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# 3.36 Application of Multivalent Mannosylated Dendrimers in Glycobiology

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## 3.36.1 Introduction

Mammalian cells expose wide arrays of complex glycoconjugates on their surfaces. These carbohydrate structures are playing critical roles in multiple key cellular events, several of which being characterized by weak but multivalent carbohydrate–protein interactions.[1] Thus, multiantennary glycans on glycoproteins, polysaccharides, or on patches of glycolipids constitute a first line of weak contacts with pathogens bearing the corresponding carbohydrate-recognition domain (CRD). Cell–cell communications also apply carbohydrates as signals. In spite of these ubiquitous phenomena, the basic carbohydrate structures recognized by carbohydrate-binding proteins are surprisingly simple, with at best a tri- or tetrasaccharide moiety being deeply involved in the protein's active sites, unless conformational epitopes are involed.[2] Additionally, several proteins or interactive mechanisms utilize a limited set of similar sugars, thus further raising the issues of selectivities. For instance, the innate immune system exploits the structures of mannosides on yeast, fungi, and bacteria through the mannose-binding protein (MBP) as the first defense mechanism against this type of infectious agents. Yet, dendritic cells, macrophages, and hepatocytes also utilize mannosides against pathogens and cellular recognition or activation. Paradoxically, bacteria, such as fimbriated *Escherichia coli* (*E. coli*), possess proteins at the tip of their fimbriae (FimH) that also recognize and bind to mannosides of host human tissues as the premise for bacterial infections. Obviously then, the sole carbohydrate structures, taken individually, could not account for the large multivalent interactions that solicit a given glycan. Consequently, valencies, geometries, and topographical saccharide arrangements must be keys for the high selectivities and affinities observed. With these questions in mind, glycodendrimers [3–6] were born to address fundamental aspects in multivalent carbohydrate–protein

interactions that could not be resolved with glycopolymers.[7–9] Moreover, dendrimers can be assembled with an infinite variety of chemical architectures and exposed number of carbohydrate moieties.[10,10a,10b,11] They may also constitute an arsenal of new therapeutic agents as well as being useful for specific cell targeting.

Bacterial resistance against established chemotherapies is a major medical issue. Novel principles for the treatment of infectious agents are thus highly attractive. One such possibility would be the inhibition of attachment of the infecting pathogens to the host's cell surfaces.[12–14] The attachment is often a prerequisite for the later stages of infection, colonization, and invasion of specific human tissues. Since the chemical structure of the adhesion inhibitors most likely would be quite similar to the natural attachment ligands used by the pathogenic agents, it is unlikely that resistance (mutations) would give them the capability to overcome the inhibitory effect of the antiadhesive drug without impairing their ability to adhere to the host cells. Glycodendrimers are therefore definitely suitable for the inhibition of bacterial adhesions to host tissues (**Figure 1**).

This review will highlight some of the most-studied carbohydrate–protein interactions involving mannosides. Obviously, the same principles should also apply to other key interactions which may be the subject of other reviews on their own, such is the case of galactose-binding proteins (e.g., galectins).[15,15a] As mentioned, glycopolymers[7–9] constitute very useful tools to study multivalent interactions;[16–17,17a,17b] however, they detract from fundamental understanding of glycoside cluster effects.[18] Again, glycodendrimers and some of their corresponding glycoclusters are perfectly designed to address the proper questions and to permit further structural refinements that ultimately could be integrated to polymers or even to dendronized glycopolymers.[19,19a,19b] This chapter will therefore also illustrate some of the synthetic strategies that have been used into the wonderful design of mannoside-bearing glycodendrimers.

## 3.36.2 Proteins that Bind D-Mannosides

A wide variety of proteins can bind D-mannopyranoside residues including high-mannose oligosaccharides.[20] Of these, plant lectins, particularly Concanavalin A (ConA), *Dioclea grandiflora*, and pea lectins, have been extensively studied from the point of view of X-ray crystallographic analysis, isothermal titration microcalorimetry (IC), as well as for fundamental aspects related to multivalent interactions and glycodendrimer bindings. Of particular interest to this review are the binding of mannosides to human monoclonal antibody 2G12 recognizing $Man_9GlcNAc_2$ from HIV-1 gp120 and the related cyanovirin-N isolated from the cyanobacterium *Nostoc ellipsosporum*; MBP of the innate immune system; DC-SIGN (dendritic cell-specific ICAM-3 grabbing nonintegrin) (ICAM-3 = intercellular adhesion molecule) present on dendritic cells and responsible for the binding of T-lymphocytes; human mannose receptors on

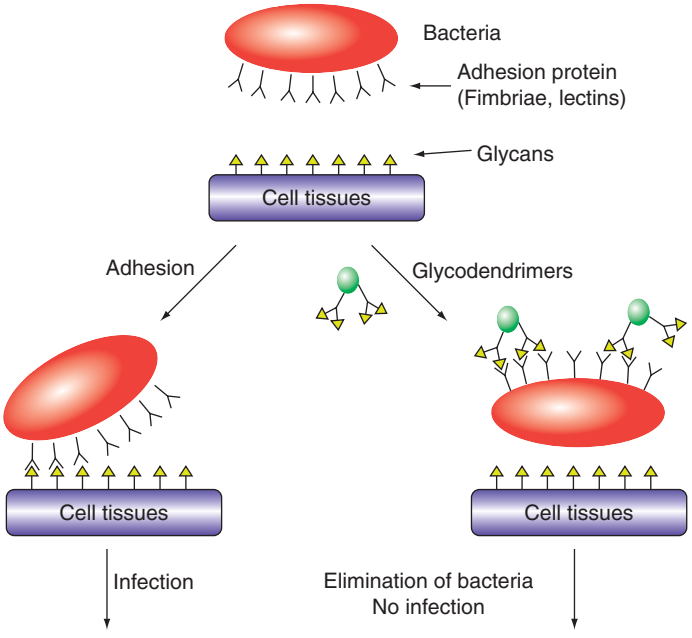



**Figure 1** Glycodendrimers are potentially useful agents to block bacterial infections.

resident macrophages and finally bacteria through their fimbriated type 1 proteins such as present in *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Serratia marcescens*, and *Shigella flexneri*. Other related mannoside-binding proteins but not discussed herein include *Urtica dioica* agglutinin (UDA), scytovirin, *Scilla campanulata* lectin (SCL), *Narcissus pseudonarcissus* lectin (NPL), *Galanthus nivalis* agglutinin (GNA), jacalin, and *Myrianthus holstii* lectin (myrianthin, MHL).

### 3.36.2.1 Human Antibody 2G12 and Cyanovirin

Figure 2 illustrates the crystal structure of the HIV-1 neutralizing human antibody 2G12 bound to the oligomannoside $Man_9GlcNAc_2$ present on the 'silent' face of the gp120 envelope glycoprotein.[21] The antibody adopts an unusual domain-swapped dimeric structure originating from interdigitation of Fab domains. Interestingly, the oligomannoside is similarly bound into the active site of cyanovirin (Figure 3).[22] While cyanovirin prefers the D1–D3 branched mannosides, the antibody 2G12 is localized in the $ManCMan D_1 Man D_2$ area. Consequently, several groups are involved in the synthetic design of properly oriented mannoside clusters for vaccination purpose.[23–25] The resulting vaccines should trigger specific, high-affinity antibodies against the carbohydrate portion of HIV's gp120.

Besides the well-studied Gal/GalNAc asialoglycoprotein hepatocyte receptors (ASGP-R), for which there is still no crystollographic data known, the case of the cyanovirin produced by the blue-green algae *Nostoc ellipsosporum* may well represent another typical role model for complex multivalent interactions.[22] This monomeric 11 kDa protein is a cyanobacterial protein that potently inactivates all strains of HIV and simian immunodeficiency virus (SIV) at the level of envelope-mediated fusion by virtue of its strong interactions with the HIV surface envelope glycoprotein gp120 and is currently under preclinical investigation as a topical antiviral microbicide.[26] The protein has a high-(13 nM) and a low-affinity binding site for high-mannose oligosaccharides. The two binding sites are 35 Å apart; thus, a single $Man_9GlcNAc_2$ residue is too small to provide a chelate effect on the two active sites simultaneously. Figure 3 illustrates the X-ray structure of the protein with bound $Man_9GlcNAc_2$ in its high-affinity site together with the bound conformation of the saccharide. The remaining low-affinity binding site can only readily accommodate smaller oligomannosides. This protein should thus constitute a good case for studying multivalent mannoside containing glycodendrimers.

### 3.36.2.2 Human Mannose-Binding Protein

The human mannose-binding protein (hMBP) is a member of the collectin family of molecules that play a role in first line host defense.[27–28] Found in the serum of many mammalian species,[29–31] this protein mediates immunoglobulin-independent defensive reactions against pathogens. Its action occurs within minutes of exposure to an infectious agent

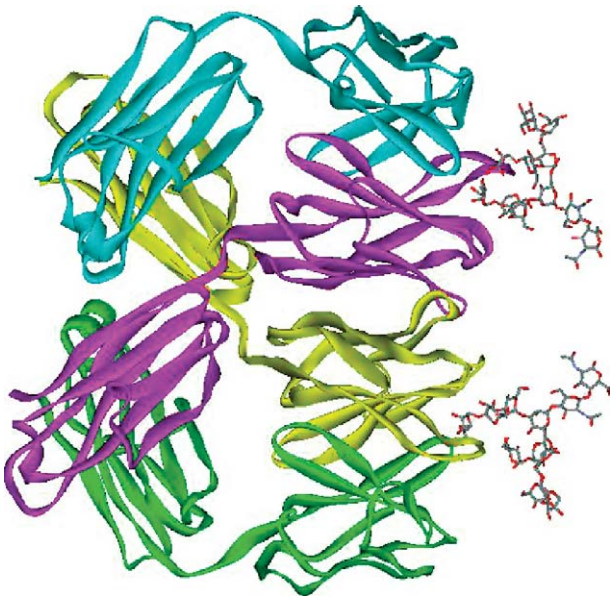


**Figure 2** Crystal structure of the HIV-1 neutralizing human antibody 2G12 bound to the oligomannoside $Man_9GlcNAc_2$ present on the 'silent' face of the gp120 envelope glycoprotein (PDB 1OP5).

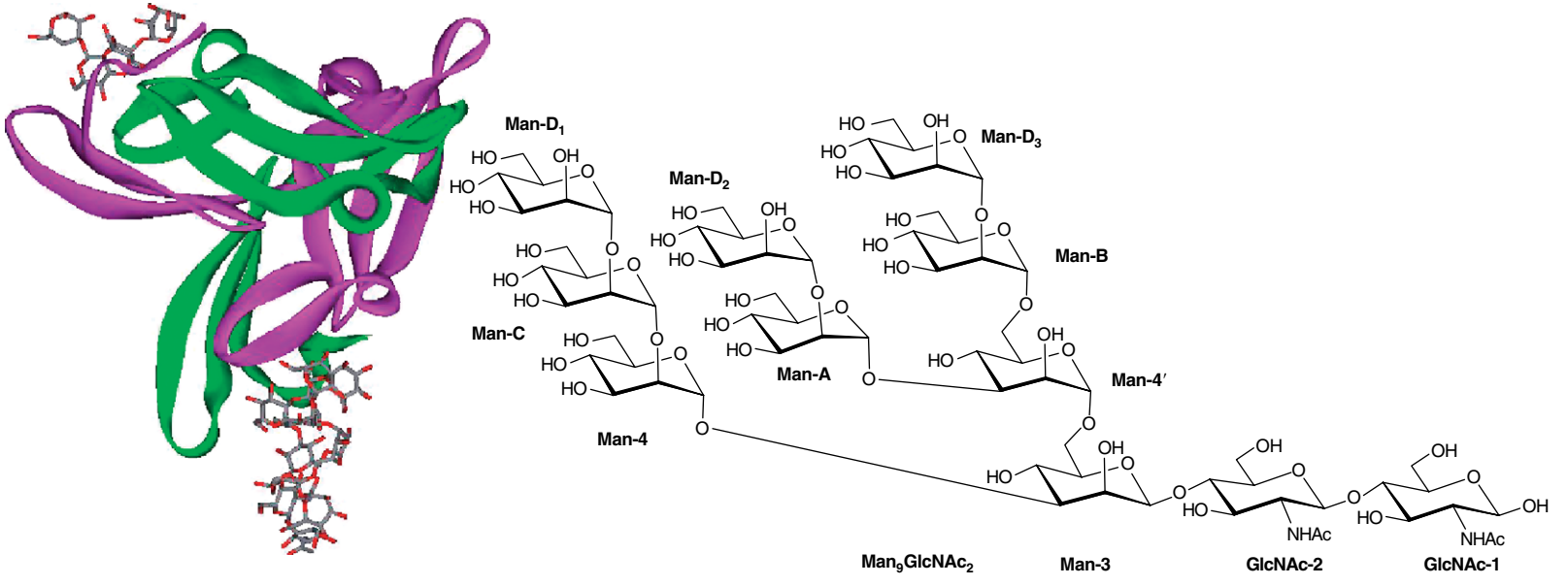



**Figure 3** Monomeric 11-kDa cyanovirin from the blue-green algae *Nostoc ellipsosporum* showing its two mannoside-binding domains with the high-affinity 13nM active site in magenta and bound $Man_9GlcNAc_2$ ligand at the bottom (PDB 1M5M).

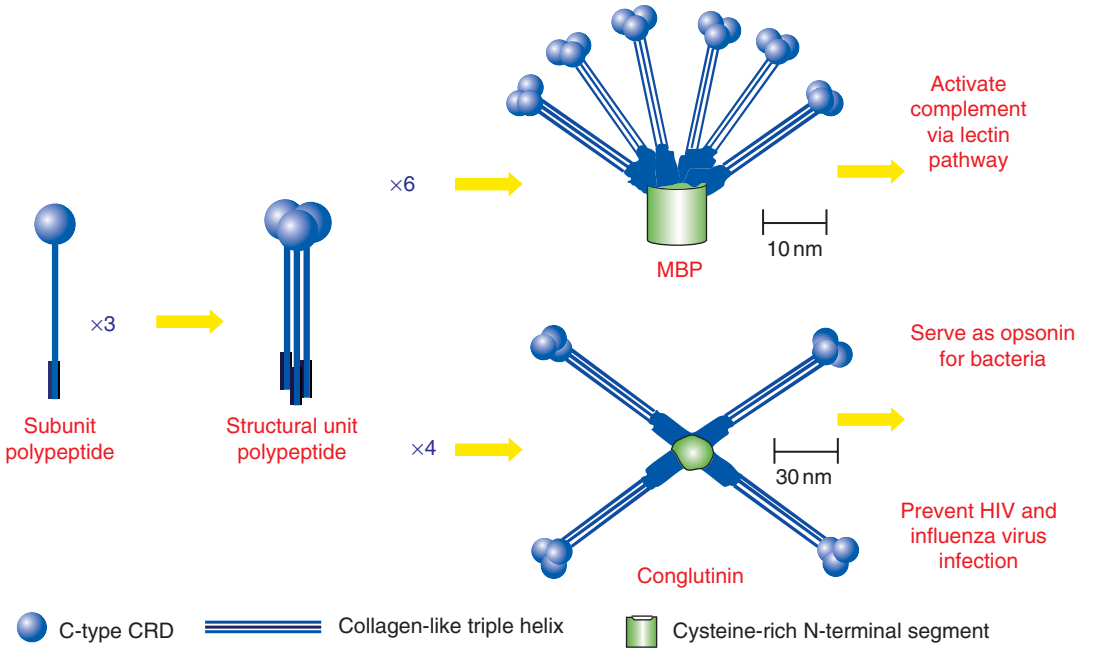



**Figure 4** Modular structures and biological activities of collectins (MBP and Conglutinin).

providing immediate defense during the 1–3 days lag period required for induction of specific antibodies.[32] The MBPs function by recognizing oligomannose on the cell surface of various bacteria and viruses. They bind and neutralize them by complement-mediated cell lysis or facilitate their recognition by phagocytes (**Figure 4**).

Upon exposure to pathogens, the concentration of MBP in the serum increases.[33] Patients with reduced levels of MBP, due to a point mutation in the MBP gene, show a propensity for repeated, severe bacterial infections.[34] MPBs also act as inhibitors of human immunodeficiency virus and influenza virus, presumably by binding to the high-mannose carbohydrates of the viral envelope glycoproteins and blocking attachment to the host cell. All these observations clearly show the importance of the MBP in the innate immune system.

The structure of the hMBP consists of 18 identical subunits arranged as a hexamer of trimers, adopting a bouquet-like structure (**Figure 4**).[35,35a–37] Each subunit's chain consists of four distinct regions. This includes a cysteine-rich region, a collagen-like region, a neck region, and a CRD that is calcium dependent, a common feature of most MBPs. The oligomerization is possible through the disulfide bonds in the cysteine-rich region, and due to the characteristic properties of the collagen-like domain and the neck region it tends to form a trimer. The clustering of three CRDs in close proximity significantly increases the affinity for small sugar clusters. The impact of further clustering can ensure that these molecules only bind with high affinity to dense sugar arrays like the one found on the surface of bacteria and viruses.

The hMBP forms oligomeric bouquets or cruciform structures of trimeric subunits each composed of three polypeptide chains coiled up in a collagenous triple-helical arrangement. This trimerization domain orients each CRD so that the ligand-binding sites are 45Å apart and 26Å from the center of the trimer (**Figure 5**). Thus, simultaneous ligation of three binding sites could not be achieved by a single molecule but rather elongated branched oligosaccharides as found on the cell walls of yeast, certain Gram-negative and Gram-positive organisms, and the envelope glycoprotein of the human immunodeficiency virus, all of which have been shown to bind to MBP. This suggests that the CRD sites are ideally configured for multivalent interactions, thereby increasing the binding affinity of hMBP to pathogens. Our group has been involved in the design of optimal mannoside trimers to provide potent chelators to hMBP and to better understand differentiating interactions between this protein and other high-mannose binding proteins (Dominique, R. and Roy, R., unpublished data).

### 3.36.2.3 Macrophages and Dendritic Cells: DC-SIGN

DC-SIGN also belongs to the family of C-type lectins able to bind high-mannose glycoproteins of HIV-gp120,[38] Ebola-gp 1,[39] or Dengue-gp E.[40] DC-SIGN, expressed exclusively at the surface of immature dendritic cells, possesses a

**Figure 5** Crystal structure of trimeric hMBP with high-mannose glycan. The yellow spheres are calcium. (S. Sheriff, personal communication.)

C-terminus CRD that, similarly to hMBP, can oligomerize into homotetramers, thus exposing four CRDs.[41,42] Additionally, DC-SIGN can organize into microdomains within lipid rafts at the plasma membrane level and can thus act as docking sites for pathogens. The interactions of this lectin with branched oligomannosides present on the pathogen glycoproteins represent another interesting and complex multivalent phenomena. Again, inhibition of these interactions may potentially represent antiviral therapies. Dendritic cells, as well as macrophages, are antigen-presenting cells that are able to stimulate $CD4^+$ T-lymphocytic responses by presenting antigens within the context of major histocompatibility (MHC) class II complexes. Hence, researchers have used these mannoside receptors to target potential vaccines.

Another representative member of this family of mannose receptors is present on the membrane-associated proteins of human peripheral and bone marrow macrophages. This 175 kDa protein is also constituted of multidomains, and interactions with mannosylated ligands are achieved through cooperative binding involving eight CRDs.[43–44] These mannoside receptors can mediate internalization of both soluble and microorganism-bearing mannoside residues. These receptors can regulate levels of endogenous glycoproteins and contribute to the clearance of potentially harmful pathogens. As such, they take part in the innate immune regulation. A linear mannoside-bearing L-lysine backbone exposing six arylated mannoside residues analogous to the glycodendrimer-based structure discussed below was shown by Biessen *et al.* to have an affinity in the low nanomolar range.[45]

### 3.36.2.4 Bacterial Lectins: *Escherichia coli* FimH

A critical step in host-tissue colonization and biofilm formation is achieved through bacterial adhesion commonly mediated by carbohydrate-binding lectin-like proteins expressed on or sheded from bacterial surfaces. Type 1 fimbriae are the most common type of adhesive appendages in *E. coli* and several other enterobacteria and mediate mannose-specific adhesion via the 30 kDa lectin-like subunit FimH.[46] The crystal structure of the FimH from uropathogenic *E. coli*, primarily causing pyelonephritis, has been solved together with methyl α-D-mannopyranoside (**1**) as well as with the hydrophobic butyl α-D-mannopyranoside.[47] The strongest monovalent inhibitor known to date for the FimH is heptyl α-D-mannopyranoside (**5**, $K_D$ 5 nM) which is thus eight times better than the known *p*-nitrophenyl α-D-mannopyranoside (**2**, PNPαMan, $K_D$ 44 nM) and four times better than methyl umbelliferyl α-D-Man (**4**, $K_D$ 20 nM). **Table 1** shows the relative affinities, $K_D$, and $\Delta G^\circ$ of a series of synthetic mannoside derivatives (**Figure 6**) against the FimH of *E. coli* K12 as measured by surface plasmon resonance.[47]

Among the series of alkyl mannosides tested from methyl up to octyl, there were 1000-fold increases from ethyl to higher homologs and a steady increase up to the heptyl mannoside (**5**) after which, the $K_D$ started to increase. It is also noticeable that the hydrophobic aglycons of the best candidates are flanked by two tyrosine residues (Tyr48 and Tyr 137) in the active site (**Figure 7**) that may provide the rationale for the observed, more than two decades ago, high affinities of *o*-chloro-*p*-nitrophenyl α-D-mannoside (**3**) against other strains of *E. coli* (346 (025) and 128).[48,48a] Indeed,

**Table 1** Relative affinity of mannosides for *Escherichia coli* K-12 isolated FimH as measured by surface plasmon resonance[47,48]

| *Ligand* | *$K_D$ SPR (nM)* | *$\Delta G°$ SPR (Kcal mol$^{-1}$)* | *Relative affinity* |
|---|---|---|---|
| Mannose | $2.3 \times 10^3$ | −7.6 | 0.96 |
| MeαMan (**1**) | $2.2 \times 10^3$ | −7.7 | 1.00 |
| EthylαMan | $1.2 \times 10^3$ | −8.1 | 1.8 |
| PropylαMan | 300 | −8.9 | 7.3 |
| ButylαMan | 151 | −9.3 | 15 |
| PentylαMan | 25 | −10.4 | 88 |
| HexylαMan | 10 | −10.9 | 220 |
| HeptylαMan (**5**) | 5 | −11.3 | 440 |
| OctylαMan | 22 | −10.4 | 100 |
| PNPαMan (**2**) | 44 | −10.0 | 50 |
| MeUmbαMan (**4**) | 20 | −10.5 | 110 |
| **6** | 113 | −9.5 | 19 |
| **7** | 55 | −9.9 | 40 |

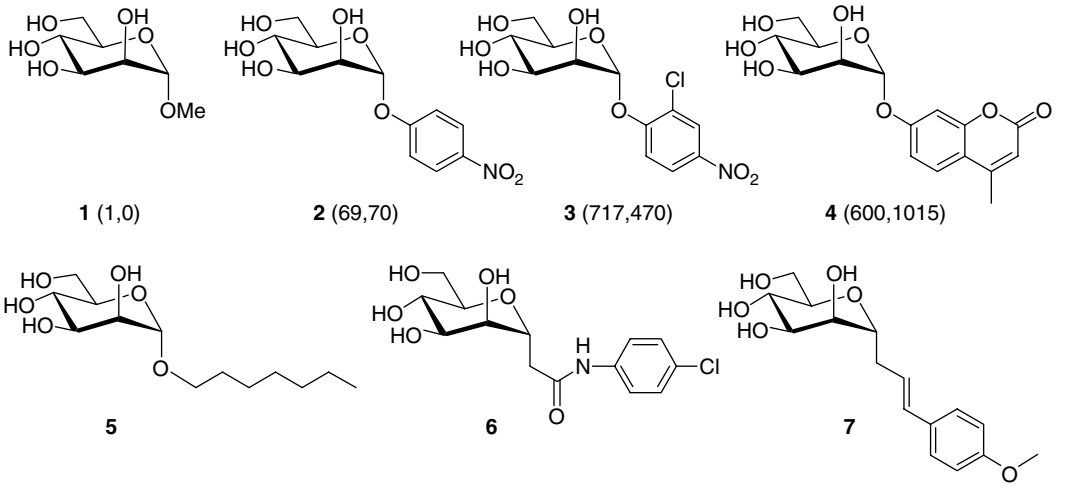



**Figure 6** Different mannoside derivatives and their relative inhibitory properties against the interaction of fimbriated *E. coli* O25 or O128 in agglutination of yeasts or adherence to epithelial cells, respectively (number in parentheses).

in inhibition of yeast aggregation by the bacteria, compound **3** showed relative inhibitory behavior that was 717 and 470 times better than the reference compound **1**, respectively (**Figure 6**). In a recent study from this laboratory (unpublished data), a small library of mannoside analogs was constructed to build a QSAR model for the FimH. The library included several C-linked mannosides, a few of which (cpds **6**, **7**) were almost equivalent to PNPαMan. These C-glycosides are valuable candidates for *in vivo* assays since they are stable under physiological conditions. Moreover, they are suitably functionalized for further manipulations such as those encountered in typical glycodendrimer syntheses. It is our opinion that the most potent glycodendrimers will be those constructed using the best monovalent aglycons together with optimized scaffolds, valencies, and linker distances. These factors should necessarily vary from one receptor to another.

