Journal of Peptide Science
J. Pept. Sci. 2008; **14**: 2–43
Published online 21 November 2007 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.931

JOURNAL OF PEPTIDE SCIENCE

Review

Glycopeptide dendrimers. Part I‡

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Received 9 August 2007; Accepted 10 August 2007

Abstract: Glycopeptide dendrimers are branched structures containing both carbohydrates and peptides. Various classes of these compounds differing in composition and structure are mentioned, together with their practical use spanning from catalysis, transport vehicles to synthetic vaccines. The main stress is given to glycopeptide dendrimers, namely multiple antigen glycopeptides (MAGs). In MAGs, the core, branches or both are composed of amino acids or peptides. Other classes of glycodendrimers (PAMAM, polypropylene imine, cyclodextrin, calixarene, etc.) are mentioned too, but to a smaller extent. Their syntheses, physicochemical properties and biological activities are given with many examples. Glycopeptide dendrimers can be used as inhibitors of cell surface protein-carbohydrate interactions, intervention with bacterial adhesion, for studying of recognition processes, diagnostics, imaging and contrast agents, mimetics, for complexation of different cationts, as site-specific molecular delivery systems, for therapeutic purposes, as immunodiagnostics and in drug design. Biomedical applications of glycopeptide dendrimers as drug and gene delivery systems are also given.

Keywords: artificial virus; calixarene dendrimers; calix[4]resorcarene dendrimers; carbopeptide dendrimers; contrast agents; cyclodextrin dendrimers; cyclotriveratrylene dendrimers; dendrimers; drug delivery; glycobiology; glycocluster; glycoconjugates; glycodendrimers; glycopeptide dendrimers; glycopeptide libraries; glycopeptides; glycotope; lectin; imaging agents; ligation chemistry; multiple antigen glycopeptide (MAG); PAMAM dendrimers; review; synthetic vaccine

INTRODUCTION

Most of the signal transduction processes in living organisms are caused by three repeating biopolymers: nucleic acids, proteins and glycoconjugates. The structure and function of nucleic acids as well as peptides and proteins have been extensively studied, including different classes of interactions (Figure 1) [1,2]. Genomics [3,4], proteomics [5–7] and glycomics [1,2,8–14] represent three interconnected areas of living organisms. In comparison with genomics and proteomics, the development in glycomics is more demanding owing to the huge increase in possible structural variations. The term glycomics was introduced to describe glycobiology and the interaction of carbohydrates with the other two major classes of biopolymers.

The capacity to store information in an oligomer was calculated for nucleic acids, proteins and carbohydrates for comparison [12,15,16]. For trimers, 64 sequence permutations (4^3) of nucleotides using the four pyrimidine and purine bases and 8000 isomers (20^3) for peptides with the standard 20 amino acids represent the total repertoire. Under these conditions, sugars afford 9 000 000 isomers. Saccharides can form branched oligomers and this must be reflected in the pool size of oligomers larger than trimers. The number of hexamers for peptides is 64 000 000 (20^6) and looks impresive when compared with 4096 (4^6) hexanucleotides, but the number of isomers of hexasaccharides is 1.44×10^{15}. Therefore, carbohydrates are the best high-density coding system. This language has been named glycocode resp. sugar code [14,17–19]. The term glycocode well represents the potential level of complex information that carbohydrate structures are able to convey. Monosaccharides, as building units for oligo- and polysaccharides constitute information-storing coding units, the third alphabet of life.

In analogy with the term 'proteome', the term 'glycome' has been coined for the glycan repertoire of an organism. Also, in the wake of 'genomics' and 'proteomics', the word 'glycomics' has become the trendy term for the characterization by function and structure of glycans in the studied system [8,10,12].

Abbreviations: Standard abbreviations have been followed throughout this paper (*J. Peptide Sci.*, 2006; **12**: 1–12). Other abbreviations are in Table. When not stated otherwise, amino acids are of L-configuration and carbohydrates are of D-configuration.

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‡ Dedicated to Professor Christian Birr (Orpegen Pharma, Heidelberg) on the occasion of his 70th birthday.

BIOGRAPHY

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Studies abroad: 1) 1988 Shemyakin Institute of Bioorganic Chemistry, Moscow, with Prof. V.T. Ivanov and T.M. Andronova, PhD., synthesis of oligosaccharide muramyl peptides and lipoglycopeptides, 2) 1989,1990 The Rockefeller University, New York, with Prof. R.B. Merrifield, glucagone analogues; Torrey Pines Institute for Molecular Studies, San Diego, with R.A. Houghten, PhD., simultaneous multiple peptide synthesis, T-bag method, 3) 1992, 1993, Institute for Biochemistry and Biophysics, Friedrich Schiller University, Jena, Germany, with Prof. S. Reissmann, bradykinine analogues with backbone-to-backbone cyclization, synthesis and structure-activity studies. Research area: SPPS, MAPs, MAGs, peptide and glycopeptide dendrimers, coupling reagents, synthetic peptide and glycopeptide vaccines. Private interests: post stamps, body building, minerals.

Dr. Petr Niederhafner: Born in 1966 in Mlada Boleslav, Czechoslovakia. In 1988, graduated the Institute of Chemical Technology, Prague teaching at secondary school (1990–1996) then research in industry (1996–2000) from 2000 until now Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, PhD. *student* (supervisor Jan Hlavacek, PhD.). Research area: Peptide synthesis on PEG carriers, synthetic vaccines. Private interests: Sport, reading.

BIOGRAPHY

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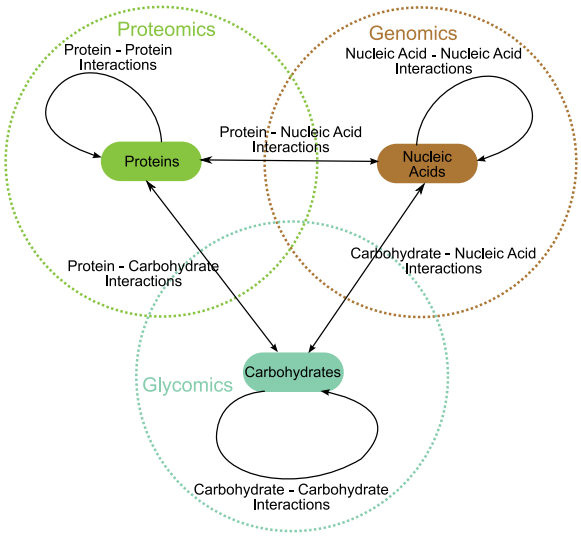

Figure 1 Different types of interactions between the three main biopolymers [1,2].

Therefore, the amount of information carried by glycopeptide dendrimers or glycodendrimers is much higher than in the case of peptide dendrimers. The same holds for the structural variability, complexity, spectrum of biological activities, etc.

Our review is focused on glycopeptide dendrimers, including MAGs. The word dendrimer stems from Greek words *dendron* meaning 'tree' or 'branch', and *meros* meaning 'part of' [20–22]. Dendritic structures emerged as a new class of polymers, first reported by Vogtle *et al.*, and were named 'cascade' molecules [23]. Development of this field led to larger dendritic structures and this class of compounds was renamed 'dendrimers' [21,22,24–33].

Dendrimers [21,27,34–46] are 'monodisperse polymers that adopt a globular three-dimensional shape as the generation number (Gn) increases. These highly-branched macromolecules possess a well-defined core, interior region layers, and an exterior corrugated surface, affording a high surface area-to-volume ratio. With each successive generation, the number of end groups multiplies and the properties of the dendrimer become more strongly influenced by the nature of the end groups. These chemical and structural attributes of dendrimers translate to unique chemical and physical properties' [35].

Another, more lyrical approach [28] describes dendrimers as 'a jungle of entangled branches traversed by winding trails which lead to sweet fruits and bright blossoms. On these trails one can reach the thicket's interior as well as find a way out. This thicket stands for

DOI: 10.1002/psc

regularly branched, densely packed structures, and the trails represent voids and channels not filled by bent back branches but by solvent. The fruit and blossoms are photochemically, electrochemically, or synthetically addressable units, catalytically active sites, etc., and the back and forth on the trails stands for transport processes.'

Dendrimers in general, including their history, classification and explanation of basic terms were reviewed by Tomalia, the father of this field [22,29,31,38]. Before 1984 there were only three polymer architectures: linear, cross-linked and branched type configurations. The 'dendritic architectural state' is a new, fourth class of polymer architecture. Dendritic polymers are classified into four dendritic (cascade) subclasses: (i) random hyperbranched polymers, (ii) dendrigraft polymers, (iii) dendrons, and (iv) dendrimers (Figure 2) [22,46]. These four subclasses reflect the degree of relative structural control present in these dendritic architectures and also their increasing symmetry.

Dendrimers are functionalized mainly at the periphery (surface). Dendrimers functionalized in the interior are also known [47], but their synthesis presents considerable hurdles.

Multivalent carbohydrate-functionalized macromolecular architectures (dendrimers) have been described and classified (Figure 3) [48–53]. Multivalent ligands include, e.g. linear, comb-branched, dendrigraft, spheroidal dendrimer, linear-dendron copolymer ligands, macrocyclic glycocluster, glyco-cyclodextrin and glyco-calixarenes. Some of these structures apply to glycodendrimers, and some to glycopeptide dendrimers. The fantasy is unlimited.

The term 'glycodendrimer' first appeared in the literature in 1993 [51,55,56]. Glycodendrimers and related glycoclusters stimulated wide interest in chemists, immunologists, etc. Glycodendrimers [51,53,56,57] are 'synthetic biomacromolecules that are made of biologically relevant carbohydrate ligands constructed at the periphery of a wide range of highly functionalized and repetitive scaffolds having varied molecular weights and structures. They were aimed to fill the gap between glycopolymers, having generally dispersed higher molecular weight, and small glycoclusters, in the study of multivalent carbohydrate protein interactions'.

The same structure with carbohydrates on the surface and peptidic branches is sometimes termed *glycodendrimer* (the author focused on the activity of the sugar epitope); others use the term glycopeptide dendrimer (respecting the whole structure of the molecule).

