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]

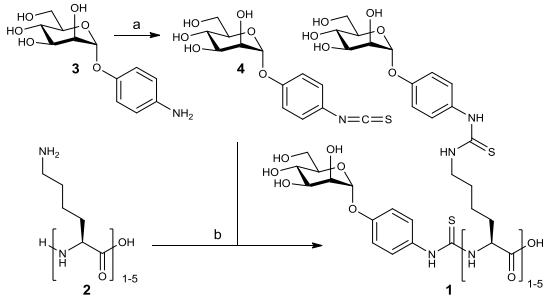
This introductory chapter describes selected examples of conjugates bearing various mannose ligands, that have been designed and synthesized to enable mannose receptor mediated uptake.

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.

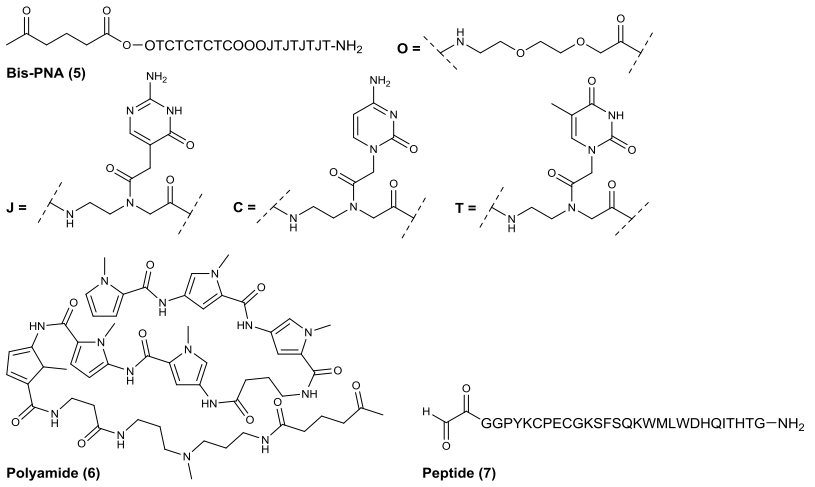
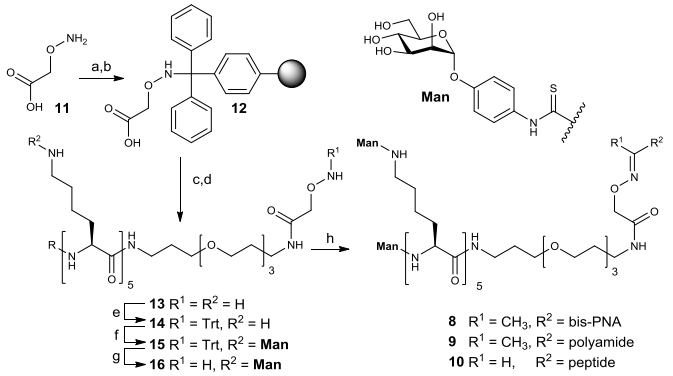


Figure 1: Structures of bis-PNA **5**, polyamide **6** and peptide **7**.

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.

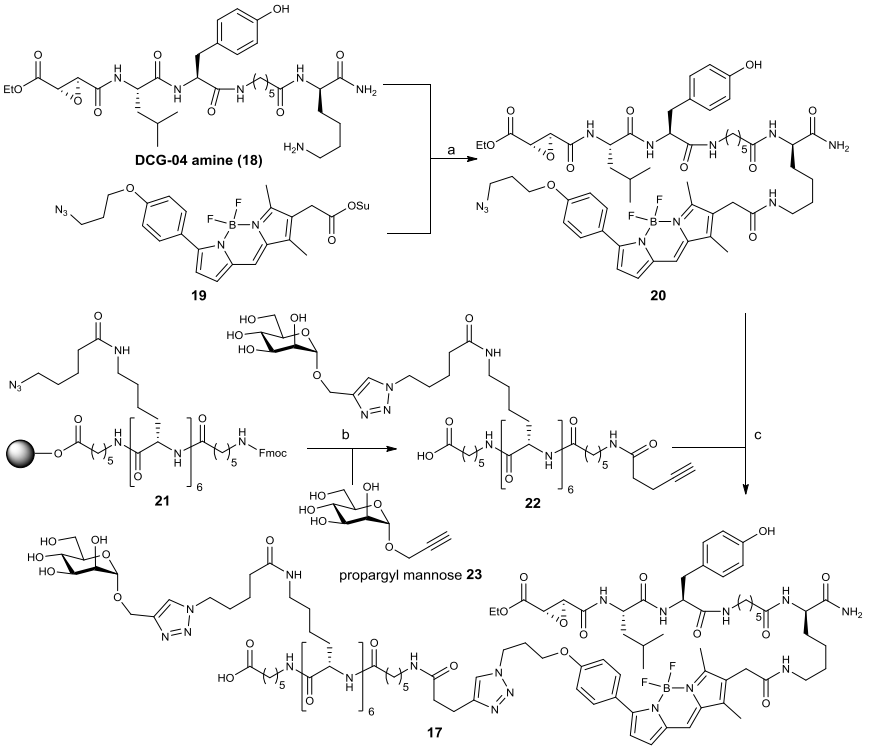
The assembly of the conjugates starts with the treatment of carboxymethoxylamine hemihydrochloride **11** with TMS-chloride and subsequent coupling to a 2-chlorotrityl resin to give the aminooxy resin **12**. Elongation of the resin with a triethylene glycol spacer was followed by five consecutive couplings 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