## 3.36.3 Glycodendrimers

### 3.36.3.1 Overview

Glycodendrimers are an interesting class of synthetic well-defined biomacromolecules that have been initially designed to address the issue of low-affinity carbohydrate–protein interactions encountered in so many biologically relevant situations.[3–6, 10,10a,10b,11] They were initially thought to expand our understanding of the 'classical glycoside

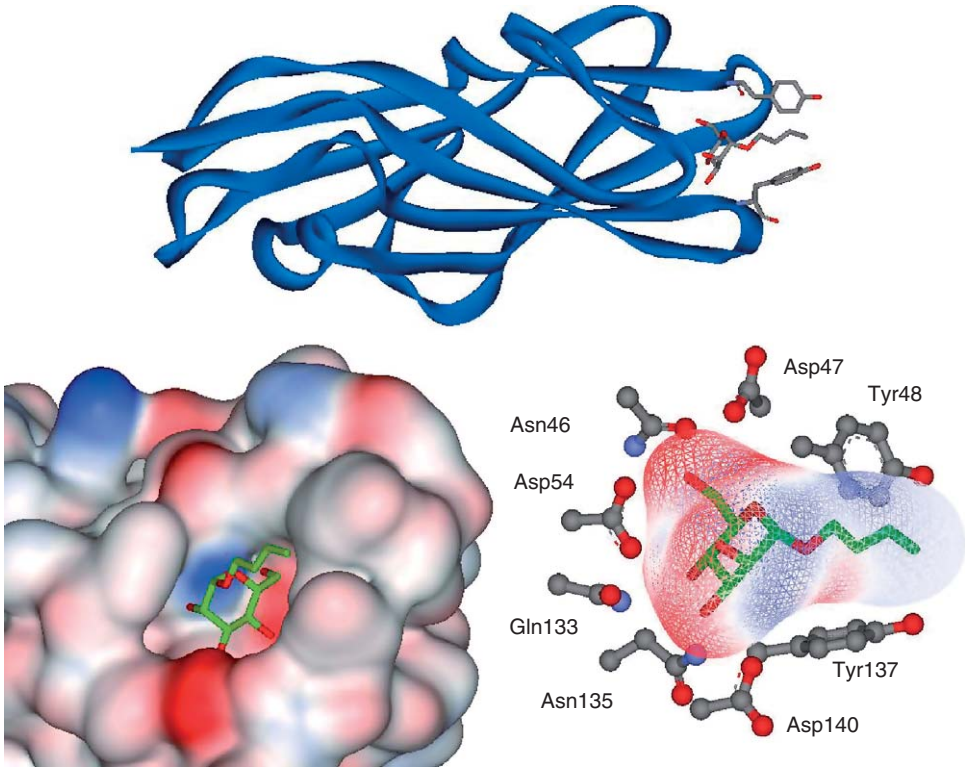



**Figure 7** Crystal structure of *E. coli* K12 FimH incorporating the potent inhibitor butyl αMan (PDB 1UWF). Top: cartoon showing the mannose ligand flanked by two tyrosines 48 and 137.

cluster effect' originally proposed by Y. C. Lee,[18] a pioneer in glycoconjugate chemistry and biochemistry. In its widespread version, it is usually assumed that the glycoside cluster effect has its source in the enhanced affinity of a given multivalent glycoside toward a CRD by fully occupying 'one active site' at a time.

Glycodendrimers, just like any other typical tree-like organic dendrimers, may take several structural architectures as they can be prepared as dendrons (wedge structures), spherical or globular dendrimers, and even polymer-dendrimer hybrids called dendronized polymers.[19,19a,19b,49,50] A few reports also describe their preparation using self-assembly techniques based on transition metal complexes with bipyridine or terpyridine cores.[51,52] Glycodendrimers can be synthesized by divergent, convergent, or double-stage convergent strategies using a hypercore molecule as the central element. Moreover, for sake of simplicity and diminished cost, the inner scaffold portion of the molecules can be synthesized by a one-pot procedure using hyperbranched polymer methodologies. This can be achieved based on $AB_2$ monomer systems such as those found in commercially available hyberbranched polyglycerols, hyperbranched polyethyleneimine (PEI), and Boltorn® dendrimers of low dispersities that were constructed using glycidol, ethylene imine, and 2,2-bis(hydroxymethyl)propionic acid (bis-MPA), respectively. Should a commercial application of glycodendrimers arise, the latter strategy will allow low-cost production, albeit at the expense of structural homogeneity.

### 3.36.3.1.1 Commercially available dendrimer cores

Several dendrimers having various surface functionalities and building blocks are commercially available, some of which are illustrated in **Figure 8**. Polyamidoamine dendrimers (PAMAM, Starburst, Dendritic Nanotechnologies), polypropylenimine (PPI, Astramol, DSM Fine Chemicals), polyglycerols, and Boltorn® dendrimers are most commonly used.[10,10a,10b] PAMAM-based dendrimers[53] having built-in amine functionalities on the surfaces have been the first and most frequently used scaffolds for sugar attachment. The very first application used amide bond formation starting from sugar lactones.[54] Although this straightforward manipulation has not yet been applied toward mannosides, it should be readily applicable to this important carbohydrate. It has the disadvantage of sacrificing the reducing sugars, which alternatively serve as extended linkers. As discussed in more details below, PAMAM (and potentially any other amine-ending dendrimers) have been functionalized with carbohydrates using: (1) thiourea linkages formed by treating the aminated dendrimers with sugar isothiocyanates; (2) amide linkages with carboxylated sugars; and

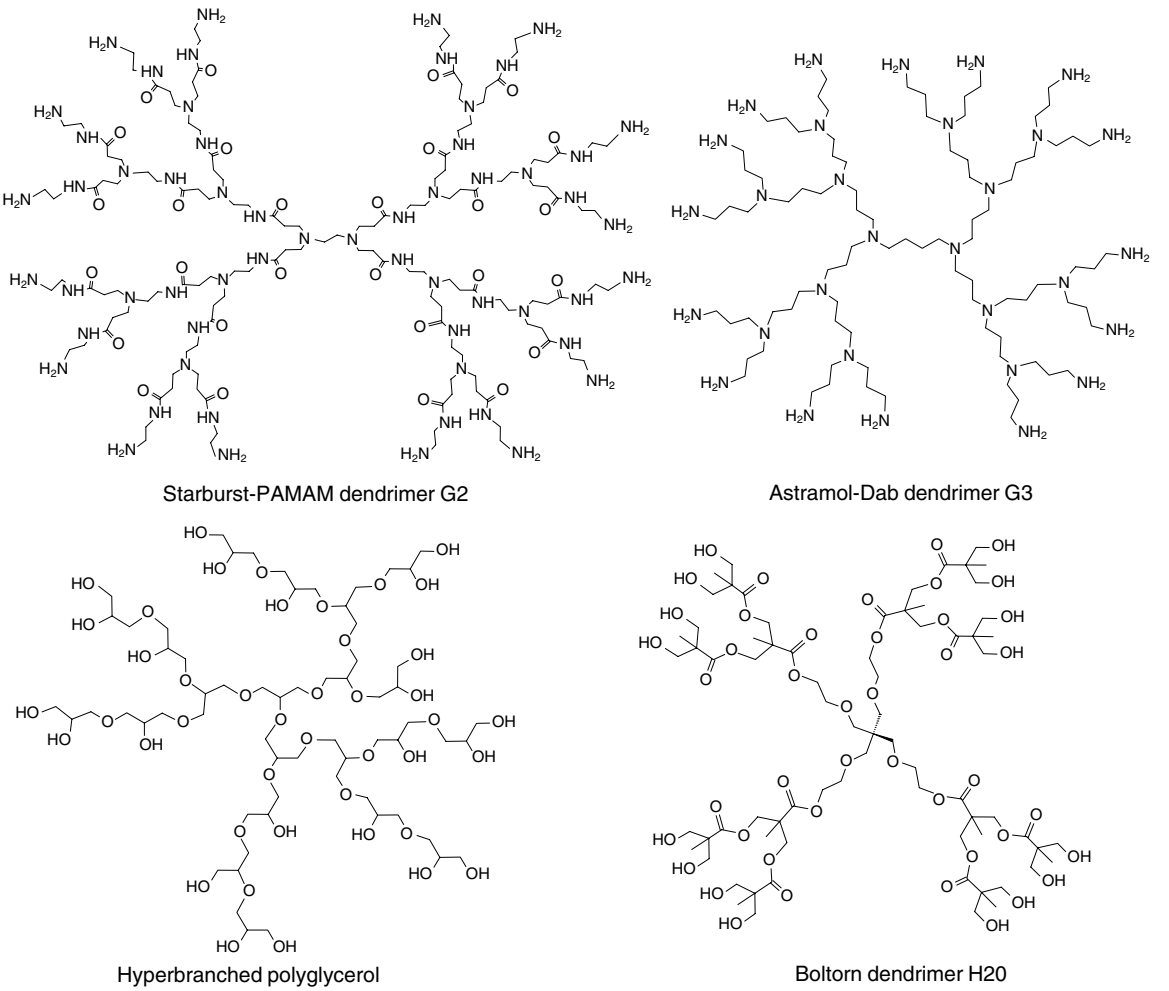



**Figure 8** Structures of commercially available dendrimer scaffolds bearing amine or alcohol functionalities.

(3) reductive amination. This last procedure can give rise to mixtures of both mono- and di-alkylation. The largest glycodendrimer built so far contained 256 mannoside residues and was prepared on generation G6-PAMAM dendrimer.[55]

### 3.36.3.1.2 Synthetic strategies

Basically, there are two key strategies for building glycodendrimers (Figure 9). The first one involves the initial synthesis of core molecules by a divergent process. In this approach, the focal and multifunctional molecules are systematically expanded outward using various chemical linkages. For instance, PAMAM-based dendrimers use α,ω-diamines as core (e.g., 1,2-ethylenediamine, 1,4-butanediamine, etc.), the amines of which are then treated with methyl acrylate by a double N-alkylation. The ensuing esters are reacted with a large excess of 1,2-ethylenediamine to provide the first generation G0 bearing four amine functionalities. This process (alkylation/amidation) is sequentially repeated from the 'heart' of the molecules until the desired external valencies are reached. The sugars are then appended at the molecules' periphery. The growth is doubly exponential ($2^n$) but the number of surface groups may vary depending on the number of functionalities on the 'seeding' molecules, for example, 2,4,8. . .; 3,6,12. . .; 4,8,16. . .; etc. The main disadvantage of the divergent growth is the necessity for an increasing number of efficacious reactions required to obtain defect-free dendrimers that are otherwise difficult to purify given the small differences in their size and number of functional groups.

The second approach, the convergent growth strategy, avoids several of the synthetic challenges inherent to the one described above. The procedure involves the construction of glycodendrons (wedges) that are useful on their own followed by their attachment to multivalent core structures. The procedure is simplified because the number of

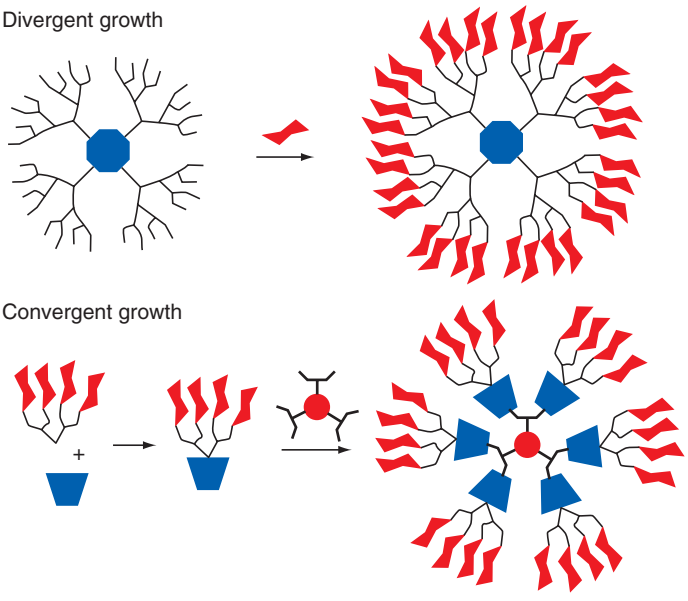



**Figure 9** The two major synthetic strategies toward glycodendrimer syntheses.

coupling partners is reduced to a minimum at any particular step. Moreover, the target molecules, by virtue of its higher molecular weight in comparison to their fragments are simpler to purify by size exclusion chromatography. The number of peripheral carbohydrate moieties that cause steric inaccessibility eventually limits the technique. Nevertheless, it is now well established that a large number of surface carbohydrate moieties are detrimental to the carbohydrate accessibility by carbohydrate-binding receptors. Doubly convergent approaches have also been described. In these cases, the core molecules are first assembled with several surface functional groups that could be directly attached to glycodendrons.

Of additional interest is the fact that glycodendrimers can be synthesized by solid-phase chemistry, combinatorial, and dynamic combinatorial methods. A recent example also illustrates the feasibility of using microwave activation for the coupling of the carbohydrate moieties. This will be discussed in the 'clicked' dendrimer section below.

### 3.36.3.2 Lysine-Based Glycodendrimers

The first glycodendrimers to appear in the literature in 1993 were built using divergent solid-phase peptide chemistry and L-lysine as repeating assemblies.[56] They were bearing exposed sialic acid residues and were constructed for the inhibition of flu virus attachment by competing with sialoside ligands present on respiratory tracts against the hemagglutinin of the virus particles (**Figure 10**). Using dendrons having eight sialosides on the surface, Roy *et al.* were able to demonstrate that each saccharide residue was 1000-fold better, on a per saccharide basis than the corresponding monomer.[57] They were however not as efficient as polymers because they could not cover efficiently the surfaces of the spherical viral particles, a property well exploited by random coiled polymers. It was later argued that dendronized polymers were even more potent in this respect.[57] When the same poly-L-lysine scaffold was utilized with mannoside residues bearing an arylated aglycon (partly optimized monomer), the resulting 8-mer glycondendron happened to show 100 000-fold increased inhibitory potency against fimbriated *E. coli* K12 on a per mannoside basis.[58] The structures of the corresponding sialo-(**Figure 10**) and manno-dendrimers (**Figure 11**) are illustrated below.

The dendronized lysines represent one of the most widely used core structures in glycodendrimers. A review by Niederhafner *et al.* describes the synthesis of peptide dendrimers and their application.[59] Glycosylated lysine dendrimers have been prepared both on solid supports[60,60a] and in solution.[61,61a] Reaction of the peripheral amino groups with a variety of electrophiles carrying pendant sugar residues leads to glycodendrimers.

Roy and co-workers have previously reported lysine glycodendrimers carrying a variety of carbohydrates via thioetherification of α-chloroacetamide attached to the N-terminal amine groups of lysine.[62] An activated ester coupling was described to provide galactoside- and *N*-acetylglucosamine-capped lysine glycoclusters.[63] The later was also further elaborated as Lewis$^x$ antigens by chemoenzymatic processes.[64]

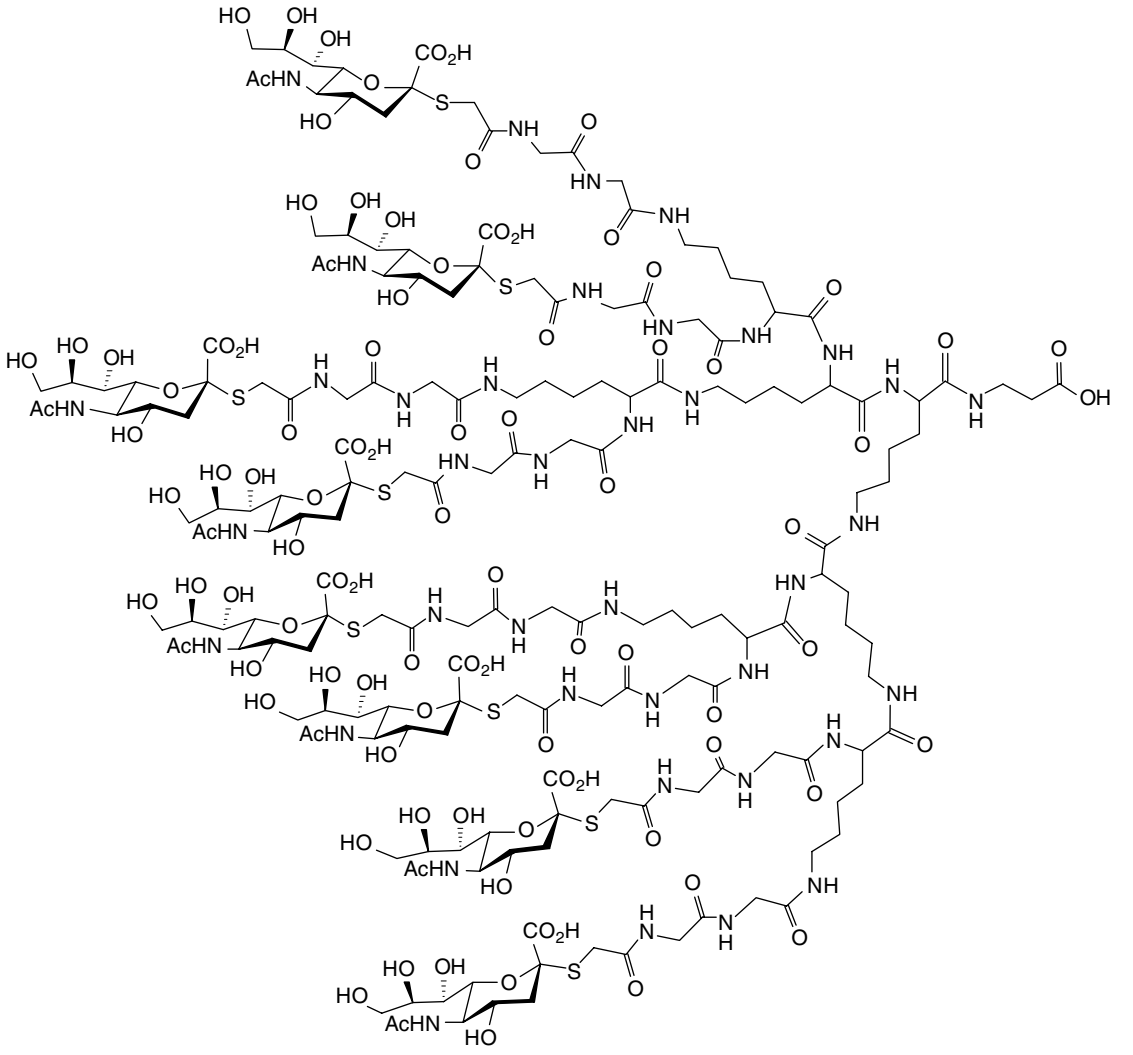


**Figure 10** Non-optimized α-thiosialo-dendrimer bearing eight residues based on a polylysine core. This structure was used for the inhibition of flu virus adhesion to human erythrocytes ($IC_{50}$ low μM).

As mentioned above, dendritic cells and macrophages are effective antigen presenting cells (APCs). Since these cells expose MBPs, they constitute powerful candidates for vaccine targeting. Thus, instead of associating short immunogenic peptides to protein carriers, such as keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA),[65] Chang and co-workers have recently investigated the possibility of using mannoside-capped polylysine glycodendrimers (**10**) constructed at the N-terminal of several immunogenic peptides (**Figure 12**).[66] Peptide sequences from HIV gp41 protein (541–555 bearing the LLSGIV motif capable of inhibiting viral fusion, 553–567),[67] SARS-CoV S2 (1081–1105, 1144–1187), and influenza hemagglutinin HA2 (1–25) were built on a Rink amide resin. The lysine moieties were then introduced as a G-3 lysine dendrimer followed by mannosylation, using 4-mannosyloxy butanoic acid (**8**) at the terminal (ε) amino groups of the lysyl-peptide dendrimer (**9**). Preliminary data from vaccine **10**, containing the LLSGIV motif, demonstrated that it could elicit polyclonal antibodies response in rabbit much stronger than the KLH construct. It was concluded that the mannosylated dendron was stabilizing the antigenicity of the peptide by protecting it from proteolysis. N-terminally mannosylated peptides carrying one to six mannose residues were also shown by Koning and co-workers to elicit immune response with efficiency up to 104-fold greater than peptide antigens alone.[68]

Mannose receptors are capable of mediating internalization of both soluble and particulate carbohydrate structures and as such they take part in innate immunity.[69,69a] The broad pattern recognition displayed by mannose receptors

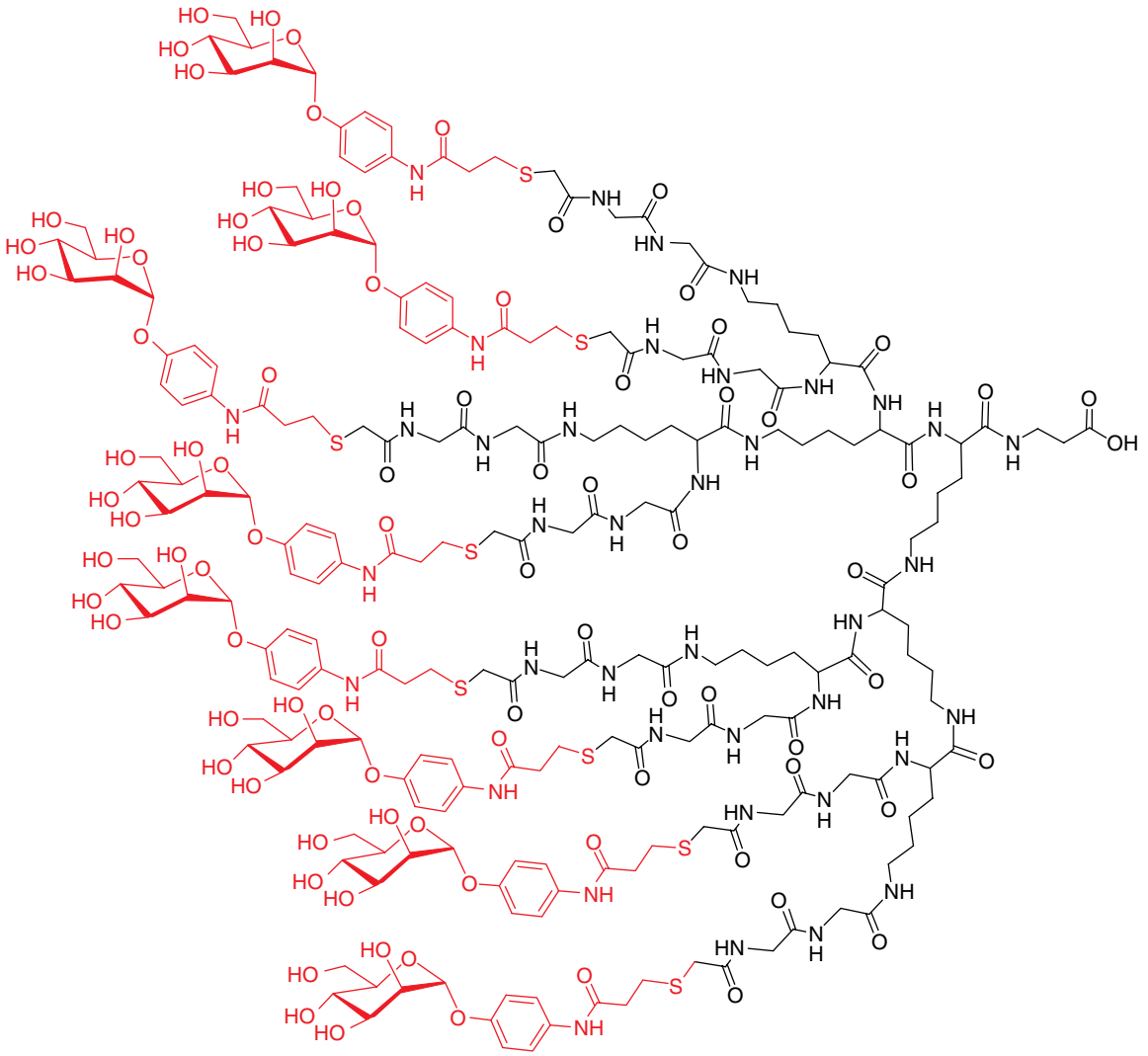


**Figure 11** Arylated glycomannoside bearing eight residues based on a polylysines core. This construct showed an $IC_{50}$ of 14 νM against *E. coli* K12.

together with their implication in adaptive immunity has stimulated considerable efforts toward the selective delivery of enzymes,[70,70a] drugs,[71,71a,71b] oligonucleotides or genes,[72,72a–72c] and antigens[73,73a,73b] to cells expressing them for therapeutic and vaccine strategies.

X-ray crystallographic data from the related rat mannose-binding lectin showed extensive hydrogen bonds and coordination bonds between two equatorial, vicinal 3- and 4-hydroxy groups for D-mannose. As shown in **Figure 13**, it becomes clear that D-(–)-quinic and shikimic acid, with their 4,5 vicinal diol in *trans*-diequatorial or pseudo-diequatorial arrangements, respectively, could function as mannose isosteres. This strategy was extensively exploited by Grandjean and co-workers to design dendrimers containing mannose mimetics.[74]

The synthetic sequence used started by the formation of shikimic or quinic acid amide bond formation with both α and ε L-lysine peptides bearing cysteine and glycine to afford dendron **11**. This was followed by the insertion of fluorescein isothiocyanate at the first ε-amino group of the bis $(ClCH_2CO)_2$-Lys-Lys$(NH_2)$-β-Ala-$NH_2$ (**12**), and finally by thioether bond formation between the two synthons **11** and **12** to provide the desired glycodendrimer 13 (**Figure 13**).

