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The Synthesis of Mannose-derived Bioconjugates and Enzyme Inhibitors

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Chung Sing Wong

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“It’s nice to be important, but it’s more important to be nice”
Scooter “*Move your ass!”* (1995)

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List of abbreviations

4MUGlc	4-methylumbelliferyl-β-D-glucopyranoside
AA	amino acid
ABP	activity-based probe
ABPP	activity-based protein profiling
Ac	acetyl
APC	antigen presenting cell
BM	bone marrow
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
BODIPY	boron- dipyrromethane
Bu	butyl
Bz	benzoyl
CBB	coomassie brilliant blue
CLRs	C-type lectin receptors
CPRG	chlorophenol β-D-galactopyranoside
CSA	camphorsulfonic acid
d	doublet
DABCO	1,4-diazabicyclo [2,2,2]octane
DC	dendritic cell
DCE	dichloroethane
DCM	dichloromethane
dd	double doublet
ddd	double double doublet
DFT	density functional theory
DIBAL-H	diisobutylaluminium hydride
DIC	*N,N*-diisopropyl carbodiimide
DiPEA	diisopropylethylamine
DMAP	4-(dimethylammino) pyridine
DMF	N,N-dimethyl formamide
DMSO	dimethylsulfoxide
dt	double triplet
EEDQ	2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline
ESI	electron spray ionization
Et	ethyl
Et_2O	diethylether
Et_3N	triethylamine
EtOAc	ethyl acetate
Fmoc	(9*H*-fluoren-9-yl) methoxycarbonyl
GBA	glucocerebrosidase
GH	glycosyl hydrolase
h	hour(s)
HCTU	(2-(6-chloro-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafruorophosphate
HOBt	*N*-hydroxybenzotriazole
HPLC	high performance liquid chromatography
IC50	inhibition concentration resulting in 50% inhibition of enzyme activity
J	coupling constant
LC-MS	liquid chromatography-mass spectrometry
m	multiplet
M	molar
m/z	mass-t-charge ratio
Man	mannose
*m*CPBA	*meta*-chloroperbenzoic acid
Me	methyl
MeCN	acetonitril
MeOH	methanol

MHC	major histocompatibility complex
MMTrt	monomethoxy trityl
MR	mannose receptor
Ms	methanesulfonyl (mesyl)
MS, Mol. Siev.	molecular sieves
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NLR	nucleotide-binding oligomerization domain-like receptor
NMP	*N*-methyl-2-pyrolidone
NMR	nuclear machnetic resonance
PAMP	pathogen associated molecular pattern
PBS	phosphate buffer saline
Ph	phenyl
ppm	parts per million
*p*Ts	*para*-toluenesulfonyl
q	quartet
RP	reverse phase
rt	room temperature
s	singlet
SDS-PAGE	sodium dodecyl sulfate-poly acrylamine gel electroforesis
Su	succinimidyl
t	triplet
TBAF	tetrabutylammonium fluoride
TBDMS	tert-butyldimethylsilyl
tert, *t*	tertiary
Tf	trifluoromethanesulfonyl (triflate)
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TLRs	toll-like receptors
TMR	tetramethylrhodamine
TMS	tetramethylsilane
Trt	trityl
UV	ultra violet

Chapter 1

General introduction

Targeted delivery of a drug to the desired site of action has clear therapeutic advantages by maximizing the efficiency of action and minimizing the systemic toxicity. Targeting of drugs can be performed by loading a drug with a specific molecular carrier.[1,2] A molecular carrier can be a ligand interacting with a specific receptor at the cell surface. Carbohydrates are key ligands in nature and carbohydrate-receptor interactions are involved in vitally important processes such as cell-cell communication and immune responses.[3] For decades naturally occurring and artificial (oligo)saccharides have been explored as carriers for drug targeting.[4]

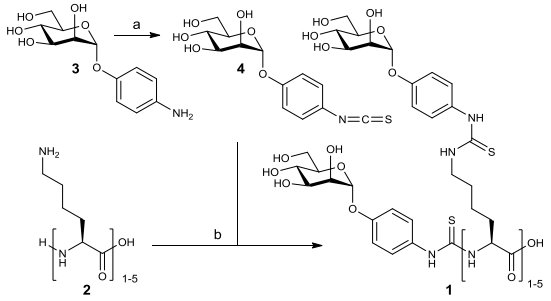
This introductory chapter describes selected examples of conjugates bearing various mannose ligands, that have been designed and synthesized to enable mannose receptor mediated uptake.

Linear scaffolds

Lysine clusters

Biesen *et al.*[5] were the first to report on the design of well-defined linear mannose clusters. In search for synthetic, high-affinity ligands for the mannose receptor (MR) they synthesized a series of oligolysines in which the lysine side chains are connected to α-mannosides ($Man_{n+1}Lys_n$) via thiourea linkages (Scheme 1, cluster **1**). The mannose clusters (**1**) were synthesized by reaction of 4-(α-D-mannopyranosyl)phenylisothiocyanate **4** with the amines in the oligolysines **2** under basic conditions. Mannoside **4** was obtained by treatment of 4-aminophenyl mannose **3** with thiophosgene in quantitative yield (Scheme 1).

Scheme 1: Synthesis of lysine mannose cluster conjugate **1**.



Reagents and conditions: a) $CSCl_2$, $EtOH/H_2O$ (80:20, v/v), quantitative; b) 0.1 mM $NaHCO_3$ (aq.), DMF, 12 - 50%.

The obtained mannose clusters **1** were subjected to competition binding studies. Isolated human MR was saturated with biotinylated ribonuclease B (bio-Rib B) and biotinylated tissue plasminogen activator (bio-t-PA), proteins with high mannose-type glycans, for competition with the mannose clusters **1**. All the clusters (Man_2Lys_1 – Man_6Lys_5) showed high affinity towards the MR and could completely outcompete both bio-Rib B and bio-t-PA ligands. Furthermore the number of mannose residues proved to have a major influence on the affinity. On average, the introduction of an additional mannose unit to the cluster led to a 10-fold increase in binding. Man_6Lys_5

was identified to be the most potent ligand with affinity in the nanomolar range, and the authors argued that this cluster may find use as a carrier device for cell-specific delivery of drugs to macrophages and endothelial liver (??) cells.

Kinzel *et al.*[6] applied the hexavalent lysine mannose cluster **1** (n = 5) for the construction of peptide-, polyamide- and peptide nucleic acids (PNA)-conjugates. They were interested in the selective delivery of these conjugates to dendritic cells (DCs) by MR mediated uptake. In the field of regulation of gene expression polyamides and PNA oligomers are explored to target a pre-determined DNA sequence.[7] Within this framework bis-PNA **5**[8] and polyamide distamycin derivative **6**[9] (Figure 1) were both conjugated to the lysine mannose cluster via flexible and water soluble spacers. Furthermore peptide **7** was conjugated to the same lysine mannose cluster for MR mediated uptake.

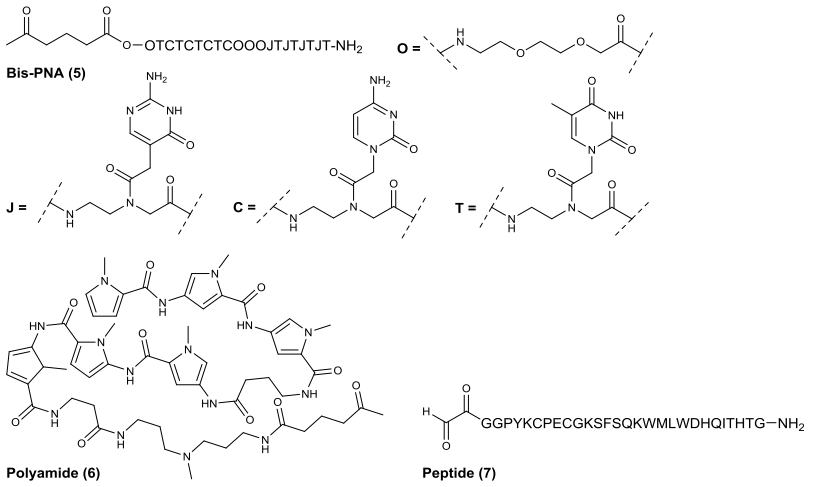
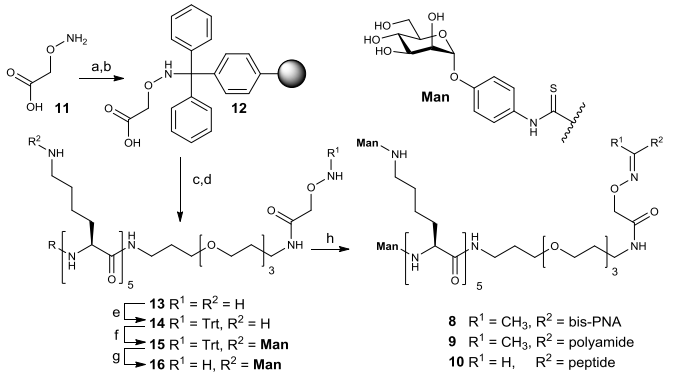


Figure 1: Structures of bis-PNA **5**, polyamide **6** and peptide **7**.

The route of synthesis to the conjugates is shown in Scheme 2 and the key conjugation reaction comprises oxime formation by condensation of an aminooxy group in spacer **16** with a ketone in bis-PNA moiety **5**, a ketone in

polyamide **6** and an aldehyde in peptide **7** to give the conjugates **8-10** respectively (Scheme 2).

Scheme 2: Synthesis of bis-PNA, polyamide and peptide conjugates **8-10**.



Reagents and conditions: (a) TMSCl, Et_3N, CH_3Cl/MeCN; (b) PS-trityl-Cl, Et_3N; (c) carbonyldiimidazole, DMF, PEG_3-diamine, HOBt; (d) *i.* Fmoc-Lys(Boc)-OH, PyBOP, HOBt, DIPEA; *ii.* piperidine/DMF (20:80); *iii.* TFA (87.5%), H_2O (5%), phenol (5%), TIPS (2.5%), 68% from resin **12**; (e) triphenylmethanol, $BF_3 \cdot Et_2O$, AcOH, DCM, 80%; (f) thioisocyanate mannoside **4**, 0.1M $NaHCO_3$ (aq.), DMF; (g) 3% TFA, 2% TIPS (in DCM), 30% from **15**; (h) bis-PNA **5** (22%), polyamide **6** (67%), peptide **7** (77), acetate buffer (0.1 M, pH 4.0), DMF.

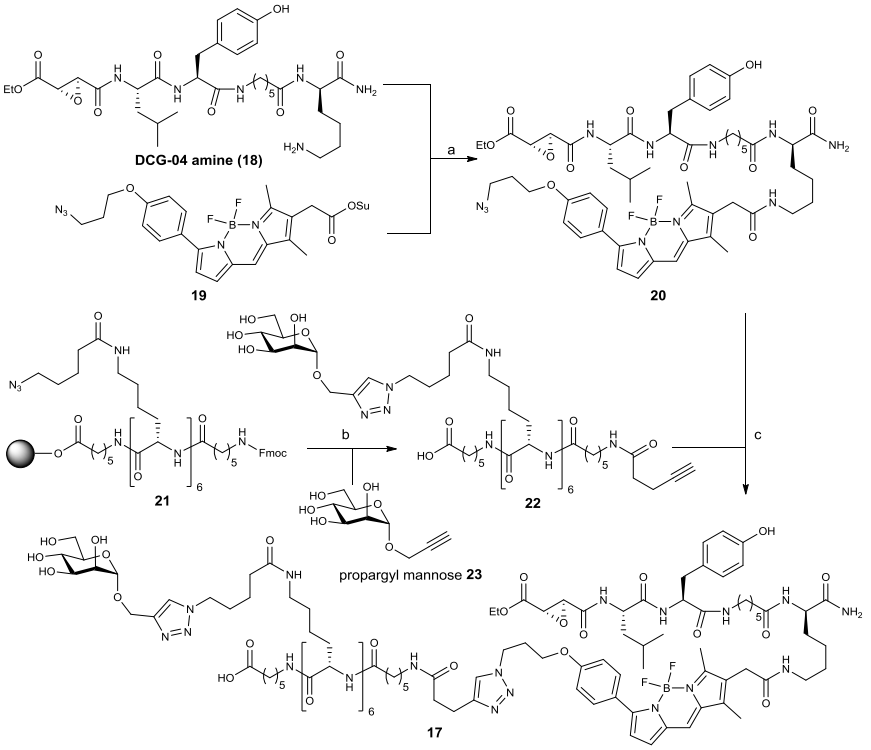
The assembly of the conjugates starts with the treatment of carboxymethoxylamine hemihydrochloride **11** with TMS-chloride and subsequent coupling to a 2-chlorotrityl resin to give the aminooxy resin **12**. Elongation of the resin with a triethylene glycol spacer was followed by five consecutive couplings of lysine residues, using standard Fmoc chemistry. Removal of the Boc protecting groups and cleavage from the solid support under acidic conditions (87.5% TFA) gave peptide **13** in 68% yield. After selective tritylation of the aminooxy functionality in **13** the mannose moieties were installed by reaction of the free amino functions in **14** with isothiocyanide mannoside **4** in the presence of sodium bicarbonate. The aminooxy functionality was released by removal of the trityl protective group under mild acidic conditions with TIPS as scavenger giving the functionalized intermediate **16** in 30% over 3 steps. Bis-PNA **5**, polyamide **6** and peptide **7**, were conjugated with cluster **16** by oxime bond formation,

providing conjugates **8** in 22%, **9** in 67% and **10** in 77%, respectively. Up to now the biological activity of conjugates **8**-**10** has not reported.

Hillaert *et al.* reported the design, synthesis and evaluation of a construct in which a fluorescent, covalent protease inhibitor is equipped with a mannose cluster.[10] This study was based on the activity-based cathepsin probe, DCG-04, a broad spectrum cysteine protease inhibitor, originally developed by the group of Bogyo.[11] DCG-04 amine derivative **18** was coupled with the activated BODIPY acid **19** to give fluorescent inhibitor **20** in 93% yield. The synthesis of hexavalent mannose cluster **22** deserves some attention. As discussed earlier, previous examples to construct mannosylated lysine clusters were performed in solution. Hillaert *et al.* successfully used the copper(I)-catalyzed Huisgen [2+3] azide-alkyne "click" cycloaddition of propargyl mannoside **23** and the six azide moieties of immobilized hexapeptide **21.** Subsequently, Fmoc removal, elongation with pent-4-ynoic acid and finally cleavage from the solid support provided mannose cluster **22**. In the second click event compound **20** was connected in solution to mannose cluster **22** to give mannosylated BODIPY-DCG-04 construct **17**.

Profiling of the activity of cathepsins with construct **17** using rat liver lysate showed a concentration dependent labeling profile similar to that previously reported for DCG04[12]. Importantly, the hexavalent mannoside cluster appeared not to interfere with the cathepsin binding. Experiments with **17** using bone-marrow derived DCs and macrophages revealed uptake and intracellular cathepsin labeling in both cell types. Blocking the MR with mannan abolished labeling, pointing towards mannose receptor dependent uptake and intracellular delivery.

Scheme 3: Synthesis of mannosylated BODIPY-DCG-04 construct **17**.



Reagents and conditions: (a) DMF, DIPEA, 93%; (b) *i.* alpha-propargyl-mannopyranoside **23**, $CuSO_4$, sodium ascorbate, *t*BuOH/DMF/H_2O (2:1:1); c) *i.* 20% piperidine (in NMP); *ii*. pent-4-ynoic acid, BOP, DIPEA, NMP; d) 20% TFA (aq.), 85%; e) $CuSO_4$, sodium ascorbate, *t*BuOH/H_2O (1:1.7), 14%.

Cyclic scaffolds

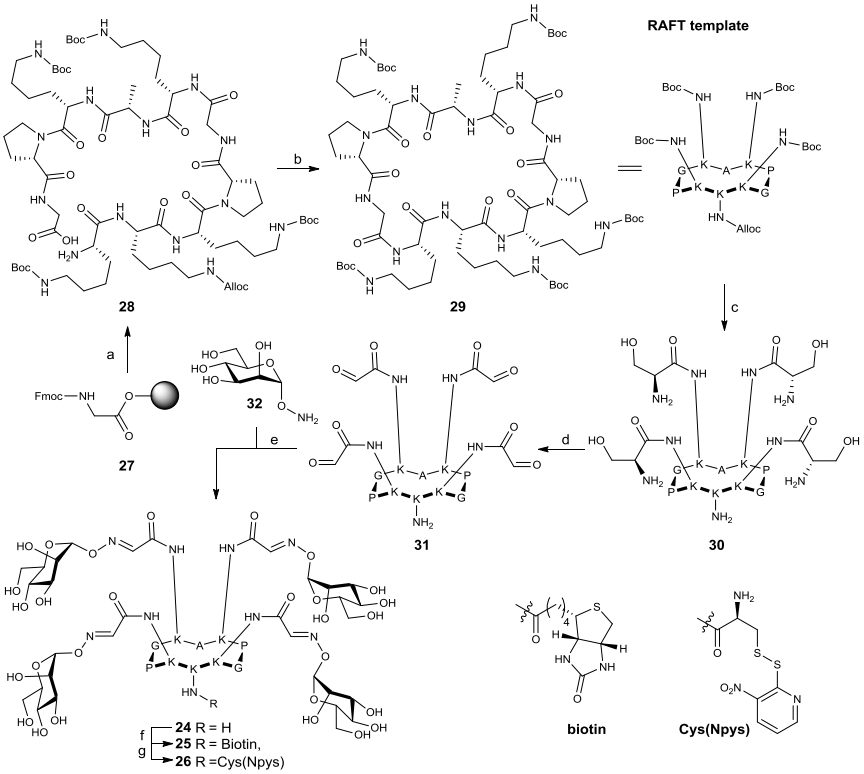
Cyclic RAFT peptides

Regioselectively addressable functionalized templates (RAFTs), are cyclic peptide templates that are commonly composed of a backbone-cyclized peptide, such as **29** (Scheme 4). This RAFT contains two proline-glycine dipeptides as β-turn inducers.[13] Renaudet *et al*. reported the synthesis of tetravalent glycoconjugates as a potential tool for cell targeting and cell-surface mimics.[14] Tetravalent mannosyl RAFT conjugate (construct **24**,

Scheme 4), was evaluated for binding with Concanavalin A (Con A), a mannose binding lectin. The mannosylated RAFT conjugate showed a 20-fold increase in potency relative to α-D-*O*-methyl mannose.[15]

A subsequent study describes the synthesis of biotin and *S*-3-nitro-2-pyridinesulfenyl (Npys) functionalized RAFT conjugates **25** and **26** (Scheme 4) to study the carbohydrate-lectin recognition.[16] The assembly of **24-26** started with the solid phase synthesis of peptide **28**, having orthogonally protected Boc-lysine and Alloc-lysine residues, using standard Fmoc chemistry. After cleavage from the resin, peptide **28** was subjected to an intramolecular cyclisation reaction under influence of PyBOP giving key cyclic peptide **29** in 87% yield.[17] Deprotection of the Boc-protective groups in **29** and coupling of the liberated amines with Boc-Ser(*t*Bu)-OH followed palladium-mediated deprotection of the alloc group[18] and removal of the Boc group in the serine residues gave compound **30** in 53% yield over 4 steps.[19] Periodate oxidation of tetravalent serine compound **30** gave the corresponding aldehyde **31** which was condensed with aminooxy mannoside **32**[20] to give the target tetravalent mannosylated RAFT conjugate **24**. The free lysine amine in **24** was used to append a biotin moiety giving biotinylated tetravalent mannosylated RAFT conjugate **25**. To immobilize the RAFT on a surface, **24** was functionalized with a cysteine residue through coupling of the free lysine with Boc-Cys(Npys)-OSu ester and subsequent removal of the Boc-group, to give tetravalent mannosylated RAFT conjugate **26**. Both **25** and **26** were immobilized on a gold surface at various surface densities. The interaction of conjugates **25** and **26** with Con A was studied by nanogravimetry and surface plasmon resonance. The conjugates show increased affinities towards Con A in comparison with the monovalent counterpart. This increase was explained by a clustering effect since the mannose ligands in **25** and **26** cannot span the saccharide binding sites within a single Con A tetramer.

Scheme 4: Synthesis of biotin-RAFT mannose conjugate **25** and Cys(Npys)-RAFT mannose conjugate **26**.



Reagents and conditions: (a) *i*) 20% piperidine/DMF (3x 20 min); *ii*) Fmoc-aa-OH, PyBOP, DIPEA, DMF; *iii*) TFA (1%) in DCM, 87%; (b) PyBOP, DIPEA, 86%; (c) *i*. 50% TFA (in DCM); *ii*. Boc-Ser(*t*Bu)-OH, PyBOP, DIPEA, DMF; *iii*. $Pd(Ph_3P)_4$, $PhSiH_3$, DCM; *iiii*. TFA/TIS/H_2O (95:2.5:2.5), 53% over 4 steps; (d) $NaIO_4$, H_2O; (e) aminooxy mannoside **32**, 70% AcOH (aq.); (f) biotin sulfone, PyBOP, DIPEA, DMF; (g) *i*. Boc-Cys(Npys)-OSu ester, DMF; *ii*. TFA/DCM (yields not given)

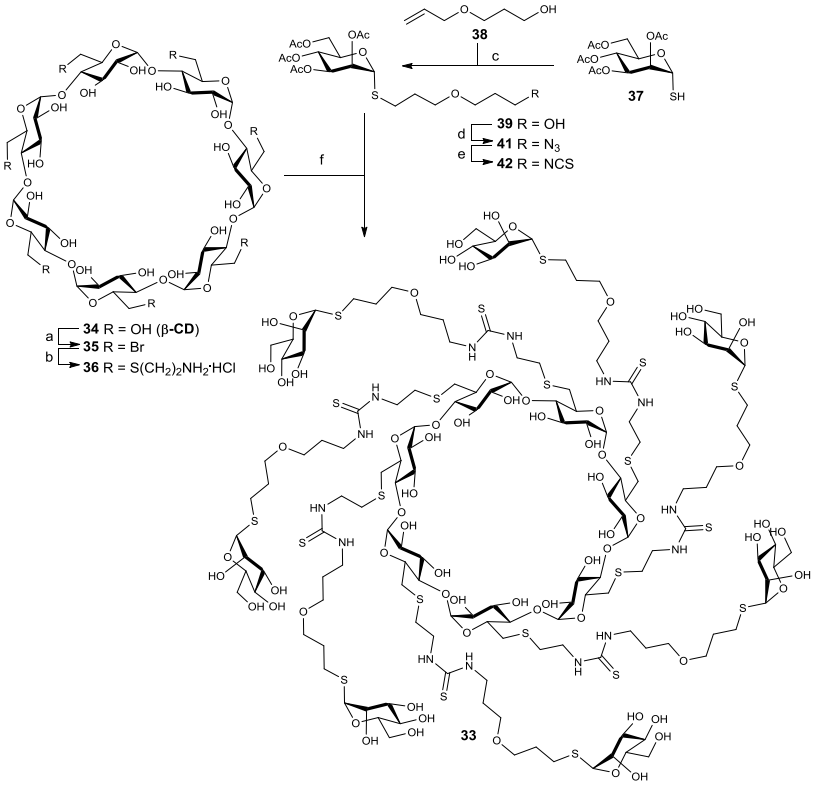
Several variations in orthogonal protection of the RAFT template and conjugation methods have been described in the literature.[21] For example, cyclic RAFT scaffolds were equipped with an alkyne or azide functionality and mannosides were conjugated using Cu(I)-catalyzed click chemistry.[22,23] Two different carbohydrate moieties could be conjugated to one RAFT scaffold in a controlled manner by using oxime and triazole formation as two chemoselective conjugation methods.[24] To improve lectin binding,

tetravalent mannose clusters were conjugated to the scaffold,[25] and later on second[26] and third[27] generation "dendri-RAFT" structures were synthesized to increase ligand valency.

Cyclodextrins

Cyclodextrins (CDs) are a family of cyclic oligosaccharides, composed of α-D-glucose moieties, that are biocompatible, non-immunogenic. They can be readily functionalized and they are able to encapsulate various hydrophobic molecules of appropriate size within their hydrophobic cavity. Therefore they have been used in several pharmaceutical applications.[28], Cyclodextrins have also been exploited for receptor-mediated glycotargeting. For instance Fernández and coworkers reported the synthesis of heptavalent mannosylated-βCD **33** as a multivalent lectin ligand.[29] They conjugated the mannose residues to the CD by the formation of a thiourea bond using the amines in heptavalent CD derivative **36** and thioisocyanate mannoside **42**. The route of synthesis starts with the conversion of β-CD **34** into per-6-(deoxybromo)-cyclodextrin **35**[30] by selective substitution of the primary alcohols using Ph_3P/NBS. Next, the bromides in **35** were substituted by 2-aminoethanethiol hydrochloride giving amino CD derivative **36** in 86% yield. Isothiocyanate mannoside **42,** bearing a heptyl spacer, was obtained from 1-thio-mannoside **37**. Thio-mannoside **37** was elongated with allyl-ether spacer **38** by a radical reaction using AIBN as initiator providing **39** in 71% yield. Next the free hydroxyl functionality was transformed into a isothiocyanate as follows: Tosylation and substitution with azide to proceeded in 65% over two steps to give mannoside **41**. Treatment of azide **41** with Ph_3P/CS_2 gave thioisocyanate **42** in 67% yield. Isothiocyanate **42** was then reacted with the amines in cyclodextrin **36** to form thiourea bonds. Subsequent saponification of the acetyl groups gave cyclodextrin conjugate **33** in 46% yield.

Scheme 5: Synthesis of mannosylated β-CD conjugate **33**.



Reagents and conditions: (a) Ph_3P, NBS, DMF, 70 °C, 96%; (b) 2-aminoethanethiol HCl salt, TEA, DMF, 86%; (c) **38**, AIBN, dioxane, 71%; (d) *i.* TsCl, DCM, DMAP, 81%; *ii.* NaN_3, DMF, 80 °C, 97%; (e) Ph_3P, CS_2, dioxane, 67%; (f) *i.* isothiocyanate mannoside **42**, $NaHCO_3$, H_2O/acetone (1:1 v/v), *ii*. 1M NaOH (aq.), 46%.

The same authors prepared 14- and 21-valent homo- and heteroglycoclusters based on CD (Figure x). All glycoclusters were evaluated for their binding affinity towards Con A, using an enzyme-linked lectin assay which provides information on the intrinsic lectin-ligand affinity and isothermal titration microcalorimetry. It turned out that the Con A-binding affinity for homogeneous mannose clusters increases when the mannose residues occur as triads. Surprisingly hetero-type glycoclusters with not only α-mannose residues but also β-glucose or β-lactose residues exhibited Con A-binding

affinities significantly higher than that of the homogeneous conjugates with identical mannose valency.

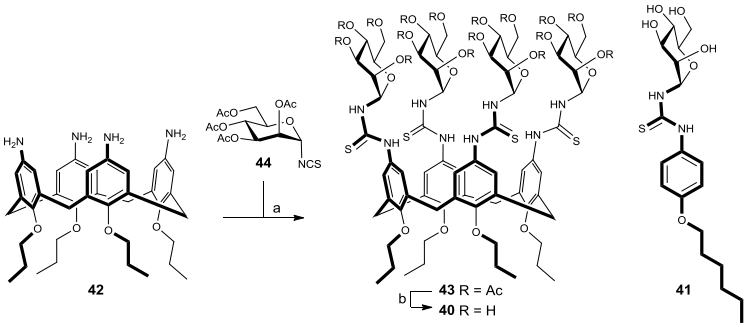
Many examples of cyclodextrin based glycoclusters have been reported, varying in the nature of the carbohydrate, the valency, and the conjugation method and strategy.[31,32] For example, Baussanne *et al.* synthesized a per-(6→6)-thiourea linked methyl-α-D-mannopyranoside β-CD cluster to study the effect of anomeric group on the binding towards Con A.[33] Surprisingly the binding affinity of this cluster towards Con A was completely abolished demonstrating the importance of all hydroxyl groups concerning lectin recognition. Carpenter *et al*. conjugated mono-mannosides and 1,3-1,6-tri-mannosides by amide bond formation with per-6-amino-β-DC.[34]

Calixarenes

Calixarenes are cyclic oligomers, which are obtained by condensation of phenols or resorcins with aromatic aldehydes (e.g. **42**, Scheme 6). Calixarenes are convenient matrices for the development of multivalent glycoclusters. The possibility to vary ring size, valency, and conformation of calixarenes allows precise control over spatial arrangement of the carbohydrate ligands in glycocalixarenes..[35]

Gold nanoparticles have found considerable use in tumour targeting applications due to their straightforward synthesis and ease of functionalization. Recently Avvakumova *et al*. reported a novel approach for non-covalent functionalization of gold nanoparticles (AuNPs) with glycocalixarenes bearing four mannose units, resulting in multivalent nanoparticles suitable for targeting.[36]

Scheme 6: Synthesis of calix-man **40** and the structure of simplified mono-man **41**.



Reagents and conditions: a) thioisocyanade mannoside **44**, TEA, DCM, 65%; b) NaOMe, MeOH, quantitative.

The assembly of these functionalized AuNPs starts with the reaction of the amino functions in calix[4]arene **42** and thioisocyanate mannoside **44** followed by global saponification to give calix-man **40** in 65% overall yield. After conversion of Au-nanoparticles into Au-dodecathiol nanoparticles, non-covalent functionalization with calix-man **40** was attained by a phase transfer procedure where Au-dodecanthiol was mixed with calix-man **40** and mono-man **41** in chloroform (Figure 2).

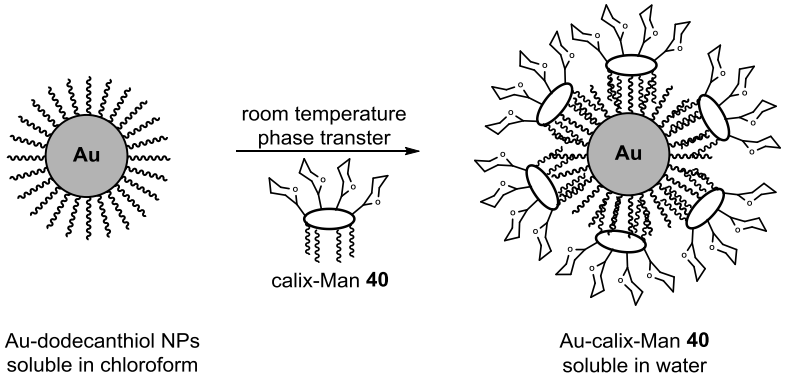


Figure 2: Schematic representation of the gold nanoparticle functionalization using a phase transfer procedure. Formation of Au-calix-Man **40**.

After removal of the solvent the calixarene-AuNPs were redispersed in water. The AuNPs functionalized with calix-man **40** and simplified AuNPs

functionalized with mono-man **41** were tested for cell uptake in HeLa cells. The results showed that the uptake of AuNPs functionalized with calix-man **40** was increased three-fold relative to the simplified AuNPs functionalized with mono-man **41** and an eight-fold increase compared to the untreated gold particles. However uptake of the untreated gold particles was also observed. Competition with dextran abolished the uptake of AuNPs functionalized with both **40** and **41**, indicating a mannose dependent uptake. Furthermore, the uptake of untreated gold particles was scarcely influenced by the presence of dextran, indicating uptake of the particles by passive endocytosis.[37]

Dendrimers and dendrons

Dendrimers are structurally defined, highly branched, symmetrical macromolecules that adopt a globular-type structure. The iterative synthesis of dendrimers permits for the introduction of multiple glycoconjugation sites to give glycodendrimers.[38,2] Two main strategies can be discerned for constructing glycodendrimers, namely a divergent or a convergent synthesis strategy.[39] In the divergent approach, the dendrimer is constructed in a stepwise manner starting from a core molecule and growing outwards with typically doubling of the number of reactive functionalities being introduced with each new generation. When the desired number of reactive functionalities has been reached carbohydrates can be introduced to form a glycodendrimer. A disadvantage of this method is the increasing number of reactions that has to be performed during each extension to a next generation. Incomplete transformation and/or side reactions give rise to the formation of mixtures of closely related compounds and separation can become impractical.[40]

In the convergent approach a glycodendrimer is constructed inwards from the periphery to the core. Dendritic segments or dendrons of selected size are synthesized and finally the obtained dendrons are attached to the core

molecule to obtain the desired dendrimer.[41] In comparison to the divergent strategy, the convergent method avoids many of the synthetic problems inherent in the divergent strategy, and it affords dendritic polymers with on average higher structural homogeneity. However, a convergent strategie requires large quantities of carbohydrate-derived materials, which typically need to be protected during dendrimer assembly (necessitating a global deprotection step at the end of the synthesis). Protecting groups can lead to steric crowding, which in turn may influence coupling efficiencies.[42]
Many studies described in the literature use commercially available dendrons and dendrimers, such as the poly(amidoamine) dendrimers (PAMAMs) (Figure 3).

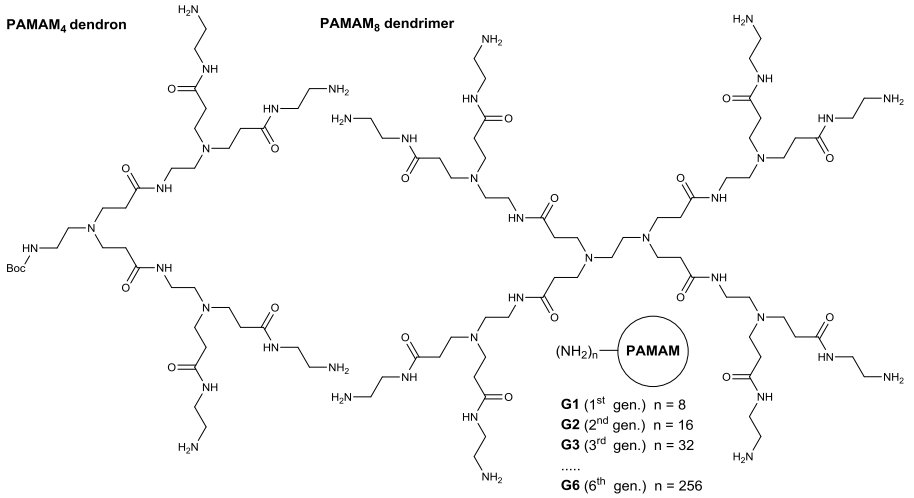
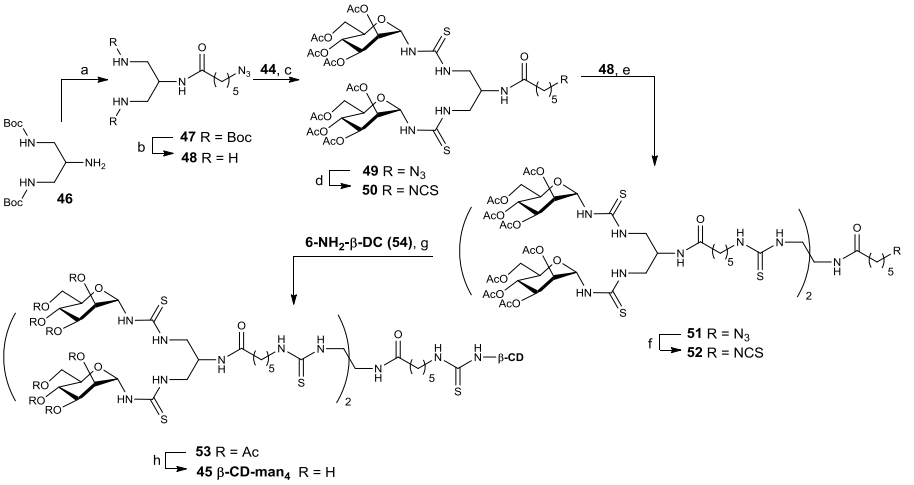


Figure 3: Structure of PAMAM dendron and dendrimer.

Mannosylated PAMAM dendrimer conjugates have been synthesized to study protein-carbohydrate interaction. Based on the X-ray structure of a complex of Con A and methyl α-D-mannopyranoside Cloninger and co-workers reasoned that a large dendrimer should be able to bind simultaneously to two binding sites in ConA, located about 65 Å apart on one side of the protein.[43,44] They synthesized 1st to 6th generations of PAMAM dendrimers in which the mannose residues are linked via thiourea bonds. Using a hemagglutination assay it was shown that ConA binding was

dependent on the density of the sugars on the dendrimer with the largest dendrimer binding best. In a subsequent study by the same group the degree of mannose functionalization was controlled by stoichiometric addition and this agreed with the lectin binding activity and the number of lectins clustered around the dendrimer.[45,46] Many other conjugation techniques has been applied to decorate the dendrimer scaffold with carbohydrates.[47,48] Fernández and co-workers described the synthesis of a row of mannose-coated β-cyclodextrin-dendrimers, such as construct **45** (Scheme 7).[49] Mannosylated dendron **51** was obtained by a convergent methodology in which isothiocyanate and amine functionalized building blocks repeatedly react to give thiourea linkages. Boc-protected triaminopropane **46** was first elongated with azidohexanoyl chloride to give **47** in 96% yield. Boc removal followed by reaction of the released amines with isothiocyanate mannoside **44** gave divalent conjugate **49**. Azide **49** was converted to isothiocyanate **50** in 72% yield using Ph_3P and CS_2. Thioisocyanate **50** was then coupled with diamine **48** giving dendron **51**.

Scheme 7: Synthesis of mannosyl-coated β-cyclodextrin-dendron construct **45**.



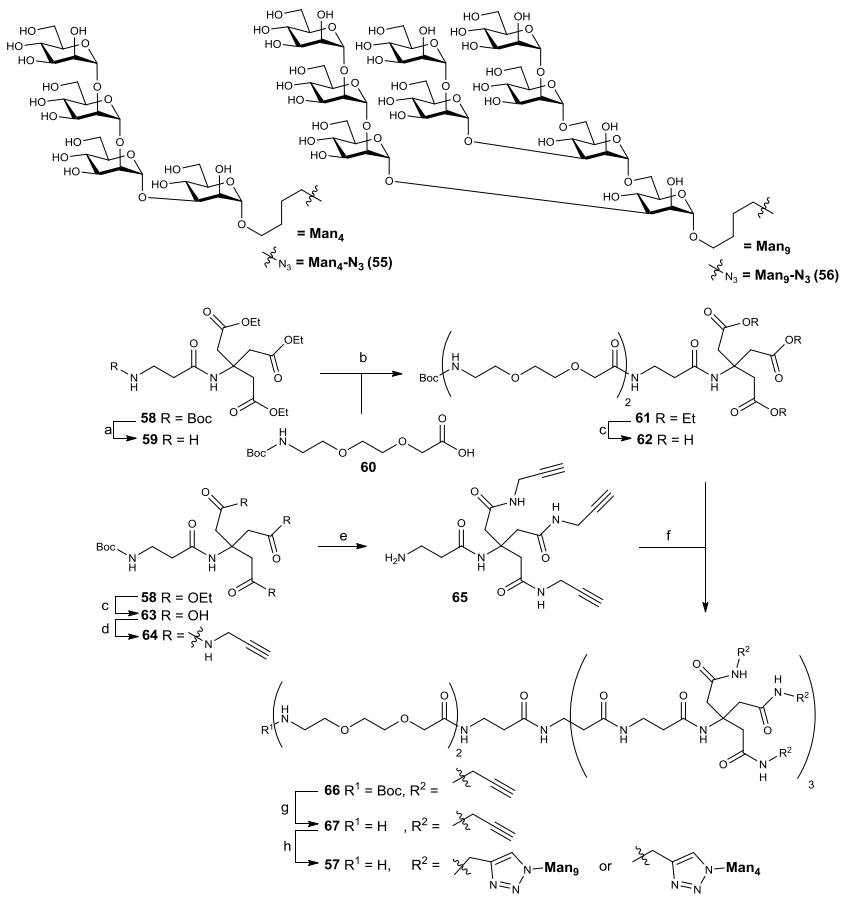
Reagents and conditions: a) azidohexanoylchloride, DMF/collidine (1:1), 96%; b) TFA/H_2O (1:1); c) thioisocyanade mannoside **44**, pyridine, 82% (over two steps); d) $(Ph)_3P$, CS_2, dioxane, 72%; e) diamine **48**, $NaHCO_3$ (aq.) pH 8, acetone, 55%; f) $(Ph)_3P$, CS_2, dioxane, 90%; g) 6-NH_2-β-CD **54**, $NaHCO_3$ (aq.) pH 8, acetone, 60%; h) NaOMe, MeOH, 89%.

A second conversion of the azide to the corresponding thioisocyanate gave dendron **52** which was coupled to β-cyclodextrin-mono-amine **54**[50] in 60% yield. Global deprotection provided mannosyl-coated β-cyclodextrin-dendrimer **45** in 89% yield. Evaluation of the mannose-functionalized β-cyclodextrin-dendrimers towards Con A with the aid of an enzyme-linked lectin assay showed that the strength of the lectin binding increased with increasing valency of the constructs. For instance, dendrimer **45** showed a 14-fold increase in binding affinity towards Con A relative to mono-mannosylated-βCD. In a following study of the same group, a hexavalent dendron was conjugated to βCD as drug carrier for the drug docetaxal (DTX, Taxotere®).[51] Complexation of the mannosylated-βCD with DTX showed a 1000-fold increase in water solubility and a 20-fold increase in uptake by peritoneal mouse macrophages compared to the unmannosylated-βCD/DTX complex. In another study, Fernández and co-workers used trivalent mannosylated β-cyclodextrin (βCD) conjugates for the targeted delivery of pharmacological chaperones for Gaucher disease to macrophages.[46] Complexes of mannosylated βCD conjugates and two nojirimicin based chaperones were tested for chaperone activity and targeted delivery to macrophages. No difference in chaperone activity was observed for the nojirimicin derivatives and its mannosylated βCD complex and uptake of the complexes by macrophage-like cells was clearly visualized by fluorescence microscopy.

Wong and co-workers studied the effect of neighbouring glycans on antibody-carbohydrate recognition using microarrays.[52] With the aim to mimic the HIV envelope glycoprotein gp120 surface using heterogeneous glycans, they hoped to find an optimal presentation of the carbohydrate epitope. In order to achieve this, high-mannose dendron conjugate **57,** having Man_9 and/or Man_4 glycans was synthesized by a convergent approach (Scheme 8), in which oligo-mannosides **55 (**Man_4) and **56 (**Man_9) (Scheme 8) containing an azide were coupled in different ratios to nona-valent alkyn dendron **67** by "click" chemistry. First, the Boc-group in tri-ester **58** was

removed to give **59**. The released amino group enabled the installation of a glycol spacer by a double elongation with acid **60** giving triester **61** which was subsequently hydrolyzed to the corresponding tri-acid **62** in 94% overall yield.

Scheme 8: Synthesis of high mannose dendron conjugate **69**.



Reagents and conditions: (a) 50% TFA (in DCM), DCM, 99%; (b) *i*. carboxylic acid **60**, EDC, HOBt, DIPEA, DMF; *ii*. 50% TFA (in DCM), DCM; *iii*. carboxylic acid **60**, EDC, HOBt, DIPEA, DMF, 96%; (c) 1M NaOH (aq.), MeOH (**62**: 99%, **64**: quantitative); (d) propargyl amine, EDC, HOBt, DIPEA, DMF, 75%; (e) TFA (50% in DCM), DCM, 96%; (f) tri-alkyn **66**, EDC, HOBt, DIPEA, DMF, 69%; (g) TFA (50% in DCM), DCM; (h) Man_4-N_3 **55** and Man_9-N_3 **56**.

Tri-alkyn amide **65** was also obtained from tri-ester **58**. Hydrolysis of **58** to tri-acid **63**, followed by coupling with propargylamine provided Boc-protected tri-alkyn **64**. Removal of the Boc-protective group, gave tri-alkyn amine **65**[53] in 72% yield starting from **58**. Next, the obtained tri-alkyn amine **65** was coupled to tri-acid **62** giving nona-alkyn dendron **66** in 69% yield. After Boc-deprotection, nona-alkyn **67** was subjected to coupling with oligomannosides Man_4-N_3 **55** and Man_9-N_3 **56** under Cu(I) catalysed "click" conditions. By varying the concentration of oligomannosides **55** and **56** Wong and co-workers could successfully control the average Man_4/Man_9 ratio conjugated to the dendron. With this method they synthesized five constructs with a Man_4/Man_9 ratio of 9:0, 0:9, 6:3, 3:6 and 5:4.
The obtained constructs were printed on N-hydroxysuccinimide (NHS) activated glass slides to form an array of conjugates with varying densities. Binding studies were performed with 2G12 antibodies that recognize a mannose containing epitope on the gp120 surface protein.[54] The results showed an increase of binding affinity with increasing glycan density of the constructs. Furthermore, the conjugate, having a Man_4/Man_9 5:4 ratio showed the highest relative binding towards the 2G12 antibody. This study showed that the use of heterogeneous glycans is a useful tool to mimic complex epitope presentation, which should benefit the future design of carbohydrate-based vaccines.

High-mannose *N*-glycan

N-linked glycans play a pivotal role in a number of vital processes such as protein folding, quality control and transportation.[55] All *N*-linked glycans contain a core pentasaccharide featuring an 1,3-1,6-α-linked trimannoside 1,4-β-linked to a GlcNAc dimer. The high mannose *N*-glycan consists of three 1,2-α-linked mannose dimers attached to the mannose trimer core (Figure 4), to give three mannose arms, termed the D1, D2 and D3 arms. Most of the approaches to engineer mimics of high-mannose

oligosaccharides rely on the synthesis of modified or truncated fragments and their multivalent presentation.

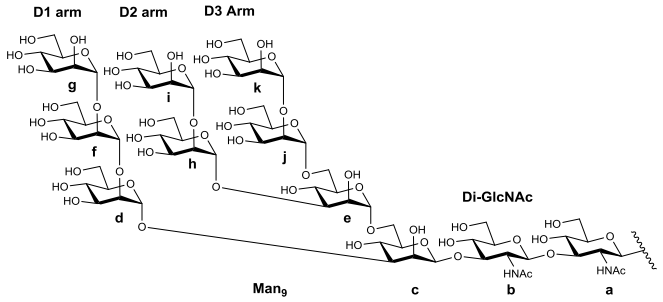


Figure 4: Structure of the high-mannose *N*-glycan.

Seeberger synthesized a set of oligomannosides derived from the natural high-mannose *N*-glycan (Figure 5) to investigate their targeting ability to DC surface receptors.[56] Oligomannoside Man_9 **70** resembles the full mannose cluster of the high mannose *N*-glycan, Man_6 **69** is truncated with three mannose units at the D1, D2 and D3 arm, respectively. Further truncation gives the branched Man_3 **68**. Mannosides **68**, **69** and **70** have a β-linked polyethylene glycol thiol spacer for further functionalization.[57]

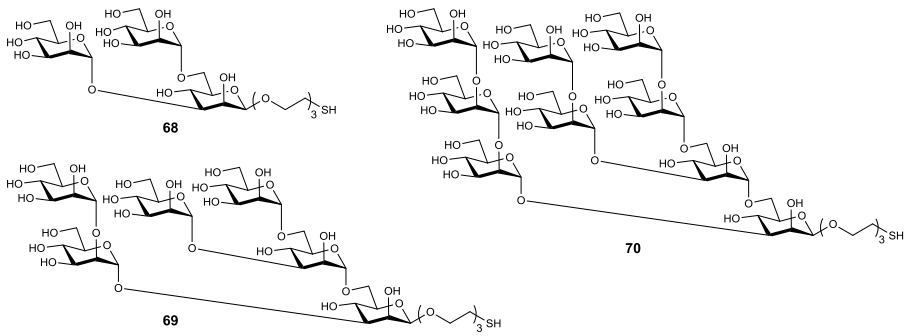
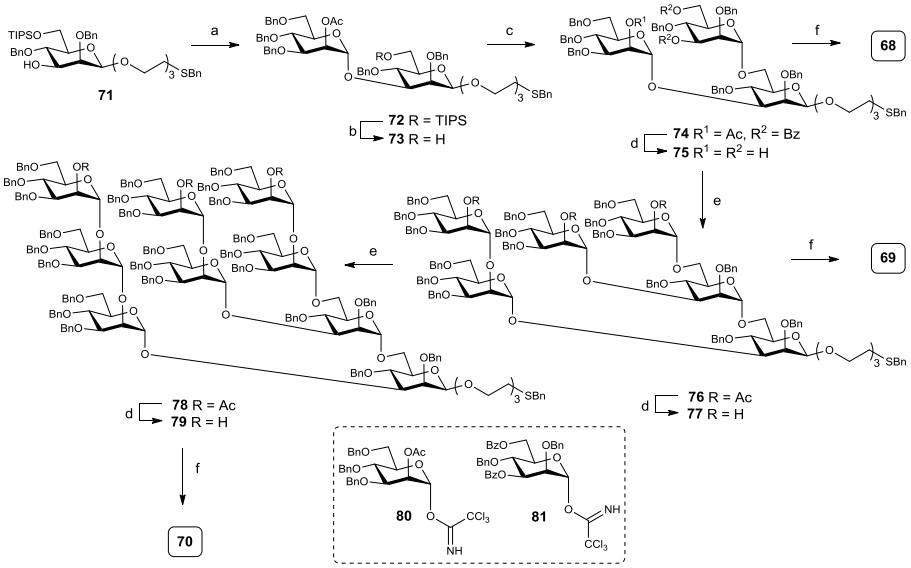


Figure 5: Structures of a set of high-mannose oligomannosides **68-70**.

The synthesis of oligomannosides **68-70** is shown in Scheme 9. Condensation of spacer containing acceptor **71**,[58] and donor **80** under influence of TBDMSOTf gave dimer **72** in 99% yield. After removal of the

silylether, the obtained dimer **73** was coupled with donor **81** yielding core trisaccharide **74** in 85% over two steps. Deacylation of **74** provided **75** and subsequent removal of all benzyl ethers by Pd/C catalysed hydrogenation gave target compound **68** in 59% yield starting from monomer **71**. Coupling of the three hydroxyl functions in trimer acceptor **75** with donor **80** using TMSOTf as activator gave fully protected hexasaccharide **76** in 94% yield. Target hexasaccharide **69** was obtained by saponification of the acetyl esters followed by hydrogenolysis of the benzyl ethers in **77** to provide hexasaccharide **69** in 64% overall yield. Finally, nonasaccharide **70** was synthesized by coupling of triol acceptor **77** with donor **80** to give fully protected nonamer **78** in 80% yield. Global deprotection provided the target nonasaccharide **70** in 53% overall yield.

Scheme 9: Synthesis of High mannose oligomannosides **68-70**.



Reagents and conditions (a) TBDMSOTf, donor **80**, DCM, -20 °C, 99%; (b) TFA/THF/H_2O (1:3:3), 91%; (c) TBDMSOTf, donor **81**, DCM, -20 °C, 93%; (d) NaOMe, MeOH/DCE (1:1) (**75**: 89%, **77**: quantitative, **79**: 90%; (e) TMSOTf, donor **80**, DCM, -20 °C (**76**: 94%, **78**: 80%); (f) Pd/C, H_2, EtOH/EtOAc, (**68**: 79%, **69**: 81%, **70**; 88%).

In a subsequent study, mannosides **68-70** were conjugated to a maleimide modified ovalbumin (OVA), a common model antigen.[59] Dendritic cells

were treated with mannosylated-OVA and antigen presentation was monitored by $CD4^+$ and $CD8^+$ response. *In vitro* data demonstrated that mannose modified-OVA conjugates all showed an increase in antigen presentation to $CD4^+$ T cells up to a 50-fold enhancement for the Man_9-OVA conjugate. A 10-fold enhancement was observed for $CD8^+$ T cells, indicating that the appendage of the oligomannosides can lead to enhanced cross-presentation of antigens on MHC class I.

