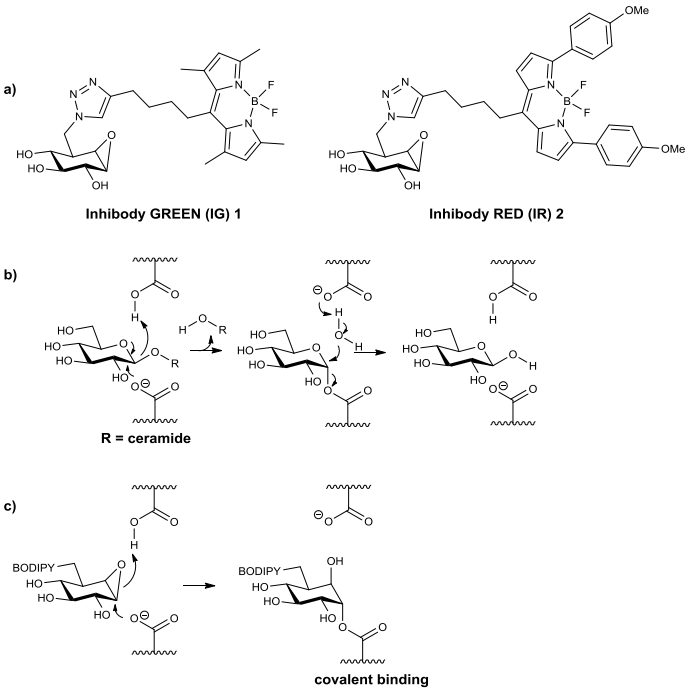


Figure 1: a) Structures of ABPs **1** and **2**. b) Double replacement mechanism of retaining glycosidase GBA. c) β-glucosidase inhibition mechanism of cyclophellitol.

In Gaucher patients storage of glucosylceramide mainly occurs in macrophages and therefore these cells are termed Gaucher cells. With the objective to transform ABP **1** into an ABP capable of specifically targeting Gaucher cells, it was envisioned that the mannose receptor, ubiquitously expressed at the cell surface of macrophages, could be exploited. A relevant example of drug targeting to Gaucher cells is presented by studies with cerezyme, a recombinant version of GBA. In the enzyme replacement therapy to treat Gaucher disease, cerezyme is chronically administered to patients and studies have shown an increased uptake and targeting to Gaucher cells of cerezyme when mannose residues were appended to the enzyme.[8,9] Apart from

this there are various reports that illustrate the decoration of specific cargo molecules with mannosides to induce targeting and internalization into macrophages. These examples of cargo molecules vary from small molecules,[10,11] peptide fragments,[12,13] macrocycles,[14,15] proteins[16,17,18] to nanoparticles,[19,20] as discussed in chapter 1.

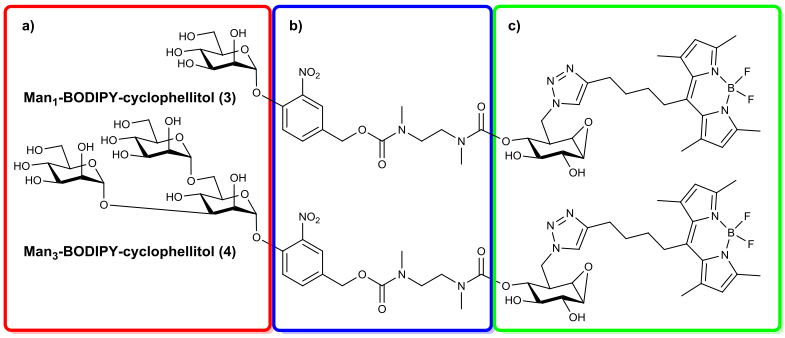


Figure 2: a-c) Design of the (tri)mannose cyclophellitol probes **3** and **4**.

Guided by these examples probes **3** and **4** were designed in which a monomannoside and trimannoside (part a, Figure 2) were selected as a homing device to target the fluorescent cyclophellitol based inhibitor (part c, Figure 2) via the mannose receptor to Gaucher cells. To liberate the BODIPY-cyclophellitol probe in the lysosome, a self-immolative linker system (part b, Figure 2) was introduced between the (tri)mannoside and the cyclophellitol. This linker system, first described by Engelhardt and co-workers,[21] has previously been used in various carbohydrate based prodrug approaches, including a strategy to selectively deliver paclitaxel (Taxol®) to necrotic tumors[22,23] and an approach described by Boons and co-workers who developed a mannostatin prodrug.[24] The projected fragmentation of the cyclophellitol prodrug-probes is depicted in Figure 3a. Following mannose mediated targeting and internalization the probe is delivered to lysosomes,[25] where the mannosidic linkages are projected to be cleaved by lysosomal α-mannosidase, setting the stage for the fragmentation of the linker and concomitant release of the probe. This chapter describes the synthesis and biological evaluation of mannose cyclophellitol probes **3** and **4**.

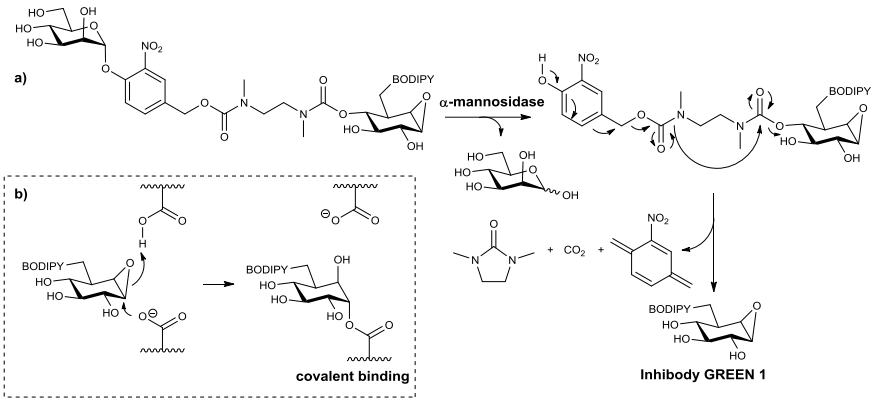
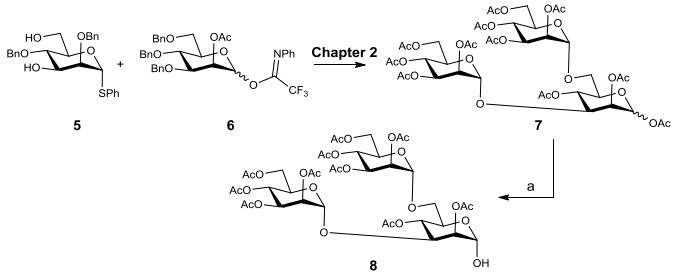


Figure 3: a) Degradation reaction of the linker liberating Inhibody GREEN **3**. b) Covalent inhibition of GBA by IG.

Results and discussion

The route of synthesis to target probes **3** and **4** is divided in four parts, namely, 1) synthesis of the mono- and trimannosides; 2) installation of the linker to the anomeric center of peracetylated (tri)mannoside; 3) construction of the conjugation ready cyclophellitol; and 4) assembly and global deprotection of the caged probe. Protected mannose trimer **7** was synthesized by condensing both alcohols in the known diol acceptor **5**[26,27] with trifluoroacetimidate **6** followed by further processing of the obtained trimer as described in chapter **2** (Scheme 1). The anomeric acetyl in **7** was selectively removed by treatment with hydrazine acetate yielding completely α-configured trimer **8** in 87%.

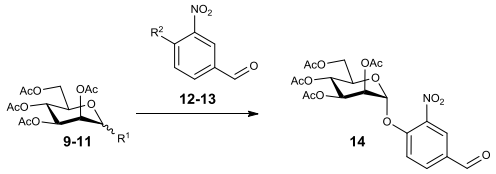
Scheme 1: trimer **8** synthesis.



Reagents and conditions: (a) Hydrazine acetate, DMF, 0 °C, 87% α-product.

The first step towards installment of the linker comprises the introduction of a phenolic α-mannosyl linkage. Acetylated mannose monomers **9-11** with different groups at the anomeric position and aldehydes **12-13** were selected to find a productive coupling to phenolic mannoside **14**. The results are summarized in Table 1.

Table 1: Synthesis of 2-Nitro-4-benzaldehyde 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (**14**).



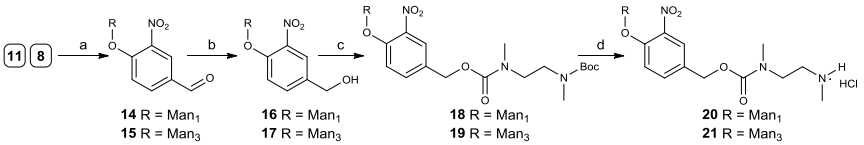
Entry	R^1	R^2	Reaction conditions	Yield[a]	α/β ratio[b]
1	I (**9**)	OH (**12**)	Ag_2O, MeCN, reflux	24	1:0
2	Br (**10**)	OH (**12**)	Ag_2O, MeCN, reflux	42	1:0
3	Br (**10**)	OH (**12**)	$Bn(Et)_3NCl$, 1.25 M NaOH (aq.), $CHCl_3$	2	1:3

4	OH (**11**)	F (**13**)	Li_2CO_3, DMAP, DCM, MS.	88	17:1
5	OH (**11**)	F (**13**)	DABCO, DMF, MS.	64	3:1
6	OH (**11**)	F (**13**)	NaH, DMF	n.i.	1:1[c]

[a)] Isolated yield. [b)] α/β ratio determined by ^{1}H-NMR of the crude. [c)] Not isolated.

Coupling of anomeric iodine **9** with nitrophenol **12**, using silver oxide as activator, gave **14** with high α-selectivity but in a low yield (24%, Entry 1). After replacing the anomeric iodide in **9** by bromine and subjecting **12** to similar conditions **14** was obtained in an improved yield (42%, Entry 2). In contrast, phase transfer conditions using 1.25M NaOH (aq.)/$CHCl_3$ and $Bn(Et)_3NCl$[28] gave hardly any product (2%) and inversion of stereoselectivity (α/β, 1:3, Entry 3). Next, nucleophilic aromatic substitution reactions were explored. Reaction of 4-fluoro-3-nitro-benzaldehyde **13** with hemiacetal **11** in DCM under influence of Li_2CO_3, a catalytic amount of DMAP[29] and molecular sieves gave, after column chromatography, the required α-product **14** in 82% yield alongside 5% of the β product (Entry 4). In this reaction DMAP functions as a shuttle base between the organic solvent and the solid lithium carbonate. Using DMF and DABCO not only reduced the yield (64 %, Entry 5) but also led to diminished stereoselectivity (α/β = 3:1). The use of sodium hydride as a base in DMF[30] gave a complex mixture of products, from which the required compound could not be isolated. Proton NMR of the crude reaction mixture showed a 1:1 mixture of α/β-anomers (Entry 6). The conditions described in Entry 4 were applied on mannotriose **8** to give α-linked Man_3-nitrobenzaldehyde **15** in 93 % yield (Scheme 2).

Scheme 2: Linker installation and elongation.