This review is a natural continuation of our earlier reviews [58,59] in 1999 and 2005 [36]. Therefore we do not explain all terms, syntheses, properties, etc. and the reader is referred to the aforementioned papers. We have included the literature since 2000, but in special cases earlier. Many topics from the area of oligosaccharides, glycopeptides and glycopeptide dendrimers have been reviewed: synthesis of oligosaccharides [9,19,60–71]; biological and immunological properties of glycopeptides and glycopeptide dendrimers [17,42,50,51,54,57,63,64,66–70,72–84]; synthesis both in solution and on the solid phase, including different coupling reagents, resins, protecting groups and discussion about side reactions [11,17,19,27,28,34,42,49,51,54,57,61–64,66,67,72, 73,77,80,84–94]; enzymatic synthesis of oligosaccharides and glycopeptides [19,60,62,66,69,71,73,95–98]; glycopeptide, multivalent glycopeptide and glycoprotein synthesis using unprotected carbohydrate (oligosaccharide) building blocks [19,60,93,99] or glycoamino acid building blocks with free sugar OH groups [100–103]; use of glycopeptides in the immunotherapy of cancer [51,63,64,67,68,70,77,78,80–82,104,105]. Examples of the use of derivatized dendrimers to inhibit viral infection (influenza, HSV 1, HSV 2, HIV, foot and mouth disease and sendai) have been reviewed [94,106–108]. Recent progress in bioinspired

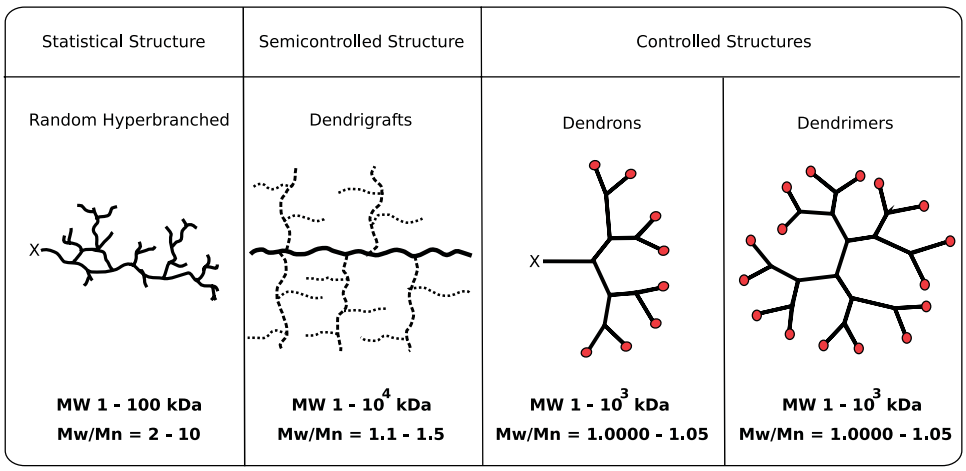

Figure 2 Dendritic polymers: subclasses of the fourth major new class of macromolecular architecture [22,46].

DOI: 10.1002/psc

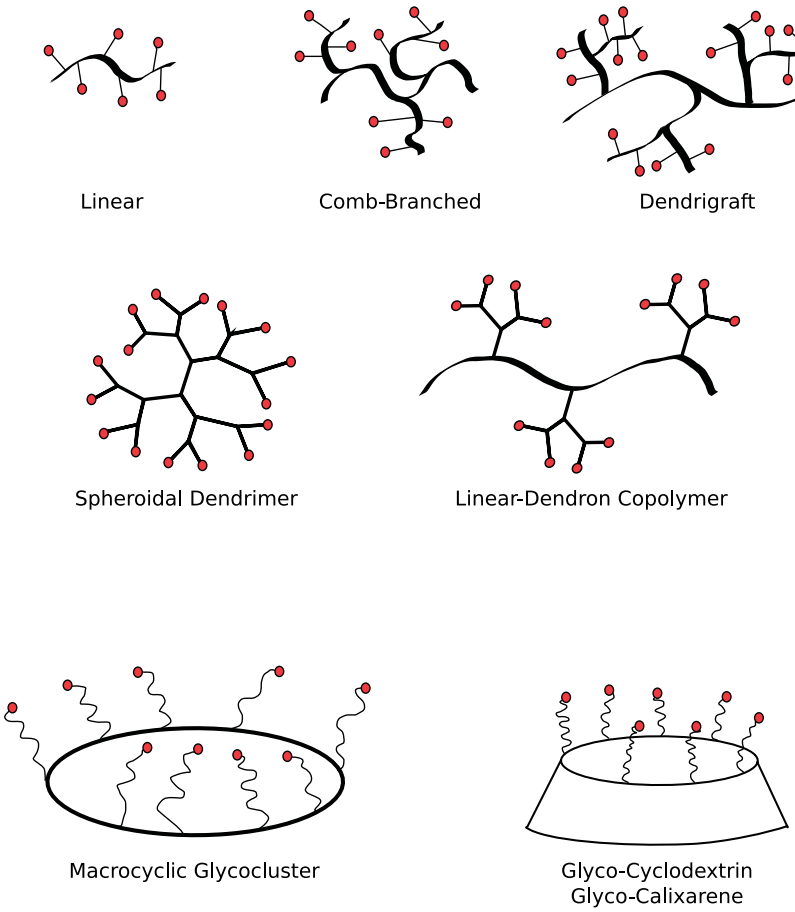

Figure 3 Multivalent carbohydrate-functionalized macromolecular architectures [50,52–54].

applications of dendrimers as protein mimics, anti-cancer or antiviral agents, vaccines, nanomaterials, drug and gene delivery vehicles has been discussed [42,83,109–113].

Scope and Limitations

The choice what, why and how to write was difficult. As chemists, we decided to choose the compounds depending on (i) the structure in general (MAGs, PAMAM, cyclodextrin based dendrimers, etc.) (ii) the branches and the core and (iii) the glycotope. The consequences are that, e.g., mannose as glycotope can be bound to different structures (with peptide, or peptide bonds or without peptide or peptide bond). As a consequence, important compounds and their activities should be omitted. For both logical and immunological reasons we decided to include compounds with interesting structure and activity, independently on the fact that they are glycodendrimers and not glycopeptide dendrimers. In exceptional cases compounds without sugar are mentioned because they have a template (core) that is (or can be) used in glycopeptide dendrimers and serve as inspirative possibility, e.g. SOCs. The unifying idea is the cluster effect. The absence of peptide (amino acid) or carbohydrate is stressed at the given examples. Such compounds represent only a minority.

In the text, the categorization was done in accordance with the chemical structure. While the advantages are clear chemical structures, unfortunately the same epitopes bound to different structures will be under different sections. The same holds for biological activities. In order to keep together some epitopes with similar activity, two exceptions were made: TACA-derived glycopeptide dendrimers, and anti-HIV and other antiviral constructs. They are described separately; see Part III of this review (Jezek *et al.*, manuscript under preparation).

In Table 1, it is more logical to arrange them first according to the activity or glycotope, e.g. mannose, glucose, different classes of tumor-associated antigens, etc., and then with the chemical structure. This will satisfy immunologists. In the table, only selected examples are given.

Glyco(peptide) dendrimers can be classified as (i) carbohydrate-coated (ii) carbohydrate-centered and (iii) fully carbohydrate-based [57,89]. The subgroup (i) contains subclasses with different arms resp. branches (peptide-based, organic, ester-based, aromatic, etc.) and different cores (cyclic peptide, sugar, cyclitol, calixarene, cyclodextrine, etc.). In some cases

DOI: 10.1002/psc

it is difficult to suborder the given structure to some category, e.g. linear peptides containing many T_N, TF or sialosyl-T_N antigens. In this case, the peptide structure is linear and contains saccharide branches (Figure 4) [63,64,77]. Other subgroups contain a cyclic peptide core, e.g. RAFT and TASP [132,186–188], to which the sugar units are bound (Figure 5) [188], or branched peptides (MAPs), to which sugar units are bound (Figure 6) [51,63,64]. This last category is termed multiple antigen glycopeptides (MAGs) [100,189]. Such structures represent a special subgroup of carbohydrate-coated dendrimers. Glycodendrimers with branches containing peptide bonds (not true peptides) will be mentioned too. We will use the term MAGs for branched compounds with sugar on the surface in analogy with MAPs [37,189–191]. For other types of compounds, the term glycopeptide dendrimers will be used. Our approach to dendrimers is not strictly limited to regular geometrical branching, but more generally to branched compounds, where the cluster effect plays an important role.

The creative potential of the nature and fantasy of organic chemists are unlimited and have overcome any attempts to put different classes of dendrimers into the nomenclature 'boxes'.

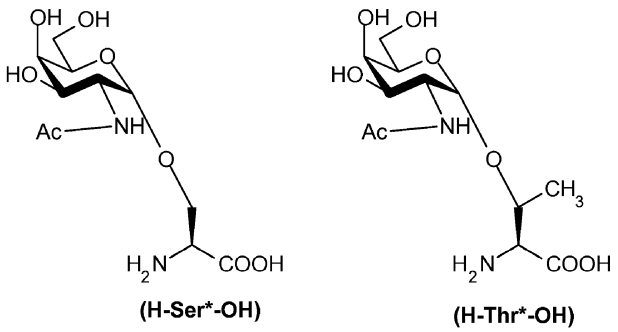

Figure 4 Linear peptide containing sugar branches [63].

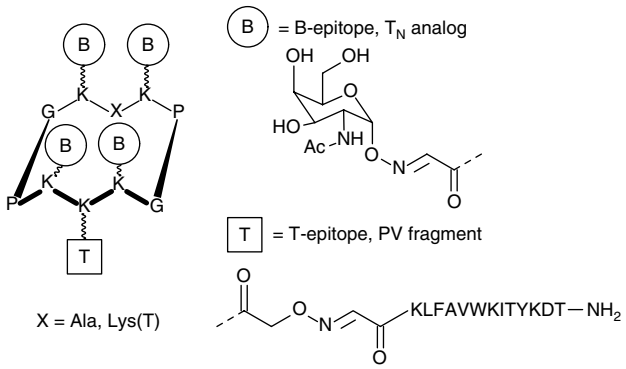

Figure 5 RAFT-based synthetic vaccine candidate [188].

EXPLANATION OF SOME TERMS

Some Terminology Must Be Unified

The terms 'multiple antigen glycopeptides' and 'multiple antigenic glycopeptides' are only seemingly the same. The second term can mean many glycopeptides that are antigenic, i.e. a mixture of different glycopeptides. The same holds for multiple antigen peptides (MAPs) *versus* multiple antigenic peptides [192]. The same abbreviation (MAG resp. MAP) can have an entirely different meaning.

The terms and nomenclature in dendrimer chemistry have been excellently covered [43–45].

Detailed nomenclature for dendritic molecules ('cascadane-nomenclature') has been developed by Vogtle *et al.* [193], based on the rules of naming dendrimers introduced by Newcome in 1993 [194]. It was undoubtedly difficult (but also necessary) to develop a nomenclature for dendrimers, like a nomenclature for carbohydrates, peptides, etc. Unfortunately for the nomenclature (and luckily for chemistry and pharmacology, immunology, etc.) the topic of denrimeric chemistry is fantastically diverse. Therefore, to push any compound into the only one right nomenclature box is very difficult or even impossible. When possible, then the name is long, complicated and difficult to understand, especially for immunologists. Besides, MAPs and MAGs belonging also to the 'dendrimer family' use their own nomenclature. The figure with a structural formula is still the clearest and most understandable information. In spite of our opinion, the authors [193] deserve appreciation for their courage, and at this moment they have reached the maximum possible.

Dendriplexes and Dendrisomes

Dendrons and dendrimers can form nanostructures with DNA. These complexes between cationic dendrons or dendrimers and DNA have been termed dendriplexes [195–197]. Schematic relationships between dendrons, dendrimers and their aggregated states (monolayers, nanoparticles, dendrisomes, dendriplexes, etc) are shown in Figure 7 [196].

Dendrisomes are small vesicular aggregates that are formed in water from cationic lipidic polylysine dendron (MAP) of the appropriate hydrophile–lipophile balance. Dendrisomes are reminiscent of cationic liposomes and are able to encapsulate negatively charged, water soluble compounds [198,199]. Both dendriplexes and dendrisomes can be used as vectors and drug delivery systems.