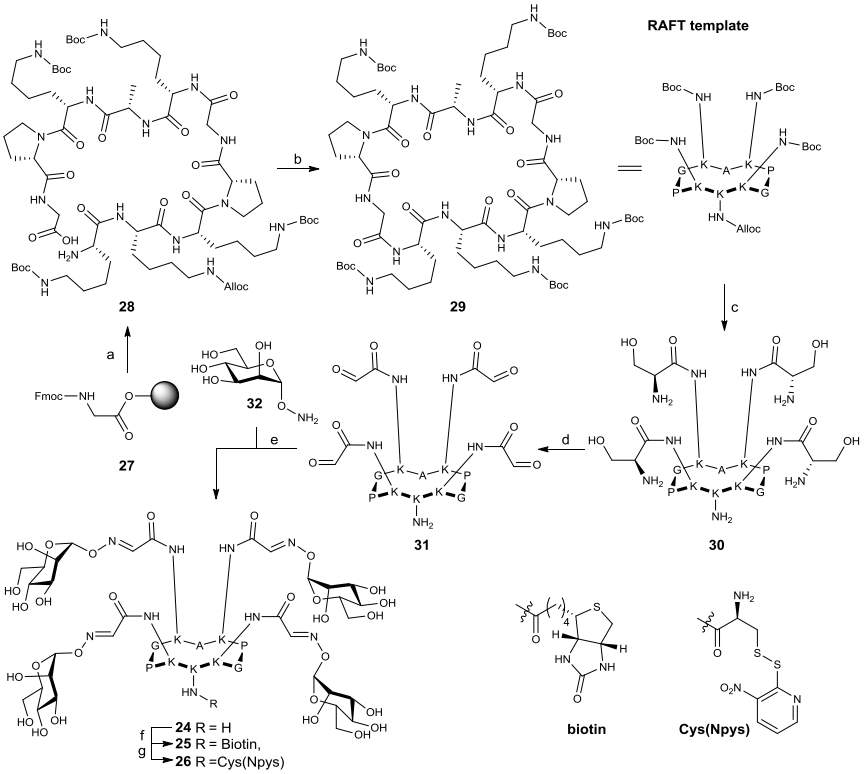
Cyclic RAFT peptides

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)