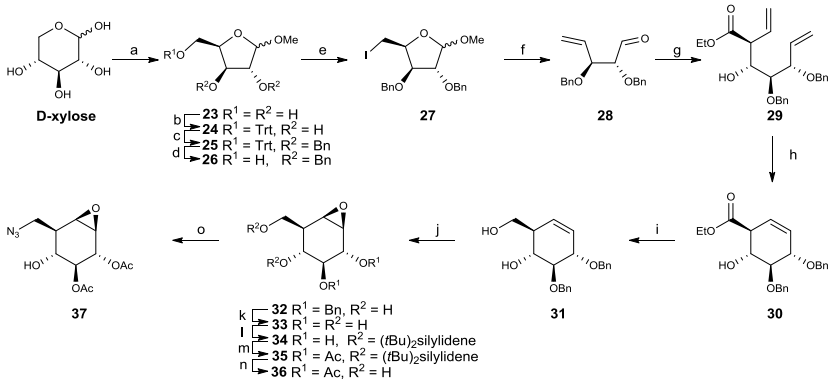
Reagents and conditions: (a) Li_2CO_3, DMAP, **13**, Mol. Siev. (Man_1 **14**: 88%, Man_3 **15**: 93%); (b) $NaBH_4$, $CHCl_3$/*i*-PrOH (4:1), silica, 0 °C (Man_1 **16**: 87%, Man_3 **17**: 59%); (c) *i*. *para*-nitrophenyl chloroformate, pyridine, DCM, 0 °C; *ii*. Mono-Boc diamine **22**, DMAP, 0 °C to rt. (Man_1 **18**: 93%, Man_3 **19**: 97%); (d) 4M HCl in EtOAc, 0 °C to rt (Man_1 **20**: quantitative, Man_3 **21**: quantitative).

Next the linker on the anomeric centre was extended by reduction of the benzaldehyde moiety in Man_1-nitrobenzaldehyde **14** and Man_3-nitrobenzaldehyde **15** to the corresponding benzyl alcohols **16** and **17**, respectively (Scheme 2). To this end $NaBH_4$ in the presence of silica was used and after complete conversion of the starting material more silica was added and the solvents were removed *in vacuo*.[31] Subsequently the adsorbed product was purified by column chromatography to yield Man_1-nitrobenzylalcohol **18** and Man_3-nitrobenzylalcohol **19** in 87% and 59% yield respectively. Treatment of **18** and **19** with 4-nitrophenyl chloroformate in DCM in the presence of 1.5 eq. pyridine gave the corresponding 4-nitrophenyl carbonates and subsequent *in situ* elongation with mono-Boc diamine **22**[21] in the presence of DMAP led to the isolation of Man_1-Boc-diamine **18** in 93% yield and Man_3-Boc-diamine **19** in 97% yield. The final step in the installation of the linker comprises the removal of the Boc-protective group by treatment of **18** and **19** with 4M dry HCl in EtOAc. Ensuing removal of the solvents provided **20** and **21** in quantitative yield.

The required 6-azido cyclophellitol derivative **40** was assembled as depicted in Scheme 3, following essentially the procedure of Madsen *et al*.[32] but with some optimizations to allow for synthesis on larger scale.[33] Methyl xylofuranose was obtained by treatment of xylose in MeOH with HCl. Selective tritylation of the primary hydroxyl of **23** was followed by benzylation of the remaining hydroxyls to give fully protected **25**. Acid mediated cleavage of the trityl ether of crude **25** provided alcohol **26** in 72%

over four steps. Using Ph_3P and I_2 the primary alcohol in **26** was converted to the corresponding iodide **27** (92% yield) and subsequent elimination with activated zinc gave aldehyde **28** in 75% yield.

Scheme 3: Synthesis of 7-azido-2,3-*O*-acetyl-cyclophellitol derivative **40**.



Reagents and conditions: (a) MeOH, AcCl, 0 ° to rt. quantitative; (b) trityl chloride, pyridine; (c) BnBr, NaH, DMF, 0 °C to rt.; (d) MeOH/DCM (1:1), *p*TsOH (72% over 4 steps); (e) *i.* Ph_3P, imidazole, THF, 70 °C; *ii*. I_2, THF, 70 °C, 92%; (f) activated Zn, THF/H_2O (9:1), sonicate, 50 °C, 75%; (g) Indium, La(OTf)$_3$, H_2O, 61%; (h) Grubbs 2nd, DCM, reflux, 85%; (i) DIBAL-H, $NaBH_4$, THF, 95%; (j) *m*CPBA, DCM 59%; (k) Pd/C, H_2, MeOH, 85%; (l) *i*. (*t*Bu)$_2$Si(OTf)$_2$, pyridine, DMF, -40 °C to 0 °C; (m) Ac_2O, pyridine 0 °C to rt., 60% over two steps; (n) HF·pyridine, THF/pyridine (2:1), 0 °C, quantitative; (o) *i.* Tf_2O, pyridine, THF, -25 °C; *ii*. NaN_3, 15-crown-5, THF, -25 °C, 90%.

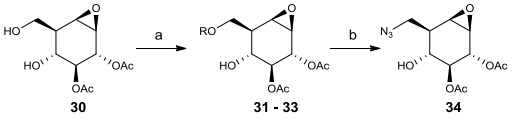
Key step in the cyclophellitol synthesis entails the indium/La(OTf)$_3$ mediated stereoselective Barbier reaction of aldehyde **28** with ethyl 4-bromocrotonate. This reaction gave target diene **29** and its L-idose configured *C*-5 epimer in a 4:1 ratio. After column purification the desired diene **29** was isolated in 61%. Ring-closing metathesis of the obtained diene **29** was accomplished by the use of Grubbs 2nd generation catalyst in boiling DCM. As gauged by TLC-MS analysis, formation of the target cyclohexene **30** ($[M+Na]^+$ 405.6) was accompanied by the formation of a side product ($[M+Na]^+$ 387.6), which likely originates from condensation of the 4-OH. Careful monitoring of the reaction progress and adjusting the amount of Grubbs catalyst led to the isolation of the desired cyclohexene **30** in 85% yield. Reduction of the ethyl

ester **30** to the corresponding alcohol proceeded uneventfully to afford partially protected cyclohexene **31**. The ensuing epoxidation of **31** using *m*CPBA proved to be difficult to monitor. Staining of the reaction TLC with anisaldehyde, in combination with TLC-MS analysis proved to be the best way to monitor the progress of the reaction, hydrogenolysis of the benzyl ethers with Pd/C, H_2 (g) provided β-cyclophellitol (**33**) in 85% yield (Scheme 3). Next, cyclophellitol **33** was further processed towards 7-azido-2,3-*O*-acetyl-cyclophellitol **40**, to enables the attachment to the (tri)mannoside. Regioselective introduction of 4,7-di-*O*-*t*butylsilylidene by treatment of **33** with $(t\text{Bu})_2\text{Si}(\text{OTf})_2$ in DMF and pyridine[34] was accompanied by the formation of an unidentified side product. Acetylation of the crude mixture and ensuing purification led to the isolation of fully protected cyclophellitol **35** in 60% over two steps. The silylidene protective group in **35** was removed with HF·pyridine in THF.[35] Because the product **36** proved to be soluble in water, aqueous work-up conditions had to be avoided and therefore the reaction was quenched with solid $NaHCO_3$, after which the mixture was filtered, concentrated, co-evaporated with toluene and purified by column chromatography to yield the partially acetylated cyclophellitol **36** in quantitative yield.

To regioselectively introduce the azide function at the primary hydroxyl of diol **36** a number of procedures were explored, the results of which are summarized in Table 2. Selective mesylation of **36** in DCM with Et_3N as base proceeded quantitatively but the subsequent substitution with sodium azide in DMF at 60 °C gave a mixture of products, from which the desired azide could not be obtained (Entry 1). The selective tosylation of **36** with tosyl chloride in the presence of pyridine[36] proceeded in low yield (14%, Entry 2), and the use of Et_3N and tosyl chloride was accompanied by migration of the acetyl groups. From this latter reaction compound **38** could be obtained in 59% yield, but the ensuing azide substitution failed (Entry 3). Therefore a more potent leaving group was explored. A first attempt to form **39** *in situ* in THF in the presence of pyridine was unsuccessful (Entry 4). In the next attempt to synthesize **40**, diol **36** was selectively triflated in DCM in the presence of pyridine to give

triflate **39**. After work-up, the crude **39** was subjected to substitution with sodium azide in DMF at -25 °C to 0 °C to give **40** in 11% yield (Entry 5). The low yield in this reaction could be partially accounted for by the water solubility of the product **40**. Avoiding the aqueous work-up procedure and the use of 18-crown-6[37,38] in the substitution reaction yielded 6-azido-cyclophellitol **40** in 78% yield (Entry 6). The yield was further improved with the aid of 15-crown-5, giving product **40** in 90% yield (Entry 7).

Table 2: Azide installation, synthesis azido-cyclophellitol **40.**



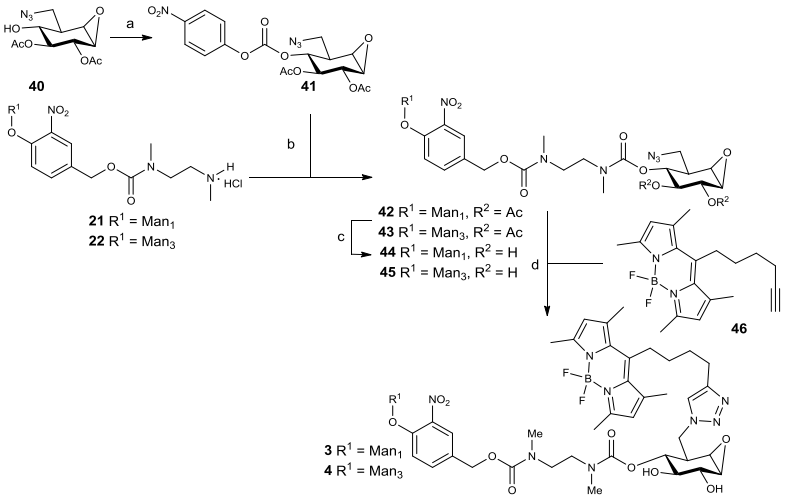
30 31 - 33 34

Entry	R	a	b	Yield
1	Ms (**37**)	MsCl, Et_3N, DCM, 0 °C.	NaN_3, DMF, 60 °C, overnight	n.i.[a]
2	Ts (**38**)	TsCl, pyridine, DCM, 0 °C to rt.	---[b]	---
3	Ts (**38**)	TsCl, Et_3N, DCM, 0 °C to rt.	NaN_3, DMF, 60 °C, overnight.	n.i.[a]
4	Tf (**39**)	THF, pyridine, Tf_2O, -25 °C to rt.	---[c]	---
5	Tf (**39**)	DCM, pyridine, Tf_2O, -25 °C.	NaN_3, DMF, -25 to 0 °C	11
6	Tf (**39**)	DCM, pyridine, Tf_2O, -25 °C.	NaN_3, 18-crown-6, THF, -25 to 0 °C	78
7	Tf (**39**)	DCM, pyridine, Tf_2O, -25 °C.	NaN_3, 15-crown-5, THF, -25 to 0 °C	90

[a] Product not isolated, ladder of products on TLC. [b] Little intermediate formation observed. [c] No intermediate formation observed.