Dendrigraft

Whereas traditional monomers are generally employed in constructing dendrimers, reactive oligomers or polymers are used to produce dendrigrafts. Therefore, dendrigrafts (Figure 2) are generally larger than dendrimers

DOI: 10.1002/psc

Table 1 Biological activities of glycopeptide dendrimers

	[14,18,51,54,76,108,114–124]
A series of galactosides containing 7 1-thio-β-lactose or β-lactosylamine residues bound to β-CD and 3 first-order dendrimers based on a β-CD core containing 14 1-thio-β-D-galactose, 1-thio-β-lactose and 1-thio-β-melibiose, respectively, (Figure 14) were tested by isothermal calorimetric titrations to measure affinity constants and thermodynamic parameters for the complex formation of β-CD dendrimers with lectin from peanut (*Arachis hypogaea*) (PNA), and guests sodium 8-anilino-1-naphtalene-sulfonate, and 2-naphtalenesulfonate. PNA forms cross-linked soluble complexes with β-CD dendrimers containing Gal and Lac, but not with derivatives containing galactopyranosylamines or melibiose. Both perbranched and hyperbranched β-CDs form stronger complexes with PNA than the monomeric analogues. The β-CD lactoside dendrimers could be used as molecular carriers for transportation of guests to specific lactoside receptors such as PNA.	[125]
Calix[4]arene-based dendrimers with T_N antigens and valencies 4, 8 and 16, respectively, (Figure 20) were tested for relative lectin-binding properties. The VVA lectin, which is known to bind α-D-GalNAc derivatives, was used. In a solid-phase competitive inhibition experiment, the hexadecavalent dendrimer was found to be a very effective inhibitor ($IC_{50} = 13.4 \times 10^{-6}$ M) of the binding of VVA with asialoglycophorin (a natural human blood group serotype).	[126,127]
PAMAM dendrimers containing TF-antigen with valencies 4, 8, 16 and 32 were tested for protein binding properties using peanut lectin from *Arachis hypogaea* and a mouse monoclonal IgG Ab. The higher valency conjugates generated stronger binding interactions, indicating a cluster effect. The inhibition increase was 460, 960, 1700 and 3800, respectively, in comparison with monomeric TF-antigen residue.	[56,128,129]
N,*N*′-Bis(acrylamido)acetic acid-based dendrimers with TF-antigen (Figure 11(a)) with valencies varying between 2 and 6 were studied by turbidimetric analysis toward the highly TF-antigen-specific plant lectin of *Arachis hypogaea* and by competitive double sandwich inhibition ELISA using mouse MAb JAA-F11 (IgG3). In the turbidimetric assay, the hexamer showed the strongest/fastest ability to precipitate the lectin. When tested as inhibitors of binding between IgG3 and a polymeric form of the TF-antigen (TF-antigen-*co*-polyacrylamide used as coating antigen), the two tetramers afforded strongest inhibition in comparison with the monovalent TF-antigen.	[56,130]
Lectin-binding properties of Cu^{2+}-self-assembling bipyridyl-glycoclusters bearing the T_N-antigen cancer marker were studied for relative inhibitory potencies against monomeric allyl α-D-GalNAc using ELLA with asialoglycophorin and horseradish peroxidase-labeled lectin from *Vicia villosa*. Inhibitory properties of the di- and tetravalent bipyridyl clusters were increased up to 87-fold in comparison with the monomer (IC_{50} 158.3 μM). The Cu^{2+} complexes were up to 259-fold more active (IC_{50} 0.61 μM), with the octamer showing the highest affinity. For details see Part II [Jezek *et al., J. Peptide Sci.*, manuscript under preparation].	[131]
Recognition assays of the tetramannosyl-RAFT with Con A using fluorescence anisotropy method afforded IC_{50} of 62 μM in comparison with methyl α-D-mannopyranoside (IC_{50} 1.2 mM), respectively.	[132]
The affinities of mannose-containing, D-glucose centered glycoclusters towards Con A were measured by competitive ELLA.	[133]
First to sixth generation of PAMAM dendrimers functionalized by α-**D**-mannose have been tested for binding with Con A and with pea lectin. Both proteins bind methyl α-**D**-mannose with specificity; Con A has four-fold higher affinity than pea lectin. Relative affinities of mannose- and glucose-substituted PAMAM dendrimers with Con A have shown difference in relative activity between glucose-functionalized and mannose-functionalized dendrimers: 14.7 for G4, 15.6 for G5 and 11.4 for G6, respectively. Multivalent affinities can be predicted on the basis of monovalent association constants.	[134–137]
Dendritic derivatives of β-CD-bearing multivalent (2, 3, 4, 6) mannosyl ligands were studied as lectin-binding inhibitors. Results of the binding inhibition of horseradish peroxidase-labeled Con A to yeast mannan by mannosylated β-CD-dendrimers were	[138]

DOI: 10.1002/psc

Table 1 (*Continued*)

expressed as IC_{50} (μM) values. Dendrimer with one CD molecule (Figure 15(b)) had the lowest IC_{50} (10 μM) with a relative efficiency (valency-corrected) of 23. The consequence of inclusion complex formation using the anticancer drug docetaxel (Taxotere) as a target guest, on biological recognition has been studied. This new type of tailor-made hexavalent CD dimer host (Figure 15(a)) for docetaxel showed high drug solubilization capability.	
Trimannosyl-peptide-β-CD dendrimers were tested for Con A binding by ELLA. Addition of 1-adamantyl-carboxylate (AC) caused a dramatic increase in Con A binding affinity for some compounds. When a suitable AC scavenger, e.g. a trimeric α,α'-trehalose-based receptor CT3, was added, "switching off" of the AC-activated samples was observed. These are the first examples of allosteric activation/deactivation of binding and of the multivalent effect.	[139]
The ability of a series of compounds with 1–4 or 6 mannosyl residues (Figure 17) to interact with Con A was measured by ELLA. IC_{50} values for inhibition of Con A yeast mannan reflected the expected amplification of lectin-binding strength for the higher-valent representatives. These mannosyl-coated β-CD dendrimers have also an ability to solubilize the anticancer drug docetaxel (Taxotére).	[126,140].
The binding interactions of glycopeptide–oligonucleotide conjugate based on cyclodecapeptide RAFT scaffold containing four lactose residues were tested with specific lectins obtained from *Arachis hypogaea* (peanut). The interactions were strong and selective. The conjugate does not bind to the nonspecific lectins (Con A from *Canavalia ensiformis*).	[141]
Thiourea-linked upper rim tetrapropoxycalix[4]arene glycoconjugates with exposed two or four glucose, galactose and lactose units have been studied. Turbidimetric analysis indicates that the tetraglucosyl and tetragalactosyl derivatives specifically bind to Con A and PNA, respectively.	[142]
Dendritic sialyloligosaccharides with α-D-Neu5Ac(2 → 3)β-D-Gal linkages were tested by competitive ELISA for inhibition of the biotinylated probe from binding to sialoadhesin, a mammalian macrophage sialic acid binding protein. The most potent compound was the divalent sialoside-cellobiosyl-based structure with IC_{50} = 0.2 mM. In contrast, the tetravalent sialoside dendrimer (IC_{50} = 2.4 mM) was less potent than the sialoside monomer (IC_{50} = 1.4 mM).	[143]
Interactions with galectins	[144]
IC_{50} values and inhibitory capacities (relative potency) of Gal, Lac and LacNAc-β-CD glycoclusters in relation to the univalent inhibitor lactose in different solid-phase assays, interactions with galectin 1, 3 and 7, discrimination between two prototypes and between prototype vs chimera-type galectins are given.	[122,145]
Lactose-containing MAGs (G1–G3) with 3,5-di(2-aminoethoxy)benzoic acid in the branches were studied as inhibitors of binding of mammalian galectins to glycoproteins, lactose maxiclusters and cell surface glycoconjugates. Inhibition of the binding of lectins (*Viscum album* lectin agglutinin, galectin-3, galectin-1, galectin-7) to immobilized glycoproteins (lactosylated BSA, laminin, galectin-3, serum amyloid P component, asialofetuin) by MAGs was studied.	[117,146]
Inhibitory effect of sialic acid on β-CD dendrimers containing sLe^x residues as a model for the interaction between E-selectin and leukocytes was studied using surface plasmon resonance method.	[147]
Bacteria	[79,123,148,149]
E. coli	[49,54,150–156]
Glycodendrimers based on glycerol and glycerol glycol polyether scaffolds with α-mannoside on the surface were tested for their ability to inhibit mannose-specific adhesion of *E. coli* (recombinant strain HB 101) expressing only type 1 fimbriae on its surface. ELISA with microtiter plates coated with yeast mannan was employed. Methyl α-D-mannoside (MeMan) inhibited the adhesion of *E. coli* HB 101 at milimolar concentrations. The di- and tetravalent glycodendrimers had IC_{50} values approximately 10× lower than that of MeMan. Their relative IC_{50} (based on MeMan = 1) were 6–13.	[151]

DOI: 10.1002/psc

Table 1 (*Continued*)

Cholera	
Lactose-containing dendrimers with 3,5-di(2-aminoethoxy)benzoic acid in the branches (2, 4, and 8 end groups) were tested by fluorescence assay for their ability to bind to the cholera toxin B subunit. The binding affinities (dissociation constants K_d) ranged from 18 000 μM for lactose to 33 μM for octavalent G3 dendrimer.	[117, 157]
Fluorescence spectroscopy and ELISA tests using GM1-coated plates were used to study the interaction of a divalent CT glycocalix[4]arene ligand 5 (Figure 21) with CT. At low inhibitor concentation (<200 μM) the efficiency of the divalent compound 5 is superior to that shown by GM1os. Roughly, 4000-fold (2000-fold per sugar mimic) affinity enhancement for the divalent ligand 5 relative to the monovalent one was estimated by fluorescence spectroscopy. This is exceptionally high in comparison with that normally measured for a divalent ligand interacting with a polyvalent receptor.	[158]
Dendrimer-based imaging and contrast agents	[27,90,159–170], see also Part II
DOTA monoamide-linked glycopeptide dendrimers (Glc, Gal, Lac) of different valencies (mono, di, and tetra) and their Sm^{3+}, Eu^{3+}, and Gd^{3+} complexes were studied as potential lectin-mediated medical imaging agents. The *in vitro* relaxivity of the Gd^{3+} glycoconjugates and their binding to the model lectin *Ricinus communis* agglutinin was measured by ^1H nuclear magnetic relaxation dispersion. The known recognition of sugars by lectins makes these DOTA glycoconjugates good candidates for medical imaging agents. For details see Part II.	[171]
In vivo biodistribution of guest–host nanodevices using the example of gold/PAMAM nanocomposites has been studied in a B16 mouse tumor model system. The biodistribution characteristics of gold/dendrimer nanocomposites *in vivo* are different from those of PAMAM dendrimers. Chemical and biological uses of these nanodevices are given with the perspective of their use in cancer imaging and therapy, in particular radiation therapy. The same authors studied biodistribution of PAMAM dendrimers also in DU145 human prostate cancer mouse tumor model.	[172–174]
Artificial viruses	[112,175–179]
Aggregation of artificial viruses based on calix[4]resorcarene glycocluster nanoparticles was studied. Each aggregation differed depending on the type of the saccharide used for derivatization of the calix[4]resorcarene scaffold. The results showed that β-D-Glc viruses are mostly monomeric, α-D-Glc viruses are highly aggregated and β-D-Gal viruses exhibit an intermediate oligomeric behavior. The resulting viruses are compactly packed, well charge-shielded and transfect cell cultures (HeLa and HepG2) via a nonspecific but highly size-regulated endocytic pathway, where only monomeric viruses possess substantial transfection activities.	
Self-aggregating (assembling) dendrimers	[180,181]
MAGs with 4–64 endgroups (Galα(1 → 3)Galβ(1 → 4)GlcNAc or lactose, both in β-D-glycosidic form) self-assemble to form noncovalent nanoparticles. The size of the particles decreases with increasing molecular weight of the individual glycodendrimer, with highest diameter for G2 (valency 4). Interestingly, these particles, and not the individual molecules, efficiently inhibit polyvalent interactions such as IgM binding to the Galα(1 → 3)Galβ(1 → 4)GlcNAc epitope (αGal), both *in vitro* and *in vivo*. Two *in vitro* assays, the inhibition of both the anti-αGal IgM binding to the xenoantigen and the anti-αGal antibody-mediated lysis of pig erythrocytes, showed highest potency for G2 and G3 (IC_{50} 0.01 μM for both assays), which form large nanoparticles. Therefore, the activity clearly correlates with the size of the aggregates but not with the size of the individual molecules. For *in vivo* profiling in cynomolgus monkeys, the most active G3 dendrimer was selected. Within 5 min after injection, the anti-αGal IgM, detected by ELISA, were reduced to 20% of the initial value. This effect lasted for more than 4 h. The anti-αGal IgM-mediated hemolytic activity was completely eliminated.	
Interactions with rat NKR-P1A and NKR-P1B receptors	
Comb-like glycodendrimers bearing mono-, di-, or tri-T_N clusters showed surprising reactivities with rat NKR-P1A and NKR-P1B receptors. Whereas monomers and dimers of T_N antigen	[182]