Outline of this Thesis

The research described in this Thesis is mainly focussed on the design and synthesis of glycoconjugates provided with oligomannoside structures or mannose clusters that can be recognized by the mannose receptor (MR) or other mannose binding lectins. **Chapter 2** describes the synthesis and biological evaluation of high-mannose conjugated cathepsin probes. Mannosides varying in size were conjugated to the probe and tested for uptake and activity in DCs. **Chapter 3** describes the synthesis and biological evaluation of two mannose conjugated cyclophellitol probes. A synthesis route is presented towards 6-azido cyclophellitol with the necessary modifications to allow for site selective conjugation at the C-4 OH. The probes were tested on macrophages to label the enzyme Glucocerebrosidase. **Chapter 4** describes the synthesis of a library of mannosylated oligopeptide epitopes. A set of propargyl mannosides were synthesized and used for conjugation to the epitope bearing various azidolysine residues to form multivalent mannosyl-clusters. To investigate how the nature and amount of mannose residues influence antigen presentation, DCs were subjected to the conjugates, and the antigen presentation measured. **Chapter 5** describes the synthesis of a number of imidate based glucosidase, galactosidase and mannosidase probes. The compounds were synthesized using a single general procedure. Both α- and β-isomers were synthesized. **Chapter 6** describes the synthesis of both the α- and β-configured mannose epimers of

aziridine cyclophellitol. **Chapter 7** summarizes the research described in this Thesis, and provides a number of future prospects.

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Chapter 2

Targeted delivery of fluorescent, oligomannose-modified cathepsin inhibitor conjugates[1]

Introduction

The targeted delivery of chemotherapeutics through the intermediacy of cell surface receptors represents an attractive means to selectively deliver cargo to target cells or subcellular compartments[2]. Conceptually distinct approaches have been developed over the years to selectively deliver therapeutics and diagnostics to specific cell types through receptor-mediated uptake. Various ligands have been used as a homing device, including antibodies[3,4] and small synthetic molecules such as folic acid,[5] peptides[6] and carbohydrates.[7,8,9] Lectins are carbohydrate-binding receptors involved in a wide variety of cellular recognition and communication processes.[10] They are abundantly expressed on dendritic cells and macrophages, the guardians of our innate immune system, to survey their surroundings and detect pathogens and danger signals.[11,12] Many of the lectins found on these cells are members of the C-type lectin family and these include the mannose receptor (MR),[12] Dectin-1 and 2 as well as DC-SIGN.[13] These carbohydrate-

binding receptors have been exploited in various antigen-targeting strategies to enable both the efficient uptake of antigens (as described in chapter 4) and simultaneous stimulation of the immune cells. Proteases play a key role in the generation of peptide antigens and as such in antigen presentation. To probe the activity of cathepsins in living DCs Hoogendoorn *et al.* reported the adaptation of the broad-spectrum cathepsin inhibitor, DCG-04 to obtain targeted activity-based cathepsin probes.[14] DCG-04 was originally reported by Bogyo and co-workers, in a seminal paper,[15] which, together with the first paper by Cravatt and co-workers on serine hydrolase probes,[16] shaped the field of activity-based protein profiling. Taking the natural product and broad-spectrum cysteine protease inhibitor, E-64, as a basis, Bogyo and co-workers appended both a biotin and – in a later contribution – a set of different fluorophores and showed that all these structures retain potency and (broad-spectrum) specificity against numerous mammalian cathepsin cysteine proteases.[17] From these studies, which yielded activity-based probes currently widely used by the chemical biology community, it became apparent that cathepsin cysteine proteases tolerate a wide variety of functional groups appended to the dipeptide epoxysuccinate core.

Functionalization of the irreversible cathepsin inhibitor, DCG-04 with a BODIPY dye and a mannose cluster gave activity-based probe (ABP) **1** (UHG392[18] as depicted in Figure 1), which was used probe the activity of cathepsins in living DCs. ABP **1** contains an artificial mannose cluster built up from mono-mannosides covalently attached through a triazole linker to a hexalysine scaffold. The nature of the mannose ligand may influence recognition by the cell surface lectins and consequently uptake and routing of the conjugates. For example, it is known that the prevalent carbohydrate binding lectins on DCs, DC-SIGN and the MR, bind oligomannosides better than monomannosides.[19,20] Glycan microarray studies have revealed that DC-SIGN strongly binds high mannose type structures[21] and available crystal structures of DC-SIGN bound to natural ligands show that a terminal branched trimannose structure, featuring α-(1,3) and α-(1,6) mannose branches on a core mannose residue, fits well in the carbohydrate binding

site of this signaling receptor.[22] The MR also binds oligomannosides and a preference for the same type of branching has been reported.[23] Because the nature of the carbohydrate ligand(s) is important for the recognition by the cell surface lectins and consequently for the uptake and routing of the molecules, a set of BODIPY-DCG-04-oligomannose conjugates, bearing oligomannosides that feature natural glycosidic connections (*i.e.* α-(1→3) and α-(1→6) linkages) were designed and synthesized. In this chapter the assembly of three BODIPY-DCG-04-mannose clusters (**2**, **3** and **4**, Figure 1), bearing either a mono-, tri- or heptamannoside targeting entity and their efficacy in labeling of cathepsins in both cell lysates and live cells is described.

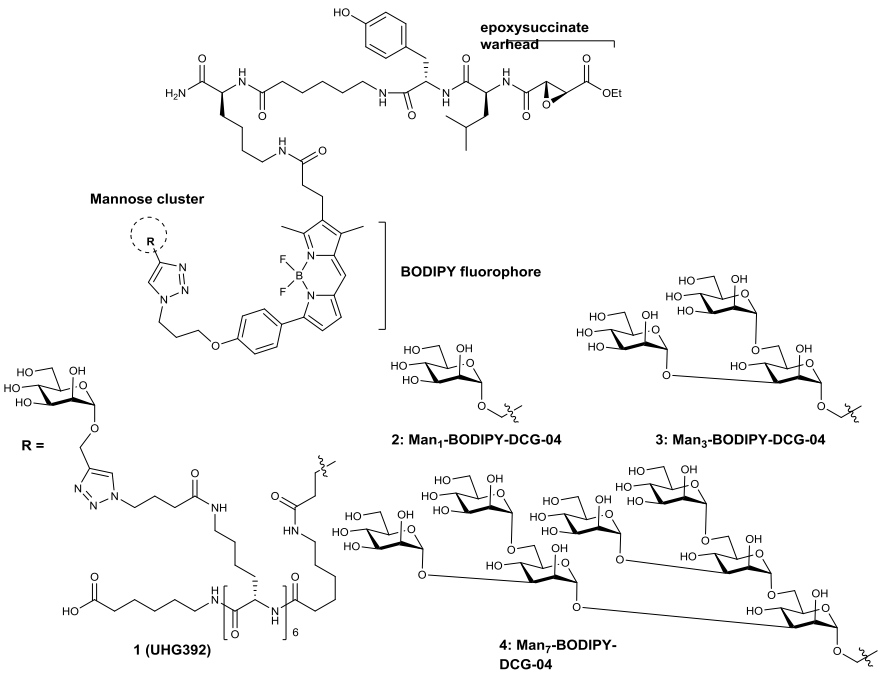


Figure 1: Structures of the known DCG-04 mannose conjugate **1** and the new DCG-04 mannose conjugates **2**, **3** and **4** described in this chapter.

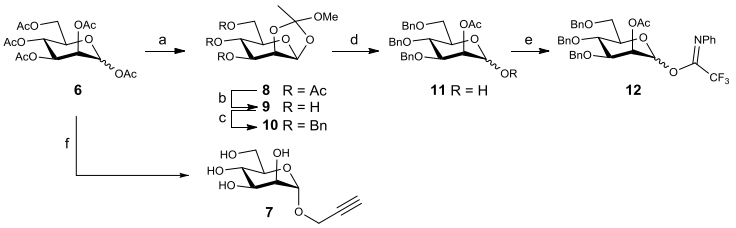
Results and discussion

Synthesis

The projected BODIPY-DCG-04 mannose conjugates **2**, **3** and **4** were assembled by conjugation of the corresponding propargyl mono-, tri- or heptamannosides **7**, **19** and **27** with azide functionalized BODIPY-epoxysuccinate **5** (Scheme 3). The synthesis of monomannoside **7** together with building block **12** and the glycosylation reactions toward propargyl tri- and heptamannoside **19** and **27** are shown in schemes 1 and 2.

Treatment of peracetylated mannose **6** with propargyl alcohol and $BF_3 \cdot Et_2O$ gave monomannoside **7** (Scheme 1).[24] Starting from peracetylated mannose **6**, orthoester **8** was obtained *via* the intermediate formation of a mannosyl iodide, as reported by Adinolfi *et al.*[25] The iodide was intramolecularly substituted to give orthoester **8**, which was deacetylated and subsequently benzylated to give orthoester **10**. Acidic hydrolysis of **10** then yielded hemiacetal **11**[26,27] in 82% over 4 steps. Treatment of this lactol with (*N*-phenyl)trifluoroacetamidoyl chloride in the presence of Cs_2CO_3 afforded (*N*-phenyl)trifluoroimidate donor **12** in 90% yield.

Scheme 1: Syntesis of propargyl mannoside **7** and donor mannoside **12**.



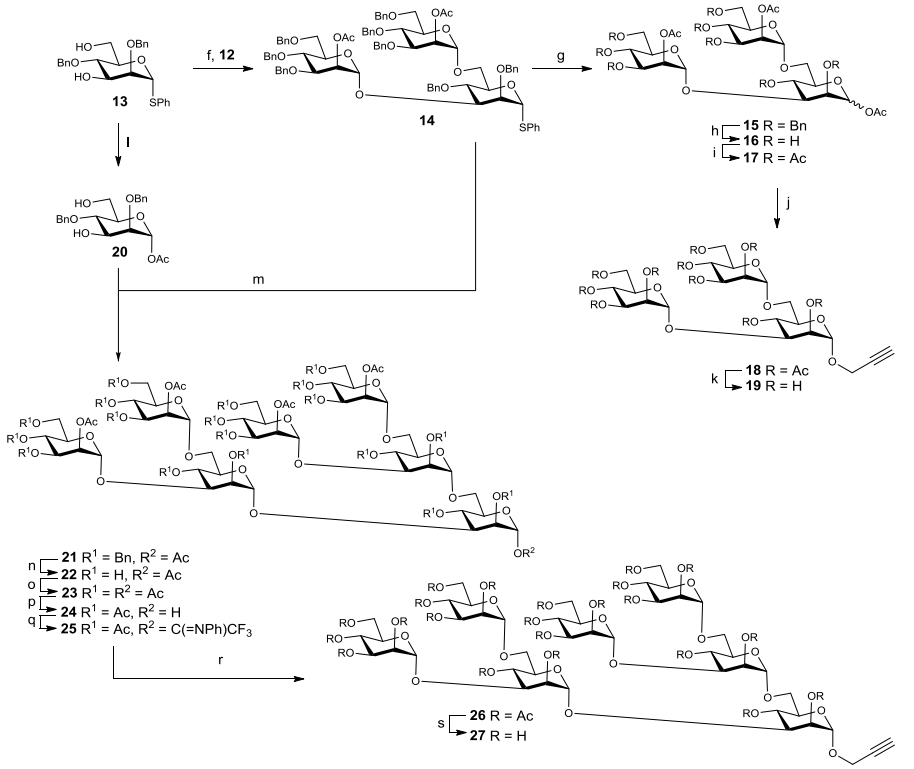
Reagents and conditions (a) i) I_2, Et_3SiH, CH_2Cl_2, reflux; ii) MeOH, 2,6-lutidine, rt; (b) K_2CO_3, MeOH, rt; (c) NaH, BnBr, DMF, 0 °C to rt; (d) DME/H_2O (10:1), *p*TsOH, 82% over 4 steps; (e) Cs_2CO_3, acetone, $(CF_3)C(NPh)Cl$, 90%; (f) propargyl alcohol, $BF_3 \cdot Et_2O$, DCM, rt, 61%.

The construction of key trisaccharide **14**, which was used as a precursor for both the propargyl trimannoside **19** and heptasaccharide **27**, was accomplished by a double glycosylation of known diol **13**[24] using donor **12**

and a catalytic amount of TfOH (Scheme 2). Next trimer **14** was converted into the corresponding anomeric acetate **15** using NIS and AcOH. Removal of all benzyl groups from this trimer required a two-step sequence. The fully protected trisaccharide was first treated with Pd/C and H_2 in a mixture of EtOAc/*t*BuOH/H_2O (1:3:4) and subsequently with Pd/C and H_2 in water to effect removal of all benzyl groups. Peracetylation of the crude trimer **16** yielded **17** in 76% over the two steps. Propargyl alcohol was then condensed with trimannosyl acetate **17** under the agency of $BF_3 \cdot Et_2O$ to provide the fully protected trimer **18**. Global deacetylation under Zemplén conditions yielded the propargyl trimannoside Man_3 **19**.

The heptasaccharide **21** was assembled using trisaccharide donor **14** and acceptor **20**. The latter building block was obtained by treatment of thio mannoside **13**[28] with NIS and AcOH (Scheme 2).[29] This led to the formation of the α-acetate **20** in 40% yield, alongside the generation of its β-anomeric counterpart (37%) and a minor by-product that was characterized as 1,6-anhydro-2,4-di-*O*-benzyl-β-D-mannose (11%). Condensation of both alcohols in acceptor **20** with trisaccharide donor **14** was achieved using the NIS/TfOH promotor couple to yield heptamer **21** as a single product in 62% yield. Hydrogenation of **21** with Pd/C and H_2 in EtOAc/MeOH/H_2O (5:4:1) was followed by a second hydrogenation in MeOH/H_2O (1:1) to give the debenzylated heptamer **22**, which was subjected without further purification to global acetylation. Attempts to introduce the propargyl moiety onto the peracetylated heptamer using $BF_3 \cdot Et_2O$ did not lead to the desired product and therefore a more potent glycosylating agent was used. To this end the anomeric acetyl was chemoselectively deblocked using hydrazine acetate and the liberated alcohol was converted into the (*N*-phenyl)trifluoroimidate donor **25**.

Scheme 2: Synthesis of the propargyl trimannoside Man_3 **19** and propargyl heptamannoside Man_7 **27**.

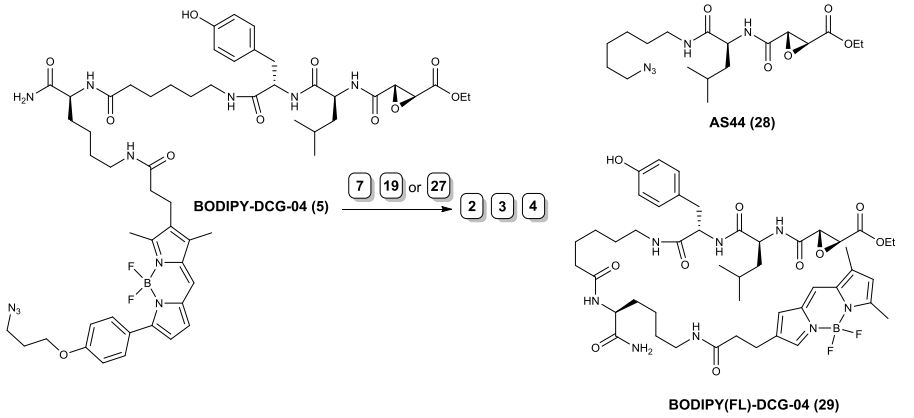


Reagents and conditions: (a) **12**, DCM, TfOH, act. Mol. Siev, -40 °C to rt, 84%; (b) NIS, AcOH, DCE/Et_2O (1:1), 94%; (c) i) Pd/C, H_2, EtOAc/*t*BuOH/H_2O (1:3:4), ii) Pd/C, H_2, H_2O; (d) Ac_2O, pyridine, 0 °C to rt, 76% over 2 steps; (e) propargyl alcohol, $BF_3{\cdot}Et_2O$, DCM, rt, 61%; (f) MeOH, NaOMe, 68%; (g) NIS, DCM/AcOH (1:1), rt, 40%; (h) **14**, NIS, TfOH, act. Mol. Siev., DCM, -40 °C to rt, 62%; (i) Pd/C, H_2, EtOAc/MeOH/H_2O (5:4:1); ii) Pd/C, H_2, MeOH/H_2O (1:1); (j) Ac_2O, pyridine, 0 °C to rt, quantitative yield over two steps; (k) $H_2NNH_2{\cdot}AcOH$, DMF, 0 °C, 79%; (l) $ClC(=NPh)CF_3$, Cs_2CO_3, acetone, quantitative; (m) propargyl alcohol, TfOH, DCM, act. Mol. Siev., -40 °C to 0 °C, 40%; (n) i) NaOMe/MeOH; ii) 0.1M NaOH (aq.), quantitative.

Glycosylation of propargyl alcohol with donor **25** under mild acid catalysis yielded the peracetylated heptamannoside **26** in 40% yield. Deacetylation under standard Zemplén conditions led to the partial removal of the acetyl groups, necessitating an extra saponification step with aqueous 0.1M NaOH to provide the target heptamer Man_7 **27**.

The BODIPY-DCG-04-mannose conjugates were obtained through a Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition[30] of azido BODIPY-DCG-04 (**5**) and the propargyl mannosides **7**, **19** and **27** (Scheme 3). After HPLC purification the three target constructs were obtained in 42% (**2**), 24% (**3**) and 32% (**4**) yield respectively.

Scheme 3: Assembly of the mannose-BODIPY-DCG-04 conjugates **2**, **3** and **4** and structures of cathepsin binding probes **5** (BODIPY-DCG-04), **28** (AS44), **29** (azido-DCG-04) and **30** (BODIPY(FL)-DCG-04).



Reagents and conditions: (a) sodium ascorbate, $CuSO_4$, DMF/H_2O (1:1), Man_1-BODIPY-DCG-04 (**2**) 42%, Man_3-BODIPY-DCG-04 (**3**) 24%, Man_7-BODIPY-DCG-04 (**4**) 32%.

Biological evaluation

To investigate labeling of cathepsins by activity-based probes **2**, **3** and **4** their activities were first evaluated in cell lysates. To this end mouse liver lysate was incubated with increasing concentrations of Man_1-BODIPY-DCG-04 (**2**), Man_3-BODIPY-DCG-04 (**3**) and Man_7-BODIPY-DCG-04 (**4**) after which the proteins in the lysates were resolved on SDS-PAGE (Figure 2A). All three mannosyl DCG-04 probes label cathepsins in a concentration-dependent manner, as is evident from Figure 2A. A small difference in gel-shift is apparent for the three different constructs and correlates to their varying molecular weight. A decrease in binding capacity was observed with increasing cluster size suggesting that the steric bulk of the heptamannosyl cluster retards binding and cathepsin inactivation. The diminished binding

efficacy of the larger mannosyl clusters, together with the difference in gel-shift of the labeled proteins indicates that mannosidases present in the cell lysate do not (effectively) trim the probes when bound to the cathepsins or when unbound in the cell extract. Incubation of immature mouse dendritic cell (DC) lysate with the probes showed a similar concentration-dependent binding of cathepsins (Figure 2B).

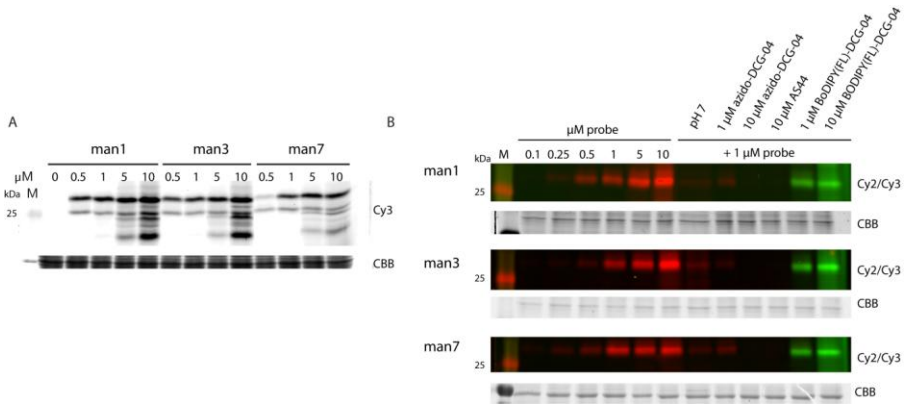


Figure 2. Cathepsin labeling experiments in mouse liver and dendritic cell lysate. Mouse liver lysate (10 µg total protein, A) or immature mouse dendritic cell (DC) lysate (8 µg total protein, B) was incubated (1 h, 37 °C) with increasing concentration of probe **2**, **3** or **4** at pH 5.5 or 1 µM at pH 7. Alternatively, lysates were incubated (1 h, 37 °C) with azido-DCG-04 (1 or 10 µM), AS44 (10 µM) or BODIPY(FL)-DCG-04 (1 or 10 µM), before treatment with probe **2**, **3** or **4** (1 µM, 1 h, 37 °C). Proteins were resolved on 12.5% SDS-PAGE, followed by fluorescence scanning (Cy2 (green): BODIPY(FL), Cy3 (red): BODIPY(TMR) and total protein staining with coomassie brilliant blue (CBB). M: dual color protein molecular weight marker.

In line with the previous findings, changing the pH of the buffer from pH 5.5 (the optimal pH for most cathepsin activity)[18] to pH 7 led to abrogation of cathepsin binding showing that active enzymes are required for labeling. Next a set of competition experiments was performed. The lysates were pre-incubated with different DCG-04 competitors, namely, AS44 **28**[14], azido-DCG-04 **29** and BODIPY(FL)-DCG-04 **30**[18] (See Scheme 3 for the structures of the competitors), followed by incubation with the probes. As

seen in Figure 2B labeling of the cathepsins with the red mannosyl BODIPY-DCG-04 conjugates was effectively prevented leading to either disappearance of the fluorescent bands in the competition experiment with non-fluorescent azido-DCG-04 and AS44, or the appearance of green fluorescent bands in the experiment with the green DCG-04 probe **30**. These competition experiments corroborate that probes **2**, **3** and **4** label active cathepsins.

Next the probes were tested for uptake and binding of cathepsins in living DCs (Figure 3). In line with the results obtained with the cell lysates, a concentration-dependent labeling pattern was observed (Figure 3A, left panel). The most efficient and selective labeling was achieved with the trimannosyl probe **3**, where the monomannosyl compound **2** showed most background fluorescence. Also in these experiments the heptamannoside probe **4** labeled the target cathepsins somewhat less efficient than its trimannoside counterpart **3**. Competition experiments with non-fluorescent cell-permeable azido-DCG-04 probe AS **8** indicated that also in living DCs active cathepsins are labeled by the probes. To test whether uptake of the probes was carbohydrate receptor mediated the DCs were pre-incubated with mannan, a mixture of mannose polysaccharides prior to exposure to the probes. Mannan, the natural substrate, binds to the mannose receptor and thus the uptake is prevented. In doing so, labeling by the tri- and heptamannosyl probes was effectively blocked showing that uptake of these ABPs is receptor dependent. The receptor-mediated uptake and labeling was confirmed by confocal microscopy. Figure 3B shows a clear uptake of Man_3-BODIPY-DCG-04 **3** and Man_7-BODIPY-DCG-04 **4** in DCs but little uptake of Man_1-BODIPY-DCG04 **2** (Figure 3B, left panels). Pre-incubation of the cells with mannan prevented uptake of probes **3** and **4**. Combined, the results indicate that Man_3-BODIPY-DCG-04 **3** and Man_7-BODIPY-DCG-04 **4** are taken up through the intermediacy of a carbohydrate binding receptor, where Man_1-BODIPY-DCG04 **2** can be internalized (at least in part) through a receptor-independent pathway. Receptor mediated internalization is clearly more efficient. Although it has previously been reported that the mannose

receptor can bind monomannosides, in the case at hand it appears that this is not enough for effective internalization of the conjugate.

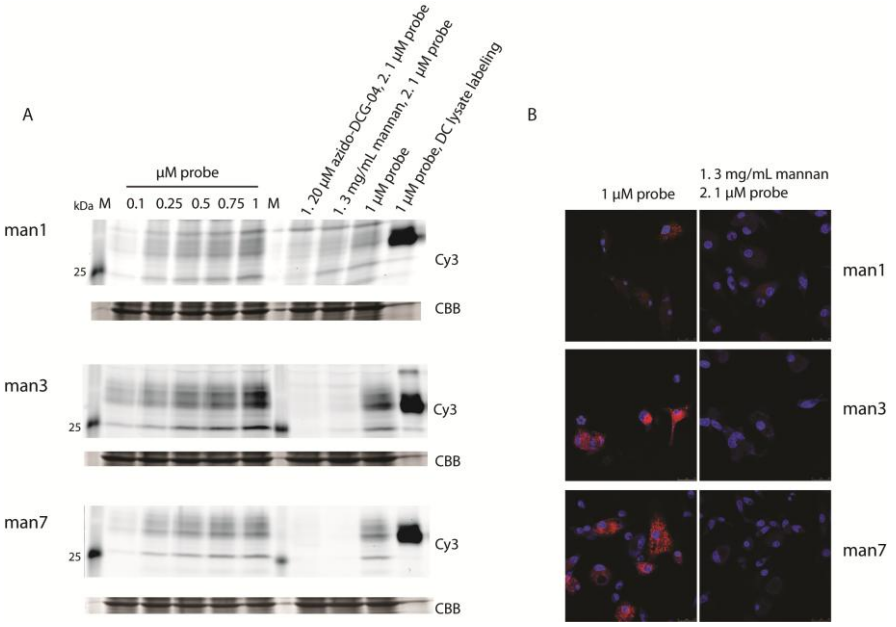


Figure 3. Uptake and cathepsin binding of the probes in live dendritic cells. A) DCs were treated with varying concentrations of **2, 3** or **4** (2 h, 37 ^{o}C) or pre-incubated (1 h, 37 ^{o}C) with azido-DCG-04 (20 μM) or mannan (3 mg/mL), followed by addition of **2, 3** or **4** (1 μM, 2 h, 37 ^{o}C), washed with PBS, lysed and resolved on 12.5% SDS-PAGE. In-gel fluorescence of BODIPY (Cy3) and total protein stain (CBB) are shown. B) Representative confocal microscope images of DCs treated with 1 μM of probes **1, 2** or **3** (left panels) or with mannan (right panels) for 1 h, followed by treatment with the probes. After treatment, cells were washed with PBS, fixed with 4% formaldehyde, nuclei stained with Draq5 and imaged using the Cy3 (λ_{ex} 532 nm) settings for BODIPY (red) and Cy5 (λ_{ex} 635 nm) settings for Draq5 as a nuclear stain (blue).

With respect to our first generation probe (**1**) it appears that DCG-04 labeling with the trimannoside probe is equally efficient. We have however observed a difference in processing of the probes. Where probe **1** seems to be processed by mannosidases in living cells (as judged from the minimal difference in gel-shift for the labeled cathepsins, indicating only a small shift

in molecular size), the current probes are more resistant to the endo/lysosomal action of mannosidases.

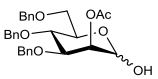
Conclusions

The assembly of three fluorescent cathepsin probes functionalized with different mannosides to investigate the role of these carbohydrate appendages on inhibition efficacy and internalization efficiency has been achieved. The size of the mannose oligosaccharides proved to influence the rate of inhibition, with the largest heptamannoside showing least effective cathepsin labeling in cell lysates at low inhibitor concentrations. The tri- and heptamannoside outcompete the monomannoside probe where it comes to effective uptake in live cells, and the trimannoside-modified DCG04 derivative proved the most effective cathepsin inhibitor in this experimental setup.

Experimental

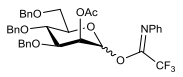
General: Traces of water in the starting materials were removed by co-evaporation with toluene for all moisture and oxygen sensitive reactions and the reactions were performed under an argon atmosphere. Dichloromethane was distilled over P_2O_5 and stored over activated 3 Å molecular sieves under an argon atmosphere. Propargyl alcohol was distilled over K_2CO_3 prior to use. 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 Sephadex 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 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 spectra 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-*O*-Acetyl-3,4,6-tri-*O*-benzyl-D-mannopyranoside (11): To a solution of peracetylated mannose **6** (114.5 g, 293.2 mmol) in DCM (750 mL) was added iodine (104.2 g, 410.5 mmol) and triethylsilane (66.3 mL, 410.5 mmol). The reaction mixture was heated till reflux. After 4 h TLC showed complete conversion of the starting material and mixture was cooled to rt. To the reaction mixture was added 2,6-lutidine (140 mL), MeOH (71.2 mL) and the reaction mixture was stirred overnight at rt. The reaction mixture was concentrated *in vacuo*, dissolved in EtOAc, washed with water (1x), 10% $Na_2S_2O_3$ (aq.) (2x), H_2O (3x), brine (2x), dried over Mg_2SO_4, filtered and concentrated *in vacuo*. The residue was dissolved in MeOH (500 mL), and to the solution was added K_2CO_3 (6.6 g, 48 mmol) and stirred for 4 h at rt. The reaction mixture was concentrated *in vacuo* and co-evaporated with toluene (3x). The product was dissolved in DMF (1.0 L) and to the solution was added BnBr (158 mL, 1.32 mol). The reaction mixture was cooled to 0 °C and to the cooled solution was added NaH (60% m/m) (31.7 g, 1.32 mol) in small portions over 6 h. The reaction mixture was

gradually warmed to rt and was stirred overnight at rt. The reaction mixture was cooled to 0 °C and quenched with MeOH. The solvent was removed *in vacuo* and the concentrate was dissolved in Et_2O, washed with H_2O (4x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was dissolved in DME/H_2O (10:1) (1.5 L) and the solution was cooled to 0 °C. To the cooled solution was added *p*TsOH (75 mmol, 14.3 g), after 3 h at 0 °C the reaction was quenched with sat. $NaHCO_3$ (aq.). Brine was added and the organic layer was separated. The product was extracted with DCM (3x) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded mannose **11** as a colourless oil (118.4 g, 240.4 mmol, 82% over 4 steps as an α/β mixture 10:1). Spectroscopic data were in accordance with known literature.[31] Spectroscopic data are reported for the major (α) isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.23 (m, 13H), 7.18 – 7.12 (m, 2H), 5.36 (dd, *J* = 3.3, 1.9 Hz, 1H), 5.19 (s, 1H), 4.85 (d, *J* = 10.8 Hz, 1H), 4.69 (d, *J* = 11.2 Hz, 1H), 4.59 (d, *J* = 12.1 Hz, 1H), 4.52 (d, *J* = 8.2 Hz, 1H), 4.49 (d, *J* = 9.1 Hz, 1H), 4.45 (d, *J* = 10.9 Hz, 1H), 4.09 – 4.05 (m, 1H), 4.03 (dd, *J* = 9.3, 3.2 Hz, 1H), 3.74 (d, *J* = 9.6 Hz, 1H), 3.69 – 3.66 (m, 2H), 2.14 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 138.4, 138.0, 137.9, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 92.5, 77.8, 77.5, 77.1, 76.8, 75.2, 74.7, 73.5, 71.9, 71.2, 69.4, 69.2, 29.8, 21.3.

2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-1-*O*-(*N*-phenyl-trifluoroacetimidoyl)-α/β-D-mannopyranoside (12): 

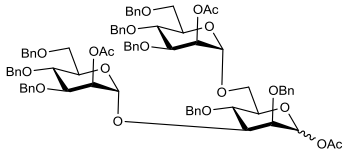
To a solution of mannose **11** (24.6 g, 50 mmol) in acetone (200 mL) was added N-(*p*-anisyl)-2,2,2-trifluoroacetimidoyl chloride (10.4 mL, 68.8 mmol) and cooled to 0 °C. To the cooled solution was added Cs_2CO_3 (20.7 g, 55 mmol) and the reaction mixture was allowed to warm to rt. After 6 h the solids were filtered over celite and the filtrate was concentrated *in vacuo*. Purification by column chromatography yielded trifluoro imidate donor **12** as a yellow oil (29.8 g, 44.9 mmol, 90% as α/β mixture 5:0.2). $[\alpha]_D^{22}$ + 26.4° (c = 1.0, DCM). FT-IR: ν_{max} (neat)/cm^{-1} 111.48, 1162.48, 1207.57, 1310.77, 1364.72, 1453.91,

1489.32, 1597.42, 1716.21, 1749.15, 2867.10, 3031.71. Spectroscopic data are reported for the major (α) isomer: ^{1}H NMR (400 MHz, $CDCl_3$, T = 328 °K) δ 7.38 – 7.01 (m, 18H), 6.79 (d, *J* = 7.4 Hz, 2H), 6.20 (s, 1H), 5.47 (dd, *J* = 3.2, 2.0 Hz, 1H), 4.87 (d, *J* = 10.9 Hz, 1H), 4.72 (d, *J* = 11.2 Hz, 1H), 4.64 (d, *J* = 12.0 Hz, 1H), 4.57 (dd, *J* = 11.3 Hz, 2H), 4.51 (d, *J* = 12.0 Hz, 1H), 4.01 (ddd, *J* = 8.3, 3.1, 1.3 Hz, 1H), 3.99 – 3.89 (m, 2H), 3.78 (dd, *J* = 7.8, 3.7 Hz, 1H), 3.72 (dd, *J* = 11.2, 1.7 Hz, 1H), 2.11 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 143.4, 138.5, 138.4, 137.8, 128.9, 128.5, 128.5, 128.3, 128.0, 127.8, 127.8, 127.7, 124.6, 119.6, 77.7, 75.4, 74.5, 74.0, 73.6, 72.4, 68.9, 67.7, 20.9. TLC-MS (*m/z*) 686.7

Phenyl 2,4-*O*-di-benzyl-3-*O*-(2-*O*-acetyl-3,4,6-*O*-tri-benzyl-α-D-mannopyranosyl)-6-*O*-(2-*O*-acetyl-3,4,6-*O*-tri-benzyl-α-D-mannopyranosyl)-1-thio-α-D-mannopyranoside (14): Trifluoro imidate donor **12** (19.9 g, 30 mmol) and acceptor **13** (4.5 g, 10 mmol) were dissolved in DCM (200 mL) and stirred over activated molecular sieves (3Å) at rt for 30 minutes. The solution was cooled to -40 °C and to the cooled solution was added Tf_2O (0.18 mL, 2.0 mmol) and the reaction mixture was gradually allowed to warm to 0 °C. At 0 °C the reaction was quenched with TEA and the mixture was filtered over celite and rinsed with DCM. The organic phase was washed with H_2O and the aqueous phase was extracted with DCM (4x). The combined organic layers were dried over $MgSO_4$, filtered, concentrated *in vacuo*. Purification by column chromatography yielded trimer **14** as a colorless oil (11.76 g, 8.4 mmol, 84%). $[\alpha]_D^{22}$ + 64.6° (c = 1.0, DCM). FT-IR: ν_{max} (neat)/cm^{-1} 978.54, 1026.58, 1049.14, 1078.97, 1232.74, 1367.72, 1453.88, 1742.59, 2866.77, 3030.51. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.08 (m, 52H), 5.54 (s, 1H), 5.51 (dd, *J* = 3.4, 1.8 Hz, 1H), 5.44 (dd, *J* = 3.0, 1.9 Hz, 1H), 5.22 (d, *J* = 1.9 Hz, 1H), 4.92 (d, *J* = 1.8 Hz, 1H), 4.88 (d, *J* = 8.8 Hz, 1H), 4.85 (d, *J* = 8.5 Hz, 1H), 4.76 (d, *J* = 11.1 Hz, 1H), 4.67 (d, *J* = 2.9 Hz, 1H), 4.64 (s, 1H),

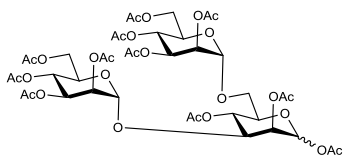
4.63 (d, *J* = 11.8 Hz, 1H), 4.62 (d, *J* = 7.8 Hz, 1H), 4.60 (d, *J* = 9.7 Hz, 1H), 4.56 – 4.40 (m, 9H), 4.38 (d, *J* = 11.2 Hz, 1H), 4.22 (dd, *J* = 9.8, 3.4 Hz, 1H), 4.13 (s, 1H), 4.09 (dd, *J* = 9.3, 3.0 Hz, 1H), 4.02 (dd, *J* = 9.1, 3.3 Hz, 1H), 3.96 – 3.86 (m, 5H), 3.83 (d, *J* = 9.4 Hz, 1H), 3.80 – 3.73 (m, 2H), 3.72 – 3.66 (m, 3H), 3.64 (dd, *J* = 11.2, 1.9 Hz, 1H), 3.60 (dd, *J* = 10.7, 1.7 Hz, 1H), 2.13 (s, 3H), 2.09 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 170.2, 138.7, 138.6, 138.3, 138.0, 137.9, 137.9, 134.8, 131.0, 129.2, 128.6, 128.6, 128.5, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.6, 127.3, 100.0, 98.3, 85.0, 79.2, 78.2, 77.9, 77.5, 77.2, 76.8, 75.3, 75.1, 75.0, 74.5, 74.2, 73.7, 73.4, 73.4, 72.4, 72.3, 72.1, 72.0, 71.6, 71.6, 71.6, 69.3, 68.9, 68.8, 68.5, 66.7, 21.3, 21.1. HRMS: $[M+H]^+$ calculated for $C_{84}H_{89}O_{17}S$ 1401.58150, found 1401.58162.

Acetyl 2,4-*O*-di-benzyl-3-*O*-(2-*O*-acetyl-3,4,6-*O*-tri-benzyl-α-D-mannopyranosyl)-6-*O*-(2-*O*-acetyl-3,4,6-*O*-tri-benzyl-α-D-mannopyranosyl)-α/β-D-mannopyranoside (15) To a suspension of NIS (1.25 g, 5.55 mmol) in DCE/THF (1:1) (27 mL) was added acetic acid (21.2 mL, 370 mmol). To the NIS mixture was added a solution of trimer **14** (5.17 g, 3.7 mmol) in DCE/THF (1:1) (5 mL) and the reaction mixture was stirred overnight at rt. The reaction mixture was diluted with EtOAc, washed with 10% $Na_2S_2O_3$ sol. (aq.) (2x), H_2O, 3x sat (1x). $NaHCO_3$ sol. (aq.) (1x), H_2O (3x), brine (2X), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded trimer **15** as a colorless oil (4.67 g, 3.5 mmol, 94%). $[\alpha]_D^{22}$ + 50.0° (c = 1.0, DCM). FT-IR: ν_{max} (neat)/cm^{-1} 975.53, 1026.80, 1048.27, 1090.94, 1231.12, 1368.81, 1453.91, 1496.72, 1743.42, 2870.34, 3031.12. ^{1}H NMR (400 MHz, $CDCl_3$) (α/β mixture, 1:0.4) δ 7.41 – 7.08 (m, 56H), 6.18 (d, *J* = 2.0 Hz, 1H), 5.59 (s, 0.4H), 5.50 (ddd, *J* = 8.3, 3.3, 1.9 Hz, 2.8H), 5.20 (d, *J* = 1.8 Hz, 1.4H), 4.96 (d, *J* = 1.9 Hz, 1H), 4.94 (d, *J* = 1.8 Hz, 0.4H), 4.89 (s, 1H), 4.86 (d, *J* = 2.8 Hz, 1.4H), 4.85 – 4.81 (m, 1.4H), 4.79 – 4.71 (m, 1.4H), 4.72 (d, *J*



= 1.7 Hz, 1H), 4.70 (d, *J* = 2.0 Hz, 0.4H), 4.67 (s, 0.4H), 4.66 – 4.63 (m, 2.4H), 4.62-4.60 (m, *J* = 1.9 Hz, 2H), 4.57 (d, *J* = 2.3 Hz, 0.4H), 4.54 (s, 0.4H), 4.51 (s, 0.4H), 4.50 – 4.38 (m, 10H), 4.12 (dd, *J* = 9.6, 3.1 Hz, 1H), 4.02 (ddd, *J* = 8.1, 5.2, 2.7 Hz, 2H), 4.00 – 3.83 (m, 8.4H), 3.84 – 3.78 (m, 1H), 3.78 – 3.65 (m, 6H), 3.64 (s, 0.4H), 3.62 (t, *J* = 2.0 Hz, 0.8H), 3.59 (s, 0.4H), 3.57 (s, 0.4H), 3.51 (ddd, *J* = 8.8, 4.4, 2.0 Hz, 0.4H), 2.35 (s, 1H), 2.15 (s, 3H), 2.14 (s, 1.2H), 2.09 (s, 3H), 2.07 (s, 1.2H), 1.99 (s, 1.2H), 1.95 (s, 3H). ^{13}C NMR (^{100} MHz, $CDCl_3$) δ 170.3, 170.3, 170.2, 170.1, 169.0, 168.9, 138.6, 138.6, 138.5, 138.3, 138.1, 137.9, 137.8, 137.8, 137.6, 129.1, 128.6, 128.5, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 127.9, 127.9, 127.9, 127.9, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 125.4, 100.1, 99.7, 98.5, 98.3, 92.8, 90.8, 79.7, 78.1, 78.0, 77.7, 76.5, 76.3, 75.3, 75.2, 75.1, 75.0, 74.7, 74.4, 74.3, 74.2, 74.1, 74.1, 74.0, 73.6, 73.5, 73.5, 73.4, 72.4, 72.2, 72.1, 72.0, 72.0, 71.7, 71.6, 71.5, 71.3, 69.2, 69.0, 68.8, 68.7, 68.6, 68.6, 68.4, 68.3, 66.4, 66.3, 21.3, 21.1, 21.0. HRMS: $[M+H]^+$ calculated for $C_{80}H_{87}O_{19}$ 1351.58361, found 1351.58399.

Acetyl 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/β-D-

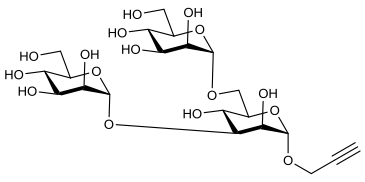


mannopyranoside (17): Trimer **15** (2.5 mmol, 3.38 g) was dissolved in an EtOAc/*t*BuOH/H_2O (1:3:4) mixture (50 mL) and the solution was purged with argon. To the solution was added cat. Pd/C (10%) and stirred at rt under H_2 (g) atmosphere. After TLC showed complete conversion to a single spot the Pd/C was filtered over a pad of celite, rinsed with MeOH and the filtrate was concentrated *in vacuo*. Proton NMR of the crude showed the presence of aromatic signals. The crude was taken up in H_2O (50 mL) and purged with argon. To the solution was added cat. Pd/C (10%) and stirred at rt under H_2 (g) atmosphere overnight. The Pd/C was filtered over a pad of celite, rinsed with H_2O and the filtrate was concentrated *in vacuo*. Proton NMR of the crude showed complete removal of aromatic signals and the crude was co-

evaporated with 1,4-dioxane (3x). The crude was dissolved in pyridine (25 mL) and the solution was cooled to 0 °C. To the cooled solution was added acetic anhydride (2.5 mL) and the reaction mixture was gradually allowed to warm to rt. After complete conversion, the reaction mixture was cooled to 0 °C, quenched with MeOH and concentrated *in vacuo.* The crude product was dissolved in EtOAc and washed with 1M HCl (aq.) (1x), sat. $NaHCO_3$ (aq.) (1x), H_2O (3x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded per-*O*-acetylated trimer **17** as a colorless oil (1.81 g, 1.9 mmol, 76%). $[\alpha]_D^{22}$ + 40.4° (c = 1.0, DCM). FT-IR: ν_{max} (neat)/cm^{-1} 975.27, 1039.52, 1139.46, 1212.30, 1368.79, 1433.73, 1741.84, 2925.08. ^{1}H NMR (300 MHz, $CDCl_3$) (α/β mixture, 1:0.25) δ 6.05 (d, *J* = 1.9 Hz, 1H), 5.81 (s, 0.25H), 5.48 (d, *J* = 3.2 Hz, 0.25H), 5.27 (dd, *J* = 8.8, 2.7 Hz, 4.4H), 5.04 (d, *J* = 2.6 Hz, 2H), 5.02 (s, 0.4H), 4.80 (s, 1.2H), 4.35 – 4.20 (m, 3.4H), 4.19 – 4.00 (m, 7.2H), 3.90 (dq, *J* = 10.0, 3.1 Hz, 1H), 3.75 (dd, *J* = 11.0, 5.5 Hz, 1H), 3.57 (dd, *J* = 10.9, 3.2 Hz, 1H), 2.27 (s, 0.8H), 2.25 (s, 2.6H), 2.18 (s, 4H), 2.16 (d, *J* = 2.1 Hz, 12H), 2.11 (s, 7H), 2.07 (s, 5.5H), 2.05 (s, 3H), 2.00 (s, 4H), 1.99 (s, 4.5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 170.6, 170.3, 170.1, 170.0, 170.0, 169.9, 169.8, 169.6, 99.2, 97.6, 90.4, 74.7, 71.5, 69.9, 69.6, 69.5, 69.3, 69.1, 68.5, 68.2, 67.9, 66.9, 65.8, 62.4, 62.3, 60.4, 20.9, 20.9, 20.8, 20.7, 20.7. HRMS: $[M+H]^+$ calculated for $C_{40}H_{55}O_{27}$ 967.29252, found 967.29269.

Propargyl 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 (18): To a solution of per-*O*-acetylated trimer **17** (29 mg, 30 µmol) in DCM (300 µL) was added a 0.6M propargyl alcohol solution (150 µL, 90 µmol) in DCM and a 0.3M $BF_3{\cdot}Et_2O$ solution (150 µl, 45 µmol) in DCM. The mixture was heated to 50 °C for 6 h after which the reaction mixture was cooled to rt, diluted with EtOAc and quenched with sat. $NaHCO_3$ (aq.). EtOAc was added till the

organic phase was transferred to the top phase. The organic phase was washed with sat. $NaHCO_3$ (2x), H_2O (3x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded per-*O*-acetylated propargyl trimer **18** as a white milky oil (17.6 mg, 18.3 µmol, 61%). $[\alpha]_D^{22}$ + 80.4° (c = 1.0, DCM). FT-IR: ν_{max} (neat)/cm^{-1} 978.00, 1038.64, 1136.67, 1214.43, 1368.90, 1433.77, 1741.73, 2926.85. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35 – 5.17 (m, 7H), 5.04 – 4.98 (m, 3H), 4.82 (d, *J* = 1.8 Hz, 1H), 4.29 – 4.25 (m, 3H), 4.24 (s, 1H), 4.21 (dd, *J* = 9.9, 3.5 Hz, 1H), 4.16 (t, *J* = 2.6 Hz, 1H), 4.13 (d, *J* = 2.4 Hz, 1H), 4.11 (d, *J* = 1.9 Hz, 1H), 4.10 – 4.06 (m, 1H), 3.88 (ddd, *J* = 9.7, 6.6, 2.5 Hz, 1H), 3.78 (dd, *J* = 10.8, 6.6 Hz, 1H), 3.53 (dd, *J* = 10.8, 2.5 Hz, 1H), 2.50 (t, *J* = 2.4 Hz, 1H), 2.23 (s, 3H), 2.16 (s, 3H), 2.14 – 2.13 (m, 9H), 2.12 (s, 3H), 2.06 (s, 3H), 2.05 (d, *J* = 1.1 Hz, 3H), 1.99 (s, 3H), 1.98 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 170.8, 170.6, 170.3, 170.2, 170.2, 170.1, 169.9, 169.74, 99.0, 97.4, 96.0, 78.1, 75.7, 74.1, 70.8, 70.2, 70.1, 69.6, 69.5, 69.2, 68.8, 68.4, 68.4, 67.0, 66.1, 66.1, 62.6, 62.5, 54.8, 21.0, 21.0, 20.9, 20.9, 20.9, 20.8, 20.8. HRMS: $[M+H]^+$ calculated for $C_{41}H_{55}O_{26}$ 963.29761, found 963.29723.

Propargyl 3-*O*-(α-D-mannopyranosyl)-6-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (19):  To a solution of per-*O*-acetylated propargyl trimer **18** (18.3 mg, 17.6 µmol) in MeOH (370 µL) was added a 5 mM sol. NaOMe (370 µL, m1.83 µmol) in MeOH. After complete conversion the reaction was quenched with Amberlite® IR-120 H^+ (pH≤7). The solids were filtered and the filtrate was concentrated *in vacuo*. Purification by column chromatography followed by lyophilisation yielded propargyl trimer **19** as a white powder (6.2 mg, 11.3 µmol, 61%). $[\alpha]_D^{22}$ + 114.0° (c = 1.0, MeOH). FT-IR: ν_{max} (neat)/cm^{-1} 981.81, 1042.80, 1131.53, 1363.00, 2490.41, 2929.08, 3285.13. ^{1}H NMR (400 MHz, MeOD) δ 5.06 (s, 1H), 4.91 (d, *J* = 1.8 Hz, 1H), 4.84 (d, *J* = 1.8 Hz, 1H), 4.26 (t, *J* = 2.3 Hz,

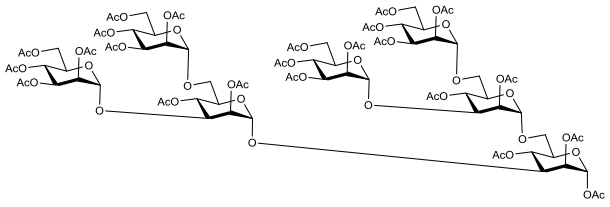
2H), 4.04 (dd, *J* = 3.1, 1.8 Hz, 1H), 3.98 (dd, *J* = 3.3, 1.7 Hz, 1H), 3.94 (dd, *J* = 11.1, 5.2 Hz, 1H), 3.89 – 3.84 (m, 3H), 3.84 – 3.81 (m, 2H), 3.81 – 3.78 (m, 2H), 3.78 – 3.65 (m, 5H), 3.65 – 3.58 (m, 3H), 2.88 (t, *J* = 2.5 Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 104.0, 101.5, 100.2, 80.7, 79.9, 76.2, 74.9, 74.4, 73.8, 72.6, 72.4, 72.1, 72.0, 71.2, 68.7, 68.5, 67.3, 67.1, 62.8, 55.0. HRMS: $[M+H]^+$ calculated for $C_{21}H_{35}O_{16}$ 543.19196, found 543.19224.

Acetyl 2,4-*O*-Benzyl-α-D-mannopyranoside (20a): To a 0 °C cooled solution of NIS (0.25 g, 1.1 mmol) in DCM/AcOH (1:1) (20 mL) was added dropwise a 0.1M solution of thio mannose **13** (10 mL, 1.0 mmol) in DCM. The reaction mixture was stirred at 0 °C for 1 h and allowed to warm to rt. After complete consumption of the starting material the reaction was quenched with 10% $Na_2S_2O_3$ (aq.) and the product was extracted with EtOAc (4x). The combined organic phases were washed with H_2O (2x), sat. $NaHCO_3$ (aq.) (3x), H_2O (3x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded α-*O*-acetyl mannose **20a** as a colourless amorphous solid (0.15 g, 0.4 mmol, 40%). $[\alpha]_D^{22}$ + 14.6° (c = 1.0, DCM). FT-IR: ν_{max} (neat)/cm^{-1} 1026.60, 1071.89, 1239.98, 1366.52, 1454.28, 1496.96, 1720.48, 2930.75, 3031.24, 3420.07. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.25 (m, 10H), 6.20 (d, *J* = 1.8 Hz, 1H), 4.90 (d, *J* = 11.1 Hz, 1H), 4.76 (d, *J* = 11.7 Hz, 1H), 4.66 (d, *J* = 11.0 Hz, 1H), 4.59 (d, *J* = 11.7 Hz, 1H), 3.99 (d, *J* = 7.0 Hz, 1H), 3.83 (dd, *J* = 12.1, 2.6 Hz, 1H), 3.80 – 3.73 (m, 2H), 3.72 – 3.65 (m, 2H), 2.52 (s, 1H), 2.32 (s, 1H), 2.05 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3, 138.2, 137.25, 128.7, 128.7, 128.3, 128.1, 128.1, 128.0, 91.0, 77.0, 75.6, 75.2, 74.0, 73.1, 71.4, 61.8, 21.0. HRMS: $[M+H]^+$ calculated for $C_{22}H_{27}O_7$ 403.17518, found 403.17527.