With the two mannosyl linkers and cyclophellitol alcohol in hand the assembly of the caged probes was undertaken (Scheme 4). Thus, azido-cylophellitol **40** was functionalized with a *p*-nitrophenyl carbonate moiety and the obtained **41** was coupled to monomannosyl linker system **21** and trimannoside **22** to give fully protected Man_1-cyclophellitol **42** (73%) and Man_3-cyclophellitol **43** (51%), respectively. Both constructs were deacetylated under standard Zémplen conditions providing Man_1-cyclophellitol **44** and Man_3-cyclophellitol **45** in 79% and 69% respectively. No degradation of the linker system and no hydrolysis of the epoxide were observed during this reaction.

Scheme 4: Assembly of final compounds **3** and **4**.



Reagents and conditions: (a) *p*-nitrophenyl chloroformate, pyridine, DCM, 0 °C to rt, quantitative; (b) **47**, Et_3N, DMF, 0 °C to rt. (Man_1-OAc-cyclophellitol **42**: 73%, Man_3-OAc-cyclophellitol **43**: 51%); (c) NaOMe, MeOH, rt. (Man_1-OH-cyclophellitol **44**: 79%, Man_3-OH-cyclophellitol **45**: 69%); (d) BODIPY-alkyne **46**, 75 mM sodium ascorbate (aq.), 50 mM $CuSO_4$ (aq.), DMF (Man_1-BODIPY-cyclophellitol **3**: 64%, Man_3-BODIPY-cyclophellitol **4**: 30%).

Finally BODIPY-alkyne **46**[39] was conjugated to azides **44** and **45** by means of Cu(I)-catalyzed azide alkyne cycloaddition providing Man_1-BODIPY-cyclophellitol **3** and Man_3-BODIPY-cyclophellitol **4**, which were purified by HPLC. Because concentration of the product containing fractions led to

degradation of the BODIPY moiety (LC-MS showed the formation of a side product having lost a BF_2 group ($[M-BF_2+H]^+$ 953.40) it proved necessary to directly lyophilize the product containing fractions. This led to Man_1-BODIPY-cyclophellitol **3** in 64% yield and Man_3-BODIPY-cyclophellitol **4** in 30% yield.

Biological results

The inhibitory potencies of **3** and **4** towards recombinant β-glucocerebrosidase (imiglucerase, GBA)[40] were determined using the green BODIPY-cyclophellitol inhibitor **1** (See Figure 1) as reference. The enzyme was incubated with varying concentrations of compounds **1**, **3** and **4**, after which residual enzyme activity was measured with the fluorogenic substrate 4-methylumbelliferyl-β-D-glucopyranoside (4MUGlc). The residual activity was plotted against the probe concentration and these curves were used to calculate the IC_{50} values (Figure 3). Caged cyclophellitol **3** showed an IC_{50} value of 33.4 μM, which is 2,000 fold higher compared to cyclophellitol derivative **1**, having an IC_{50} of 17.1 nM. At a concentration of 100 μM compound **1** did not completely inhibit the enzyme. Probe **4** showed hardly any inhibition of the enzyme and an IC_{50} value could therefore not be determined.

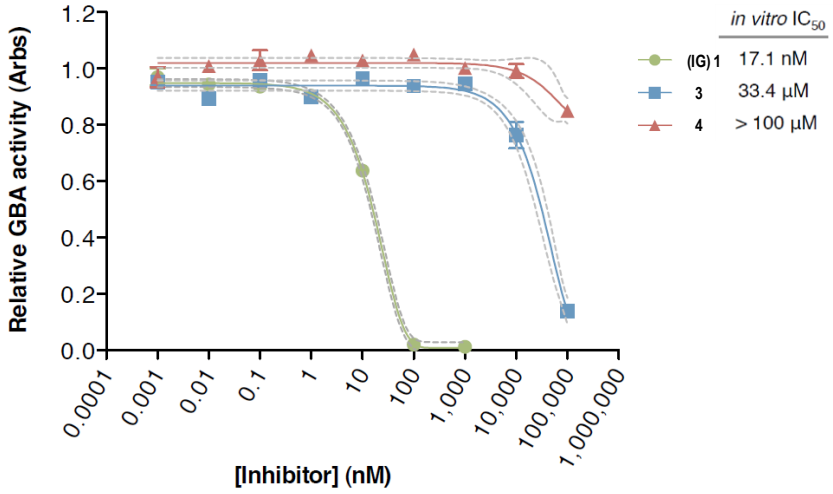


Figure 4: Inhibition of recombinant GBA by **1**, **3** and **4**. Imiglucerase was treated with the probes for 30 min at 37 °C after which residual activity was determined using 4MUGlc.

Next the detection limit of both probes was tested by pre-incubation of 2 pmol of imiglucerase with different concentrations of **3** and **4**. The residual enzyme activity was probed with the red BODIPY-cyclophellitol **2** and resolved on SDS-page (Figure 4).

For probe **3** (Figure 5a) a band was visible at probe concentrations ranging from 100 µmol to 5 µmol and clear yellow bands were formed in the overlay. Probe **4** (Figure 5b) showed hardly any labelling. Only faint bands were observed at high concentration (100 and 50 µmol) and no labeling was observed at lower concentrations.

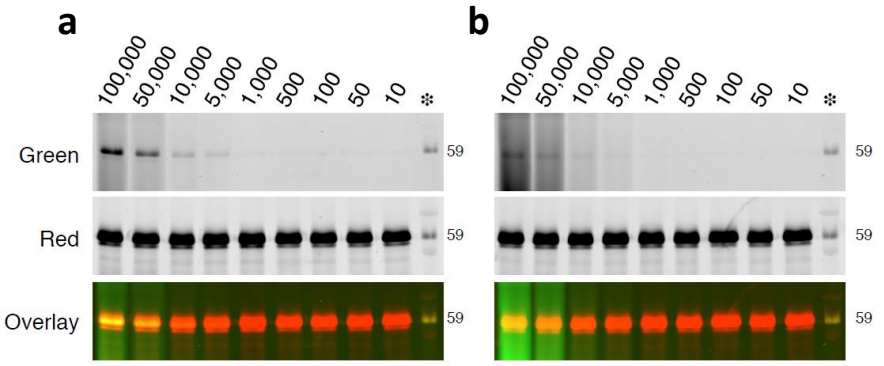


Figure 5: Detection limit of **3** and **4** for GBA. Imiglucerase (2 pmol) was incubated with **3** (a) or **4** (b) in different concentrations (nm) for 60 min at 37 °C. Residual enzyme activity was determined by incubation with IR for 30 min at 37 °C. All gels are 10% SDS-PAGE with fluorescent readout.

Next it was investigated whether mannosidases present in Gaucher cell lysate were capable of liberating the cyclophellitol probes. To this end **3** and **4** were incubated with varying concentrations of Gaucher spleen lysate. Subsequently imiglucerase was added and allowed to react for 60 min. Residual imiglucerase activity was then determined by treatment of the mixtures with red-BODIPY cyclophellitol **2**. Figure 6 depicts the SDS-PAGE gels of the cyclophellitol-lysate-imiglucerase mixtures. As can be seen in Figure 6a, no imiglucerase binding of probe **3** could be detected, regardless of the amount of Gaucher cell lysate added as only red-BODIPY labeling of the enzyme was seen. The faintly labelled band running above imiglucerase was assigned as ovalbumine, present in the used cell lysate. For probe **4** an identical picture is seen, and no effective labeling of imiglucerase by the green probe **4** could be detected.

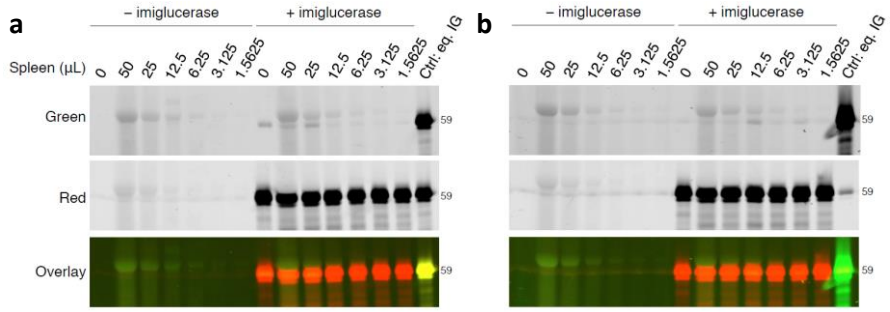


Figure 6: 3 (a) and **4** (b) processing with α-mannosidase present in Gaucher spleen lysate prior to labelling with recombinant GBA. a-b) 10µmol of **3** and **4** were incubated with different amount of spleen lysate and incubated for 60 min at 37 °C. Recombinant GBA was incubated with the lysate mixture for 60 min at 37 °C and the residual enzymes were incubated with IR for 30 min at 37 °C. All gels are 10% SDS-PAGE with fluorescent readout.

To improve α-mannosidase activity of the Gaucher spleen lysate, the $ZnCl_2$ and $ZnSO_4$ concentrations as well as the pH were optimized. After having established that these conditions did not adversely affect the activity of imiglucerase, the probes **3** and **4** were subjected to the optimal mannosidase conditions (1 or 10 M $ZnCl_2$ or $ZnSO_4$, pH 3.5) as depicted in Figure 7. In line with the above-described experiments, the probes were first subjected to Gaucher spleen lysate before the addition of imiglucerase and finally red BODIPY-probe **2**. This time very faint bands were observed in the Gaucher spleen lysate, indicative of residual GBA activity. The intensity of the band did not change with different concentrations of zinc salts. They did become more intense upon addition of red-BODIPY probe **2**. Adding imiglucerase to the mixtures led to more intense bands, but again no dependence on the concentration of zinc salts was observed. With trimannoside probe **4** no labeling in spleen lysate was observed under any of the used conditions.

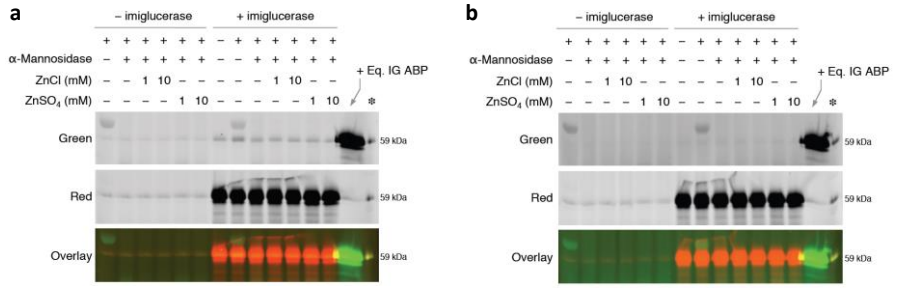


Figure 7: 3 (a) and **4** (b) processing with α-mannosidase (with- or without different zinc salts) present in Gaucher spleen lysate prior to labelling of imiglucerase. a-b) **3** and **4** were treated with Gaucher spleen lysate (with or without different zinc salts) and incubated for 60 min at 37 °C in a pH 3.5 buffer. The mixture was incubated with imiglucerase for 60 min at 37 °C and the residual enzymes were labelled by incubation with IR for 30 min at 37 °C. All gels are 10% SDS-PAGE with fluorescent readout.