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Table 1 (*Continued*)

reacted equally with both isoforms of NKR-P1 receptor, the trimer of T_N antigen reacted exclusively with the rat NKR-P1B isoform. For details see Part II.	
Binding and inhibition experiments of PAMAM glycodendrimers and MAGs (containing Man and GlcNAc, respectively) with recombinant extracellular ligand-binding domains of the rat NKR-P1A (activating) and NKR-P1B (inhibitory) receptors showed no differences in the binding of monosaccharide ligands, but dramatic differences in the binding of oligosaccharides and glycodendrimers were revealed. NKR-P1A seems to be one of the most important receptors for the glycodendrimers *in vitro* and *in vivo*. Fluorescein-labeled $GlcNAc_4$-PAMAM dendrimers were used to study the *in vitro* and *in vivo* fate of GlcNAc coated PAMAM dendrimers in white blood cells, tumors and other tissues.	183,184
Octavalent β-D-GlcNAc-PAMAM dendrimers with high *in vitro* affinity for the NKR-P1A receptor were studied as biological modulators in the B16F10 melanoma model *in vivo* in C57BL/6 mice. Reduction of tumor growth and prolonged survival were observed in a dose-dependent manner. The $(\beta$-D-GlcNAc$)_8$-PAMAM dendrimers can stimulate an antitumor immune response including both innate and acquired immunity.	185

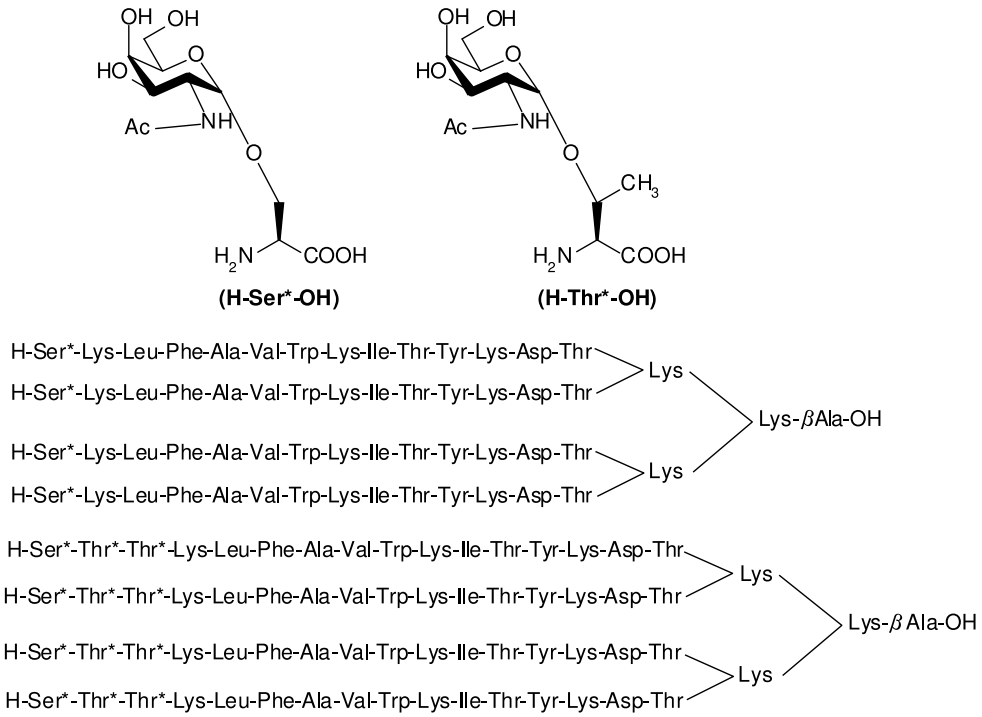

Figure 6 Tetravalent MAGs with T_N antigens [63].

[22,29,31,200]. Sometimes the term *dendronized polymer* is applied [54].

Glycotope

The term 'epitope' is used often in peptide and protein chemistry and immunology. In the fields of glycobiology, glycopeptides, glycodendrimers and MAGs the term 'glycotope' is used. Glycotope is a spatial carbohydrate epitope in which saccharides play a decisive role in immunological recognition processes (self–nonself, interaction with T and B cells, antibodies, etc.). Its activity depends not only on the carbohydrate part, but also on the peptide or protein backbone and also on the amino acid, to which the glycotope is bound (Ser, Thr) [56,101–103,114,201–207]. Other known authors [54,63,64,89,93,208] do not use the term at all. The terms glycotope, carbohydrate epitope, sugar epitope, glycopeptide epitope, glycocluster, polyvalent glycotope, glycodomain, etc. are more or less overlapping. We will employ the term glycotope.

'Smart' Glycodendrimers

Glyco- and glycopeptide dendrimers with labile functionalities that can rapidly interconvert in solutions

DOI: 10.1002/psc

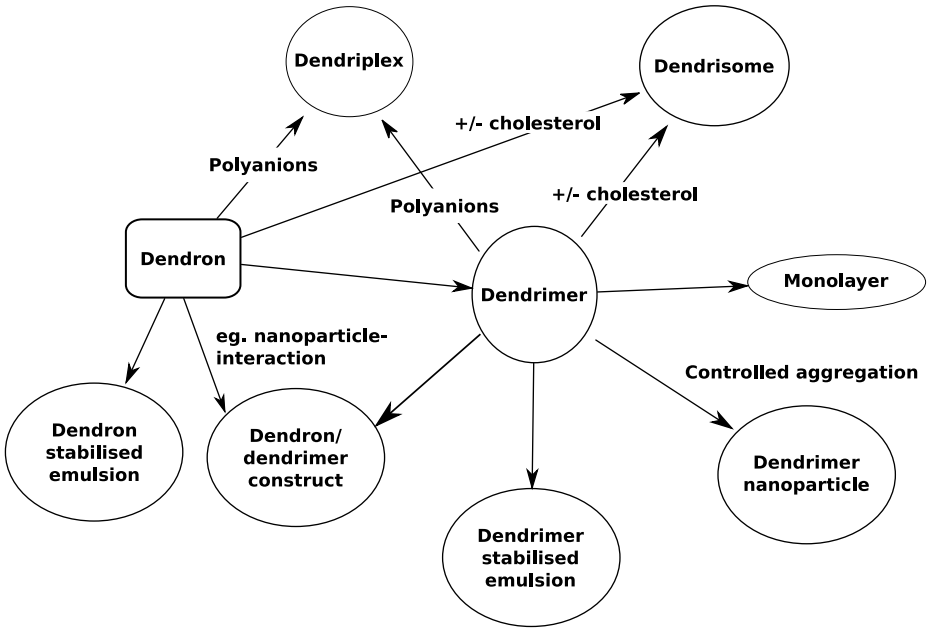

Figure 7 A schematic of the variety of relationships between the primary dendron and dendrimer structures and their aggregated states. Adapted from [196].

(hydrazones, disulfides, imines, metal coordination, etc.) are left to equilibrate to select one another to best fit to the binding site of interest [51]. This 'dynamic combinatorial chemistry' strategy was used in the preparation of dynamic combinatorial libraries (see later).

Glycocluster

Glycocluster is a sterical arrangement of glycotopes (two and more), e.g., in the form of dendron or dendrimer, leading to the amplification of the given biological or physicochemial activity. The amplification factor is a few orders of magnitude higher than the sum of the individual contributions. For more details see 'cluster effect' [17,49,51,54,56,89,115,131,133,141,143,145,150, 175–178,209–219].

Multivalency

'Multivalency is a prerequisite to attain biologically useful affinities between carbohydrate ligands and their protein receptors' [49,50,54,57,68,79,106,111,116, 134,138,157,204,220,221]. Presentation of the sugar epitopes as multiple copies on an appropriate scaffold (molecular, dendritic, polymeric) creates a multivalent display that can effectively mimic the nature of affinity enhancement, which results in higher affinities than expected from the addition of the individual interactions. For this concept, the term 'cluster effect' is used. Multivalency and cooperativity in dendrimer and supramolecular chemistry have been reviewed [222]. Synthetic multivalent architectures, using noncovalent bonding interactions as the supramolecular 'glue', provide (i) well-defined systems for studying the concept of multivalency in nature and (ii) building blocks for nanomaterials.

Cluster Effect

The interactions between saccharides and peptides or proteins are weak. Isolated carbohydrate–protein interactions are typically very weak with K_D values of the order of 10^{-3}–10^{-6} M. Nature compensate for the weekness of these isolated interactions by tending to cluster together multiple copies of carbohydrate ligands and their receptors. In this way, stronger cooperative binding takes place, known as 'multivalent effect' or 'cluster effect' [19,51,53,54,126,134,223]. With simple cluster glycosides a logarithmic increase of affinity for multimeric hepatic lectins was observed in a hemagglutination assay upon a linear increase of scaffolded carbohydrate ligands which were varied from one to three. This effect was termed *the cluster effect* [19,54,57,224].

The most striking improvement was achieved using the multivalency concept (cluster effect) [51,55,116]. Roy *et al.* [55] prepared a sialosyl MAG with valency of 2, 4, 8 and 16. The sialic acid was bound to the lysine branches by S-CH_2-CO-Gly-Gly spacer. The binding properties of the sialylated MAGs were studied using the plant lectin wheat germ agglutinin (WGA) in a direct ELLA in microtiter plates using horseradish peroxidase (HRPO) labeled WGA. The octa- and hexadecavalent MAG showed best binding properties. In an inhibition test using sialylated glycopolymer as coating antigen and HRPO-WGA, all MAGs showed excellent inhibitory capacities

DOI: 10.1002/psc

(10^6 times better than a monosialoside). The hexadecavalent MAG was the most powerful inhibitor. The effect of multivalency concept has been clearly proven [17,49,50,54,57,117,118,130,132,144,146,158,204, 221,223,225–227].