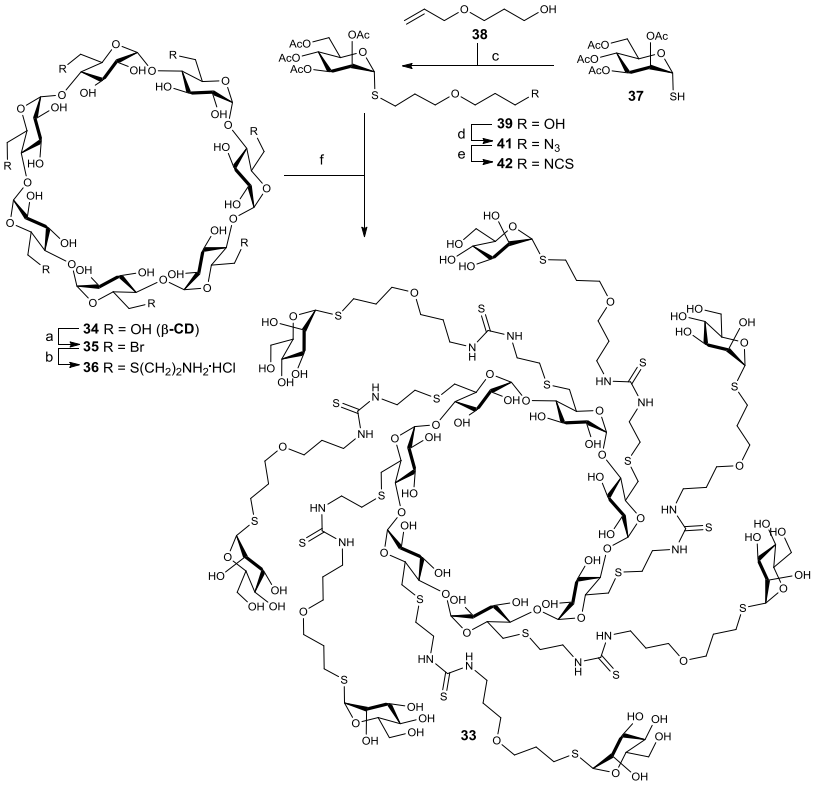
Several variations in orthogonal protection of the RAFT template and conjugation methods have been described in the literature.[21] For example, cyclic RAFT scaffolds were equipped with an alkyne or azide functionality and mannosides were conjugated using Cu(I)-catalyzed click chemistry.[22,23] Two different 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.

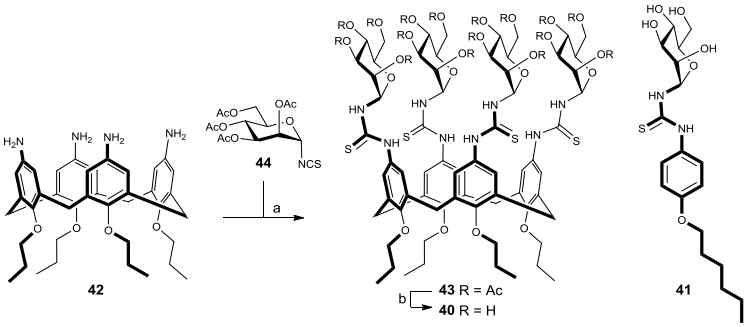
Many examples of cyclodextrin based glycoclusters have been reported, varying in the nature of the carbohydrate, the valency, and the conjugation method and strategy.[31,32] For example, Baussanne *et al.* synthesized a per-(6→6)-thiourea linked methyl-α-D-mannopyranoside β-CD cluster to study the effect 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).

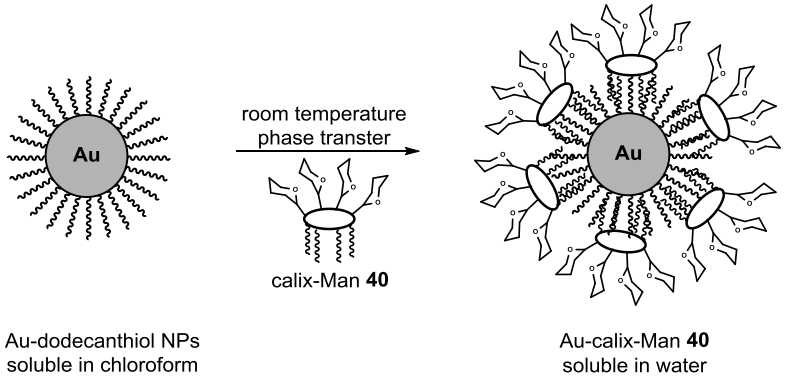


Figure 2: Schematic representation of the gold nanoparticle functionalization using a phase transfer procedure. Formation of Au-calix-Man **40**.

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).