Fluorescein-labeled pseudo-glycodendrimers with valencies of two to eight were tested by competitive inhibition assays with mannan, which was evaluated by confocal microscopy analysis using mannose receptors expressed in transfected Cos-1 cells. Cells expressing mannose receptor-mediated uptake was assayed on monocyte-derived human

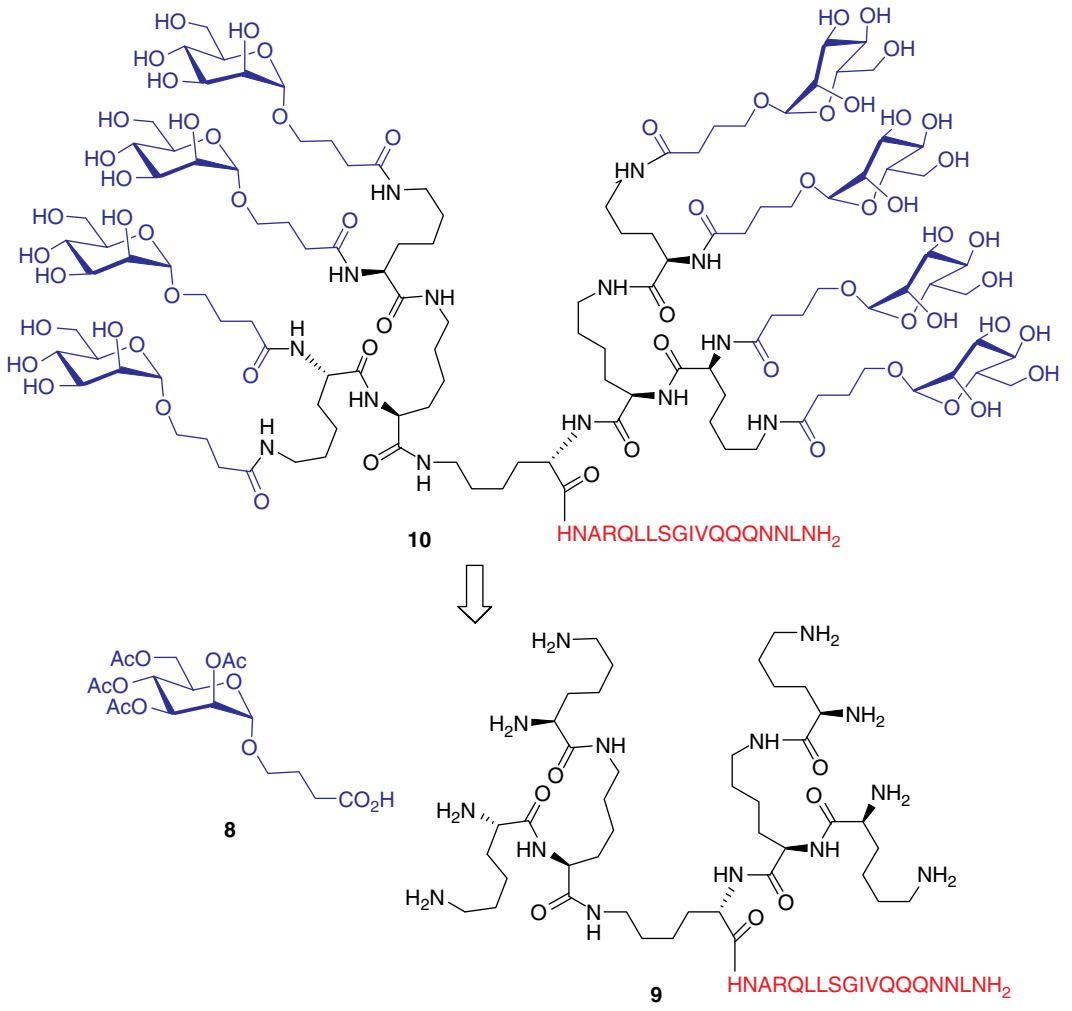



**Figure 12** Retrosynthetic strategies for the synthesis of octamannosylated third-generation polylysine dendrimer–peptide conjugate used as vaccine against HIV-1.

dendritic cells by cytofluorimetric analysis. The synthetic clusters were shown to be effective ligands against the dendritic cells, with an optimum affinity toward clusters having a valency of four. The glycomimetics did not perform as well as the natural mannosides. However, these results indicated that the mannose receptor could accommodate structural variations significantly divergent from the natural ligand.

In the same vein, Kiessling and co-workers have synthesized a solid-phase library of glycomimetics from shikimic acid.[75] Rink amide resin was utilized as the solid support. The immobilized amine was coupled to several different amino acids, the N-terminal of which was then coupled to shikimic acid through amide bond formation, as above. Conjugate nucleophilic addition of thiolates, cleavage from the resin, and removal of the protecting groups from the amino acid side chains furnished a library of 192 compounds. The relative binding potency of the library members toward the mannose-binding protein (MBP-A) using fluorescein-labeled MBP-A was measured. Ten compounds were identified with potencies comparable or even slightly better than that of MeαMan (**Figure 14**).

### 3.36.3.3 PAMAM-Based Glycodendrimers

Tomalia and co-workers have pioneered the synthesis of poly(amidoamine) (PAMAM) dendrimers (**Figure 8**) using the divergent growth procedure described above.[53,76] These attractive molecules constitute an exciting new class of macromolecular architectures and have drawn a vast interest in several research areas.[77,77a–77g] As they are commercially available, these scaffolds were extensively used by many groups of glycochemists.

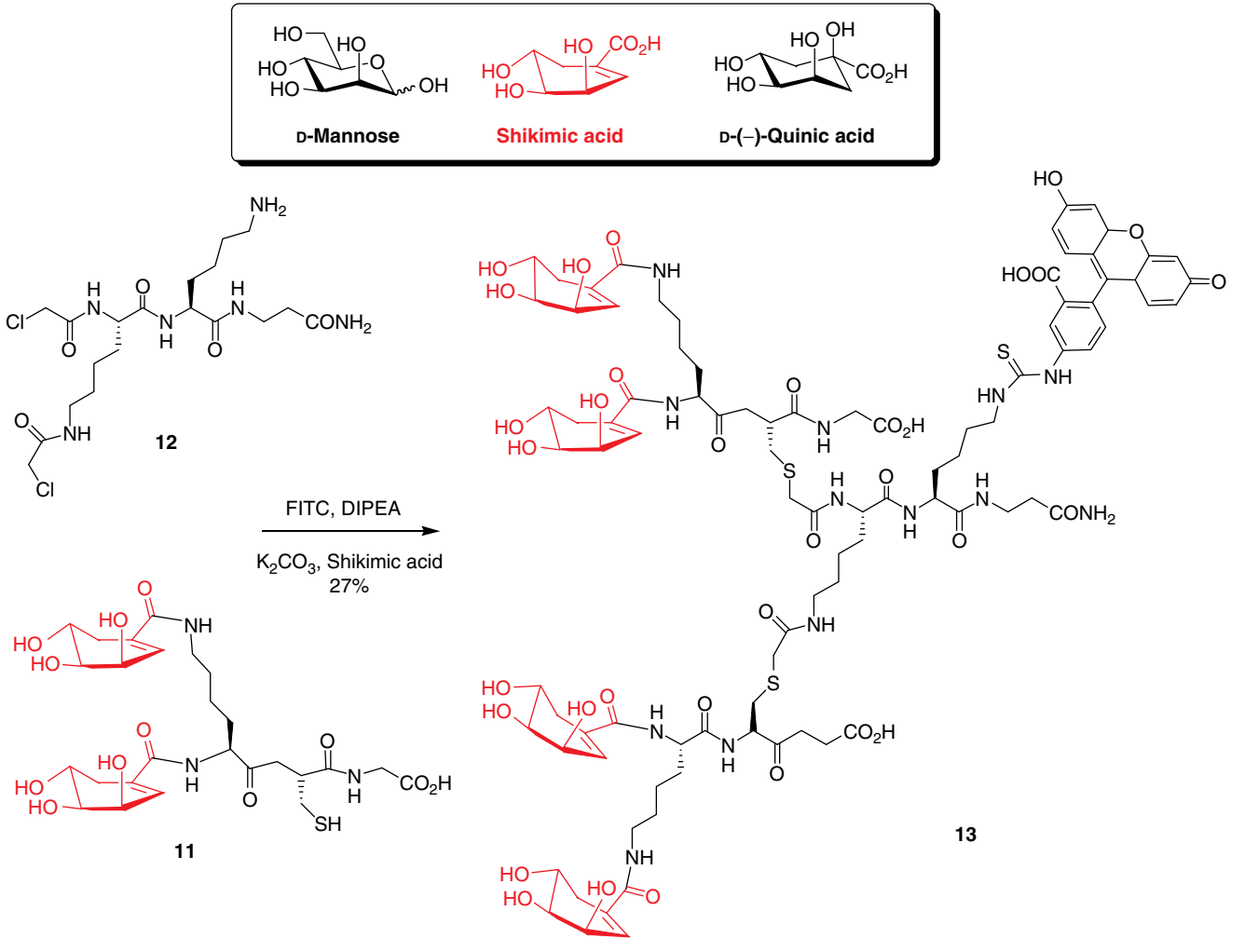



**Figure 13** Mannose isosters (shikimic and quinic acids) used on a polylysine dendrimer scaffold for the improved targeting of macrophages and dendritic cells.

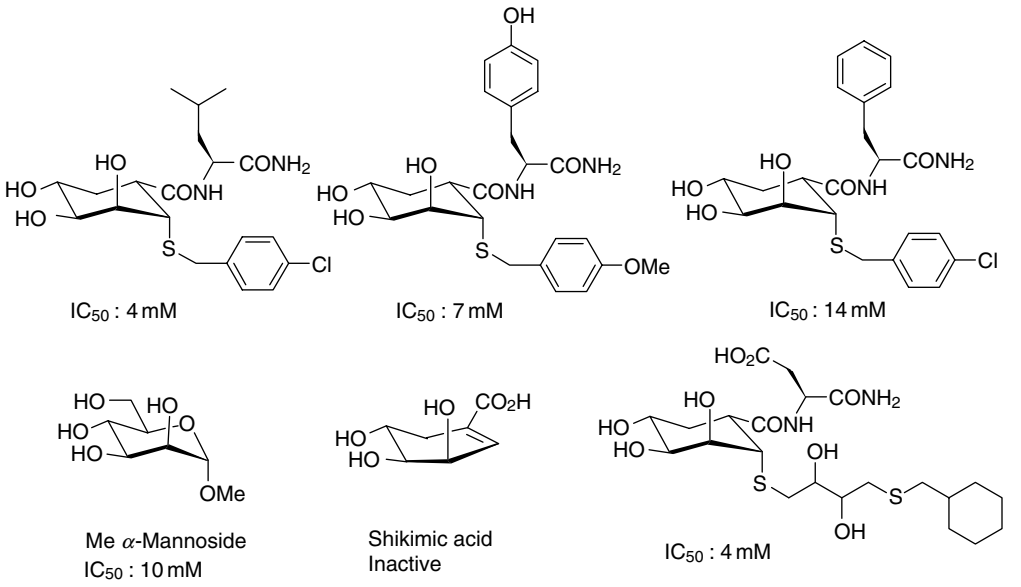



**Figure 14** Shikimic acid derivatives with relative activity in a MBP-A inhibition assay.

Our group has previously described the easy and efficient coupling of several amino-ending PAMAM generations to *p*-isothiocyanatophenyl α-D-mannopyranoside.[78,78a–78e] **Figure 15** shows an example of a resulting a 32-mer mannodendrimer (**14**). This useful and efficient mode of coupling through isothiourea linkages was therefore used by several other groups and was nicely exploited by Lindhorst[5] and Cloninger.[6]

The first four generations of this novel class of monodispersed neoglycoconjugates having up to 32 mannoside units were evaluated as ligands for the phytohemagglutinins from ConA and *Pisum sativum* (pea lectin) using enzyme-linked lectin assay (ELLA) and turbidimetric analysis. The binding properties of these glycodendrimers, together with reference monosaccharides, were determined using yeast mannan as a coating antigen and peroxidase-labeled lectins. These mannosylated dendrimers were demonstrated to be potent inhibitors with $IC_{50}$ values 400 times better than those of monomeric methyl α-D-mannopyranoside taken as a standard.

Lindhorst and co-workers have also used the same thiourea-bridge, with protected and unprotected mannoside derivatives (**15**) (**Figure 16**)[79,79a,79b] to investigate their potencies in the inhibition of hemagglutination of guinea pig erythrocytes by type 1 fimbriated *E. coli*. With the trivalent cluster, an increase in binding potency of over 100-fold was achieved; however, this inhibitory behavior was surpassed by hexa- and octa-mers. The nonspecific interactions such as hydrophobic interactions and stacking effects have to be considered for the interpretation of these results.

Reductive amination was used by Lindhorst and co-workers to provide access to glycoclusters from a tris(aminoethyl)amine core (**17**).[80] The glycoclusters were obtained from (2-mannosyloxy)ethanal (**16**), which was obtained from allyl α-D-mannopyranoside by ozonolysis, followed by treatment with sodium triacetoxyborohydride ($NaBH(OAc)_3$) (**Figure 17**).[80] This procedure may give rise to double N-alkylation, so excess aldehydes must be used for completion. In more complex carbohydrate-based glycodendrimers, this situation may lead to undesired partial structures. To circumvent it, Stoddart *et al.* in their sialodendrimers syntheses targeting siglecs, used an *N*-methyl amine for the reductive amination.[81,81a]

Similarly, Okada and co-workers have used unprotected sugar-lactones (**18**) which can form amide bonds directly with amine-ending PAMAM dendrimers (**19**) in DMSO[54] to afford the second- to fourth-generation lactodendrimers **20** (**Figure 18**). As discussed, this strategy can be equally applied to mannosides.

Pieters and co-workers have described the preparation and evaluation of multivalent mannosides, including small divalent systems, glycodendrimers, and glycopolymers, as inhibitors of type 1 fimbriated uropathogenic *E. coli*. The mannosylated surface groups were obtained by an amide bond formation between 3-aminopropyl α-mannopyranoside that was pretreated with diglycolic anhydride. Typical BOP or TBTU peptide coupling reagents were used to furnish the desired PAMAM-dendrimers (**21**) (**Figure 19**).[82]

A novel and easy bioassay was set for this purpose. The mannosylated dendrimers to be tested were used in an ELISA-based assay for their ability to inhibit the binding of mannose-binding type 1 fimbriated *E. coli* (FimH) to a

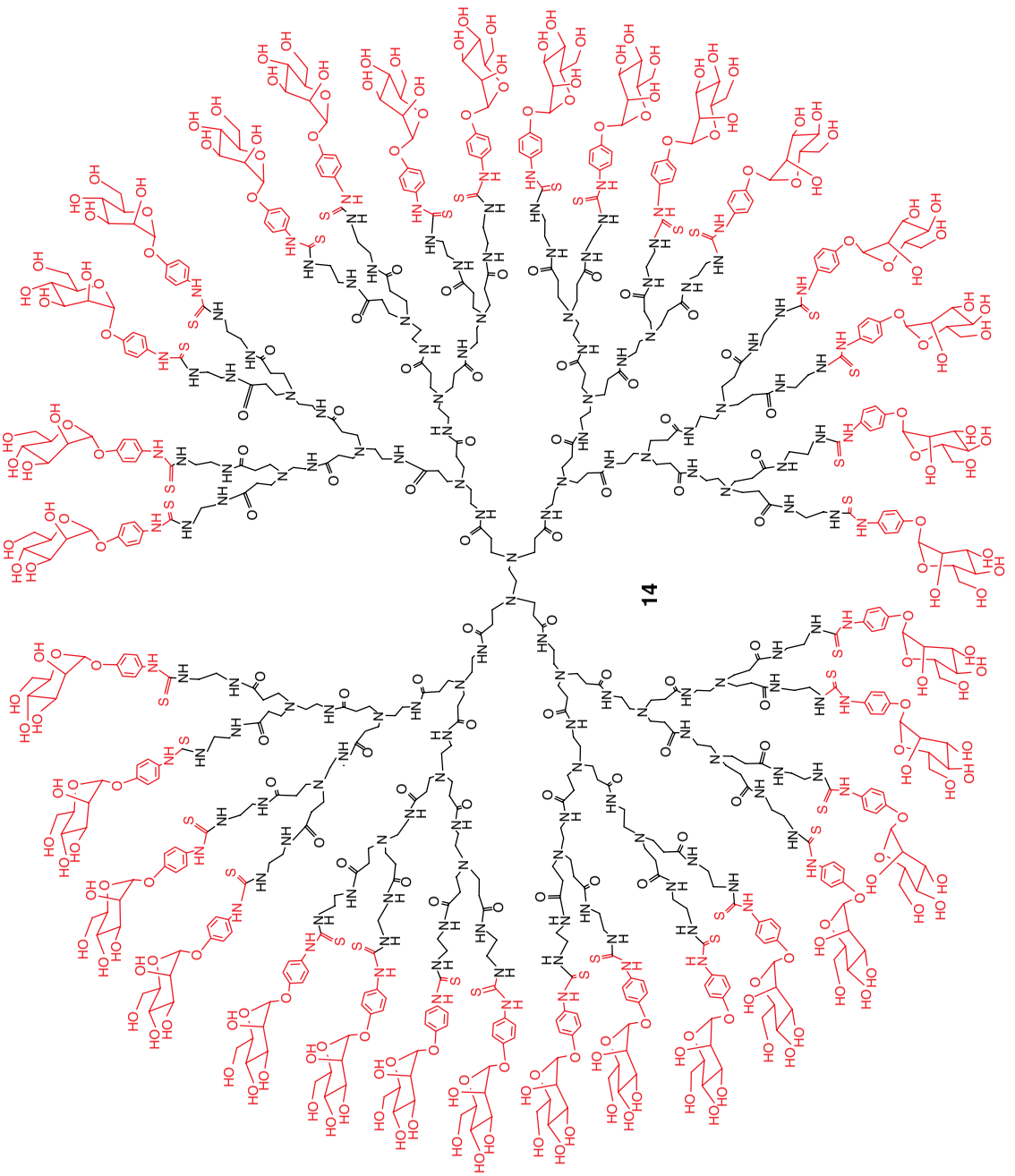



**Figure 15** 32-Mer mannodendrimer **14** with isithiourea linkages based on a PAMAM core.

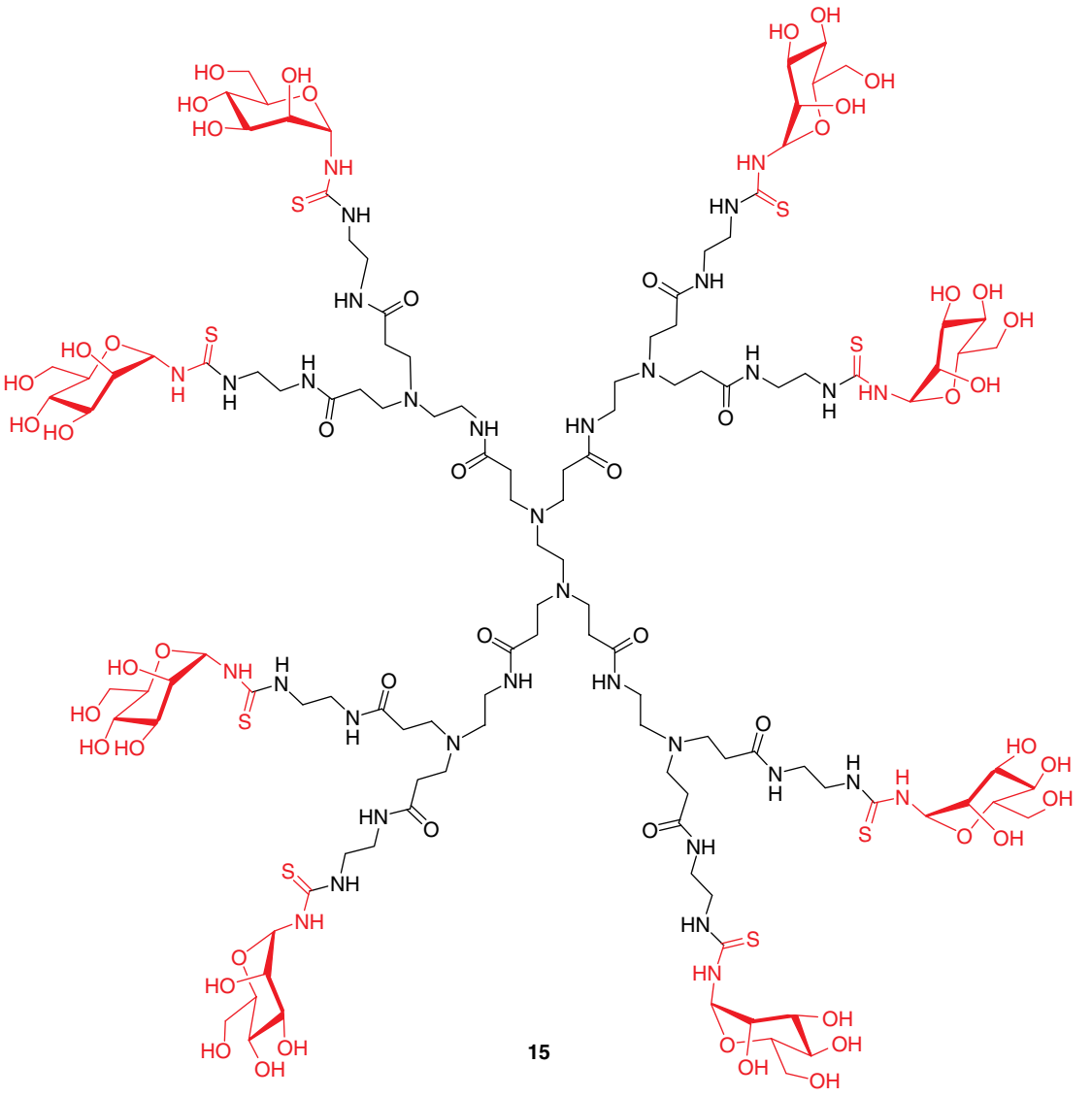



**Figure 16** Thiourea-bridged PAMAM-based dendrimer **15** obtained using unprotected mannopyranosyl thioisocyanate.

monolayer of T24 cell lines derived from human urinary bladder epithelium. The PAMAM mannodendrimers displayed the highest affinity towards the target, although their relative potency per mannose was rather low. Glycopolymers with 3–21 mannose residues per polymer showed enhanced activity with increasing mannose substitution up to an $IC_{50}$ as low as 12μM. The relative potency of the polymer series on a per mannoside basis was relatively constant at around 30–40.

Cloninger and co-workers have similarly described first-through sixth-generation PAMAM dendrimers (256 surface groups) with mannoside residues and thiourea linkers.[6,55,83] When compared to methyl α-D-mannoside taken as control monomer, dendrimers G1 and G2 did not show any increase in activity toward the phytohemagglutinin ConA and the G3 dendrimer bound roughly one order of magnitude better than G1, G2, or methyl mannoside. This is suggestive of a glycoside clustering effect (enhanced local concentration). As with G1 and G2, G3 was too small for multivalent binding to occur (chelate effect). Dendrimers G4–G6 all showed increased activity against the tetrameric Con A of two orders of magnitude, indicating that multivalent binding was occurring. It is also possible that the change in shape from circular (G(1)- to G(3)-PAMAM) to spherical (G4–G6) caused the observed binding enhancement. It is to be noted that each of the four mannose-binding sites in ConA is ~73Å apart from each other (calcium to calcium

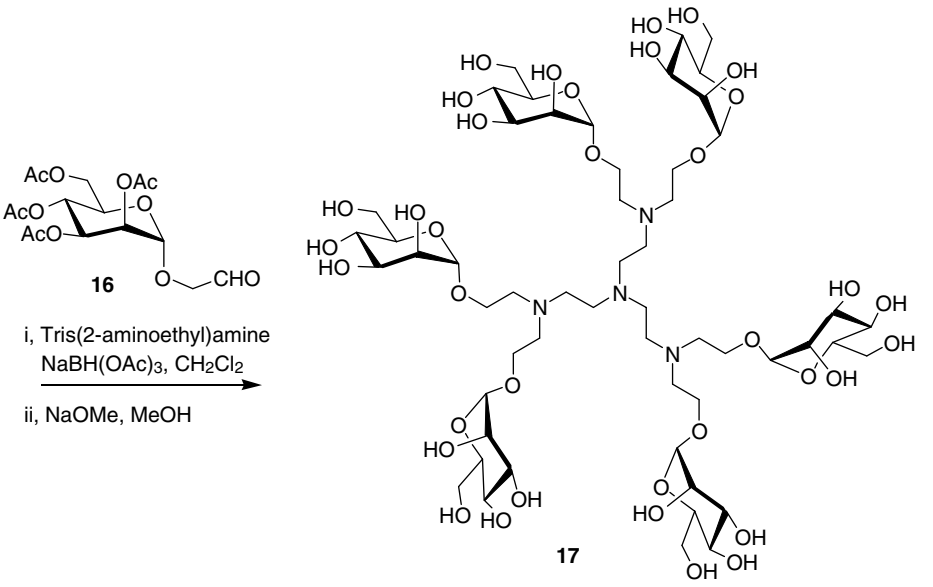



**Figure 17** Reductive amination for glycoclusters synthesis.

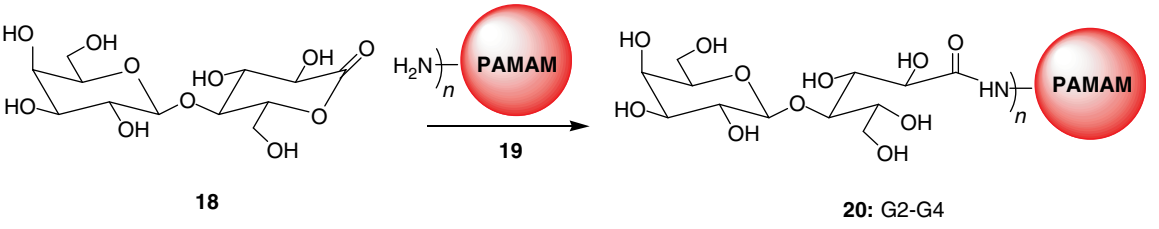



**Figure 18** Lactose-PAMAM dendrimers obtained by amidation of lactonolactone.

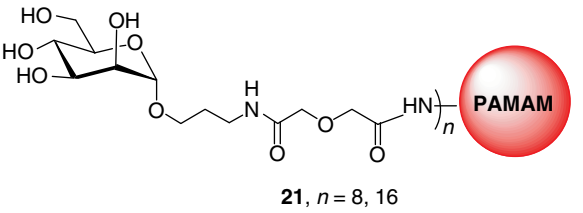
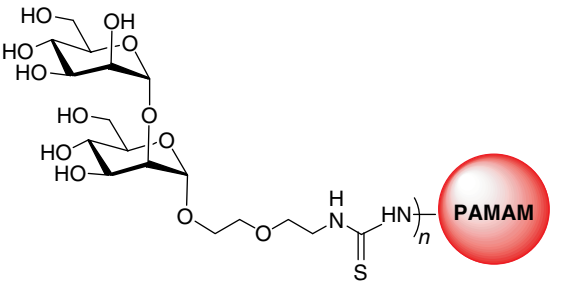



**Figure 19** PAMAM glycodendrimers **21** obtained from 3-aminopropyl α-D-mannopyranoside.

distances). Lectin cross-linking that only requires clusters to be <10Å apart may also cause the increase in affinity. Noteworthy is the fact that dimeric lectin only requires a dimeric cluster for this to happen.