Acetyl 2,4-*O* -di-Benzyl-3-*O*-(2,4-*O*-di-benzyl-3-*O*-(2-*O*-acetyl-3,4,6-*O*-tri-benzyl-α-D-mannopyranosyl)-6-*O*-(2-*O*-acetyl-3,4,6-*O*-tri-benzyl-α-D-mannopyranosyl)-α-D-mannopyranosyl)-6-*O*-(2,4-*O*-di-benzyl-3-*O*-(2-*O*-acetyl-3,4,6-*O*-tri-benzyl-α-D-mannopyranosyl)-6-*O*-(2-*O*-acetyl-3,4,6-*O*-tri-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (21): Trimer donor **14** (4.2 g, 3 mmol) and acceptor **20a** (402 mg, 1 mmol) were dissolved in DCM (20 mL) and stirred over activated molecular sieves (3Å) at rt for 30 minutes. To the solution was added NIS (0.74 g, 3.3 mmol) and stirred at rt. After 15 minutes the reaction mixture was cooled to -40 °C and TfOH (0.3 mmol, 27 µL) was added to the mixture. The reaction mixture was gradually warmed to rt and quenched with Et_3N. The mixture was filtered over celite and diluted with DCM. The organic phase was washed with 10% $Na_2S_2O_3$ (aq.) and the aqueous phase was extracted with DCM (5x). The combined organic layers were washed with H_2O (1x), brine (1x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by size exclusion (DCM/MeOH, 1:1) yielded benzylated heptamer **21** as a colourless oil (1.835 g, 0.62 mmol, 62%). $[\alpha]_D^{22}$ + 43.6 (c = 1.0, DCM). FT-IR: ν_{max} (neat)/cm^{-1} 977.68, 1026.77, 1051.75, 1232.44, 1368.31, 1453.93, 1496.62, 1742.78, 2868.56, 3032.00. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.07 (m, 90H), 6.17 (d, *J* = 1.8 Hz, 1H), 5.54 – 5.46 (m, 4H), 5.21 (s, 3H), 4.97 (d, *J* = 1.8 Hz, 1H), 4.93 (s, 1H), 4.90 (s, 1H), 4.84 (dt, *J* = 10.5, 5.2 Hz, 5H), 4.75 – 4.69 (m, 3H), 4.64 (d, *J* = 3.8 Hz, 1H), 4.63 – 4.46 (m, 12H), 4.46 – 4.35 (m, 14H), 4.34 (d, *J* = 2.2 Hz, 2H), 4.24 (d, *J* = 12.1 Hz, 1H), 4.15 (ddd, *J* = 18.1, 9.2, 3.0 Hz, 2H), 4.05 (dd, *J* = 9.2, 3.2 Hz, 2H), 3.95 (d, *J* = 8.9 Hz, 2H), 3.93 – 3.82 (m, 10H), 3.83 – 3.71 (m, 3H), 3.71 – 3.46 (m, 15H), 3.39 (dd, *J* = 10.8, 5.9 Hz, 2H), 2.12 (s, 6H), 2.06 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 170.2, 170.1, 170.1, 169.0,

138.8, 138.7, 138.6, 138.5, 138.3, 138.3, 138.2, 138.2, 138.2, 138.0, 138.0, 137.9, 137.9, 137.9, 137.8, 137.2, 128.7, 128.6, 128.6, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 127.5, 127.4, 127.4, 127.2, 127.1, 99.7, 98.5, 98.3, 97.2, 90.7, 78.3, 78.2, 78.0, 77.5, 77.2, 76.8, 76.4, 75.2, 75.1, 74.9, 74.9, 74.6, 74.5, 74.2, 74.1, 74.1, 73.8, 73.45, 73.4, 73.4, 72.3, 72.1, 72.0, 72.0, 71.9, 71.8, 71.8, 71.6, 71.6, 71.3, 71.0, 68.9, 68.8, 68.8, 68.6, 68.3, 68.2, 66.3, 21.2, 21.2, 21.1, 21.1, 21.1.

Acetyl 2,4-*O*-di-acetyl-3-*O*-(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)-6-*O*-(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)-α-D-mannopyranoside (23): Benzylated heptamer



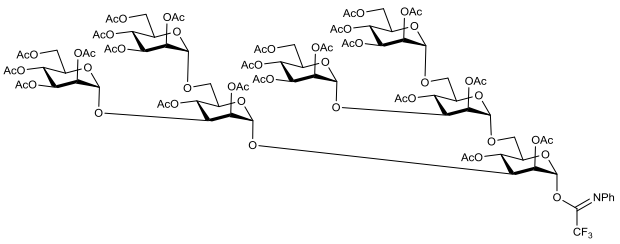
21 (896 mg, 0.3 mmol) was dissolved in EtOAc/MeOH/H_2O (5:4:1) (6 mL) and the solution was purged with argon. To the solution was added cat. Pd/C (10%) and the mixture was stirred overnight at rt under H_2 (g) atmosphere. The Pd/C was filtered over celite, rinsed with methanol and concentrated *in vacuo*. Proton NMR of the crude showed the presence of aromatic signals. The crude was taken up in MeOH/H_2O (1:1) (6 mL) and purged with argon. To the solution was added cat. Pd/C (10%) and stirred at rt under H_2 (g) atmosphere overnight. the Pd/C was filtered over a pad of celite, rinsed with MeOH and the filtrate was concentrated *in vacuo*. Proton NMR of the crude showed complete removal of aromatic signals. The debenzylated intermediate was co-evaporated with pyridine (3x), dissolved in pyridine (10 mL) and the solution was cooled to 0 °C. To the cooled solution was added Ac_2O (1 mL) dropwise and the reaction mixture was

allowed to warm to rt. After complete conversion of the starting material the mixture was cooled to 0 °C and the reaction was quenched with MeOH. The reaction mixture was concentrated *in vacuo* and the crude was dissolved in EtOAc. The organic phase was washed with 1M HCl (aq.) (1x), sat. $NaHCO_3$ (aq.) (2x), H_2O (3x), brine (3x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded per-acetylated heptamer **23** as a colourless amorphous solid (635.2 mg, 0.3 mmol, quantitative yield). FT-IR: ν_{max} (neat)/cm^{-1} 976.88, 1038.56, 1084.01, 1138.17, 1213.21, 1369.06, 1432.60, 1742.41, 2935.07. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.98 (d, *J* = 1.9 Hz, 1H), 5.37 – 5.17 (m, 13H), 5.15 (s, 1H), 5.10 – 5.04 (m, 2H), 5.01 (s, 2H), 4.99 (s, 2H), 4.93 (s, 1H), 4.88 – 4.78 (m, 2H), 4.28 (dtd, *J* = 18.4, 10.1, 8.2, 4.0 Hz, 4H), 4.20 – 3.86 (m, 13H), 3.85 – 3.65 (m, 4H), 3.62 – 3.52 (m, 2H), 3.50 (d, *J* = 2.5 Hz, 1H), 2.27 – 1.92 (m, 69H). ^{13}C NMR (^{100} MHz, $CDCl_3$) δ 170.7, 170.6, 170.6, 170.6, 170.5, 170.5, 170.4, 170.4, 170.3, 170.2, 170.0, 170.0, 169.9, 169.9, 169.8, 169.8, 169.8, 169.7, 169.7, 169.6, 169.6, 169.6, 169.5, 169.5, 168.4, 168.0, 99.6, 99.2, 99.0, 98.7, 97.4, 97.3, 90.5, 90.5, 77.5, 76.0, 75.6, 75.3, 75.2, 75.0, 74.7, 73.4, 71.4, 70.9, 70.8, 70.7, 70.6, 70.1, 70.0, 69.8, 69.8, 69.6, 69.5, 69.4, 69.4, 69.3, 69.1, 69.0, 69.0, 68.8, 68.7, 68.5, 68.5, 68.4, 67.7, 67.5, 67.5, 67.2, 66.9, 66.3, 66.0, 65.9, 65.8, 65.7, 65.6, 62.3, 62.2, 62.1, 62.0, 20.8, 20.8, 20.8, 20.8, 20.7, 20.7, 20.6, 20.6, 20.6, 20.5. MALDI: $[M+H]^+$ calculated for $C_{88}H_{119}O_{59}$ 2119.63059, found 2119.63084.

2,4-*O*-di-acetyl-3-*O*-(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)-6-*O*-(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)-α/β-D-mannopyranoside (24): To a 0 °C cooled solution of per-acetylated heptamer **23** (0.498 g,

0.235 mmol) in DMF (2.8 mL) was added hydrazine acetate (23.3 mg, 0.259 mmol). The reaction was stirred at 0 °C for 1 h and 30 minutes at rt. After TLC showed complete conversion the reaction was quenched with acetone and the reaction mixture was concentrated *in vacuo*. The crude was dissolved in Et_2O and the organic phase was washed with brine (3x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded 1-OH acetylated heptamer **24** as a white amorphous solid (0.384 g, 0.186 mmol, 79%). FT-IR: ν_{max} (neat)/cm^{-1} 978.58, 1038.62, 1081.66, 1137.06, 1215.09, 1369.26, 1433.38, 1741.35, 2926.28. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.37 (dd, *J* = 3.4, 1.6 Hz, 1H), 5.33 – 5.24 (m, 8H), 5.21 (dd, *J* = 10.4, 3.2 Hz, 2H), 5.17 (d, *J* = 3.0 Hz, 2H), 5.11 (t, *J* = 10.1 Hz, 1H), 5.07 – 5.02 (m, 3H), 4.99 (dd, *J* = 4.8, 1.8 Hz, 2H), 4.95 (s, 1H), 4.86 – 4.82 (m, 3H), 4.76 (d, *J* = 5.0 Hz, 1H), 4.37 – 4.22 (m, 4H), 4.18 (dd, *J* = 9.8, 3.4 Hz, 1H), 4.15 – 3.96 (m, 12H), 3.94 (ddd, *J* = 9.7, 5.0, 2.2 Hz, 1H), 3.86 (ddd, *J* = 10.2, 6.0, 2.5 Hz, 1H), 3.76 (tt, *J* = 7.6, 2.7 Hz, 3H), 3.55 (dd, *J* = 11.4, 3.2 Hz, 1H), 3.51 (dd, *J* = 11.4, 3.2 Hz, 1H), 3.47 (d, *J* = 9.5 Hz, 1H), 2.21 (s, 3H), 2.18 (s, 3H), 2.17 – 2.13 (m, 24H), 2.13 – 2.11 (m, 12H), 2.06 (s, 3H), 2.05 – 2.03 (m, 9H), 2.00 – 1.97 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 170.9, 170.8, 170.8, 170.7, 170.5, 170.5, 170.5, 170.4, 170.3, 170.2, 170.2, 170.1, 170.1, 167.0, 169.9, 169.9, 169.8, 169.7, 169.7, 99.5, 99.2, 99.0, 97.9, 97.6, 97.4, 91.4, 77.5, 77.2, 76.8, 75.3, 75.0, 74.5, 72.7, 70.9, 70.8, 70.1, 70.0, 69.9, 69.7, 69.6, 69.6, 69.5, 69.4, 69.3, 69.2, 68.6, 68.5, 68.5, 68.4, 68.2, 68.1, 67.7, 67.3, 66.8, 66.7, 66.3, 66.0, 65.7, 62.4, 62.3, 62.2, 62.1, 21.2, 21.1, 21.0, 21.0, 20.9, 20.8, 20.8, 20.7. HRMS: $[M+H]^+$ calculated for $C_{86}H_{117}O_{58}$ 2077.62003, found 2077.62039.

2,4-*O*-di-acetyl-3-*O*-(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)-6-*O*-(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)-1-*O*-(*N*-phenyl-trifluoroacetimidoyl)-α/β-D-mannopyranoside

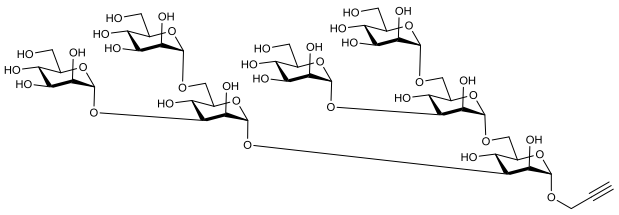


(25): To a solution of 1-OH acetylated heptamer **24** (56.9 mg, 27.4 µmol) in acetone (274 µL) was added a 0.15M *N*-phenyl trifluoroacetimidoyl chloride solution (274 µL, 41.1 µmol) in acetone. To the reaction mixture was added Cs_2CO_3 (15.5 mg, 41.1 µmol) and the mixture was stirred at rt till TLC showed complete conversion of the starting material. The reaction mixture was concentrated *in vacuo* and directly purified without further work up. Purification by column chromatography yielded heptamer imidate donor **25** as a slightly yellow oil (61.6 mg, quantitative yield). FT-IR: ν_{max} (neat)/cm^{-1} 1040.72, 1084.61, 1138.28, 1215.66, 1370.23, 1435.52, 1674.33, 1743.57, 2854.33, 2924.27. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (t, *J* = 7.5 Hz, 2H), 7.09 (t, *J* = 7.5 Hz, 1H), 6.85 (d, *J* = 7.7 Hz, 2H), 5.40 – 5.16 (m, 16H), 5.10 – 5.05 (m, 2H), 5.04 – 4.95 (m, 4H), 4.91 (s, 1H), 4.84 (d, *J* = 1.7 Hz, 1H), 4.81 (s, 1H), 4.36 – 4.22 (m, 4H), 4.22 – 3.88 (m, 13H), 3.87 – 3.79 (m, 1H), 3.79 – 3.70 (m, 3H), 3.60 – 3.46 (m, 3H), 2.25 – 2.01 (m, 54H), 2.01 – 1.95 (m, 9H), 1.92 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 170.7, 170.7, 170.7, 170.5, 170.2, 170.1, 170.1, 170.1, 167, 167.0, 169.9, 169.8, 169.8, 169.7, 169.7, 169.5, 143.2, 128.9, 124.7, 119.5, 99.6, 99.4, 99.1, 97.6, 97.6, 75.5, 75.2, 71.9, 70.8, 70.8, 70.0, 69.9, 69.9, 69.8, 69.6, 69.5, 69.5, 69.4, 69.1, 68.7, 68.7, 68.6, 68.6, 67.8, 67.4, 66.8, 66.4, 66.1, 66.0, 65.9, 65.7, 62.5, 62.2, 62.1, 29.8, 21.0, 20.9, 20.9, 20.8, 20.8, 20.7, 20.7, 20.6.

Propargyl 2,4-*O*-di-acetyl-3-*O*-(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)-6-*O*-(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)-α-D-mannopyranoside (26): heptamer imidate donor **25** (43.9 mg, 19.5 µmol) was dissolved in a 0.274M propargyl alcohol solution (356 µL, 97.5 µmol) in DCM and the mixture was stirred over activated molecular sieves (3Å) for 30 min. at rt. After 30 min. the mixture was cooled to -40 °C and to the cooled mixture was added a 0.1M TfOH solution (36 µL, 3.9 µmol) in DCM and the reaction mixture was gradually warmed to 0 °C. The reaction was quenched with a 0.1M Et_3N solution (0.1 mL) in DCM and the solution was filtered over celite and concentrated *in vacuo*. Purification by column chromatography yielded per-acetylated propagyl mannose heptamer **25** as a white milky oil (16.5 mg, 7.8 µmol, 40%). FT-IR: ν_{max} (neat)/cm^{-1} 1037.06, 1136.93, 1214.09, 1369.36, 1433.95, 1740.68, 2926.41. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.35 (dd, *J* = 9.9, 3.5 Hz, 1H), 5.33 – 5.32 (m, 1H), 5.29 – 5.22 (m, 7H), 5.21 (dd, *J* = 10.0, 3.4 Hz, 2H), 5.19 – 5.16 (m, 1H), 5.07 (dd, *J* = 3.0, 1.9 Hz, 1H), 5.05 (dd, *J* = 3.4, 1.8 Hz, 1H), 5.00 – 4.97 (m, 3H), 4.97 – 4.94 (m, 3H), 4.92 – 4.89 (m, 1H), 4.85 (d, *J* = 1.8 Hz, 1H), 4.84 (d, *J* = 1.9 Hz, 1H), 4.32 – 4.23 (m, 6H), 4.17 (td, *J* = 10.0, 3.4 Hz, 2H), 4.14 – 4.09 (m, 2H), 4.08 (s, 1H), 4.07 – 3.99 (m, 4H), 3.97 (dd, *J* = 9.9, 3.4 Hz, 2H), 3.97 – 3.88 (m, 3H), 3.87 (ddd, *J* = 10.3, 5.2, 2.7 Hz, 1H), 3.75 (dq, *J* = 10.8, 5.5 Hz, 3H), 3.71 (d, *J* = 5.6 Hz, 1H), 3.56 (dd, *J* = 11.4, 2.2 Hz, 1H), 3.53 (d, *J* = 2.6 Hz, 1H), 3.52 (d, *J* = 2.6 Hz, 1H), 2.52 (t, *J* = 2.4 Hz, 1H), 2.21 (s, 3H), 2.19 (s, 3H), 2.17 (s, 3H), 2.16 – 2.13 (m, 21H), 2.12 – 2.11 (m, 12H), 2.06 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H), 1.98 (s, 3H), 1.97 (d, *J* = 1.3 Hz, 6H). ^{13}C

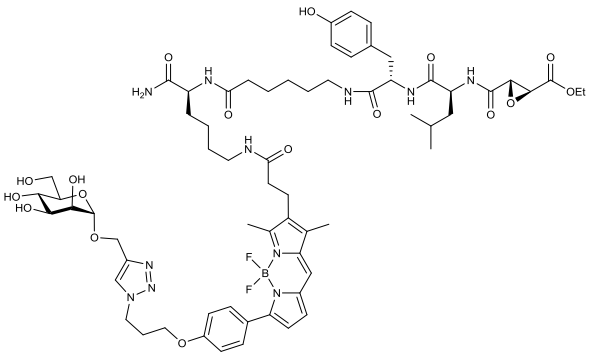
NMR (150 MHz, $CDCl_3$) δ 171.1, 171.0, 170.9, 170.8, 170.8, 170.8, 170.8, 170.8, 170.6, 170.6, 170.6, 170.5, 170.4, 170.3, 170.3, 170.2, 170.2, 170.2, 170.2, 170.1, 170.1, 170.1, 170.0, 170.0, 170.0, 170.0, 169.9, 169.9, 169.8, 169.8, 169.8, 169.7, 99.4, 99.2, 97.8, 97.7, 97.3, 96.6, 78.8, 75.6, 75.5, 75.3, 75.0, 71.2, 71.0, 70.9, 70.3, 70.0, 70.0, 69.8, 69.6, 69.6, 69.5, 69.5, 69.5, 69.3, 69.2, 68.8, 68.7, 68.6, 68.6, 68.0, 67.8, 67.6, 66.8, 66.8, 66.4, 66.1, 66.1, 66.0, 66.0, 65.8, 62.5, 62.3, 62.2, 55.0, 21.0, 21.0, 21.0, 21.0, 20.9, 20.9, 20.9, 20.9, 20.8, 20.8, 20.8, 20.8. HRMS: $[M+H]^+$ calculated for $C_{89}H_{119}O_{58}$ 2115.63568, found 2115.63581.

Propargyl 3-*O*-(3-*O*-(α-D-mannopyranosyl)-6-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyl)-6-*O*-(3-*O*-(α-D-mannopyranosyl)-6-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyl)-α-D-mannopyranoside (27): To



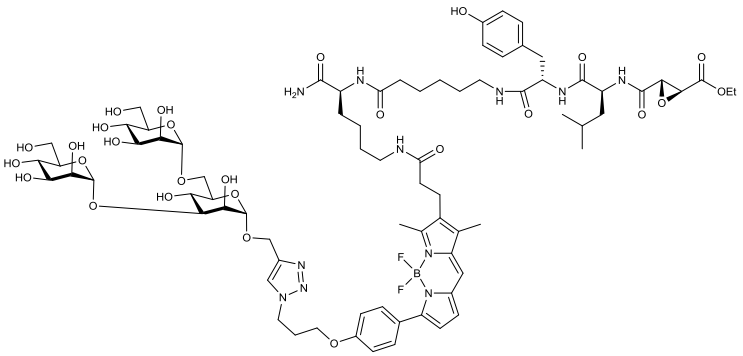
a solution of per-*O*-acetylated propargyl mannose heptamer **26** (16.5 mg, 7.8 µmol) in MeOH (156 µL) was added a 125mM NaOMe (156 µL, 3.9 µmol) solution in MeOH and the reaction was stirred overnight at rt. TLC-MS analysis showed incomplete deacetylation of the starting material and the reaction was quenched with Amberlite® IR-120 H^+ (pH≤7). The crude was taken up in H_2O (0.5 mL) and a 0.2M NaOH (aq.) (0.5 mL) solution was added to the solution. The reaction was followed on TLC-MS and after completion the reaction was quenched with Amberlite® IR-120 H^+ (pH≤7). Solids were filtered and the filtrate was concentrated *in vacuo*. The product was lyophilized from H_2O without further purification yielding propargyl mannose heptamer **27** as a white powder (9.3 mg, 7.8 µmol, quantitative yield). ^{1}H NMR (600 MHz, D_2O) δ 5.17 (d, *J* = 1.8 Hz, 1H), 5.14 (d, *J* = 1.8 Hz, 1H), 5.07 (d, *J* = 1.8 Hz, 1H), 5.02 (d, *J* = 1.8 Hz, 1H), 4.92 (d, *J* = 1.8 Hz, 1H), 4.91 (d, *J* = 1.8 Hz, 1H), 4.88 (d, *J* = 1.9 Hz, 1H), δ 4.36 (s, 2H), 4.26 (dd, *J* = 3.3, 1.8 Hz, 1H), 4.16 (dd, *J* = 3.3, 1.8 Hz,

1H), 4.11 (dd, *J* = 3.2, 1.7 Hz, 1H), 4.08 (dt, *J* = 3.6, 1.9 Hz, 2H), 4.03 (dd, *J* = 9.6, 3.5 Hz, 1H), 4.01 – 3.97 (m, 5H), 3.96 – 3.92 (m, 2H), 3.92 – 3.87 (m, 11H), 3.87 – 3.83 (m, 2H), 3.80 – 3.72 (m, 8H), 3.70 (ddd, *J* = 12.6, 7.3, 2.1 Hz, 4H), 3.67 (s, 2H), 3.65 (s, 1H). ^{13}C NMR (150 MHz, D_2O) δ 103.8, 103.7, 103.5, 100.8, 100.7, 100.6, 100.3, 80.1, 79.7, 79.7, 74.70, 74.6, 74.0, 73.9, 73.1, 72.7, 72.2, 71.9, 71.9, 71.7, 71.4, 71.3, 71.3, 71.2, 70.9, 70.9, 70.8, 68.1, 68.1, 68.0, 67.1, 67.1, 66.8, 66.7, 66.6, 66.4, 62.4, 62.2, 56.2. HRMS: $[M+H]^+$ calculated for $C_{45}H_{75}O_{36}$ 1191.40325, found 1191.40308.



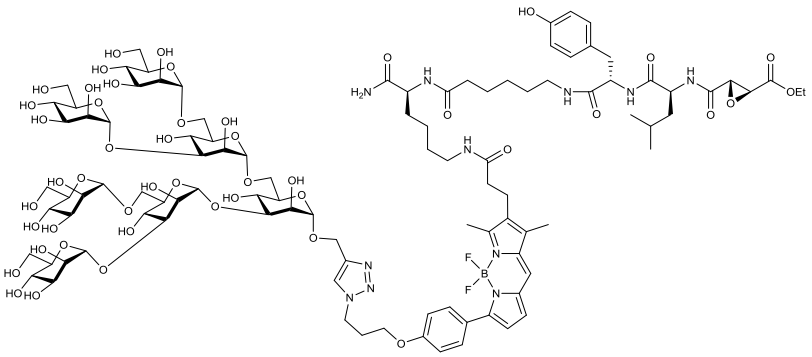
Man$_1$-BODIPY-DCG-04 (2): To a solution of propargyl mannose **7** (1.75 mg, 8 µmol) and BODIPY-DCG-04 **(5)** (8.6 mg, 7.6 µmol) in DMF/H_2O (1:1) (3 mL) was added 0.1 M sodium ascorbate (aq.) (160 µL, 16 µmol) and 0.1M $CuSO_4$ (aq.) (16 µL, 1.6 µmol). The resulting mixture was stirred for 2 h at room temperature, before being concentrated and co-evaporated with toluene. Purification by HPLC-MS (A: 25 mM NH_4OAc, B: linear gradient 20→ 35% ACN in12') followed by lyophilization from H_2O yielded Man$_1$-BODIPY-DCG-04 (**2**) (4.3 mg, 3.2 µmol, 42%). ^{1}H NMR (600 MHz, $CDCl_3$/MeOD): δ 8.02 (s, 1H), 7.92 (t, *J* = 5.6 Hz, 1H), 7.86 (d, *J* = 7.5 Hz, 2H), 7.83 (d, *J* = 7.6 Hz, 1H), 7.76 (t, *J* = 5.6 Hz, 1H), 7.40 (s, 1H), 7.06 (d, *J* = 4.1 Hz, 1H), 7.01 (d, *J* = 8.5 Hz, 2H), 6.95 (d, *J* = 8.9 Hz, 2H,), 6.69 (d, *J* = 8.5 Hz, 2H, 2), 6.59 (d, *J* = 4.1 Hz, 1H), 4.80 (d, *J* = 12.4 Hz, 1H), 4.67 - 4.60 (m, 3H, CH_2), 4.45 (t, *J* = 7.5 Hz, 1H), 4.40 - 4.36 (m, 1H), 4.30 - 4.19 (m, 3H), 4.08 (t, *J* = 5.8 Hz, 2H), 3.85 (dd, *J* = 11.8, 2.1 Hz, 1H), 3.82 - 3.77 (m, 1H), 3.74 - 3.65 (m, 3H), 3.64 - 3.55 (m, 3H), 3.15 - 3.11 (m, 3H), 3.06 - 3.01 (m, 1H), 2.99 - 2.93 (m, 1H), 2.86 - 2.80 (m, 1H), 2.75 (t, *J* = 7.4 Hz, 2H), 2.50 (s, 3H), 2.42 (p, *J* = 6.5 Hz, 2H), 2.33 (t, *J* = 7.3 Hz,

2H), 2.25 (s, 3H), 2.21 - 2.16 (m, 2H), 1.78 - 1.69 (m, 1H), 1.62 - 1.48 (m, 6H), 1.46 - 1.41 (m, 2H), 1.41 - 1.35 (m, 2H), 1.31 - 1.29 (m, 5H), 1.22 - 1.15 (m, 2H), 0.92 (d, *J* = 6.4 Hz, 3H), 0.88 (d, *J* = 6.4 Hz, 3H,). ^{13}C NMR (150 MHz, $CDCl_3$/MeOD): δ 177.02, 176.02, 174.68, 173.56, 172.96, 168.66, 168.31, 160.68, 160.59, 157.16, 156.37, 145.22, 141.69, 136.49, 135.67, 131.84, 131.81, 131.78, 131.68, 131.30, 129.27, 128.81, 127.14, 125.60, 124.63, 119.12, 116.19, 115.14, 100.70, 74.84, 72.42, 71.93, 68.54, 65.67, 63.19, 62.90, 60.64, 56.36, 54.34, 54.11, 53.33, 53.14, 48.49, 41.56, 40.15, 40.12, 38.14, 36.86, 36.58, 32.64, 30.93, 29.87, 29.74, 27.32, 26.39, 25.80, 24.17, 23.29, 22.02, 21.29, 14.35, 13.28, 9.62. HRMS: $[M+H]^+$ calculated for $C_{65}H_{89}BF_2N_{11}O_{17}$ 1344.64935, found 1344.65139.



Man$_3$-BODIPY-DCG-04 (3): To a solution of propargyl mannoside **19** (2.4 mg, 4.5 µmol) and BODIPY-DCG-04 (**5**) (5.12 mg, 4.5 µmol) in DMF/H_2O (1:1) (2 mL) was added 0.1M sodium ascorbate (aq.) (90 µl, 9 µmol) and 0.1M $CuSO_4$ (aq.) (2.2 µL, 0.22 µmol). The resulting mixture was stirred for 1 h at room temperature, before being concentrated and co-evaporated with toluene. Purification by HPLC-MS (A: 25 mM NH_4OAc, B: linear gradient 20 → 35% ACN in 12') followed by lyophilization from H_2O yielded Man$_3$-BODIPY-DCG-04 **(3)** (1.8 mg, 1.1 µmol, 24 %) ^{1}H NMR (600 MHz, MeOD): δ 8.08 (s, 1H), 7.88 (d, *J* = 8.9 Hz, 2H,), 7.43 (s, 1H), 7.07 (d, *J* = 4.0 Hz, 1H), 7.01 (d, *J* = 8.5 Hz, 2H), 6.97 (d, *J* = 8.9 Hz, 2H), 6.69 (d, *J* = 8.5 Hz, 2H), 6.62 (d, *J* = 4.1 Hz, 1H), 5.05 (s, 1H), 4.85 - 4.77 (m, 3H), 4.69 - 4.61 (m, 3H), 4.45 (t, *J* = 7.6 Hz, 1H), 4.38 (dd, *J* = 9.3, 5.7 Hz, 1H), 4.29 -

4.23 (m, 3H), 4.09 (t, *J* = 5.9 Hz, 2H), 4.05 (d, *J* = 2.5 Hz, 1H), 3.98 - 3.90 (m, 2H), 3.88 - 3.54 (m, 17H), 3.16 - 3.09 (m, 3H), 3.07 - 2.99 (m, 1H), 2.99 - 2.93 (m, 1H), 2.86 - 2.80 (m, 1H), 2.76 (t, *J* = 7.3 Hz, 2H), 2.51 (s, 3H), 2.42 (p, *J* = 6.5 Hz, 2H), 2.33 (t, *J* = 7.3 Hz, 2H), 2.26 (s, 3H), 2.20 - 2.14 (m, 2H), 1.77 - 1.70 (m, 1H), 1.64 - 1.47 (m, 6H), 1.47 - 1.41 (m, 2H), 1.41 - 1.35 (m, 2H), 1.32 - 1.29 (m, 5H), 1.20 - 1.15 (m, 2H), 0.92 (d, *J* = 6.4 Hz, 3H), 0.88 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (150 MHz, MeOD): δ 177.14, 176.13, 174.79, 173.69, 173.08, 168.74, 168.43, 165.61, 163.01, 160.81, 160.67, 157.29, 156.48, 150.29, 145.18, 142.19, 136.59, 135.75, 131.90, 131.77, 131.36, 129.35, 128.91, 127.23, 125.71, 124.73, 119.16, 116.24, 115.22, 103.97, 101.33, 100.79, 80.67, 74.96, 74.39, 73.70, 72.66, 72.46, 72.10, 71.27, 68.80, 68.64, 67.48, 67.14, 65.75, 63.23, 62.91, 60.71, 56.47, 54.38, 54.21, 53.42, 53.19, 41.62, 40.20, 38.19, 36.91, 36.63, 32.71, 31.05, 29.95, 29.82, 27.40, 26.48, 25.88, 24.25, 23.30, 22.02, 21.34, 14.35, 9.59. LC/MS analysis (linear gradient 10% → 90% ACN) t*R*: 6.53 min, ESI-MS (*m/z*): [M + H]+: 1668.40.



Man$_7$-BDP-DCG-04 (4): To a solution of propargyl mannoside **27** (4 mg, 3.4 µmol) and BODIPY-DCG-04 (**5**) (3.8 mg, 3.4 µmol) in DMF/H_2O (1:1) (2 mL) was added 0.1M sodium ascorbate (aq.) (68 µL, 6.8 µmol) and 0.1M $CuSO_4$ (aq.) (6.8 µL, 0.68 µmol). The resulting mixture was stirred for 8 h at room temperature, before being concentrated and co-evaporated with toluene. Purification by HPLC-MS (A: 25 mM NH_4OAc, B: linear gradient 20 → 35% ACN in 12') followed by lyophilization from H_2O yielded Man$_7$-

BDP-DCG-04 (**4**) (2.5 mg, 1.1 μmol, 32%). ^{1}H NMR (600 MHz, MeOD): δ 8.28 (s, 1H), 8.09 (d, *J* = 8.1 Hz, 1H), 7.95 (s, 1H), 7.90 - 7.85 (m, 2H), 7.79 (t, *J* = 5.6 Hz, 1H), 7.42 (s, 1H), 7.07 (d, *J* = 4.1 Hz, 1H), 7.01 (d, *J* = 8.5 Hz, 2H), 6.98 (d, *J* = 8.9 Hz, 2H), 6.69 (d, *J* = 8.5 Hz, 2H), 6.62 (d, *J* = 4.1 Hz, 1H), 5.11 (s, 1H), 5.07 (s, 1H), 4.99 (s, 1H), 4.81 - 4.64 (m, 8H), 4.50 - 4.43 (m, 1H), 4.41 - 4.36 (m, 1H), 4.31 - 4.23 (m, 3H), 4.19 (d, *J* = 2.8 Hz, 1H), 4.12 - 4.07 (m, 4H), 4.03 - 3.53 (m, 41H), 3.16 - 3.10 (m, 3H), 3.07 - 3.02 (m, 1H), 3.00 - 2.94 (m, 1H), 2.88 - 2.80 (m, 1H), 2.76 (t, *J* = 7.3 Hz, 2H), 2.51 (s, 3H), 2.44 (q, *J* = 6.4 Hz, 2H), 2.34 (t, *J* = 7.2 Hz, 2H), 2.26 (s, 3H), 2.21 - 2.17 (m, 2H), 1.78 - 1.71 (m, 1H), 1.66 - 1.47 (m, 6H), 1.47 - 1.41 (m, 2H), 1.41 - 1.36 (m, 2H), 1.33 - 1.28 (m, 5H), 1.23 - 1.17 (m, 2H), 0.92 (d, *J* = 6.4 Hz, 3H), 0.88 (d, *J* = 6.4 Hz, 3H). HRMS: $[M+H]^+$ calculated for $C_{101}H_{149}BF_2N_{11}O_{47}$ 2317.96965, found 2317.97256.

Cell culture of primary cells. Immature dendritic cells were obtained from the bone marrow of C75BL/6 mice and were a gift from the Biopharmaceutical Department (Leiden University). The use of animals was approved by the ethics committee of Leiden University. Mice were sedated, bone marrow of tibiae and femurs was flushed out and washed with PBS. Cells were grown in dendritic cell selection medium (IMDM containing granulocyte-macrophage colony stimulating factor (GM-CSF) (2:1 v/v) containing 8% FCS, penicillin/streptomycin (100 units/mL), glutamax (2 mM) and β-mercaptoethanol (20 μM). Cells were selected for 10 days (37 °C; 5% CO_2) and subcultured every 2-3 days before use in the assays.

Labeling of cathepsins in mouse liver and immature dendritic cell lysate. Lysates (~8-10 μg total protein, determined on a Qubit 2.0 fluorometer, Life Technologies-Invitrogen) in 50 mM sodium citrate pH 5.5 or pH 7 (as indicated), 5 mM DTT, 0.2% CHAPS, and 0.1% Triton X-100, were incubated with the indicated concentration of probe (total volume: 10 μL) for 1 h at 37 °C. For competition experiments, lysates were first incubated with N_3-DCG-04 (1 or 10 μM), AS44 (10 μM) or BODIPY(FL)-DCG-04 (1 or 10

μM) for 1 h, 37 °C, before addition of the probe and continued incubation for 1 h. After treatment, 5x Laemli's sample buffer (including β-mercaptoethanol) was added and the samples were boiled (100 °C, 5 min) and resolved on 12.5% SDS-PAGE. Gels were scanned on a Typhoon 2000 imager (GE Healthcare) using the Cy2 (λ_{ex} 532 nm; λ_{em} 526 nm) and Cy3 (λ_{ex} 532 nm; λ_{em} 580 nm) settings. Total protein loading was determined by staining with Coomassie brilliant blue and subsequent scanning on a BioRad GS800 calibrated densitometer. Image processing was done with ImageJ, representative gels from at least three independent experiments are shown.

Labeling of cathepsins in live immature mouse dendritic cells. Cells were seeded onto tissue-culture coated 24-wells plates (200.000 cells/well, 250 μL medium) and allowed to attach for 2 h (37 °C; 5% CO_2), before addition of inhibitor or probe to the medium. Pre-incubations with N_3-DCG-04 (20 μM) or mannan (3 mg/mL) were conducted for 1 h, followed by addition of compound **2**, **3** or **4** (1 μM) and continued incubation for 2 h. For direct labeling experiments, cells were cultured for 2 h (37 °C; 5% CO_2) in the presence of probes **2**, **3** or **4** (0.1, 0.25, 0.5, 0.75 or 1 μM). After incubation, cells were washed with PBS (2x), lysed (35 μL Invitrogen complete cell extraction buffer) and proteins resolved on 12.5% SDS-PAGE, followed by fluorescence scanning (Cy3 settings) and CBB staining. Image processing was done with ImageJ, representative gels from at least three independent experiments are shown.

Confocal fluorescence microscopy. Experiments were conducted on a Leica TCS SPE confocal microscope, using dsRed filter settings for BODIPY (λ_{ex} 532 nm) and Cy5 settings for Draq5 (λ_{ex} 635 nm). Cells (30-75 x 10^4 cells/well) were seeded onto sterile Labtek II 4- or 8-chamber borosilicate coverglass systems (Fisher Emergo). Dendritic cells were allowed to attach for 2 h before pre-incubation with mannan (3 mg/mL) (1 h, 37 °C, 5% CO_2) and subsequent probe incubation (1 μM, 2 h). Cells were then thoroughly washed (PBS), fixed (4% formaldehyde in PBS), washed

again with PBS, nuclei stained with Draq5 (Thermo Scientific) and imaged. All experiments were performed at least in duplicate.

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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).

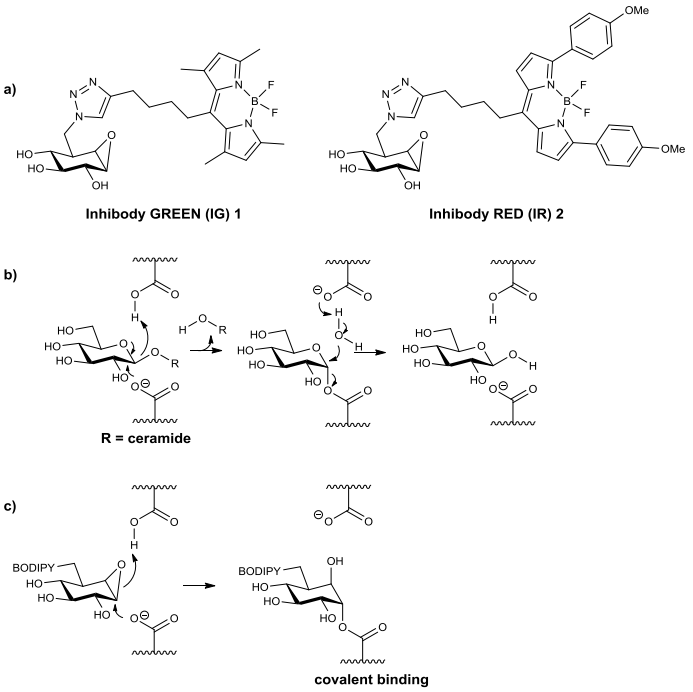


Figure 1: a) Structures of ABPs **1** and **2**. b) Double replacement mechanism of retaining glycosidase GBA. c) β-glucosidase inhibition mechanism of cyclophellitol.

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.

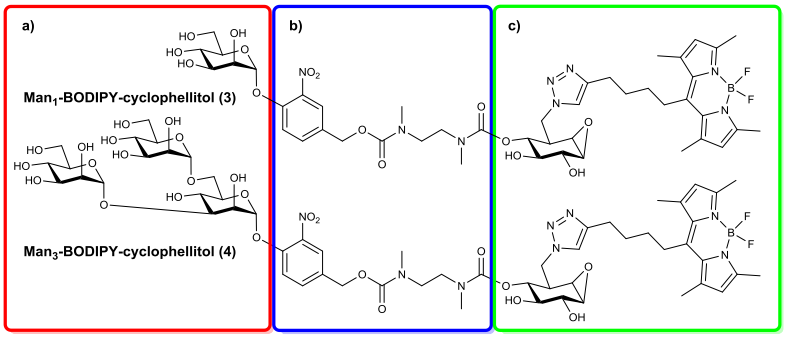


Figure 2: a-c) Design of the (tri)mannose cyclophellitol probes **3** and **4**.

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**.

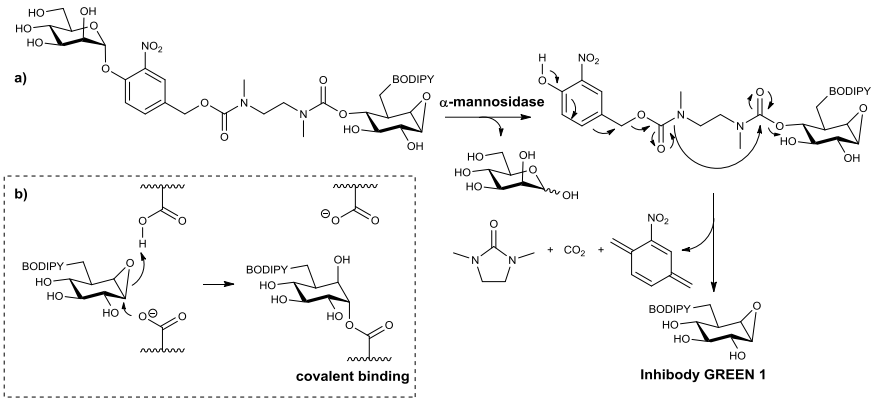
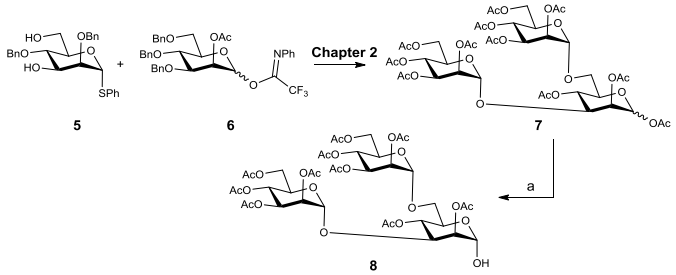


Figure 3: a) Degradation reaction of the linker liberating Inhibody GREEN **3**. b) Covalent inhibition of GBA by IG.

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%.

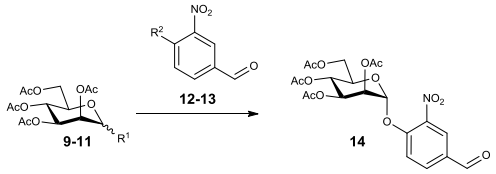
Scheme 1: trimer **8** synthesis.



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**).



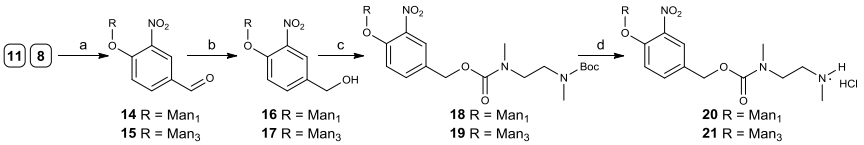
Entry	R^1	R^2	Reaction conditions	Yield[a]	α/β ratio[b]
1	I (**9**)	OH (**12**)	Ag_2O, MeCN, reflux	24	1:0
2	Br (**10**)	OH (**12**)	Ag_2O, MeCN, reflux	42	1:0
3	Br (**10**)	OH (**12**)	$Bn(Et)_3NCl$, 1.25 M NaOH (aq.), $CHCl_3$	2	1:3

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.



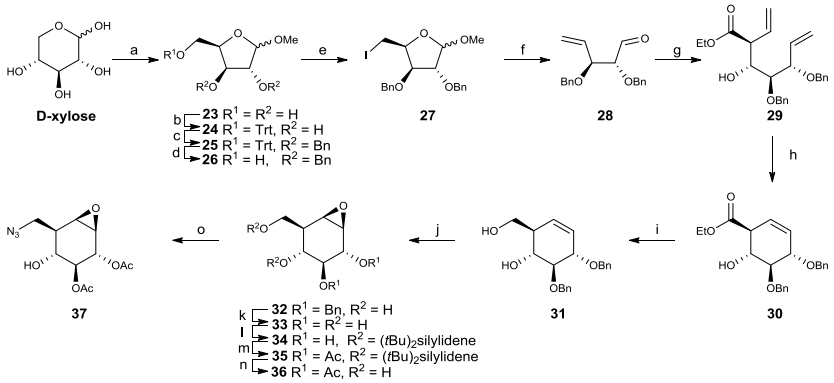
Reagents and conditions: (a) Li_2CO_3, DMAP, **13**, Mol. Siev. (Man_1 **14**: 88%, Man_3 **15**: 93%); (b) $NaBH_4$, $CHCl_3$/*i*-PrOH (4:1), silica, 0 °C (Man_1 **16**: 87%, Man_3 **17**: 59%); (c) *i*. *para*-nitrophenyl chloroformate, pyridine, DCM, 0 °C; *ii*. Mono-Boc diamine **22**, DMAP, 0 °C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

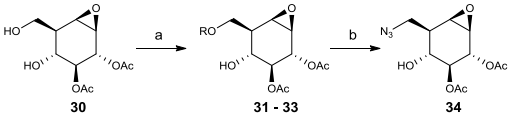
Key step in the cyclophellitol synthesis entails the indium/La(OTf)$_3$ mediated stereoselective Barbier reaction of aldehyde **28** with ethyl 4-bromocrotonate. This reaction gave target diene **29** and its L-idose configured *C*-5 epimer in a 4:1 ratio. After column purification the desired diene **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.**



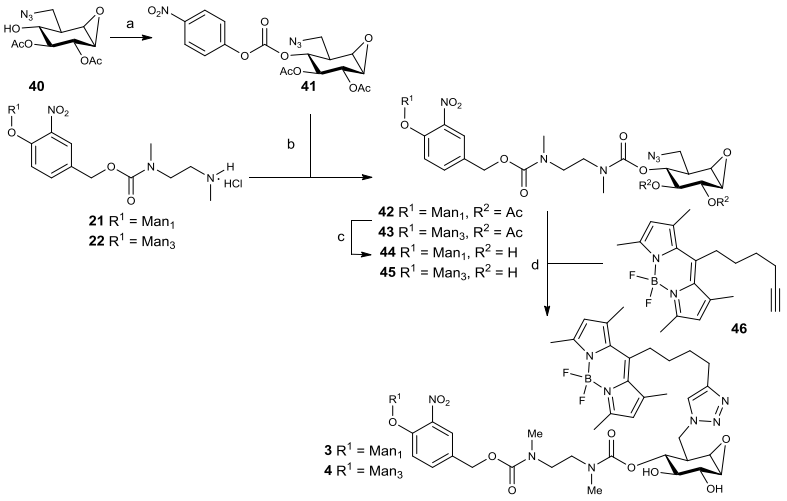
30 31 - 33 34

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.

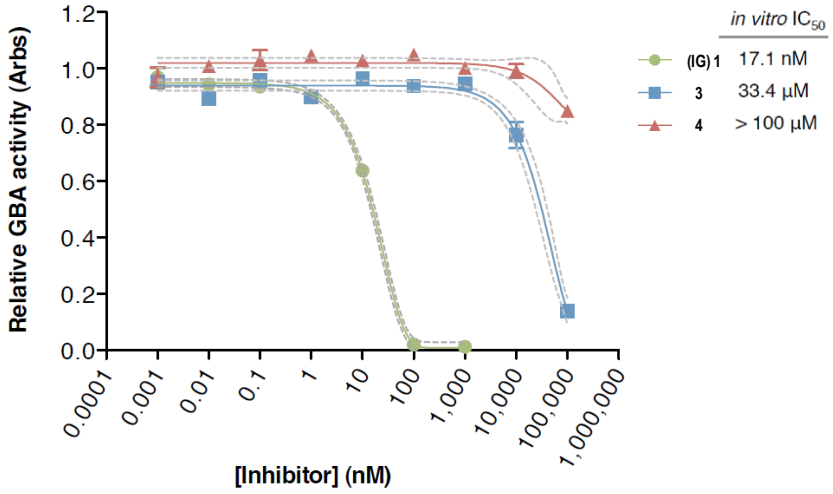


Figure 4: Inhibition of recombinant GBA by **1**, **3** and **4**. Imiglucerase was treated with the probes for 30 min at 37 °C after which residual activity was determined using 4MUGlc.

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.

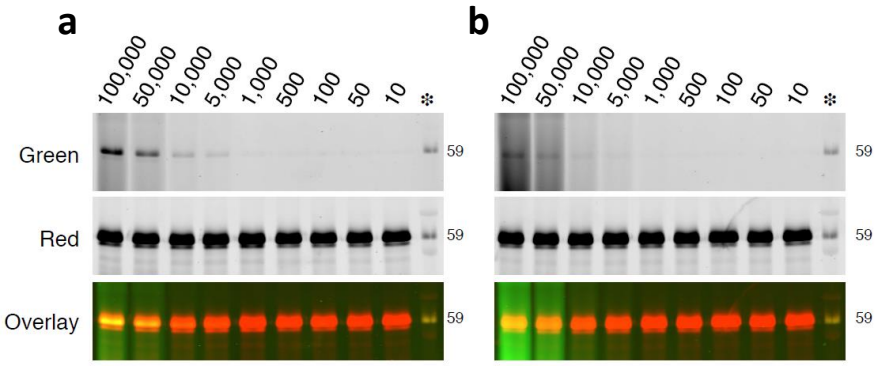


Figure 5: Detection limit of **3** and **4** for GBA. Imiglucerase (2 pmol) was incubated with **3** (a) or **4** (b) in different concentrations (nm) for 60 min at 37 °C. Residual enzyme activity was determined by incubation with IR for 30 min at 37 °C. All gels are 10% SDS-PAGE with fluorescent readout.

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.

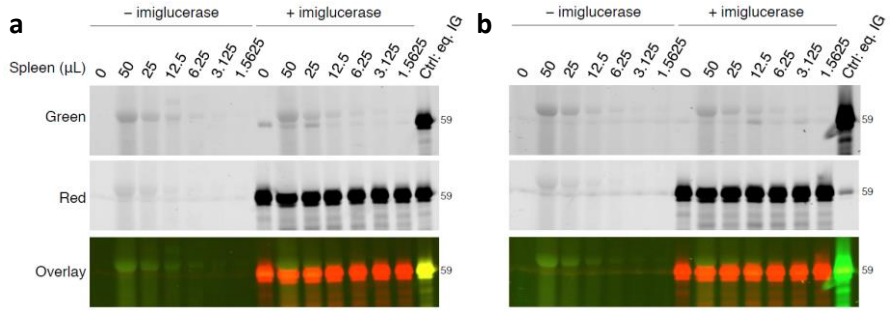


Figure 6: 3 (a) and **4** (b) processing with α-mannosidase present in Gaucher spleen lysate prior to labelling with recombinant GBA. a-b) 10µmol of **3** and **4** were incubated with different amount of spleen lysate and incubated for 60 min at 37 °C. Recombinant GBA was incubated with the lysate mixture 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.