The cluster glycoside effect was studied also by calorimetric analysis of multivalent glycodendrimer ligands [228,229]. Multivalent glycosides (dendrimers) bind to their polyvalent protein receptors with affinities greater than those that can be rationalized solely on the basis of valency. This was studied on mannosylated dendritic ligands and their performance in competitive and non-competitive binding assays, e.g. hemagglutination inhibition, ELLA and isothermal titration microcalorimetry (ITC). The thermodynamic parameters of association are consistent with nonspecific aggregation rather than enhanced lectine–ligand affinity. Molecular interpretation of cluster glycoside effect has been reviewed [115,117,210,220,229], including many examples of different ligands, epitopes, valency, assays, magnitude of enhancement, maximum enhancement (enhancement corrected for the valency of the ligand) and reference ligand. The authors consider three mechanisms for the cluster effects: intramolecular, intermolecular and steric stabilization.

In general, we can say with some exaggeration that in the case of cluster effect 1×8 is a few orders of magnitude higher than 8×1. The optimal results depend on the structure of the dendrimer, its surface groups, methodology used, model (*in vivo, in vitro*) and many others. In some cases, for the given activity, valency 4 is better than 16, in other cases it can be the opposite (steric reasons) [54,56]. In general, tetravalent dendrimers often give the best results. The optimal valency must be determined for any dendrimer and its application separately. In any case, some optimization is necessary for successful biological output. Other terms like multivalent effect [126,202] or multivalent glycotope [203] have also been coined.

Minicluster (Microcluster) and Macrocluster (Maxicluster)

Sometimes the terms minicluster (microcluster) and macrocluster (maxicluster) effect have beeen used in the literature [138,146,171] to refer to the observed increases on lectin-binding affinities for small glycoclusters and large glycopolymers, respectively.

Multiple Antigen Glycopeptides (MAGs)

The term multiple antigen glycopeptide was first coined in 1997 by Cantacuzene *et al.* [100]. They prepared tetravalent MAG containing four T_N antigens bound to peptide T epitopes. Since then, this term is used routinely [189,230] for branched compounds such as MAPs [36,37], to the surface of which glycopeptides or saccharides are bound.

SYNTHESIS OF GLYCOPEPTIDE DENDRIMERS – GENERAL CONSIDERATIONS

Key coupling reactions for the synthesis of glyco- and glycopeptide dendrimers include amide, thiourea and thioether formation [49,51,150,183,185,211,227,231, 232], glycosylation, photoaddition to allyl ethers and reductive amination [52,57,89,233–235].

The chemical and chemoenzymatic synthesis of glycopeptides and glycoproteins can also be carrieed out using unprotected carbohydrates as intermediates [93,98,236]. The three main strategies for glycopeptide synthesis using free glycans are: (i) chemical ligation of glycans and peptides, (ii) free glycosyl amino acids and (iii) elongation of the glycans of simple glycopeptides. The use of unprotected carbohydrates circumvents the final deprotection step and leads to highly convergent synthetic design.

Glycopeptide dendrimers can be synthetized stepwise in solution or stepwise on the solid phase (divergent strategy) or by fragment condensation or ligation of pre-purified fragments (convergent strategy) [19,41,44,45,80,89,200,231,237]. Different classes of chemoselective ligations (hydrazone, maleimide, thioether, oxime, Se–S) have been used for the synthesis of glycocluster–peptide conjugates [37,51,132,141, 180,181,190,191,213,226,238–245]. At the beginning, mainly SPPS has been applied for glycopeptide synthesis. With the growing complexity of the prepared compounds and problems connected with their purification and characterization, more and more ligation strategies have been used. For this purpose the fragments can be prepared in solution or SP, purified and then condensed together. Many classes of ligations have been described, both natural (native) where true peptide bond is created or thiol, hydrazone, oxime, etc. ligations. This topic has been reviewed [19,37,49,51,66,74,80,84,94,190,244–248] and will be not discussed here.

CLASSES OF GLYCOPEPTIDE DENDRIMERS

Depending on the composition and structure of the core, branches and surface groups and their substitution, we can distinguish, e.g., multiple antigen glycopeptides (MAGs) [42,53,54,58,76,89,100,103,230, 232,249] cyclodextrin-based dendrimers [115,119–122,125,126,138,145,147,210,249–255], cyclotriveratrylene-scaffold-based dendrimers [256], calixarene-based dendrimers [115,126,142,158,249,257–260], calix[4]resorcarene-based dendrimers [175–177,219, 249,261], carbopeptide dendrimers [54,159,227,240, 241,253,262–265], 'fully carbohydrate mannodendrimers' [266] and RAFT structures [132,141,188,267]. Other examples are linear polymers with variable valency, e.g. sequential oligopeptide carriers SOCn-I

DOI: 10.1002/psc

and SOCn-II-based dendrimers [268–271], chitosan-based dendrimers [272,273], brush dendrimers or comb dendrimers [39,54,69,116,182,274,275]. Other examples are porphyrin-containing glycodendrimers [40,276,277], fullerene glycoconjugates [278], Cu^{2+} bipyridyl-glycoclusters [131], carbosilane-based glycodendrimers [279–283], silsesquioxane glycoclusters [212], glycerol and glycerol glycol glycodendrimers [151], PEGylated MAPs [284,285] and polyphenylene glycodendrimers [286]. For other dendrimer types, see Refs [33,34,38,43–45,47,255,277,287–290].

DIFFERENT STRUCTURES OF MAGS – SYNTHESES AND ACTIVITIES

These glycopeptide dendrimers have carbohydrates on the surface. The peptide branches in MAGs must contain a trifunctional amino acid. In most cases it is Lys [36,53,56,76,100,189,232,249,277]. Other AAs have been used too: Orn [291],2,3-diaminopropionic acid [292], Pro in combination with nonnatural trifunctional AAs *cis*-4-amino-L-proline and imidazolidine-2-carboxylic acid [293–295], α,α-disubstituted β-alanine [296], and *N*,*N*′-bis(acrylamido)acetic acid [51,56,130]. Alternative types are PAMAM (poly (amidoamine)) dendrimers [22,27,32,44,45,49,51,53,56,57,69,76,89,90, 108,109,113,116,128,160–163,183,200,249,277,289, 290,297–311], aminobis (polypropylamine) MAGs (Figure 8) [56,210], poly (propyleneimine) dendrimers [49,53,56,57,210,277,289,290,297,304,312] and many other types [51,56,63,289].

In the following paragraphs, detailed examples of different MAGs are given.

Lysine MAGs

Reactions in solution have been used to prepare (β-Ala_8-Lys_4-Lys_2-Lys-NH-CH_2-CH_2-)$_2$. β-Ala has been introduced in order to equalize the reactivity of the 16 amino groups. Reductive alkylation with borane–pyridine complex in the presence of excess of maltose, lactose, cellobiose, maltotriose or a mixture of lactose and maltose afforded MAGs with 32 oligosaccharide residues (sugar$_{16}$-β-Ala_8-Lys_4-Lys_2-Lys-NH-CH_2-CH_2-)$_2$, where sugar = C1-reduced maltose, lactose, cellobiose, maltotriose or a mixture of lactose and maltose, respectively. No significant difference in reactivity was observed among maltose, lactose, cellobiose or maltotriose when a strong reducing agent borane–pyridine complex has been used [314]. NMR spectra support the given structure. MALDI-TOF MS afforded experimental values of 13472.5 Da (for maltose-MAG), 13507.28 Da (lactose-MAG), 13418.36 Da (cellobiose-MAG) and 13384.36 for lactose/maltose-MAG. The calculated value for all these compounds is 13453.87 Da. Although the mass value was either 18.63 Da (for maltose-MAG) to 53.41 Da (lactose-MAG) higher or 35.51 Da (cellobiose-MAG) lower than the calculated value, the authors assume that such mass differences might be attributed to the errors usually occuring from salt, solvent, matrix, desorption/ionization efficiency and the detector response [314]. The final goal of these dendrimers is the synthesis of a glycopeptide-type HIV vaccine by binding an antigen from HIV components to the dendrimer surface. No biological data were given.

Analogous hemisphere-type oligosaccharide Lys_4-Lys_2-Lys MAGs (without β Ala) have been prepared. Since the eight amino groups included in the dendrimer had different reactivity, the number of oligosaccharide residues connected to the dendrimer ranged from 13 to 16 [315].

Di-, tetra- and octavalent glycoside–antigen conjugates have been synthesized by two orthogonal, hydrazone/thioether ligations, performed by using thio derivatives of D-mannose, D-galactose, or D(−)-quinic acid, glyoxylyl (or hydrazino)-*N*-chloroacetylated lysyl trees, and *N*-terminal hydrazino (or glyoxylyl) peptide antigens [226]. As peptide antigens, fragments of $TT^{830-846}$ and $HA^{307-319}$ were used. These MAGs were developed for selective targeting of the dendritic cell mannose receptor. As an example of many prepared compounds, the octavalent MAG (Manα-O-CH_2-CH_2-S-CH_2-CO)$_8$-Lys_4-Lys_2-Lys-Lys-β-Ala-NH-$(CH_2)_3$-NHCO-CH=N-Gly-$HA^{307-319}$-amide is given. The *N*-chloroacetylated lysyl trees, modified with either a hydrazino or a glyoxylyl group, proved to be valuable intermediates. No biological activities were given.