Finally an *in situ* labelling was performed on human monocyte-derived macrophages. The macrophages were incubated with green-BODIPY probes **1**, **3** and **4** and chased over a period of time (Figure 8).

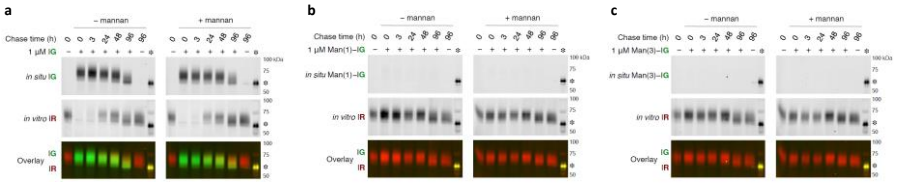


Figure 8: *in situ* labelling of GBA in human monocyte-derived macrophages. a-c) Human monocyte-derived macrophages, matured for 7 d, were incubated with IG (a), **3** (b) and **4** (c) in the presence or absence of mannan for 2 h at 37 °C. Cells were washed with PBS and chased in fresh medium over 96 h. The cells were lysed and the residual enzymes were labelled by incubation with IR for 60 min at 37 °C. All gels are 10% SDS-PAGE with fluorescent readout.

After incubation the cells were lysed and the unbound enzymes were probed with red-BODIPY probe **2**. The experiments were performed in the absence or presence of mannan to block the mannose receptor, thereby prohibiting the uptake of the mannose conjugates. As can be seen in Figure 8a green-BODIPY probe **1** labels endogenous GBA almost immediately after addition to the tissue culture, with no red-BODIPY labelling observed. The intensity

decreases over time due to production of new GBA, which is labelled by the red probe. Neither **3** nor **4** did showed any labelling of GBA in the cell (Figure 8b,c).

Conclusion

The successful synthesis of Man_1-BODIPY-cyclophellitol **3** and Man_3-BODIPY-cyclophellitol **4** as caged activity based probes for glucocerebrosidase, the key enzyme in Gaucher disease, was accomplished. In a convergent synthetic route the mannose building blocks were fused with a suitably protected azido cyclophellitol through a degradable linker system. Global deprotection and instalment of a BODIPY fluorophore completed the synthesis of the porbes. The obtained Man_1-BODIPY-cyclophellitol **3** and Man_3-BODIPY-cyclophellitol **4** were designed for specific targeting to Gaucher cells. After uptake via the mannose receptor, the probes have to be trimmed by intracellular mannosidases to allow for labelling of glucocerebrosidase. However, no effective labelling of (recombinant) glucocerebrosidase by **3** and **4** was achieved, which most likely is due to ineffective cleavage of the mannosyl residues from the probes. Hydrolysis of the mannose moieties from probes **3** and **4** is an essential prerequisite for their inhibitory action, as judged from the minimal inhibition of imiglucerase by the probes.

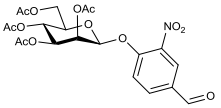
Experimental

General: Traces of water in the starting materials were removed by co-evaporation with toluene for all moisture and oxygen sensitive reactions and the reactions were performed under an argon atmosphere. Dichloromethane was distilled over P_2O_5 and stored over activated 3 Å molecular sieves under an argon atmosphere. All other solvents and chemicals (Acros, Fluca, Merck)

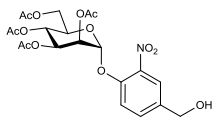
were of analytical grade and used as received. Column chromatography was performed on Screening Device silica gel 60 (0.040-0.063 mm). Size exclusion was performed on Sepadex LH20 (eluent DCM/MeOH, 1:1). TLC analysis was conducted on HPTLC aluminium sheet (Merck, TLC silica gel 60, F_{254}). Compounds were visualized by UV absorption (λ = 254 nm), staining with *p*-anisaldehyde (3.7 mL in 135 mL EtOH, 1.5 mL AcOH and 5 mL H_2SO_4), 20% H_2SO_4 in EtOH or with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (25g/l) in 10% H_2SO_4 in H_2O followed by charring at +/- 140 °C. ^1H and ^{13}C NMR were recorded on a Bruker DPX 300 (300 and 75 MHz respectively), Bruker AV 400 (400 and 100 MHz respectively), Bruker DMX 400 (400 and 100 MHz respectively), or Bruker DMX 600 (600 and 125 MHz respectively). Chemical shifts are given in ppm (δ) relative to the residual solvent peak or TMS (0 ppm) as internal standard. *J* couplings are given in Hz. Optical rotations were measured on a Propol automatic polarimeter. IR spectra (thin film) were conducted on a Perkin Elmer FTIR Spectrum Two UATR (Single reflection diamond). LC-MS measurements were conducted on a Thermo Finnigan LCQ Advantage MAX ion-trap mass spectrometer (ESI+) coupled to a Thermo Finnigan Surveyor HPLC system equipped with a standard C_{18} (Gemini, 4.6 mm x 50 mm, 5µm particle size, Phenomenex) analytical column and buffers A: H_2O, B: MeCN, C: 0.1% TFA (aq.). High-resolution mass spectra were recorded on a LTQ Orbitrap (Thermo Finnigan) mass spectrometer.

2-Nitro-4-benzaldehyde 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (14): To a solution of mannose **11** (2.61 g, 7.5 mmol) in DCM (50 mL) was added Li_2CO_3 (1.11 g, 15.0 mmol) and 4-fluoro-3-nitrobenzaldehyde **13** (1.52 g, 9 mmol) under argon atmosphere. To the solution was added activated mol. siev. (3 Å) and DMAP solution 0.27 g, 2.25 mmol). The reaction mixture was stirred for 4 hours at rt and solids were formed. The solids were filtered over a pad of celite and the filtrate was concentrated *in vacuo*. Purification by column chromatography yielded benzaldehyde mannose **14** as a slightly yellow

amorphous solid (3.25 g, 6.5 mmol, 87 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.99 (s, 1H), 8.44 (d, *J* = 2.0 Hz, 1H), 8.11 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.50 (d, *J* = 8.7 Hz, 1H), 5.82 (d, *J* = 1.9 Hz, 1H), 5.56 (dd, *J* = 10.0, 3.4 Hz, 1H), 5.50 (dd, *J* = 3.5, 1.9 Hz, 1H), 5.43 (t, *J* = 10.0 Hz, 1H), 4.29 (dd, *J* = 12.3, 5.0 Hz, 1H), 4.16 – 4.11 (m, 1H), 4.08 (dd, *J* = 12.3, 2.3 Hz, 1H), 2.23 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.7, 170.5, 170.0, 169.8, 169.6, 152.7, 140.6, 134.5, 131.1, 127.8, 117.2, 96.3, 70.7, 68.9, 68.4, 65.4, 61.9, 20.9, 20.8, 20.8, 20.7. HRMS: $[M+H]^+$ calculated for $C_{21}H_{24}NO_{13}$ 498.12422, found 498.12421.

2-Nitro-4-benzaldehyde 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranoside (β-by-product): 

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.98 (s, 1H), 8.38 (d, *J* = 2.0 Hz, 1H), 8.09 (dd, *J* = 8.6, 2.0 Hz, 1H), 7.38 (d, *J* = 8.7 Hz, 1H), 5.71 (dd, *J* = 3.5, 1.7 Hz, 1H), 5.55 (d, *J* = 1.7 Hz, 1H), 5.32 (t, *J* = 8.8 Hz, 1H), 5.23 (dd, *J* = 9.2, 3.4 Hz, 1H), 4.34 (dd, *J* = 12.2, 6.4 Hz, 1H), 4.25 (dd, *J* = 12.2, 3.2 Hz, 1H), 3.95 (ddd, *J* = 8.3, 6.4, 3.2 Hz, 1H), 2.25 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.7, 170.5, 170.1, 170.1, 169.6, 153.4, 140.7, 134.1, 131.1, 127.6, 117.5, 96.0, 73.2, 69.5, 67.4, 66.0, 62.4, 20.8, 20.8, 20.7, 20.7. HRMS: $[M+H]^+$ calculated for $C_{21}H_{24}NO_{13}$ 498.12422, found 498.12422.

2-Nitro-4-(hydroxymethyl)phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (16): 

To a solution of benzaldehyde mannose **14** (0.124 g, 0.25 mmol) in $CHCl_3$/*i*-PrOH 4:1 (2.5 mL) was added silica (0.19 g, 0.75g/mmol) under argon atmosphere. The reaction mixture was cooled to 0 °C and $NaBH_4$ (113 mg, 3 mmol) was added in small portions. The mixture was allowed to warm to rt, after complete conversion of the startingmaterial more silica was added (0.5 g). The solvent was removed *in vacuo* and the immobilized product was purified by column chromatography yielding benzylalcohol mannose **16** as a yellow amorphous solid (0.110 g, 0.22 mmol,

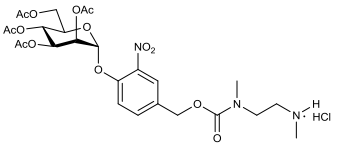
88 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, *J* = 2.4 Hz, 1H), 7.55 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.29 (d, *J* = 8.6 Hz, 1H), 5.66 (d, *J* = 2.1 Hz, 1H), 5.55 (dd, *J* = 10.0, 3.4 Hz, 1H), 5.50 (dd, *J* = 3.5, 1.9 Hz, 1H), 5.40 (t, *J* = 10.0 Hz, 1H), 4.73 (s, 2H), 4.28 (dd, *J* = 12.3, 5.1 Hz, 1H), 4.18 (ddd, *J* = 10.2, 5.1, 2.2 Hz, 1H), 4.08 (dd, *J* = 12.4, 2.3 Hz, 1H), 2.21 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 170.1, 169.9, 169.8, 147.8, 140.6, 136.6, 132.4, 124.1, 117.9, 96.8, 70.3, 69.2, 68.7, 65.7, 63.5, 62.1, 21.0, 20.8, 20.8, 20.8. HRMS: $[M+H]^+$ calculated for $C_{21}H_{26}NO_{13}$ 500.13987, found 500,1986.

Mono-Boc-diamine 23: To an ice cooled solution of N,N'-dimethylethylenediamine (3.7 mL, 34 mmol) in DCM (40 mL) was added a Boc_2O solution (2.35 mL, 11 mmol) in DCM (20 mL) dropwise by a mechanical syringe (3 mL/h). After addition of the Boc_2O the reationmixture was allowed to warm to rt and stirred overnight. The reaction mixture was diluted with DCM, washed with brine (1x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (EtOAc/MeOH, 80:20, 1% Et_3N) yielded mono-Boc-diamine **20** as a slightly yellow oil (1.65 g, 8.5 mmol, 26 %). Spectroscopic data were in accordance with published data.[21] ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.36 (t, *J* = 6.1 Hz, 2H), 3.23 (s, 1H), 2.88 (s, 3H), 2.76 (t, *J* = 6.6 Hz, 2H), 2.47 (s, 3H), 1.46 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.3, 156.0, 100.1, 79.5, 53.4, 49.4, 48.2, 47.9, 35.9, 34.7, 28.4, 23.4.