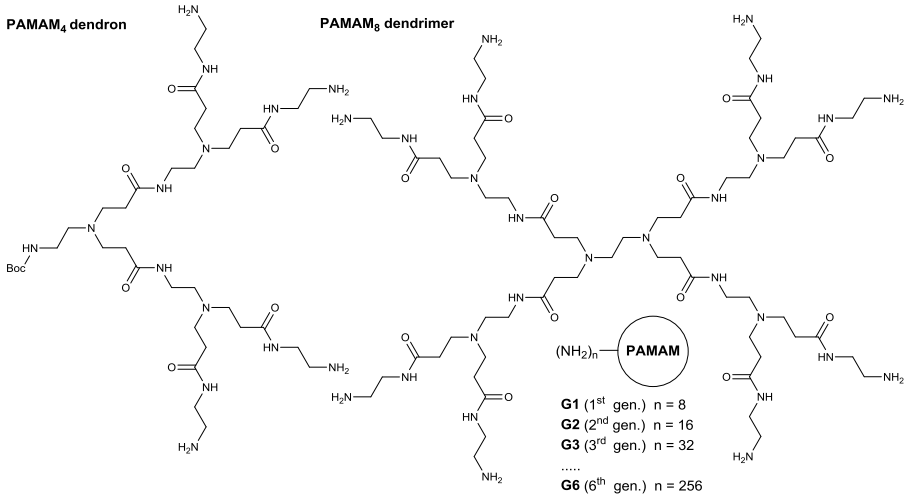
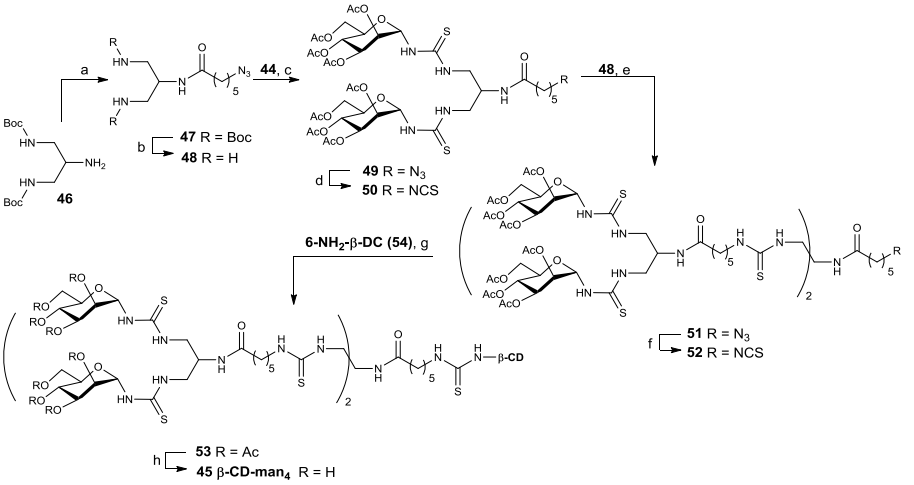


Figure 3: Structure of PAMAM dendron and dendrimer.

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**.



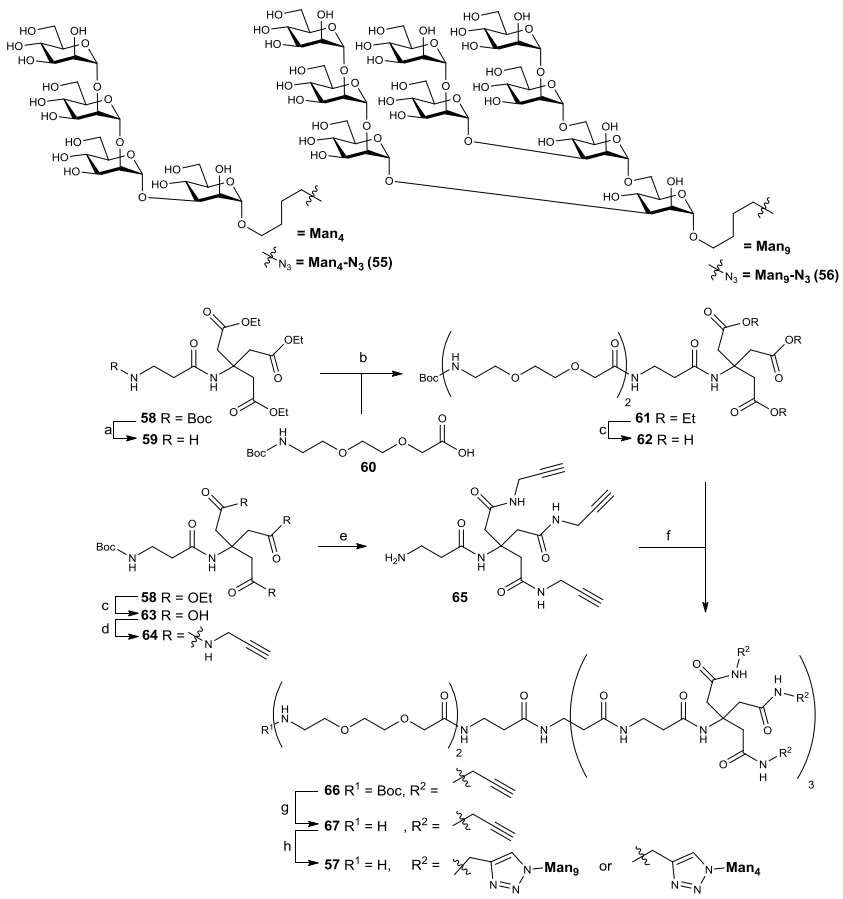
Reagents and conditions: a) azidohexanoylchloride, DMF/collidine (1:1), 96%; b) TFA/H_2O (1:1); c) thioisocyanade mannoside **44**, pyridine, 82% (over two steps); d) $(Ph)_3P$, CS_2, dioxane, 72%; e) diamine **48**, $NaHCO_3$ (aq.) pH 8, acetone, 55%; f) $(Ph)_3P$, CS_2, dioxane, 90%; g) 6-NH_2-β-CD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