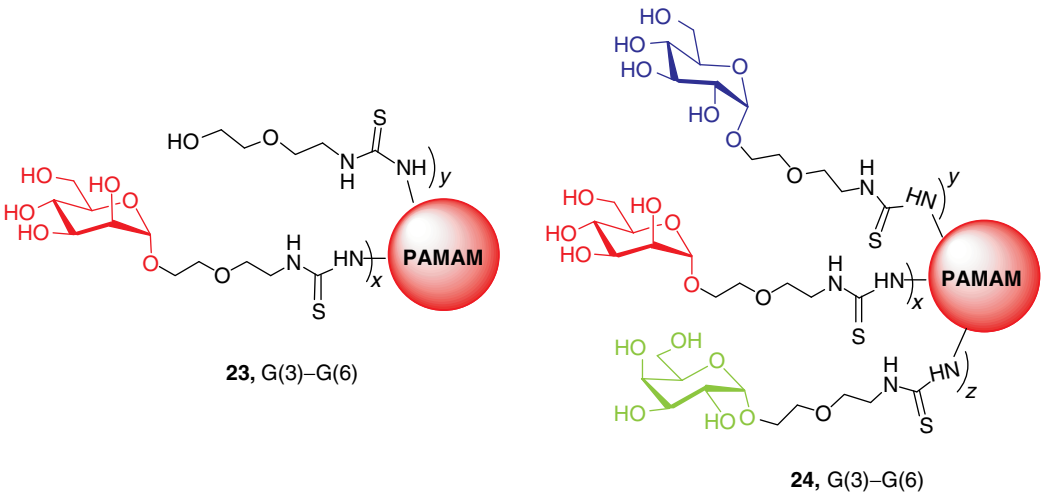
Cyanovirin-N (CV-N) recognizes Manα1-2Man disaccharide and higher homologs (see discussion above) that are present on terminal branches of high oligomannose structures present on viruses and other microbes. Cloninger and co-workers have investigated the relative inhibitory properties of fuctionalized Manα1-2Manα-PAMAM dendrimers (**22**) against their ability to bind CV-N (**Figure 20**).[84]

In order to evaluate the mannosylated dendrimer–CV-N interactions, precipitation assays were performed. PAMAM-functionalized dendrimers induced precipitation of CV-N–dendrimer complexes. Titration of the soluble CV-N with increasing amounts of dendrimers showed a linear decrease in the absorbance, that is, an increase in the total amount of protein precipitated. These data translated to apparent stoichiometries of 8:1 and 11:1 for CV-N: dendrimer complexes for G3 and G4, respectively.




**22**, $n = 32, 64$

**Figure 20** Dimannosylated PAMAM dendrimers **22** used for Cyanovirin-N binding studies.




**23,** G(3)–G(6)

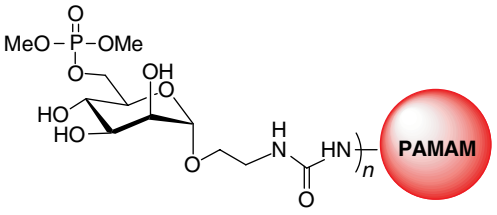
**24,** G(3)–G(6)

**Figure 21** Heterogeneous PAMAM mannodendrimers **23** and **24** bearing interspacing hydroxyl functionalities (left) or 'diluting' unrelated glucosides and galactosides (right) residues for ConA binding studies.

The study of variously loaded saccharide-functionalized dendrimers provided other valuable information regarding multivalent binding. A number of constructs have been reported that vary the loading densities of carbohydrate antigens. In general, the most highly functionalized scaffolds were not the ones with the highest activity.[3,10,10a,17,17a,17b,51,56,62,78] Often, less-functionalized ligands can exhibit optimal activities. This was attributed to the fact that at high loading, the saccharide portions became less accessible due to an increase in steric hindrance. Another hypothesis is that with aromatic aglycons, $\pi$-stacking might further tighten the saccharide units on the dendrimer surfaces. In fact, PAMAM-dendrimers bearing more than 64 arylated mannoside moieties such as those seen in **Figure 15** were experimenting solubility problems in aqueous environment.

Analogous to the experiments on PAMAM-based sialosides in flu virus interactions, wherein the saccharide portions were interspaced with nonsaccharidic functionalities,[57] Cloninger and co-workers described the synthesis of heterogeneously functionalized PAMAM manno dendrimers **23** and **24** (**Figure 21**).[85,86]

A hemagglutination assay was performed by adding rabbit erythrocytes to preincubated solutions of Con A and varying concentrations of dendrimers. There was a significant increase in activity for the fourth, fifth, and sixth dendrimer generations. Maximum activity occurred at just over 50% sugar loading for all generations. The area available per sugar at maximum activity varied slightly for the different generations. The trends were similar in each case. The most striking observation was that the highest activity did not correlate with the maximum sugar loading, but rather occurred at slightly closer packing of the sugars as the generation increased. By analogy, the loading distributions of dendrimers bearing heterogeneous structures were evaluated. Hence, mannoside/TEMPO-functionalized PAMAM dendrimers were synthesized.[87] EPR analysis of these dendrimers suggested that all dendrimers had random distributions of surface functional groups. Comparison of hemagglutination assays between mannoside/TEMPO- and mannoside/hydroxy-functionalized dendrimers suggests that the presence of the spin label had no influence on the dendrimer binding to ConA.




**25,** $n = 4, 8, 16, 32$

**Figure 22** Mannose-6-phosphate fuctionalized PAMAM dendrimers **25**.

Further derivatives were also recently described using PAMAM simultaneously functionalized with mannose, galactose, and glucose residues (**24**) (**Figure 21**).[86,88] MALDI-TOF MS was used to determine the number of carbohydrate residues of each type on the dendrimers. Both the change in MW after each sequential addition and the change in MW after deacylation were used. The association of these dendrimers with ConA was studied using precipitation and hemagglutination assays. Increasing the number of mannose residues while decreasing the number of glucose residues caused an increase in the relative activity toward ConA. As with the dendrimers bearing 50% mannose/glucose loading, a linear relationship between Man/Glc loading and assay activity was observed for compounds of generations 4–6. However, the difference between fully mannose-functionalized dendrimers and fully glucose-functionalized dendrimers never approached 16. For compounds of generation 3, mannose-functionalized dendrimers had a fivefold higher relative activity toward Con A than did the glucose-functionalized analogs.

Mannose-1-phosphate (Man-1-*P*) and galactose$\beta$1-4Man-1-*P* residues are constituents of the repeating units of some phosphoglycans acting as biological signals. Man-6-*P*, in particular, is known to be involved in the selective targeting of newly synthesized enzymes to lysosomes.[89,89a–89d] It is known that the presence of multiple Man-6-*P* residues on N-linked oligosaccharides leads to binding affinity enhancements to the cation-independent Man-6-*P* receptors in macrophages. Such enhanced binding affinities have been attributed to the cluster effect. In light of the importance of the glycoside cluster effect in carbohydrate–protein interactions, Jayaraman and co-workers investigated the synthesis and properties of mannose-6-phosphate-functionalized PAMAM dendrimers (**25**) (**Figure 22**).[90]

The synthesis of dendritic Man-6-*P* was initiated from 2-*N*-(benzyloxycarbonylamino)ethyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranoside, that was modified sequentially by (1) deprotection of the benzoyl esters; (2) tritylation of the primary hydroxyl group; and (3) benzoylation of the remaining hydroxy groups. Detritylation, phosphorylation of the free hydroxyl group with chlorodimethyl phosphate, and hydrogenolysis provided the desired Man-6-*P* derivative which was subjected to amide bond formation with pre-formed PAMAM dendrimers exposing carboxylic acid end groups in the presence of diisopropylcarbodiimide (DIC)/1-hydroxybenzotriazole (HOBt).

The mannose-6-*O*-phosphate substitution on the dendrimers was confirmed by a microcolorimetric determination using the modified resorcinol-sulfuric acid assay.[91] This microtiter plate assay was widely used for the estimation of the percentage of sugar substitutions in neoglycoproteins, glycolipids, and macromolecules such as glycopolymers. The monomeric, tetrameric, and octameric polymers were immobilized unto Seralose gels, denoted as Seralose-M, Seralose-T, and Seralose-O gels, respectively. The purified goat MPR 300 protein (cation-independent receptor) was separately incubated with these gels. SDS-PAGE analysis of the eluted samples revealed that the receptor was bound on these gels. This experiment confirmed previous results in the authors' laboratory in which glycodendrimers can be effectively used as adsorbents in related affinity chromatography.[92]

### 3.36.3.4 Boltorn®-Based Glycodendrimers (Hyperbranched Polymers)

Since the first synthesis of dendritic polymers in the late 1970s, growing interests in these hyperbranched materials have always extended for their unique and specific properties relative to their conventional linear and branched homologs.[93] They are obtained by reacting a poly-functional core with ABx monomers, typically $AB_2$ monomers, yielding amorphous structures. The obtained macromolecules are thus characterized by an exponential growth in both molecular weight and in end-group functionalities.

Dendritic polymers have traditionally been classified into two categories: dendrimers and hyperbranched polymers. A dendrimer is characterized by a perfect symmetrical globular shape that results from a stepwise controlled process giving a monodisperse molecular weight distribution. The second category, the hyperbranched polymers are attractive because they resemble dendrimers (their difference lies in their polydispersity and the less-perfect globular shape) but

they can be produced more easily on a larger scale and at a reasonable cost, thus making them commercially available in large quantities nowadays. Unlike conventional polymers, the high number of end groups and their nature participate actively in the physical properties (solubility, glass transition temperature, and viscosity) in combination with the backbone structures. This characteristic is exceptional because it leads to the possibility of designing the macromolecule with the combination of many different end groups.

Rojo and co-workers have selected hyperbranched dendritic polymers named Boltorn® (**Figure 8**) for which second, third, and fourth generations are commercially available at a very low cost. This family of dendrimers varies by the numbers of hydroxyl surface groups: they are BoltornH20 (16 OH groups) and BoltornH30 (32 OH groups, on average). These dendritic polymers, which are based on the monomer 2,2-bis(hydroxymethyl)propionic acid, have been functionalized with the mannosides.[94] These dendritic alcohols were transformed into carboxylic acid surface groups by treating BoltornH20 or H30 polymers with succinic anhydride in the presence of DMAP in pyridine. The resulting acids (**27**) were then mannosylated via an amide bond with unprotected 2-aminoethyl α-D-mannopyranoside (**26**) and DCC to provide mannodendrimer **28** (**Figure 23**). The ester functionality of the backbone precluded the use of peracetylated sugars.

The ensuing hyperbranched dendrimers were soluble under physiological conditions and were nontoxic against several cell lines. The third-generation dendrimers showed nanomolar inhibitory properties against the binding of Ebola virus (EBOV) to DC-SIGN.[95,95a] It is highly likely that EBOV, in a manner similar to HIV, subverts the physiological role of DC-SING and liver/lymph nodes SIGN (L-SIGN) to achieve important steps in its infectious process. These receptors are critical for the initial steps of viral infection at the mucosal level and subsequent dissemination throughout the body. DC-SIGN and L-SIGN also bind ligands containing oligomannose glycans through their C-terminal CRD.

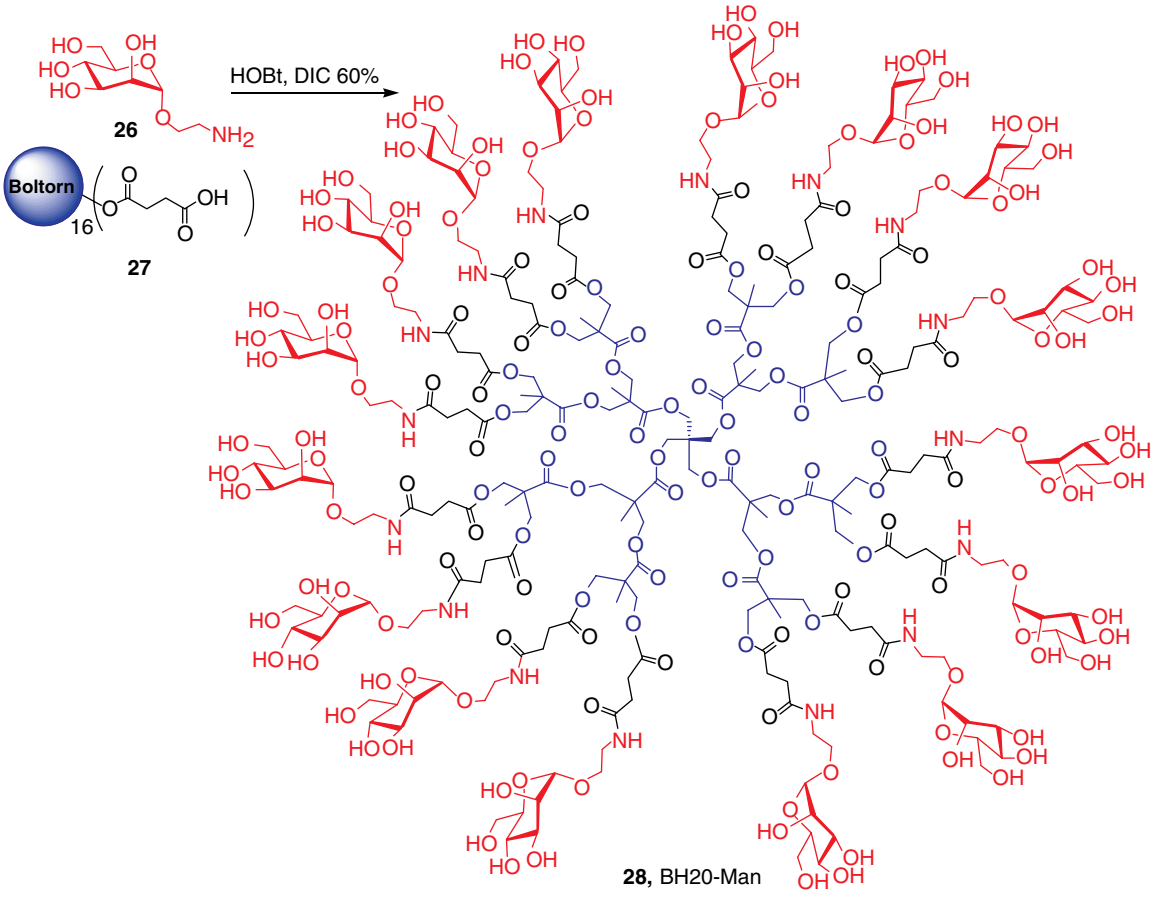



**Figure 23** Boltorn® H20 mannodendrimer **28** obtained by amide bond coupling and used against Ebola binding to DC-SIGN.

Mannosylated BH30 dendrimer was able to selectively inhibit DC-SIGN-mediated EBOV infection in an efficient dose-dependent manner ($IC_{50}$, 337 nM) and showed no inhibitory effect in infection experiments using DC-SIGN-negative cell lines. In another assay the third mannosylated dendrimer inhibited the DC-SIGN binding to the HIV glycoprotein gp120 with an $IC_{50}$ in the micromolar range.[96] It is clearly established that C-type lectins can be considered potential new targets for the design of compounds acting as viral entry inhibitors such as EBOV and HIV. In a model study with the mannose-binding lectin, using the lectin from *Lens culinaris*, Arce *et al.* have demonstrated again that such glycodendrimers were perfectly designed for cross-linking soluble receptors.[97] It is also obvious that membrane receptors could be cross-linked with glycodendrimers, thus leading to signalization, a phenomenon previously observed with glycopolymers[9] and other glycodendrimers.[98] The polyhydroxylated nature of the scaffolds was demonstrated to play no role in the binding interactions.

In another application of hyperbranched Boltorn® polymers, our group applied the versatile 'click chemistry' conditions to an azido-functionalized Boltorn® dendrimer (**30**) (BH20) (Touaibia and Roy, unpublished data). Dendrimer **30** was obtained by treatment of the hydroxylated BH20 core with azido acetic anhydride (**Figure 24**). IR analysis was used to demonstrate complete hydroxyl group transformation and azide function introduction. The 'clicked' hyperbranched dendrimer **31** was obtained under typical reaction conditions ($CuSO_4$ and sodium ascorbate) using nonprotected propargyl α-mannoside **29**. The relative inhibitory potency of the clicked dendrimer in the inhibition of agglutination of *E. coli* by yeast mannan was approximately 400 times higher than that of the respective methyl mannoside (Benhamioud and Roy, unpublished data). In the next section that discusses the use of 'clicked' glycodendrimers, further examples will be illustrated in which analogous structures were systematically prepared by a stepwise convergent approach.[99]

### 3.36.3.5 'Clicked' Glycodendrimers

Triazoles (**32**, **33**) are important five-member nitrogen heterocycles involved in a wide range of industrial applications such as agrochemicals, corrosion inhibitors, dyes, optical brighteners, as well as biologically active agents.[100] The well-established approach for the preparations of [1,2,3]-triazole ring systems relies on the thermal 1,3-dipolar Huisgen cycloaddition between alkynes and azides (**Figure 25**).[101,101a,101b] However, this noncatalyzed process exhibits several disadvantages, including (1) the requirement for high temperature conditions with the potential for the decomposition of labile products, (2) the production of the desired [1,2,3]-triazoles generally in low yields, and (3) poor regioselectivity, given the fact that the noncatalyzed cycloaddition affords mixtures of 1,4-disubstituted triazole **32** and 1,5-disubstituted triazole **33**, unless the alkyne is substituted with an electron-withdrawing group.[102,102a]

Over the years, several efforts for the control of 1,4-versus 1,5-regioselectivity have been reported.[103,103a–103c] However, the regioselective and high-yielding synthesis of 1,4-disubstituted triazoles (**32**) via a Cu(I)-catalyzed [3+2]-cycloaddition of terminal alkynes and azides has only recently been described (**Figure 25**).[104,105,105a] It is postulated that this copper-catalyzed click reaction proceeds via a copper-acetylide intermediate, generated from Cu(I) and the terminal alkyne, which then participates in an annealing process upon its coordination with the reacting azide.[105,105a] Although Cu(I) could be introduced directly in the form of different copper salts, the presence of a nitrogen-containing base as well as prior exclusion of oxygen from the reactions are usually required in order to minimize the formation of undesired by-products, primarily diacetylenes. Alternatively, the catalytic Cu(I) species could be generated *in situ* from $CuSO_4$ and sodium ascorbate.[105,105a] The latter method eliminates the problem of by-product formation and has been used successfully in several different solvent systems, including water. In this method, the need for prior exclusion of oxygen or the presence of a nitrogen base could be abolished.

A recent application of this reaction between azides and terminal alkynes has led to many interesting applications of click reactions including the synthesis of natural product analogs. Although azides and alkynes display high mutual reactivity, individually these functional groups are two of the least reactive in organic synthesis. They have been termed 'bioorthogonal' because of their stability and inertness toward the functional groups typically found in biological molecules.[106] This bioorthogonality has allowed the use of the azide-alkyne [3+2]-cycloaddition in various biological applications including target-guided synthesis[107] and activity-based protein profiling.[108]

Riguera and co-workers have described a quick, efficient, and reliable multivalent conjugation of unprotected alkyne-derived carbohydrates to three generations of azido-terminated gallic acid-triethylene glycol dendrimers (**Figure 26**).[109] Azide-ending dendrimer **36** of first generation and bearing nine azide functionalities was obtained via successive peptide coupling reactions between amine **34** and acid **35** that was obtained by reduction of azide precursors by hydrogenolysis under Pd-catalyzed conditions. This order of coupling was preferred to those incorporating terminal alkynes because of the potential Cu(II)-catalyzed alkynes oxidative homocoupling that may give rise to intramolecular reactions. Under aqueous conditions and under typical click chemistry, glycodendrimers containing up

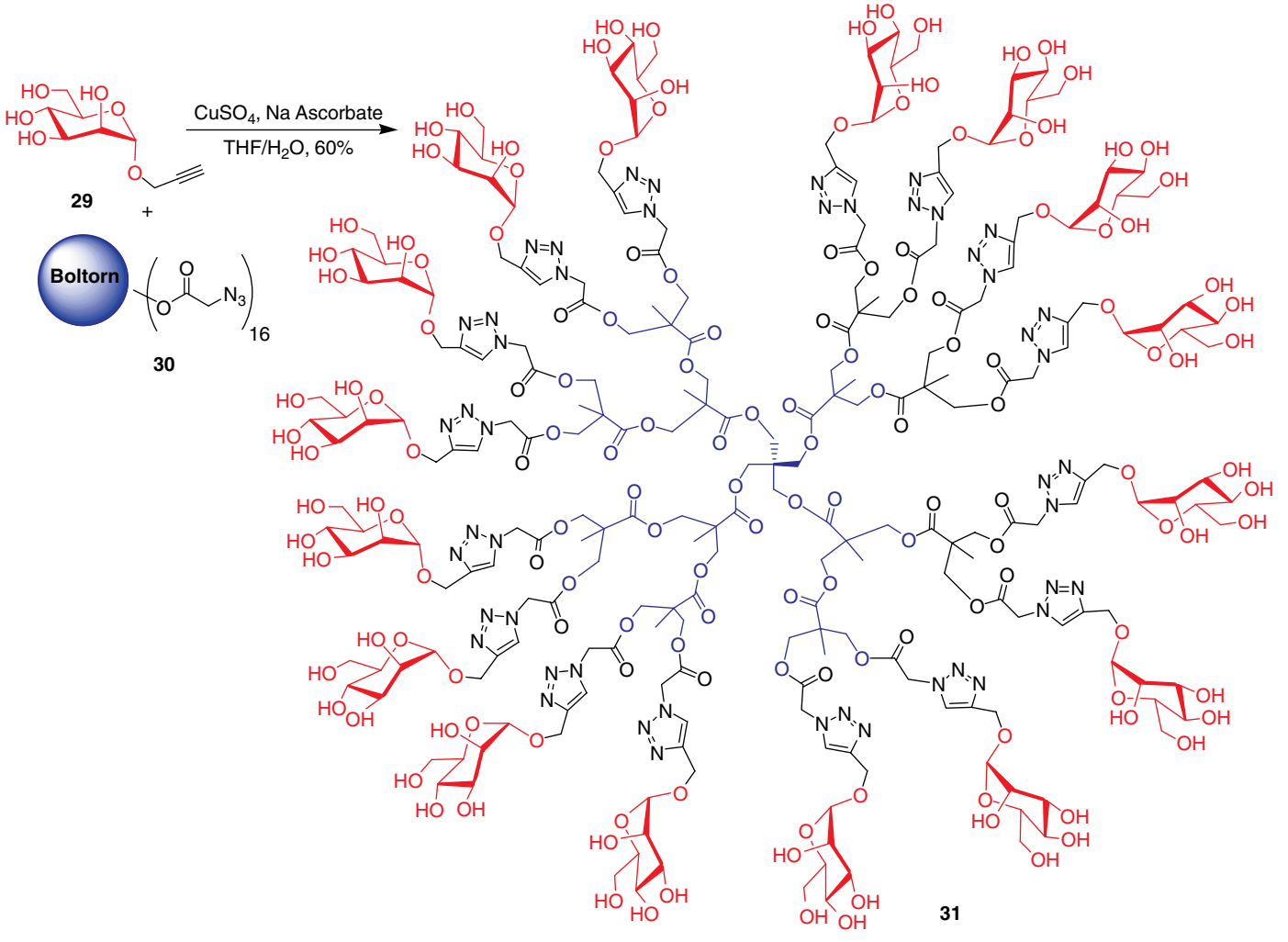



**Figure 24** Boltorn® H20 mannodendrimer **31** obtained by click chemistry (Roy *et al*., unpublished data).

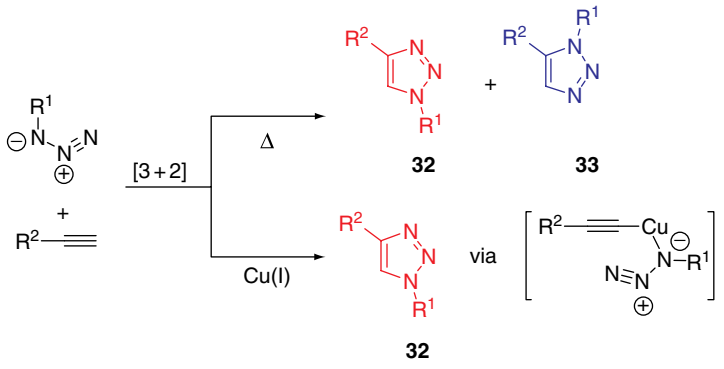



**Figure 25** Thermal and copper(I)-catalyzed [3+2] cycloaddition between alkyne and azide used for the 'click' chemistry.

to 27 unprotected mannose (**37**, G2Man), fucose, and lactose residues were incorporated, respectively using the same propargyl mannoside **29** described above.