Uryu *et al.* [233] synthesized octavalent MAG with eight cellobiose units containing free hemiacetal groups in a study to prepare a synthetic vaccine against HIV. First, the lysine MAP was prepared and then to the eight free aminogroups, benzyl 2,2′,3,3′,6,6′-hexa-*O*-benzyl-4′-(1-carboxyethyl)-β-cellobioside was condensed by BOP reagent. The obtained compound was hydrogenolyzed over $Pd(OH)_2$/C to give the free cellobioside-MAG [4′-(1-carboxyethyl)-β-cellobioside]$_8$-Lys_4-Lys_2-Lys-β-Ala-OMe (**1**) (Figure 9). This MAG was characterized by ^{1}H, ^{13}C NMR and MALDI-TOF. The data indicate that a fully substituted, monodispersed cellobiose dendrimer was successfully prepared. To this MAG, tripeptide Gly-Pro-Leu as a model compound was connected by reductive amination (BH_3/pyridine). From ^{1}H NMR spectra it follows that only two tripeptide moieties on average were bound to the dendrimer. In the next step, a cyclic peptide designed according to the V3 loop sequence of HIV gp120, cyclo-KNNTRK(ClZ)SIRIQRGPGRAFVTIGK(ClZ)IGNMG was bound to compound **1**, with the aim to prepare a glycopeptide conjugate by reductive amination. In this case, NMR measurements in solution could not be carried out because of the very poor solubility of the

DOI: 10.1002/psc

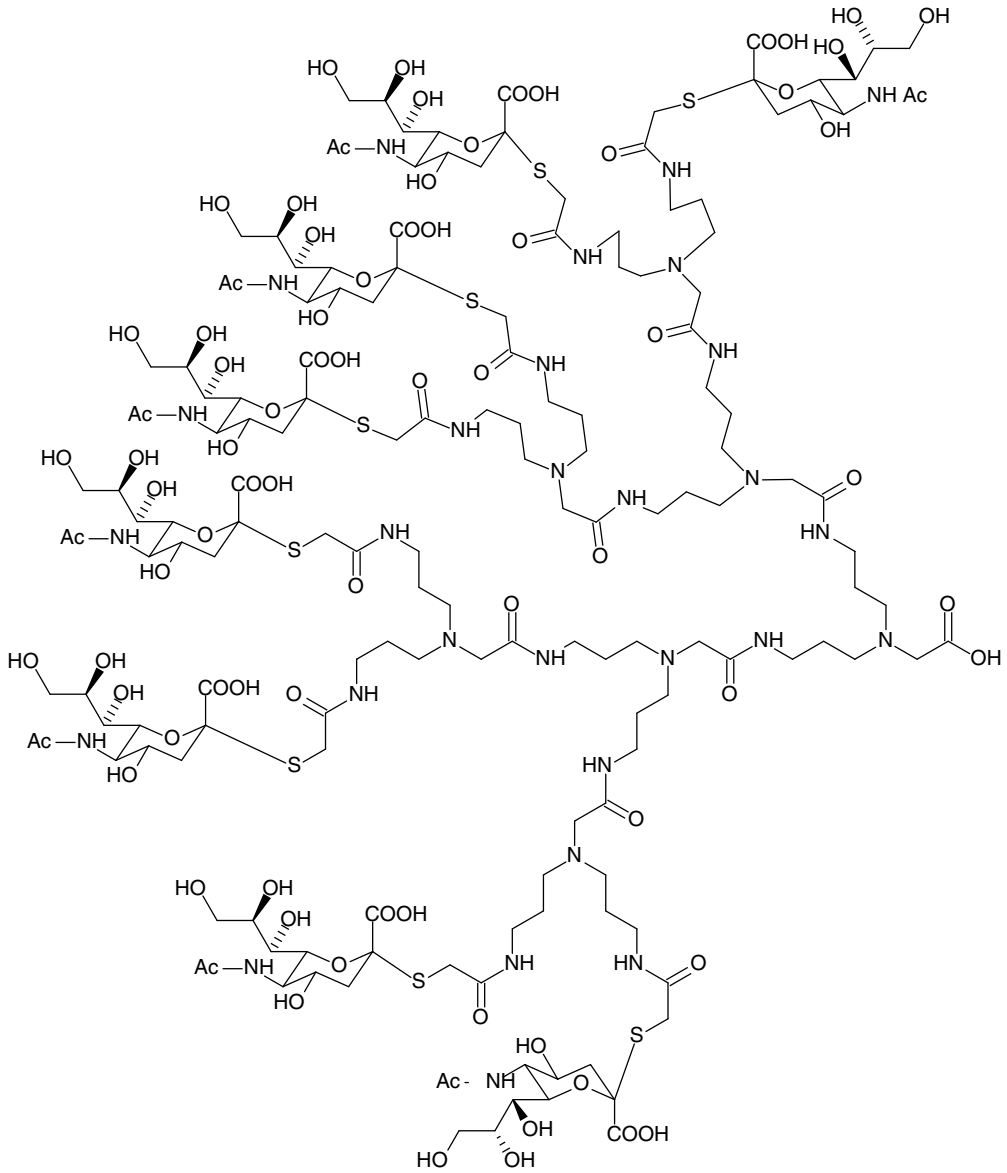

Figure 8 Sialic acid-containing dendron with an aminobis (polypropylamine) backbone [210,313].

product in any solvent. Some information about the structure was obtained from IR spectra (KBr pellet), which showed the presence of the V3 loop in the product. The loop contains four Arg residues with free guanidino groups. It has been found that these groups react in the same way as an amino group in reductive amination with BH_3/pyridine. The authors assumed that cross-linking occurred, which caused the solubility problems. The search for a new coupling reaction conditions is necessary. For the above-mentioned reasons, no biological data are given.

The same authors [232] described improved synthesis of lysine MAGs containing 24 or 8 terminal cellobiose units, prepared from tris(2-ethylamino)amine and β-alanine methyl ester as core compounds, respectively. The MAGs were characterized by NMR, IR and MALDI-TOF MS.

The topic of lysine MAGs has been reviewed [53,76,249,277]. For other examples see also the section on TACAs in Part III.

Ornithine MAGs

MAGs containing ornithine instead of lysine with lactose or maltose as antigen have been prepared in the framework of a synthetic AIDS vaccine study [291]. The reactions were carried out in solution. The hemispherical ornithine dendrimer Orn_4-Orn_2-Orn-β-Ala-OH was prepared and used to investigate the reactivity during reductive alkylation with lactose or maltose by means of the borane–pyridine complex. When the molar ratio of maltose to the amino group was in the range of 2 : 1 to 20 : 1, the number of maltose residues to the dendrimer having eight amino groups was 9–15.

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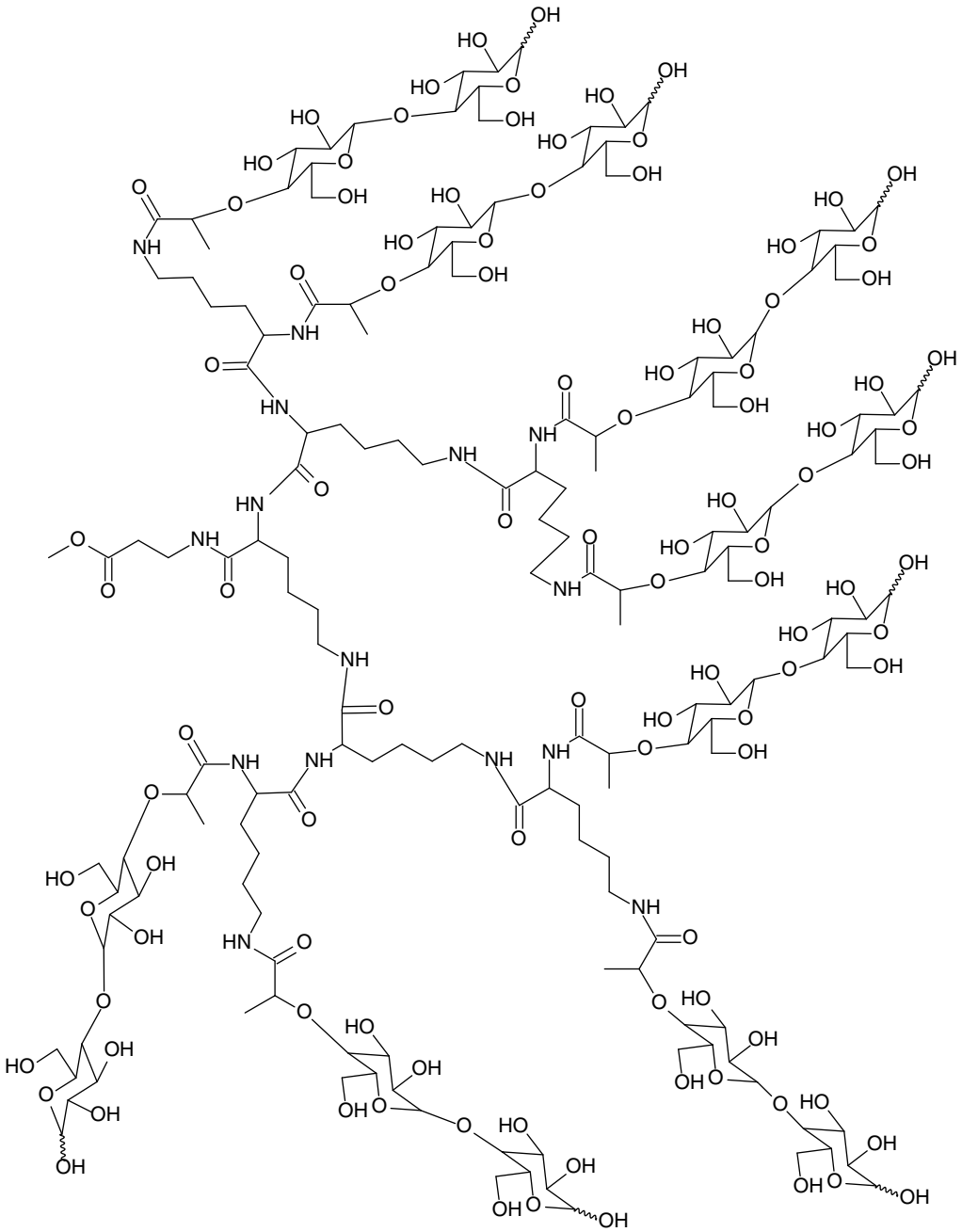

Figure 9 Octavalent MAG **1** containing cellobiose [233].

The same authors [291] prepared ornithine glycopeptide dendrimers with or without a β-Ala periphery [(β-Ala$_8$-Orn$_4$-Orn$_2$-Orn-NH-CH$_2$-CH$_2$-)$_2$ or (Orn$_4$-Orn$_2$-Orn-NH-CH$_2$-CH$_2$-)$_2$] starting from a 1,4-diaminobutane core, followed by preparing lactose or maltose MAGs (Figure 10). The hexadecavalent MAP afforded MAGs with 32 molecules of reduced lactose or maltose, respectively. That means, that under suitable conditions double alkylation takes place. β-Ala was used in order to equalize the reactivity of the 16 amino groups. The spherical MAP without β-Ala after alkylation with maltose afforded a mixture, which according to MALDI-TOF MS contained 28, 29, 30, 31 and 32 maltose residues, respectively. The homogeneity and properties of these MAGs were investigated by NMR and MALDI-TOF MS. The results of the MALDI-TOF MS measurements were dependent on the molecular mass. For low-molecular-weight dendrimers (3–4 kDa), the found mass was in good accordance with the corresponding calculated mass. As the mass increased (10.8–13.3 kDa), such as in the case of (Mal$_{16}$-β-Ala$_8$-Orn$_4$-Orn$_2$-Orn-NH-CH$_2$-CH$_2$-)$_2$ (Mal = C1-reduced maltose), a considerable difference was seen between the found (13357.37 Da) and calculated (13264.95 Da) masses. For analogous problem with lysine-MAGs, see above [314].

DOI: 10.1002/psc

MAGs with 2,3-Diaminopropionic Acid

MAGs containing 4 or 8 identical glycoside moieties at their surface (β-D-Glc, α-D-Gal, α-D-GalNAc, or lactose), natural amino acids within the branches (Ser, Thr, His, Asp, Glu, Leu, Val, Phe), 2,3-diaminopropionic acid as the branching unit and a Cys residue at the core have been synthesized [292] as drug-delivery devices for colchicine (Colch). A series of MAG–colchicine conjugates have been prepared using different strategies for glucoside attachment: oxime bond formation to *N*-terminal glyoxamide generated by periodate cleavage of *N*-terminal Ser or Thr residues, reductive alkylation with lactose/$NaBH_3CN$ and amide bond formation with an acetylated carboxypropyl glycoside directly on the SP followed by cleavage and deacetylation. The last strategy is the most practical, because only the conjugation of colchicine is necessary after the SP synthesis. Colchicine was attached to the MAG at the Cys SH group through a disulfide or thioether linkage. As example, {[(D-GalNAc-α-O-$(CH_2)_3$-CO-Ser-Thr)$_2$-Dap-Gly-Glu]$_2$ Dap-Leu-His}$_2$-Dap-Cys(S-$(CH_2)_2$-Colch)-Asp-NH_2 is given. Biological activities of MAGs were evaluated in HeLa tumor cells and nontransformed mouse embryonic fibroblasts (MEFs). The concentrations of MAG necessary for inhibition of cell proliferation by interference with the tubulin system were found to be higher ($IC_{50} > 1$ μM) than that for colchicine. On the contrary, MAGs inhibited the proliferation of HeLa cells 20–100 times more effectively than the proliferation of MEFs. MAPs (nonglycosylated) and colchicine itself had a selectivity of 10-fold or less for HeLa cells. Therefore, these MAGs provide a suitable selective vehicle for the delivery of cytotoxic compounds to cancer cells.