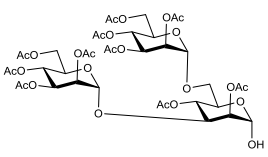
***N,N'*-Dimethyl-(4-(2,3,4,6-tetra-*O*-acetyl-α-D-mannosyl)-3-nitrobenzyl-oxycarbonyl)-*N'*-(*tert*-butyloxycarbonyl)-ethylendiamine (18):** To a solution of benzylalcohol mannose **16** (0.25 g, 1 mmol) in DCM (18 mL) was added dry pyridine (0.12 mL, 1.5 mmol) under argon atmosphere and the mixture was cooled to 0 °C. To the cooled solution was added *p*-nitrophenyl chloroformate (0.302 g, 1.5 mmol) and the reaction was stirred for 30 min at 0 °C and 1.5 h at rt.

The reaction mixture was cooled back to 0 °C and a mono-Boc-diamine solution (0.320 g, 1.7 mmol) in DCM (2 mL) and DMAP (208 mg, 1.7 mmol) were added to the reaction mixture. The mixture was allowed to warm to rt and was stirred overnight. The reaction mixture was diluted with DCM and washed with H_2O. The aquaeous phase was extracted with DCM (3x) and the combined organic layers were washed with H_2O (3x), brine (3x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded mannose-Boc-diamine **18** as a yellow amorphous solid (0.666 g, 0.93 mmol, 93 %). FT-IR: ν_{max} (neat)/cm^{-1} 975.14, 1218.86, 1365.49, 1535.22, 1693.00, 1747.76, 2974.90. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.55 (d, *J* = 7.3 Hz, 1H), 7.30 (d, *J* = 8.7 Hz, 1H), 5.67 (s, 1H), 5.56 (dd, *J* = 10.1, 3.4 Hz, 1H), 5.49 (d, *J* = 2.6 Hz, 1H), 5.41 (t, *J* = 10.0 Hz, 1H), 5.11 (s, 2H), 4.29 (dd, *J* = 12.3, 5.0 Hz, 1H), 4.17 (ddd, *J* = 10.1, 5.0, 2.2 Hz, 1H), 4.08 (d, *J* = 13.2 Hz, 1H), 3.50 – 3.27 (m, 4H), 2.96 (s, 3H), 2.88 (s, 1.6H rotamer), 2.82 (s, 1.4H rotamer), 2.21 (d, *J* = 0.9 Hz, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.03 (d, *J* = 0.9 Hz, 3H), 1.44 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 170.0, 169.8, 169.6, 155.7, 148.3, 140.5, 140.5, 134.0, 133.8, 132.6, 132.5, 132.4, 125.6, 125.4, 117.7, 96.7, 79.8, 70.3, 69.2, 68.6, 65.6, 65.5, 65.3, 62.1, 47.4, 47.2, 46.9, 46.6, 46.0, 35.5, 34.9, 34.6, 28.5, 20.9, 20.8, 20.8, 20.7. HRMS: $[M+NH_4]^+$ calculated for $C_{31}H_{47}N_4O_{16}$ 732.30131, found 732.30177.

***N*,*N*'-Dimethyl-(4-(2,3,4,6-tetra-*O*-acetyl-α-D-mannosyl)-3-(nitrobenzyloxycarbonyl)-ethylendiamine HCl salt (20):** A freshly prepared 4M HCl solution in EtOAc (37.5 mL) was cooled to 0 °C and to the cooled solution was added dropwise a Mannose-Boc-diamine **18** (357 mg, 0.5 mmol) solution in EtOAc (12.5 mL). After 15 min at 0 °C the solution was gradually warmed to rt and stirred for 1 h. The reaction mixture was concentrated *in vacuo* and co-evaporated with toluene (3x) and Et_2O (1x) yielding mannose-diamine HCl salt **20** as a yellow solid (325 mg, 0.5 mmol, quantitative). ^{1}H NMR (300 MHz, D_2O) δ



8.24 – 7.83 (m, 1H), 7.66 (d, *J* = 8.7 Hz, 1H), 7.34 (d, *J* = 8.7 Hz, 1H), 7.13 (s, 3H), 6.87 – 6.58 (m, 1H), 5.84 (s, 1H), 5.45 (d, *J* = 8.4 Hz, 2H), 5.29 (t, *J* = 9.9 Hz, 1H), 5.09 (d, *J* = 7.1 Hz, 2H), 4.25 (dd, *J* = 12.0, 4.2 Hz, 1H), 4.15 (d, *J* = 10.3 Hz, 0H), 4.03 (d, *J* = 12.3 Hz, 1H), 3.58 (dd, *J* = 13.7, 7.6 Hz, 2H), 3.27 – 3.22 (m, 1H), 3.18 (t, *J* = 5.7 Hz, 2H), 2.89 (d, *J* = 18.2 Hz, 3H), 2.66 (d, *J* = 8.4 Hz, 3H), 2.18 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.95 (s, 3H).



2,4-*O*-Di-acetyl-3-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-6-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (8): To a 0 °C cooled solution of peracetylated mannose trimer **7** (1.45 g, 1.5 mmol) in DMF (7.5 mL) was added hydrazine acetate (0.145 g, 1.65 mmol) and stirred till complete conversion. The reaction was quenched with acetone and concentrated *in vacuo*. The crude was dissolved in Et_2O and washed with H_2O (2x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded mannose trimer **8** as a white amorphous solid (1.20 g, 1.30 mmol, 87 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.32 (dd, *J* = 10.1, 3.5 Hz, 1H), 5.30 – 5.17 (m, 4H), 5.04 (dd, *J* = 3.2, 1.8 Hz, 1H), 5.01 (d, *J* = 1.9 Hz, 1H), 4.84 – 4.80 (m, 2H), 4.77 (d, *J* = 4.0 Hz, 1H), 4.30 (dd, *J* = 6.8, 3.3 Hz, 2H), 4.28 (t, *J* = 6.1 Hz, 1H), 4.25 (dd, *J* = 12.3, 5.2 Hz, 1H), 4.14 (dd, *J* = 10.1, 2.1 Hz, 2H), 4.13 – 4.05 (m, 4H), 3.76 (dd, *J* = 11.2, 6.2 Hz, 1H), 3.56 (dd, *J* = 11.2, 2.9 Hz, 1H), 2.21 (s, 3H), 2.15 (s, 3H), 2.14 (d, *J* = 2.1 Hz, 6H), 2.12 (s, 3H), 2.11 (s, 3H), 2.06 (d, *J* = 1.8 Hz, 6H), 1.99 (s, 3H), 1.98 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.9, 170.8, 170.6, 170.2, 170.1, 170.0, 169.9, 169.9, 169.8, 169.6, 98.8, 97.6, 91.9, 74.1, 71.5, 70.0, 69.4, 69.3, 69.1, 69.1, 68.6, 68.5, 68.3, 67.5, 66.2, 66.0, 62.4, 62.4, 20.9, 20.8, 20.8, 20.8, 20.7, 20.7, 20.7, 20.6. HRMS: $[M+H]^+$ calculated for $C_{38}H_{53}O_{26}$ 925.28196, found 925.28199.

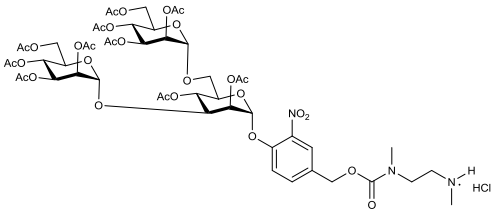
2-Nitro-4-benzaldehyde 2,4-*O*-di-acetyl-3-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-6-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (15): To a solution of mannose trimer **8** (0.783 g, 0.725 mmol) in DCM (7.25 mL) was added Li_2CO_3 (0.112 g, 1.52 mmol) and 4-fluoro-3-nitrobenzaldehyde **13** (0.147 mg, 0.87 mmol) under argon atmosphere. To the solution was added activated molecular sieves (3 Å) and a 0.25M DMAP solution (1 mL, 0.25 mmol) in DCM. The reaction mixture was stirred for 4 hours at rt and solids were formed. The solids were filtered over a pad of celite and the filtrate was concentrated *in vacuo*. Purification by size exclusion (DCM/MeOH, 1:1) yielded benzaldehyde mannose trimer **15** as a slightly yellow amorphous solid (0.296 g, 0.134 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.94 (s, 1H), 8.44 (s, 1H), 8.21 (d, *J* = 8.8 Hz, 1H), 7.55 (d, *J* = 8.8 Hz, 1H), 5.87 (s, 1H), 5.52 (dd, *J* = 3.1, 1.4 Hz, 1H), 5.35 – 5.17 (m, 4H), 5.12 (s, 1H), 5.08 (dd, *J* = 3.2, 1.6 Hz, 1H), 5.02 – 4.96 (m, 1H), 4.85 (dd, *J* = 10.2, 3.6 Hz, 1H), 4.69 (s, 1H), 4.48 (dd, *J* = 9.8, 3.4 Hz, 1H), 4.38 – 4.31 (m, 1H), 4.26 (d, *J* = 11.7 Hz, 1H), 4.16 (dd, *J* = 6.5, 5.1 Hz, 1H), 4.14 – 4.01 (m, 3H), 3.97 (t, *J* = 9.4, 8.3 Hz, 1H), 3.77 (dd, *J* = 10.8, 7.8 Hz, 1H), 3.43 (d, *J* = 10.4 Hz, 1H), 2.27 (d, *J* = 1.3 Hz, 3H), 2.22 (d, *J* = 1.3 Hz, 3H), 2.17 (d, *J* = 1.6 Hz, 3H), 2.17 (s, 3H), 2.14 (d, *J* = 1.2 Hz, 3H), 2.11 (d, *J* = 1.3 Hz, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.01 (d, *J* = 1.3 Hz, 3H), 1.94 (d, *J* = 1.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.9, 170.8, 170.6, 170.1, 170.1, 170.0, 169.9, 169.8, 169.7, 169.7, 169.5, 151.9, 140.0, 134.4, 131.0, 128.2, 117.2, 99.1, 96.9, 95.3, 73.7, 71.4, 69.9, 69.7, 69.6, 69.1, 68.6, 68.2, 66.2, 62.6, 62.3, 20.8, 20.8, 20.8, 20.7, 20.7, 20.6, 20.6, 20.6, 20.5. HRMS: $[M+H]^+$ calculated for $C_{45}H_{56}NO_{29}$ 1074.29325, found 1074.29321.