For most practical and biological applications of glycodendrimers, three functional units are usually required: a targeting moiety, a biologically active agent, and a probe. A general and facile strategy for functional groups introduction at defined positions on dendrimers is best achieved when dendrimers are built by stepwise syntheses (**Figure 27**). Hawker and co-workers have described a fashion-controlled strategy toward glycodendrimers in which two distinct moieties (targeting and detection probe) were placed at the chain ends.[99] Click chemistry was used as the key step, thus allowing simple and original buildup of these bifunctional dendrimers.

The synthetic approach was based on 2,2-bis(hydroxymethyl)propionic acid (bis-MPA). This acid is also present in the mannosylated Boltorn® hyperbranched polymers proposed by Rojo and co-workers[94–96] used as potential antiviral drug (see Boltorn-based glycodendrimers above). The corresponding bis-MPA anhydride 38 provided access to alkyne ester **39** and azide ester **40** by condensation with appropriate alcohol (**Figure 27**). Removal of the protecting groups and subsequent condensation by cycloaddition allowed the generation growth of the core to afford **41** having a diol as the focal point. Two molecules of the fluorescent dye (7-diethylaminocoumarin **43**) were then introduced by an esterification of the two free hydroxyl groups of **41** with pent-4-ynoic anhydride **42** followed by [3+2] cycloaddition to the resulting bisalkyne. Acetal hydrolysis and subsequent introduction of the 16 alkynes via esterification followed by [3+2] cycloaddition with an unprotected 2-azidoethyl α-D-mannopyranoside (**44**) in $THF/H_2O$ furnished the asymmetrical heterobifunctional dendrimer **45**.

Dendrimer **45** was tested in a standard hemaglutination assay using the MBP ConA and rabbit red blood cells.[110] When compared to the mannose activity, the mannosylated dendrimer exhibited 240-fold greater potency. The relative activity was 15 per sugar moiety.

The use of microwave irradiation in organic synthesis has become increasingly popular within the pharmaceutical and academic arenas, because of its new enabling technology for drug discovery and development.[111] Santoyo-Gonzalez and co-workers have used organic-soluble copper(I) complexes that can act as a catalyst allowing homogeneous reactions in [3+2]-cycloadditions.[112] Among the copper catalysts already reported, $(Ph_3P)_3$,CuBr,[113] and $(EtO)_3$P,CuI[114] were chosen due especially to their air stability and easy and inexpensive preparations. In order to improve the cycloaddition and to shorten reactions times, reactions were also simultaneously assisted by the use of microwave irradiation (**Figure 28**).

The synthesis of tri-, tetra-, and hexavalent mannopyranosylated 1,4-disubstituted 1,2,3-triazole ligands (**48**) were readily obtained by using carbohydrates, noncarbohydrates, and aromatic azides (**47**) with peracetylated propargyl mannoside (**46**). Although earlier experiments revealed that the reactions of peracetylated mono-(C-6)-azido and per-(C-6)-azido-β-cyclodextrin with propargyl and thiopropargyl mannosides were quite slow in the presence of $(Ph_3P)_3$. CuBr-DIPEA, this difficulty was overcome by the addition of a catalytic amount of CuI (10% mol) or by the use of $(EtO)_3$P.CuI-DIPEA. Under these conditions, C-6-branched β-cyclodextrins were formed in high yields. The experimental procedure for the microwave-assisted reactions was simple, requiring only the microwave irradiation, and the evaporation of the solvent prior to direct purification. Formation of undesired by-products was never observed.

More recently, Pieters *et al.* have used the extensively used catalysts ($CuSO_4$, vitamin C) in a high-yielding microwave-assisted triazole-linked glycodendrimer synthesis (**Figure 29**).[115] The required anomeric glycosyl azides (**50**) were prepared from their respective peracetylated counterparts by reaction with HBr in AcOH under microwave irradiation. The resulting glycosyl bromides were treated with trimethylsilyl azide again using microwaves to afford

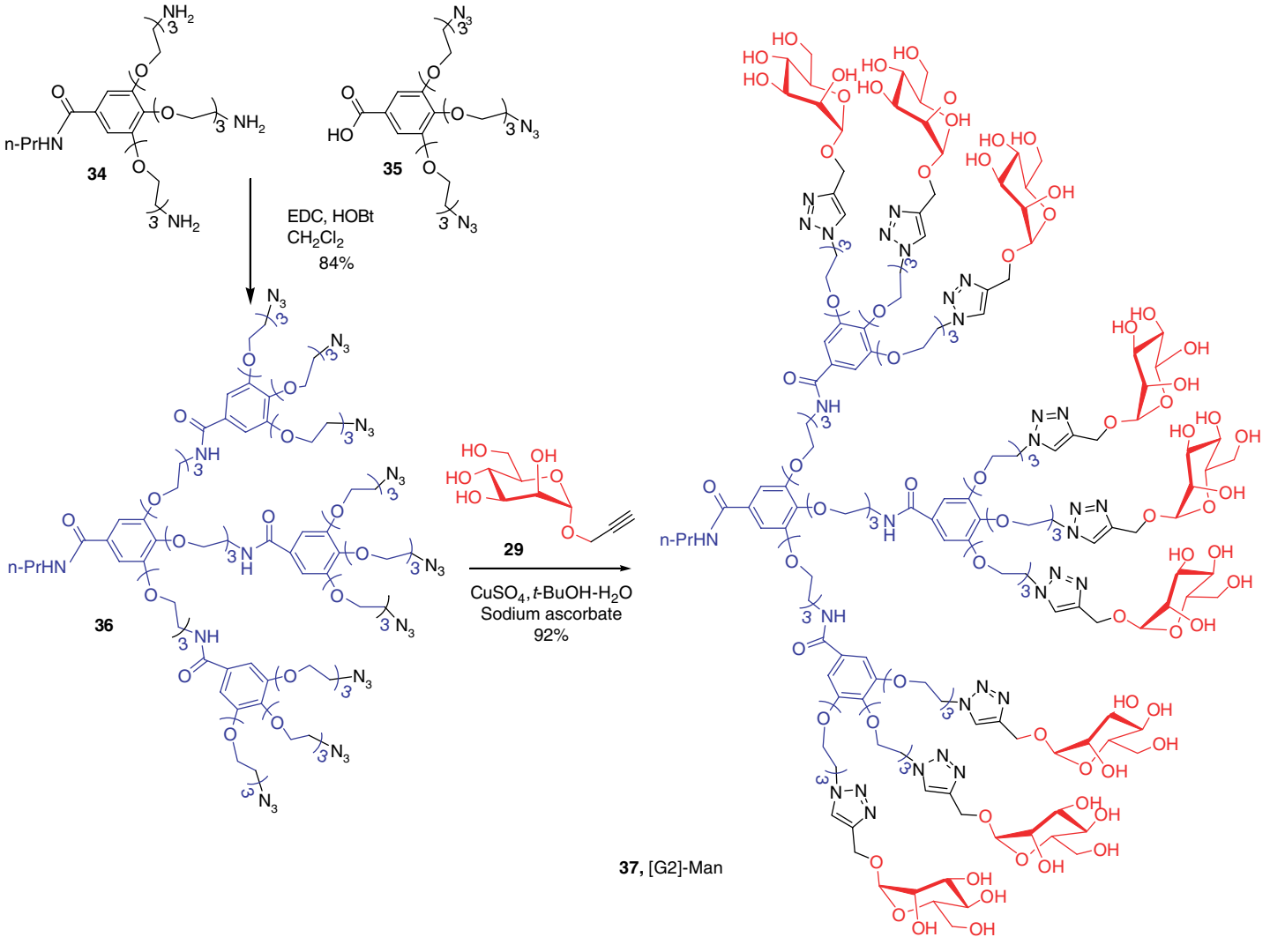



**Figure 26** Glycodendrimer **37** with unprotected mannoside prepared click chemistry.

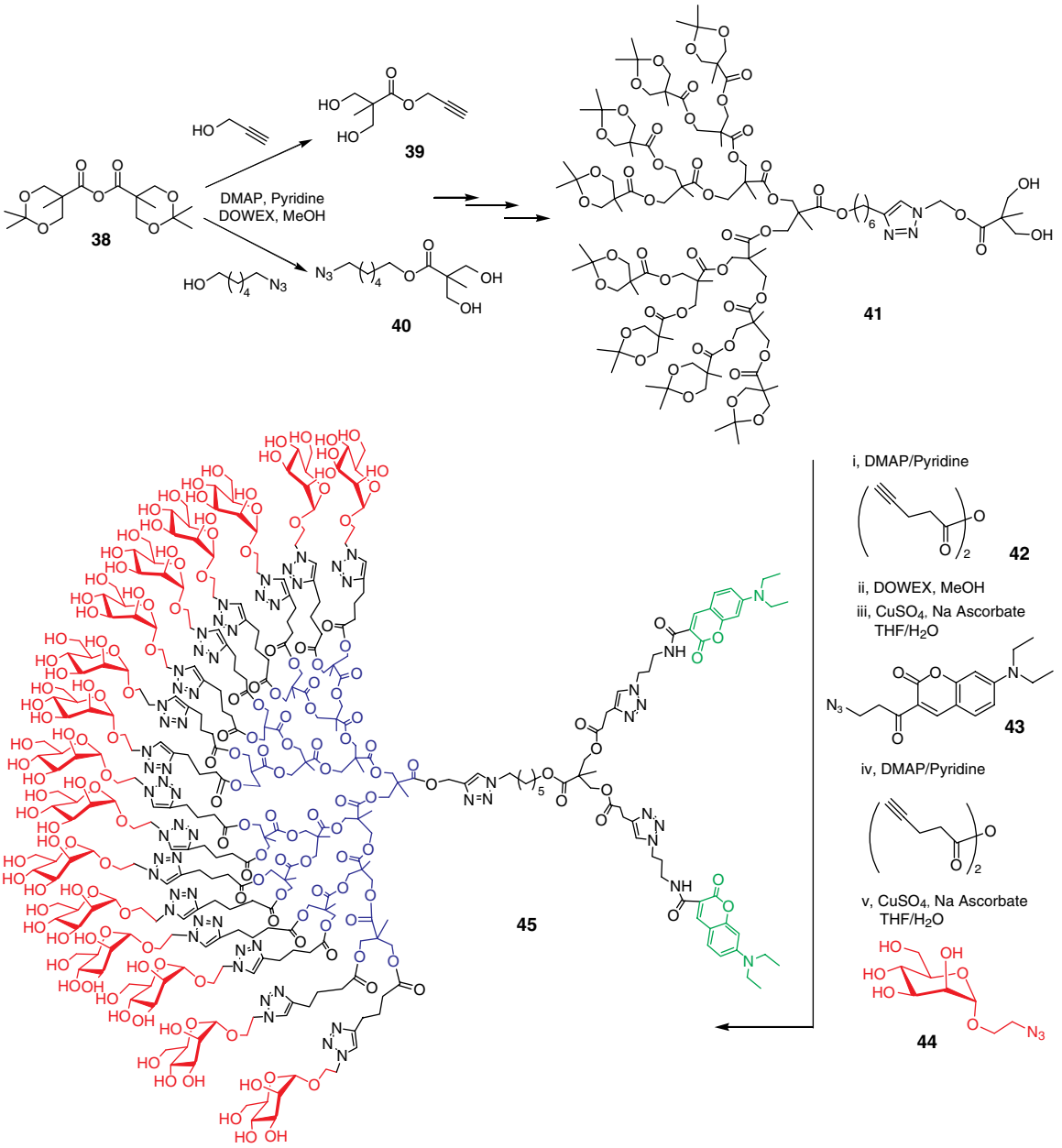



**Figure 27** Synthetic strategy toward asymmetrical glycodendrimer **45** bearing two coumarin chromophores.

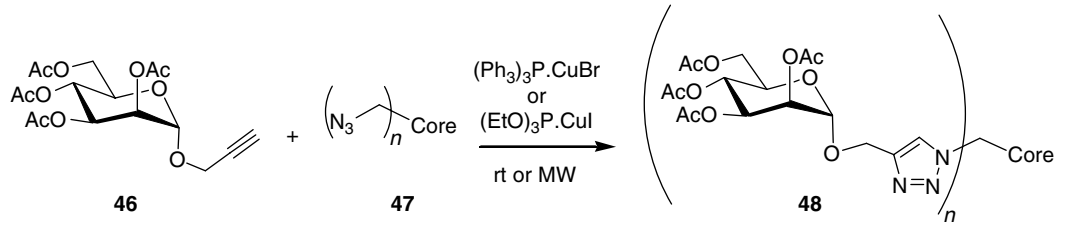



**Figure 28** Clicked mannoclusters **48** obtained with and without microwave irradiation.

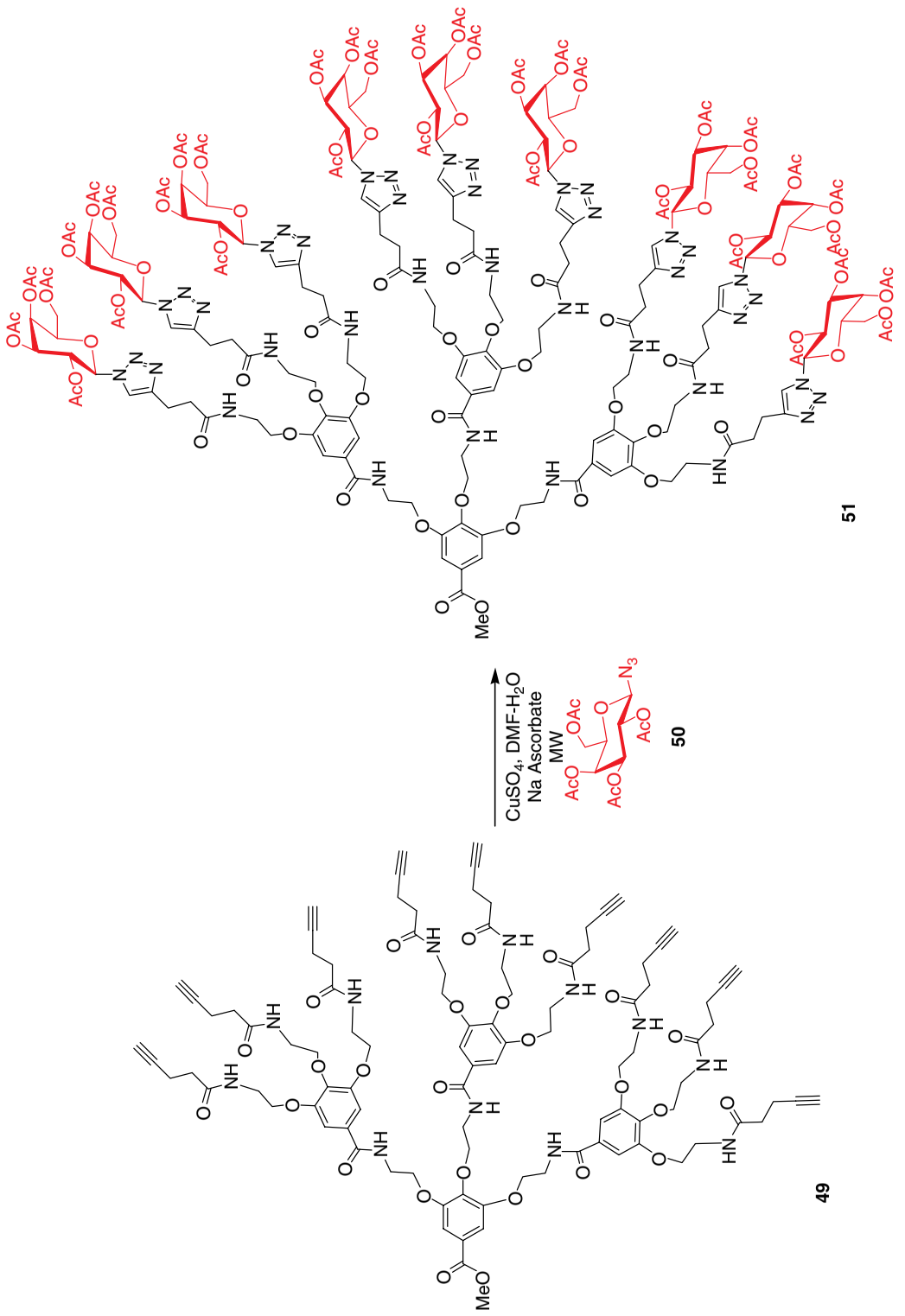



**Figure 29** Gallic acid-based clicked glycodendrimer **51**.

$\beta$-azido sugars **50**. The cycloadditions with dendron **49** bearing nine propargyl amides were achieved with galactose, glucose, cellobiose, and lactose. Nonavalent glycodendrimers such as **51** were obtained and for biological evaluations a divalent glycoconjugate containing a fluorescent probe was similarly obtained in high yield. Obviously, this approach is also applicable to mannoside-bearing dendrimers. In fact, for analogous architectures comprising mannosides but not prepared by microwave-assisted reactions, see the next section.

Gallic acid (3,4,5-trihydroxybenzoic acid) (see **Figure 26**) is a common scaffold for the construction of glycodendrimers and other related architectures.[109,115] It has been initially used in our group for the construction of both sialodendrimers[116] and for the synthesis of lactosylated dendrimers.[117] It has the advantages of permitting a faster growth of the dendrimer generation since the exposed functional group valencies start from 3 (G0), 9 (G1), 27 (G2), and so on (3″). Other interesting 'seeding molecules' are calix[4]arenes, calyx[4]resorcarenes, porphyrins, triphenylene, and hexasubstituted benzenes (see following section). Another interesting structure based on polyphenylene has also been recently published by Sakamoto and Mullen using a key Diels–Alder reaction.[118]

### 3.36.3.6 Glycodendrimers Built on Aromatic Scaffolds

Using an aromatic core, the group of Pieters described the synthesis of multivalent mannosides as inhibitors of type 1 fimbriated uropathogenic *E. coli*.[82] A 3,5-di-(2-aminoethoxy)-benzoic acid scaffold (**53**) was used as repeating unit and a carboxylic acid moiety was introduced onto the carbohydrate (**52**), thus enabling peptide couplings with mono-, bis- and multivalent amino-functionalized aromatic scaffolds to give dendrons such as **54** and **55** (**Figure 30**).

The mannosylated dendrimers were tested for their relative inhibitory potencies using a developed ELISA test discussed in the PAMAM section above. Mannose itself was a poor inhibitor of binding of *E. coli* to urinary cell lines with an $IC_{50}$ of only 7.6 mM. Increasing the number of mannoside residues from 2 to 16, greatly improved the affinities, both in absolute value ($IC_{50}$, 51 μM for the tetramer **55**) and in relative terms when expressed on a per mannoside basis. A divalent compound, **54** having an elongated spacer, showed the highest relative potency on per sugar basis with a 141-fold enhancement ($IC_{50}$, 27 μM). Mannosylated polymers of analogous structures were not as good as the dimer above.

The convergent methodology (see **Figure 9**) used by Stoddart and co-workers involved essentially the synthesis of dendritic wedges possessing (9–32) α-D-mannopyranosides.[119] The structural components that constituted the

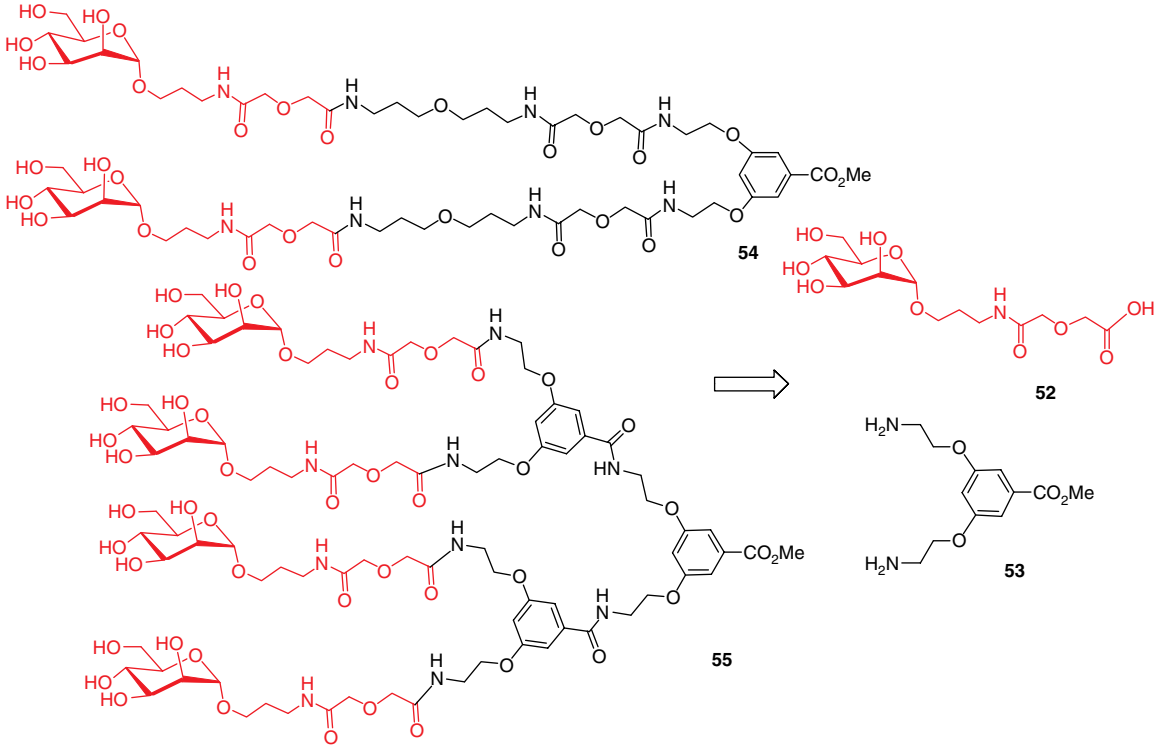



**Figure 30** Aromatic core as scaffold for multivalent mannoside clusters used in the inhibition of binding of *E. coli* to human urothelial cell lines.

branching regions of the dendritic wedges have been derived from tris(hydroxymethyl)aminomethane, for which the glycosylation of the three hydroxyl groups gave a tris-branched mannoside **56** possessing an amine end group. Further dendronization with tetraacid **57** and deprotection afforded dendron **58** having 12 mannoside residues. Finally, the branched wedge was attached to an aromatic core **59**, derived from 1,3,5-benzenetricarbonyl chloride, once again by the formation of amide bonds in presence of DCC/HOBt (**Figure 31**). The resulting glycodendrimer **60**, having 36-exposed mannoside residues, was next evaluated.

The biological potencies of these glycodendrimers have been evaluated by an ELLA, involving the inhibition of ConA binding to a purified yeast mannan fraction Sc500.[120] This fraction is of interest because of its strong binding to antibodies found in the serum of patients with Crohn's disease. The 9-mer was 4 times that of the 3-mer and methyl α-D-mannopyranoside with an $IC_{50}$ of 0.65 mM. The 18-mer and the 36-mer both have similar activities when compared on a molar basis with each other. Their activities were not much greater than the 9-mer, suggesting that with the homogeneous, monodisperse, dendrimers the clustering effect was most pronounced between the 9-mer and the 18-mer. The glycodendrimers did not show any inhibition of the binding of antibodies found in the serum of Crohn's patients that recognize the yeast mannan fraction Sc500.

As mentioned above, other aromatic scaffolds used as 'seeding molecules' have been extensively used calix[4]arenes,[121,121a–121c] calyx[4]resorcarenes,[122,122a] porphyrins,[123] triphenylene,[124] and hexasubstituted benzenes.[125,125a,126,126a] The calix[4]arenes and calyx[4]resorcarenes are of particular interest since they have the potentials to act as carriers by virtue of their cavities.