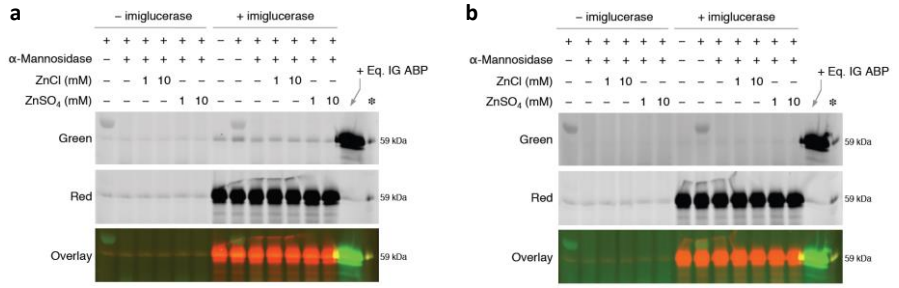


Figure 7: 3 (a) and **4** (b) processing with α-mannosidase (with- or without different zinc salts) present in Gaucher spleen lysate prior to labelling of imiglucerase. a-b) **3** and **4** were treated with Gaucher spleen lysate (with or without different zinc salts) and incubated for 60 min at 37 °C 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).

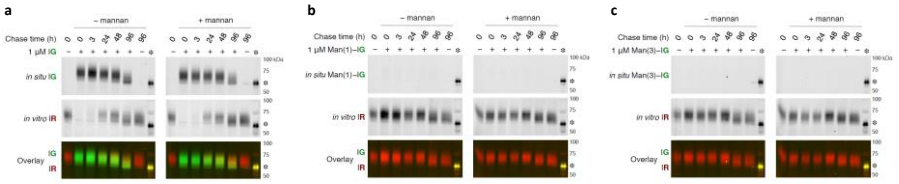


Figure 8: *in situ* labelling of GBA in human monocyte-derived macrophages. a-c) Human monocyte-derived macrophages, matured for 7 d, were incubated with IG (a), **3** (b) and **4** (c) in the presence or absence of mannan for 2 h at 37 °C. Cells were washed with PBS and chased in fresh medium over 96 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.

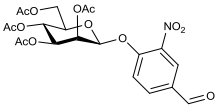
Experimental

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)

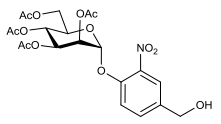
were of analytical grade and used as received. Column chromatography was performed on Screening Device silica gel 60 (0.040-0.063 mm). Size exclusion was performed on Sepadex LH20 (eluent DCM/MeOH, 1:1). TLC analysis was conducted on HPTLC aluminium sheet (Merck, TLC silica gel 60, F_{254}). Compounds 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,

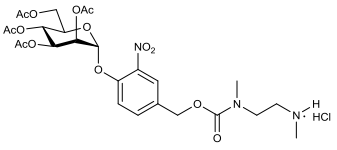
88 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, *J* = 2.4 Hz, 1H), 7.55 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.29 (d, *J* = 8.6 Hz, 1H), 5.66 (d, *J* = 2.1 Hz, 1H), 5.55 (dd, *J* = 10.0, 3.4 Hz, 1H), 5.50 (dd, *J* = 3.5, 1.9 Hz, 1H), 5.40 (t, *J* = 10.0 Hz, 1H), 4.73 (s, 2H), 4.28 (dd, *J* = 12.3, 5.1 Hz, 1H), 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.

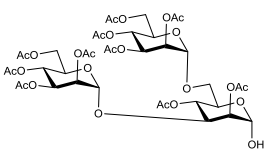
***N,N'*-Dimethyl-(4-(2,3,4,6-tetra-*O*-acetyl-α-D-mannosyl)-3-nitrobenzyl-oxycarbonyl)-*N'*-(*tert*-butyloxycarbonyl)-ethylendiamine (18):** To a solution of benzylalcohol mannose **16** (0.25 g, 1 mmol) in DCM (18 mL) was added dry pyridine (0.12 mL, 1.5 mmol) under argon atmosphere and the mixture 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.

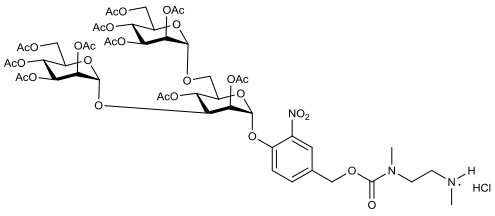
2-Nitro-4-benzaldehyde 2,4-*O*-di-acetyl-3-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-6-*O*-(2,3,4,6-*O*-tetra-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (15): To a solution of mannose trimer **8** (0.783 g, 0.725 mmol) in DCM (7.25 mL) was added Li_2CO_3 (0.112 g, 1.52 mmol) and 4-fluoro-3-nitrobenzaldehyde **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,

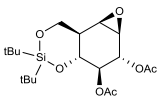
169.6, 155.7, 155.7, 148.1, 148.1, 140.1, 140.1, 134.3, 134.3, 132.4, 132.4, 125.5, 125.5, 125.3, 125.3, 117.5, 117.5, 99.2, 99.2, 97.6, 97.6, 96.1, 96.1, 74.3, 74.3, 71.0, 71.0, 70.3, 70.3, 69.8, 69.8, 69.6, 69.6, 69.3, 69.3, 68.9, 68.9, 68.7, 68.7, 68.4, 67.4, 67.4, 66.5, 66.5, 66.1, 66.1, 65.9, 65.9, 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):**



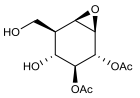
A freshly prepared 4M HCl solution in EtOAc (6.67 mL) was cooled to 0 °C and to the cooled solution was added dropwise a mannose trimer-Boc-diamine **19** (129.0 mg, 0.1 mmol) solution in EtOAc (3.33 mL). After 15 min. at 0 °C the solution was gradually warmed to rt and stirred for 1 h. The reaction 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):



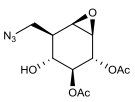
To a solution of cyclophellitol (**33**) (47.1 mg, 0.25 mmol) in DMF (2.3 mL) was added pyridine (19.8 mg, 0.2 mL, 2.5 mmol) and the solution was cooled to -40 °C under argon atmosphere. To the cooled solution was added $(t\text{Bu})_2\text{Si}(\text{OTf})_2$ (0.138 g, 102 µl, 0.313 mmol)

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.

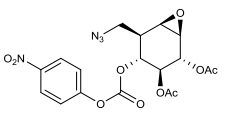


2,3-Di-*O*-acetyl-cyclophellitol (36): To a solution of silylidene protected cyclophellitol **33** (38.4 mg, 0.1 mmol) in THF (0.5 mL) was added pyridine (0.25 mL). and the mixture was cooled to 0 °C. To the cooled reaction mixture was added 1M HF·pyridine (0.25 mL, 0.25 mmol) and stirred for 1 h at 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,

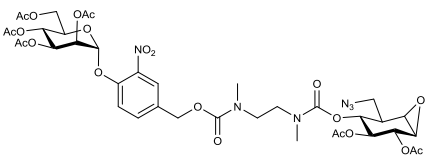
1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.3, 170.3, 71.0, 67.0, 63.0, 55.2, 53.3, 43.7, 21.0, 20.9. HRMS: $[M+H]^+$ calculated for $C_{11}H_{17}O_7$ 261.09622, found 261.09695.



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.

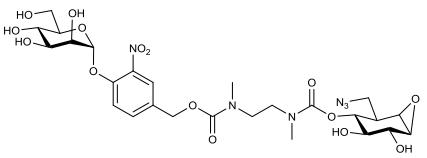


2,3-Di-*O*-acetyl-4-*O*-(4-nitrophenoxycarbonyl)-7-deoxy-7-azido-cyclophellitol (41): 2,3-acetylated azido cyclophellitol **40** (0.1 mmol, 28.5 mg) was dissolved in a 0.2M pyridine solution in DCM (1 mL, 0.2 mmol) and the solution was cooled to 0 °C. To the cooled solution was added *para*-nitrophenyl 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.



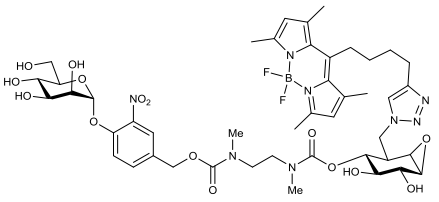
Man$_1$-OAc-azido-cyclophellitol (42): *p*-Nitro phenyl oxycarbonyl cyclophellitol **41** (14.9 mg, 33 µmol) was dissolved in 0.3M Et_3N sol. in DMF (333 µl, 100 µmol, 3 eq.) and cooled to 0 °C. To the solution was added a solution of mannose-diamine HCl salt **18** in a 0.01M Et_3N sol. in DMF (333 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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Chapter 4

Synthesis and immunological evaluation of a small library of mannosylated peptides[1]

Introduction

Antigen presenting cells (APCs), such as dendritic cells (DCs) play a pivotal role in the mammalian innate and adaptive immune system and thereby in the defence against external challenges, such as bacteria, viruses and toxins, but also internal challenges, such as malignantly transformed self-cells.[2] APCs recognize pathogen related molecular structures with the aid of various classes of receptors such as Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NLRs). This recognition event can result in the upregulation of pro-inflammatory signalling molecules (both surface-bound and soluble), that alert the immune system to the presence of danger.[3] Concomitantly, exogenous material is taken up by APCs through endocytosis, phagocytosis or marcopynocitic internalization. After uptake, the cargo is proteolytically degraded in the endo-lysosomal system by a family of proteases[4] and further processed in the lysosomes.[5] The degradation of proteins ultimately results in presentation of a peptide antigen on major

histocompatibility complex II (MHC class II) at the outer membrane of APCs.[6] The combination of the expression of pro-inflammatory receptors and MHC-II loaded with exogenous peptides will lead to the activation of CD4+ T-helper cells.

Malignantly transformed self-cells and cells harbouring pathogens in their cytosol are cleared using a different pathway: the MHC-I-pathway of antigen presentation. On the MHC-I molecule 8-9-mer peptides derived from cytosolic and ER-associated proteins are continually expressed from various stages of the protein life cycle.[7] In this cytosolic pathway to antigen presentation, proteins from within the cell are processed by proteasomes and downstream aminopeptidases and presented on MHC class I.[8,9] In a process referred to as antigen cross-presentation[10] DCs take up exogenous antigen and let this antigenic material 'escape' from the endosomally-restricted MHC-II pathway into their own MHC-I-loading pathway.[11] Cross-presentation is the subject of intense study due to its relevance to disease and its mechanistic complexity. Besides the above mentioned PRRs that detect pathogen associated molecular patterns (PAMPs), APCs express various C-type lectin receptors (CLRs) which bind specific carbohydrates of pathogens but also carbohydrates structures of self-glycoproteins.[12] It has been hypothesized that these lectins play a crucial role in antigen cross-presentation.[13] Binding of a glycoconjugate by CLRs on APCs is followed by internalization thereby facilitating the processing and finally antigen presentation. There are multiple carbohydrate receptors on the surface of APCs and their expression varies between cell types. They have been shown to have overlapping binding specificity, which has complicated their structure. Despite this absence of knowledge about the exact structure of the carbohydrate-ligands for specific CLRs, considerable attention is directed to explore CLRs with the aid of natural and artificial glycoconjugates. Examples of CLRs that recognise mannose-containing structures are Dectin-2, Mincle, DC-SIGN, SIGNR1, Langerin, and the Mannose Receptor (MR). As discussed in Chapters 2 and 3 (oligo)mannose conjugates are regularly exploited in targeting strategies to enable improved uptake of specific molecules, such as (fluorescently) labeled

inhibitors.[14,15] In addition (oligo)mannose conjugates are used to target antigens to APCs to improve the uptake of antigens.[16]

Recently it has been shown that the MR can strongly enhance antigen cross-presentation by routing glycoprotein ligands to a very mild endosome (low proteolysis, near neutral pH), from which cross-presentation is favoured.[17] In line with these results, it was recently reported that a bismannosylated synthetic long peptide showed enhanced cross-presentation in an MR-dependent manner[18] and the chemical introduction of additional glycans onto the glycoprotein ovalbumin was shown to further enhance cross-presentation.[19] However, it is still unclear how different glycans exactly enhance cross-presentation, which in part is due to the extensive heterogeneity of glycoproteins that have been studied.[20,21] The position, branching, spacing and multivalency of (oligo)mannoses in a glycoconjugate are decisive for uptake by a mannose recognising CLR, the subsequent routing and finally for antigen presentation.

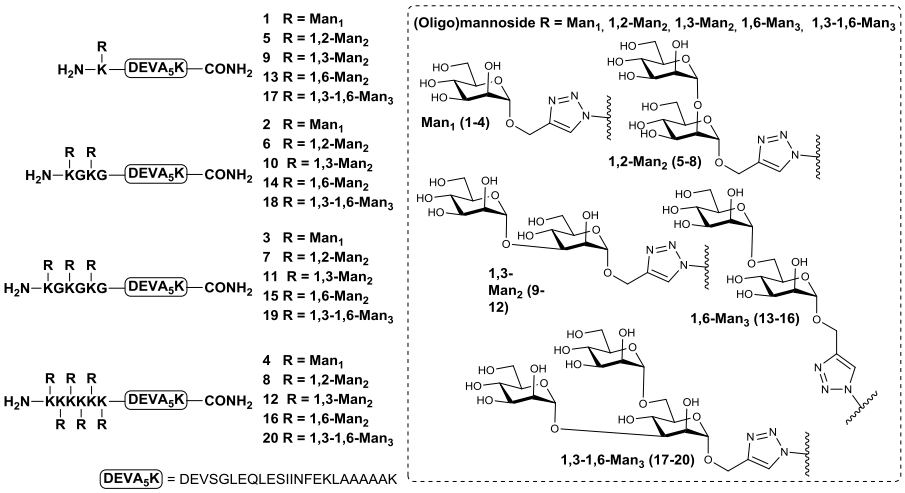


Figure 1: Target compounds 1-20 discussed in this chapter.

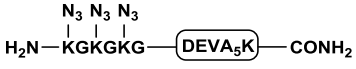
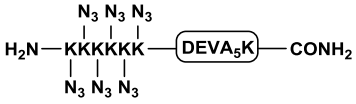
To gain more insight which carbohydrate structures promote cross-presentation, this chapter presents the synthesis and initial immunological evaluation of a library of mannosylated peptides, based on the MHC-class-I

epitope DEVSGLEQLESIINFEKLAAAAAK ($DEVA_5K$).[22] A set of 5 different (oligo)mannosides were attached to the side chain of azido lysine residues. The resulting library of twenty mannosylated peptides (**1-20**, Figure 1) has been evaluated on their ability to promote antigen presentation.

Result and discussion

The assembly of the mannosylated peptides **1-20** entails the following stages; i) the solution phase synthesis of five propargylated mono-, di- and tri-mannosides (Scheme 1), ii) the automated solid phase synthesis of four oligopeptides containing one, two, three or six azide functions **26-29** (Table 1), iii) the assembly of the library of twenty mannosylated peptides by the solution phase conjugation of the propargylated mannosides and azide containing peptides as depicted in Figure 2.

Table 1: Peptide sequence of azidolysine-$DEVA_5K$ peptides **26-29**.

Compound		Peptide Sequence
26	Az_1DEVA_5K	
27	Az_2DEVA_5K	
28	Az_3DEVA_5K	
29	Az_6DEVA_5K	

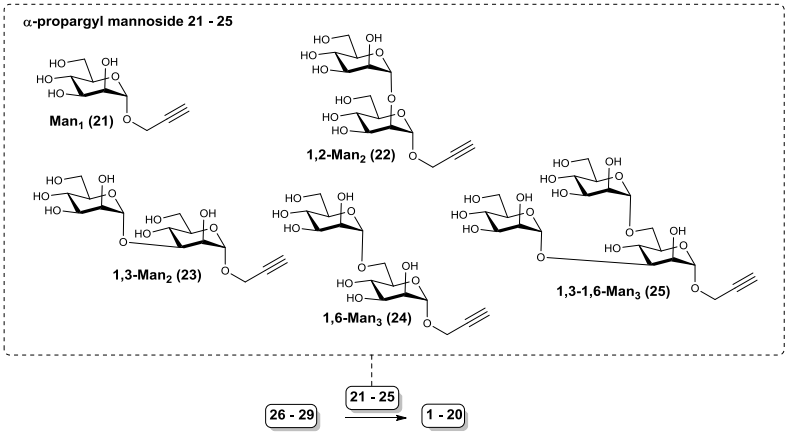
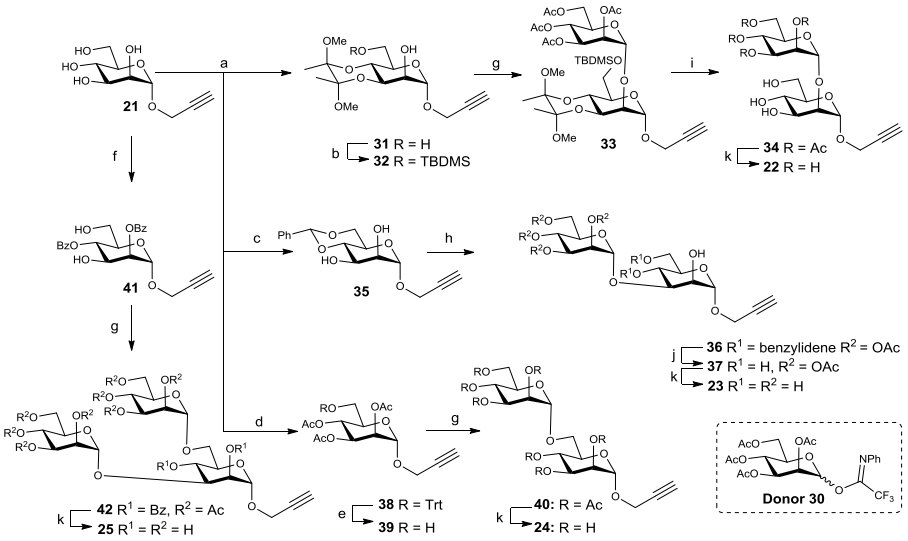


Figure 2: Peptide conjugate assembly.

Propargyl oligomannoside synthesis

The propargyl mannopyranosides **21-25** were prepared using propargyl α-D-mannopyranoside **21**[23] as starting compound (Scheme 1). In order to keep the anomeric alkyne moiety in the mannosides intact reductive transformations were avoided and acid/base labile protective groups were applied. En route to α-linked 1,2-di-mannoside (1,2-Man_2) **(22)** the equatorial hydroxyl functions in propargyl α–D-mannopyranose **21** were selectively masked with a 1,2-butane diacetal moiety to give **31** in 66% yield. Subsequent silylation of the primary alcohol in **31** using TBDMS chloride and imidazole gave acceptor **32** in quantitative yield. Condensation of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate **30** and acceptor **32** under influence of TfOH provided fully protected α-(1-2) linked dimer **33** in 71% yield. Both the butane diacetal and the silyl ether in dimer **33** were removed by treatment with 90% TFA. Cleavage of the glycosidic bonds could not be detected under these acidic conditions. Subsequently dimer **34** was globally deacetylated under standard Zémplen conditions providing 1,2-Man_2 **22** in 58% yield over two steps.

Scheme 1: Synthesis of α-propargyl (oligo)mannosides **21**-**25**.



Reagents and conditions: (a) 2,3-butanedione, $HC(OMe)_3$, MeOH, CSA, reflux, 66%; (b) TBDMSCl, imidazole, DMF, quantitative; (c) $PhCH(OMe)_2$, CSA, DMF, 200 mBar, 60 °C 55%; (d) *i*. TrtCl, DABCO, DCM; *ii*. Ac_2O, DMAP, 97%; (e) $BF_3{\cdot}Et_2O$, MeOH/toluene (1:1) 70%; (f) *i*. $PhC(OMe)_3$, CSA, MeCN; *ii*. H_2O, 52%; (g) Donor **30**, TfOH, DCM, activated molecular sieves, -25 to 0 °C, (**33**: 82%, **40**: 79%, **42**: 82%); (h) Donor **30**, TMSOTf, DCM, activated molecular sieves, -20 to 0 °C, 80%; (i) TFA/H_2O (9:1), 71%; (j) 70% AcOH (aq.), 55 °C, 84%; (k) NaOMe, MeOH, (**22**: 82%, **23**: 84%, **24**: 66%, **25**: 75%).

To obtain α-linked 1,3-di-mannoside (1,3-Man_2) **23**, imidate donor **30** and propargyl 4,6-*O*-benzylidene mannopyranose **35** were coupled in a regio and stereoselective fashion to give target dimer **36** in 80% yield together with the corresponding mannose trimer as minor by-product. Acidolysis of the benzylidene acetal and NaOMe mediated removal of the acetyl groups provided 1,3-Man_2 **23**.

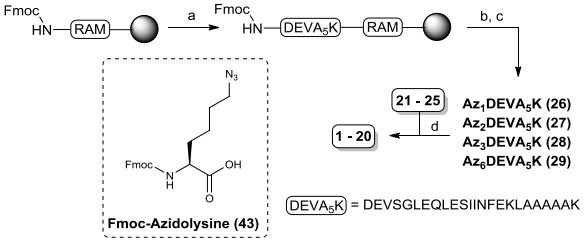
Acceptor **39**, needed to obtain α-linked 1,6-di-mannoside (1,6-Man_2) **24**, was synthesized by a two-step one-pot tritylation/acetylation procedure[24] followed by detritylation. Ensuing coupling with mannopyranosyl trichloroacetimidate **30** led to the isolation of fully acylated 1,6-Man_2 dimer **40** in 79%. Deprotection using NaOMe in MeOH gave 1,6-Man_2 **24** in 66% yield.

Propargyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (1,3-1,6-Man_3) **25** was obtained by double mannosylation of diol acceptor **41**. Treatment of propargyl α–D-mannopyranose **21** with trimethyl orthobenzoate in presence of a catalytic amount of CSA followed by *in situ* hydrolysis of the formed orthoesters delivered after purification 2,4-di-*O*-benzoylated mannose **41** in 52% yield and 2,6-di-*O*-benzoylated mannose in 35% yield. The 2,4-di-*O*-benzoylated acceptor **41** was coupled with imidate donor **30** giving the peracylated mannose trimer **42** in 82% yield. Subsequently the trimer was saponificated under standard Zémplen conditions providing 1,3-1,6-Man_3 **25** in 75%. With all the propargyl mannosides in hand attention was directed to the solid phase synthesis of the azide containing oligopeptides (**26**-**29**, Figure 2).

Peptide synthesis

The synthesis of the oligopeptides was carried out with an automated peptide synthesizer using standard Fmoc-chemisty (Scheme 2). Tentagel Rink amide was used as solid support together with commercially available Fmoc-protected amino acids and HCTU as condensing agent. Fmoc-protected azido lysine **43**[25] was prepared using a reported procedure. The four target oligopeptides **26**-**29**, contain one, two, three or six azide functions at the N-terminal end of the common DEVSGLEQLESIINFEKLAAAAAK ($DEVA_5K$) sequence. After construction of the immobilized protected $DEVA_5K$ elongation with azido lysine was undertaken. Incorporation of one azido lysine gave oligopeptide **26** and incorporation of six azido lysines resulted in oligopeptide **29**. The remaining immobilized oligopeptide **27** and **28** having two or three azide lysine residues were obtained by alternating coupling of azido lysine and glycine.

Scheme 2: Synthesis of mannosyl-DEVA$_5$K peptide conjugates **1-20**



Reagens and conditions: (a) SPPS using repetitive cycle of: *i*. 20% piperidine/DMF; *ii*. Fmoc-AA-OH, HCTU, DiPEA, NMP; *iii*. Ac_2O, DiPEA, HOBt, NMP; (b) SPPS using repetitive cycle of: *i*. 20% piperidine/DMF; *ii*. Fmoc-azidolysine **43** or glycine, HCTU, DiPEA, NMP; *iii*. Ac_2O, DiPEA, HOBt, NMP; (c) *i*. 95%TFA, 2.5% H_2O, 2.5% TIS. *ii*. RP-HPLC purification; (d) 0.05 M propargyl mannose sugar (aq.), 0.05 M sodium ascorbate (aq.), 0.05 M $CuSO_4$ (aq.), rt.

All protected immobilized peptides were cleaved from the solid support by treatment with 95% TFA and purified by RP-HPLC to yield Az_1DEVA_5K **26**, Az_2DEVA_5K **27**, Az_3DEVA_5K **28** and Az_6DEVA_5K **29**. With the peptide backbones in hand the peptides were subjected to conjugation with the set of propargyl mannosides.

Propargyl mannosides Man_1 (**21**), 1,2-Man_2 (**22**), 1,3-Man_2 (**23**), 1,6-Man_2 (**24**) and 1,3-1,6-Man_3 (**25)** were conjugated to oligopeptides Az_1DEVA_5K (**26),** Az_2DEVA_5K (**27**), Az_3DEVA_5K (**28**) and Az_6DEVA_5K (**29**) by Cu-catalyzed azide-alkyne cycloaddition. In this reaction 1 equivalent of $CuSO_4$ and (n + 2) equivalents of both the propargyl mannoside and sodium ascorbate with respect to the number of azides in the oligopeptide were used and each reaction was monitored by LCMS. After completion of the reaction, the mixture was subjected to gel filtration (HW-40) to remove compounds of low molecular weight. The retention time of the target mannosylated peptides prove to be shorter than expected and Cu-cluster formation of the products was assumed. Subsequent HPLC purification gave the mannosylated peptides conjugated with Man_1 **1-4**, 1,2-Man_2 **5-8** or 1,3-1,6-Man_3 **17-20** in relatively low yields (Table 1). In an alternative procedure HW-40 gel filtration of the crude cyclo-addition products was replaced by treatment with Cu-ion exchange resin CupriSorb^{TM} prior to HPLC purification.[26] This procedure led

to the isolation of a set of mannosylated peptides in an increased yield (Table 1).

Table 2: Results assembly of mannosyl-DEVA$_5$K conjugates **1-20**.

Peptide	Man$_1$[a]	1,2-Man$_2$[a]	1,3-Man$_2$[b]	1,6-Man$_2$[b]	1,3-1,6-Man$_3$[a]
26	**(1)** 13%	**(5)** 9%	**(9)** 25%	**(13)** 48%	**(17)** 12%
27	**(2)** 5%	**(6)** 8%	**(10)** 17%	**(14)** 22%	**(18)** 6%
28	**(3)** 7%	**(7)** 8%	**(11)** 20%	**(15)** 14%	**(19)** 5%
29	**(4)** 7%	**(8)** 14%	**(12)** 43%	**(16)** 35%	**(20)** 12%

[a)] Size-exclusion followed by HPLC purification. [b)] Treated with CupriSorb™ followed by HPLC purification.

Preliminary biological results

Immunological evaluation

In order to study whether the different glycan glycosylation pattern lead to different cross-presentation, the conjugates (**1-20**) were analysed for their ability to activate CD8+ T-cells, which reflects the degree of antigen presentation. Mouse bone marrow dendritic cells (BM-DCs) were incubated for 3h at 37 ^{0}C with an aliquot of each conjugate.[27] After this incubation the DCs were washed and the B3Z T-cell hybridoma were added.[28] These T-cells express the B3 T-cell receptor, which specifically recognises the peptide SIINFEKL presented in the H2-K^b subtype of MHC-I. Upon recognition of this peptide MHC-I complex, the B3Z is activated resulting in IL-2 production, which can be quantified. To allow for spectrophotometric activation of the T-cells, expression of a β-galactosidase reporter protein has been coupled to the IL-2 promotor. The expressed β-Gal can be quantified using chlorophenol β-D-galactopyranoside (CPRG) as β-galactosidase

substrate, that delivers a red colour upon hydrolysis by the enzyme.[29] The T-cell responses to conjugate **1**-**20** are plotted in Figure 4.

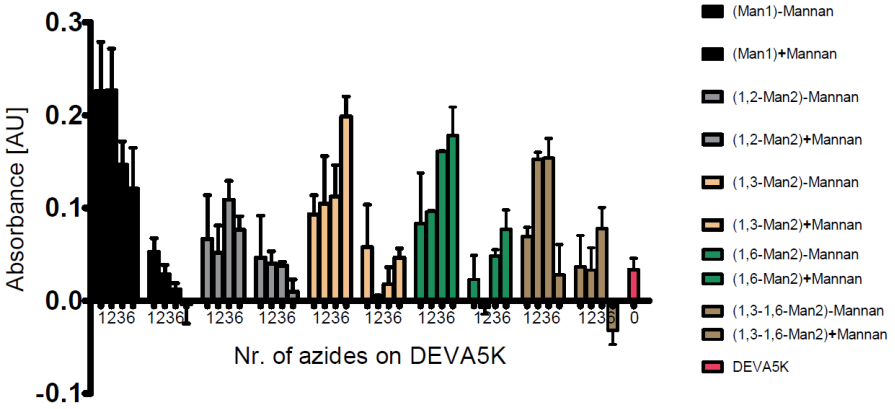


Figure 3: T-cell response of mannosyl-DEVA$_5$K conjugates **1**-**20**.

As shown in Figure 4 monomannoside conjugate $(Man_1)_1$ **1** and $(Man_1)_2$ **2** provided with one or two monomannosides showed the highest T cell response compared to the other constructs. Increasing the number of monomannose copies (Man_1) to three $(Man_1)_3$ **3** or six $(Man_1)_6$ **4** led to a decrease of T cell response. Surprisingly, the highest mannosylated conjugate $(1,3\text{-}1,6\text{-}Man_3)_6$ **20**, having six copies of the trimannoside gave the lowest T cell response of all. Conjugates provided with dimannosides (1,2-Man_2, 1,3-Man_2, 1,6-Man_2) **5**-**16** and in particular (1,2-Man_2) **5**-**8** showed a lower T cell response in comparison with the monomannoside conjugates (Man_1). Whereas increasing the number of monomannosides $(Man_1)_x$ to three (x=3) or six (x=6) copies led to a lower T cell response, an opposite trend was found for dimannosides $(1,3\text{-}Man_2)_x$ **9**-**12** and $(1,6\text{-}Man_2)_x$ **13**-**16**. By increasing the number of dimannosides (x= 2, 3, 6) an increase in T cell response was observed. Varying the number of dimannosides in the conjugate $(1,2\text{-}Man_2)_x$ did not show a significant difference in T cell response. The T cell response of the conjugates having a trimannosides $(1,3\text{-}1,6\text{-}Man_3)_x$ showed the highest T cell response with two (x=2, **18**) or three (x=3, **19**) copies. Surprisingly, T cell activation of the conjugate $(1,3\text{-}1,6\text{-}man_3)_x$ was completely diminished by the presence of

six trimannosides (x = 6, **20**). To assess the involvement of the mannose receptor in the uptake of the conjugates analogous experiments were performed in the presence of mannan, to block mannose receptor mediated uptake. Incubation in the presence of mannan reduced the T cell response to a level close to parent peptide $DEVA_5K$. This strongly indicates a mannose receptor mediated uptake of the mannosylated peptide conjugates. As control, DCs were incubated with all the unconjugated azido peptides (**26-29**) and with the short peptide $DEVA_5K$. All peptides lacking mannose sugars showed similar T cell response as the $DEVA_5K$ peptide (data not shown). Furthermore the presence of mannan did not affect the response of the unconjugated peptides indicating a mannose receptor independent mediated uptake of the control peptides.

Conclusion

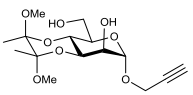
In this chapter the synthesis and preliminary immunological evaluation of a set of twenty mannosylated peptide conjugates is described. A mono-mannoside, tri di-mannosides and one tri-mannoside (**21-25**) equipped with an alkyne were synthesized uneventfully on mmole scale. The propargylated (oligo)mannosides were conjugated to MHC-class-I epitope DEVSGLEQLESIINFEKLAAAAAK provided with one, two, three or six azido lysines via Cu(I) catalysed click chemistry. The isolation procedure and hence the yield of the conjugates could be considerably improved by the application of CupriSorb^{TM} prior before HPLC purification. All mannosylated conjugates showed an increase in T cell response in comparison with a parent peptide. Reduction of T cell response of the conjugates in the presence of mannan to the level of the parent peptide is an indication of mannose receptor mediated uptake. The monomannoside conjugate $(Man_1)_1$ **1** and $(Man_1)_2$ **2** provided with one or two mono-mannosides showed the highest T cell response compared to the other constructs. The relation of the structure of the mannosylated conjugates and the degree of T cell response is rather

unforeseen. The effectiveness of both the mannose receptor mediated uptake and the subsequent processing of the conjugates are the main issues that contribute to the degree of T cell response. Both a low mannose mediated uptake of a specific conjugate and/or a reduced proteolytic susceptibility and processability may explain a decreased T cell response.

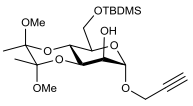
Experimental

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. Propargyl alcohol was distilled over K_2CO_3 prior to use. 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 Sephadex LH20 (eluent DCM/MeOH, 1:1). TLC analysis was conducted on HPTLC aluminium sheet (Merck, TLC silica gel 60, F_{254}). Copper was removed using CupriSorb™ (Seachem) chelating resin. 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, ^{13}C and 2D-HMBC (^1J-coupling) 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, 5 µm 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.



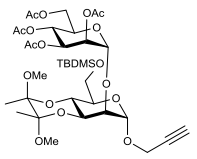
Propargyl 3-*O*,4-*O*-(2′,3′-Dimethoxybutan-2′,3′-diyl)-α-D-mannopyranoside (31): To a solution of propargyl mannose **21** (2.2 g, 10 mmol) in MeOH (100 mL) was added butane-2,3-dione (1.1 mL, 12 mmol), trimethyl orthoformate (6 mL, 55 mmol), CSA (0.232 g, 1 mmol) and the reaction mixture was refluxed overnight. The reaction mixture was cooled to rt., neutralized with Et_3N, concentrated *in vacuo* and directly purified by column chromatography without further aqueous workup. Purification by column chromatography yielded butane-2,3-acetal protected mannose **31** as a colourless amorphous solid (2.2 g, 6.6 mmol, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.06 (s, 1H), 4.24 (d, *J* = 2.4 Hz, 2H), 4.13 (t, *J* = 9.8 Hz, 1H), 4.03 (dd, *J* = 10.3, 3.1 Hz, 1H), 3.96 (s, 1H), 3.87 – 3.74 (m, 3H), 3.28 (s, 3H), 3.27 (s, 3H), 2.46 (t, *J* = 2.4 Hz, 1H), 1.33 (s, 3H), 1.29 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 100.5, 100.0, 98.7, 78.8, 75.1, 71.3, 69.6, 68.1, 62.9, 61.2, 54.5, 48.3, 48.0, 17.9. HRMS: $[M+H]^+$ calculated for $C_{15}H_{25}O_8$ 333.15439, found 333.15444.



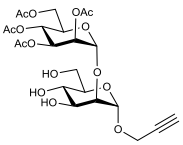
Propargyl 3-*O*,4-*O*-(2′,3′-Dimethoxybutan-2′,3′-diyl)-6-*O*-*tert*butyldimethylsilyl-α-D-mannopyranoside (32): To a solution of butane-2,3-acetal protected mannose **31** (332 mg, 1,0 mmol) in DMF (10 mL) was added TBDMSCl (0.19 g, 1.25 mmol) and the mixture was cooled to 0 °C. To the mixture was added imidazole (85 mg, 1.25 mmol) and the reaction mixture was allowed to warm to rt. After complete conversion of the starting material the reaction was quenched with MeOH. The mixture was concentrated *in vacuo* and dissolved in Et_2O. The organic layer was washed with H_2O (3x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded mannose acceptor **32** as a colourless oil (473 mg, 1 mmol,

quantitative). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.98 (s, 1H), 4.20 (d, *J* = 2.4 Hz, 2H), 4.03 – 3.94 (m, 2H), 3.91 (s, 1H), 3.82 (dd, *J* = 11.3, 2.0 Hz, 1H), 3.77 (dd, *J* = 11.3, 5.0 Hz, 1H), 3.68 (ddt, *J* = 7.3, 4.8, 2.2 Hz, 1H), 3.25 (s, 3H), 3.22 (s, 3H), 2.48 (s, 1H), 2.40 (t, *J* = 2.4 Hz, 1H), 1.30 (s, 3H), 1.26 (s, 3H), 0.86 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 100.4, 99.9, 98.3, 79.0, 74.7, 72.1, 69.6, 68.4, 62.8, 61.6, 53.8, 48.2, 48.0, 25.9, 18.4, 17.9, 17.8, -5.0, -5.3. HRMS: $[M+H]^+$ calculated for $C_{21}H_{39}O_8Si$ 447.24087, found 447.24090.

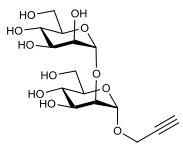
Propargyl 2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-3-*O*,4-*O*-(2′,3′-Dimethoxybutan-2′,3′-diyl)-6-*O*-*tert*butyldimethylsilyl-α-D-mannopyranoside (33): 2,3-Acetal protected mannose acceptor **32** (223 mg, 0.5 mmol) and trichloro imidate donor **30** (389 mg, 0.75 mmol) were dissolved in DCM (10 mL) and stirred over activated molecular sieves for 0.5h at rt. The mixture was cooled to -25 °C and TfOH (4.4 µl, 50 µmol) was added. The reaction mixture was allowed to gradually warm up and the reaction was quenched with Et_3N at 0 °C. The mixture was diluted with DCM and the organic layer was washed with H_2O. The aqueous layer was extracted with DCM (5x) and the combined organic layers was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by size-exclusion chromatography yielded dimer **33** as an colourless oil (320 mg, 0.41 mmol, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.43 (dd, *J* = 3.5, 1.9 Hz, 1H), 5.35 (dd, *J* = 10.0, 3.4 Hz, 1H), 5.24 (t, *J* = 9.9 Hz, 1H), 5.20 (d, *J* = 1.9 Hz, 1H), 4.97 (s, 1H), 4.23 (dd, *J* = 12.1, 5.0 Hz, 1H), 4.20 (d, *J* = 1.5 Hz, 2H), 4.13 – 4.07 (m, 1H), 4.09 – 4.01 (m, 1H), 4.01 – 3.97 (m, 2H), 3.91 (s, 1H), 3.84 (dd, *J* = 11.3, 2.0 Hz, 1H), 3.77 (dd, *J* = 11.3, 5.8 Hz, 1H), 3.71 – 3.61 (m, 1H), 3.22 (s, 3H), 3.20 (s, 3H), 2.41 (t, *J* = 2.4 Hz, 1H), 2.10 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H), 1.95 (s, 3H), 1.22 (s, 3H), 1.18 (s, 3H), 0.86 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 169.9, 169.6, 169.6, 100.1, 100.1, 99.6, 99.0, 97.3, 78.7, 75.8, 74.9, 72.8, 69.5, 69.1, 68.9, 68.5, 66.4, 63.1, 62.5, 61.8, 53.7, 48.2, 47.9, 25.9, 20.9, 20.9, 20.8, 20.8, 18.3, 17.7, 17.5,



-5.1, -5.3. HMBC: $^{1}J_{\alpha\text{-}1,2\text{-}man\ C\text{-}H}$ = 176 Hz. HRMS: $[M+H]^+$ calculated for $C_{35}H_{57}O_{17}Si$ 777.33595, found 777.33598.

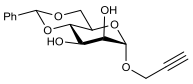


Propargyl 2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (34): Dimer **33** (311 mg, 0.4 mmol) was dissolved in TFA/H_2O (9:1, 12.3 mL) and stirred at rt. After 30 min. the reaction mixture was diluted with toluene and concentrated *in vacuo*. The crude was co-evaporated with toluene (3x) and directly purified by column chromatography without further aqueous workup. Purification by column chromatography yielded dimer **34** as a colourless amorphous solid (159 mg, 0.29 mmol, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.41 (dd, *J* = 3.3, 1.8 Hz, 1H), 5.31 (dd, *J* = 10.0, 3.3 Hz, 1H), 5.24 (d, *J* = 1.6 Hz, 1H), 5.20 (t, *J* = 10.0 Hz, 1H), 5.05 (d, *J* = 1.8 Hz, 1H), 4.29 (dd, *J* = 6.7, 1.6 Hz, 1H), 4.25 (s, 1H), 4.24 – 4.21 (m, 2H), 4.16 – 4.12 (m, 1H), 4.10 (d, *J* = 6.9 Hz, 1H), 3.99 – 3.87 (m, 3H), 3.83 (dd, *J* = 12.3, 3.0 Hz, 1H), 3.57 (dt, *J* = 9.7, 3.3 Hz, 1H), 3.37 (s, 3H), 2.49 (t, *J* = 2.4 Hz, 1H), 2.14 (s, 6H), 2.08 (s, 3H), 2.00 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 170.7, 170.1, 170.0, 99.6, 96.9, 80.3, 78.6, 75.3, 73.1, 70.7, 69.4, 69.2, 68.9, 67.4, 66.2, 63.1, 61.7, 54.5, 21.0, 20.9, 20.8, 20.8. HRMS: $[M+H]^+$ calculated for $C_{23}H_{33}O_{15}$ 549.18140, found 549.18138.

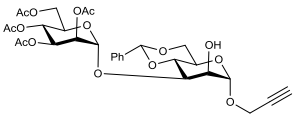


Propargyl 2-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (22): To a solution of mannose dimer **34** (137 mg, 0.25 mmol) in MeOH (2.5 mL) was added NaOMe (1.3 mg, 0.025 mmol) and the mixture was stirred at room temperature. After 4h the reaction was quenched with Amberlite IR-120 H^+ till pH ≤7 and the solids were filtered. The filtrate was concentrated *in vacuo* and the crude was dissolved in methanol. The solution was added dropwise to vigorously stirred cold Et_2O. The formed precipitate was filtered, rinsed with cold Et_2O and dried over a stream of air yielding 1,2-Man_2 dimer **22** as a white powder (78 mg, 0.21 mmol, 82%). ^{1}H NMR (400 MHz, MeOD) δ 5.20 (d, *J* = 1.7 Hz, 1H), 4.98 (d, *J* = 1.8 Hz, 1H), 4.26 (dd, *J* = 4.3, 2.4 Hz, 2H), 3.99

(dd, *J* = 3.1, 1.7 Hz, 1H), 3.88 – 3.76 (m, 4H), 3.73 (t, *J* = 4.2 Hz, 1H), 3.73 – 3.57 (m, 5H), 3.50 (ddd, *J* = 8.9, 5.5, 1.9 Hz, 1H), 2.86 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 104.2, 98.6, 80.3, 80.1, 76.0, 75.0, 74.8, 72.4, 72.0, 71.8, 68.7, 68.4, 62.8, 62.7, 55.1. HRMS: $[M+H]^+$ calculated for $C_{15}H_{25}O_{11}$ 381.13914, found 381.13911.

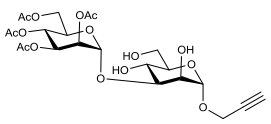


Propargyl 4,6-*O*-benzylidene-α-D-mannopyranoside (35): To a solution of propargyl mannose **21** (436 mg, 2 mmol) in MeCN (10 mL) was added benzaldehyde dimethyl acetal (0.36 mL, 2.4 mmol), trichlorotriazine (0.1 g, 0.6 mmol) and sonicated at 60 °C. After complete conversion of the starting material the reaction mixture was concentrated *in vacuo*. Purification by column chromatography yielded compound **35** as a white amorphous solid (330 mg, 1.1 mmol, 55%). ^{1}H NMR (400 MHz, MeOD) δ 7.50 (dd, *J* = 6.7, 3.1 Hz, 2H), 7.34 (dd, *J* = 5.1, 2.0 Hz, 3H), 5.60 (s, 1H), 4.99 (d, *J* = 1.3 Hz, 1H), 4.29 (dd, *J* = 2.4, 0.9 Hz, 2H), 4.21 (ddd, *J* = 9.3, 4.1, 1.5 Hz, 1H), 4.00 – 3.89 (m, 2H), 3.92 – 3.85 (m, 1H), 3.84 – 3.77 (m, 1H), 3.75 (dd, *J* = 10.3, 4.0 Hz, 1H), 2.91 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 139.2, 129.9, 129.0, 127.5, 103.3, 101.1, 80.0, 76.2, 72.4, 69.6, 69.5, 65.6, 55.2. HRMS: $[M+H]^+$ calculated for $C_{16}H_{19}O_6$ 307.11761, found 307.11763.



Propargyl 3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-4,6-*O*-benzylidene-α-D-mannopyranoside (36): Benzylidene acceptor **35** (153 mg, 0.5 mmol) and trichloro imidate donor **30** (271 mg, 0.55 mmol) were dissolved in DCM (5 mL) and stirred over activated molecular sieves for 0.5 h at rt. The mixture was cooled to -20 °C and TMSOTf (4.5 µl, 25 µmol) was added. The reaction was stirred for 2 h at -20 °C and quenched with Et_3N (0.1 mL) at that temperature. The mixture was filtered and concentrated *in vacuo*. Purification by size-exclusion yielded dimer **36** as a colourless amorphous foam (253 mg, 0.4 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.40 (m, 2H), 7.33 (dd, *J* = 5.0, 1.9 Hz, 3H), 5.59 (s, 1H), 5.39 (dd, *J* =

3.5, 1.8 Hz, 1H), 5.34 (dd, *J* = 9.8, 3.5 Hz, 1H), 5.27 (d, *J* = 1.8 Hz, 1H), 5.22 (t, *J* = 9.9 Hz, 1H), 5.09 (d, *J* = 1.5 Hz, 1H), 4.28 (d, *J* = 5.3 Hz, 1H), 4.26 (d, *J* = 2.5 Hz, 2H), 4.25 – 4.19 (m, 1H), 4.20 – 4.13 (m, 2H), 4.13 – 4.04 (m, 2H), 3.86 (d, *J* = 9.6 Hz, 1H), 3.85 (d, *J* = 9.8 Hz, 1H), 2.46 (t, *J* = 2.4 Hz, 1H), 2.11 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 1.99 (s, 3H), 1.60 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 170.2, 169.8, 169.7, 137.2, 128.9, 128.2, 126.1, 101.5, 99.0, 98.5, 78.5, 78.3, 75.3, 73.7, 70.9, 69.2, 69.1, 69.0, 68.6, 66.4, 64.1, 62.8, 54.6, 20.8, 20.8. HMBC: $^{1}J_{\alpha\text{-}1,3\text{-}man\ C\text{-}H}$ = 176 Hz. HRMS: $[M+H]^+$ calculated for $C_{30}H_{37}O_{15}$ 637.21270, found 637.21264.

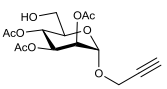


Propargyl 3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (37): Dimer **36** (0.172 g, 0.27 mmol) was dissolved in 70% AcOH (aq.) (2.7 mL) and stirred for 4h at 55 °C. The mixture was cooled to rt, diluted with H_2O and transferred to a separation funnel. To the solution was added solid $NaHCO_3$ till neutral pH after which the aqueous layer was extracted with DCM (5x). The combined organic layers was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded dimer **37** as a colourless oil (0.124 g, 0.23 mmol, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.47 – 5.36 (m, 2H), 5.30 – 5.20 (m, 2H), 5.04 (s, 1H), 4.31 – 4.18 (m, 4H), 4.13 (d, *J* = 10.3 Hz, 1H), 4.06 (s, 1H), 3.99 (d, *J* = 13.5 Hz, 1H), 3.94 (d, *J* = 11.5 Hz, 1H), 3.84 (d, *J* = 11.8 Hz, 1H), 3.62 (d, *J* = 9.5 Hz, 1H), 3.11 (s, 4H), 2.41 (t, *J* = 2.1 Hz, 1H), 2.15 (s, 3H), 2.11 (s, 3H), 2.07 (s, 3H), 2.01 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 170.9, 170.7, 169.9, 99.5, 98.6, 79.5, 78.8, 75.0, 73.0, 70.7, 69.7, 69.5, 69.1, 66.3, 65.6, 62.9, 61.4, 54.6, 21.1, 21.0, 20.9. HRMS: $[M+H]^+$ calculated for $C_{23}H_{33}O_{15}$ 549.18140, found 549.18142.

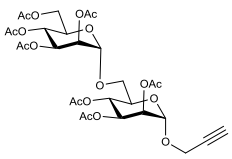
Propargyl 3-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (23): To a solution of mannose dimer **37** (91.1 mg, 0.17 mmol) in MeOH (1.7 mL) was added NaOMe (0.9 mg, 17 µmol) and the mixture was stirred at room temperature. After 6h the reaction was quenched with Amberlite IR-120 H^+ till pH ≤7 and the solids were filtered. The filtrate was concentrated *in vacuo* and the crude was dissolved in methanol. The solution was added drop wise to vigorously stirred cold Et_2O. The product precipitated and the formed solids were filtered, rinsed with cold Et_2O and dried over a stream of air yielding dimer **23** as a white powder (61 mg, 0.16 mmol, 95%). ^{1}H NMR (400 MHz, MeOD) δ 5.07 (d, *J* = 1.7 Hz, 1H), 4.95 (d, *J* = 1.8 Hz, 1H), 4.28 (d, *J* = 2.4 Hz, 2H), 4.04 (dd, *J* = 2.9, 1.9 Hz, 1H), 3.97 (dd, *J* = 3.4, 1.8 Hz, 1H), 3.85 (t, *J* = 2.4 Hz, 1H), 3.82 (d, *J* = 2.3 Hz, 1H), 3.81 – 3.75 (m, 4H), 3.73 (dd, *J* = 5.8, 2.4 Hz, 1H), 3.69 (dd, *J* = 5.8, 1.8 Hz, 1H), 3.62 (t, *J* = 9.5 Hz, 1H), 3.55 (ddd, *J* = 6.7, 3.8, 3.0 Hz, 1H), 2.87 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 103.9, 99.9, 80.5, 79.9, 76.1, 75.3, 74.9, 72.4, 72.1, 71.3, 68.7, 67.4, 62.8, 62.7, 54.8. HRMS: $[M+H]^+$ calculated for $C_{15}H_{25}O_{11}$ 381.13914, found 381.13915.

Propargyl 2,3,4-tri-*O*-acetyl-6-*O*-trityl-α-D-mannopyranoside (38): To a solution of propargyl mannose **21** (1.6 g, 7.5 mmol) in pyridine (30 mL) was added trityl chloride (2.6 g, 9.4 mmol) and the mixture was stirred till complete conversion of the starting material. The reaction mixture was cooled to 0 °C and Ac_2O (3.2 mL, 33.8 mmol) was added dropwise. The reaction mixture was allowed to warm to rt and the reaction was stirred overnight at rt. The mixture was cooled to 0 °C and the reaction was quenched with MeOH. Solvents were removed *in vacuo* and the crude was dissolved in EtOAc. The organic layer was washed with 1M HCl (aq.) (1x), sat. $NaHCO_3$ (aq.) (1x), H_2O (3x), brine (3x), dried over $MgSO_4$, filtered and concentrated *in vacuo* yielding propargyl mannose **38** in qunantitative yield. The crude trityl mannose **38** was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, *J* = 7.2

Hz, 6H), 7.29 (t, *J* = 7.4 Hz, 6H), 7.22 (t, *J* = 7.2 Hz, 3H), 5.31 (d, *J* = 2.4 Hz, 1H), 5.30 – 5.26 (m, 2H), 5.08 (d, *J* = 1.6 Hz, 1H), 4.33 (d, *J* = 1.0 Hz, 2H), 3.90 (tt, *J* = 5.6, 2.9 Hz, 1H), 3.23 (dd, *J* = 10.3, 2.6 Hz, 1H), 3.18 (dd, *J* = 10.3, 5.1 Hz, 1H), 2.47 (t, *J* = 2.3 Hz, 1H), 2.17 (s, 3H), 1.96 (s, 3H), 1.73 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 170.0, 169.4, 143.8, 128.8, 127.9, 127.1, 95.8, 86.7, 78.3, 75.5, 70.7, 69.7, 69.3, 66.6, 62.4, 54.5, 21.0, 20.8, 20.6. HRMS: $[M+H]^+$ calculated for $C_{34}H_{35}O_9$ 587.22756, found 587.22762.