2-Nitro-4-(hydroxymethyl)phenyl 2,4-*O*-di-acetyl-3-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-6-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (17): To a solution of benzaldehyde mannose trimer **15** (0.537 g, 0.5 mmol) in $CHCl_3$/*i*-PrOH 4:1 (5 mL) was added silica (0.375 g, 0.75g/mmol) under argon atmosphere. The reaction mixture was cooled to 0 °C and $NaBH_4$ (0.227 g, 6 mmol) was added in small portions. The mixture was allowed to warm to rt, after complete conversion of the startingmaterial more silica was added (1 g). The solvent was removed *in vacuo* and the immobilized product was purified by column chromatography yielding benzylalcohol mannose trimer **17** as a yellow amorphous solid (0.306 g, 2.9 mmol, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, *J* = 2.1 Hz, 1H), 7.65 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.34 (d, *J* = 8.6 Hz, 1H), 5.77 – 5.73 (m, 1H), 5.50 (dd, *J* = 3.5, 1.8 Hz, 1H), 5.29 (t, *J* = 10.0, 9.6 Hz, 1H), 5.27 – 5.21 (m, 2H), 5.19 (dd, *J* = 10.2, 4.4 Hz, 2H), 5.10 (d, *J* = 1.8 Hz, 1H), 5.07 (dd, *J* = 3.2, 1.8 Hz, 1H), 5.03 (dd, *J* = 10.0, 3.6 Hz, 1H), 4.96 (dd, *J* = 3.7, 1.6 Hz, 1H), 4.70 – 4.60 (m, 2H), 4.48 (dd, *J* = 9.9, 3.5 Hz, 1H), 4.34 (dd, *J* = 12.3, 5.5 Hz, 1H), 4.22 – 4.18 (m, 2H), 4.14 – 4.01 (m, 4H), 3.96 (ddd, *J* = 10.4, 8.6, 1.8 Hz, 1H), 3.76 (dd, *J* = 10.9, 8.4 Hz, 1H), 3.42 (dd, *J* = 11.0, 1.9 Hz, 1H), 2.26 (s, 3H), 2.17 (s, 3H), 2.16 (s, 3H), 2.13 (d, *J* = 1.9 Hz, 9H), 2.07 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 170.9, 170.4, 170.3, 170.2, 170.2, 169.9, 169.8, 169.8, 146.8, 139.9, 136.8, 132.9, 124.4, 116.6, 99.1, 97.1, 94.9, 73.9, 70.9, 70.3, 69.9, 69.6, 69.2, 69.1, 68.5, 68.4, 67.7, 66.6, 66.4, 65.9, 63.8, 62.5, 62.5, 21.0, 20.9, 20.9, 20.9, 20.8, 20.7. HRMS: $[M+H]^+$ calculated for $C_{45}H_{58}NO_{29}$ 1076.30890, found 1076.30891.

***N*,*N*'-Dimethyl-*N*-(4-(2,4-*O*-di-acetyl-3-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-6-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl)-3-nitrobenzyl-oxycarbonyl)-*N*'-(*t*butyloxycarbonyl)-ethylendiamine (19):** To a solution of benzylalcohol mannose trimer **17** (0.251 g, 0.233 mmol) in DCM (2.3 mL) was added dry pyridine (28.3 µl, 0.35 mmol) under argon atmosphere and the mixture was cooled to 0 °C. To the cooled solution was added p-nitrophenyl chloroformate (70.5 mg, 0.35 mmol) and the reaction was sitrred for 30 min at 0 °C and 1.5 h at rt. The reaction mixture was cooled back to 0 °C and a mono-Boc-diamine solution (74.6 mg, 0.4 mmol) in DCM (0.4 mL) and DMAP (48.9 mg, 0.40 mmol) were added to the reaction mixture. The mixture was allowed to warm to rt and was stirred for 1h. The reaction mixture was diluted with EtOAc and washed with sat. $NaHCO_3$ (aq.) (7x), H_2O (5x), brine (2x), dried over $MgSO_4$, filtered and concentrated in vacuo. Purification by size exclusion chormatography (DCM/MeOH, 1:1) yielded mannose trimer-Boc-diamine **19** as a slightly yellow amorphous solid (0.293 g, 0.227 mmol, 97%). FT-IR: ν_{max} (neat)/cm^{-1} 1043.26, 1219.78, 1367.97, 1536.33, 1700.42, 1745.90, 2938.17. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.69 – 7.61 (m, 1H), 7.35 (d, *J* = 8.6 Hz, 1H), 5.70 (d, *J* = 1.9 Hz, 1H), 5.47 (dd, *J* = 3.3, 1.9 Hz, 1H), 5.35 (t, *J* = 9.9 Hz, 1H), 5.30 (d, *J* = 3.1 Hz, 1H), 5.25 (ddd, *J* = 10.1, 6.6, 2.9 Hz, 1H), 5.21 – 5.11 (m, 2H), 5.09 (d, *J* = 2.5 Hz, 2H), 4.76 (s, 1H), 4.45 (dd, *J* = 9.8, 3.4 Hz, 1H), 4.35 (dd, *J* = 12.2, 5.3 Hz, 1H), 4.25 (dd, *J* = 12.3, 5.5 Hz, 1H), 4.16 (d, *J* = 2.4 Hz, 1H), 4.14 – 4.03 (m, 3H), 4.00 (t, *J* = 7.3 Hz, 1H), 3.77 (dd, *J* = 11.1, 6.5 Hz, 1H), 3.46 (d, *J* = 10.7 Hz, 1H), 3.44-3.33 (m, 4H), 2.97 (s, 3H), 2.88 (s, 1.6H rotamer), 2.83 (s, 1.4H rotamer), 2.27 (s, 3H), 2.17 (s, 3H), 2.16 (s, 3H), 2.14 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.44 (s, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 170.8, 170.8, 170.8, 170.4, 170.4, 170.1, 170.1, 170.0, 170.0, 169.9, 169.9, 169.8, 169.8, 169.6,

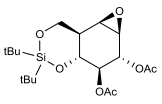
169.6, 155.7, 155.7, 148.1, 148.1, 140.1, 140.1, 134.3, 134.3, 132.4, 132.4, 125.5, 125.5, 125.3, 125.3, 117.5, 117.5, 99.2, 99.2, 97.6, 97.6, 96.1, 96.1, 74.3, 74.3, 71.0, 71.0, 70.3, 70.3, 69.8, 69.8, 69.6, 69.6, 69.3, 69.3, 68.9, 68.9, 68.7, 68.7, 68.4, 67.4, 67.4, 66.5, 66.5, 66.1, 66.1, 65.9, 65.9, 65.5, 65.5, 65.3, 65.3, 62.5, 62.5, 62.4, 62.4, 47.4, 47.4, 47.2, 47.2, 46.9, 46.9, 46.6, 46.6, 46.1, 46.1, 35.5, 35.0, 34.6, 28.5, 21.0, 21.0, 20.9, 20.9, 20.9, 20.9, 20.8. HRMS: $[M+H]^+$ calculated for $C_{55}H_{79}N_4O_{32}$ 1307.46719, found 1307.46724.

***N,N*'-Dimethyl-(4-(2,4-*O*-di-acetyl-3-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-6-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl)-3-nitrobenzyl-oxycarbonyl)-ethylendiamine HCl salt (21):**



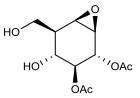
A freshly prepared 4M HCl solution in EtOAc (6.67 mL) was cooled to 0 °C and to the cooled solution was added dropwise a mannose trimer-Boc-diamine **19** (129.0 mg, 0.1 mmol) solution in EtOAc (3.33 mL). After 15 min. at 0 °C the solution was gradually warmed to rt and stirred for 1 h. The reaction mixture was concentrated *in vacuo* and co-evaporated with toluene (3x) and Et_2O (1x) yielding mannose trimer-diamine HCl salt **21** as a yellow solid (122.6 mg, 0.1 mmol, quantitative). ^{1}H NMR (400 MHz, Deuterium Oxide) δ 7.98 (d, *J* = 15.3 Hz, 1H), 7.68 (s, 1H), 7.39 (s, 1H), 5.91 (d, *J* = 8.9 Hz, 1H), 5.52 (s, 1H), 5.39 (d, *J* = 9.2 Hz, 1H), 5.34 – 4.95 (m, 8H), 4.55 – 4.22 (m, 3H), 4.23 – 3.95 (m, 6H), 3.84 – 3.42 (m, 3H), 3.25 (d, *J* = 6.9 Hz, 2H), 3.04 – 2.86 (m, 3H), 2.82 – 2.63 (m, 3H), 2.52 – 1.88 (m, 32H).

2,3-Di-*O*-acetyl-4,7-*O*-(di-*tert*-butylsilylidene)-cyclophellitol (35):



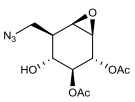
To a solution of cyclophellitol (**33**) (47.1 mg, 0.25 mmol) in DMF (2.3 mL) was added pyridine (19.8 mg, 0.2 mL, 2.5 mmol) and the solution was cooled to -40 °C under argon atmosphere. To the cooled solution was added $(t\text{Bu})_2\text{Si}(\text{OTf})_2$ (0.138 g, 102 µl, 0.313 mmol)

and the reaction mixture was gradually allowed to warm to 0 °C. At 0 °C the mixture was diluted with EtOAc and washed with H_2O. The aquaeous phase was extracted with EtOAc (4x) and the combined organic layers was washed with H_2O (3x), brine (2x), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude was dissolved in pyridine (2.5 mL) and cooled to 0 °C. To the cooled solution was added Ac_2O (0.25 mL) dropwise and the reaction mixture was allowed to warm to rt. After complete conversion the reaction mixture was cooled to 0 °C and quenched with MeOH. The reaction mixture was concentrated *in vacuo* and traces of pyridine were removed by co-evaporation with toluene. Purification by column chromatography yielded protected cyclophellitol **35** as a colorless oil (57.7 mg, 0.15 mmol, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.14 (d, *J* = 8.4 Hz, 1H), 5.08 (t, *J* = 9.1 Hz, 1H), 4.25 (dd, *J* = 10.6, 4.4 Hz, 1H), 4.16 (t, *J* = 10.9 Hz, 1H), 3.93 (t, *J* = 9.8 Hz, 1H), 3.09 (d, *J* = 3.2 Hz, 1H), 3.05 (dd, *J* = 3.7, 1.2 Hz, 1H), 2.40 (dt, *J* = 11.1, 4.3 Hz, 1H), 2.11 (s, 3H), 2.05 (d, *J* = 1.0 Hz, 3H), 1.00 (s, 9H), 0.97 (s, 9H). ^{13}C NMR (^{100} MHz, $CDCl_3$) δ 170.3, 170.0, 74.3, 70.7, 70.2, 66.5, 53.8, 52.9, 42.0, 27.3, 26.9, 22.7, 20.9, 20.8, 19.9. HRMS: $[M+H]^+$ calculated for $C_{19}H_{33}O_7Si$ 401.19901, found 401.19883.