### 3.36.3.7 Pentaerythritol-Based Glycodendrimers

Pentaerythritol is a simple five-carbon tetraol used in the fabrication of resins, alkylated resins, varnishes, PVC stabilizers, tall oil esters, and olefin antioxidants. It is also known under names Hercules P6, monopentaerythritol, tetramethylolmethane, THME, PETP, pentaerythrite, Pentek, etc. Pentaerythritol is an interesting compound that allows for the attachment of four (similar or different) groups and, hence, the construction of highly branched structures. Accordingly, this compound has received considerable interest as an orthogonally protected handle useful for the generation of combinatorial libraries and as a building block that fits well in the general structure of oligonucleotides and peptides, providing additional functionalities.

For the inhibition of mannose-specific bacterial adhesion, Lindhorst and co-workers designed a pentaerythritol-based cluster-mannoside **65** as shown in **Figure 32**, in which pentaerythritol itself, as well as the included C3 spacers, were used as structural elements of the monosaccharide moiety.[127]

In the first route, a hydroxy linker was introduced within the aglycon moiety (**62**) and a Williamson ether synthesis with commercially available pentaerythritol tetrabromide (**61**) led to a mixture of mono-, di-, tri-, and tetradentate (**65**) products, even when an eightfold excess of alcohol and forcing reaction conditions were used (**Figure 32**). In the best case, the tetravalent cluster was isolated in 65% yield. An alternative route for the targeting of tetrameric cluster was investigated, in which pentaerythritol was modified to serve as a spacer-equipped tetrafunctional core molecule (**63**) for the subsequent glycosylation step. To this end, pentaerythritol was initially per allylated and the extended tetraol (**63**) was obtained by oxidative ozonolysis (9-BBN, NaOH, $H_2O_2$). Then, perbenzoylated mannosyl trichloroacetimidate (**64**) was used as the glycosyl donor. The protected tetramannoside (**65**) was isolated in excellent yield.

Lindhorst and co-workers have also synthesized a series of trisaccharide mimetics to serve as ligands for the type 1 fimbrial adhesin.[128] These glycoclusters were either obtained through a 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ)-assisted peptide-coupling reaction with triacid **67** and mannosides **66** or **68**, without further protection to provide trivalent mannosides **69–71** (**Figure 33**). This procedure was successfully used to obtain clusters bearing methyl (**69**) and *p*-nitrophenyl (**70**) aglycons after branching through the C-6 amino position. The latter is of interest as aromatic moieties can increase the affinity of a given carbohydrate ligand to its lectin receptor by hydrophobic interactions. The inhibitory potency of *p*-nitrophenyl α-D-mannoside (*p*NPMan) was approximately 100 times higher than that of the respective methyl mannoside. Cluster **69** ($R=CH_3$) showed an $IC_{50}$ of 4 nM and has surpassed the inhibitory potency of the cluster containing *p*NPMan. Only the spacer-modified mannosides **71** revealed interesting successful inhibition of type-1 fimbriae-mediated adhesion of *E. coli* to high mannose-type surfaces. None of these compounds reached the inhibitory potency of **69** ($R=CH_3$). These results were however somewhat surprising given the fact that fimbriated *E. coli* (FimH) prefers to bind mannosides from the nonreducing end (see **Figure 7**), thus providing no room for the clusters to enter in the active site by their reducing end.

As mentioned above, high-mannose type N-glycans represent valuable targets for a wide range of biological applications. The linear trimannoside Manα1-2Manα1-6Manα has been introduced by a sequential one-pot reaction onto extended pentaerythritol by Langer *et al.*[129] The sequence of reiterative reactions toward the extended tetraol

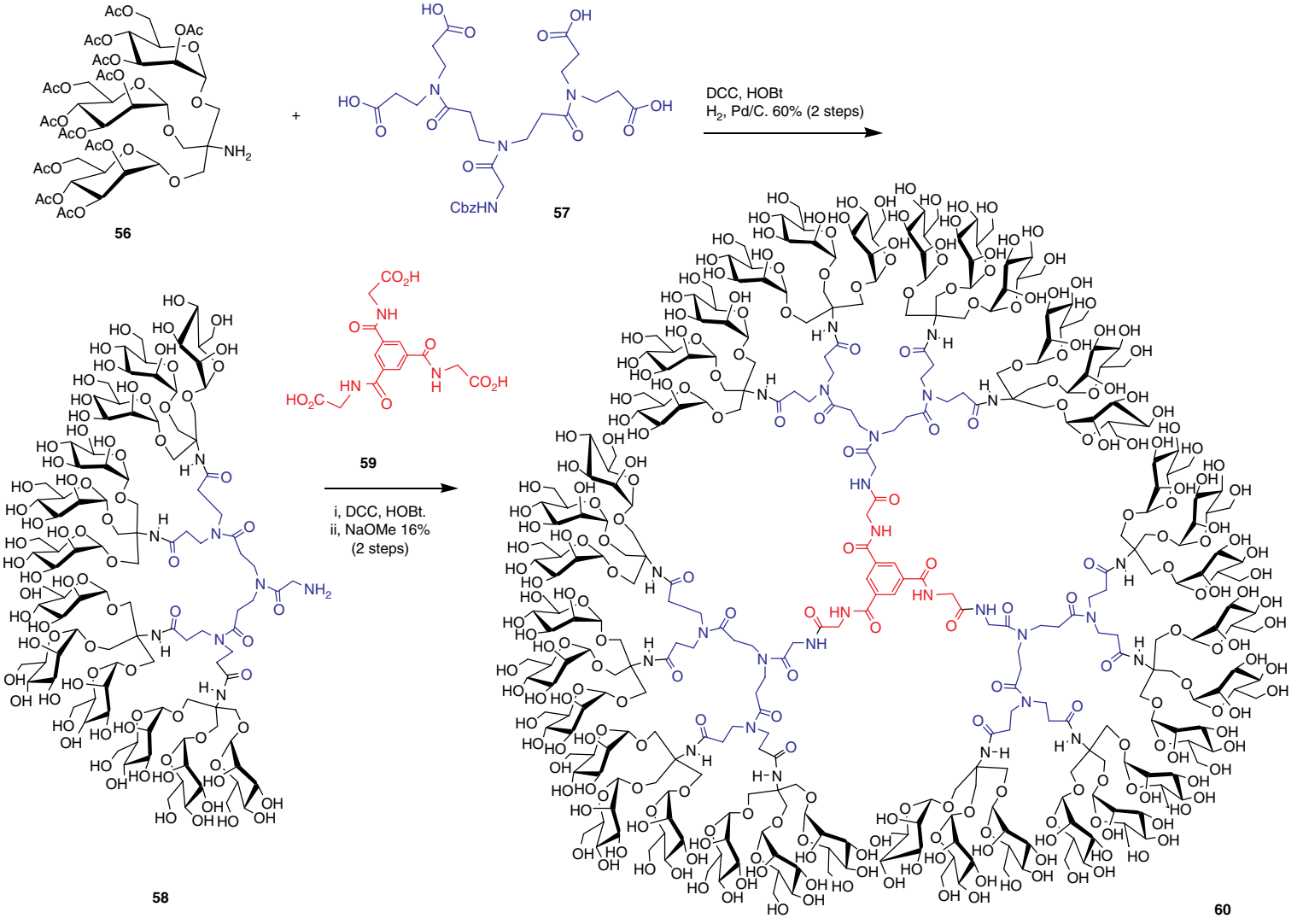



**Figure 31** Convergent methodology for a 36-mer mannoside **60**.

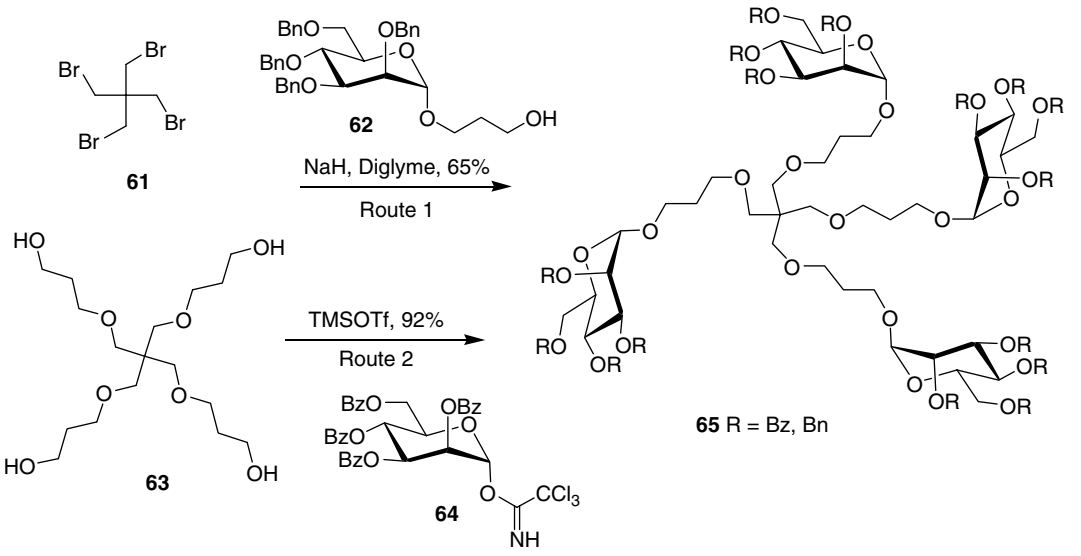



**Figure 32** Pentaerythritol-based mannocluster.

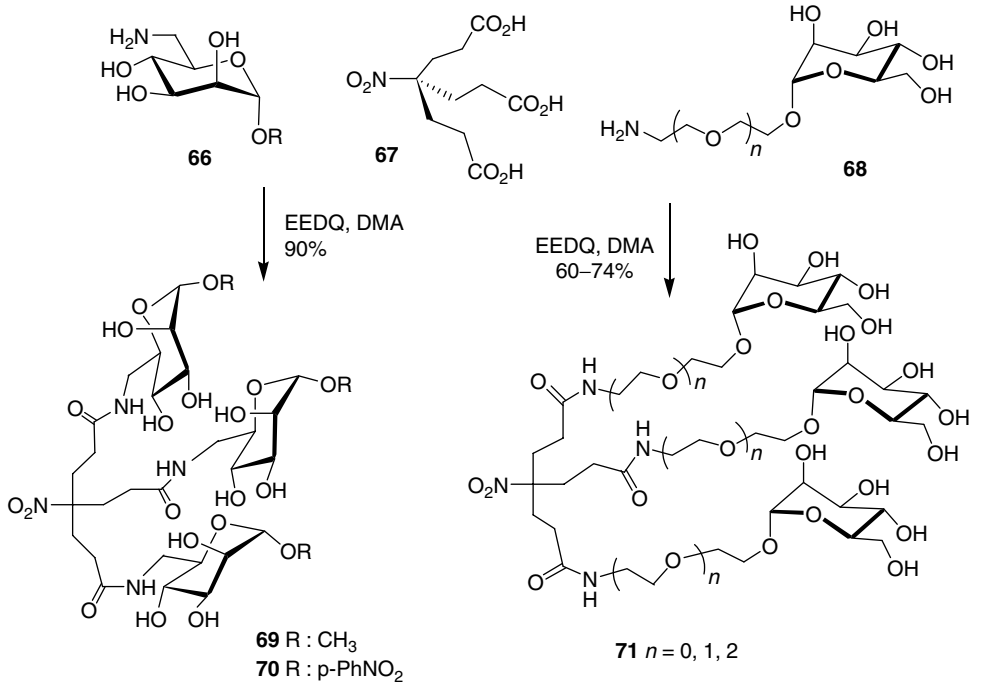



**Figure 33** Anomeric and 6-linked trimannoclusters used in binding studies against *E. coli*.

precursor involved two cycles of pentaerythritol allylation, followed by oxidative hydroboration. Mannosylation (50%) of the tetraol by a protected trimannosyl selenide donor provided, after deprotection (65%), the valuable tetramer **72** shown below in Figure 34. Unfortunately, no biological data was given for **72**.

Preparations of glycosylamines by direct condensation of amines with reducing sugars is well described in the literature and condensation of a small range of reducing sugars with diamines has also been previously reported to allow efficient and rapid access to divalent carbohydrate derivatives in excellent yields.[130] Hayes and co-workers have described a one-pot methodology that allowed the synthesis of higher valent derivatives through reaction of more highly functionalized amine clusters with α-D-mannose **73**.[131] This strategy represents an attractive entry toward

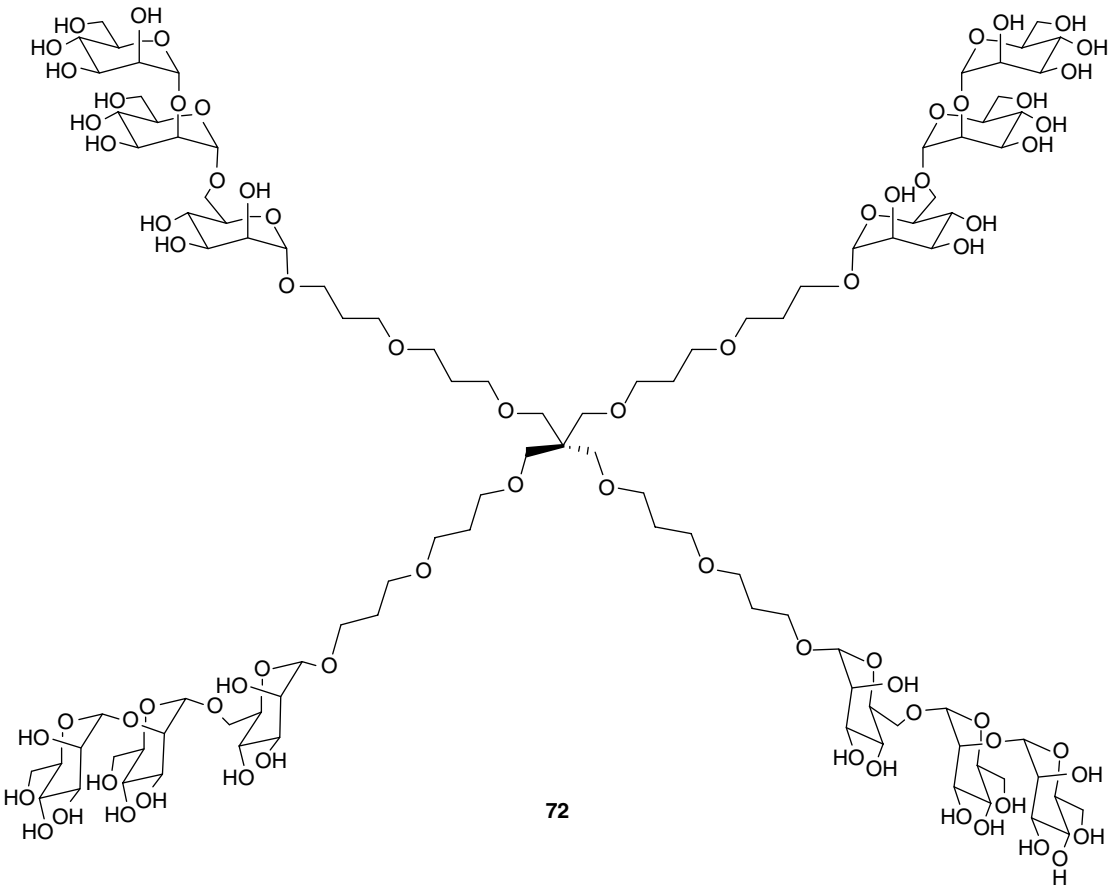



**Figure 34** Trimannoside pentaerythritol-based cluster **72** using one-pot assembly and extended pentaerythritol scaffold.

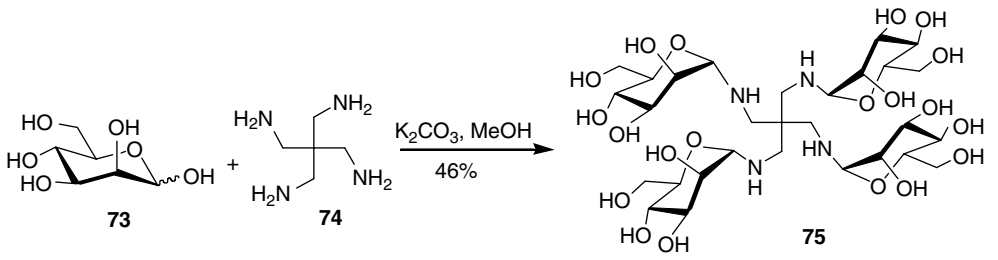



**Figure 35** Glycosamine preparation by direct condensation of reducing mannose **73** with tetaramino pentaerythritol **74**.

multivalent carbohydrates. Several linkers of different lengths, flexibility, and valency were readily incorporated with each allowing one-pot entry to the desired targets in good yields. Pentaerythritol tetraamine (**74**), which was prepared by the reduction of tetraazido pentaerythritol with hydrogen on 10% Pd/C, gave the tetramannoside cluster **75** with reducing mannose (**73**) (**Figure 35**).

By an extension of the above reductive amination strategy, it is also possible to construct hexamer such as **17** (**Figure 17**) by combining aldehyde **16** with tris(ethylamino)amine with sodium triacetoxyborohydride.[80]

A more recent report describes the synthesis of a nonavalent glycodendrimer with α-D-mannopyranoside units.[132] The trivalent mannosylated dendron **77** was prepared by glycosylation of a pentaerythritol derivative with three hydroxyl groups. The triple Sonogashira coupling reaction of dendron **77** and the tri-*O*-propargyl ether **76** gave the nonavalent glycodendrimer **78** in excellent yields (**Figure 36**). This approach represents another versatile extension to the synthesis of more rigid glycodendrimers using transition metal catalysts.[126,126a]

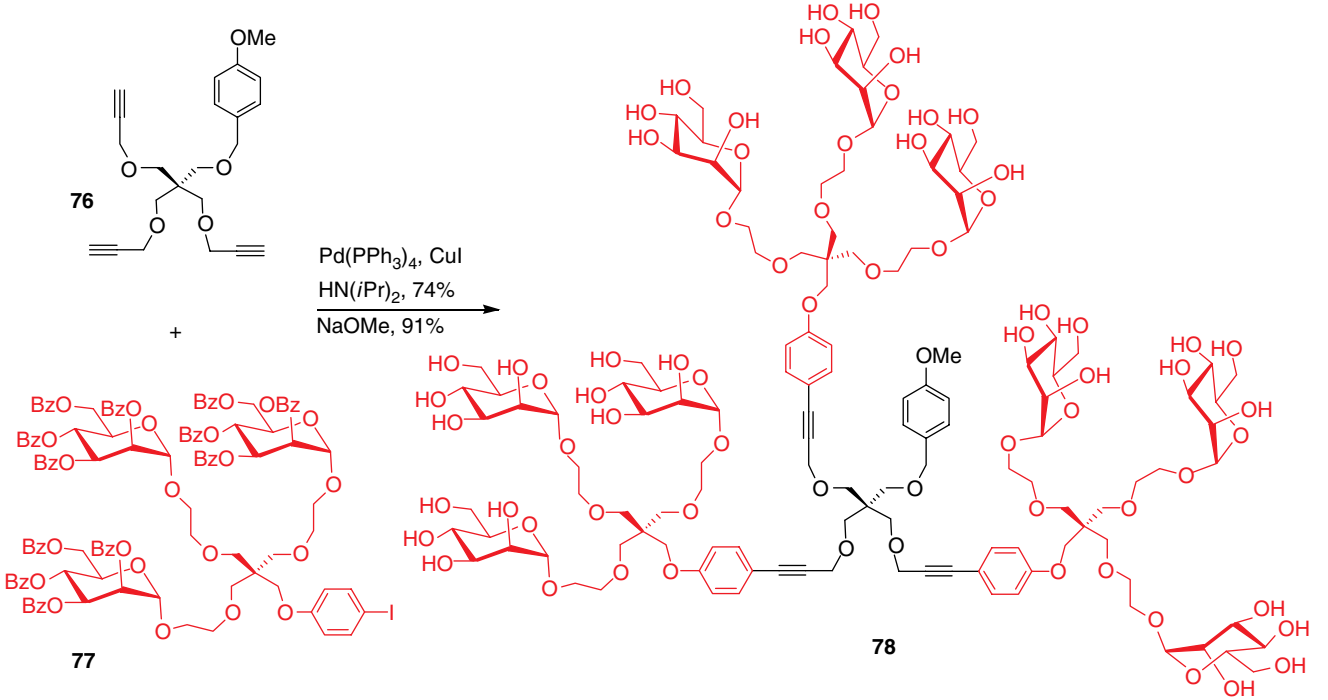



**Figure 36** Synthesis of a nonavalent glycodendrimer **78** using triple Sonogashira coupling.

### 3.36.3.8 Sugar Scaffolds

The multifunctional nature of monosaccharides or oligosaccharides, in combination with the inherent stereochemistry of the glycosidic linkages increases the potential structural diversity attainable from sugars when used as scaffolds. In this respect, carbohydrates by far surpass the potential of peptide or nucleotide oligomers as multivalent building blocks.

Lindhorst and co-workers have described the synthesis of carbohydrate-centered glycoclusters with incorporated spacer arms that allowed further conjugation of oligosaccharide mimetics (Figure 37). Thus, D-glucose and trehalose were used as core molecules.[133,133a] Starting from glucose pentaacetate, tetrasubstituted glucoside **79** was obtained through a sequence of reactions involving glycosylation of 6-bromohexanol catalyzed by $BF_3$.etherate, followed by bromide substitution with azide anions, acetate deprotection, and perallylation under PTC conditions. The resulting tetraallyl glucoside was then treated under oxidative hydroboration conditions (9-BBN, NaOH, $H_2O_2$) as above to give **79**. The desired glucose-based mannocluster **82** was then obtained after glycosidation and deprotection with perbenzoylated mannosyl trichloroacetimidate **80**. The terminal amino function of the tethered mannoside cluster allowed further attachment of probes such as biotin, fluorescent dyes, or coupling to solid supports in order to provide matrices for affinity chromatography.

Attempts toward similar treatment of the nonreducing disaccharide trehalose to construct analogous octameric dendron **83** was met with several difficulties. As an alternative, octaol **81** could be easily obtained by hydroboration of octa-*O*-allyl-trehalose. Mannosylation, as in the monosaccharide approach, was followed by the cleavage of the interglycosidic linkage at an intermediate stage of the synthesis using $BF_3$-etherate to afford two equivalents of the reducing glucose-centered mannocluster which after appropriate transformations, gave the same amino tetramer **82** (Figure 37).