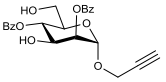
Propargyl 2,3,4-tri-*O*-acetyl-α-D-mannopyranoside (39): To a solution of trityl mannose **38** (4.4 g, 7.5 mmol) in a MeOH/toluene mixture (1:1, 75 mL) was added dropwise $BF_3{\cdot}Et_2O$ (1.0 mL, 8.25 mmol) and stirred till complete conversion of the starting material. The reaction mixture was diluted with EtOAc and washed with H_2O (3x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded propargyl mannose acceptor **39** as a white amorphous solid (1.81 g, 5.25 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40 (dd, *J* = 10.2, 3.4 Hz, 1H), 5.29 (dd, *J* = 3.6, 1.8 Hz, 1H), 5.26 (t, *J* = 10.2 Hz, 1H), 5.05 (d, *J* = 1.8 Hz, 1H), 4.28 (d, *J* = 2.4 Hz, 2H), 3.82 (ddd, *J* = 10.1, 4.1, 2.3 Hz, 1H), 3.72 (ddd, *J* = 12.8, 8.5, 2.3 Hz, 1H), 3.63 (ddd, *J* = 12.8, 5.6, 4.1 Hz, 1H), 2.47 (t, *J* = 2.4 Hz, 1H), 2.40 (dd, *J* = 8.5, 5.7 Hz, 1H), 2.16 (s, 3H), 2.08 (s, 3H), 2.01 (s, 3H), 1.66 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 170.1, 170.0, 96.5, 78.2, 75.7, 71.3, 69.5, 68.8, 66.5, 61.3, 55.1, 21.0, 20.9, 20.8. HRMS: $[M+H]^+$ calculated for $C_{15}H_{21}O_9$ 345.11801, found 345.11804.



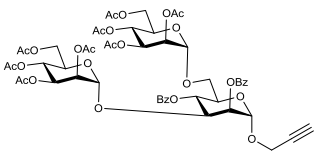
Propargyl 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (40): Mannose acceptor **39** (0.479 g, 0.156 mmol) and trichloro imidate donor **30** (0.373 g, 0.72 mmol) were dissolved in DCM (9.6 mL) and stirred over activated molecular sieves for 0.5h at rt. The mixture was cooled to -25 °C and TfOH (13 µl, 0.144 µmol) was added. The reaction mixture was allowed to gradually warm up and the

reaction was quenched with Et_3N at 0 °C. The mixture was diluted with DCM and the organic layer was washed with H_2O. The aqueous layer was extracted with DCM (5x) and the combined organic layers was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by size-exclusion yielded dimer **40** as an colourless oil (0.257 g, 0.38 mmol, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.37 – 5.31 (m, 3H), 5.30 – 5.26 (m, 3H), 5.02 (d, *J* = 1.7 Hz, 1H), 4.86 (d, *J* = 1.7 Hz, 1H), 4.30 (d, *J* = 2.4 Hz, 2H), 4.27 (dd, *J* = 12.2, 5.2 Hz, 1H), 4.13 (dd, *J* = 12.3, 2.4 Hz, 1H), 4.11 – 4.04 (m, 1H), 3.99 (ddd, *J* = 10.1, 5.7, 2.4 Hz, 1H), 3.79 (dd, *J* = 11.0, 5.7 Hz, 1H), 3.59 (dd, *J* = 11.0, 2.5 Hz, 1H), 2.52 (t, *J* = 2.4 Hz, 1H), 2.17 (s, 3H), 2.16 (s, 3H), 2.12 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 170.2, 170.1, 170.0, 169.9, 169.9, 169.9, 97.6, 96.1, 78.1, 75.7, 69.9, 69.4, 69.4, 69.1, 69.1, 68.7, 66.7, 66.5, 66.0, 62.5, 55.0, 21.0, 20.9, 20.9, 20.8, 20.8, 20.8. HMBC: $^1J_{\alpha\text{-}1,6\text{-man C-H}}$ = 173 Hz. HRMS: $[M+H]^+$ calculated for $C_{29}H_{40}O_{18}$ 675.21309, found 675.21300.

Propargyl 6-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (24): To a solution of mannose dimer **40** (0.257 g, 0.38 mmol) in MeOH (3.8 mL) was added NaOMe (5 mg, 0.095 mmol) and the mixture was stirred at room temperature. After 1h the reaction was quenched with Amberlite IR-120 H^+ till pH ≤7 and the solids were filtered. The filtrate was concentrated *in vacuo* and the crude was dissolved in methanol. The solution was added dropwise to vigorously stirred cold Et_2O. The product precipitated and the formed solids were filtered, rinsed with cold Et_2O and dried over a stream of air yielding 1,6-Man_2 dimer **24** as a white powder (94 mg, 0.25 mmol, 66%). ^{1}H NMR (400 MHz, MeOD) δ 4.93 (d, *J* = 1.6 Hz, 1H), 4.84 (d, *J* = 1.8 Hz, 1H), 4.25 (t, *J* = 2.2 Hz, 2H), 3.92 (dd, *J* = 11.0, 5.2 Hz, 1H), 3.87 (d, *J* = 2.8 Hz, 1H), 3.83 (d, *J* = 11.9 Hz, 2H), 3.78 – 3.69 (m, 3H), 3.67 (d, *J* = 3.4 Hz, 2H), 3.67 – 3.61 (m, 3H), 2.88 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 101.4, 100.0, 80.0, 76.1, 74.3, 73.5, 72.6, 72.0, 71.9, 68.5, 68.3, 67.2, 62.8, 54.9. HRMS: $[M+H]^+$ calculated for $C_{15}H_{25}O_{11}$ 381.13914, found 381.13913.

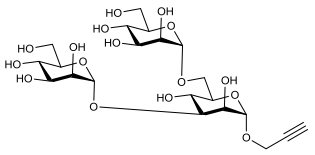


Propargyl 2,4-di-*O*-benzoyl-α-D-mannopyranoside (41): To a suspension of propargyl mannose **21** (0.218 g, 1.15 mmol) in MeCN (11.5 mL) was added $PhC(OMe)_3$ (0.6 mL, 3.5 mmol) and CSA (26 mg, 115 µmol) and the mixture was stirred rt. After 2h H_2O (0.5 mL) was added and the mixture was stirred overnight at rt. The reaction was mixture was neutralized wit Et_3N, concentrated *in vacuo* and co-evaporated wit toluene (3x). Purification by column chromatography yielded benzoylated mannose **41** as a white amorphous solid (254 mg, 0.6 mmol, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 – 8.03 (m, 4H), 7.65 – 7.53 (m, 2H), 7.51 – 7.41 (m, 3H), 5.52 (t, *J* = 9.9 Hz, 1H), 5.44 (dd, *J* = 3.5, 1.7 Hz, 1H), 5.24 (d, *J* = 1.7 Hz, 1H), 4.44 (dd, *J* = 9.9, 3.5 Hz, 1H), 4.32 (d, *J* = 2.5 Hz, 2H), 3.99 (ddd, *J* = 10.1, 4.2, 2.3 Hz, 1H), 3.81 (dd, *J* = 12.6, 2.4 Hz, 1H), 3.73 (dd, *J* = 12.6, 4.2 Hz, 1H), 2.51 (t, *J* = 2.4 Hz, 1H), 2.40 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4, 166.1, 133.8, 133.7, 130.1, 129.3, 129.1, 128.7, 128.7, 96.7, 78.4, 75.6, 72.8, 71.2, 70.3, 68.7, 61.5, 55.4. HRMS: $[M+H]^+$ calculated for $C_{23}H_{24}O_8$ 427.13874, found 427.13869.



Propargyl 2,4-di-*O*-benzoyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (42): Benzoylated mannose acceptor **41** (0.107 g, 0.25 mmol) and trichloro imidate **30** (390 mg, 0.75 mmol) were dissolved in DCM (5 mL) and stirred over activated molecular sieves for 0.5h at rt. The mixture was cooled to -25 °C and TfOH (4.4 µl, 50 µmol) was added. The reaction mixture was allowed to gradually warm up and the reaction was quenched with Et_3N at 0 °C. The mixture was diluted with DCM and the organic layer was washed with H_2O. The aqueous layer was extracted with DCM (5x) and the combined organic layers was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by size-exclusion yielded trimer **42** as an colourless oil (223 mg, 0.2 mmol, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (dd, *J* = 8.5, 1.5 Hz, 2H), 8.03 (dd, *J* = 8.5, 1.3 Hz, 2H), 7.67 – 7.50 (m, 4H), 7.45 (t, *J* = 7.8 Hz, 2H), 5.65 (t, *J* =

10.0 Hz, 1H), 5.55 (dd, *J* = 3.5, 1.8 Hz, 1H), 5.34 (dd, *J* = 10.1, 3.4 Hz, 1H), 5.26 (d, *J* = 10.1 Hz, 2H), 5.23 (dd, *J* = 3.4, 1.8 Hz, 1H), 5.12 – 5.07 (m, 2H), 4.98 (d, *J* = 1.8 Hz, 1H), 4.88 (dd, *J* = 2.8, 1.8 Hz, 1H), 4.81 (d, *J* = 1.7 Hz, 1H), 4.49 (dd, *J* = 9.8, 3.4 Hz, 1H), 4.37 (d, *J* = 2.4 Hz, 2H), 4.24 – 4.04 (m, 5H), 4.03 (t, *J* = 2.2 Hz, 1H), 4.00 (t, *J* = 2.3 Hz, 1H), 3.91 (dd, *J* = 10.8, 6.7 Hz, 1H), 3.62 (dd, *J* = 10.8, 2.2 Hz, 1H), 2.56 (t, *J* = 2.4 Hz, 1H), 2.14 (s, 3H), 2.12 (s, 3H), 2.05 (s, 3H), 1.98 (s, 3H), 1.94 (d, *J* = 0.9 Hz, 6H), 1.85 (s, 3H), 1.83 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 170.6, 170.0, 169.8, 169.7, 169.2, 169.1, 166.0, 165.3, 133.7, 133.7, 130.1, 130.0, 129.1, 128.9, 128.8, 128.6, 99.5, 97.3, 96.2, 78.1, 75.8, 75.0, 71.6, 70.1, 69.4, 69.3, 69.1, 68.8, 68.7, 68.3, 66.8, 66.1, 65.9, 62.4, 62.4, 55.1, 20.9, 20.8, 20.8, 20.7, 20.7, 20.5, 20.5. HRMS: $[M+H]^+$ calculated for $C_{51}H_{59}O_{26}$ 1087.32891, found 1087.32883.



Propargyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (25): To a solution of mannose trimer **42** (223 mg, 0.2 mmol) in MeOH (4 mL) was added NaOMe (5.4 mg, 0.1 mmol) and the mixture was stirred at room temperature. After 1h the reaction was quenched with Amberlite IR-120 H^+ untill pH ≤7 and the solids were filtered. The filtrate was concentrated *in vacuo* and the crude was dissolved in methanol. The solution was added dropwise to vigorously stirred cold Et_2O. The product precipitated and the formed solids were filtered, rinsed with cold Et_2O and dried over a stream of air yielding 1,3-1,6-Man_3 trimer **25** as a white powder (82 mg, 0.15 mmol, 75%). ^{1}H NMR (400 MHz, MeOD) δ 5.07 (d, *J* = 1.7 Hz, 1H), 4.92 (d, *J* = 1.8 Hz, 1H), 4.84 (d, *J* = 1.7 Hz, 1H), 4.26 (t, *J* = 2.4 Hz, 2H), 4.04 (dd, *J* = 2.9, 1.7 Hz, 1H), 3.99 (dd, *J* = 3.4, 1.7 Hz, 1H), 3.94 (dd, *J* = 11.1, 5.3 Hz, 1H), 3.88 (dd, *J* = 3.4, 1.7 Hz, 1H), 3.86 – 3.81 (m, 10H), 3.80 (t, *J* = 3.6 Hz, 1H), 3.78 – 3.71 (m, 2H), 3.71 – 3.68 (m, 1H), 3.66 – 3.61 (m, 2H), 2.89 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 104.0, 101.4, 100.1, 80.6, 79.9, 76.2, 74.9, 74.3, 73.8, 72.6, 72.4, 72.0, 72.0, 71.2, 68.7,

68.5, 67.2, 67.0, 62.8, 55.0. HRMS: $[M+H]^+$ calculated for $C_{21}H_{35}O_{16}$ 543.19196, found 543.19200.

General procedure peptide synthesis

The solid-phase peptide synthesis was performed on 100 µmol scale according to established methods on an ABI 433A (Applied Biosystems) automated instrument applying an Fmoc based protocol[30] starting from Tentagel-S-RAM resin (loading 0.23 mmol/g). The consecutive steps performed in each cycle for HCTU chemistry on µmol scale: 1) Deprotection of the Fmoc-group with 20% piperidine in NMP for 15 min; 2) NMP wash; 3) Coupling of the appropriate amino acid using a five-fold excess. Generally, the Fmoc amino acid (0.25 mmol) was dissolved in 0.25 M HCTU in NMP (1 mL), the resulting solution was transferred to the reaction vessel followed by 0.5 mL of 1.0 M DiPEA in NMP to initiate the coupling. The reaction vessel was shaken for 30 min; 4) NMP wash; 5) capping with 0.5 M acetic anhydride in NMP in presence of 0.5 mmol DiPEA; 6) NMP wash; 7) DCM wash.

H-Lys(N_3)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (26): Az_1DEVA_5K **26** (49.0 mg, 18.1 µmol, 18% based on theoretical loading of 0.23mmol/g). ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{118}H_{197}N_{33}O_{39}$ 1350.72, found 1350.73.

H-Lys(N_3)-Gly-Lys(N_3)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (27): Az_2DEVA_5K **27** (49.0 mg, 16.8 µmol, 17% based on theoretical loading of 0.23mmol/g). ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{126}H_{210}N_{38}O_{41}$ 1456.27, found 1456.27.

H-Lys(N_3)-Gly-Lys(N_3)-Gly-Lys(N_3)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (28): Az_3DEVA_5K **28** (31.1 mg, 10.0 µmol, 10% based on theoretical

loading of 0.23mmol/g). ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{134}H_{223}N_{43}O_{43}$ 1561.82, found 1561.87.

H-Lys(N_3)-Lys(N_3)-Lys(N_3)-Lys(N_3)-Lys(N_3)-Lys(N_3)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (29): Az_6DEVA_5K **29** (18.0 mg, 5.2 µmol, 5% based on theoretical loading of 0.23mmol/g). ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{148}H_{247}N_{53}O_{44}$ 1735.93, found 1735.93.

General procedure (oligo)mannoside conjugation

The peptide was dissolved in a 0.05M propargyl mannose sugar solution (n_{azide} + 2 eq.) (aq.). To the solution was added a 0.05M sodium ascorbate solution (n_{azide} + 2 eq.) (aq.) and a 0.05M $CuSO_4$ solution (2 eq.) (aq.) and the reaction was monitored by HPLC. After full conversion to the product, two workup procedures were performed prior before purification by RP-HPLC. Gel filtration: The reaction mixture was diluted with MiliQ and purified by gel filtration (HW-40). The combined frations were concentrated, centrifuged and the supernatant was purified by RP-HPLC. Resin purification: The reaction mixture was diluted with MiliQ water and CupriSorb™ (50 mg) was added to the reaction mixture for 2 in which the solution turned from light blue to colourless. The resin was filtered and rinsed with MiliQ water. The filtrate was centrifuged and the supernatant was directly purified by RP-HPLC.

H-Lys(Man_1)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (1): $(Man_1)_1DEVA_5K$ **1** was conjugated following the general procedure using: Az_1DEVA_5K (6.80 mg, 2.52 µmol), 0.05M propargyl mannose **21** sol. (151.2 µl, 7.56 µmol), 0.05M sodium ascorbate sol. (151.2 µl, 7.56 µmol) and 0.05M $CuSO_4$ (50.4 µl, 2.52 µmol). Gel filtration followed by RP-HPLC (linear gradient 20 → 40% MeCN in 12 min) followed by lyophilisation yielded $(Man_1)_1DEVA_5K$ **1** as a white powder (0.97 mg, 0.33 µmol, 13%). LC-MS analysis (linear gradient 10 → 90% MeCN in 12 min), t_R: 5.067 min, ESI/MS (*m/z*): $[M+2H]^{2+}$

calculated for $C_{127}H_{211}N_{33}O_{45}$ 1459.76, found 1459.73; $[M+3H]^{3+}$ calculated for $C_{127}H_{212}N_{33}O_{45}$ 973.50, found 973.80.

H-Lys(Man$_1$)-Gly-Lys(Man$_1$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (2): (Man$_1$)$_2$DEVA$_5$K **2** was conjugated following the general procedure using: Az$_2$DEVA$_5$K **27** (8.35 mg, 2.87 µmol), 0.05M propargyl mannose **21** sol. (229.6 µl, 11.48 µmol), 0.05M sodium ascorbate sol. (229.6 µl, 11.48 µmol) and 0.05M $CuSO_4$ (5.74 µl, 114.8 µmol). Gel filtration followed by RP-HPLC purification by RP-HPLC (linear gradient 20 → 45% MeCN in 12 min) followed by lyophilisation yielded (Man$_1$)$_2$DEVA$_5$K **2** as a white powder (0.50 mg, 0.15 µmol, 5%). LC-MS analysis (linear gradient 10 → 90% MeCN in 12 min), t_R: 5.033 min, ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{144}H_{238}N_{38}O_{53}$ 1673.35, found 1674.27; $[M+3H]^{3+}$ calculated for $C_{144}H_{239}N_{38}O_{53}$ 1116.57, found 1116.67.

H-Lys(Man$_1$)-Gly-Lys(Man$_1$)-Gly-Lys(Man$_1$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (3): (Man$_1$)$_3$DEVA$_5$K **3** was conjugated following the general procedure using: Az$_3$DEVA$_5$K **28** (4.85 mg, 1.55 µmol), 0.05M propargyl mannose **21** sol. (155.0 µl, 7.75 µmol), 0.05M sodium ascorbate sol. (155.0 µl, 7.75 µmol) and 0.05M $CuSO_4$ (4.65 µl, 93.0 µmol). Gel filtration followed by RP-HPLC (linear gradient 20 → 45% MeCN in 12 min) followed by lyophilisation yielded (Man$_1$)$_3$DEVA$_5$K **3** as a white powder (0.42 mg, 0.11 µmol, 7%). LC-MS analysis (linear gradient 10 → 90% MeCN in 12 min), t_R: 5.027 min, ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{161}H_{265}N_{43}O_{61}$ 1888.94, found 1889.13; $[M+3H]^{3+}$ calculated for $C_{161}H_{266}N_{43}O_{61}$ 1259.63, found 1259.53.

H-Lys(Man$_1$)-Lys(Man$_1$)-Lys(Man$_1$)-Lys(Man$_1$)-Lys(Man$_1$)-Lys(Man$_1$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (4): (Man$_1$)$_6$DEVA$_5$K **4** was conjugated following the general procedure using: Az$_6$DEVA$_5$K **29** (2.40 mg, 0.69 µmol),

0.05M propargyl mannose **21** sol. (110.4 µl, 5.52 µmol), 0.05M sodium ascorbate sol. (110.4 µl, 5.52 µmol) and 0.05M $CuSO_4$ (4.14 µl, 82.8 µmol). Gel filtration followed by RP-HPLC (linear gradient 20 → 45% MeCN in 12 min) followed by lyophilisation yielded $(Man_1)_6DEVA_5K$ **4** as a white powder (0.22 mg, 0.05 µmol, 7%). LC-MS analysis (linear gradient 10 → 90% MeCN in 12 min), t_R: 4.900 min, ESI/MS (*m/z*): $[M+3H]^{3+}$ calculated for $C_{202}H_{333}N_{53}O_{80}$ 1594.11, found 1594.20.

H-Lys(1,2-Man$_2$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (5): $(1,2\text{-}Man_2)_1DEVA_5K$ **5** was conjugated following the general procedure using: Az_1DEVA_5K **26** (9.06 mg, 3.36 µmol), 0.05M 1,2-Man_2 **22** sol. (201.6 µl, 10.08 µmol), 0.05M sodium ascorbate sol. (201.6 µl, 10.08 µmol) and 0.05M $CuSO_4$ (3.36 µl, 67.2 µmol). Gel filtration followed by RP-HPLC (linear gradient 20 → 45% MeCN in 12 min) followed by lyophilisation yielded $(1,2\text{-}Man_2)_1DEVA_5K$ **5** as a white powder (0.98 mg, 0.32 µmol, 9%). LC-MS analysis (linear gradient 10 → 90% MeCN in 12 min), t_R: 5.060 min, ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{133}H_{221}N_{33}O_{50}$ 1540.78, found 1540.80; $[M+3H]^{3+}$ $C_{133}H_{222}N_{33}O_{50}$ 1027.52, found 1027.40.

H-Lys(1,2-Man$_2$)-Gly-Lys(1,2-Man$_2$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (6): $(1,2\text{-}Man_2)_2DEVA_5K$ **6** was conjugated following the general procedure using: Az_2DEVA_5K **27** (8.41 mg, 2.89 µmol), 0.05M 1,2-Man_2 **22** solution (231.2 µl, 11.56 µmol), 0.05M sodium ascorbate sol. (231.2 µl, 11.56 µmol) and 0.05M $CuSO_4$ (5.78 µl, 115.6 µmol). Gel filtration followed by RP-HPLC (linear gradient 20 → 45% MeCN in 12 min) followed by lyophilisation yielded $(1,2\text{-}Man_2)_2DEVA_5K$ **6** as a white powder (0.83 mg, 0.23 µmol, 8%). LC-MS analysis (linear gradient 10 → 90% MeCN in 12 min), t_R: 4.990 min, ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{156}H_{258}N_{38}O_{63}$ 1836.40, found 1836.27; $[M+3H]^{3+}$ calculated for $C_{156}H_{259}N_{38}O_{63}$ 1224.60, found 1224.53.

H-Lys(1,2-Man$_2$)-Gly-Lys(1,2-Man$_2$)-Gly-Lys(1,2-Man$_2$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (7): (1,2-Man$_2$)$_3$DEVA$_5$K **7** was conjugated following the general procedure using: Az$_3$DEVA$_5$K **28** (5.37 mg, 1.72 µmol), 0.05M 1,2-Man$_2$ **22** sol. (172.0 µl, 8.60 µmol), 0.05M sodium ascorbate sol. (172.0 µl, 8.60 µmol) and 0.05M $CuSO_4$ (5.16 µl, 103.2 µmol). Gel filtration followed by RP-HPLC (linear gradient 20 → 45% MeCN in 12 min) followed by lyophilisation yielded (1,2-Man$_2$)$_3$DEVA$_5$K **7** as a white powder (0.55 mg, 0.13 µmol, 8%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 4.937 min, ESI/MS (*m/z*): $[M+3H]^{3+}$ calculated for $C_{179}H_{296}N_{43}O_{76}$ 1422.02, found 1422.07.

H-Lys(1,2-Man$_2$)-Lys(1,2-Man$_2$)-Lys(1,2-Man$_2$)-Lys(1,2-Man$_2$)-Lys(1,2-Man$_2$)-Lys(1,2-Man$_2$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (8): (1,2-Man$_2$)$_6$DEVA$_5$K **8** was conjugated following the general procedure using: Az$_6$DEVA$_5$K **29** (3.20 mg, 0.92 µmol), 0.05M 1,2-Man$_2$ **22** sol. (147.2 µl, 7.36 µmol), 0.05M sodium ascorbate sol. (147.2 µl, 7.36 µmol) and 0.05M $CuSO_4$ (5.52 µl, 110.4 µmol). Gel filtration followed by RP-HPLC (linear gradient 20 → 45% MeCN in 12 min) followed by lyophilisation yielded (1,2-Man$_2$)$_6$DEVA$_5$K **8** as a white powder (0.72 mg, 0.13 µmol, 14%). LC-MS analysis (linear gradient 10 → 90% MeCN in 12 min), t_R: 4.990 min, ESI/MS (*m/z*): $[M+3H]^{3+}$ calculated for $C_{238}H_{391}N_{53}O_{110}$ 1918.22, found 1918.33.

H-Lys(1,3-Man$_2$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (9): (1,3-Man$_2$)$_1$DEVA$_5$K **9** was conjugated following the general procedure using: Az$_1$DEVA$_5$K **26** (7.32 mg, 2.71 µmol), 0.05M 1,3-Man$_2$ **23** sol. (162.6 µl, 8.13 µmol), 0.05M sodium ascorbate sol. (162.6 µl, 8.13 µmol) and 0.05M $CuSO_4$ (2.71 µl, 54.2 µmol). Resin purification followed by RP-HPLC (linear gradient 20 → 40% MeCN in 12 min) followed by lyophilisation yielded (1,3-

$Man_2)_1DEVA_5K$ **9** as a white powder (2.11 mg, 0.68 µmol, 25%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 5.017 min, ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{133}H_{221}N_{33}O_{50}$ 1540.78, 1540.73; $[M+3H]^{3+}$ $C_{133}H_{222}N_{33}O_{50}$ 1027.52, found 1027.53.

H-Lys(1,3-Man$_2$)-Gly-Lys(1,3-Man$_2$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (10) (1,3-$Man_2)_2DEVA_5K$ **10** was conjugated following the general procedure using: Az_2DEVA_5K **27** (7.41 mg, 2.55 µmol), 0.05M 1,3-Man_2 **23** sol. (204.0 µl, 10.2 µmol), 0.05M sodium ascorbate sol. (204.0 µl, 10.2 µmol) and 0.05M $CuSO_4$ (102.0 µl, 5.1 µmol). Resin purification followed by RP-HPLC (linear gradient 20 → 45% MeCN in 12 min) followed by lyophilisation yielded (1,3-$Man_2)_2DEVA_5K$ **10** as a white powder (1.61 mg, 0.44 µmol, 17%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 4.957 min, ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{156}H_{258}N_{38}O_{63}$ 1836.40, found 1836.33; $[M+3H]^{3+}$ calculated for $C_{156}H_{259}N_{38}O_{63}$ 1224.60, found 1224.67.

H-Lys(1,3-Man$_2$)-Gly-Lys(1,3-Man$_2$)-Gly-Lys(1,3-Man$_2$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (11): (1,3-$Man_2)_3DEVA_5K$ **11** was conjugated following the general procedure using: Az_3DEVA_5K **28** (5.09 mg, 1.63 µmol), 0.05M 1,3-Man_2 **23** sol. (163.0 µl, 8.15 µmol), 0.05M sodium ascorbate sol. (163.0 µl, 8.15 µmol) and 0.05M $CuSO_4$ (97.8 µl, 4.89 µmol). Resin purification followed by RP-HPLC (linear gradient 20 → 40% MeCN in 12 min) followed by lyophilisation yielded (1,3-$Man_2)_3DEVA_5K$ **11** as a white powder (0.1.42 mg, 0.33 µmol, 20%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 4.903 min, ESI/MS (*m/z*): $[M+3H]^{3+}$ calculated for $C_{179}H_{296}N_{43}O_{76}$ 1422.02, found 1422.67.

H-Lys(1,3-Man$_2$)-Lys(1,3-Man$_2$)-Lys(1,3-Man$_2$)-Lys(1,3-Man$_2$)-Lys(1,3-Man$_2$)-Lys(1,3-Man$_2$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-

Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (12): (1,3-$Man_2)_6$DEVA$_5$K **12** was conjugated following the general procedure using: Az_6DEVA$_5$K **29** (2.7 mg, 0.78 µmol), 0.05M 1,3-Man_2 **23** sol. (124.8 µl, 6.24 µmol), 0.05M sodium ascorbate sol. (124.8 µl, 6.24 µmol) and 0.05M $CuSO_4$ (93.6 µl, 4.68 µmol). Resin purification followed by RP-HPLC (linear gradient 10 → 90% MeCN in 12 min) followed by lyophilisation yielded (1,3-$Man_2)_6$DEVA$_5$K **12** as a white powder (1.93 mg, 0.34 µmol, 43%). LC-MS analysis (linear gradient 10 → 90% MeCN in 12 min), t_R: 4.813 min, ESI/MS (*m/z*): $[M+3H]^{3+}$ calculated for $C_{238}H_{391}N_{53}O_{110}$ 1918.22, found 1918.07.

H-Lys(1,6-Man_2)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (13): (1,6-$Man_2)_1$DEVA$_5$K **13** was conjugated following the general procedure using: Az_1DEVA$_5$K **26** (7.40 mg, 2.74 µmol), 0.05M 1,6-Man_2 **24** sol. (164.4 µl,8.22 µmol), 0.05M sodium ascorbate sol. (164.4 µl, 8.22 µmol) and 0.05M $CuSO_4$ (54.8 µl, 2.74 µmol). Resin purification followed by RP-HPLC (linear gradient 20 → 40% MeCN in 12 min) followed by lyophilisation yielded (1,6-$Man_2)_1$DEVA$_5$K **13** as a white powder (3.99 mg, 1.3 µmol, 48%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 5.013 min, ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{133}H_{221}N_{33}O_{50}$ 1540.78, found 1540.73, $[M+3H]^{3+}$ $C_{133}H_{222}N_{33}O_{50}$ 1027.52, found 1027.87.

H-Lys(1,6-Man_2)-Gly-Lys(1,6-Man_2)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (14): (1,6-$Man_2)_2$DEVA$_5$K **14** was conjugated following the general procedure using: Az_2DEVA$_5$K **27** (8.11 mg, 2.79 µmol), 0.05M 1,6-Man_2 **24** sol. (223.2 µl, 11.16 µmol), 0.05M sodium ascorbate sol. (223.2 µl, 11.16 µmol) and 0.05M $CuSO_4$ (111.6 µl, 5.58 µmol). Resin purification followed by RP-HPLC (linear gradient 20 → 40% MeCN in 12 min) followed by lyophilisation yielded (1,6-$Man_2)_2$DEVA$_5$K **14** as a white powder (2.30 mg, 0.63 µmol, 22%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 4.970 min, ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{156}H_{258}N_{38}O_{63}$

1836.40, found 1836.27; $[M+3H]^{3+}$ calculated for $C_{156}H_{259}N_{38}O_{63}$ 1224.60, found 1224.47.

H-Lys(1,6-Man$_2$)-Gly-Lys(1,6-Man$_2$)-Gly-Lys(1,6-Man$_2$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (15): (1,6-Man$_2$)$_3$DEVA$_5$K **15** was conjugated following the general procedure using: Az$_3$DEVA$_5$K **28** (5.02 mg, 1.61 µmol), 0.05M 1,6-Man$_2$ **24** sol. (161.0 µl, 8.0.5 µmol), 0.05M sodium ascorbate sol. (161.0 µl, 8.05 µmol) and 0.05M $CuSO_4$ (69.6 µl, 3.48 µmol). Resin purification followed by RP-HPLC (linear gradient 20 → 40% MeCN in 12 min) followed by lyophilisation yielded (1,6-Man$_2$)$_3$DEVA$_5$K **15** as a white powder (0.93 mg, 0.22 µmol, 14%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 4.893 min, ESI/MS (*m/z*): $[M+3H]^{3+}$ calculated for $C_{179}H_{296}N_{43}O_{76}$ 1422.02, found 1421.93.

H-Lys(1,6-Man$_2$)-Lys(1,6-Man$_2$)-Lys(1,6-Man$_2$)-Lys(1,6-Man$_2$)-Lys(1,6-Man$_2$)-Lys(1,6-Man$_2$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (16): (1,6-Man$_2$)$_6$DEVA$_5$K **16** was conjugated following the general procedure using: Az$_6$DEVA$_5$K **29** (2.6 mg, 0.75 µmol), 0.05M 1,6-Man$_2$ **24** sol. (120.0 µl, 6.0 µmol), 0.05M sodium ascorbate sol. (120.0 µl, 6.0 µmol) and 0.05M $CuSO_4$ (90.0 µl, 4.5 µmol). Resin purification followed by RP-HPLC (linear gradient 10 → 90% MeCN in 12 min) followed by lyophilisation yielded (1,6-Man$_2$)$_6$DEVA$_5$K **16** as a white powder (1.33 mg, 0.23 µmol, 35%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 4.810 min, ESI/MS (*m/z*): $[M+3H]^{3+}$ calculated for $C_{238}H_{391}N_{53}O_{110}$ 1918.22, found 1918.27.

H-Lys(1,3-1,6-Man$_3$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (17): (1,3-1,6-Man$_3$)$_1$DEVA$_5$K **17** was conjugated following the general procedure using: Az$_1$DEVA$_5$K **26** (7.45 mg, 2.76 µmol), 0.05M 1,3-1,6-Man$_3$ **25** sol. (165.6 µl, 8.28 µmol), 0.05M sodium ascorbate sol. (165.5 µl, 8.28 µmol) and 0.05M

$CuSO_4$ (55.2 µl, 2.76 µmol). Gel filtration followed by RP-HPLC (linear gradient 20 → 40% MeCN in 12 min) followed by lyophilisation yielded (1,3-1,6-Man_3)$_1$$DEVA_5K$ **17** as a white powder (1.11 mg, 0.34 µmol, 12%). LC-MS analysis (linear gradient 10 → 90% MeCN in 12 min), t_R: 4.993 min, ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{139}H_{231}N_{33}O_{55}$ 1621.31, found 1621.53; $[M+3H]^{3+}$ calculated for $C_{139}H_{231}N_{33}O_{55}$ 1081.54, found 1081.20.

H-Lys(1,3-1,6-Man_3)-Gly-Lys(1,3-1,6-Man_3)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (18): (1,3-1,6-Man_3)$_2$$DEVA_5K$ **18** was conjugated following the general procedure using: Az_2DEVA_5K **27** (8.34 mg, 2.87 µmol), 0.05M 1,3-1,6-Man_3 **25** sol. (229.6 µl, 11.48 µmol), 0.05M sodium ascorbate sol. (229.6 µl, 11.48 µmol) and 0.05M $CuSO_4$ (114.8 µl, 5.74 µmol). Gel filtration followed by RP-HPLC (linear gradient 20 → 45% MeCN in 12 min) followed by lyophilisation yielded (1,3-1,6-Man_3)$_2$$DEVA_5K$ **18** as a white powder (0.73 mg, 0.18 µmol, 6%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 4.903 min, ESI/MS (*m/z*): $[M+2H]^{2+}$ calculated for $C_{168}H_{278}N_{38}O_{73}$ 1998.45, found 1998.87; $[M+3H]^{3+}$ calculated for $C_{168}H_{279}N_{38}O_{73}$ 1332.64, found 1332.93.

H-Lys(1,3-1,6-Man_3)-Gly-Lys(1,3-1,6-Man_3)-Gly-Lys(1,3-1,6-Man_3)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH_2 (19): (1,3-1,6-Man_3)$_3$$DEVA_5K$ **19** was conjugated following the general procedure using: Az_3DEVA_5K **28** (4.90 mg, 1.57 µmol), 0.05M 1,3-1,6-Man_3 **25** sol. (157.0 µl, 7.85 µmol), 0.05M sodium ascorbate sol. (157.0 µl, 7.85 µmol) and 0.05M $CuSO_4$ (94.2 µl, 4.71 µmol). Gel filtration followed by RP-HPLC (linear gradient 25 → 50% MeCN in 12 min) followed by lyophilisation yielded (1,3-1,6-Man_3)$_3$$DEVA_5K$ **19** as a white powder (0.36 mg, 0.08 µmol, 5%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 4.837 min, ESI/MS (*m/z*): $[M+3H]^{3+}$ calculated for $C_{197}H_{326}N_{43}O_{91}$ 1584.07, found 1584.60.

H-Lys(1,3-1,6-Man$_3$)-Lys(1,3-1,6-Man$_3$)-Lys(1,3-1,6-Man$_3$)-Lys(1,3-1,6-Man$_3$)-Lys(1,3-1,6-Man$_3$)-Lys(1,3-1,6-Man$_3$)-Asp-Glu-Val-Ser-Gly-Leu-Glu-Gln-Leu-Glu-Ser-Ile-Asn-Phe-Glu-Lys-Leu-Ala-Ala-Ala-Ala-Ala-Lys-NH$_2$ (20): (1,3-1,6-Man$_3$)$_6$DEVA$_5$K **20** was conjugated following the general procedure using: Az$_6$DEVA$_5$K **29** (2.50 mg, 0.72 µmol), 0.05M 1,6-Man$_2$ **25** sol. (115.2 µl, 5.76 µmol), 0.05M sodium ascorbate sol. (115.2 µl, 5.76 µmol) and 0.05M $CuSO_4$ (86.4 µl, 4.32 µmol). Gel filtration followed by RP-HPLC (linear gradient 10 → 90% MeCN in 12 min) followed by lyophilisation yielded (1,3-1,6-Man$_3$)$_6$DEVA$_5$K **20** as a white powder (0.58 mg, 0.09 µmol, 12%). LC-MS analysis (linear gradient 10 →90% MeCN in 12 min), t_R: 4.717 min, ESI/MS (*m/z*): $[M+3H]^{3+}$ calculated for $C_{274}H_{452}N_{53}O_{140}$ 2243.0, found 2242.9; $[M+4H]^{4+}$ calculated for $C_{274}H_{453}N_{53}O_{140}$ 1682.5, found 1683.1; $[M+5H]^{5+}$ calculated for $C_{274}H_{453}N_{53}O_{140}$ 1346.2, found 1346.5.

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Chapter 5

Synthesis of mannose configured cyclophellitol and its aziridine derivative[1]

Introduction

Carbohydrates are structurally the most diverse class of biopolymers. They not only occur as oligosaccharides and polysaccharides but are also constituents of glycoproteins and glycolipids.[2] These glycoconjugates are involved in numerous fundamental biological processes, such as communication processes[3] and cell-cell recognition.[4,5] Gaining insight into the role of carbohydrates and glycoconjugates in these biological processes is complicated by their complex structure, their transient occurrence and their biosynthesis that is not directly controlled by the genetic code.[6] The metabolism of glycoconjugates is guided by glycosyltransferases, enzymes that specifically introduce glycosidic bonds, and glycosidases, enzymes that specifically cleave glycosidic bonds.[7] Establishment of the activity of these enzymes in a defined biological context is an important approach to elucidate their role and the function of the corresponding glycans. In this respect activity-based protein profiling (ABPP) has became an important and

rapidly advancing field of research in the past decade.[8] Central and vital for this type of research is the development of activity based probes (ABPs). These probes are characterized by the presence of an irreversible activity based inhibitor for a specific (class of) enzyme(s) and a reporter group or ligation handle.[9]

Recently, major advances in the development of APBs for glycosidases have been made.[10] Key to the success of these ABPs is the classical Koshland double-replacement mechanism of retaining glycosidases.[11] As shown in Figure 1a two carboxylic acid residues (Asp or Glu) in the active site of the enzyme are involved in the two-step process.[12] In the first step protonation by one carboxylic acid residue of the glycan substrate and nucleophilic displacement by the second carboxylate leads to a covalent enzyme-glycosyl intermediate with inversion of configuration. In the next step the formed carboxylate anion assists in the hydrolysis of the enzyme-glycosyl intermediate to give the stripped glycan with retention of configuration with respect to the substrate. Fluorinated glycosides and cyclitol epoxides are two classes of covalent inhibitors, qualified for the development of ABPs for retaining glycosidases.

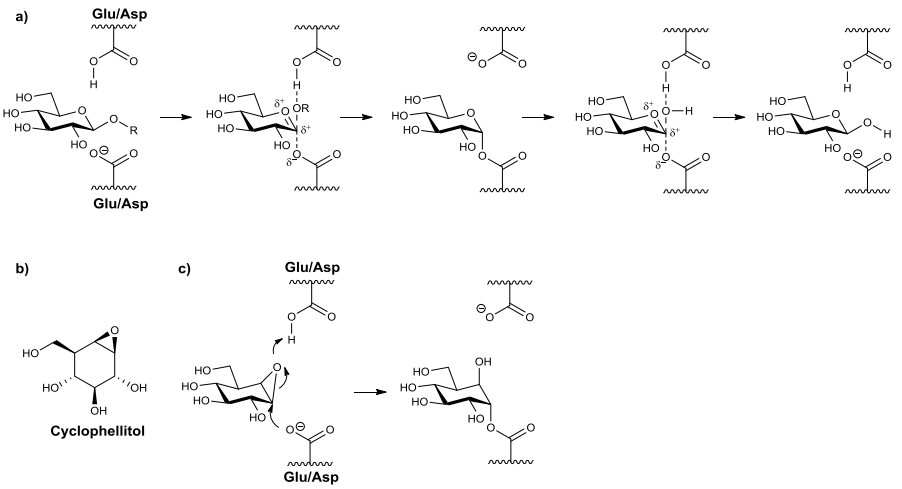


Figure 1: a) Classical Koshland double replacement mechanism of retaining beta-glucosidases. b) Structure of cyclophellitol. c) Proposed mechanism of cyclophellitol binding to retaining beta-glucosidases.

Cyclophellitol (Figure 1b), the cyclitol analogue of D-glucopyranose with an β-configured epoxide, is an irreversible and naturally occurring β-glucosidase inhibitor.[13] The inhibition mechanism comprises protonation of the epoxide in the active site by the general acid/base catalyst, followed by nucleophilic attack of the carboxylate to give a covalent cyclophellitol-enzyme adduct (Figure 1c).[14]

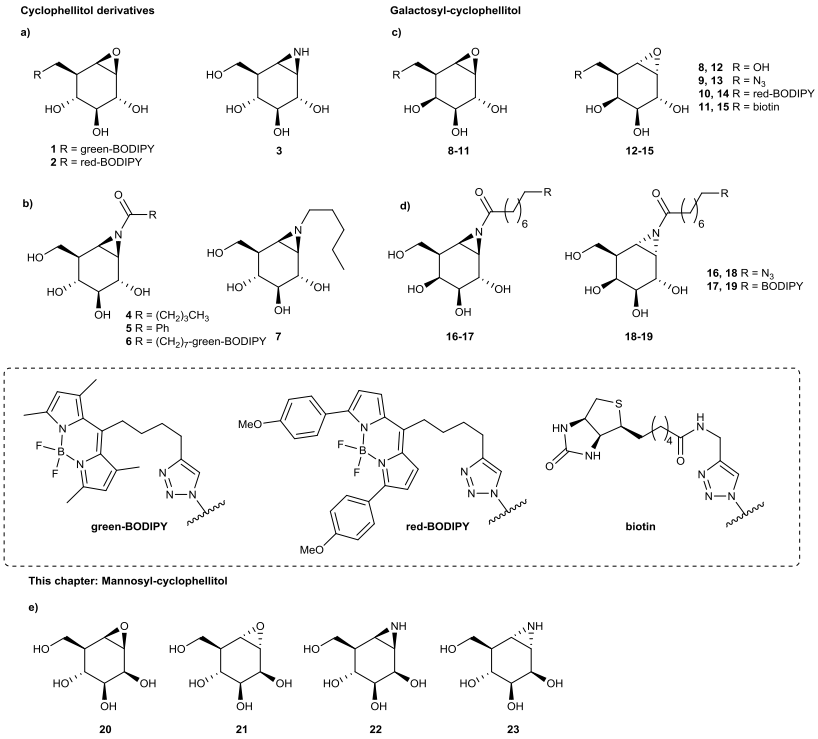


Figure 2: Structures of cyclophellitol based ABPs. a) Structures of BODIPY conjugated cyclophellitol **1** and **2** and its aziridine derivative **3**. b) Structures of functionalized aziridine cyclophellitol **4**-**7**. c) Structures of α/β-galactose configured cyclophellitol **8**-**15**. d) Structures of α/β-galactose configured functionalized aziridine cyclophellitol **16**-**19**. e) Structures of mannose configured α/β-cyclophellitol **20**, **21** and α/β-mannose configured aziridine cyclophellitol derivatives **22** and **23**.

Based on the mode of action of cyclophellitol, Witte *et al.*[15] developed two ABPs (**1** and **2**, Figure 2a) allowing highly specific and efficient labelling of active glucocerebrosidase, key enzyme in Gaucher disease.[16] Chapter 3

describes the synthesis of Man_1- and Man_3-BODIPY-cyclophellitol probes designed to selectively target the probes to Gaucher macrophages. Next to the epoxide based probes, broad spectrum ABPs for β-glucosidases were developed[17] by replacement of the epoxide electrophilic trap in cyclophellitol by functionalized aziridines[18,19] (as in **4**-**7**, Figure 2b**)**. In this manner the reporter group or ligation handle could be varied and installed at a position in the ABP pointing towards the aglycon site, where most glycosyl hydrolases (GHs) are more relaxed in their substrate specificity.

The synthesis of cyclophellitol analogs derived from the common monosaccharides found in mammalian and bacterial glycans will provide an ABP toolbox that can be used to interrogate different GHs that use the Koshland double-replacement mechanism. Willems *et al*. synthesized both α- and β-galactopyranose configured cyclophellitol analogues **8**-**15** and the α-aziridine derivatives **18** and **19** (Figure 2c-d).[20,21] Synthesis of β-Aziridine derivative **16** and **17** is currently in progress. With these probes GH27 human retaining α-galactosidases could be detected.[22]

This chapter describes the synthesis of four cyclophellitol analogs having the D-mannose configuration (2-*epi*-cyclophellitol), bearing either an α- or β-configured epoxide (**20**, **21**, Figure 2e) or a α- or β- aziridine function (**22**, **23**), to use these both as covalent inhibitors (the epoxides and non-functionalized aziridines) and as a starting point for the generation of ABPs.

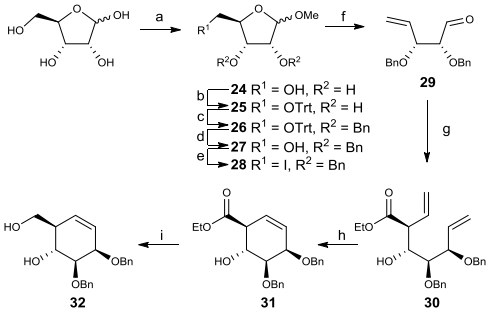
Results and discussion

Up to now only one low yielding synthesis has been described for mannose configured cyclophellitol,[23] whereas the corresponding aziridine has not been reported. To develop a straightforward route of synthesis towards these 2-*epi*-cyclophellitol targets, it was reasoned that the Madsen route towards cyclophellitol[24] (see Chapter 3) could be adapted, starting from the appropriate epimeric starting material, that is, D-ribose instead of D-xylose. Scheme 1 shows the synthesis of cyclohexene **32**, the common precursor for

the 2-*epi*-cyclophellitol target epoxides and aziridines. Treatment of D-ribose with acetyl chloride in methanol under kinetic conditions gave methyl-D-ribofuranose (**24**) as an α/β anomeric mixture. Selective tritylation of the primary alcohol in **24** and ensuing benzylation of the *cis*-diol to give fully protected ribose **26** followed by acid mediated detritylation furnishing ribose **27**[25] in 72% yield over 4 steps. Substitution of the primary alcohol in **27** by iodine and subsequent reductive ring opening of **28** with activated zinc yielded aldehyde **29**[26], the required starting compound for the ensuing indium catalyzed Barbier reaction.

In this key reaction the indium reagent derived from ethyl-4-bromocrotonate was formed *in situ* and added to aldehyde **29**. Following this procedure diene **30** was formed with excellent stereoselectivity (95:5 with respect to the undesired C-4 epimer).

Scheme 1: Synthesis of cyclohexene precursor **32**.



Reagents and conditions: (a) acetyl chloride, MeOH, 0 °C to rt.; (b) trityl chloride, pyridine, rt.; (c) BnBr, NaH, DMF, 0 °C to rt.; (d) *p*TsOH, DCM/MeOH (1:1), rt, 72% over four steps; (e) *i*. $(Ph)_3P$, imidazole, THF, reflux; *ii*. I_2, THF, reflux, 93%; (f) act. Zn, THF/H_2O (9:1), 60 °C, sonicate, 40%; (g) ethyl 4-bromocrotonate, indium (powder), $La(OTf)_3$, 61%; (h) Grubbs II, DCM, reflux 93%; (i) *i*. DIBAL-H, THF, 0 °C to rt. *ii*. $NaBH_4$, EtOAc/H_2O (2:1), 95%.

This stereoselectivity can be explained by a similar transition state as proposed by Madsen and co-workers in the Barbier reaction leading to the cyclophellitol octadiene precursor. As depicted in Figure 3, a 6-membered ring transition state, in which the aldehyde and benzyl ether moieties in **29** coördinate to indium, explain the observed stereochemistry at the two new

stereocenters (C-4 and C-5) in **30**. Purification by column chromatography resulted in the isolation of homogenous **30** in 61% yield. RCM of diene **30** using Grubbs 2nd generation catalyst gave cyclohexene **31** in 93% yield. Reduction of the ethyl ester in **31** was accomplished by treatment with DIBAL-H and sodium borohydride to furnish primary alcohol **32**, the common precursor to both the target α/β-epoxides and α/β-aziridines.

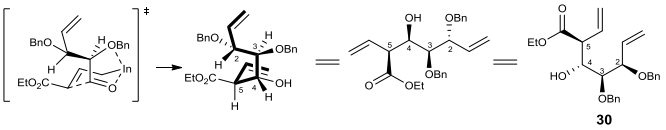
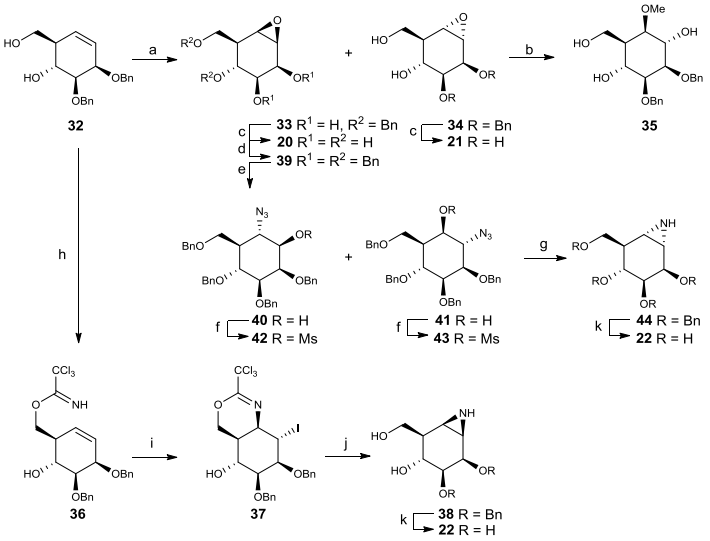


Figure 3: Possible transition state of the indium catalyzed Barbier reaction of aldehyde **29** with ethyl bromocrotonate leading to diene **30**.

The transformation of cyclohexene **32** into the four target compounds is shown in Scheme 2. Epoxidation of cyclohexene **32** using *m*CPBA resulted in a mixture of the α- and β-epoxides **33** and **34** in a 3:2 α/β-ratio and a combined 64% yield. Column chromatography gave the individual epoxides, the identity of which was ascertained by NMR experiments in combination with DFT calculations. The latter calculations were performed because the stereochemistry of the newly formed epoxides cannot be simply derived from the coupling constants of the ring protons.

Scheme 2: Scheme overview synthesis of target compounds **20**, **21**, **22** and **23**.