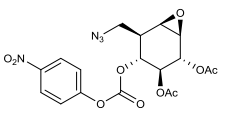


2,3-Di-*O*-acetyl-cyclophellitol (36): To a solution of silylidene protected cyclophellitol **33** (38.4 mg, 0.1 mmol) in THF (0.5 mL) was added pyridine (0.25 mL). and the mixture was cooled to 0 °C. To the cooled reaction mixture was added 1M HF·pyridine (0.25 mL, 0.25 mmol) and stirred for 1 h at 0 °. The reaction was quenched with $NaHCO_3$ (s). The excess of solids were filtered over a plug of cotton wool, rinsed with acetone and *in vacuo*. Purification by column chromatography yielded 2,3-acetylated cyclophellitol **36** as a colorless amorphous solid (26.8 mg, quant.). FT-IR: ν_{max} (neat)/cm^{-1} 1031.61, 1233.01, 1372.86, 1749.37, 3443.82. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.05 (d, *J* = 8.4 Hz, 1H), 4.99 (t, *J* = 8.4 Hz, 1H), 4.05 (ddd, *J* = 10.5, 6.3, 4.2 Hz, 1H), 3.95 (ddd, *J* = 10.8, 6.6, 4.2 Hz, 1H), 3.63 (dd, *J* = 9.6, 5.4, Hz, 1H), 3.56 (t, *J* = 5.4 Hz, 1H), 3.38 – 3.34 (m, 2H), 3.10 (d, *J* = 3.2 Hz, 1H), 2.24 (dt, *J* = 8.4, 6.0 Hz, 1H), 2.10 (s, 3H), 2.09 (s,

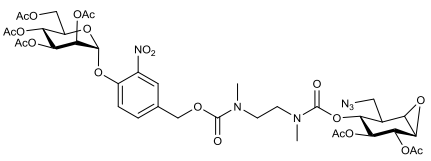
1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.3, 170.3, 71.0, 67.0, 63.0, 55.2, 53.3, 43.7, 21.0, 20.9. HRMS: $[M+H]^+$ calculated for $C_{11}H_{17}O_7$ 261.09622, found 261.09695.



2,3-Di-*O*-acetyl-7-deoxy-7-azido-cyclophellitol (40): To a solution of 2,3-acetylated cyclophellitol **34** (65.3 mg, 0.25 mmol) in DCM (9.8 mL) was added pyridine (0.2 mL, 2.5 mmol) and the solution was cooled to -25 °C under argon atmosphere. To the solution was added triflic anhydride (52.5 µl, 0.36 mmol) and the reaction was stirred for 1 h at -25 °C. The reaction mixture was diluted with EtOAc and washed with 0.1 M HCl (aq.) (5 mL, pH ≤ 6), sat. $NaHCO_3$ (aq) (pH ≥ 6), H_2O (3x) and brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo* (30 °C, 100 mbar). Triflated product **39** was co-evaporated once with toluene and used in the next step without further purification. The triflated product was dissolved in THF (2.5 mL) and cooled to -25 °C under argon atmosphere. To the cooled solution was added NaN_3 (53.3 mg, 0.75 mmol) and 15-crown-5 (46.25 µl, 0.25 mmol,) and the reaction mixture was gradually allowed to warm to 0 °C. After 20 min the reaction was complete and the reaction mixture was diluted with Et_2O, silica gel was added and the solvents were removed under reduced pressure. The silica-immobilized product was purified by column chromatography yielding 2,3-acetylated azido cyclophellitol **40** as a colorless amorphous solid (64.1 mg, 0.225 mmol, 90 %). FT-IR: ν_{max} (neat)/cm^{-1} 1078.90, 1033.96, 1115.73, 1237, 94, 1370.57, 1451.48, 1749.61, 2103.16, 2873.00, 2923.53, 3456.26. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.07 (d, *J* = 8.5 Hz, 1H), 4.96 (dd, *J* = 10.2, 8.4 Hz, 1H), 3.88 (dd, *J* = 12.2, 4.1 Hz, 1H), 3.59 (dd, *J* = 12.2, 8.5 Hz, 1H), 3.51 (t, *J* = 10.1 Hz, 1H), 3.41 (d, *J* = 3.5 Hz, 1H), 3.14 (d, *J* = 3.6 Hz, 1H), 2.24 (dddd, *J* = 10.0, 8.5, 4.1, 1.6 Hz, 1H), 2.10 (s, 3H), 2.09 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 170.1, 75.3, 70.8, 66.4, 54.9, 54.1, 51.0, 42.2, 21.0, 20.9. HRMS: $[M+H]^+$ calculated for $C_{11}H_{16}N_3O_6$ 286.10336, found 286.10344.

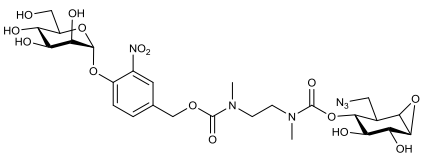


2,3-Di-*O*-acetyl-4-*O*-(4-nitrophenoxycarbonyl)-7-deoxy-7-azido-cyclophellitol (41): 2,3-acetylated azido cyclophellitol **40** (0.1 mmol, 28.5 mg) was dissolved in a 0.2M pyridine solution in DCM (1 mL, 0.2 mmol) and the solution was cooled to 0 °C. To the cooled solution was added *para*-nitrophenyl chloroformate (40.4 mg, 0.2 mmol) and the solution was allowed to warm to rt. After 2 h the reaction mixture turned clear and TLC showed full conversion of the starting material. The reaction mixture was diluted with DCM, silica was added to immobilize the product. After removal of the solvents, the immobilized product was directly purified by column chromatography yielding *p*-nitro phenyl oxycarbonyl cyclophellitol **41** as a slightly yellow amorphous solid (46.6 mg, 0.1 mmol, quantitative yield). FT-IR: ν_{max} (neat)/cm^{-1} 1210.93, 1348.92, 1492.95, 1526.21, 1749.96, 2105.51, 2854.81, 2925.12. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, *J* = 2.2 Hz, 1H), 8.29 (d, *J* = 2.1 Hz, 1H), 7.37 (d, *J* = 2.2 Hz, 1H), 7.35 (d, *J* = 2.1 Hz, 1H), 5.27 (dd, *J* = 10.3, 8.1 Hz, 1H), 5.15 (d, *J* = 8.1 Hz, 1H), 4.87 (t, *J* = 10.2 Hz, 1H), 3.72 (dd, *J* = 12.4, 5.0 Hz, 1H), 3.66 (dd, *J* = 12.4, 7.9 Hz, 1H), 3.50 (d, *J* = 3.2 Hz, 1H), 3.23 (d, *J* = 3.5 Hz, 1H), 2.58 (dddd, *J* = 9.8, 7.9, 5.0, 1.5 Hz, 1H), 2.12 (s, 3H), 2.05 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 169.9, 155.3, 152.1, 145.7, 125.6, 121.6, 73.7, 72.1, 71.0, 54.9, 53.8, 50.8, 40.0, 20.9, 20.8. HRMS: $[M+H]^+$ calculated for $C_{18}H_{19}N_4O_{10}$ 451.10957, found 541.10934.



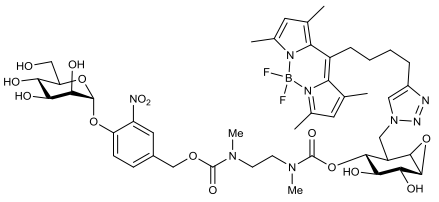
Man$_1$-OAc-azido-cyclophellitol (42): *p*-Nitro phenyl oxycarbonyl cyclophellitol **41** (14.9 mg, 33 µmol) was dissolved in 0.3M Et_3N sol. in DMF (333 µl, 100 µmol, 3 eq.) and cooled to 0 °C. To the solution was added a solution of mannose-diamine HCl salt **18** in a 0.01M Et_3N sol. in DMF (333 µl, 3.3 µmol, 0.1 eq.) and the reaction was allowed to warm to rt. After 2 h TLC showed full conversion of the starting material and the reaction mixture was diluted with Et_2O. Upon addition of sat. $NaHCO_3$ (aq.) the organic phase turned yellow. The aqueous phase was extracted with Et_2O (3x) and the

combined organic phase was washed with sat. $NaHCO_3$ (aq.) (3x), H_2O (4x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by size-exclusion chromatography yielded peracetylated-mannose-cyclophellitol construct **42** as a colorless amorphous solid (22.3 mg, 24.1 µmol, 73%). FT-IR: ν_{max} (neat)/cm^{-1} 1220.07, 1368.04, 1536.15, 1707.27, 1749.40, 2105.56, 2927.98. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 – 7.89 (m, 1H), 7.61 – 7.52 (m, 1H), 7.31 (d, *J* = 8.6 Hz, 1H), 5.68 (d, *J* = 2.0 Hz, 1H), 5.56 (dd, *J* = 10.0, 3.4 Hz, 1H), 5.49 (dd, *J* = 3.4, 1.9 Hz, 1H), 5.41 (t, *J* = 10.0 Hz, 1H), 5.23 – 5.04 (m, 4H), 4.86 – 4.70 (m, 1H), 4.29 (dd, *J* = 12.4, 5.0 Hz, 1H), 4.19 (dd, *J* = 10.3, 5.2 Hz, 1H), 4.09 (dd, *J* = 12.3, 2.3 Hz, 1H), 3.72 – 3.39 (m, 5H), 3.39 – 3.26 (m, 1H), 3.18 (t, *J* = 2.9 Hz, 1H), 2.99 – 2.92 (m, 4H), 2.85 (t, *J* = 13.5 Hz, 3H), 2.42 – 2.29 (m, 1H), 2.21 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H), 2.01 – 1.97 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 170.2, 170.0, 169.9, 169.9, 169.7, 156.1, 156.0, 155.6, 155.4, 155.1, 155.1, 148.4, 140.6, 134.3, 133.9, 133.6, 132.4, 132.4, 132.3, 132.2, 125.9, 125.6, 125.5, 125.4, 117.8, 96.8, 72.4, 72.3, 72.3, 71.0, 70.9, 70.4, 69.2, 69.1, 68.9, 68.8, 68.6, 65.7, 65.5, 65.4, 62.1, 54.8, 54.2, 50.7, 50.6, 47.4, 47.4, 47.0, 46.8, 46.6, 46.6, 46.1, 41.1, 41.0, 35.8, 35.5, 35.4, 34.8, 34.6, 21.0, 20.9, 20.8, 20.8, 20.8. HRMS: $[M+H]^+$ calculated for $C_{38}H_{49}N_6O_{21}$ 925.29453, found 925.29558.