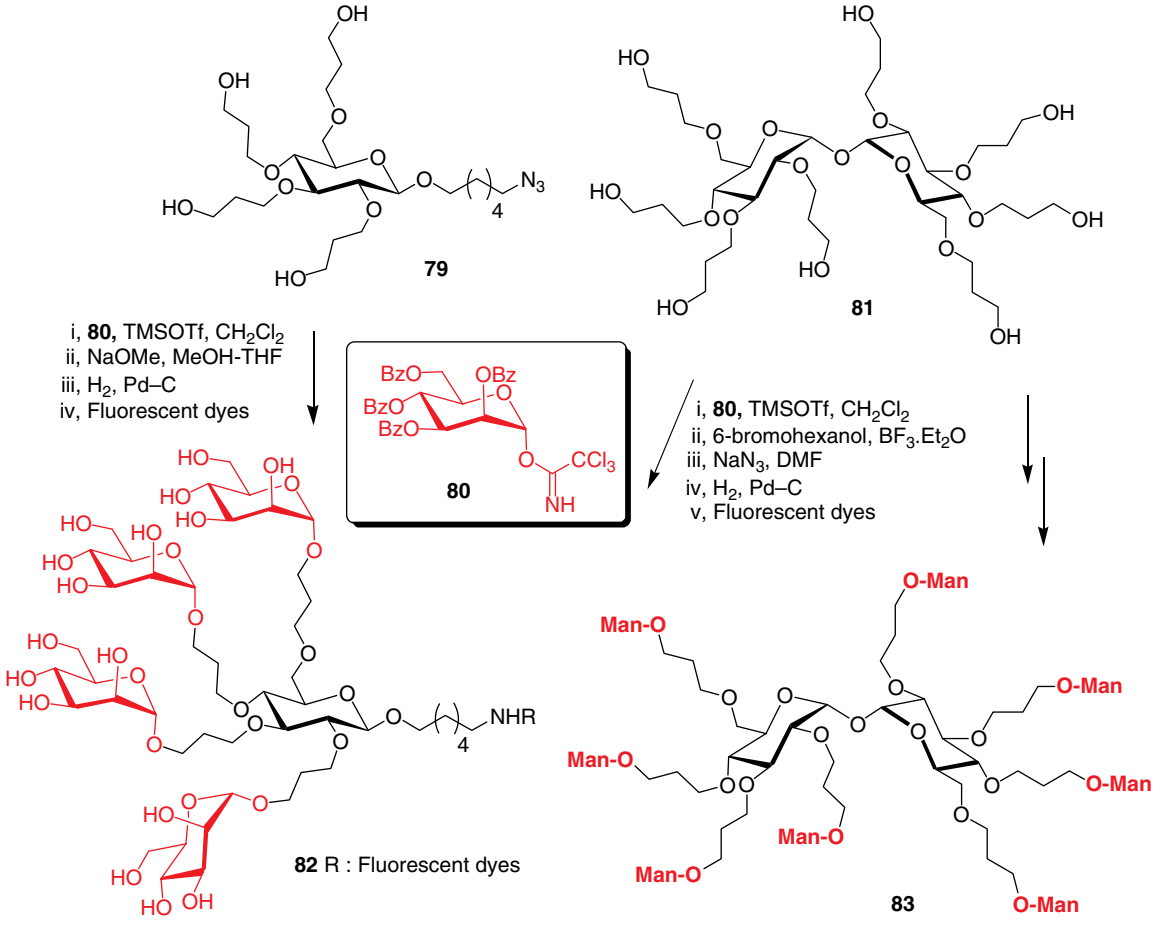



**Figure 37** Glucose **82** and trehalose-based **83** mannocluster syntheses.

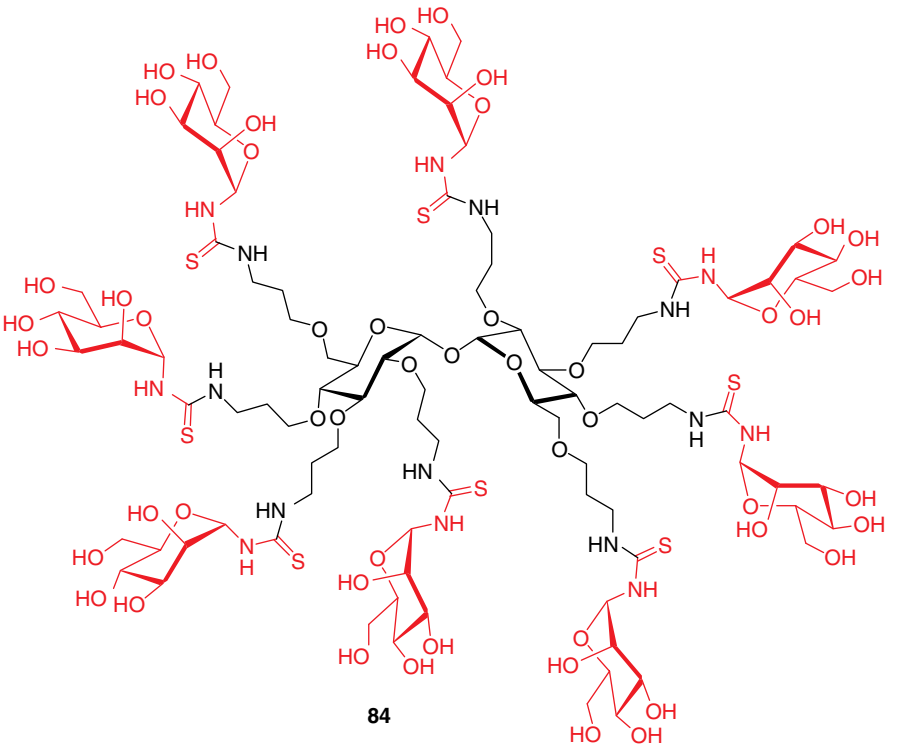



**Figure 38** Trehalose-based mannocluster **84** prepared using direct thiourea linkages.

Alternatively, amino-functionalized trehalose core has been obtained from the same group by reductive ozonolysis of the octaallylated trehalose derivative followed by reductive amination of the ensuing octaaldehyde.[134] When the resulting amino trehalose was treated with 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl isothiocyanate, octavalent thiourea-bridged mannose cluster **84** was obtained after deprotection (**Figure 38**). For other examples including cyclodextrins as scaffolds, see discussion below.

An interesting variant of this elegant strategy was put forward by Wang and co-workers in their synthesis of a candidate vaccine against HIV-1.[24] Based on the high recognition of multiantennary mannosides on gp120 by neutralizing human antibody 2G12 (see above), they constructed hyperbranched mannosides on the four positions of a galactoside derivative. Four maleimido groups were introduced onto perallylated galactoside bearing a T-helper cell epitope at the anomeric position. The ligation of the maleimido-functionalized scaffold with the known SH-tagged $Man_9GlcNAc_2Asn$ provided the key immunogen. The obtained anti-sera was weakly cross-reactive to HIV-gp120. The majority of the IgG antibodies were directed against the linkers of the glycoconjugate.

### 3.36.3.9 Silicon-Based Glycodendrimers

Although, with a few exceptions, silanes are still considered specialty products for the synthesis of biologically active compouds and the industrial-scale production of agrochemicals; the growth of literature reports on applications and patents regarding their utilization suggests that demand in other applications will rise in the next few years.

Carbosilanes are compounds in which the elements carbon and silicon occupy alternate positions in the molecular framework. Glycoconjugates possessing carbosilane-based saccharides on their surface were first introduced by Matsuoka and co-workers[135] and Lindhorst and co-workers have reported a heteroatom-free connection of carbohydrates to carbosilane scaffolds.[136]

Carbosiloxanes are generally formed by treatment of a chlorosilane with an alcohol component in the presence of a base.[137] The moderate yields of this type of reaction are likely due to steric hindrance of the second reactive chlorine on one silicon atom after the initial attack of the first alcohol derivative. A better procedure involving a hydrosilylation reaction was put forward. By using the well-known platinum-catalyzed hydrosilylation reaction of alkenes, suitable

hydrosilane derivatives (**86**),[138] together with the powerful catalyst Silopren (Bayer AG, platinum-siloxane complex 67–69%),[139] and an allylated mannoside acetal (**85**), the desired isopropylidene-protected siladendrimer was obtained in just 21%. Deprotection of the acetals with methanolic HCl provided the unprotected carbosilane glycocluster **87** (**Figure 39**).

For the introduction of mannose derivatives, Terunuma and co-workers have used three carbosilane dendrimer scaffolds having: three-(**88**), four-(**89**), and six-branches (**90**) (**Figure 40**).[140] Bromides **88** and **89** were the zero-generation scaffolds, which were respectively prepared from triallylphenylsilane and tetraallylsilane by following three reaction steps: hydroxylation, mesylation, and bromination.[141,141a] On the other hand, bromide **90** represents the first-generation carbosilane dendrimer scaffold, prepared by allylation of dichlorodimethylsilane followed by hydrosilylation[139] with the first-generation skeleton.

Protected mannose disaccharide **93** was synthesized starting from D-mannose using standard protocol (**Figure 40**). Glycosylation of **92** with acetobromomannose **91** in the presence of AgOTf proceeded stereoselectively. Deprotection of the resulting disaccharide with aqueous TFA, acetylation, allylation with $BF_3$ dietherate, and radical-induced thioacetylation provided the desired disaccharide **93**. The unprotected Manα1-3Man disaccharide was coupled to the carbosilane dendrimer scaffolds such as **90** after complete deacetylation using sodium methoxide and nucleophilic displacement of the bromides by the sugar thiolate. Re-O-acetylation was required for purification purpose. By means

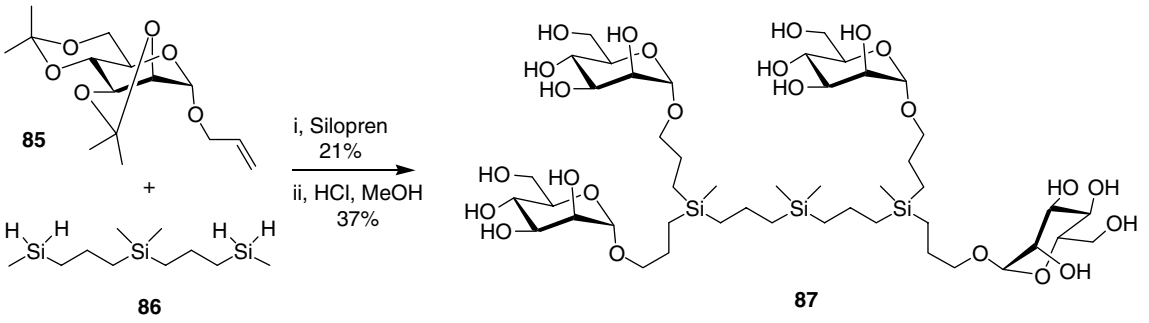



**Figure 39** Carbosilanes-based mannocluster **87** obtained by hydrosilylation.

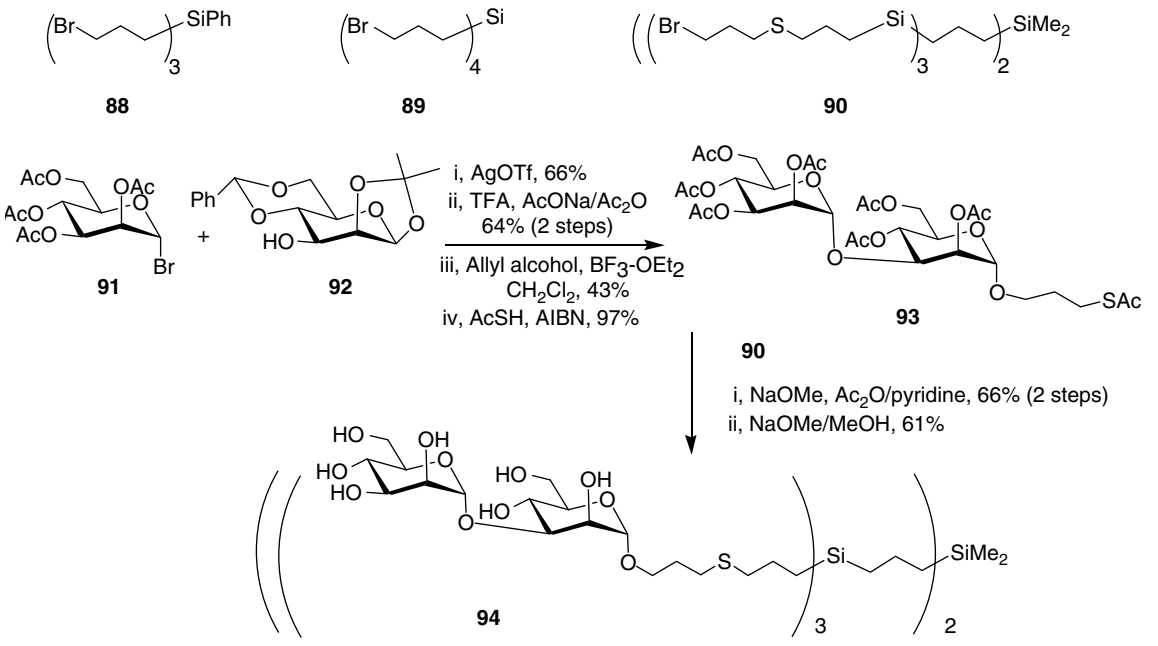



**Figure 40** Carbosilanes-based dimannoside clusters used in binding studies with ConA.

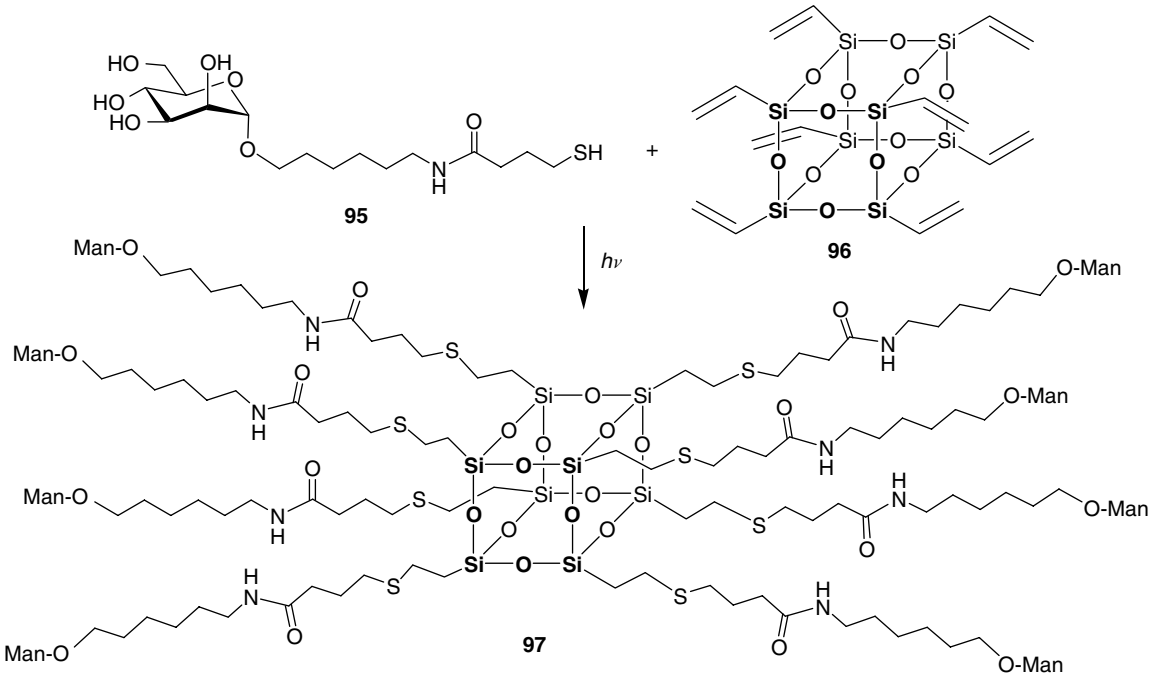



**Figure 41** Silsesquioxanes-based glycodendrimers obtained by radical addition.

of gel permeation chromatography (GPC), mannose-coated carbosilane dendrimers were obtained and disulfide by-product (Manα1-3ManαR-S)$_2$ was removed.

The bindings of the corresponding tri-, tetra-, and hexavalent ligands **88**-Man, **89**-Man, and **90**-Man to dimeric lectin ConA (pH 5.2) were evaluated by titration microcalorimetry (ITC).[142,142a] A soluble protein was titrated with aliquots of a soluble ligand in these measurements. The heat produced during ligand addition served as reporter signal for binding, giving a binding constant, which, in turn could be related to the free Gibbs energy of binding. Since this technique also directly measures binding enthalpies, entropy of binding could be evaluated.[142,142a]

All of the carbosilane dendrimers showed higher binding constant values with Con A, than the nondendritic mannose derivatives, MeαMan and Manα1-3ManαMe, thus demonstrating the cluster glycoside effect. The magnitude of the effects depended on the amount of mannose in a given dendrimer. In the case of three-branched dendrimers with peripheral mannoside, the value of the carbosilane dendrimer was substantially higher than that of the non-carbosilane dendrimer.

Another recent report by Gao *et al.* described the facile and efficient preparation of glycoclusters from silsesquioxanes **96**.[143] Unprotected thiolated mannoside **95** was directly coupled by radical reaction to octavinyl POSS core **96** to provide glycocluster **97** in approximately 70% yield after gel filtration purification (**Figure 41**). The strategy was equally applied to galactoside and lactoside. Using a galactose specific *Ricinus communis* agglutinin (RCA120), the octavalent cluster was shown to completely inhibit the binding of RCA120 to asialo-oligosaccharides from human $\alpha_1$-acid glycoprotein at 10μM. Thus, the glycoside cluster effect of this family of compounds showed 200 times better affinity than the corresponding monomers.

### 3.36.3.10 Combinatorial and Solid-Phase Synthesis of Glycodendrimers

Solid-phase organic synthesis is a rapidly expanding area of synthetic chemistry that is being widely exploited in the search for new biologically active compounds by combinatorial techniques. It was originally developed for peptide synthesis and then oligonucleotide synthesis but is now widely applied to oligosaccharide chemistry. Combinatorial chemistry applied to solid-phase techniques is now broadly used in organic synthesis. Takahashi and co-workers have reported an elegant solid-phase combinatorial synthesis of a carbohydrate cluster on a tree-type linker, where subsequent orthogonal cleavage provided dimer and tetramer **105** and **106** (**Figure 42**).[144] Ether formation between

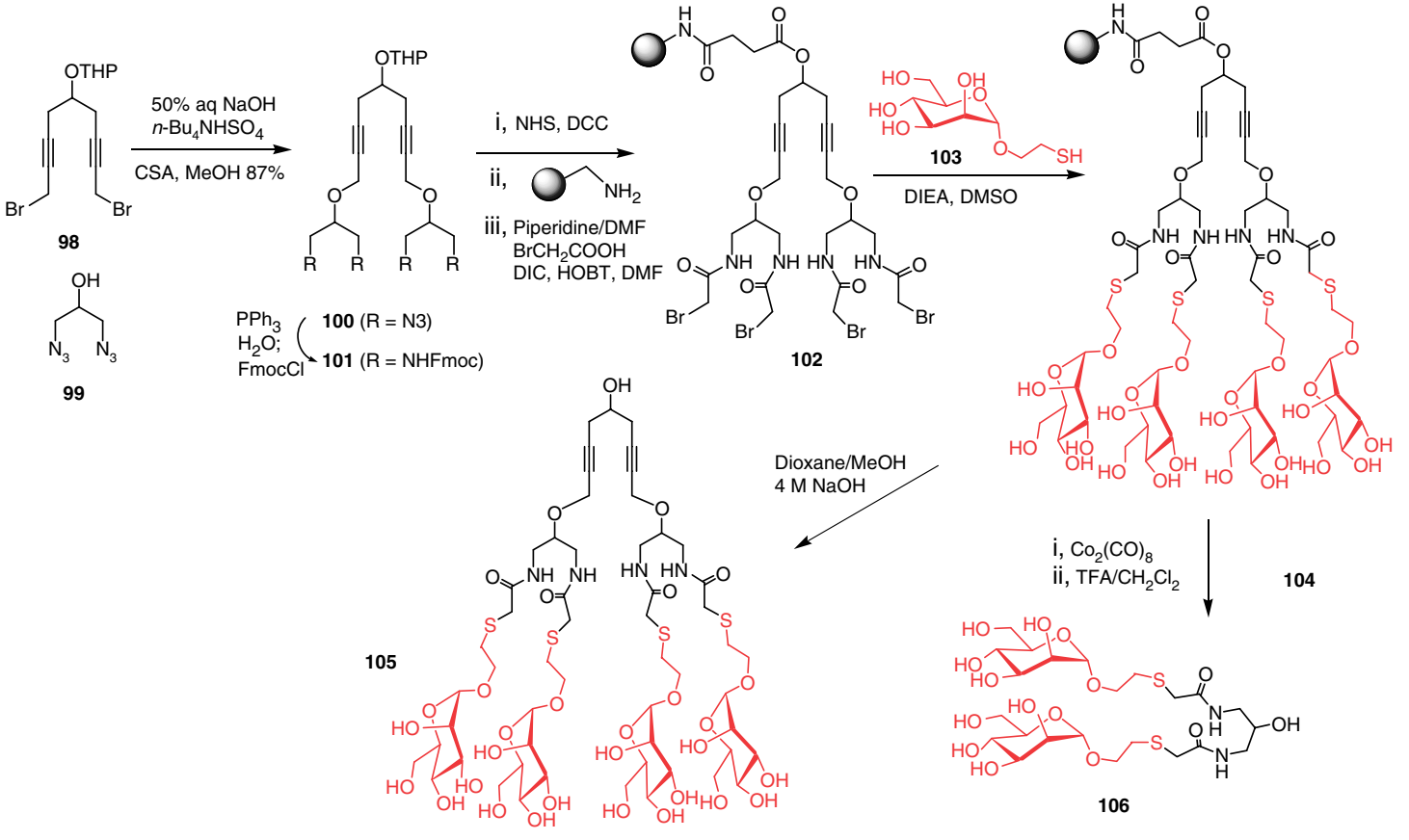



**Figure 42** Combinatorial solid-phase strategy and orthogonal cleavage for the preparation of dimannoside **106** and tetramannoside **105** clusters.

bromide **98** and bisazido alcohol **99** under two phase conditions gave the desired tetraazide **100** which upon Staudinger reduction with aqueous $PPh_3$ followed by Fmoc protection of the resulting amine provided intermediate **101**. The formation of the resin-loaded tree-type dendron precursor **102** was successfully achieved by THP deprotection, attachment to an aminated resin through succinylation, and treatment with bromoacetic acid (DIC, HOBt) after Fmoc deprotection with 20% piperidine in DMF. Tetrameric mannosylated cluster **104** was then obtained under standard alkaline conditions using thioether formation from thiomannoside **103**. Finally, the tetrameric glycocluster was released from the resin by alkaline treatment to provide **105**. Interestingly, the dimeric glycocluster **106** could also be released from the resin under acidic treatment of the acetylenic-$Co_2(CO)_8$ complex (Nicholas reaction). The tetramer inhibited 40% of the binding of fluorescein-labeled mannosylated bovine serum albumin (BSA) onto peritoneal exudative macrophages.

Of particular interest in this section, is the finding by Ramström and Lehn of a very appealing dynamic combinatorial carbohydrate library.[145,145a] The library was generated from an array of thiolated mannosides in which the structures of the linkers were varied and the resulting thiol derivatives were allowed to randomly oxidize into disulfides of varied composition. A library of up to 21 members was thus generated and the mannodimers evaluated against ConA.

### 3.36.3.11 Other Glycodendrimers

Inositol derivatives[146] are becoming increasingly recognized as important building units of dozens of new aminocyclitol antibiotics[147] and as intracellular messengers.[148] Historically, they have been featured so prominently in the development of modern ideas about conformational energies and symmetry properties that it is hard to give a course in stereochemistry without discussing them.[149] Scyllo-inositol which is the all-equatorial stereoisomer of myo-inositol was selected by Chung and co-workers as a scaffold for glycodendrimers synthesis.[150]

Amino scyllo-inositol scaffold **107** constituted the key intermediate for subsequent glycosylation as well as for chain elongation and multiplication to the desired dendrimeric generation (**Figure 43**). Conjugate-1,4 addition between triamine **107** and excess methyl acrylate followed by amidation of the resulting esters with ethylenediamine afforded an amidoamine dendrimer under conditions previously seen for the synthesis of PAMAM dendrimers. Reiteration of this two-step reaction sequence successfully resulted in the doubling of peripheral amino functionalities toward a G2-dendrimer **109**. Glycoconjugation of the dendrimers was then achieved using previously discussed thiourea linkages based on mannosyl isothyocyanate **108**.

Another option for making glycodendrimers is to take a convergent route, starting with a monosaccharide or small glycoclusters which are attached to branching units and then finally to a suitable core. As shown in **Figure 44**, Fernandez and co-workers have described the synthesis of a glycodendrimer–cyclodextrin structure and then provided its biological evaluation with ConA.[151] Several oligosaccharide-branched cyclodextrins have also been reported in the past few years.[152,152a,152b] Cyclodextrins are particularly well-suited for glycocluster-lectin studies since they have the ability to complex small hydrophobic molecules such as drugs in their cavities.[153]

The key template was a $6^I$-amino-$6^I$-deoxy-$\beta$-cyclodextrin,[154] the modified 1,2,3-triaminopropane branching element,[155] and an isothiocyanato-functionalized α-D-mannopyranosyl cluster prepared from trisamine (TRIS). Coupling of the isothiocyanate and the amine-functionalized trimannoside and the branching element gave thiourea-bridged glycodendrimer–cyclodextrin conjugates **110** (**Figure 44**). The monosubstituted hexavalent $\beta$-cyclodextrin mannodendrimer showed a strong cluster effect toward Con A-yeast mannan interaction with an $IC_{50}$ of 10 μM that represented up to a 22-fold increase on a molar basis compared to monovalent derivative. The cyclodextrin derivatives exhibited extremely high water solubility, above 20-fold higher as compared to the parent cyclodextrin (15 mM). Up to 4.5 and 4.7 $g l^{-1}$ of Taxol was solubilized in 25 mM aqueous solutions of a trivalent cyclodextrin derivative at 25 °C, respectively, that is, more than a 1000-fold solubility enhancement as compared to the water solubility of the drug (0.004 $g l^{-1}$).[156] This result illustrates the superior potential of dendritic cyclodextrins as drug carriers.