Reagents and conditions: (a) *m*CPBA, DCE, reflux (**33**: 38%, **34**: 26%); (b) Pd/C, H_2 (g), MeOH; (c) Pd/C, H_2 (g), 1,4-dioxane/*t*BuOH (9:1), rt. (**20**: 40%, **21**: 36%); (d) BnBr, NaH, DMF, 0 °C to rt (52%); (e) NaN_3, $LiClO_4$; (f) MsCl, pyridine (**40**: 88%, **41**: 72%); (g) $LiAlH_4$, THF, 0 °C (31%); (h) Cl_3CCN, DBU, DCM, 0 °C to rt, 94%; (i) I_2, $NaHCO_3$, THF/H_2O (4:1), 60 °C, 80%; (j) *i.* 1,4-dioxane/H_2O/AcOH (1:1:8), rt.; *ii.* $NaHCO_3$, MeOH, 93%; (k) Li, NH_3 (l), -60 °C, quantitative.

The spectroscopic data and calculated coupling constants for both the $^4H_3$ and the opposite $^3H_4$ half chair α- and β- epoxides are summarized in Table 1. Comparison of the experimental ^3J coupling constants of the two epoxide isomers with the calculated values show that the coupling constants for the protons of epoxide **34** match best with the series of coupling constants calculated for the α-epoxide in a $^4H_3$ conformation, where there is also good agreement between the recorded coupling constants of epoxide **35** and the calculated β-epoxide in a $^4H_3$ half chair. The measured and calculated coupling constants are summarized in Figure 4.

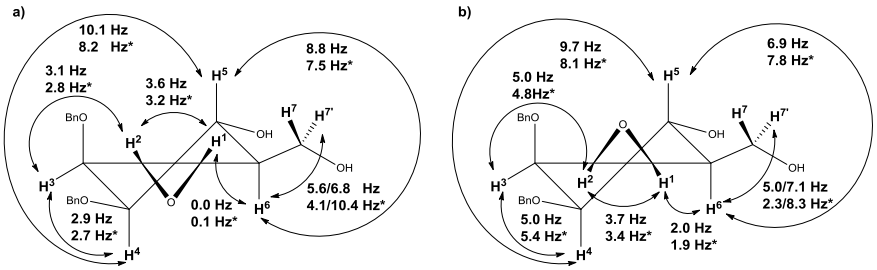


Figure 4: Absolute configuration of a) α-epoxide **34** b) and β-epoxide **35**. *Calculated coupling constants.

Table 1: Calculated and experimental *J* coupling α-epoxide **34** and β-epoxide **35**.

entry	correlation	$^{3}H_4$ *J* calc. (Hz)		$^{4}H_3$ *J* calc. (Hz)		*J* exp. (Hz)	
		α-**34**	β-**35**	α-**34**	β-**35**	α-**34**	β-**35**
1	H1-H2	3.6	3.6	3.2	3.4	3.6	3.7
2	H2-H3	0.01	3.8	2.8	4.8	3.1	5.0
3	H3-H4	4.8	8.5	2.7	5.4	2.9	5.0
4	H4-H5	4.4	8.2	8.2	8.1	10.1	9.7
5	H5-H6	1.6	7.9	7.5	7.8	8.8	6.9
6	H6-H7	0.5	2.3	4.1	2.3	5.6	5.0
7	H6-H7’	6.4	8.3	10.4	8.3	6.8	7.1
8	H6-H1	1.3	1.1	0.1	1.9	0.0	2.0

To conclude the synthesis of the epoxides, the removal of the benzyl groups in epoxides **33** and **34** was undertaken (Scheme 2). Hydrogenolysis of the benzyl groups in **34** with Pd/C and H_2 in MeOH gave a mixture of products. With the aid of NMR spectroscopy and mass spectrometry it was revealed

that the crude reaction mixture contained the desired compound **21** next to side product **35**, originating from ring opening of the epoxide by methanol. Changing the solvent mixture of the hydrogenolysis to 1,4-dioxane/*t*BuOH (9:1) prevented the undesired epoxide ring opening and led to the isolation of target epoxides **20** and **21** in 40% and 36% yield, respectively, after crystallization.

β-Configured aziridine (**22)** was obtained by adaptation of the procedure that was used to prepare β-cyclophellitol aziridine[18] (Scheme 2). First, the trichloroacetimidate function was regioselectively introduced at the primary hydroxyl group by treatment of cyclohexene **32** with trichloroacetonitrile and DBU to give **36**. Next, stereospecific iodo-cyclisation gave oxazine **37** in 80% yield. Hydrolysis of oxazine could be effected by treatment with 80% AcOH (1,4-dioxanes/H_2O/AcOH, 1:1:8) at ambient temperature. It is of interest to note that for the same opening of cyclophellitol oxazine heating at 60 °C in an HCl solution is prescribed. Base treatment of the crude 1,2-*trans* amino iodide provided β-aziridine **38** in 93% yield. The removal of the benzyl protective groups required some optimization. Using a reported procedure, in which Birch reduction is followed by treatment with Amberlite IR-H^+ to remove the lithium salts, gave an inseparable mixture of compounds. Treating the crude product with Amberlite IR-NH_4^+ was successful and β-manno-aziridine **22** was isolated in 70% overall yield starting from cyclohexene **38**.

α-Mannose configured aziridine cyclophellitol **23** was obtained from β-manno-epoxide **33** as depicted in Scheme 2. Perbenzylation of β-epoxide **33** was followed by epoxide opening by the azide anion in presence of $LiClO_4$ to afford a 1:1 mixture of azido alcohol regioisomers **40** and **41**. Separation by column chromatography gave the individual isomers **40** and **41** in 48% and 52% yield, respectively. Mesylation of the free hydroxyl in **40** and **41** yielded compound **42** and **43**, suitable for α-aziridine formation. To this end cyclitol **43** was subjected to $LiAlH_4$ treatment. Monitoring of the reaction by TLC-MS showed formation of the amine product, the desired aziridine and hydrolyzed aziridine. Unfortunately, a prolonged reaction time, to convert

more of the amine into the desired aziridine was accompanied by an increase of hydrolyzed side product and decrease of yield. After 4h the mixture was quenched and purification by column chromatography provided the benzylated α-aziridine **44** in a yield of 31%. Finally, removal of the benzyl groups by Birch reduction and treatment of resulting mixture with Amberlite NH_4^+ IR-120 as described for the β-aziridine yielded α-manno-aziridine **23** in 14% overall yield starting from β-epoxide **32**.

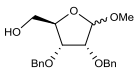
Conclusion

This chapter describes the synthesis of the α- and β-mannose configured cyclophellitol derivatives **20** and **21** and the corresponding aziridine analogues **22** and **23**. The key indium catalyzed Barbier reaction, in which two new stereocenters were introduced, proceeded with excellent stereoselectively. Central intermediate cyclohexene **32** was used for the synthesis of both the α- and β-epoxides **20** and **21** and the β-aziridine **22**, while the α-aziridine **23** was constructed from the β-epoxide **20**.
The obtained epoxides and aziridines can be explored as mechanism based covalent inhibitors for α- and β-mannosidases, that hydrolyze mannosidic linkages with retention of configuration, such as the glycosyl hydrolases from CAZy GH-family 38[27,28], 47[29], 92[30,31] and 99[32,33]. Aziridines **22** and **23** can also be further processed by installation of *N*-alkyl and *N*-acyl groups to deliver ABPs that can report on α- and β-mannosidase activity.

Experimental

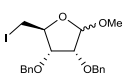
General: Traces of water in the starting materials were removed by co-evaporation with toluene for all moisture and oxygen sensitive reactions and the reactions were performed under an argon atmosphere. Dichloromethane was distilled over P_2O_5 and stored over activated 3 Å molecular sieves under

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.

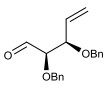


methyl 2,3-di-*O*-benzyl-D-ribofuranoside (27): To a 0 °C cooled solution of D-(-)-ribose (37.5 g, 250 mmol) in MeOH (500 mL) was added dropwise AcCl (3.5 mL, 50 mmol) and the reaction mixture was allowed to warm to rt. After complete conversion of the starting material the reaction was quenched with Et_3N till pH $\geq$7 and the mixture was concentrated *in vacuo* giving OMe-ribose **24** as an α/β mixture (1:0.3). The crude OMe-ribose **24** was co-evaporated with toluene and dissolved in pyridine (500 mL). To the solution was added trityl chloride (76.7 g, 275

mmol) and the mixture was stirred overnight at rt. The reaction was quenched with MeOH and the mixture was concentrated *in vacuo*. The product was dissolved in EtOAc and the organic phase was washed with H_2O (3x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude tritylated OMe-ribose **25** was taken up in DMF (1 L) and to the solution was added BnBr (90 mL, 750 mmol) and the mixture was cooled to 0 °C. To the cooled mixture was added (60%) NaH (25 g, 625 mmol) in small portions over a period of 6h. The reaction was gradually allowed to warm to rt and stirred overnight. The reaction mixture was cooled to 0 °C and quenched with MeOH after which the solvents were removed *in vacuo*. The product was dissolved in Et_2O and the organic phase was washed with H_2O (3x), brine (2x), and dried over $MgSO_4$. The crude was filtered over a bed of silica to remove the bulk of impurities, concentrated and dissolved in DCM/MeOH (1:1) (1 L). To the solution was added *p*TsOH (4.8 g, 25 mmol) and the reaction mixture was stirred overnight at rt. The mixture was neutralized with Et_3N and concentrated *in vacuo*. Purification by column chromatography yielded benzylated OMe-ribose **27** as a colourless oil (57.4 g, 179 mmol, 72%). Spectroscopic data were in accordance with known literature data.[25] (α-product) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.23 (m, 10H), 4.88 (s, 1H), 4.65 (d, *J* = 12.0 Hz, 1H), 4.60 (d, *J* = 12.0 Hz, 1H), 4.56 (d, *J* = 11.7 Hz, 1H), 4.47 (d, *J* = 11.7 Hz, 1H), 4.27 (dt, *J* = 6.9, 3.4 Hz, 1H), 4.11 (dd, *J* = 7.0, 4.7 Hz, 1H), 3.85 (d, *J* = 4.7 Hz, 1H), 3.78 (d, *J* = 12.2 Hz, 1H), 3.55 (ddd, *J* = 10.9, 7.3, 3.5 Hz, 1H), 3.34 (s, 3H), 2.15 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.7, 137.7, 128.5, 128.0, 127.9, 127.9, 106.8, 82.3, 80.1, 77.3, 72.7, 72.5, 62.8, 55.6.
(β-product) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.27 (m, 10H), 4.85 (d, *J* = 4.1 Hz, 1H), 4.74 (d, *J* = 12.7 Hz, 1H), 4.66 (d, *J* = 12.3 Hz, 1H), 4.65 – 4.54 (m, 2H), 4.17 (q, *J* = 3.5 Hz, 1H), 3.84 (dd, *J* = 6.9, 3.5 Hz, 1H), 3.73 (dd, *J* = 6.9, 4.2 Hz, 1H), 3.63 (dd, *J* = 12.0, 3.3 Hz, 1H), 3.45 (s, 3H), 3.39 (dd, *J* = 12.7, 3.5 Hz, 1H), 1.97 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.2, 137.8, 128.4, 128.4, 128.2, 128.0, 127.9, 127.8, 102.7, 83.2, 78.1, 74.7, 72.7, 72.6, 62.7, 55.6.

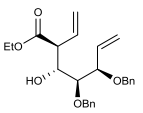


methyl 6-deoxy-6-iodo-2,3-di-*O*-benzyl-D-ribofuranoside (28): To a solution of benzylated α-OMe-ribose **27** (51.7 g, 113.8 mmol) in THF (455 mL) was added imidazole (15.5 g, 227.6 mmol), Ph_3P (44.8 g, 170.7 mmol) and the mixture was heated till reflux. After complete consumption of the starting material a 1M I_2 solution (170.7 mL, 170 mmol) in THF was added dropwise to the boiling reaction mixture and refluxed overnight. The mixture was cooled to rt and Et_2O was added, upon addition of Et_2O crystalline precipitate was formed. The mixture was cooled to -20 °C and the solids were filtered. The filtrate was washed with 10% $Na_2S_2O_3$ (aq.) (2x), H_2O (3x), brine (2x) dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was immobilized on silica and purification by column chromatography yielded iodo ribose **28** as a colourless oil (47.8 g, 105.4 mmol, 93%). Spectroscopic data were in accordance with known literature data.[34] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 – 7.28 (m, 10H), 4.92 (s, 1H), 4.65 (d, *J* = 11.7 Hz, 1H), 4.57 (d, *J* = 12.0 Hz, 1H), 4.57 (d, *J* = 12.0 Hz, 1H), 4.48 (d, *J* = 12.0 Hz, 1H), 4.14 (t, *J* = 6.6 Hz, 1H), 3.94 (dd, *J* = 6.6, 4.5 Hz, 1H), 3.88 (d, *J* = 4.5 Hz, 1H), 3.38 – 3.33 (m, 4H), 3.26 (dd, *J* = 10.5, 6.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.7, 128.6, 128.1, 128.0, 106.3, 81.8, 20.4, 80.2, 72.7, 72.5, 55.4, 8.8.

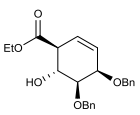


(2R,3R)-2,3-dibenzyloxypent-4-enal (29): Iodo ribose **28** (47.8 g, 105.4 mmol) was dissolved in THF/H_2O (9:1) (1 L) and the solution was purged with argon under sonication. To the solution was added activated zinc (65.4 g, 1.0 mol) and the mixture was further sonicated at 60 °C under argon atmosphere. After complete conversion of the starting material the excess of zinc was filtered and rinsed with DCM. The crude mixture was diluted with brine and the product was extracted with DCM (5x). The combined organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded aldehyde **29** as a colourless oil (12.47 g, 42 mmol, 40%). Spectroscopic data were in accordance with known literature data.[35] ^{1}H NMR (400 MHz,

$CDCl_3$) δ 9.63 (d, *J* = 2.1 Hz, 1H), 7.39 – 7.24 (m, 10H), 5.87 (dt, *J* = 17.6, 10.5, 7.6 Hz, 1H), 5.38 (d, *J* = 9.1 Hz, 1H), 5.34 (d, *J* = 16.9 Hz, 1H), 4.69 (d, *J* = 12.0 Hz, 1H), 4.64 (d, *J* = 12.2 Hz, 2H), 4.40 (d, *J* = 12.0 Hz, 1H), 4.16 (dd, *J* = 7.6, 4.6 Hz, 1H), 3.89 (dd, *J* = 4.6, 2.2 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.7, 137.8, 137.3, 134.1, 128.6, 128.5, 128.1, 127.8, 120.2, 85.0, 80.3, 73.1, 70.6.

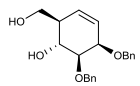


ethyl (2*S*,3*R*,4*S*,5*R*)-4,5-bis(benzyloxy)-3-hydroxy-2-vinylhept-6-enoate (30) To a solution of aldehyde **29** (1.01 g, 3.41 mmol) in H_2O (15.4 mL) was added 75% ethyl 4-bromocrotonate (2.04 mL, 11.1 mmol), $La(OTf)_3$ (4.00 g, 6.83 mmol), indium powder (0.90 g, 7.85 mmol) and the mixture was vigorously stirred overnight at rt. The reaction mixture turned into a white slurry in which sand was added till small balls were formed. The mixture was filtered over a pad of celite and rinsed with Et_2O. The layers were separated and the aqueous layer was extracted with Et_2O (3x). The combined organic phase was washed with H_2O (3x), brine (3x), dried over $MgSO_3$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded manno diene **30** as a colourless oil (0.853 g, 2.078 mmol, 61%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.26 (m, 10H), 5.89 (ddd, *J* = 17.5, 10.4, 7.2 Hz, 1H), 5.72 (ddd, *J* = 17.1, 10.3, 9.4 Hz, 1H), 5.41 (dt, *J* = 9.2, 1.3 Hz, 1H), 5.37 (t, *J* = 1.2 Hz, 1H), 5.14 (dd, *J* = 10.3, 1.4 Hz, 1H), 5.08 (d, *J* = 17.1 Hz, 1H), 4.68 (d, *J* = 11.2 Hz, 1H), 4.64 (d, *J* = 11.7 Hz, 1H), 4.44 (d, *J* = 11.3 Hz, 1H), 4.40 (d, *J* = 11.7 Hz, 1H), 4.24 (ddd, *J* = 9.5, 6.8, 1.3 Hz, 1H), 4.17 (t, *J* = 6.6 Hz, 1H), 4.13 (q, *J* = 7.3 Hz, 2H), 3.44 (dd, *J* = 5.8, 1.3 Hz, 1H), 3.34 (t, *J* = 9.5 Hz, 1H), 3.14 (d, *J* = 6.9 Hz, 1H), 1.24 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 137.9, 137.9, 135.5, 133.1, 128.5, 128.5, 128.1, 128.0, 128.0, 127.8, 120.0, 119.6, 80.2, 79.0, 73.2, 72.0, 71.0, 60.9, 55.0, 14.2. HRMS: $[M+H]^+$ calculated for $C_{25}H_{31}O_5$ 411.21660, found 411.21653.



ethyl (1*S*,4*R*,5*S*,6*R*)-4,5-bis(benzyloxy)-6-hydroxycyclohex-2-enecarboxylate (31) To a solution of manno diene **30** (0.575 g, 1.4 mmol) in DCM (56 mL) was added Grubbs 2nd catalyst (95 mg, 0.11 mmol, 8 mol%) and the reaction mixture was refluxed in the dark for 2 h. The mixture was concentrated *in vacuo* and directly purified by column chromatography without further workup yielding cyclohexene ethyl ester **31** as a slightly brown oil (0.498 g, 1.302 mmol, 93%). $[\alpha]_D^{22}$ + 42.8° (c = 1.0, DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.27 (m, 10H), 5.89 (ddd, *J* = 9.9, 5.2, 2.8 Hz, 1H), 5.82 (dd, *J* = 9.9, 2.4 Hz, 1H), 4.74 (d, *J* = 11.9 Hz, 1H), 4.69 (s, 2H), 4.62 (d, *J* = 11.8 Hz, 1H), 4.55 (ddd, *J* = 10.4, 8.8, 1.8 Hz, 1H), 4.21 (qd, *J* = 7.1, 0.8 Hz, 2H), 4.09 (t, *J* = 4.4 Hz, 1H), 3.46 (dd, *J* = 10.2, 4.0 Hz, 1H), 3.13 (ddt, *J* = 8.9, 2.5, 0.9 Hz, 1H), 2.91 (d, *J* = 1.9 Hz, 1H), 1.28 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 138.6, 138.1, 128.6, 128.5, 128.1, 128.0, 128.0, 127.8, 127.5, 126.7, 80.3, 72.2, 71.9, 69.6, 67.3, 61.4, 51.2, 14.3. HRMS: $[M+H]^+$ calculated for $C_{23}H_{27}O_5$ 383.18530, found 383.18548.

(1*S*,2*R*,5*S*,6*S*)-5,6-bis(benzyloxy)-2-(hydroxymethyl)cyclohex-3-enol

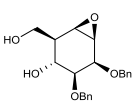


(32): To a 0 °C cooled solution of cyclohexene ethyl ester **31** (0.463 g, 1.2 mmol) in THF (40 mL) was added a 1M DIBAL-H sol. (6 mL, 6 mmol) in THF dropwise and the mixture was warmed to rt. After 30 min the mixture was cooled to 0 °C and to the mixture was added EtOAc (2.4 mL, 24.4 mmol), H_2O (1.2 mL) and $NaBH_4$ (0.295 g, 7.8 mmol) in small portions. After stirring for 20 min at 0 °C TLC showed full conversion of the starting material and the mixture was diluted with EtOAc. The mixture was transferred to a separation funnel and H_2O was added giving a white slurry. 1M HCl (aq.) was added till a clear two phase system was formed. The layers were separated and the aqueous layer was extracted with EtOAc (3x). The combined organic phase was washed with sat. $NaHCO_3$ (aq.), H_2O (3x), brine (3x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded manno cyclohexene **32** as a white amorphous solid $[\alpha]_D^{22}$ + 48.5° (c = 1.0,

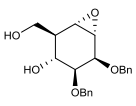
DCM). (0.387 g, 1.137 mmol, 95%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.27 (m, 10H), 5.88 (ddd, *J* = 9.9, 5.3, 2.8 Hz, 1H), 5.64 (dd, *J* = 9.9, 2.3 Hz, 1H), 4.72 (d, *J* = 11.6 Hz, 1H), 4.68 (d, *J* = 12.3 Hz, 1H), 4.67 (d, *J* = 12.1 Hz, 1H), 4.52 (d, *J* = 11.7 Hz, 1H), 4.13 – 4.08 (m, 2H), 3.81 – 3.73 (m, 2H), 3.46 (dd, *J* = 10.2, 3.9 Hz, 1H), 2.97 (s, 1H), 2.64 (s, 1H), 2.44 – 2.37 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.6, 137.9, 130.7, 128.7, 128.5, 128.1, 128.1, 128.1, 127.9, 81.1, 71.8, 71.7, 70.1, 69.4, 65.9, 46.6. HRMS: $[M+H]^+$ calculated for $C_{21}H_{25}O_4$ 341.17474, found 341.17501.

Epoxidation

To a solution of manno cyclohexene **32** (0.953 g, 2.8 mmol) in DCE (48 mL) was added *m*CPBA (55%) (1.32 g, 4.2 mmol) and the mixture was heated to reflux. After complete conversion of the starting material the mixture was cooled to rt and silica was added to the mixture after which the solvents were removed *in vacuo*. The immobilized product was directly purified by column chromatography yielding benzylated β-manno cyclophellitol **33** (0.283 g, 0.794 mmol, 29%) and benzylated α-manno cyclophellitol **34** (0.180 g, 0.505 mmol, 18%) both as a white amorphous solid.

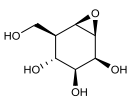


β-2,3-*O*-dibenzyl-2-*epi*-cyclophellitol (33): $[\alpha]_D^{22}$ + 64.7° (c = 1.0 DCM). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45 – 7.41 (m, 2H), 7.39 – 7.27 (m, 8H), 4.85 (d, *J* = 12.1 Hz, 1H), 4.65 (d, *J* = 11.6 Hz, 1H), 4.63 (d, *J* = 12.1 Hz, 1H), 4.42 (d, *J* = 11.6 Hz, 1H), 4.10 – 4.05 (m, 2H), 3.93 (dd, *J* = 10.7, 5.1 Hz, 2H), 3.91 (t, *J* = 9.7 Hz, 1H), 3.28 (dd, *J* = 3.7, 2.0 Hz, 1H), 3.24 (dd, *J* = 4.9, 3.7 Hz, 1H), 3.20 (dd, *J* = 10.1, 5.0 Hz, 1H), 2.76 (s, 1H), 2.61 (s, 1H), 2.10 (dddd, *J* = 9.1, 7.1, 5.2, 2.0 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 137.8, 137.5, 128.7, 128.6, 128.5, 128.2, 128.1, 79.7, 71.5, 71.4, 68.5, 67.1, 64.5, 54.6, 50.4, 44.8. HRMS: $[M+H]^+$ calculated for $C_{21}H_{25}O_5$ 357.16965, found 357.16965.

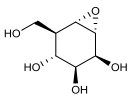


α-2,3-*O*-dibenzyl-2-*epi*-cyclophellitol (34): $[\alpha]_D^{22}$ -32.3° (c = 1.0 DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.27 (m,

10H), 4.83 (d, *J* = 12.0 Hz, 1H), 4.65 (d, *J* = 11.9 Hz, 1H), 4.62 (d, *J* = 11.6 Hz, 1H), 4.51 (d, *J* = 11.6 Hz, 1H), 4.28 (t, *J* = 2.9 Hz, 1H), 3.93 (dd, *J* = 9.8, 3.4 Hz, 1H), 3.91 (d, *J* = 10.2 Hz, 1H), 3.83 (dd, *J* = 10.7, 5.8 Hz, 1H), 3.53 (dd, *J* = 10.1, 3.1 Hz, 1H), 3.26 (t, *J* = 3.1 Hz, 1H), 3.07 (d, *J* = 3.6 Hz, 1H), 2.69 (s, 2H), 2.18 (dt, *J* = 8.8, 5.9 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.2, 137.8, 128.8, 128.6, 128.2, 128.1, 128.1, 128.0, 79.6, 74.0, 72.6, 71.6, 67.5, 63.7, 54.5, 53.7, 44.1. HRMS: $[M+H]^+$ calculated for $C_{21}H_{25}O_5$ 357.16965, found 357.16967



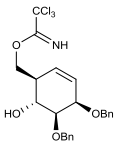
β-5-*epi*-cycloophellitol (20): Benzylated β-manno cyclophellitol **33** (41 mg, 0.115 mmol) was dissolved in dioxanes/*t*BuOH (9:1) (2.5 mL) and purged with argon gas. To the solution was added Pd/C (10%) and the mixture was stirred under a H_2 atmosphere. After complete conversion of the starting material to the fully debenzylated product the mixture was filtered over a pad of celite and rinsed with H_2O. The filtrate was concentrated *in vacuo*. The product was crystallized in MeOH yielding β-manno cyclophellitol **20** as a colourless crystalline solid (8.1 mg, 46 µmol, 40%). mp 164 °C. ^{1}H NMR (500 MHz, D_2O) δ 4.37 (t, *J* = 5.1 Hz, 1H), 3.99 (dd, *J* = 11.2, 4.2 Hz, 1H), 3.83 (dd, *J* = 11.2, 8.0 Hz, 1H), 3.56 (dd, *J* = 4.0, 1.9 Hz, 1H), 3.52 (dt, *J* = 8.3, 3.7 Hz, 2H), 3.46 (dd, *J* = 10.1, 8.9 Hz, 1H). ^{13}C NMR (126 MHz, D_2O) δ 72.3, 65.9, 65.3, 60.8, 56.1, 53.6, 44.1. HRMS: $[M+H]^+$ calculated for $C_7H_{13}O_5$ 177.07575, found 177.07576

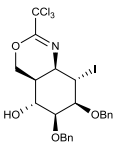


α-5-*epi*-cyclophellitol (21): Benzylated β-manno cyclophellitol **34** (71.2 mg, 0.2 mmol) was dissolved in dioxanes/*t*BuOH (9:1) (5 mL) and purged with argon gas. To the solution was added Pd/C (10%) and the mixture was stirred under a H_2 atmosphere. After complete conversion of the starting material to the fully debenzylated product, the mixture was filtered over a pad of celite and rinsed with H_2O. The filtrate was concentrated *in vacuo*. The product was crystallized in MeOH yielding α-manno cyclophellitol **21** as a white solid

(12.6 mg, 72 µmol, 36%). mp 135-137 °C. ^{1}H NMR (400 MHz, MeOD) δ 4.30 – 4.26 (m, 1H), 3.91 (dd, *J* = 10.8, 4.0 Hz, 1H), 3.67 (dd, *J* = 10.8, 7.9 Hz, 1H), 3.46 (dd, *J* = 8.2, 2.0 Hz, 1H), 3.41 (dd, *J* = 10.2, 3.3 Hz, 1H), 3.24 (t, *J* = 3.1 Hz, 1H), 3.19 (dd, *J* = 3.6, 0.8 Hz, 1H), 1.94 (td, *J* = 8.2, 3.9 Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 72.2, 69.1, 67.5, 62.4, 56.9, 55.1, 46.6. HRMS: $[M+H]^+$ calculated for $C_7H_{13}O_5$ 177.07575, found 177.07575

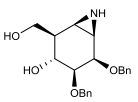
(1*S*,2*R*,5*S*,6*S*)-5,6-bis(benzyloxy)-2-(methylthrichloroacetimidate)cyclohex-3-enol (36) To a solution of manno cyclohexene **32** (68.1 mg, 0.2 mmol) in DCM (4.25 mL) was added a 0.6 M Cl_3CCN (0.5 mL, 0.3 mmol) in DCM and the mixture was cooled to 0 °C. To the cooled solution was added dropwise a 0.4 M DBU (0.25 mL, 0.1 mmol) solution in DCM to the reaction mixture. The mixture was warmed to rt and stirred for 30 min. The mixture was diluted with DCM and silica was added. The solvents were removed *in vacuo* and the immobilized product was directly purified by column chromatography yielding cyclohexene imidate **36** as a colourless oil (75.4 mg, 0.16 mmol, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.39 – 7.26 (m, 10H), 5.95 – 5.84 (m, 2H), 4.74 (d, *J* = 11.7 Hz, 1H), 4.67 (d, *J* = 12.0 Hz, 1H), 4.64 (d, *J* = 12.0 Hz, 1H), 4.61 (dd, *J* = 6.5, 4.1 Hz, 1H), 4.56 (d, *J* = 11.7 Hz, 1H), 4.16 – 4.06 (m, 2H), 3.48 (dd, *J* = 10.1, 3.8 Hz, 1H), 2.87 (s, 1H), 2.62 (ddd, *J* = 8.3, 7.8, 3.5 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0, 138.6, 138.0, 131.0, 128.6, 128.5, 128.1, 128.1, 128.0, 127.8, 125.8, 81.2, 71.8, 71.3, 69.5, 69.5, 66.8, 44.3. TLC-MS: $[M + Na]^+$ 508.0





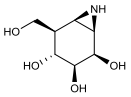
iodo oxazine (37) To a solution of cyclohexene imidate **36** (0.484 g, 1 mmol) in THF/H_2O (4:1) (25 mL) was added $NaHCO_3$ (0.294 g, 10 mmol), I_2 (0.888 g, 3.5 mmol) and the mixture was heated till reflux. After complete conversion of the starting material the mixture was cooled to rt and diluted with EtOAc. To the solution was added 10% $Na_2S_2O_3$ (aq.) till the organic phase was colourless.

The two phases were separated and the aqueous phase was extracted with EtOAc (3x). 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 iodo oxazine **37** as a yellow oil (0.459 g, 0.75 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (ddq, *J* = 9.6, 6.9, 2.1 Hz, 10H), 5.05 (t, *J* = 2.4 Hz, 1H), 4.88 (dd, *J* = 11.2, 1.6 Hz, 1H), 4.79 (d, *J* = 12.3 Hz, 1H), 4.53 (d, *J* = 11.5 Hz, 1H), 4.45 (d, *J* = 12.3 Hz, 1H), 4.41 (d, *J* = 11.6 Hz, 1H), 4.19 (dd, *J* = 7.0, 2.6 Hz, 1H), 4.16 (dd, *J* = 5.6, 2.7 Hz, 1H), 4.13 – 4.11 (m, 1H), 4.10 (d, *J* = 2.7 Hz, 1H), 4.07 (q, *J* = 2.6 Hz, 1H), 2.63 (s, 1H), 2.50 (ddt, *J* = 9.9, 4.7, 2.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.6, 137.5, 128.7, 128.5, 128.4, 128.2, 128.1, 127.9, 79.3, 77.1, 71.8, 71.1, 67.2, 64.7, 59.3, 33.8, 26.9. TLC-MS: $[M + Na]^+$ 633.8. HRMS: $[M+H]^+$ calculated for $C_9H_{14}INO_4$ 328.00403, found 328.00409.

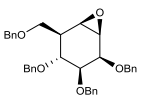


β-2,3-*O*-dibenzyl-2-*epi*-cyclophellitol azirine (38) Iodo oxazine **37** (0.459 g, 0.75 mmol) was dissolved in a 1,4-dioxane/H_2O/AcOH (1:1:8) mixture (30 mL) and stirred overnight at rt. The mixture was concentrated *in vacuo*, co-evaporated with toluene (3x) and the residue was dissolved in MeOH (30 mL). To the solution was added $NaHCO_3$ (1.26 g, 15 mmol) and stirred overnight at rt. The solids were filtered over a pad of celite and the filtrate was concentrated *in vacuo*. The crude was dissolved in DCM and washed with H_2O (1x). The aqueous layer was extracted with DCM (5x) and the combined organic layers was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography using neutral and activated silica followed by precipitation in cold Et_2O yielded benzylated aziridine **38** as a white powder (0.155 g, 0.437 mmol, 58%). ^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.46 – 7.25 (m, 10H), 4.77 (d, *J* = 11.5 Hz, 1H), 4.62 (d, *J* = 11.5 Hz, 1H), 4.49 (d, *J* = 11.6 Hz, 1H), 4.43 (d, *J* = 11.5 Hz, 1H), 4.18 (t, *J* = 5.2 Hz, 1H), 3.92 (dd, *J* = 10.7, 5.7 Hz, 1H), 3.84 (dd, *J* = 10.8, 4.8 Hz, 1H), 3.77 (t, *J* = 9.8 Hz, 1H), 3.23 (dd, *J* = 10.1, 4.7 Hz, 1H), 2.82 (s, 2H), 2.42 – 2.29 (m, 2H), 2.05 (s, 1H). ^{13}C NMR (100 MHz, CD_2Cl_2) δ 138.6, 128.9, 128.9,

128.7, 128.5, 128.3, 81.2, 71.6, 71.5, 69.7, 66.1, 64.9, 44.7. HRMS: $[M+H]^+$ calculated for $C_{21}H_{26}NO_4$ 356.18563, found 356.18570.



β-5-*epi*-cycloophellitol aziridine (22) NH_3 gas was condensated at -60 °C and liquid NH_3 was collected (± 2.5 mL). To the liquid NH_3 was added lithium (16.5 mg, 2.5 mmol), upon addition of the lithium the solution turned dark blue. The mixture was stirred till all the lithium was completely dissolved and a solution of benzylated aziridine **38** (35.5 mg, 0.1 mmol) in THF (2 mL) was added drop wise. The reaction was stirred for 30 min at -60 °C and H_2O (1.5 mL) was added dropwise to the reaction mixture. The mixture was gradually warmed to rt and co-evaporated with H_2O (3x). The crude product was dissolved in H_2O and treated with Amberlite IR-120 NH_4^+ for 2 h. The resin was filtered, the filtrate was concentrated *in vacuo* and retreated with Amberlite IR-120 NH_4^+ (3x). The product was dissolved in MeOH and precipitated in 0 °C ether under vigorous stirring. The precipitate was filtered and dried over a stream of air yielding β-aziridine **22** as a white powder (17.4 mg, 0.1 mmol, quantitative). ^{1}H NMR (400 MHz, Deuterium Oxide) δ 4.27 (t, *J* = 5.3 Hz, 1H), 3.89 (dd, *J* = 10.9, 4.5 Hz, 1H), 3.70 (dd, *J* = 10.9, 8.7 Hz, 1H), 3.44 (dd, *J* = 9.3, 4.9 Hz, 1H), 3.35 (t, *J* = 8.8 Hz, 1H), 2.66 – 2.52 (m, 3H), 2.01 (ddd, *J* = 8.3, 4.6, 3.6 Hz, 1H). ^{13}C NMR (100 MHz, D_2O) δ 73.4, 66.4, 65.5, 62.3, 43.6, 33.1, 32.6. HRMS: $[M+H]^+$ calculated for $C_7H_{13}NO_4$ 176.09173, found 176.09170.



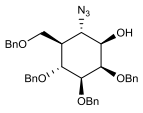
β-3,4,5,7-*O*-tetrabenzyl-5-*epi*-cyclophellitol (39): To a 0 °C cooled solution of β-manno cyclophellitol **33** (0.104 g, 0.29 mmol) in DMF (2.9 mL) was added BnBr (86 µl, 0.725 mmol) and NaH (60%) (29 mg, 0.725 mmol) in small portions and the reaction mixture was allowed to warm to rt. After complete conversion of the starting material the mixture was cooled to 0 °C and quenched with MeOH. The solvent was removed *in vacuo* and the crude was dissolved in Et_2O. The product was washed with H_2O (3x), brine (2x), dried over $MgSO_4$, filtered

and concentrated *in vacuo*. Purification by column chromatography yielded per-benzylated β-manno cyclophellitol **39** as a white amorphous solid (80.6 mg, 0.15 mmol, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.41 (m, 2H), 7.40 – 7.26 (m, 16H), 7.24 – 7.18 (m, 2H), 4.83 (d, *J* = 12.5 Hz, 1H), 4.79 (d, *J* = 11.2 Hz, 1H), 4.72 (d, *J* = 12.5 Hz, 1H), 4.60 (s, 2H), 4.56 (d, *J* = 12.1 Hz, 1H), 4.51 (d, *J* = 12.0 Hz, 1H), 4.42 (d, *J* = 11.2 Hz, 1H), 4.04 (t, *J* = 4.6 Hz, 1H), 3.77 (dd, *J* = 8.8, 4.9 Hz, 1H), 3.69 (t, *J* = 8.8 Hz, 1H), 3.60 (dd, *J* = 8.7, 7.6 Hz, 1H), 3.47 (dd, *J* = 4.8, 2.1 Hz, 1H), 3.46 (t, *J* = 4.9 Hz, 1H), 3.27 (t, *J* = 4.0 Hz, 1H), 2.30 (dddd, *J* = 8.8, 7.6, 4.9, 2.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.5, 138.5, 138.4, 138.3, 128.6, 128.5, 128.5, 128.5, 128.3, 128.2, 128.0, 128.0, 127.8, 127.8, 127.8, 127.7, 79.7, 74.4, 73.6, 73.4, 72.7, 71.5, 70.9, 69.4, 54.7, 52.0, 42.6. HRMS: $[M+H]^+$ calculated for $C_{35}H_{36}O_5$ 537.26355, found 537.26347.

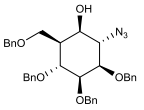
Azido ringopening

To a solution of per-benzylated β-manno cyclophellitol **39** (53.7 mg, 0.1 mmol) in MeCN (2 mL) was added $LiClO_4$ (16 mg, 0.15 mmol), NaN_3 (65 mg, 1.0 mmol) and the reaction mixture was stirred overnight at 80 °C under an argon atmosphere. The reaction mixture was cooled to rt and quenched with H_2O. The product was extracted with DCM (5x) from the water and the combined organic layers were dried over $MgSO_4$, filtered and concentred *in vacuo* giving a 1:1 mixture of two products. Purification by column chromatography yielded 0-azido-1-hydroxy **40** (28.0 mg, 0.048 mmol, 48%) and 0-hydroxy-1-azido **41** (30.9 mg, 0.052 mmol, 52%) (cyclophellitol numbering).

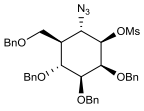
(1*R*,2*S*,3*R*,4*R*,5*S*,6*R*)-2-azido-4,5,6-tris(benzyloxy)-3-((benzyloxy)methyl) cyclohexan-1-ol (40) ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.15 (d, *J* = 11.6 Hz, 1H), 4.90 (d, *J* = 10.7 Hz, 1H), 4.75 (d, *J* = 11.8 Hz, 1H), 4.71 (d, *J* = 11.9 Hz, 1H), 4.67 (d, *J* = 11.7 Hz, 1H), 4.54 (d, *J* = 10.6 Hz, 1H), 4.51 (d, *J* = 12.1 Hz, 1H), 4.45 (d, *J* = 12.1 Hz, 1H), 4.10 (t, *J* = 10.2 Hz, 1H), 4.05 (t, *J* = 2.7 Hz, 1H), 3.87 – 3.77 (m, 2H), 3.63



(dd, *J* = 9.2, 2.4 Hz, 1H), 3.44 (dd, *J* = 9.8, 2.1 Hz, 1H), 3.40 (dd, *J* = 10.2, 2.8 Hz, 1H), 1.34 (tt, *J* = 11.2, 2.3 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.6, 138.6, 138.5, 138.3, 128.7, 128.6, 128.5, 128.2, 128.0, 128.0, 127.9, 127.8, 127.7, 127.7, 84.2, 77.8, 75.8, 75.7, 74.8, 74.2, 73.2, 73.2, 65.1, 62.4, 45.0. TLC-MS $[M+Na]^+$ 602.5. HRMS: $[M+H]^+$ calculated for $C_{35}H_{37}N_3O_5$ 580.28060, found 580.28062.

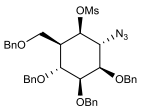
(1*R*,2*S*,3*R*,4*R*,5*S*,6*R*)-2-azido-4,5,6-tris(benzyloxy)-3-((benzyloxy)methyl) cyclohexan-1-ol (41) 

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.41 – 7.14 (m, 20H), 4.72 – 4.37 (m, 8H), 3.98 – 3.84 (m, 3H), 3.75 (t, *J* = 4.7 Hz, 1H), 3.72 (t, *J* = 3.9 Hz, 1H), 3.66 (dd, *J* = 8.1, 2.9 Hz, 1H), 3.62 (dd, *J* = 9.5, 5.5 Hz, 1H), 3.45 (s, 1H), 2.57 (dq, *J* = 9.8, 4.6 Hz, 1H). TLC-MS $[M+Na]^+$ 602.5. HRMS: $[M+H]^+$ calculated for $C_{35}H_{37}N_3O_5$ 580.28060, found 580.28056.

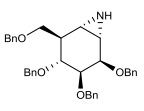
(1*R*,2*S*,3*R*,4*R*,5*S*,6*S*)-2-azido-4,5,6-tris(benzyloxy)-3-((benzyloxy)methyl) cyclohexyl methanesulfonate (42) 

To a 0 °C cooled solution of **40** (22.9 mg, 39.5 µmol) in pyridine (1.2 mL) was added dropwise a 0.5M mesyl chloride solution (0.237 mL, 119 µmol) in toluene and the mixture was allowed to warm to rt. After complete conversion of the starting material the reaction was quenched with H_2O and the product was extracted with EtOAc (5x). The combined organic phase was washed with brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded mesylated compound **42** (18.7 mg, 28.4 µmol, 72%). FT-IR: ν_{max} (neat)/cm^{-1} 962.12, 991.55, 1075.14, 1091.00, 1179.40, 1360.29, 1454.53, 1496.68, 2112.75, 2918.66, 3030.95. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.22 (m, 16H), 7.21 – 7.15 (m, 4H), 4.69 (dd, *J* = 10.3, 6.2 Hz, 1H), 4.58 (d, *J* = 11.6 Hz, 1H), 4.47 (q, *J* = 6.3, 5.8 Hz, 5H), 4.38 (d, *J* = 11.7 Hz, 1H), 4.31 (d, *J* = 11.8 Hz, 1H), 4.08 (t, *J* = 3.3 Hz, 1H), 4.03 (t, *J* = 10.0 Hz, 1H), 3.87 (t, *J* = 9.5 Hz, 1H), 3.77 (dd, *J* = 9.4, 5.9 Hz, 1H), 3.73 (t, *J* = 3.2 Hz, 1H), 3.67 (dd, *J* = 9.7, 3.2 Hz, 1H), 3.06 (s, 3H), 2.93 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.3, 137.7,

137.6, 128.6, 128.6, 128.5, 128.5, 128.2, 128.1, 128.1, 128.0, 127.7, 79.7, 78.5, 74.8, 73.3, 73.2, 72.8, 72.8, 66.3, 61.5, 42.8, 38.4. TLC-MS $[M+Na]^+$ 580.9. HRMS: $[M+H]^+$ calculated for $C_{36}H_{39}N_3O_7S$ 658.25815, found 658.25808.

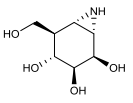
(1*R*,2*R*,3*R*,4*S*,5*R*,6*R*)-2-azido-3,4,5-tris(benzyloxy)-6-(benzyloxy)methyl) cyclohexyl methanesulfonate (43) 

To a 0 °C cooled solution of **41** (21.0 mg, 36.2 µmol) in pyridine (1.0 mL) was added dropwise a 0.5M mesyl chloride solution (0.217 mL, 108 µmol) in toluene and the mixture was allowed to warm to rt. After complete conversion of the starting material the reaction was quenched with H_2O and the product was extracted with EtOAc (5x). The combined organic phase was washed with brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded mesylated compound **43** (21.0 mg, 32 µmol, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.39 (m, 2H), 7.37 – 7.25 (m, 16H), 7.17 (dd, *J* = 7.4, 2.2 Hz, 2H), 4.94 (d, *J* = 11.8 Hz, 1H), 4.89 (d, *J* = 10.8 Hz, 1H), 4.86 (d, *J* = 11.9 Hz, 1H), 4.60 (d, *J* = 11.7 Hz, 1H), 4.58 (d, *J* = 11.7 Hz, 1H), 4.54 – 4.44 (m, 2H), 4.43 (d, *J* = 12.0 Hz, 1H), 4.37 (dd, *J* = 10.5, 2.5 Hz, 1H), 4.30 (t, *J* = 2.4 Hz, 1H), 4.24 (t, *J* = 10.8 Hz, 1H), 4.10 (dd, *J* = 10.8, 9.5 Hz, 1H), 3.79 (dd, *J* = 9.4, 1.9 Hz, 1H), 3.61 (dd, *J* = 9.4, 2.5 Hz, 1H), 3.43 (dd, *J* = 9.6, 2.3 Hz, 1H), 3.07 (s, 3H), 1.38 (dt, *J* = 11.4, 2.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.5, 138.4, 138.2, 138.0, 128.6, 128.5, 128.5, 128.4, 128.3, 128.0, 127.8, 127.8, 127.7, 127.6, 83.1, 82.3, 76.3, 75.8, 75.1, 75.0, 73.1, 72.6, 64.7, 59.1, 45.1, 38.5. TLC-MS $[M+Na]^+$ 581.0. HRMS: $[M+H]^+$ calculated for $C_{36}H_{39}N_3O_7S$ 658.25815, found 658.25822.

α-3,4,5,7-*O*-tetrabenzyl-5-*epi*-cyclophellitol aziridine (44) 

A solution of mesyl **43** (21 mg, 32 µmol) in THF (0.3 mL) was added dropwise to a 0 °C 0.1 M $LiAlH_4$ solution (0.5 mL, 50 µmol) in THF. After 4 h the reaction was diluted with THF and quenched with 3M NaOH (aq.) (0.167 mL, 0.5 mmol). The mixture was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column

chromatography yielded perbezylated α-manno aziridine **44** (5.5 mg, 10 µmol, 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.37 (m, 2H), 7.37 – 7.24 (m, 16H), 7.23 – 7.19 (m, 2H), 4.93 (d, *J* = 12.2 Hz, 1H), 4.84 (d, *J* = 11.3 Hz, 1H), 4.73 (d, *J* = 12.4 Hz, 1H), 4.70 (d, *J* = 11.9 Hz, 1H), 4.64 (d, *J* = 11.8 Hz, 1H), 4.52 (d, *J* = 12.1 Hz, 1H), 4.46 (t, *J* = 11.2 Hz, 2H), 4.22 (t, *J* = 2.5 Hz, 1H), 3.76 (dd, *J* = 9.7, 2.6 Hz, 1H), 3.71 (dd, *J* = 9.8, 7.7 Hz, 1H), 3.61 (dd, *J* = 9.2, 4.0 Hz, 1H), 3.51 (t, *J* = 8.7 Hz, 1H), 2.46 (dd, *J* = 6.0, 2.4 Hz, 1H), 2.36 (d, *J* = 5.8 Hz, 1H), 2.26 (td, *J* = 8.0, 4.1 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.3, 139.1, 139.0, 138.6, 128.5, 128.5, 128.4, 128.4, 128.2, 127.8, 127.8, 127.7, 127.6, 127.5, 80.8, 75.7, 75.2, 74.7, 73.3, 73.1, 73.1, 70.9, 43.4, 34.6, 31.9. TLC-MS $[M+H]^+$ 536.3 HRMS: $[M+H]^+$ calculated for $C_{35}H_{37}NO_4$ 536.27954, found 536.27957.



α-5-*epi*-cyclophellitol aziridine (23) NH_3 gas was condensated at -60 °C and liquid NH_3 was collected (± 2.5 mL). To the liquid NH_3 was added lithium (3 mg, 0.43 mmol), upon addition of the lithium the solution turned dark blue. The mixture was stirred till all the lithium was completely dissolved and a solution of perbenzylated α-manno-aziridine **44** (5.5 mg, 10 µmol) in THF (1.0 mL) was added drop wise. The reaction was stirred for 30 min at -60 °C and H_2O (1.0 mL) was added dropwise to the reaction mixture. The mixture was gradually warmed to rt and co-evaporated with H_2O (3x). The crude product was dissolved in H_2O and treated with Amberlite IR-120 NH_4^+ for 2 h. The resin was filtered, the filtrate was concentrated *in vacuo* and retreated with Amberlite IR-120 NH_4^+ (3x) yielding α-aziridine **23** (1.8 mg, 10 µmol, quantitative). ^{1}H NMR (600 MHz, D_2O) δ 3.89 (s, 1H), 3.47 (dd, *J* = 11.0, 3.9 Hz, 1H), 3.27 (dd, *J* = 11.0, 7.7 Hz, 1H), 3.04 (t, *J* = 9.7 Hz, 1H), 3.01 (dd, *J* = 10.3, 3.3 Hz, 1H), 2.08 (d, *J* = 6.0 Hz, 1H), 1.90 (d, *J* = 5.8 Hz, 1H), 1.43 (td, *J* = 8.3, 3.8 Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 72.1, 69.1, 68.3, 63.2, 46.2, 36.8, 32.3. HRMS: $[M+H]^+$ calculated for $C_7H_{13}NO_4$ 176.09173, found 176.09169.

DFT calculations

The calculated ^{1}H NMR coupling constants were obtained by first finding the lowest energy conformation of both epoxide isomers, for which a library of gas phase conformations was generated using conformer distribution option included in Spartan 04 program employing DFT/B3LYP 6-31G(d). All conformers were further optimized by Gaussion 03 at DFT/B3LYP 6-311G(d,p), their zero-point energies were calculated and the energies corrected for solvent by another optimization step employing a Polarizable Continuum Model set for water. The energies of these conformers, corrected for their zero-point energies, were compared and of the lowest energy conformer an NMR calculation was performed using Gauche-Independent Atomic Orbital (GIAO) method with added spin-spin coupling calculation.

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Chapter 6

Tuning the imidate leaving group of 2-deoxy-2-fluoro glycoside-based glycosidase inhibitors[1]

Introduction

Glycoconjugates are a highly diverse class of biomolecules, playing an important role in many biological processes.[2] The metabolism of glycoconjugates and the enzymes involved are extensively studied. Glycosidases, enzymes that hydrolyse glycosidic linkages, are engaged in a number of diseases, including metabolic storage disorders such as Gaucher's disease,[3,4] cancer,[5,6,7] HIV/AIDS,[8] Parkinson's disease,[9,10] Alzheimer's disease[11] and influenza.[12] Specific inhibitors of glycosidases are therefore interesting targets as potential therapeutics, as well as useful tools for structural and mechanistic characterisation of these enzymes.[13,14] In this framework attention has been focussed on the development of mechanism based covalent inhibitors and activity-based probes (ABPs), which are increasingly being used as research tools (see chapter 3 and 5).[15,16,17]

The classical Koshland double-replacement mechanism of retaining glycosidases operates in two steps, the first of which is the formation of a glycosyl-enzyme intermediate (the "glycosylation" step), which is hydrolysed in the second step (the "deglycosylation" step, Figure 1). In 1987

Withers *et al.* introduced the 2-deoxy-2-fluoroglucosides **1** and **2** (Figure 2) as mechanism based inhibitors for retaining β-glucosidases.[18] The design of inhibitors such as **1** and **2** is based on the stabilization of the inhibitor-enzyme intermediate by the introduction of an electron-withdrawing fluorine substituent at the C-2 position, which retards the deglycosylation step.[16]

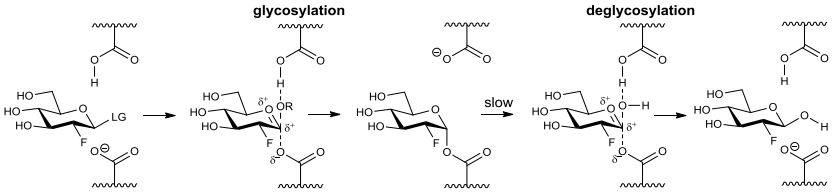


Figure 1: Mechanism-based inhibition of retaining beta-glucosidases with 2-deoxy-2-fluoroglucosides.