Man$_1$-OH-azido-cyclophellitol (44): To a solution of peracetylated-mannose-cyclophellitol construct **42** (10.1 mg, 10.9 µmol) in MeOH (109 µl) was added a 0.1M NaOMe sol. in MeOH (109 µl, 10.9 µmol) and the mixture was stirred for 2 h at rt. The reaction was quenched with Amberlite® IR-120 H^+ till pH ≤7. The solids were filtered and the filtrate was concentrated *in vacuo*. Purification by column chromatograpy yielded mannose-cyclophellitol **42** as a white powder after lyophilizing (12.6 mg, 18.7 µmol, 79%). FT-IR: ν_{max} (neat)/cm^{-1} 973.37, 1067.35, 1131.21, 1175.14, 1131.21,

1254.16, 1354.23, 1405.68, 1486.46, 1533.84, 1692.93, 2104.78, 2925.93, 3384.84. ^{1}H NMR (600 MHz, D_2O) δ 8.04 – 7.92 (m, 1H), 7.75 – 7.66 (m, 1H), 7.57 – 7.48 (m, 1H), 5.84 (s, 0.4H rotamer), 5.81 (d, *J* = 6.8 Hz, 0.6H rotamer), 5.20 – 5.05 (m, 2H), 4.46 (q, *J* = 9.7 Hz, 0.5H rotamer), 4.39 (t, *J* = 10.2 Hz, 0.5H rotamer), 4.21 (dd, *J* = 3.7, 1.9 Hz, 1H), 4.04 (ddd, *J* = 9.9, 3.4, 1.7 Hz, 1H), 3.85 (dd, *J* = 8.6, 4.6 Hz, 5H), 3.71 – 3.61 (m, 2H), 3.56 – 3.34 (m, 4H), 3.34 – 3.15 (m, 3H), 2.98 (s, 1H), 2.93 (s, 1H), 2.91 (s, 1H), 2.89 (s, 0.5H rotamer), 2.84 (s, 0.5H rotamer), 2.82 (s, 1H), 2.46 – 2.30 (m, 0.6H rotamer), 2.09 – 2.02 (m, 0.4H rotamer). ^{13}C NMR (150 MHz, D_2O) δ 158.4, 158.2, 158.1, 158.0, 149.5, 149.4, 149.3, 149.2, 140.5, 140.5, 140.4, 136.1, 135.7, 135.0, 134.7, 132.3, 131.9, 131.9, 126.8, 126.4, 126.3, 125.6, 125.3, 118.9, 118.9, 118.8, 99.5, 99.4, 99.4, 75.3, 75.3, 75.3, 75.1, 75.1, 75.0, 72.2, 72.0, 71.9, 71.8, 71.7, 71.6, 71.6, 71.1, 70.5, 67.2, 67.0, 67.0, 66.9, 66.6, 61.5, 57.1, 57.0, 56.9, 56.9, 56.9, 56.8, 56.7, 51.2, 51.1, 51.0, 50.9, 47.4, 47.3, 47.2, 47.2, 47.0, 46.9, 40.9, 40.8, 40.7, 35.6, 35.4, 35.3, 35.2, 35.0, 34.9, 34.8. HRMS: $[M+H]^+$ calculated for $C_{26}H_{37}N_6O_{15}$ 673.23184, found 673.23186.



Man$_1$-BODIPY-cyclophellitol (3): To a solution of Man$_1$-OH-azido cyclophellitol **44** (744 µg, 1.1 µmol) and BODIPY-alkyn **46** (540 µg, 1.65 µmol) in DMF (110 µl) was added a 0.075M sodium ascorbate (aq.) solution (11 µl, 0.825 µmol) and a 0.05M $CuSO_4$ (aq.) solution (11 µl, 0.55µmol) under argon atmosphere. The reaction was stirred overnight at rt and LC-MS showed full consumption of the starting material. Purification by HPLC followed by lyophilization yielded Man$_1$-BODIPY-cyclophellitol **1** as an orange-red powder (700 µg, 0.7 µmol, 64%). FT-IR: ν_{max} (neat)/cm^{-1} 971.93, 1119.40, 1450.71, 2073.18, 2242.73, 2362.56, 2486.52, 3363.59. LC-MS: R_t 6.37 in 12.5 min, 10→90% H_2O/MeCN, ESI/MS $[M+H]^+$ 1001.13. HRMS: $[M+H]^+$ calculated for $C_{45}H_{60}BF_2N_8O_{15}$ 1001.42415, found 1001.42433.

Man_3-OAc-azido-cyclophellitol (43):. *p*-Nitro phenyl oxycarbonyl cyclophellitol **41** (450 mg, 0.1 mmol) was dissolved in 0.3M Et_3N sol. in DMF (1 mL, 0.3 mmol) and cooled to 0 °C. To the solution was added a solution of mannose trimer-diamine HCl salt **21** in a 0.01M Et_3N sol. in DMF (1 mL, 10 mmol) and the reaction was allowed to warm to rt. After 2 h TLC showed full conversion of the starting material and the reaction mixture was diluted with Et_2O. Upon addition of sat. $NaHCO_3$ (aq.) the organic phase turned yellow. The aqueous phase was extracted with Et_2O (3x) and the combined organic phase was washed with sat. $NaHCO_3$ (aq.) (3x), H_2O (4x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by size-exclusion yielded peracetylated-mannose trimer-cyclophellitol construct **43** as a colorless amorphous solid (76.6 mg, 51 µmol, 51%). FT-IR: ν_{max} (neat)/cm^{-1} 1041.62, 1086.21, 1135.84, 1219.53, 1369.13, 1536.51, 1709.80, 1747.60, 2105.92, 2852.59, 2925.64. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 – 7.89 (m, 1H), 7.63 (d, *J* = 9.0 Hz, 1H), 7.35 (d, *J* = 8.6 Hz, 1H), 5.70 (d, *J* = 2.2 Hz, 1H), 5.47 (dt, *J* = 3.8, 2.0 Hz, 1H), 5.34 (t, *J* = 10.1, 9.2 Hz, 1H), 5.32 – 5.20 (m, 4H), 5.22 – 5.02 (m, 8H), 4.86 – 4.68 (m, 2H), 4.44 (dd, *J* = 9.8, 3.4 Hz, 1H), 4.35 (dd, *J* = 12.5, 5.4 Hz, 1H), 4.25 (dd, *J* = 12.3, 5.5 Hz, 1H), 4.16 (d, *J* = 2.4 Hz, 1H), 4.14 – 4.03 (m, 3H), 4.02 – 3.97 (m, 1H), 3.77 (dd, *J* = 11.2, 6.4 Hz, 1H), 3.71 – 3.39 (m, 6H), 3.33 (dt, *J* = 13.0, 7.2 Hz, 1H), 3.18 (d, *J* = 3.4 Hz, 1H), 3.00 – 2.91 (m, 4H), 2.89 – 2.80 (m, 2H), 2.35 (h, *J* = 5.0 Hz, 1H), 2.26 (s, 3H), 2.16 (s, 3H), 2.16 (s, 3H), 2.14 (s, 3H), 2.12 (s, 3H), 2.10 (s, 6H), 2.08 (s, 3H), 2.06 (s, 3H), 2.00 (s, 3H), 1.98 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 170.7, 170.4, 170.2, 170.1, 170.0, 169.8, 169.8, 169.6, 156.0, 155.9, 155.7, 155.5, 155.4, 155.0, 155.0, 155.0, 148.3, 148.2, 140.2, 134.8, 134.4, 134.1, 132.4, 132.3, 132.1, 125.8, 125.4, 125.1, 117.5, 99.2, 97.6, 96.2, 96.1, 74.2, 72.3, 72.3, 72.2, 70.9, 70.3, 69.8, 69.6, 69.3, 68.9, 68.8, 68.7, 68.4, 67.4, 66.5, 66.1, 65.9, 65.6, 65.4, 62.5, 62.4,

54.7, 54.1, 50.6, 50.6, 50.5, 47.4, 47.3, 47.0, 46.8, 46.6, 46.5, 46.1, 41.0, 40.9, 35.7, 35.3, 34.7, 34.6, 34.6, 29.8, 20.9, 20.9, 20.9, 20.9, 20.9, 20.8, 20.8, 20.7. HRMS: $[M+H]^+$ calculated for $C_{62}H_{81}N_6O_{37}$ 1501.46356, found 1501.46367.

Man_3-OH-azido-cyclophellitol (45): To a solution of peracetylated-mannose-trimer-cyclophellitol construct **43** (30.8 mg, 20 µmol) in MeOH (200 µl) was added a 0.1M NaOMe sol. in MeOH (200 µl, 20 µmol) and stirred overnight at rt. The reaction was quenched with Amberlite® IR-120 H^+ till pH ≤7. The solids were filtered and the filtrate was concentrated *in vacuo*. Purification by column chromatograpy yielded mannose-cyclophellitol **45** as a white powder after lyophilizing (13.72 mg, 13.76 µmol, 69%). FT-IR: ν_{max} (neat)/cm^{-1} 1053.15, 1131.99, 1253.41, 1353.55, 1405.62, 1486.69, 1533.03, 1622.70, 1684.90, 2104.39, 2927.58, 3363.18. ^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.03 (t, *J* = 9.1 Hz, 1H), 7.76 (t, *J* = 8.8 Hz, 1H), 7.51 (t, *J* = 9.1 Hz, 1H), 5.88 – 5.75 (m, 1H), 5.22 (d, *J* = 1.8 Hz, 1H), 5.21 – 5.06 (m, 2H), 4.40 – 4.33 (m, 1H), 4.15 (dd, *J* = 9.5, 3.3 Hz, 1H), 4.12 (dd, *J* = 3.4, 1.7 Hz, 1H), 4.04 – 3.89 (m, 5H), 3.87 (s, 1H), 3.85 – 3.76 (m, 3H), 3.76 – 3.61 (m, 6H), 3.60 – 3.33 (m, 3H), 3.29 (q, *J* = 3.9 Hz, 1H), 3.26 – 3.16 (m, 1H), 2.99 (d, *J* = 2.5 Hz, 1H), 2.96 – 2.88 (m, 3H), 2.86 (d, *J* = 7.4 Hz, 2H), 2.32 (d, *J* = 18.5 Hz, 0.6H), 2.00 (t, *J* = 9.6 Hz, 0.4H), 1.29 (s, 1H). ^{13}C NMR (100 MHz, D_2O) δ 174.0, 157.5, 139.7, 131.3, 117.9, 102.3, 99.1, 98.3, 77.6, 74.4, 74.4, 73.3, 72.7, 72.5, 71.0, 70.6, 70.4, 70.1, 69.9, 69.1, 66.7, 66.7, 66.1, 65.7, 65.6, 65.0, 60.9, 60.8, 56.0, 50.0, 46.4, 46.3, 39.9, 39.8, 34.7, 34.4. LC-MS: R_t 4.12 in 12.5 min, 10→90% H_2O/MeCN, ESI/MS $[M + H]^+$ 996.93. HRMS: $[M+H]^+$ calculated for $C_{38}H_{57}N_6O_{25}$ 997.33679, found 997.33677.

Man_3-BODIPY-cyclophellitol (4): To a solution of Man_3-OH-azido cyclophellitol **45** (7.8 mg, 7.8 µmol) and BODIPY-alkyn **46** (3.9 mg, 11.7 µmol) in DMF (624 µl) was added a 0.075M sodium ascorbate (aq.) solution (78 µl, 5.85 µmol) and a 0.05M $CuSO_4$ (aq.) solution (78 µl, 3.9 µmol) under argon atmosphere. The reaction was stirred overnight at rt and LC-MS showed full consumption of the starting material. Purification by HPLC followed by lyophilization yielded Man_3-BODIPY-cyclophellitol **2** as an orange-red powder (3.136 mg, 2.37 µmol, 30%). FT-IR: ν_{max} (neat)/cm^{-1} 1681.21, 2074.42, 2229.80, 2342.27, 2360.78, 3373.05.. LC-MS: R_t 5.81 in 12.5 min, 10→90% H_2O/MeCN, ESI/MS $[M + H]^+$ 1325.27. HRMS: $[M+H]^+$ calculated for $C_{57}H_{80}BF_2N_8O_{25}$ 1325.52998, found 1325.52970.

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