In a different approach, radical photoaddition of glycothiols **112** to per-2,6-diallyl-$\beta$-cyclodextrin **111** allowed Stoddart and co-workers to achieve the simultaneous attachment of up to 14 saccharide moieties as shown in structure **113** (**Figure 45**).[157]

Calixarenes are cyclic molecules containing a cavity useful in host–guest chemistry.[158] Their intrinsic amphiphilic architecture also makes them ideal candidates for the study of water–monolayer surface interactions. In this respect, they surpass their cyclodextrin counterparts.[159] Kim and Roy have described the first synthesis of dendritic, water-soluble,

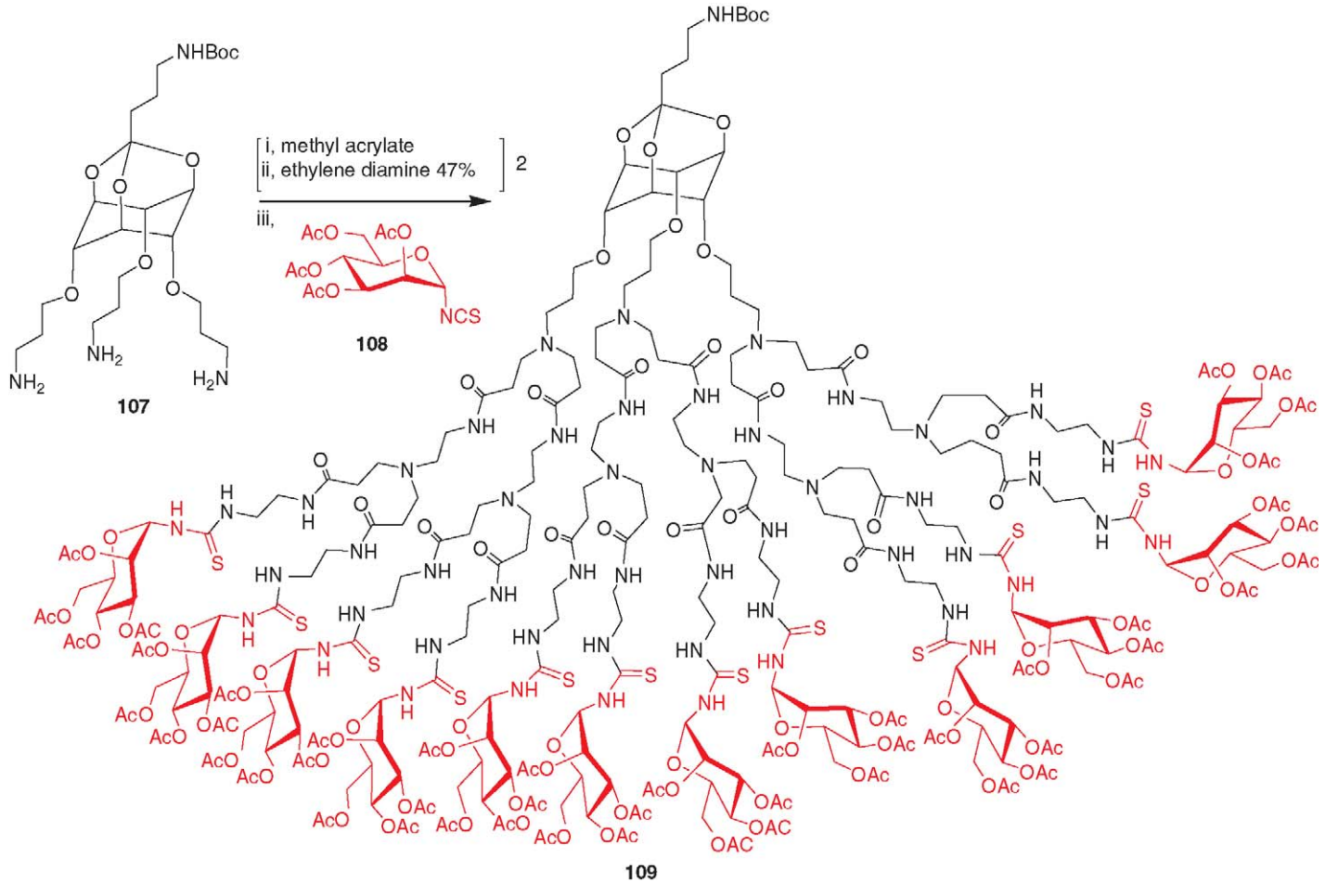



**Figure 43** Scyllo-inositol-based glycodendrimer using thiourea linkages.

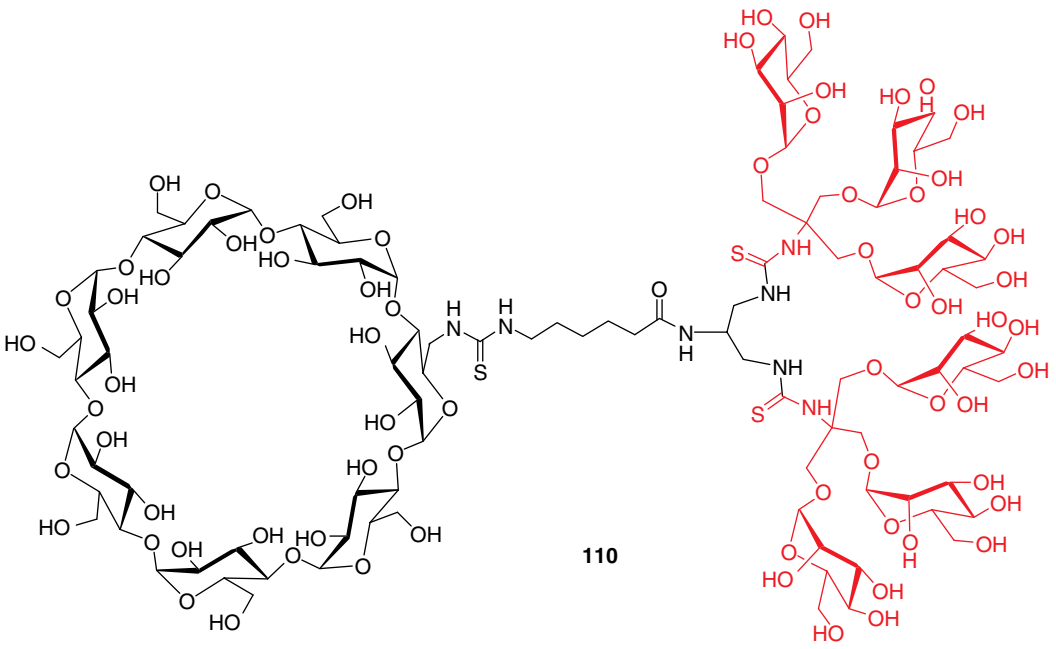



**Figure 44** Monosubstituted $\beta$-cyclodextrin as scaffold for a unique hexameric mannoside cluster **110**. The cavity portion of $\beta$-CD was used to carry the anticancer drug taxol for which the water solubility was greatly improved by the construct.

carbohydrate-containing *p*-tert-butylcalix[4]arene and their lectin-binding properties.[121,121a–121c] These carbohydrate-containing calix[4]arenes can serve as models to further investigate factors influencing multivalent carbohydrate–protein interactions at the molecular level. The lipophilic *p*-tert-butyl substituents provided the driving force for stable assembly and/or adhesion of a calixarene monolayer to a hydrophobic surface, while the hydrophilic carbohydrate tail mimicked cells saccharide-rich surfaces present on cancer tissues ($T_N$-antigen, **115**). This new type of hybrid molecules can serve as coating materials for carbohydrate ligands in competitive solid-phase immunoassays. The hexadecameric glycocalix[4]arene **116** was obtained from octameric amine **114** and bromoacetamido-GalNAc derivative **115** after de-O-acetylation (**Figure 46**).

The tetra-, octa- and hexaocta-calix[4]arene glycodendrimers were evaluated for their relative lectin-binding properties against *Vicia villosa* agglutinin (VVA). This plant lectin has been used previously for binding studies against α-D-GalNAc derivatives.[160] The direct binding abilities and cross-linking behavior calix[4]arene ligands toward VVA were determined by turbidimetric analysis. Hypervalent glycocalix[4]arene dendrimers demonstrated direct binding to VVA by the rapid formation of insoluble precipitates. The efficiency of glycocalixarenes to inhibit the binding of asialoglycophorin, a natural glycoprotein of human erythrocytes-blood group serotype, was measured by ELLA. The best result was obtained from the hexadecavalent conjugate ($IC_{50}$, 13.4 mM), which represents a 12-fold increase in potency over that of allyl α-D-GalNAc monomer ($IC_{50}$, 158.3 mM). The prepared glycocalixarene derivatives were also directly adsorbed onto the lipophilic surface of polystyrene microtiter plates and thus should be useful in bioanalytical devices. *p-Tert*-butylcalix[4]arene was also used by Roy and Meunier for the synthesis of α-sialylated-calix[4]arene.[121] Evidences for strong cross-linking ability with tetrameric wheat germ agglutinin (WGA), a plant lectin known to bind sialosides, was detected with this tetramer.

Glycerol has also been used as scaffold for glycodendrimer syntheses. A report describes the synthesis of up to four mannose-containing residues **117** and **118** (**Figure 47**).[161] These compounds were evaluated for their capacity to inhibit mannose-specific adhesion of *E. coli* using a recombinant strain, *E. coli* HB 101 (pPK14), expressing only type 1 fimbriae on its surface. Unfortunately, none of the tested clusters was a better inhibitor than *p*NPMan.

Stoddart and co-workers described multivalent glycodendrimers **119** based on a triphenylene core (**Figure 48**). The dendrimer, having 10 methylene linkers between the core and the glycodendron end groups, formed hexagonal columnar structures which gave rise to a fluid birefringent texture between 165–220 °C.[124]

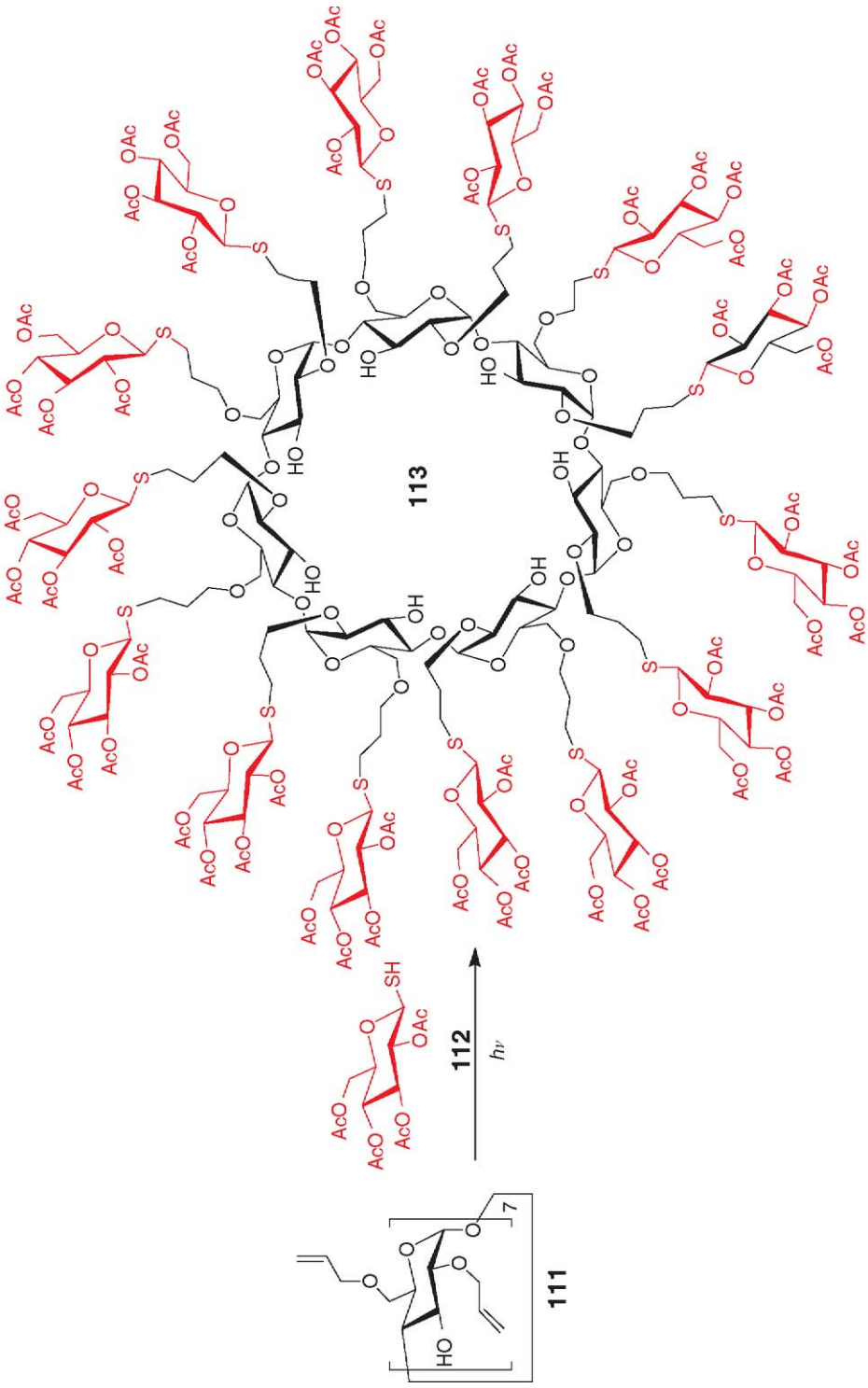



**Figure 45** Per-disubstituted $\beta$-cyclodextrin-based glycodendrimer **113** obtained by radical photoaddition of thiolated sugar **112**.

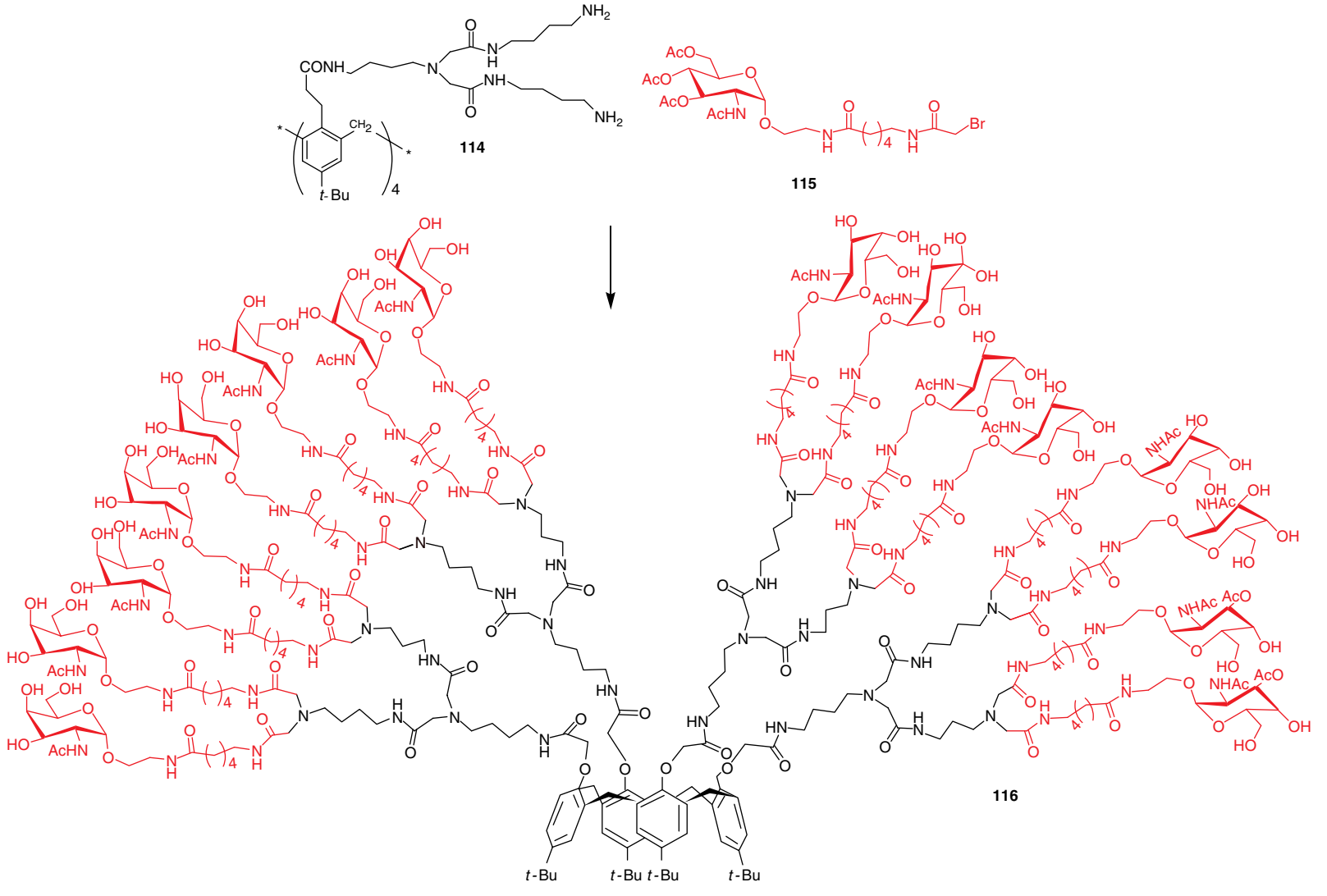
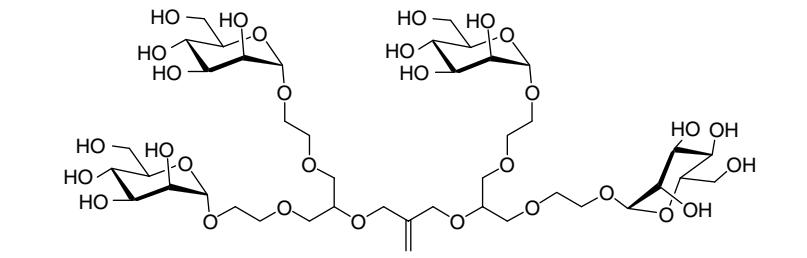
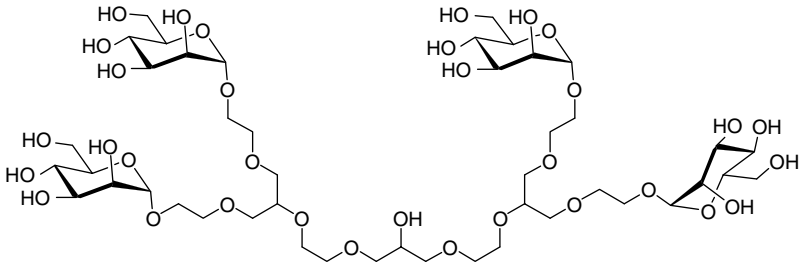



**Figure 46** Calix[4]arene-based glycodendrimer **116** obtained by amide bond and bearing the cancer marker $T_N$-antigen **115**. This glycodendrimer, by virtue of its poly-hydrophobic *t*-butyl head groups could bind to an hydrophobic polystyrene surface.

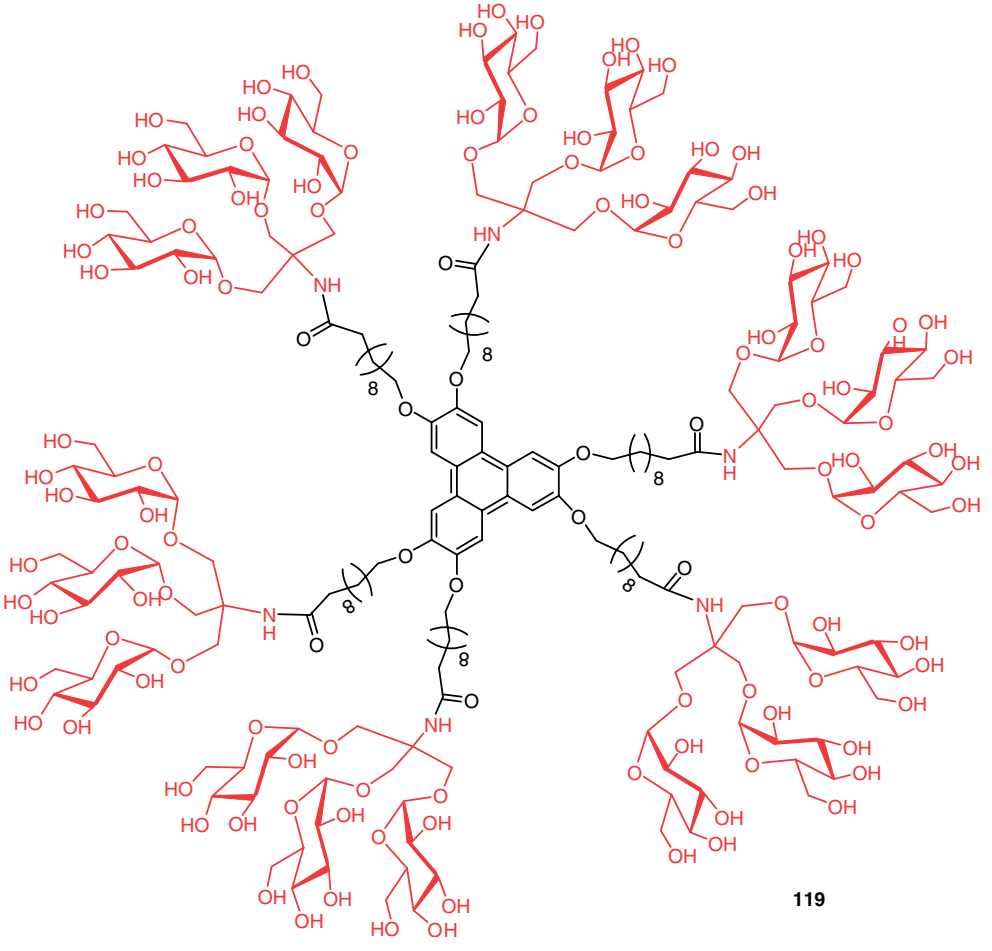


**117**



**118**

**Figure 47** Glycerol-based mannoclusters.



**119**

**Figure 48** Glycodendrimer bearing a triphenylene core with 18 sugar appendages and showing liquid crystal behavior.

## 3.36.4 Conclusions

The enormous creativity that has been injected into the field of glycodendrimer syntheses has allowed the preparation of many varied structural and beautiful architectures. Unless otherwise stated, this report has only presented those involving mannoside residues with the hope to sensitize the community with the broad arsenal of molecules available and perhaps to stimulate others to come. In spite of the fact that this relatively novel family of small macromolecules has been discovered in 1993, it is somewhat surprising that there are still no architectural rules governing the synthesis of the best candidates for any given applications. Through our own personal experience, we could conclude that, for now, glycopolymers still represent the most efficient candidates for targeting viral particles. However, with the scarce information available, it appeared that dendronized glycopolymers might offer clear advantages. The situation seems to be dramatically different when dealing with fimbriated bacteria and certainly so, to multimeric soluble carbohydrate-binding proteins such as lectins, galectins, antibodies, and so on. It is also somewhat surprising that the community has not yet concented for the use of the same bacterial strains when dealing with the inhibition of adhesion of fimbriated *E. coli* and related pathogens. This situation will have to be remedied in the near future if one wants to establish governing rules for the design of better and improved adhesion inhibitors.

The general information at hand tends to indicate that glycodendrimers are better than glycopolymers for binding to FimH on fimbriated *E. coli*. Additionally, longer and rigid linkers have brought some noticeable improvements. It is also clear, that for any given situation, optimization of the key monovalent sugars will have to be investigated, prior to or in parallel to making multivalent clusters.

The ever-increasing field of nanotechnology as it is now applied to glycobiology will certainly allow novel architectures to come into reality. In this regard, fullerenes, carbon nanotubes, gold nanoparticles, quantum dots, and the like represent stimulating carrier candidates for particular applications. The inherent physical and spectroscopic properties of these novel basic materials will likely, when properly conjugated to suitable carbohydrate motifs, afford 'glycomaterials' useable in nanomedicine, vaccines, drug delivery, and topical cures.

With the vast access and information now available regarding new glycodendrimers, more thorough biological investigations will have to be designed. Cellular toxicity is certainly an area behind schedule for glycodendrimers. Cell permeability with varying structural motifs and sugar composition also remains to be further investigated. With the merging of several glycoscientists working together, the future looks very bright for glycodendrimers and, as stated by N. Sharon and co-workers more than two decades ago, the realm of using 'sugars' as bacterial antiadhesion molecules is finally touchable.

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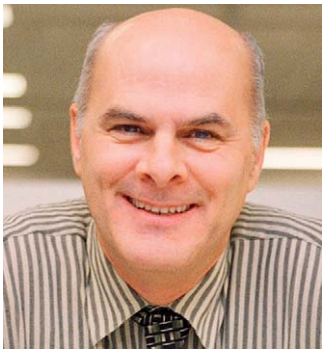
## Biographical Sketch



René Roy, Professor in the Department of Chemistry of the Université du Québec à Montréal (UQAM), is the recipient of the 2003 Melville L.Wolfrom Award for his outstanding contributions in the design of novel neoglycoconjugates and glycodendrimers. He is a native of Montreal where he received his education. He obtained his Ph.d. in chemistry in 1980 from the University of Montreal under the supervision of Prof. S. Hanessian. Prof. Roy joined the Division of the Biological Sciences of the National Research Council of Canada where he worked from 1980 to 1985 as a Research Fellow. He has contributed to the design of new bacterial polysaccharide vaccines and he is the co-inventor of the recently commercialized *Neisseria meningitidis* vaccine. He then joined the Department of Chemistry of the University of Ottawa, Ontario, where he worked until December 2002 before returning to his hometown in Montreal. In 1997, he received the Hoffmann–LaRoche award for his distinguished contribution to medicinal chemistry; in 2001, the Ottawa Life Science Council Award and the Paul Harris Fellowship. Prof. Roy has used modern synthetic chemistry to tackle problems associated with the synthesis and applications of neoglycoconjugates and polymers. He developed the concept of active and latent glycosidation methods applied to thioglycosides. His design of novel carbohydrate architectures, glycodendrimers, has paved the way for a better understanding of multivalent carbohydrate–protein interactions at the molecular level. He has recently contributed to the first semi-synthetic bacterial polysaccharide vaccine against *H. influenza* type b that was shown successful in infants. The clinical trials were done in Cuba and vaccines has been approved in 2003. Since 2003 he holds a Canadian Research Chair in Therapeutic Chemistry.

Mohamed Touaibia was born and received his elementary education in Ténes, Algeria. He received a B.S. and Masters degree in organic chemistry from the University of Algiers in 1995 and Tlemcen in 1998, respectively. From there, Mohamed studied at the University of Paris 7 in France and received a Ph.D. in pharmacochemistry in 2002. His doctoral work was centered on the development of $PAL_2$ inhibitors. He is currently a research assistant in Prof. Roy's laboratory and is actually working on glycomimetics and glycodendrimers as potent FimH inhibitors.