Proline MAPs

Giralt *et al.* [293–295] established efficient synthetic protocols for the preparation of polyproline dendrimers based on of a convergent SPPS strategy. Fmoc-Pro$_n$-L-Amp(Fmoc-Pro$_n$)-OH ($n = 5$, 14) building blocks have been assembled on two different orthogonally protected cores: spermidine and cyclic Lys–Lys (2,5-DKP) using PyAOP as coupling reagent. The obtained branched polyproline peptides and dendrimers with $n = 14$ have been analyzed by CD spectroscopy. Fluorescence spectroscopy experiments proved an interaction of dendrimer H-(Pro$_{14}$)$_2$Amp-NH-$(CH_2)_4$-NH-$(CH_2)_3$-NH ← Amp ←$_2$ (Pro$_{14}$)-H and ciprofloxacin (a synthetic 6-fluoroquinolone antibiotic currently in clinical use for the treatment of infections caused by Gram-positive and Gram-negative bacteria) in 99.5% propanol with a 1 : 2 stoichiometry and an association constant of 2.0×10^6 M^{-1}. The complex decomposes in water. These results show the possibility of using polyproline dendrimers as new drug delivery systems.

Analogous polyproline dendrimers generation 2, 3, and 4 with 4, 8, and 16 free amino groups, respectively, (e.g. G4 = [H-Gly-(Pro)$_5$]$_{16}$-[Imd-Gly-(Pro)$_5$]$_8$-[Imd-Gly-(Pro)$_5$]$_4$-[Imd-Gly-(Pro)$_5$]$_2$-Imd-Gly-OH) with imidazolidine-2-carboxylic acid (Imd) as the branching point were synthesized by convergent SPPS [295]. As a key building block (Fmoc-Gly-Pro$_5$)$_2$-Imd-OH was used. The sterically unhindered imidazolidine ring as a branching unit and flexible Gly residue at the *N*-terminal position of the building block were crucial for the success of the synthesis. In this way it was possible to prepare dendrimers up to fourth generation. The conformation of polyproline dendrimers was studied by CD spectroscopy. There are two distinct conformations of polyproline oligomers. In organic solvents they exist in a conformation known as polyproline I (PPI), i.e. a right-handed helix with *cis*-oriented peptide bonds ($\omega = 0°$). In aqueous solvents, they have a conformation known as polyproline II (PPII), a left-handed helix with all peptide bonds *trans*-oriented ($\omega = 180°$). Transition PPI → PPII leads to considerable increase of the long dimension of the helix from 1.9 to 3.1 A. The prepared proline MAPs in organic solvents (*n*-propanol, TFE, EtOH) existed only in PPII conformation. The solubility of proline MAPs in both aqueous and organic solvents and their conformational plasticity make them good candidates for biomedical applications. The described synthesis provides a new class of protein-like globular biopolymers, in which the compactness is created by dendrimeric composition. The well-defined branched secondary structure is maintained by proline-rich sequences. This 'dendritic effect' leads to the stabilization of a specific helical structure owing to the dendrimer. 'To the best of our knowledge, this is the first case where a peptide sequence is part of the dendritic structure and that shows a clear dendritic effect on its secondary structure' [295]. Till now, this group of proline-based dendrimers has not been used as a sugar carrier.

MAGs Based on α,α-Disubstituted β-Alanine

The synthesis of an orthogonally protected building block, *N*-Alloc-*N*′-Boc-*N*″-Fmoc-α,α-bis(aminomethyl)-β-alanine and its use in the synthesis of triantennary peptide glycoclusters on SP have been reported [296]. The protecting groups were removed in the order Fmoc, Boc and Alloc, and subsequently coupled with peracetylated *O*-(glycopyranosyl)-*N*-Fmoc-L-Ser-OPfp esters (glycopyranosyl = galactose, glucose, mannose and ribose) to each amino group exposed. Glycoclusters with different combinations of sugars were prepared. When three different sugars are present, the dendrimer contains an additional chiral centre in the branching unit and therefore exists as a mixture of two diastereomers. Unfortunately, they could not be separated by HPLC. No biological activity given.

DOI: 10.1002/psc

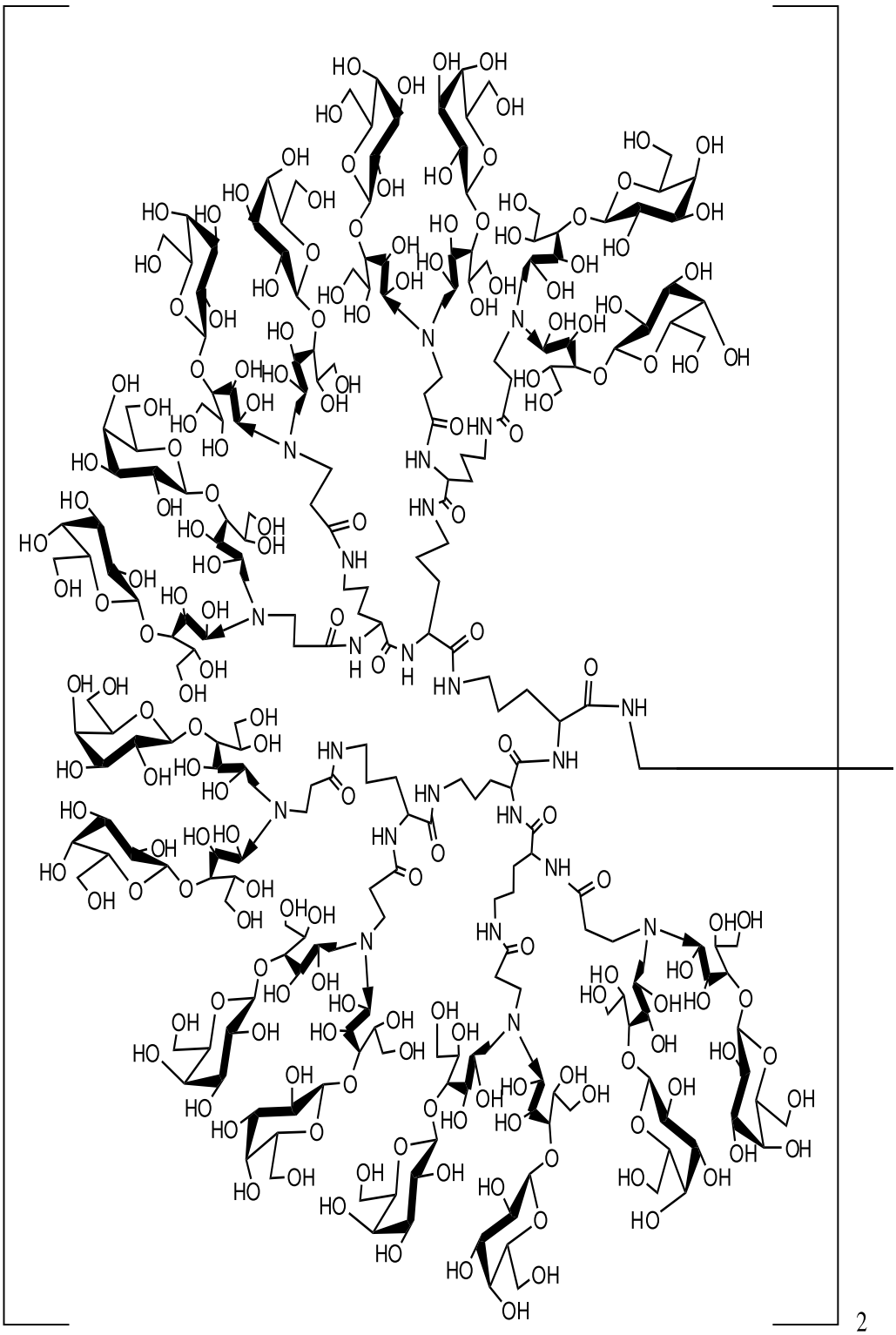

Figure 10 Ornithine-based MAG containing 32 lactose units [291].

N,N′-bis(Acrylamido)Acetic Acid-based MAGs

Roy *et al.* [51,56,130] synthesized *N*,*N*′-bis(acrylamido) acetic acid-based dendrimers with TF-antigen (for a tetravalent MAG see Figure 11(a)) with valencies varying between 2 and 6. The cluster effect of these TF-antigen dendrimers was studied by turbidimetric analysis toward the highly TF-antigen-specific plant lectin of *A. hypogaea* and by competitive double sandwich inhibition ELISA using mouse MAb JAA-F11 (IgG3). Monomeric TF-antigen (in the form of α-allyl glycoside) was used as standard. Turbidimetric

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analysis with 2–6 valent dendrimers showed maximum optical density (OD) within 1 h. The hexamer showed the strongest/fastest ability to precipitate the lectin (OD 0.43). The tetramers (OD 0.13 and 0.12) and the dimer (OD 0.1) showed lower precipitation ability. When tested as inhibitors of binding between IgG3 and a polymeric form of the TF-antigen (TF-antigen-*co*-polyacrylamide used as coating antigen), di-, tetra-, hexa-, and tetravalent glycodendrimers showed IC_{50} values (nM) of 174, 19, 48, and 18, respectively. The two tetramers were 120–128-fold stronger inhibitors in comparison with the monovalent TF-antigen (IC_{50} = 2.3 mM). These data imply that not all of the TF-antigen residues participate properly in the inhibition reaction. The above data show that for the same compounds in different tests optimal results are obtained with different valencies. No universal optimal valency exists. Heterobifunctional TF_2-glycodendrimer-bearing biotin probe was prepared for cancer cell labeling [130]. These glycodendrimers can be used for therapeutic purposes, as immunodiagnostics and in drug design.

Heterobifunctional TF-antigen conjugates with biotine or fluorescein were used for receptor screening [56].