The electron-withdrawing fluorine substituent at the C-2 position of the inhibitor also reduces the rate of the formation of the inhibitor-enzyme intermediate. To counterbalance this effect, potent anomeric leaving groups are installed on the inhibitors. The 2-deoxy-2-fluoroglucosides **1** and **2**, provided with dinitrophenol or fluoride as leaving groups, were converted and evaluated as ABPs by Witte *et al.* (Figure 2a). Introduction of an azide at the C-6 of **1** and **2** gave two-step labelling probes **3** and **4**, which in turn were coupled to a BODIPY dye to give the fluorescent labelled ABPs **5** and **6**.[19] These labelled 2-deoxy-2-fluoroglucosides probes completely labelled the glucosidase GBA-1, provided that prolonged reaction times (6 h) and relatively high concentrations were used. Increasing the rate of formation of the inhibitor-enzyme intermediate can be achieved by tuning the ability of the leaving group on the anomeric position of the 2-deoxy-2-fluoro probes. Walvoort *et al*. investigated the influence of the leaving group at the anomeric centre by the synthesis and evaluation of APBs **7-10** (Figure 2b). It was shown that both phosphate probe **9** and imidate probe **10** label GBA-1 more efficiently than probes **5** and **6**. It was also shown that imidate probe **10** is more hydrolytic stabile than phosphate probe **9**.[20] Rempel *et al.* reported the synthesis and evaluation of various 2-deoxy-2-fluorinated glycosides

bearing an dialkyl phosphate or phosphonate as leaving group (**11**-**16**, Figure 2c).[21,22] In agreement with the pKa of the leaving groups it was shown that phosphate probes such as **14** are less hydrolytic stabile than phosphonate probes such as **15**. The β-D-gluco-, β-D-manno- and β-D-galacto-configured phosphonate derivatives function as efficient inhibitors of the corresponding β-D-gluco-, β-D-manno- and β-D-galactosidases. Contrary, the α-D-gluco- and α-D-manno-configured phosphonate derivatives proved to be less efficient covalent inhibitors. The finding that the inhibitory potency of a 2-fluoroglycoside based inhibitor can be fine tuned by varying the nature of the leaving group at the anomeric centre of the inhibitor and the activity of *N*-phenyl trifluoroacetimide imidate **10**, was an incentive to further explore *N*-phenyl trifluoroacetimide imidate ABPs.

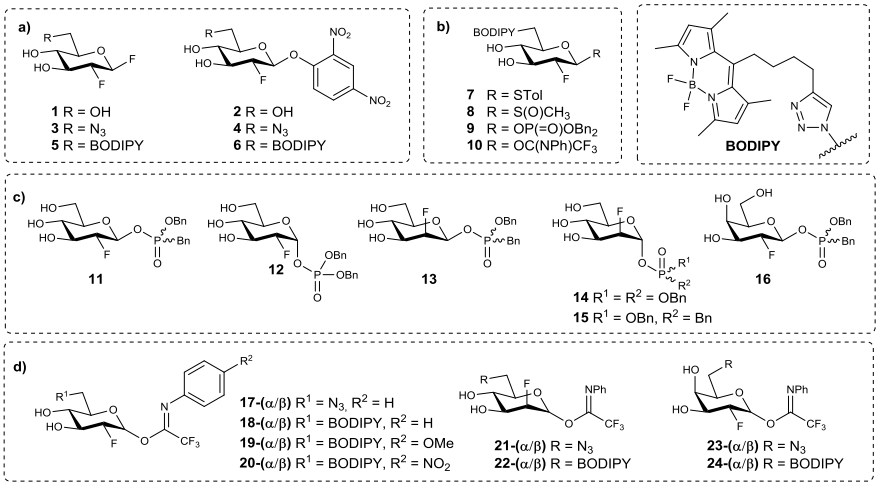


Figure 2: a) First generation 2-deoxy-2-fluoro glucosyl inhibitors **1-2**, modified 2-deoxy-2-fluoro glucosyl ABPs **3-6**. b) 2-Deoxy-2-fluoro glucosyl probes with varying leaving groups. c) Phosphate-/phosphonate- 2-deoxy-2-fluoro glucosyl **11-12**, mannosyl **13-15** and galactosyl **16** inhibitors. d) 2-Deoxy-2-fluoro glucosyl probes bearing various imidate leaving groups **17-20**, 2-deoxy-2-fluoro mannosyl ABPs **21-22**, 2-deoxy-2-fluoro galactosyl ABPs **23-24**.

Because several retaining glycosidases, processing different epimeric glycans following the same two-step mechanism described in Figure 1, are naturally occurring, stereoisomers of known 6-azido-β-D-gluco *N*-phenyl

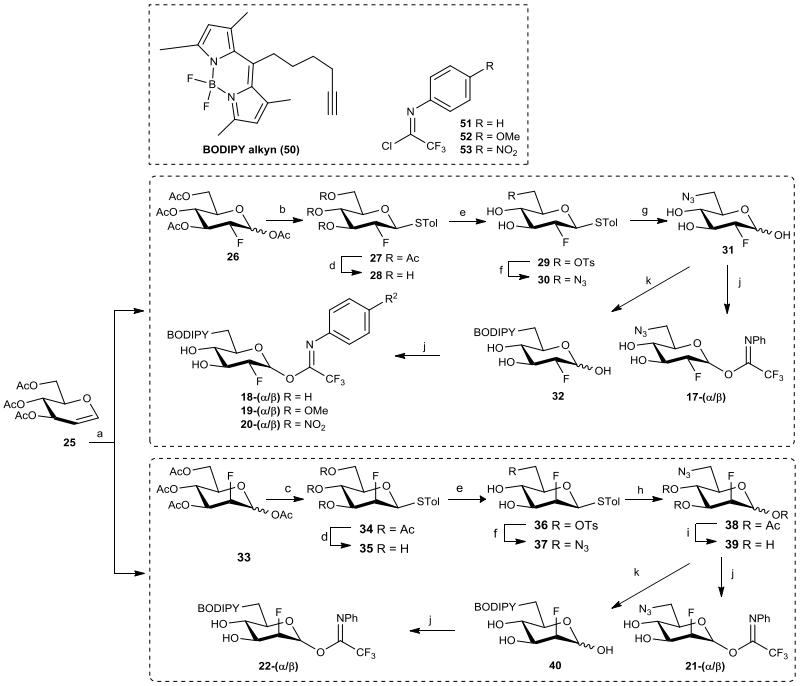
trifluoroacetimide probe **17-β** can potentially function as ABPs. Therefore this chapter describes a study to the synthesis of *N*-phenyl trifluoroacetimide imidate probes to give probes in the α- gluco- (**17-α**), the α-manno- (**21-α**) and the α- (**23-α**) and β-galacto (**23-β**) configuration (Figure 2d). Because the imidate substituent can also be readily adapted, thereby potentially further fine-tuning the reactivity of the probes, also different groups on the imidate nitrogen were explored. An electron donating methoxy substituent and an electron withdrawing nitro substituent were installed on the phenyl ring of the *N*-phenyl trifluoroacetimide imidate ABPs (**19-20**) having either an α- or β-gluco configuration. Some of the prepared 6-azido derivatives (**17**, **21** and **23**) were transformed in the corresponding fluorescently labelled ABPs (**18**, **22** and **24**) by the installation of a BODIPY group.

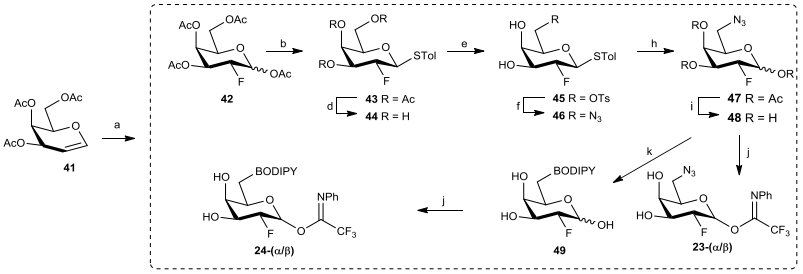
Results and discussion

All *N*-phenyl trifluoroacetimide imidate ABPs (**17-24**) were accessed through a similar route of synthesis, passing by the corresponding thioglycoside precursors (**28, 35** and **44**) as depicted in Scheme 1. Per-acetylated glucal **25** was used as a starting compound for both gluco- and manno-configured target compounds. Using the same procedure as described by Walvoort *et al.*[20] commercially available glucal **25** was treated with Selectfluor® to provide, after anomeric acetylation and column chromatography, 2-fluoro glucose **26** and 2-fluoro mannose **33** in 14% and 28% yield respectively (Scheme 1). The *p*-thiocresol was introduced at the anomeric centre of the gluco-configured **26** by preparing the anomeric bromide and subsequent treatment of this bromide with thiocresol under phase transfer conditions to give, after global deacetylation using NaOMe in MeOH, 2-fluoroglucoside **28**. Selective tosylation of the primairy hydroxyl followed by substitution of the tosylate with an azide yielded thioglucoside **30** in 88% over two steps. To access 2-fluoromannoside **34**, the anomeric acetate in 2-fluoro mannose **33** was first converted into the α-bromide,

which was treated with sodium *p*-thiocresolate to give β-thiomannoside **34**. Deacetylation, selective tosylation and azide substitution as described for the gluco-configured epimer, gave 6-azido thiomannoside **37** in 38% over five steps. The synthesis of 2-fluoro-6-azido thiogalactoside **46** starts from peracetylated galactal **41** and follows the same sequence of events as described for glucose epimer **30**. The reaction of galactal with Selectfluor® provided only the product with the galactose configuration, as formation of the talo-epimer was not observed. Having the three epimeric 2-fluoro-6-azido thioglycosides (**30**, **37** and **46**) in hand the syntheses of the respective two- step ABPs (**17**, **21** and **23**) and the BODIPY labelled ABPs (**18**-**20**, **22** and **24**) were undertaken.

Scheme 1: Synthesis of 2-deoxy-2-fluoro glycosyl ABPs **17-23**.





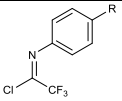
Reagents and conditions: (a) *i*. Selectfluor®, $MeNO_2/H_2O$, (5:1); *ii*. Ac_2O, pyridine 0 °C to rt **26**: 14%, **33**: 28%, **42**: 66%; (b) *i.* HBr (33% in AcOH), DCM, 0 °C to rt; *ii*. *P*-thiocresol, TBABr, KOH, $CHCl_3$, H_2O, 0 °C to rt, **27**: 82%, **43**: 68; (c) *i.* HBr (33% in AcOH), DCM, 0 °C to rt; *ii*. *p*-thiocresol, NaH (60%), DMF, 0 °C to rt, 79%; (d) NaOMe, MeOH, rt, **28**, **35** and **44** quantitatively; (e) TsCl, pyridine, 0 °C to rt, **29**: 88%, **36**: 63%, **45**: 83%; (f) NaN_3, DMF, 80 °C, **30** quantitatively, **37**: 76%, **46**: 74%; (g) NBS, acetone/H_2O (3:1), 0 °C to rt, 68%; (h) *i*. NBS, acetone/H_2O (3:1), 0 °C to rt; *ii*. Ac_2O, pyridine, 0 °C to rt, **38**: 51%, **47**: 32%; (i) NaOMe, MeOH, **39**, **48 (**quantitative); (j) Cs_2CO_3, imidate reagents, acetone, rt (results are summarized in Table 1); (k) 0.075M Sodium ascorbate (aq.), 0.05M $CuSO_4$ (aq.), DMF, **32**: 90%, **40**: 97%, **49**: 90%.

Treatment of 2-fluoro-6-azido thioglucoside **30** with NBS in a mixture of acetone and water gave the corresponding hemiacetal **31**. The same procedure was used to hydrolyse 2-fluoro-6-azido-mannoside **37** and 2-fluoro-6-azido galactoside **46** to their corresponding hemiacetals. Unfortunately the desired products could not be purified by column chromatography. After acetylation of the crude reaction products to give peracetylated 2-fluoro-6-azido mannoside **38** and peracetylated 2-fluoro-6-azido galactose purification could be accomplished. Saponification of **38** and **47** under Zémplen conditions provided 2-fluoro-6-azido mannoside **39** and 2-fluoro-6-azido galactoside **48** in 51% and 32%, respectively, starting from thiomannoside **37** and thiogalactoside **46**.

Next, the obtained 2-fluoro-6-azido glycosides **31**, **39** and **48** were subjected to a Cu-catalyzed azide alkyne cycloaddition with BODIPY alkyne **50**[23] giving 2-fluoro-6-BODIPY glucose **32** (90%), 2-fluoro-6-BODIPY mannose **40** (97%) and 2-fluoro-6-BODIPY galactose **49** (90%). Finally, the imidates were introduced on the 2-fluoro glycosides using the relevant

imidoylchloride reagents (**51-53**) in combination with Cs_2CO_3. The results are summarized in Table 1.

Table 1: Results imidate formation

Entry	Compound	 Imidate reagents	α-product	β-product
1	**31**	R = H (**51**)	3% (**17-α**)	2% (**17-β**)
2	**32**	R = H (**51**)	2% (**18-α**)	<1% (**18-β**)
3	**32**	R = OMe (**52**)	1% (**19-α**)	1% (**19-β**)
4	**32**	R = NO_2 (**53**)	2% (**20-α**)	3% (**20-β**)
5	**39**	R = H (**51**)	10% (**21-α**)	--- (**21-β**)
6	**40**	R = H (**51**)	6% (**22-α**)	--- (**22-β**)
7	**48**	R = H (**51**)	--- (**23-α**)	--- (**23-β**)
8	**49**	R = H (**51**)	--- (**24-α**)	--- (**24-β**)

[a] α/β-ratio determined by ^{1}H-NMR of the crude based on the anomeric signal of the α- and β-product.

The projected imidates prove to be unstable and very sensible towards acid. Purification by HPLC using 100 mM $(NH_4)_2CO_3$ (aq.) as eluens proceeded uneventful but decomposition of both the crude and purified products led to a dramatic loss of product. Decomposition of the purified products could be suppressed by cooling of the collected fractions to -80 °C and immediate lyophilisation. Following this procedure, the treatment of 2-fluoro-6-azido glucoside **31** with *N*-phenyl trifluoroacetimide **51** led to product **17**. The individual α- and β-products were separated by the aid of RP-HPLC yielding

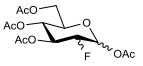
17-α in 3% and **17-β** in 2%. Three different imidates (**51**-**53**) were coupled to BODIPY glucoside **32** (Entry 2-4) leading to the individual α and β anomers (**18**-**20**). All glucosyl imidates were isolated in low yields, ranging from <1% for **18-β** (Entry 2) to 3% for **20-β** (Entry 4). 2-Fluoro-6-azido mannoside **21-α** was obtained as a single anomer from the reaction of **39** with imidate **51** in 10% after HPLC purification (Entry 5). A similar reaction using 2-fluoro-6-BODIPY mannoside **40** gave the α-product **22-α** in a somewhat lower yield of 6% (Entry 6). Finally, the 2-fluoro galactoside **48** and 2-fluoro-6-BODIPY galactoside **49** were subjected to a base mediated reaction with imidate reagent **51**. Unfortunately, TLC and HPLC analysis did not show the formation of the desired products (Entry 7-8). This can be explained by the relatively high instability/reactivity of galactosyl imidates.[24]

Conclusion

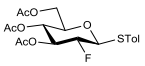
In summary, the synthesis of 2-deoxy-2-fluoro glycoside probes **17**-**22** is described. The probes turned out to be rather unstable and therefore purification was very difficult leading to poor overall yields. Nevertheless six new imidate probes were successfully prepared. In the glucose series, probes having the α- and β-anomeric configuration were obtained. In the manno series only the α-anomers were obtained. Unfortunately the corresponding galactosyl probes could not be obtained. Possibly this is the result of the higher reactivity of galactose probes with respect to the other epimers. The probes that were successfully synthesized can be evaluated for their inhibitory properties on relevant glycosidases (glucosidases, mannosidases) and be probed as possible chaperones, for example to stabilize glucosylcerebrosidase.[25,26,27]

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.

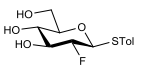


Acetyl 2-deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-α/β-D-glucopyraniside (26) and Acetyl 2-deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-α/β-D-mannopyraniside (33): To a 0 °C solution of acetylated glucal **25** (35.9 g, 131.9 mmol) in nitromethane/H_2O (5:1) (360 mL) was added Selectfluor® (59.8 g, 169 mmol) and the reaction mixture was allowed to warm to rt and stirred overnight. The mixture was heated to 100 °C for 1 h and concentrated *in vacuo*. The concentrate was dissolved in DCM and washed with sat. $NaHCO_3$ (1x), H_2O (1x), brine (1x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was dissolved in pyridine (200 mL) and cooled to 0 °C. To the cooled solution was added dropwise Ac_2O (15 mL) and the mixture was allowed to rt. After completion the reaction was quenched with MeOH and the mixture was concentrated *in vacuo*. The product was dissolved in EtOAc, washed with 1M HCl (aq.) (3x), sat. $NaHCO_3$ (3x), H_2O (3x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo* giving a mixture of 2-deoxy-2-fluoro-glucose **26** and 2-deoxy-2-fluoro-mannose **33**. Purification by column chromatography yielded 2-deoxy-2-fluoro-glucose **26** (6.3 g, 18.0 mmol, 14%) and 2-deoxy-2-fluoro-mannose **33** (10.7 g, 37.6 mmol, 28%) both as a colourless oil. Spectroscopic data were in accordance with known literature data for both compounds.[28]

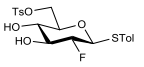


Tolyl 2-deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (27): To a 0 °C cooled solution of 2-deoxy-2-fluoro glucose **26** (3.26 g, 8.79 mmol) in DCM (6 mL) was added dropwise 33% HBr in AcOH (7.6 mL, 44.0 mmol) and the reaction was stirred at 4 °C overnight, followed by stirring for 2h at rt. The mixture was poured in ice-water and diluted with EtOAc. The two phases were separated and the organic phase was washed with H_2O (2x), brine (2x), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude bromide was taken up in $CHCl_3$ (10 mL) and to the solution was added *p*-thiocresol (1.64 g, 13.2 mmol) and a solution of TBABr (0.567 g, 1.76 mmol) in H_2O (11.9 mL). The mixture was cooled to 0 °C and under vigorous stirring was added

dropwise a KOH (1.0 g, 17.6 mmol) solution in H_2O (11.9 mL) over a period of 10 minutes. The reaction mixture was allowed to warm to rt and was vigorously stirred overnight. The two phases were separated and the organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded peracetylated 2-deoxy-2-fluoro-thio glucoside **27** as a white amorphous solid (2.97 g, 7.17 mmol, 82%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46 (d, *J* = 8.4 Hz, 2H), 7.15 (d, *J* = 8.0 Hz, 2H), 5.31 (dt, , *J* = 14.0, 9.6 Hz, 1H), 4.93 (t, *J* = 10.0 Hz, 1H), 4.62 (dd, *J* = 9.6, 1.6 Hz, 1H), 4.16-4.22 (m, 2H), 4.11 (dt, *J* = 46.4, 9.6 Hz, 1H), 3.72 (ddd, *J* = 10.0, 4.4, 3.2 Hz, 1H), 2.38 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 170.1, 169.7, 139.5, 135.0, 129.9, 126.1, 87.0, 84.4, 75.9, 74.0 (d, *J* = 20 Hz), 68.1 (d, *J* = 7 Hz), 62.1, 21.4, 20.9, 20.8, 20.7; HRMS: $[M+H]^+$ calculated for $C_{19}H_{24}FO_7S$ 415.12213, found 415.12207.

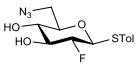


Tolyl 2-deoxy-2-fluoro-1-thio-β-D-glucopyranoside (28): To a solution of **27** (1.53 g, 3.70 mmol) in MeOH (30 mL) was added NaOMe (200 mg, 3.7 mmol) and stirred for 3 h. The reaction was quenched with Amberlite-H^+ IR-120 till pH≤7, filtered and concentrated *in vacuo* yielding 2-deoxy-2-fluoro thio glucose **28** as a white amorphous solid without further purification (1.07 g, 3.70 mmol, quantitatively). Spectroscopic data were in accordance with known literature data.[19]

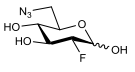


Tolyl 2-deoxy-2-fluoro-6-*O*-tosyl-1-thio-β-D-glucopyranoside (29): To a 0 °C cooled solution of 2-deoxy-2-fluoro thio glucose **28** (634 mg, 2.2 mmol) in pyridine (11 mL) was added TsCl (641 mg, 2.4 mmol), the mixture was allowed to warm to rt and stirred overnight. The reaction was quenched with MeOH and concentrated *in vacuo* followed by co-evaprated with toluene (3x) of the crude. Purification by column chromatography yielded tosylated 2-deoxy-2-fluoro thio glucose **29** as a white amorphous solid (859 mg, 1.94 mmol, 88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (d, *J* = 8.4 Hz, 2H), 7.35-7.40 (m, 4H), 7.09 (d, *J* = 8.0

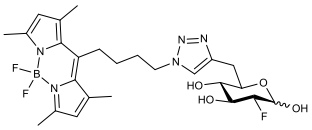
Hz, 2H), 4.53 (dd, *J* = 9.6, 1.6 Hz, 1H), 4.30 (s, 2H), 3.97 (dt, J = 49.6, 8.8 Hz, 1H), 3.70-3.78 (m, 1H), 3.49 (d, *J* = 4.8 Hz, 2H), 2.45 (s, 3H), 2.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 145.3, 139.1, 134.4, 132.7, 130.1, 129.9, 128.2, 126.9, 89.3 (d, *J* = 186 Hz), 84.5 (d, *J* = 24 Hz), 76.8, 76.4 (d, *J* = 19 Hz), 69.1 (d, *J* = 8 Hz), 68.3, 21.8, 21.4.



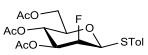
Tolyl 6-azido-2,6-di-deoxy-2-fluoro-1-thio-β-D-glucopyranoside (30): To a solution of tosylated glucose **29** (0.929 g, 2.1 mmol) in DMF (25 mL) was added NaN_3 (0.410 g, 6.3 mmol) and the mixture was stirred overnight at 80 °C. The reaction mixture was diluted with EtOAc and the product was washed with sat. $NaHCO_3$ (aq.) (2x), H_2O (2x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded 6-azido-2-deoxy-2-fluoro thio glucose **30** as a colourless amorphous solid (0.651 g, 2.1 mmol, quantitatively). Spectroscopic data were in accordance with known literature data.[19]



6-Azido-2,6-dideoxy-2-fluoro-α/β-D-glucopyranose (31): To a 0 °C cooled solution of 6-azido-2-deoxy-2-fluoro thio glucose **30** (0.392 g, 1.25 mmol) in a acetone/H_2O mixture (3:1, 12.5 mL) was added NBS (1.33 g, 7.5 mmol). The reaction mixture was allowed to warm to rt and was stirred overnight. During the reaction the mixture turned from orange to a colorless clear solution. The reaction was quenched with 10% $Na_2S_2O_3$ (aq.) and diluted with brine. The water layer was extracted with EtOAc (5x) and the combined organic layers were washed with brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded deprotected 6-azido-2-deoxy-2-fluoro glucose **31** as a white amorphous solid. Spectroscopic data were in accordance with known literature data.[19]

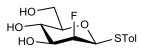


BODIPY 2-fluoro glucoside (32): Deprotected 6-azido-2-deoxy-2-fluoro glucose **31** (0.142 g, 0.687 mmol) was dissolved in DMF (55 mL) and the solution was purged with argon for 30 min. To the solution was added a 0.075 M sodium ascorbate solution (aq.) (6.87 mL, 0.52 mmol), a 0.05M $CuSO_4$ (aq.) (6.87 mL, 0.34 mmol) and the reaction was stirred for 2h. The mixture was taken up in brine and the product was extracted with EtOAc (2x). The combined organic layers were washed with brine (3x) dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded 2-deoxy-2-fluoro BODIPY glucose **32** as an orange solid (0.332 g, 0.619 mmol, 90%). LC-MS: R_t 6.55 min (C_{18} column, linear gradient 10 → 90% B in 15 min). Spectroscopic data were in accordance with known literature data.[19]

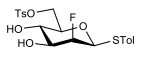


Tolyl 2-deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-1-thio-β-D-mannopyranoside (34): To a 0 °C cooled solution of 2-deoxy-2-fluoro mannose **33** (7.32 g, 20.9 mmol) in dry DCM (14 mL) was added 33% HBr in AcOH (18 mL, 60 mmol) dropwise. Ac_2O (0.2 mL, 2.2 mmol) was added and the mixture was allowed to warm to rt and stirred overnight. The reaction was quenched with ice water and the product extracted with EtOAc (3x). The combined organic layers were washed with sat. $NaHCO_3$ (3x), H_2O (3x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude bromide was dissolved in DMF (42 mL) and *p*-thiocresol (3.89 g, 31.45 mmol) was added to the solution. The mixture was cooled to 0 °C and to the cooled mixture was added 60% NaH (1.05 g, 26.13 mmol) in small portions. The mixture was allowed to warm to rt and stirred overnight. The reaction was quenched with 0.02M HCl (aq.) and taken up in EtOAc. The two phases were separated and the organic phase was washed with sat. $NaHCO_3$ (aq.) (1x), H_2O (3x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded 2-deoxy-2-fluoro-β-thio mannose **34** as a white

amorphous solid (6.9 g, 16.6 mmol, 79%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.38 (t, *J* = 10.0 Hz, 1H), 5.06 (dd, *J* = 47.2, 2.4 Hz, 1H), 4.91-5.04 (m, 1H), 4.79 (d, *J* = 26.4 Hz, 1H), 4.27 (dd, *J* = 12.4, 5.6 Hz, 1H), 4.16 (dd, *J* = 12.4, 2.8 Hz, 1H), 3.64-3.69 (m, 1H), 2.35 (s, 3H), 2.03-2.12 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9, 170.5, 169.6, 138.7, 132.8, 130.0, 129.5, 89.0 (d, *J* = 186 Hz), 85.8 (d, *J* = 18 Hz), 76.4, 72.5 (d, *J* = 18 Hz), 65.7, 62.6, 21.3, 20.9, 20.9, 20.8; FT-IR: ν_{max} (neat)/cm^{-1} 1742, 1368, 1218, 1092, 1049, 960, 916, 834, 811, 776; HRMS: $[M+H]^+$ calculated for $C_{19}H_{24}FO_7S$ 415.12213, found 415.12281.

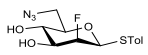


Tolyl 2-deoxy-2-fluoro-1-thio-β-D-mannopyranoside (35): To a solution of **34** (860 mg, 2.1 mmol) in MeOH (20 mL) was added NaOMe (0.108 g, 2.0 mmol) and stirred overnight. The reaction was quenched with Amberlite-H^+ IR-120 till pH≤7, filtered and concentrated *in vacuo* yielding 2-deoxy-2-fluoro thio mannose **35** as a white amorphous solid without further purification (606 mg, 2.1 mmol, quantitatively). ^{1}H NMR (400 MHz, MeOD): δ 4.99 (d, *J* = 28 Hz, 1H), 4.86 (dd, *J* = 49.2, 2.8 Hz, 1H), 3.89 (dd, *J* = 12.0, 2.4 Hz, 1H), 3.71 (dd, *J* = 12.0, 6.0 Hz, 1H), 3.55-3.66 (m, 2H), 3.28-3.35 (m, 1H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, MeOD): δ 138.6, 132.1, 130.8, 93.9 (d, *J* = 181 Hz), 86.7 (d, *J* = 18 Hz), 82.5, 74.8 (d, *J* = 18 Hz), 68.4 (d, *J* = 8 Hz), 62.8, 21.1; FT-IR: ν_{max} (neat)/cm^{-1} 3357, 1493, 1090, 1058, 1005, 849, 805, 766, 689, 487; HRMS: $[M+H]^+$ calculated for $C_{13}H_{18}FO_4S$: 289.34406, found 289.34409.

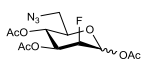


Tolyl 2-deoxy-2-fluoro-6-*O*-tosyl-1-thio-β-D-mannopyranoside (36): To a 0 °C cooled solution of 2-deoxy-2-fluoro thio mannose **35** (606 mg, 2.1 mmol) in pyridine (10.5 mL) was added TsCl (0.478 g, 2.51 mmol), the mixture was allowed to warm to rt and stirred overnight. The reaction was quenched with MeOH and concentrated *in vacuo* followed by co-evaprated with toluene (3x) of the crude. Purification by column chromatography yielded tosylated 2-deoxy-2-fluoro thio mannose **36** as a white amorphous solid (0.592 g, 1.34 mmol, 63%). ^{1}H

NMR (400 MHz, $CDCl_3$): δ 7.82 (d, *J* = 8.0 Hz, 2H), 7.33-7.38 (m, 4H), 7.10 (d, *J* = 8.0 Hz, 2H), 4.94 (dd, *J* = 49.2, 2.4 Hz, 1H), 4.72 (d, *J* = 28.4 Hz, 1H), 4.37 (d, *J* = 11.2 Hz, 1H), 4.32 (dd, *J* = 11.2, 5.4 Hz, 1H), 3.78 (t, *J* = 9.4 Hz, 1H), 3.59 (ddd, *J* = 27.2, 9.6, 2.6 Hz, 1H), 3.45-3.48 (m, 1H), 2.43 (s, 3H,), 2.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 145.3, 139.1, 134.4, 132.7, 130.1, 129.9, 128.2, 126.9, 89.3 (d, *J* = 186 Hz), 84.5 (d, *J* = 24 Hz), 76.8, 76.4 (d, *J* = 19 Hz), 69.1 (d, *J* = 8 Hz), 68.3, 21.8, 21.4. ;FT-IR: ν_{max} (neat)/cm^{-1} 3367, 1494, 1358, 1190, 1175, 1079, 983, 946, 810, 687, 760.

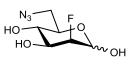


Tolyl 6-azido-2,6-di-deoxy-2-fluoro-1-thio-β-D-mannopyranoside (37): To a solution of tosylated mannose **36** (0.593 g, 1.34 mmol) in DMF (13.4 mL) was added NaN_3 (260 mg, 4.0 mmol) and the mixture was stirred overnight at 80 °C. The reaction mixture was diluted with EtOAc and the product was washed with sat. $NaHCO_3$ (aq.) (2x), H_2O (2x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded 6-azido-2-deoxy-2-fluoro thio mannose **37** as a colourless amorphous solid (318 mg, 1.0 mmol, 76%). ^1H NMR (400 MHz, MeOD): δ 7.42 (d, *J* = 8.0 Hz, 2H), 7.14 (d, *J* = 8.0 Hz, 2H), 4.97 (d, *J* = 28.0 Hz, 1H), 4.86 (dd, *J* = 50.0, 2.0 Hz, 1H), 3.37-3.64 (m, 5H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, MeOD): δ 139, 132.9, 131.8, 130.7, 93.7 (d, *J* = 181 Hz), 86.8 (d, *J* = 18 Hz), 80.8, 74.6 (d, *J* = 18 Hz), 69.1, 52.9, 21.1; FT-IR: ν_{max} (neat)/cm^{-1} 3356, 2093, 1493, 1278, 1060, 1018, 998, 982, 951, 869, 843, 807, 767, 689, 573; HRMS $[M+H]^+$ calculated for $C_6H_{10}FN_3O_3$ 191.07007, found 191.07010.



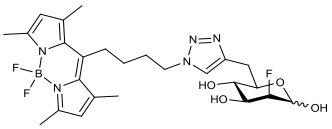
Acetyl 6-azido-2,6-dideoxy-2-fluoro-3,4-di-*O*-acetyl-α/β-D-mannopyraniside (38): To a 0 °C cooled solution of 6-azido-2-deoxy-2-fluoro thio mannose **37** (305 mg, 1.0 mmol) in a acetone/H_2O mixture (3:1, 12.5 mL) was added NBS (1.33 g, 7.5 mmol). The reaction mixture was allowed to warm to rt and was stirred overnight. During the reaction the mixture turned from orange to a colourless clear solution. The

reaction was quenched with 10% $Na_2S_2O_3$ (aq.) and diluted with brine. The product was extracted with EtOAc (5x) and the combined organic layers were washed with brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The concentrate was taken up in pyridine (4 mL), cooled to 0 °C and Ac_2O (1.0 mL) was added to the cooled solution. The mixture was allowed to warm to rt and stirred overnight. The reaction was quenched with MeOH, concentrated *in vacuo* and dissolved in EtOAc. The product was washed with 1M HCl (aq.) (2x), sat. $NaHCO_3$(aq.) (1x), H_2O (3x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded acetylated 6-azido-2-deoxy-2-fluoro mannose **38** as a colourless oil (0.165 g, 0.495 mmol, 51%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.28 (d, *J* = 6.8 Hz, 1H), 5.36 (t, *J* = 10.0 Hz, 1H), 5.27 (ddd, *J* = 28.0, 10.0, 2.0 Hz, 1H), 4.77 (dd, *J* = 48.8, 1.8 Hz, 1H), 4.00-4.04 (m, 1H), 3.33-3.42 (m, 2H), 2.19 (s, 3H), 2.12 (s, 3H), 2.07 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 169.4, 168.0, 89.9 (d, *J* = 31 Hz), 86.0 (d, *J* = 181 Hz), 71.9, 69.3 (d, *J* = 16 Hz), 66.3, 50.8, 20.8, 20.7, 20.6; FT-IR: ν_{max} (neat)/cm^{-1} 2105, 1751, 1372, 1214, 1147, 1050 ,1021, 975, 927, 601; HRMS $[M+H]^+$ calculated for $C_{12}H_{17}FN_3O_7$ 334.10450, found 334.10476.

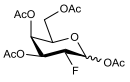


6-Azido-2,6-dideoxy-2-fluoro-α/β-D-mannopyranose (39): To a solution of acetylated 6-azido-2-deoxy-2-fluoro mannose **38** (0.143 g, 0.428 mmol) in MeOH (10 mL) was added NaOMe (4 mg, 0.04 mmol) and stirred overnight. The reaction was quenched with Amberlite-H^+ IR-120 till pH≤7, filtered and concentrated *in vacuo* yielding 6-azido-2-deoxy-2-fluoro mannose **39** as a colorless oil without further purification as an α/β mixture (α/β = 9:1, 87.4 mg, 0.422 mmol, quantitatively). ^{1}H NMR (400 MHz, MeOD): δ 5.22 (dd, *J* = 7.2, 2.0 Hz, 1H-α), 4.83 (d, *J* = 20.0 Hz, 1H-β), 4.60 (dd, *J* = 51.6, 2.2 Hz, 1H-β), 4.56 (dt, *J* = 50.4, 2.2 Hz, 1H-α), 3.87-3.91 (m, 1H, H-α), 3.79 (ddd, *J* = 30.8, 9.6, 2.4 Hz, 1H-α), 3.61 (td, *J* = 9.6, 0.8 Hz, 1H-α), 3.52 (dd, *J* = 13.2, 2.4 Hz, 1H-α), 3.41 (dd, *J* = 13.2, 6.0 Hz, 1H-α); ^{13}C NMR (100 MHz, MeOD): δ 94.3 (d, *J* = 16 Hz, C-β), 93.1 (d, *J* = 33 Hz, C-α), 92.7 (d, *J* = 182 Hz, C-β), 92.1 (d, *J* = 177 Hz, C-α), 73.1

(C-α), 71.2 (d, *J* = 17 Hz, C-α), 69.5 (C-α), 52.7 (C-α); FT-IR: ν_{max} (neat)/cm^{-1} 3354, 2107, 1283, 1064.

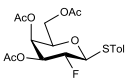


BODIPY 2-fluoro mannoside (40): Deprotected 6-azido-2-deoxy-2-fluoro mannose **39** (32.7 mg, 0.157 mmol) was dissolved in DMF (12 mL) and the solution was purged with argon for 30 min. To the solution was added a 0.075M sodium ascorbate solution (aq.) (1.50 mL, 0.113 mmol), a 0.05M $CuSO_4$ (aq.) (1.50 mL, 0.075 mmol) and the reaction was stirred for 2h. The mixture was taken up in brine and the product was extracted with EtOAc (2x). The combined organic layers were washed with brine (3x) dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded BODIPY 2-deoxy-2-fluoro mannoside **40** as an orange solid (82.0 mg, 0.153 mmol, 97%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (s, 1H), 7.70 (s, 1H), 6.07 (s, 2H), 5.17 (dd, *J* = 7.0, 1.4 Hz, 1H), 4.47-4.89 (m, 3H), 4.07 (ddd, *J* = 9.6, 9.6, 2.0 Hz, 1H), 3.83 (ddd, *J* = 30.8, 9.6, 2.4 Hz, 1H), 3.42 (t, *J* = 9.6 Hz, 1H), 2.80-2.85 (m, 2H), 2.68 (t, *J* = 7.6 Hz, 2H), 2.42 (s, 6H), 2.29 (s, 6H), 1.81 (p, *J* = 7.4 Hz, 2H), 1.56-1.61 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.8, 148.3, 147.9, 142.3, 132.6, 124.6, 122.6, 93.1 (d, *J* = 29 Hz), 91.9 (d, *J* = 174 Hz), 72.2, 71.1, 69.7, 52.3, 32.1, 30.8, 28.9, 25.8, 16.4, 14.5.



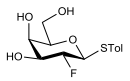
Acetyl 2-deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-α/β-D-galactopyraniside (42): To a 0°C solution of acetylated galactal **41** (7.5 g, 27.5 mmol) in nitromethane/H_2O (5:1) (83 mL) was added Selectfluor® (11.7 g, 33 mmol) and the reaction mixture was allowed to warm to rt and stirred for 70 h. The mixture was heated to 100 °C for 30 min and cooled to rt. The mixture was diluted with brine and extracted with DCM (5x). The combined organic layers were washed with sat. $NaHCO_3$ (1x), H_2O (x), brine (1x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was dissolved in DCM (65 mL) and cooled to 0 °C. To the

cooled solution was added pyridine (4.2 mL), Ac_2O (3.2 mL, 34 mmol) and the mixture was allowed to rt. After stirring overnight, the mixture was cooled to 0 °C and additionally pyridine (3 mL), Ac_2O (2 mL, 21 mmol) was added and the mixture was allowed to warm to rt. After 2 h the reaction was quenched with MeOH and the mixture was concentrated *in vacuo*. The product was dissolved in EtOAc, washed with 1M HCl (aq.) (3x), sat. $NaHCO_3$ (3x), H_2O (3x), brine (2x) dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded 2-deoxy-2-fluoro galactoside **42** as a yellow oil (8.7 g, 18.0 mmol, 66%). Spectroscopic data were in accordance with known literature data.[29]

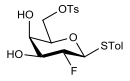


Tolyl 2-deoxy-2-fluoro-3,4,6-tri-*O*-acetyl-1-thio-β-D-galactopyranoside (43): To a 0 °C cooled solution of 2-deoxy-2-fluoro galactoside **42** (3.35 g, 9.56 mmol) in dry DCM (6.4 mL) was added 33% HBr in AcOH (8.2 mL, 9.6 mmol) dropwise. Ac_2O (0.1 mL, 1.1 mmol) was added and the mixture was allowed to warm to rt and stirred overnight. The reaction was quenched with ice water and the product extracted with EtOAc (3x). The combined organic layers were washed with sat. $NaHCO_3$ (3x), H_2O (3x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude bromide was taken up in $CHCl_3$ (100 mL) and to the solution was added *p*-thiocresol (1.8 g, 14.3 mmol) and a solution of TBABr (0.616 g, 1.91 mmol) in H_2O (13.5 mL). The mixture was cooled to 0 °C and under vigorous stirring was added dropwise a KOH (1.1 g, 19.1 mmol) solution in H_2O (13.5 mL) over a period of 10 minutes. The reaction mixture was allowed to warm to rt and was vigorously stirred overnight. The two phases were separated and the organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded peracetylated 2-deoxy-2-fluoro-thio galactoside **43** as a white amorphous solid (2.68 g, 6.46 mmol, 68%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49 (d, *J* = 8.0 Hz, 2H), 7.15 (d, *J* = 7.6 Hz, 2H), 5.43 (s, 1H), 5.12 (ddd, *J* = 13.2, 9.2, 3.6 Hz, 1H), 4.68 (dd, *J* = 10.0, 2.6 Hz, 1H), 4.46 (dt, *J* = 49.6, 9.6 Hz, 1H), 4.18 (dd, *J* = 11.2, 6.8 Hz, 1H), 4.10 (dd, *J* = 11.2,

6.4 Hz, 1H), 2.36 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.1, 170.1, 170.1, 139.1, 134.2, 129.9, 127.4, 85.7 (d, *J* = 187 Hz), 85.6 (d, *J* = 24 Hz), 74.5, 72.2 (d, *J* = 20 Hz), 68.1 (d, *J* = 8 Hz), 61.5, 21.4, 20.8, 20.8, 20.6; HRMS: $[M+H]^+$ calculated for $C_{19}H_{24}FO_7S$ 415.12213, found 415.12222.



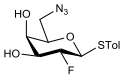
Tolyl 2-deoxy-2-fluoro-1-thio-β-D-galactopyranoside (44): To a solution of (860 mg, 2.1 mmol) in MeOH (20 mL) was added NaOMe (108 mg, 2.0 mmol) and stirred overnight. The reaction was quenched with Amberlite-H^+ IR-120 till pH≤7, filtered and concentrated *in vacuo* yielding 2-deoxy-2-fluoro thio mannose **44** as a white amorphous solid without further purification (597 mg, 2.1 mmol, quantitatively). ^{1}H NMR (400 MHz, MeOD): δ 7.45 (d, *J* = 8.0 Hz, 2H), 7.14 (d, *J* = 8.0 Hz, 2H), 4.66 (dd, *J* = 9.6, 2.0 Hz, 1H), 4.30 (dt, *J* = 50.8 Hz, 1H), 3.92 (t, *J* = 3.2 Hz, 1H), 3.68-3.78 (m, 3H), 3.58 (t, *J* = 6.0 Hz, 1H), 2.32 (s, 3H,); ^{13}C NMR (100 MHz, MeOD): δ 133.9, 130.6, 90.7, 86.7 (d, *J* = 24 Hz,), 80.8, 74.3, 62.4; HRMS: $[M+H]^+$ calculated for $C_{13}H_{18}FO_4S$: 289.34406, found 289.34461.



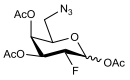
Tolyl 2-deoxy-2-fluoro-6-*O*-tosyl-1-thio-β-D-galactopyranoside (45):

To a 0 °C cooled solution of 2-deoxy-2-fluoro-thio galactoside **44** (0.591 g, 2.05 mmol) in pyridine (10 mL) was added TsCl (0.434 g, 2.25 mmol), the mixture was allowed to warm to rt and stirred overnight. The reaction was quenched with MeOH and concentrated *in vacuo* followed by co-evaprated with toluene (3x) of the crude. Purification by column chromatography yielded tosylated 2-deoxy-2-fluoro-thio galactoside **45** as a white amorphous solid (0.750 g, 1.369 mmol, 83%). ^{1}H NMR (400 MHz, MeOD): δ 7.79 (d, *J* = 8.0 Hz, 2H), 7.41 (d, *J* = 8.0 Hz, 2H), 7.36 (d, *J* = 8.0 Hz, 2H), 7.10 (d, *J* = 8.0 Hz, 2H), 4.61 (d, *J* = 9.2 Hz, 1H), 4.09-4.32 (m, 3H), 3.80-3.84 (m, 2H), 3.72 (ddd, *J* = 14.0, 8.8, 3.6 Hz, 1H), 2.43 (s, 3H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, MeOD): δ 146.6, 139.1, 133.8, 131.1, 130.6, 130.2, 129.1, 90.3

(d, *J* = 182 Hz), 86.1 (d, *J* = 25 Hz), 77.5, 73.9 (d, *J* = 18 Hz), 70.9, 70.9 (d, *J* = 6 Hz), 21.6, 21.1.

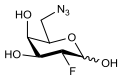


Tolyl 6-azido-2,6-di-deoxy-2-fluoro-1-thio-β-D-galactopyranoside (46): To a solution of tosylated galactoside **45** (650 mg, 1.5 mmol) in DMF (20 mL) was added NaN_3 (390 mg, 6.0 mmol) and the mixture was stirred overnight at 80 °C. The reaction mixture was diluted with EtOAc and the product was washed with sat. $NaHCO_3$ (aq.) (2x), H_2O (2x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded 6-azido-2-deoxy-2-fluoro-thio galactoside **46** as a colourless amorphous solid (342 mg, 1.1 mmol, 74%). ^{1}H NMR (400 MHz, MeOD): δ 7.45 (d, *J* = 8.0 Hz2H), 7.14 (d, *J* = 8.0 Hz, 2H), 4.68 (dd, *J* = 9.6, 2.0 Hz, 1H), 4.29 (dt, *J* = 50.4, 9.2 Hz, 1H), 3.69-3.83 (m, 3H), 3.60 (dd, *J* = 12.8, 8.8 Hz, 1H), 3.27-3.31 (m, 1H), 2.32 (s, 3H); ^{13}C- NMR (100 MHz, MeOD): δ 139.4, 134.3, 130.6, 130.1, 90.5 (d, *J* = 182 Hz), 86.8 (d, *J* = 24 Hz), 79.1 (C-5), 74.1 (d, *J* = 18 Hz), 71.4 (d, *J* = 9 Hz), 52.6, 21.1; HRMS: $[M+H]^+$ calculated for $C_{13}H_{17}FN_3O_3S$ 314.09692, found 314.09701.

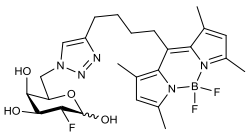


Acetyl 6-azido-2,6-dideoxy-2-fluoro-3,4-di-*O*-acetyl-α/β-D-galactopyraniside (47): To a 0 °C cooled solution of 6-azido-2-deoxy-2-fluoro thio galactoside **46** (325 mg, 1.0 mmol) in a acetone/H_2O mixture (3:1, 10.3 mL) was added NBS (1.1 g, 6.2 mmol). The reaction mixture was allowed to warm to rt and was stirred overnight. During the reaction the mixture turned from orange to a colourless clear solution. The reaction was quenched with 10% $Na_2S_2O_3$ (aq.) and diluted with brine. The product was extracted with EtOAc (5x) and the combined organic layers were washed with brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The concentrate was taken up in pyridine (4 mL), cooled to 0 °C and Ac_2O (1.0 mL) was added to the cooled solution. The mixture was allowed to warm to rt and stirred overnight. The reaction was quenched with MeOH, concentrated *in vacuo* and dissolved in EtOAc. The product was washed

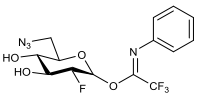
with 1M HCl (aq.) (2x), sat. $NaHCO_3$(aq.) (1x), H_2O (3x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded acetylated 6-azido-2-fluoro galactoside **47** as a colourless oil (0.110 g, 0.329 mmol, 32%). ^{1}H NMR (400 MHz, MeOD): δ 6.49 (d, *J* = 4.0 Hz, 1H-α), 5.83 (d, *J* = 8.0, 4.0 Hz, 1H-β), 5.51 (t, *J* = 3.0 Hz, 1H-α), 5.43-5.45 (m, 1H, H-β), 5.39 (dd, *J* = 10.8, 3.6 Hz, 1H-α), 5.20 (ddd, *J* = 13.4, 9.6, 3.6 Hz, 1H-β), 4.90 (ddd, *J* = 49.2, 10.4, 4.0 Hz, 1H-α), 4.66 (ddd, *J* = 51.6, 9.6, 8.0 Hz, 1H-β), 4.21 (t, *J* = 6.4 Hz,H-α), 4.00 (t, *J* = 6.4 Hz, 1H-β), 3.51 (dd, *J* = 12.8, 7.2 Hz, 1H-β), 3.43 (dd, *J* = 12.8, 7.2 Hz, 1H-α), 3.20-3.25 (m, 2H); ^{13}C NMR (100 MHz, MeOD): δ 170.1, 170.0, 169.8, 168.8, 168.8, 91.6 (d, *J* = 25 Hz), 88.9 (d, *J* = 23 Hz), 86.7 (d, *J* = 187 Hz), 84.1 (d, *J* = 190 Hz), 73.7, 71.0 (d, *J* = 27 Hz), 70.9, 68.2, 68.2, 50.2, 50.0, 20.9, 20.8, 20.7, 20.6, 20.6; HRMS $[M+H]^+$ calculated for $C_{12}H_{17}FN_3O_7$ 334.10450, found 334.10444.



6-Azido-2,6-dideoxy-2-fluoro-α/β-D-galactopyranose (48): To a solution of acetylated 6-azido-2-deoxy-2-fluoro galactoside **47** (0.110 g, 0.329 mmol) in MeOH (10 mL) was added NaOMe (3 mg, 0.06 mmol) and stirred overnight. The reaction was quenched with Amberlite-H^+ IR-120 till pH≤7, filtered and concentrated *in vacuo* yielding 6-azido-2-fluoro galactoside **48** as a colourless oil without further purification (68.1 mg, 0.33 mmol, quantitatively). ^{1}H NMR (400 MHz, MeOD): δ 5.32 (d, *J* = 4.0 Hz, 1H-α), 4.68 (dd, *J* = 7.6, 3.2 Hz, 1H-β), 4.65 (ddd, *J* = 50.4, 10.0, 4.0 Hz, 1H-α), 4.25 (ddd, *J* = 52.0, 9.2, 3.6 Hz, H-β), 4.13-4.17 (m, 1H), 3.98-4.05 (m, 1H-α), 3.86 (dt, *J* = 3.6, 1.2 Hz, 1H-α), 3.80 (dt, *J* = 3.8, 1.0 Hz, 1H-β), 3.68-3.76 (m, 2H), 3.49-3.60 (m, 2H), 3.29-3.42 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 96.1 (d, *J* = 24 Hz), 94.0 (d, *J* = 180 Hz), 91.8 (d, *J* = 22 Hz), 90.4 (d, *J* = 183 Hz), 75.2, 73.2 (d, *J* = 17 Hz), 72.0 (d, *J* = 8 Hz), 71.3 (d, *J* = 9 Hz), 70.4, 69.0 (d, *J* = 17 Hz), 52.4, 52.4.

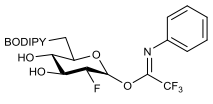


BODIPY 2-fluoro galactoside (49): Deprotected 6-azido-2-deoxy-2-fluoro galactoside **48** (33.6 mg, 0.16 mmol) was dissolved in DMF (12 mL) and the solution was purged with argon for 30 min. To the solution was added a 0.075M sodium ascorbate solution (aq.) (1.5 mL, 0.11 mmol), a 0.05M $CuSO_4$ (aq.) (1.5 mL, 0.075 mmol) and the reaction was stirred for 1h. The mixture was taken up in brine and the product was extracted with EtOAc (2x). The combined organic layers were washed with brine (3x) dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded BODIPY 2-deoxy-2-fluoro galactoside **49** as an orange solid (85.3 mg, 0.16 mmol, 98%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 (s, 1H), 7.69 (s, 1H), 6.07 (s, 4H), 5.26 (d, *J* = 3.6 Hz, 1H-α), 4.42-4.68 (m, 6H), 4.40 (t, *J* = 6.8 Hz, 1H-β), 4.30 (ddd, *J* = 52, 9.2, 8.0 Hz, 1H-β), 3.96-4.06 (m, 2H), 3.90 (t, *J* = 3.0 Hz, 1H-α), 3.83 (t, *J* = 2.8 Hz, 1H-β), 3.71-3.77 (m, 1H), 2.80-2.84 (m, 4H), 2.68 (t, *J* = 7.4 Hz, 2H), 2.41 (s, 12H), 2.28 (s, 12H), 1.81 (p, *J* = 7.5 Hz, 4H), 1.52-1.60 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.8, 148.4, 147.9, 142.3, 132.6, 124.3, 124.2, 122.6, 96.0 (d, *J* = 23 Hz), 93.8 (d, *J* = 179 Hz), 91.8 (d, *J* = 22 Hz), 90.3 (d, *J* = 183 Hz), 74.8, 72.9 (d, *J* = 17 Hz), 71.9 (d, *J* = 8 Hz), 71.3 (d, *J* = 9 Hz), 70.2, 68.9 (d, *J* = 18 Hz), 52.1, 52.0, 32.1, 30.8, 28.9, 25.8, 16.4, 14.5.