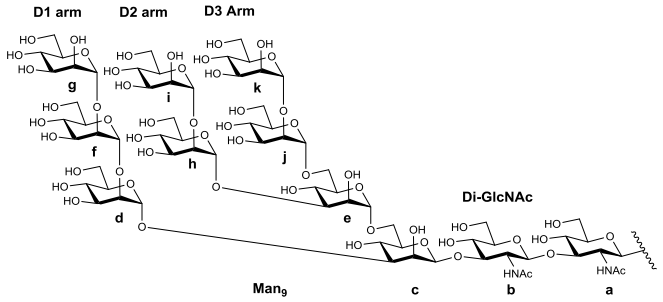


Figure 4: Structure of the high-mannose *N*-glycan.

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]

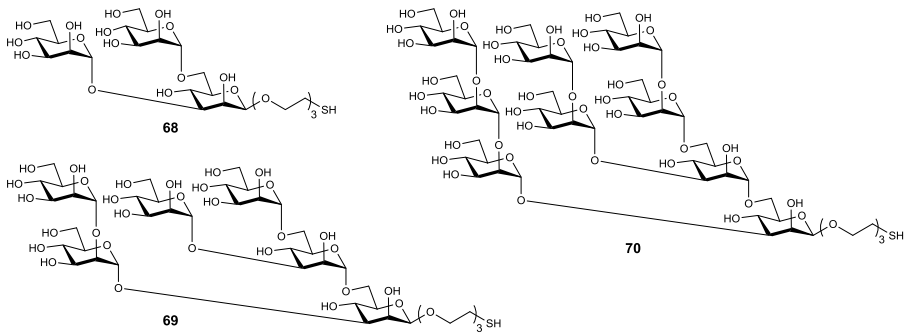
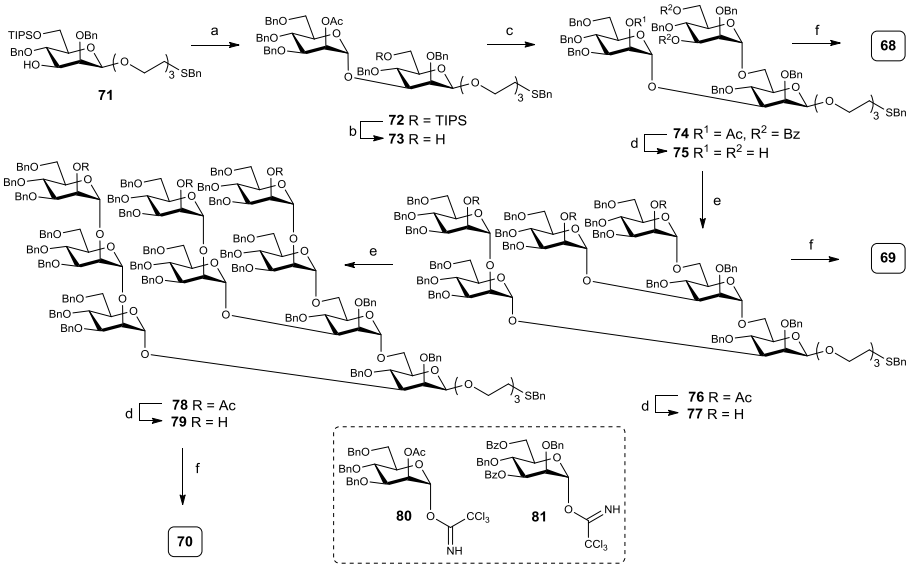


Figure 5: Structures of a set of high-mannose oligomannosides **68-70**.

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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