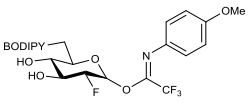
α/β-6-Azido-2-fluoro-glucosyl imidate (17): To a 0 °C cooled solution of 6-azido-2-deoxy-2-fluoro glucosyl **31** (26.2 mg, 126 µmol) in acetone (6 mL) was added trifluoro aniline imidate **51** (52.5 mg, 253 µmol) and Cs_2CO_3 (100 mg, 280 µmol). The reaction was gradually warmed to rt and stirred overnight. The solids were filtered and the filtrate was concentrated *in vacuo* Purificaton by column chromatography yielded glucose aniline imidate **17** as a α/β mixture (α/β = 3:2, 1.08 mg, 2.86 µmol, 2%). Spectroscopic data for the α-anomer **17-α**: ^{1}H NMR (CD_3CN, 600 MHz): δ 7.31 (t, 2H, *J* = 7.8 Hz), 7.12 (t, 1H, *J* = 7.2 Hz), 6.85 (d, 2H, *J* = 7.2 Hz), 6.41 (bs, 1H), 4.50 (d, 1H, *J* = 48.0 Hz), 3.77-3.82 (m, 2H), 3.51-3.58 (m, 1H), 3.36-3.41 (m, 2H); LC-MS: R_t 7.68

min (C18 column, linear gradient 10 → 90% B in 15 min); FT-IR: ν_{max} (neat)/cm^{-1} 3352, 2104, 1720, 1312, 1211, 1155, 1116, 1031, 117, 695.
Spectroscopic data for the β-anomer **17-β**: ^{1}H NMR (CD_3CN, 850 MHz): δ 7.31 (t, 2H, *J* = 7.7 Hz), 7.13 (t, 1H, *J* = 7.2 Hz), 6.87 (bs, 2H), 5.92 (bs, 1H), 4.31(d, 1H, *J* = 50.2 Hz), 3.50-3.75 (m, 2H), 3.41-3.44 (m, 2H), 3.33-3.39 (m, 1H); LC-MS: R_t 8.18 min (C18 column, linear gradient 10 → 90% B in 15 min); FT-IR: ν_{max} (neat)/cm^{-1} 3356, 2103, 1721, 1316, 1212, 1163, 1001, 695.



α/β-2-Fluoro-BODIPY-glucosyl imidate (18): To a 0 °C cooled solution of BODIPY 2-deoxy-2-fluoro glucosyl **32** (51.1 mg, 95.4 µmol) in acetone (6 mL) was added trifluoro aniline imidate **51** (39.6 mg, 191 µmol) and Cs_2CO_3 (47.0 mg, 140 µmol). The reaction was gradually warmed to rt and stirred overnight. The solids were filtered and the filtrate was concentrated *in vacuo* Purificaton by column chromatography yielded glucose aniline imidate **18** as a α/β mixture (α/β = 1:2, 22.3 mg, 31.6 µmol, 33%) . Purification by RP-HPLC followed by lyophilisation yielded α-2-deoxy-2-fluoro-BODIPY-glucosyl-aniline-imidate **18-α** (1.63 mg, 2.30 µmol, 2%) and β-2-deoxy-2-fluoro-BODIPY-glucosyl-aniline imidate **18-β** (97 µg, 0.14 µmol, 0.1%) both as an orange powder. Spectroscopic data for the α-anomer **18-α**: ^{1}H NMR (CD_3CN, 600 MHz): δ 7.50 (s, 1H, CH_{arom} triazole), 7.27 (t, 2H, *J* = 8.1 Hz, CH_{arom} phenyl), 7.09 (t, 1H, *J* = 7.5 Hz, CH_{arom} phenyl), 6.55 (d, 2H, *J* = 7.2 Hz, CH_{arom} phenyl), 6.26 (bs, 1H, H-1), 6.14 (s, 2H, CH_{arom} pyrrole), 4.72 (d, 1H, *J* = 13.8 Hz, H-6), 4.40-4.48 (m, 2H, H-2, H-6), 3.87-4.01 (m, 4H, H-3, H-5, OH), 3.26 (bs, 1H, H-4), 2.97 (t, 2H, *J* = 8.7 Hz, CH_2), 2.67-2.77 (m, 2H, CH_2), 2.43 (s, 6H, CH_3), 2.37 (s, 6H, CH_3), 1.81-1.84 (m, 2H, CH_2), 1.52-1.76 (m, 2H, CH_2); LC-MS: R_t 9.80 min (C18 column, linear gradient 10 → 90% B in 15 min); FT-IR: ν_{max} (neat)/cm^{-1} 3383, 1719, 1550, 1510, 1310, 1203, 1159, 1075, 986. HRMS $[M + H]^+$ calculated for $C_{33}H_{37}BF_6N_6O_4$: 707.29463, found 707.29547.

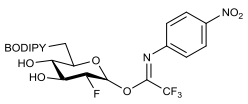
Spectroscopic data for the β-anomer **18-β**: NMR (CD_3CN, 600 MHz): δ 7.57 (s, 1H, CH_{arom} triazole), 7.26 (t, 2H, J = 7.8 Hz, CH_{arom} phenyl), 7.08 (t, 1H, J = 7.5 Hz, CH_{arom} phenyl), 6.70 (t, 2H, J = 7.2 Hz, CH_{arom} phenyl), 6.15 (s, 2H, CH_{arom} pyrrole), 5.66 (bs, 1H, C-1), 4.79 (d, 1H, J = 14.4 Hz, H-6), 4.32-4.36 (m, 2H, H-2, H-6), 4.06 (bs, 1H, OH), 3.96 (bs, 1H, OH), 3.71 (bs, 2H, H-3, H-5), 3.33 (bs, 1H, H-4), 2.92 (t, 2H, J = 8.4 Hz, CH_2), 2.54-2.61 (m, 2H, CH_2), 2.44 (s, 6H, CH_3), 2.34 (s, 6H, CH_3), 1.70-1.75 (m, 2H, CH_2), 1.52-1.54 (m, 2H, CH_2); HRMS $[M + H]^+$ calculated for $C_{33}H_{37}BF_6N_6O_4$: 707.29463, found 707.29547.



α/β-2-Flouro-BODIPY-glucosyl imidate (19): To a 0 °C cooled solution of 2-deoxy-2-fluoro BODIPY glucose **32** (53.2 mg, 99.4 µmol) in acetone (6 mL) was added trifluoro *p*OMe-aniline imidate reagens **52** (47.2 mg, 199 µmol) and Cs_2CO_3 (48.9 mg, 150 µmol). The reaction was gradually warmed to rt and stirred overnight. The solids were filtered and the filtrate was concentrated *in vacuo* Purificaton by column chromatography yielded *p*OMe-aniline imidate glucosyl **19** as a α/β mixture (α/β = 1:2, 19.8 mg, 26.7 µmol, 27%). Purification by RP- followed by lyophilisation yielded α-2-fluoro-BODIPY-glucose-*p*OMe-aniline-imidate **19-α** (0.374 mg, 0.51 µmol, 0.5%) and β-2-fluoro-BODIPYglucose-*p*OMe-aniline imidate **19-b** (1.03 mg, 1.4 µmol, 1.4%) both as an orange powder. Spectroscopic data for the α-anomer **19-α**: ^{1}H NMR (600 MHz, CD_3CN): δ 7.49 (s, 1H), 6.82 (d, J = 9.0 Hz, 2H), 6.60 (bs, 2H), 6.23 (bs, 1H), 6.14 (s, 2H), 4.71 (d, J = 14.4 Hz, 1H), 4.37-4.46 (m, 2H), 3.88-3.95 (m, 4H), 3.71 (s, 3H), 3.26 (bs, 1H), 2.97 (t, J = 8.7 Hz, 2H), 2.66-2.78 (m, 2H), 2.43 (s, 6H), 2.37 (s, 6H), 1.80-1.85 (m, 2H), 1.52-1.59 (m, 2H); LC-MS: R_t 3.92 min (C18 column, linear gradient 50 → 90% B in 15 min); FT-IR: ν_{max} (neat)/cm^{-1} 3356, 2925, 1551, 1508, 1203, 1159, 1067; HRMS $[M + H]^+$ calculated for $C_{34}H_{39}BF_6N_6O_5$: 737.30519, found 737.30615.

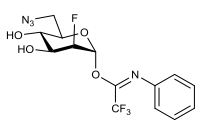
Spectroscopic data for the β-anomer **19-β**: NMR (600 MHz, CD_3CN): δ 7.55 (s, 1H,), 6.81 (d, J = 6.6 Hz, 2H), 6.67 (bs, 2H), 6.15 (s, 2H), 5.65 (bs, 1H),

4.79 (d, *J* = 14.4 Hz, 1H), 4.31-4.35 (m, 2H), 4.05 (bs, 1H), 3.94 (bs), 3.68 (bs 5H), 3.32 (bs, 1H), 2.91 (t, *J* = 8.4 Hz, 2H), 2.54-2.59 (m, 2H), 2.44 (s, 6H), 2.34 (s, 6H), 1.74 (bs, 2H), 1.51-1.54 (m, 2H); LC-MS: R_t 4.14 min (C18 column, linear gradient 50 → 90% B in 15 min); LC-MS: R_t 9.73 min (C18 column, linear gradient 10 → 90% B in 15 min); FT-IR: ν_{max} (neat)/cm^{-1} 3356, 2971, 1551, 1508, 1409, 1310, 1203, 1160, 1080, 986, 836; HRMS $[M + H]^+$ calculated for $C_{34}H_{39}BF_6N_6O_5$: 737.30519, found 737.30613.

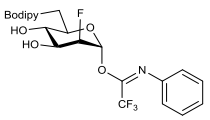


α/β-2-Fluoro-BODIPY-glucosyl imidate (20): To a 0 °C cooled solution of 2-deoxy-2-fluoro BODIPY glucose **32** (75.1 mg, 140 µmol) in acetone (6 mL) was added trifluoro *p*NO_2 aniline imidate **53** (70.7 mg, 240 µmol) and Cs_2CO_3 (68.2 mg, 210 µmol). The reaction was gradually warmed to rt and stirred overnight. The solids were filtered and the filtrate was concentrated *in vacuo*. Purification by column chromatography yielded glucosyl *p*OMe-aniline imidate **20** as an α/β mixture (α/β = 2:3, 47.5 mg, 63.2 µmol, 45%) . Purification by RP-HPLC followed by lyophilisation yielded α-2-deoxy-2-fluoro-BODIPY-glucosyl-*p*NO_2-aniline-imidate **20-α** (2.52 mg, 3.35 µmol, 2%) and β-2-deoxy-2-fluoro-BODIPY-glucosyl-*p*NO_2-aniline imidate **20-β** (3.03 mg, 4.03 µmol, 2.9%) both as an orange powder. Spectroscopic data for the α-anomer **20-α**: NMR (CD_3CN, 600 MHz): δ 8.06 (dd, 2H, *J* = 6.9, 2.1 Hz), 7.50 (s, 1H), 6.73 (dd, 2H, *J* = 6.9, 2.1 Hz), 6.15 (s, 1H), 6.12 (s, 2H), 4.75 (dd, 1H, *J* = 14.4, 2.4 Hz), 4.49 (ddd, 1H, *J* = 48 Hz), 4.39 (dd, 1H, *J* = 14.4, 3.0 Hz), 4.10 (dt, 1H, *J* = 9.6, 2.4 Hz), 3.40 (bs, 1H), 3.90-3.93 (m, 1H) 3.31-3.33 (m, 1H), 2.87-2.90 (m, 2H), 2.68-2.80 (m, 2H), 2.43 (s, 6H), 2.32 (s, 6H), 1.80-1.83 (m, 2H), 1.45-1.47 (m, 2H); LC-MS: R_t 3.57 min (C18 column, linear gradient 50 → 90% B in 15 min); LC-MS: R_t 9.36 min (C18 column, linear gradient 10 → 90% B in 15 min); FT-IR: ν_{max} (neat)/cm^{-1} 3360, 2972, 1551, 1511, 1408, 1343, 1311, 1203, 1161, 1066, 986; HRMS $[M + H]^+$ calculated for $C_{33}H_{36}BF_6N_7O_6$: 752.27971, found 752.28037.

Spectroscopic data for the β-anomer **20-β**: NMR (CD3CN, 600 MHz): δ 8.09 (dt, 2H, J = 9.0, 2.7 Hz), 7.58 (s, 1H), 6.83 (dt, 2H, J = 9.0, 2.4 Hz), 6.14 (s, 2H), 5.59 (bs, 1H), 4.80 (dd, 1H, J = 14.7, 1.5 Hz), 4.30-4.43 (m, 2H), 4.08 (bs, 1H), 4.00 (bs, 1H), 3.62-3.76 (m, 2H), H-52.84-2.88 (m, 2H), 2.52-2.69 (m, 2H), 2.43 (s, 6H), 2.29 (s, 6H), 1.85-1.98 (m, 2H), 1.63-1.80 (m, 2H); LC-MS: Rt 3.95 min (C18 column, linear gradient 50 → 90% B in 15 min); LC-MS: Rt 9.55 min (C18 column, linear gradient 10 → 90% B in 15 min); FT-IR: ν_{max} (neat)/cm^{-1} 3360, 2972, 1551, 1511, 1408, 1311, 1202, 1161, 1079, 986. HRMS [M + H]$^+$ calculated for $C_{33}H_{36}BF_6N_7O_6$: 752.27971, found 752.28052.



α-6-Azido-2-Flouro-mannosyl imidate (21): To a 0 °C cooled solution of 2-deoxy-2-fluoro azido mannose **39** (27.5 mg, 133 µmol) in acetone (6 mL) was added trifluoro aniline imidate **51** (55.0 mg, 265 µmol) and Cs_2CO_3 (67.0 mg, 0.2 mmol). The reaction was gradually warmed to rt and stirred overnight. The solids were filtered and the filtrate was concentrated *in vacuo*. Purificaton by column chromatography yielded α-glucose aniline imidate **21** (α/β = 1:0, 18.0 mg, 47.5 µmol, 36%). Purification by RP-HPLC followed by lyophilisation yielded α-6-azido-2-deoxy-2-fluoro-mannosyl-aniline-imidate **21** as an orange powder (5.25 mg, 13.9 µmol, 10%). LC-MS: Rt 7.64 min (C18 column, linear gradient 10 → 90% B in 15 min); Spectroscopic data for the α-anomer **21-α:** ^{1}H NMR (CD_3CN, 600 MHz): δ 7.36-7.30 (m, 2H), 7.12 (t, 1H, *J* = 7.5 Hz), 6.86 (d, 2H, *J* = 7.2 Hz), 6.25 (bs, 1H), 4.82 (d, 1H, *J* = 46.2 Hz), 3.73-3.83 (m, 2H), 3.60-3.65 (m, 1H), 3.51-3.56 (m, 2H), 3.36-3.45 (m, 2H); R_t 7.64 min (C18 column, linear gradient 10 → 90% B in 15 min); FT-IR: ν_{max} (neat)/cm^{-1} 3369, 2103, 1717, 1307, 1211, 1165, 1111, 951, 755, 694; HRMS [M + H]$^+$ calculated for $C_{14}H_{14}F_4N_4O_4$: 455.14225, found 455.15176.



α-2-Flouro-BODIPY-mannosyl imidate (22): To a 0 °C cooled solution of 2-deoxy-2-fluoro BODIPY mannose **40** (59.6 mg, 111 µmol) in acetone (6 mL) was added trifluoro aniline imidate **51** (46.2 mg, 223 µmol) and Cs_2CO_3 (55.9 mg, 0.167 mmol). The reaction was gradually warmed to rt and stirred overnight. The solids were filtered and the filtrate was concentrated *in vacuo* Purificaton by column chromatography yielded α-mannose-BODIPY aniline imidate **22-α** (α/β = 1:0, 16.8 mg, 23.8 µmol, 21%). Purification by RP-HPLC (linear gradient 50 → 90 % MeCN in 12min) followed by lyophilisation yielded α-2-deoxy-2-fluoro-BODIPY-mannose-aniline-imidate **22-α** as an orange powder (4.33 mg, 6.13 µmol, 5.5%). ^{1}H NMR (600 MHz, CD_3CN): δ 7.53 (s, 1H), 7.27 (t, *J* = 8.1 Hz, 2H), 7.10 (t, *J* = 7.5 Hz, 1H), 6.68 (d, *J* = 7.2 Hz, 2H), 6.15 (bs, 1H), 6.14 (s, 2H), 4.80 (d, *J* = 48.0 Hz, 1H), 4.4.74 (d, *J* = 14.4 Hz, 1H), 4.48 (dd, J = 14.4, 7.8 Hz, 1H), 4.00 (bs, 1H), 3.70-3.84 (m, 3H), 2.97 (t, J = 8.7 Hz, 2H), 2.68-2.77 (m, 2H), 2.43 (s, 6H), 2.38 (s, 6H), 1.80-1.85 (m, 2H), 1.54-1.66 (m, 2H); Rt 9.96 min (C18 column, linear gradient 10 → 90% B in 15 min); FT-IR: ν_{max} (neat)/cm-1 3368, 2972, 1550, 1510, 1409, 1309, 1202, 1161, 1117, 1079, 985.; HRMS $[M + H]^+$ calculated for $C_{33}H_{37}BF_6N_6O_4$: 707.29463, found 707.29553.

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Chapter 7

Summary and future prospects

Glycoconjugates are involved in numerous fundamental biological processes and are the subject of many studies. Glycoconjugates can bind on the basis of their specific carbohydrate structures to lectin receptors. Lectin receptors can be either membrane bound or soluble and play a key role in a wide variety of cellular recognition and communication processes. Many of the lectins, in particular those found on cells of the immune system are members of the C-type lectin family and these include the mannose receptor (MR), Dectin-1 and 2 as well as DC-SIGN. These carbohydrate-binding receptors have been exploited in immunological studies and targeting strategies. Significant attention has been directed to the design and synthesis of artificial glycoconjugates for binding to specific lectins. The research in this thesis is mainly focussed on glycoconjugates that carry oligomannosides and artificial mannose clusters that can be recognized by the mannose receptor (MR) or other mannose binding lectins.

In **Chapter 1** a selection of different mannose binding lectin-targeting strategies is described. Although carbohydrate protein interactions vary depending on the lectin of interest, the majority is considered to be multivalent. The synthesis of selected examples of linear and dendritic

multivalent mannosyl conjugates is discussed. In addition examples of the synthesis of high mannose *N*-glycan conjugates are described.

Chapter 2 describes the design, synthesis and evaluation of three conjugates comprising the irreversible cathepsin inhibitor DCG-04, a BODIPY dye and a mono-, tri- or heptamannoside, featuring natural *O*-glycosidic linkages (**1**, **2**, **3**, Figure 1). In a modular synthesis approach the oligomannosides were introduced via a Cu(I)-catalyzed "click" reaction in these conjugates. It was shown that the heptamannoside conjugate showed the least effective cathepsin labelling in cell lysates, whereas the uptake of the tri- and heptamannoside conjugates outcompeted the monomannoside conjugate in living cells. Also in live cells the trisaccharide proved to be a more effective inhibitor than its heptasaccharide counterpart. This outcome could be related to the resistance of the conjugates to the endo/lysosomal removal of the mannose residues by mannosidases and further research is needed to clarify this finding. For example the naturally occurring nonamannoside conjugate **4** can be probed. On the basis of the existence mannose-6-phosphate receptors and the targeting study of Hoogendoorn *et al.*[1] it is proposed to synthesize and evaluate structurally related conjugates of which the 6-hydroxyl groups in the oligomannosides is provided with a phosphate monoesters (**5**, Figure 1) The potential hydrolysis of the phosphate functions by phosphatases can be prevented by replacement of the 6-phosphate groups by 6-*C*-phosphonates (**6**, Figure 1).

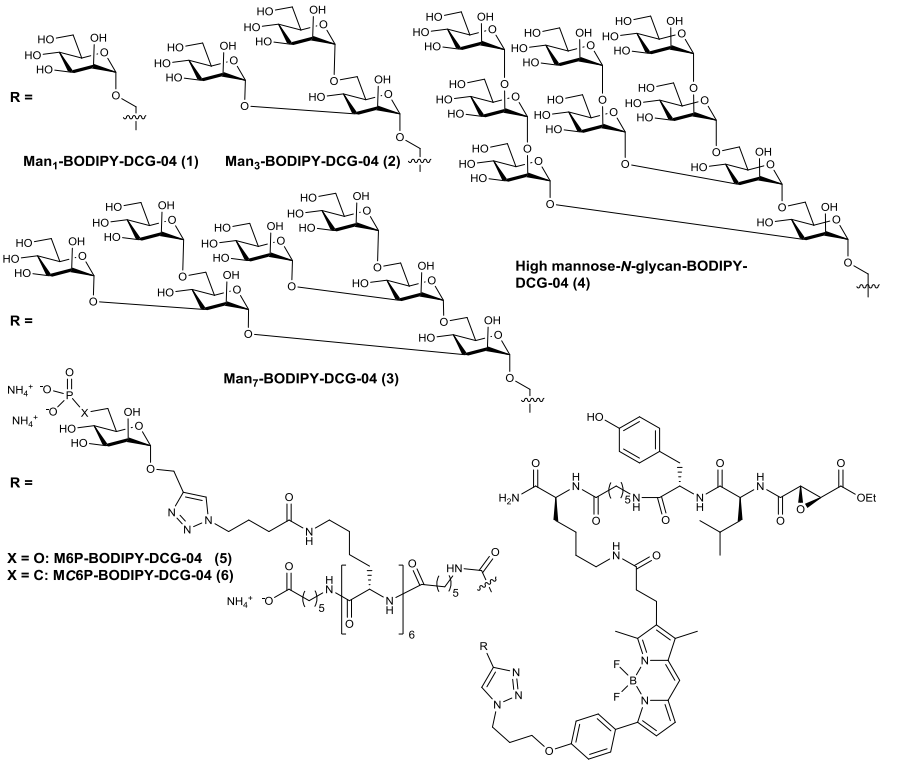


Figure 1: The structures of the conjugates **1**, **2** and **3** presented in Chapter 2 and the structures of the proposed high mannose-*N*-glycan- (**4**), mannose 6-*O*-phosphate- (**5**) and mannose-6-*C*-phosphonate-DCG-04 (**6**) conjugates.

In **Chapter 3** the synthesis and evaluation of Man_1-BODIPY-cyclophellitol **7** and Man_3-BODIPY-cyclophellitol **8** as "caged" activity based probes for glucocerebrosidase, the key enzyme in Gaucher disease, is described (Figure 2). The mannosides in these probes should function as homing device to target the fluorescent cyclophellitol inhibitor via the mannose receptor to Gaucher cells. The self-immolative linker system was installed between the mannoside and the cyclophellitol to allow liberation of the probe in the lysosome through the action of lysosomal mannosidases.

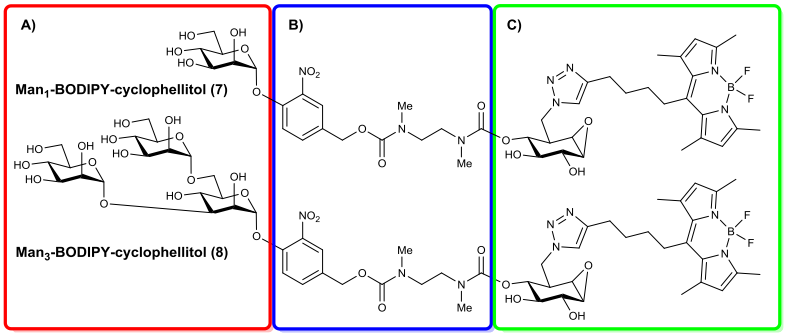


Figure 2: The structures of the conjugates presented in Chapter 3.

In a convergent approach the mono- and trimannosides were prepared and the self-immolative linker was introduced at the reducing end of the mannosides by nucleophilic aromatic substitution. An alternative and high yielding route of synthesis to a partially protected azido-cyclophellitol was developed in which the stereoselective introduction of the epoxide was followed by the introduction of the azide. Having the mannosylated linker systems and the partially protected azido-cyclophellitol and BODIPY-alkyne in hand the assembly of the probes went uneventful. Biological evaluation of the probes showed that they were significantly less effective inhibititors of the target enzyme than their BODIPY-cyclophellitol counterparts without the targeting device. This indicates ineffective cleavage of the mannosyl residues from the probes by intracellular mannosidases.

Guided by the successful uptake of the probes with the aid of the (oligo)mannosides as targeting device it is proposed to apply a disulfide based sulfhydryl-assisted self- immodulative linker.[2] As depicted in Figure 3, after internalization of the probe by Gaucher cells, cyclophellitol can be liberated via reduction of the disulfide bond by cellular glutathione,[3] thioredoxin,[4,5] or γ-interferon-inducible lysosomal thiol reductase and ensuing intramolecular ring formation.[6]

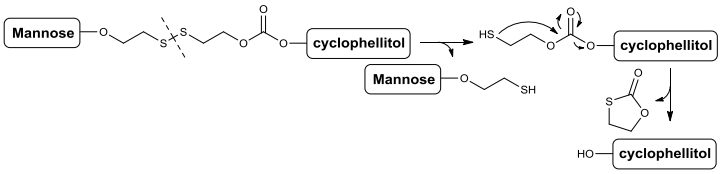
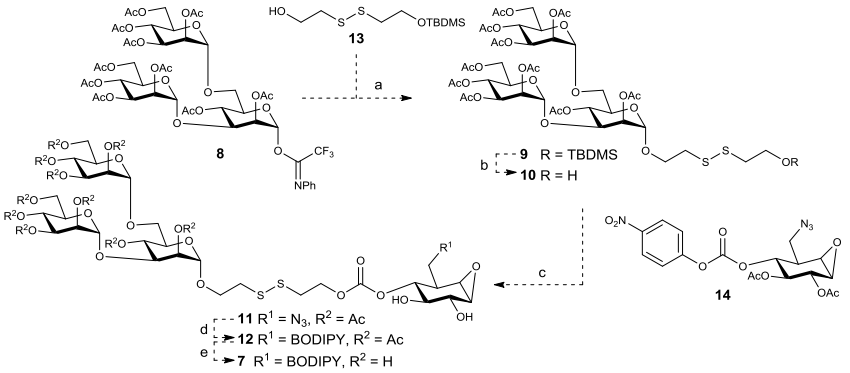


Figure 3: Proposed mechanism of a disulfide based sulfhydryl-assisted self immodulative linker.

The first biodegradable disulfide based conjugate, which is proposed, contains the same oligomannose homing device as described in Chapter 3 (Scheme 1).

Scheme 1: Proposed synthesis of disulfide bridged mannose-cyclophellitol construct **7**.



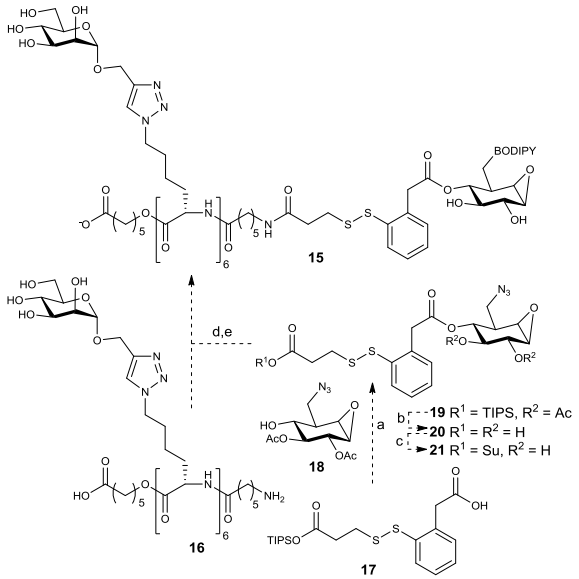
Reagents and conditions: (a) Disulfide **13**, TfOH, DCM; (b) TBAF, DCM/MeOH; (c) **14**, TEA, DMF, rt.; d) NaOMe/MeOH; e) Alkyne-BODIPY, $CuSO_4$, sodium ascorbate DMF/H_2O.

Based on the work of Bhuniya *et al.*[2] the partially protected TBDMS-2-hydroxyethyl disulphide linker **13**[7] is condensed with trimannoside (*N*-phenyl)trifluoroimidate **8** to give disulfide trimer **9**. Removal of the TBDMS group in **9** allows coupling with 4-nitrophenyloxycarbonate azidocyclophellitol derivative **14** to give compound **11**. Further processing by global deacetylation[8] and conjugation of alkyne-BODIPY with Cu(I)-cat. "click" chemistry leads to the proposed conjugate **7**.

In the same vein other mannose based targeting devices can be used. For instance, it is proposed to combine the self-immolative disulfide linker

reported by Chen *et al*.[9] with the synthetic mannose cluster, capable for lysosomal targeting of a cathepsin inhibitor, as developed by Hillaert *et al*.[10] A potential route of synthesis to this conjugate **15** is presented in Scheme 2. Azido-cyclophellitol derivative **18** can be coupled with TIPS-protected disulfide linker **17**[11]. Protective group manipulations followed by conversion of the carboxylic acid into the OSu-ester **21** allows the installation of mannose cluster **16**[10]. In the final event the BODIPY tag is introduced by "click" chemistry affording the proposed conjugate **15**.

Scheme 2: Proposed synthesis of disulfide bridged mannose cluster-cyclophellitol construct **15**.



Reagents and conditions: (a) **18**, DIC, DMAP, THF, 0 °C to rt.; (b) *i.* HF/pyr, pyridine/MeCN, 0 °C to rt; *ii.* NaOMe/MeOH; (c) DIC, HOSu, 0 °C to rt.; (d) **8**, DMSO, rt.; (e) Alkyne-BODIPY, $CuSO_4$, sodium ascorbate DMF/H_2O.

Chapter 4 describes the synthesis and preliminary immunological evaluation of twenty mannosylated peptide conjugates. One, two, three and six copies of a mono-mannoside, three different dimannosides and a trimannoside, were conjugated to a peptide containing the MHC-class-I epitope SIINFEKL. The respective target peptides were provided with the

required number of azidolysines, while all (oligo)mannosides were equipped with an alkyne allowing an efficient Cu(I) catalyzed "click" mediated synthesis of the twenty mannosylated peptide conjugates. Worth noting is the increase in overall yield of the conjugates by the use of CupriSorb® resin to remove copper from the reaction mixture after the Cu(I) catalyzed "click" reaction. Preliminary immunological evaluation showed an increase in T cell response of almost all mannosylated conjugates in comparison with the non-mannosylated parent peptide. In addition, the uptake of the conjugates was likely mannose receptor mediated. Surprisingly, the monomannoside conjugates with one or two monomannosides showed the highest T cell response compared to the other constructs and no clear correlation between the type and number of (oligo)mannosides in the conjugates and the T cell response was apparent. A possible explanation for this finding is the reduced proteolytic susceptibility and processability of the mannosylated epitope conjugates. It is also unclear at present which lectins are involved in binding and or transporting the conjugates. It is proposed to introduce biodegradable linkers between the (oligo)mannose clusters and the epitope using the same chemistry as proposed for the modified cyclophellitol conjugates (*vide supra*, Scheme **1** and **2**) to influence the processing the conjugates.

As discussed above, mannose-6-phosphate conjugates are promising devices for receptor-mediated uptake. To study the relation between mannose-6-phosphate-receptor mediated uptake and antigen presentation, multivalent mannose-6-phosphate/phosphonate epitope conjugates are proposed (Figure **4**).

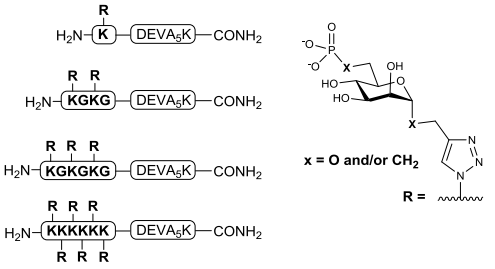
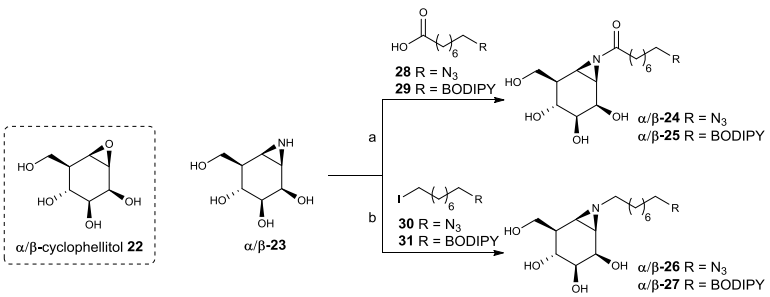


Figure 4: Schematic representation of mannose-6-phosphate/phosponate *O*/*C*-mannoside conjugates.

Chapter 5 describes the synthesis of mannose configured α- and β-cyclophellitol **22**, and their aziridine counterparts **23**. The chiral cyclohexene, the common intermediate for both epoxides and aziridines, was obtained by adaptation of the procedure of the group of Madsen for the synthesis of cylcophellitol.[12] Using the procedure of Li *et al.*[13] for the conversion of the α-epoxide to the β-aziridine, the *manno*-configured β-aziridine cyclophellitol was generated. The synthesis of the α-aziridine started with the β-epoxide, the minor product of the cyclohexene epoxidation reaction. Opening of the epoxide with sodium azide and mesylation of the formed hydroxyl was followed by reduction of the azide with $LiAlH_4$ to produce the α-aziridine cyclophellitol. The absolute configurations of the α- and β benzyl-protected epoxides compounds were elucidated by NMR spectroscopy. Both the α- and β-epoxide adopt a $^4H_3$ conformation as corroborated by DFT calculations.

Both aziridine α-**23** and β-**23** can be converted into two-step labelling activity based probes **24** and **26** by introduction of an azide ligation handle via either acylation or alkylation of the aziridine nitrogen (Scheme 3).[14] These two-step probes can be further converted into direct activity based probes **25** and **27** by introduction of a BODIPY reporter group, using a Cu(I) catalyzed "click" reaction.

Scheme 3: Proposed functionalization of mannose configured aziridine cyclophellitol **23**.

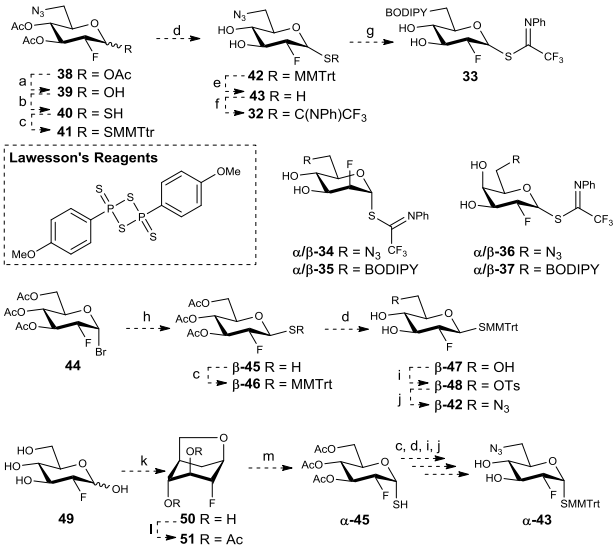


Reagents and conditions: a) acid **28** or **29**, EEDQ, DMF, 0 °C; b) iodide **30** or **31**, K_2CO_3, DMF, 90 °C.

Chapter 6 deals with the synthetic accessibility of differently configured 2-deoxy-2-fluoro glycosides with various (substituted) *N*-phenyltrifluoroacetimidate leaving groups at the anomeric centre as potential glycosidase inhibitors. The research in this chapter is based on the work of Walvoort *et al.*[15] who showed that 2-deoxy-2-fluoro-β-glucose bearing an anomeric *N*-phenyltrifluoroacetimidate has an improved inhibitory profile towards the enzyme glucocerebrosidase in comparison with commonly used inhibitors featuring other leaving groups such as fluoride, 2,4-dinitrophenyl and diphenylphosphate. Various naturally occurring glycosidases are known that hydrolyze different epimeric glycans using the Koshland double-replacement mechanism and therefore glucose- mannose- and galactose configured probes were targeted in this chapter. Unfortunately it turned out that all the 2-deoxy-2-fluoroglycosyl imidates were highly unstable and degraded partly or completely during HPLC purification. Only α-2-deoxy-2-fluoro-mannosyl *N*-phenyltrifluoroacetimidate and a few α- and β-2-deoxy-2-fluoroglucosyl N-phenyltrifluoroacetimidate derivatives could be prepared in low yields. All 2-fluoro-galactose configured imidates were too instable and could not be isolated.

It is proposed to use thioimidates as more stable leaving groups in the 2-deoxy-2-fluoro glycosides probes. Several possible routes of synthesis to 2-deoxy-2-fluoro-glucosyl thioimidate probes can be envisaged (Scheme 4).

Scheme 4: Proposed synthesis of thioimidate glycosidase probes **32-37**.



Reagents and conditions: (a) Piperidine/DMF; (b) Dioxane, Lawesson's reagent, 80 °C; (c) MMTrtCl, pyridine; (d) NaOMe/MeOH; (e) TFA, TES-H, DCM; (f) $ClC(=NPh)CF_3$, K_2CO_3, acetone; (g) Alkyn-BODIPY, $CuSO_4$, sodium ascorbate, DMF/H_2O; (h) *i.* thiourea; *ii.*$Na_2S_2O_5$, DCM/H_2O; (i) TsCl, pyridine; (j) NaN_3, DMF; (k) *i.* TsCl, pyridine; *ii.* 3N NaOH (aq.); (l) Ac_2O, pyridine; (m) *i.* $(TMS)_2S$, DCM, TMSOTf; *ii.* Ac_2O, pyridine *iii* KSAc/MeOH.

In the first route 2,6-dideoxy-2-fluoro-6-azido-glucose derivative **38** is converted to the corresponding thiol **40** with the aid of the Lawesson's reagents.[16] Protection of the thiol function with an monomethoxytrityl (MMTrt) group can give the individual α- and β-anomers **α-41** and **β-41**.[17] Deacetylation and deprotection of the MMTrt-group is followed by reaction with *N*-phenyl trifluoroacetimidoyl chloride under basic conditions[18] to give the individual α- and β-anomers of the potential thio-imidate probe **32**. A BODIPY fluorophore can be introduced at the azide of compound **32** with alkyn-BODIPY using "click" chemistry giving probe **33**.

In the second approach 2-deoxy-2-fluoro-glucose derivatives **44** and **49** are used as starting compounds, thereby postponing the introduction of the azide to a later stage of the synthesis (Scheme 4). Treatment of anomeric bromide **44** with potassium thioacetate followed by selective deacetylation of the

thioester gives **β-45**. Ensuing, protection of the thiol with a MMTrt group and deacetylation of the remaining acetyl groups sets the stage for the successive introduction of the azide at the 6-position and the thio-imidate at the anomeric centre to furnish β-probe **43**. The corresponding α-probe is accessible by opening of known 1,6-anhydro-glucose derivative **51**[23] with $(TMS)_2S$[19] followed by global acetylation and selective deacetylation of the anomeric thioester to give **α-45**. Using the procedure described above, **α-45** can be transformed into the α-thio-imidate probe **32**.

The proposed 2-deoxy-2-fluoro-glucosyl thioimidate inhibitors can be evaluated as pharmacological chaperones of the enzyme GBA for the treatment of the Gaucher's disease.[20] It is also proposed to evaluate lipophilic thio-imidate **53**, **54** and **55** as a closer mimic of the natural substrate glucosylceramide (**52**, Figure 5).

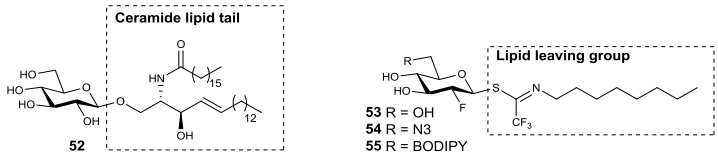


Figure 5: Structure of glucosylceramide (**52**) and its imidate based mimic inhibitor **53**-**55**.

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Samenvatting

Glycoconjugaten zijn betrokken bij een groot aantal biologische processen en onderwerp van vele studies. Glycoconjugaten kunnen aan lectine receptoren binden op basis van hun specifieke suiker structuur. Veel van deze lectines, en voornamelijk diegene aanwezig op cellen van het immuunsysteem. zijn lid van de C-type lectin familie. De mannose receptor, Dectrin-1 en 2 maar ook DC-SIGN vallen hieronder. Binnen immunologische studies en "targeting" strategien zijn deze suiker-bindende receptoren interessante doelen om gebruik van te maken. Veel aandacht is besteed aan het ontwerp en synthese van artificiële glycocnjugaten om specifieke lectines te binden. Het onderzoek beschreven in dit proefschrift richt zich voornamelijk op glycoconjugaten bestaande uit oligomannosides en kunstmatige mannose clusters die herkend worden door de mannose receptor of andere mannose bindende lectines.

Hoofdstuk 1 geeft een overzicht van verschillende mannose lectine-binding strategieën. Ondanks dat suiker eiwit interactie verschilt per lectine, worden de meeste beschouwd als multivalent. Enkele geselecteerde voorbeelden van lineaire en dendritische multivalente mannosyl conjugaten worden verder toegelicht toegelicht waarbij de toepassing, synthese en biologische evaluatie in detail wordt behandeld. Tot slot wordt de synthese van de natuurlijke mannose *N*-glycaan conjugaten beschreven.

Hoofdstuk 2 beschrijft de synthese en biologische evaluatie van drie (oligo)mannosyl-DCG-04-conjugaten. Om de invloed van mannose op de cel opname te onderzoeken zijn verschillende mannoses variërend in grootte gekoppeld aan de irreversibele cathepsine remmer DCG-04 met een BODIPY fluorofoor. Het betreft een mono- tri- en heptamannoside waarbij

de natuurlijke *O*-glycosidische band in tact gehouden is. Middels een koper gecatalyseerde "click" reactie is deze verbonden met BODIPY-DCG-04. Behandeling van de constructen met cel lysaat toonde aan dat de hexamannoside conjugaat het slechts bond met cathepsines in cel lysaat. Echter, hexamannoside en trimannoside conjugaten vertoonde een hoge opname door levende cellen, in tegenstelling tot de monomannose conjugaat wat nauwelijks opname vertoonde. Verder was de trimannoside conjugaat een betere inhibitor in levende cellen dan de heptameermannoside variant.

In **Hoofdstuk 3** wordt de synthese van een mono- en tri-mannosyl-BODIPY-cyclophellitol construct beschreven. Deze constructen zijn ontworpen om selectief herkend en opgenomen te worden door Gacher cellen (macrophagen) door middel van mannose suiker herkenning. Het construct wordt opgenomen waarna het getransporteerd wordt naar het lysosoom. Door de aanwezigheid van α-mannosidase wordt het construct afgebroken waarna BODIPY-cyclophellitol, een "retaining β-glucosidase" probe vrij komt en bindt aan het enzym Glucocerebrosidase aanwezig in het lysosoom. De synthese kan verdeeld worden in vier onderdelen. 1) Mannose suiker synthese; 2) linker synthese aan de suikers; 3) de synthese van gedeeltelijk bescherm cyclophellitol en tot slot het assembleren van het constructen. Een nieuwe synthese route naar azidocyclophellitol wordt beschreven waarbij de azine pas in een laat stadium wordt geïntroduceerd. Biologische evaluatie toonde een opname van beide probes aan door de cel echter een lage binding was geobserveerd. De lage binding efficiëntie komt vermoedelijk door een ineffectieve hydrolyse van de mannose residuen.

Hoofdstuk 4 beschrijft de synthese en eerste immunologische resultaten van twintig gemannosyleerde peptiden conjugaten. Hierbij is gekeken naar de invloed van verschillende mannose clusters op de opname en antigeen presentatie van levende cellen. De peptide, SIINFEKLDEVA_5K, een MHC-I epitope, wordt opgenomen via de mannose receptoren waarna het epitope via de MHC-II route gepresenteerd wordt aan de T cel. Dit wordt ook wel "cross-presentation" genoemd. Aan het peptiden zijn mannose residuen geconjugeerd variërend in eenheden (1,2,3, en 6 eenheden) en verschillende

grootte (mono- di- en tri-mannosiden). Door een strategie toe te passen waarbij eerst het volledige peptide gesynthetiseerd wordt, kan zo het gewenste mannose residuen erop gekoppeld. Door deze strategie toe te passen kan uit één enkel peptide eenvoudig verschillende conjugaten verkregen worden. Cluster vorming van het product met het aanwezige Cu(I) zorgde voor product verlies tijdens het eerste zuiveringsproces. Dit is opgelost door gebruik te maken van de ionenwisselaar CupriSorb® waarna de opbrengst aanmerkelijk is verhoogd. Verrassend genoeg vertoonde de conjugaten met één of twee mono mannose residuen het hoogste T cel response. Echter een duidelijke trend tussen aantal mannose residuen en mannose grootte is niet zichtbaar.

Hoofdstuk 5 beschrijft de synthese van mannose geconfigureerd α- en β-cyclophellitol en de corresponderende aziridine derivaat. Deze verbindingen kunnen gebruikt worden als selectieve probes om retaining α- en β-mannosidase te remmen. Voor de synthese is een beschreven route gevolgd waarna de absolute conformatie van benzyl ether beschermd α- en β-aziridine cyclophellitol is gemeten met behulp van NMR spectroscopie. Zowel α- en β-aziridine cyclophellitol begaven zich in een $^4H_3$ conformatie wat bevestigd is met DFT berekeningen.

Het werk beschreven in **Hoofdstuk 6** is gebaseerd op voorgaand onderzoek van Walvoort die aantoonde dat 2-deoxy-fluoro-β-glucose met een *N*- phenyltrifluoroacetimidate gesubstitueerde groep op het anomere centrum een verbeterde binding gaf met het enzym glucocerebrosidase dan zijn voorgangers. Dit hoofdstuk beschrijft de synthetische toegankelijkheid van verschillende geconfigureerde 2-deoxy-fluoro suikers met verschillende *N*-phenyltrifluoroacetimidate substituenten als vertrekkende groep op de anomere positie. Helaas blijken de 2-deoxy-fluoro imidaat suikers zeer instabiel te zijn en gedeeltelijke tot volledige degradatie van het product tijdens HPLC zuiveringen kon niet worden voorkomen. Enkel α-2-deoxy-2-fluoro-mannosyl *N*-phenyltrifluoroacetimidate en enkele α- en β-2-deoxy-2-fluoro-glucosyl gesubstitueerde *N*-phenyltrifluoroacetimidate derivaten konden geproduceerd worden in lage opbrengst. Alle 2-deoxy-2-fluoro-

galactose geconfigureerde imidaten bleken te instabiel waardoor er geen product geïsoleerd is.

Tot slot wordt in **Hoofdstuk 7** een samenvatting gegeven van de voorgaande hoofdstukken en worden een aantal toekomstperspectieven behandeld, waaronder de introductie van een dithiol linker om afgifte van BODIPY-cyclophellitol te bevorderen en de conjugatie van het natuurlijke mannose *N*-glycaan oligomeer aan de cathepsine remmer DCG-04. Verder wordt het gebruik van mannose-6-fosfaten voorgesteld om opname van de SIINFEKLDEVA$_5$K te verhogen en worden er alternatieve synthese routes voorgesteld naar de productie van thioimidaat suikers als potentiële glucocerebrosidase remmers.

List of publications

"Targeted Delivery of Fluorescent High-Mannose-Type Oligosaccharide Cathepsin Inhibitor Conjugates"
Wong, C.S.; Hoogendoorn, S.; van der Marel, G.A.; Overkleeft, H.S.; Codeé, J.D.C. *Chempluschem*, **2015**, *80*, 928-937.

"Synthesis of Cyclophellitol, Cyclophellitol Aziridine, and Their Tagged Derivatives"
Li, K.Y., Jiang, J.B., Witte, M.D., Kallemeijn, W.W., van den Elst, H., Wong, C.S., Chander, S.D., Hoogendoorn, S., Beenakker, T.J.M., Codee, J.D.C., Aerts, J.M.F.G., van der Marel, G.A., Overkleeft, H.S *Eur.J. Org. Chem.* **2015**, 6030-6043.

Curriculum Vitae

Chung Sing Wong werd geboren op 14 december 1982 te Gouda. Na het behalen van het MAVO diploma aan het Petrus Canisius te Gouda in 1995 werd in hetzelfde jaar begonnen met de MBO opleiding Middelbare Laboratorium Onderwijs analytische chemie aan het Zadkine college te Rotterdam. Als onderdeel van deze opleiding werden er in het laatste studiejaar twee stages uitgevoerd. De eerste stage deed hij onder begeleiding van H. van den Elst en dr. P. van Swieten in de werkgroep Bio-Organische Synthese van de universiteit van Leiden onder leiding van wijlen prof. dr. J.H. van Boom. Tijdens deze stage werd onderzoek verricht naar de synthese van isotoop gelabelde peptiden. Zijn tweede stage heeft hij gedaan aan de technische universiteit van Delft bij de vakgroep Toegepaste Organische Chemie en Katalyse van prof. dr. T. Maschmeyer onder begeleiding van dr. D. Klomp. Dit betrof onderzoek naar epimerisatie van secundaire alcoholen via een Meerwein-Ponndorf-Verley reductie/Oppenauer oxidatie voor de ontwikkeling van een dynamische kinetische resolutie systeem. Na het behalen van het MLO diploma in 2003, is hij in hetzelfde jaar begonnen aan de HBO opleiding Hoger Laboratorium Onderwijs organische chemie aan de Hogeschool Leiden. Deze studie heeft hij gecombineerd met werkzaamheden als parttime QC-technicus bij het bedrijf OctoPlus B.V. Hier was hij verantwoordelijk voor het inklaren van chemicaliën, het kalibreren van analyse apparatuur, het uitvoeren van water kwaliteitscontrole en het bijhouden van een database met veiligheidsvoorschriften. Als onderdeel van de HBO opleiding heeft hij een onderzoeksstage uitgevoerd bij TNO Defensie & Veiligheid in Rijswijk. Het project betrof het ontwikkelen van antimicrobiële polymeren. Na het behalen van zijn HLO diploma in 2006 heeft

hij twee maanden fulltime gewerkt bij dezelfde TNO afdeling aan de synthese van explosieve adducten als interne standaard voor analyse methoden. Aansluitend is hij begonnen met de Master opleiding Chemistry aan de Universiteit van Leiden met als specialisatie Design and Synthesis. Als onderdeel van deze masteropleiding heeft hij twee onderzoeksstages volbracht. Gedurende het eerste jaar heeft hij onder begeleiding van dr. J. Dinkelaar in de vakgroep Bio-Organische Synthese onderzoek verricht naar het gebruik van lactonen als bouwstenen voor de synthese van pectine oligosacchariden. In het kader van de bijvakstage werd in 2009 onderzoek verricht aan de Universidade de Vigo (Spanje) in de groep van prof. dr. A.R. DeLera. Dit onderzoek was gericht op de totaalsynthese van retinoide zuur derivaten als interne standaard voor het vaststellen van de absolute configuratie. In 2010 behaalde hij het Master diploma. In datzelfde jaar werd hij aangesteld als assistent in opleiding en werd het in dit proefschrift onderzoek beschreven uitgevoerd in de vakgroep Bio-Organische Synthese onder de begeleiding van prof. dr. H.S. Overkleeft, prof. dr. G.A. van der Marel en dr. J.D.C. Codée.

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