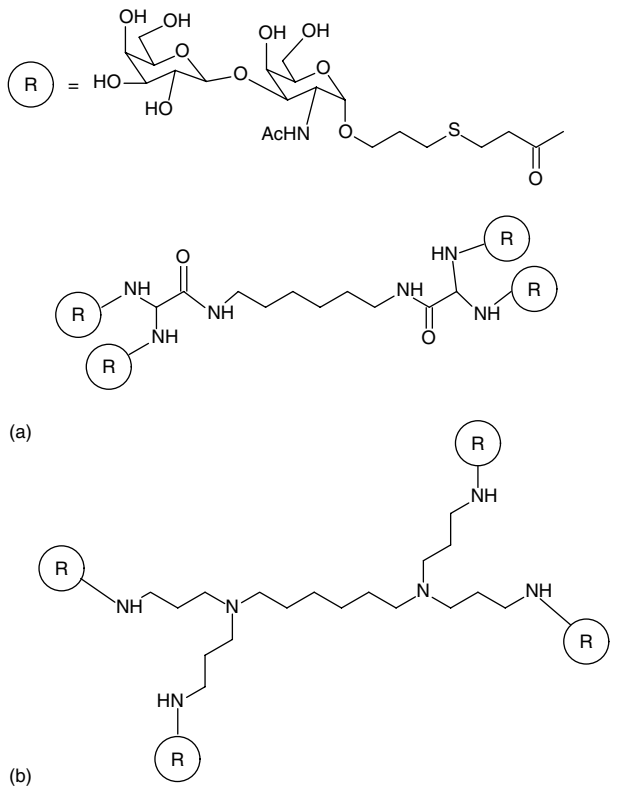

Figure 11 (a) TF-antigen built on tetravalent *N*,*N*′-bis(acrylamido)acetic acid core [56] (b) tetrameric poly (propyleneimine) dendrimer with TF-antigen [56].

PAMAM Dendrimers

The structure of poly (amidoamine) dendrimers containing 3-amino-propionic acid is given in Figure 12 [22,38,51,56,113].

First- to sixth-generation PAMAM dendrimers have been functionalized with α-D-mannose residues [135]. The binding tests of these mannose-derivatized dendrimers with Con A and with pea lectin [18,136,137] were performed and compared. Con A and pea lectin (mitogenic lectin isolated from *Pisum sativum*) are legume lectins containing one highly conserved sugar binding site on each monomeric unit. At biological pH, Con A is a homotetramer, while pea lectin is a homodimer. Both proteins bind methyl α-D-mannoside with specificity; Con A has fourfold higher affinity than pea lectin. The hemagglutination assays showed the relative activity of PAMAM dendrimers, generations 1–6 for pea lectin and for Con A, respectively, compared to methyl α-D-mannoside. Mannose-functionalized G(1) and G(2)-PAMAMs were bound to Con A with affinities comparable to that of methyl α-D-mannoside, but significant increases in affinity were observed for G3, and mainly for G4, G5 and G6. The high affinity of G4, G5 and G6 to Con A is probably caused by the cluster effect. Hemagglutination experiments for all generations of PAMAM dendrimers with pea lectin showed no increase in binding affinity (on a per mannose basis) with increasing dendrimer generation. This diference can be explained by the lack of the concave surface in pea lectins. Therefore pea lectin is unable to accomodate a multivalent binding motif of dendrimers. Isothermal titration calorimetry (ITC) was also studied. By controling the number of sugars on the dendrimer surface, it is possible to control both the lectin-binding activity (hemagglutination assay) and the number of lectins clustered around the dendrimer (precipitation assay).

The same group [134] investigated relative affinities of mannose- and glucose-substituted PAMAM dendrimers with Con A. The difference in relative activity between glucose-functionalized and mannose-functionaliced dendrimers is 14.7 for G4, 15.6 for G5 and 11.4 for G6. One can predict that exchanging mannose for glucose would cause a 16-fold reduction in binding to Con A. Hemagglutination assays with Con A and mannose/glucose-functionalized PAMAM dendrimers show that multivalent affinities can be predicted on the basis of monovalent association constants.

TF-antigen PAMAM dendrimers (Figure 12) [56,128, 129] with valency 4, 8, 16 and 32 were prepared. The relative efficiency of the various glycodendrimers to inhibit the binding of mouse monoclonal IgG Ab to coating copolyacrylamide TF-α-O-$(CH_2)_2$-S-$(CH_2)_2$-NH_2 was determined using goat anti-mouse MAb IgG. The degree of inhibition for TF-antigen PAMAM was proportional to the valency. Maximum inhibition was obtained for dendrimer with 32 valencies. Turbidimetric analysis and

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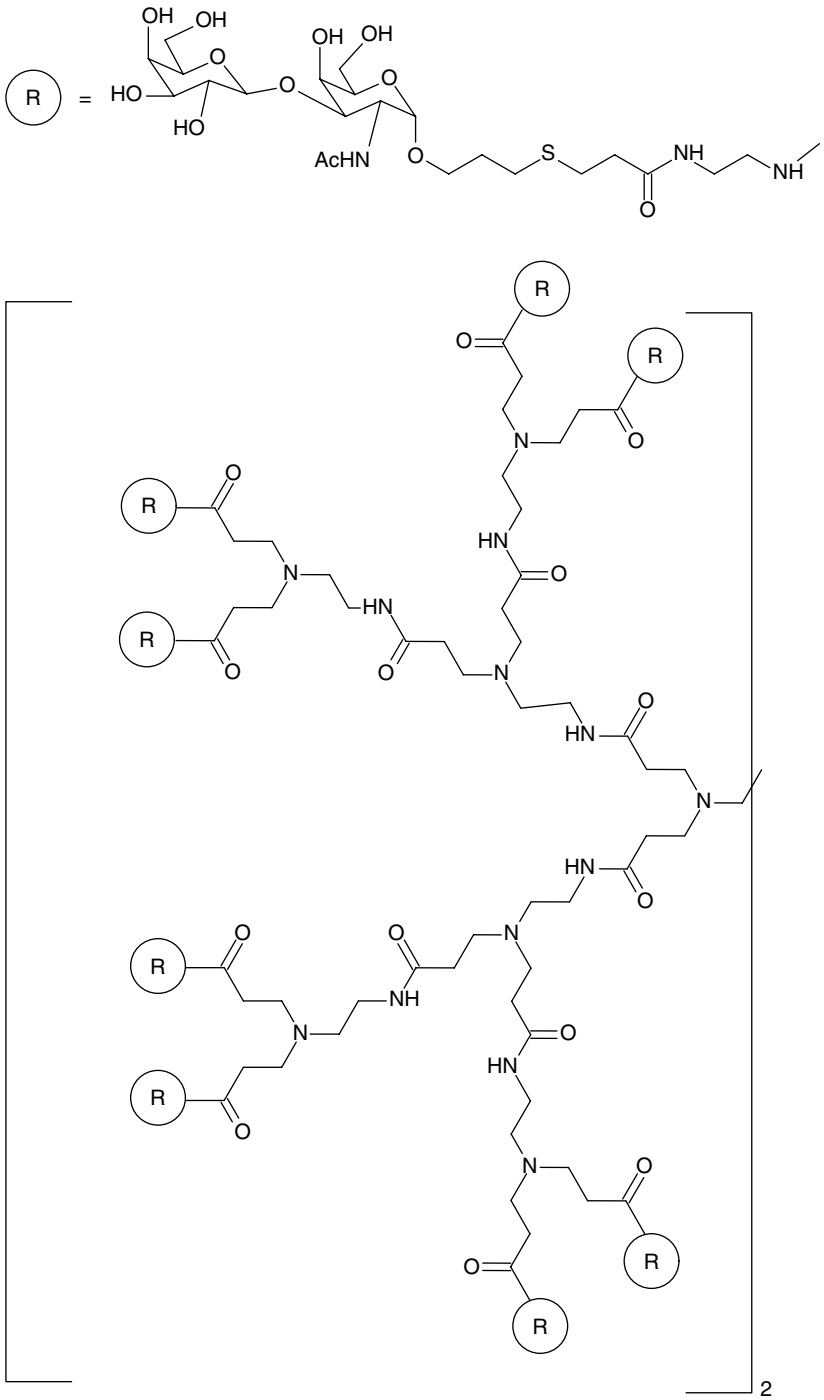

Figure 12 Example of 16-mer PAMAM TF-antigen dendrimer [56,129].

ELLA are consistent with these results. These results represent 460, 960, 1700 and 3800-fold enhancement of inhibitory potentials in relation to TF-α-*O*-allyl. In the series of TF-antigen on *N*,*N*′-bis(acrylamido)acetic acid dendrimer, the dimer showed the lowest inhibitory value (120), tetramers were approximately equipotent (120, 128), while hexamer showed a dramatic decrease (48). The activity of TF-antigen PAMAM dendrimers [56,128,129] was compared also with the analogous poly (propylene imine) dendrimers (Figure 11(b)). These dendrimers differ only by a three carbons shorter distance between the anomeric oxygen and the branching amine residues and by one less amide bond in the poly (propylene imine) dendrimers, which should confer less polar behavior. The PAMAM dendrimers were more active.

Mannose-6-phosphate-coated PAMAM dendrimers (G0.5-G3.5) with 4, 8, 16 and 32 α-D-mannopyranosyl-6-phosphate residues, respectively, at the peripheries of the dendrimer have been synthesized [316]. The

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structure was assessed by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy, and the sugar content by a colorimetric resorcinol–sulfuric acid assay. Preliminary biological studies showed that the mannose-6-phosphate dendrimers could bind purified goat liver mannose-6-phosphate receptor MPR 300 protein (cation-independent receptor).

In vivo biodistribution of dendrimers and dendrimer nanocomposites has been studied in connection with cancer imaging and therapy [172–174,317]. The composition, surface chemistry and 3D structure of guest–host nanodevices using the example of gold/PAMAM nanocomposites determine their *in vivo* biodistribution. The biodistribution characteristics of gold/dendrimer nanocomposites *in vivo* are different from PAMAM dendrimers in a B16 mouse tumor model system. Chemical and biological uses of these nanodevices have been reviewed with the perspective of their use in cancer imaging and therapy, in particular radiation therapy. Besides biodistribution, the toxicity assessment and biodegradability are also of utmost importance. The same authors [173] studied biodistribution of PAMAM dendrimers also in DU145 human prostate cancer mouse tumor model.

PAMAM dendrimer-based multifunctional conjugates were used for cancer therapy [298]. A conjugate containing fluoresceine (imaging agent), folic acid (targets overexpressed folate receptors on cancer cells) and Taxol (chemotherapeutic drug) has been prepared. This multifunctional dendrimer conjugate has been tested *in vitro* for targeted delivery to specific cancer cells.

Binding and inhibition experiments with recombinant extracellular ligand-binding domains of the rat natural killer receptor protein 1 (NKR-P1A; activating) and NKR-P1B (inhibitory) were carried out [184]. No differences in the binding of monosaccharide ligands to these receptors were observed, but dramatic differences in the binding of oligosaccharides and glycodendrimers were revealed. The PAMAM glycodendrimers used were 4 and 8 valent with GlcNAc and mannose, respectively. The tested MAGs were 4 or 8 valent with GlcNAc only. These data allow understanding why NKR-P1A seems to be one of the most important receptors for the glycodendrimers *in vitro* and *in vivo*.

To study the *in vitro* and *in vivo* fate of GlcNAc coated PAMAM dendrimers, the same authors [183] prepared fluorescein-labeled $GlcNAc_4$-PAMAM dendrimers. The fluorescent tag enabled the localization of the dendrimers in white blood cells, tumors and other tissues by using fluorescence, confocal microscopy and other imaging techniques.

Octavalent β-D-GlcNAc-PAMAM dendrimers with high *in vitro* affinity for the NKR-P1A receptor were studied as biological modulators in the B16F10 melanoma model *in vivo* [185]. Tumor development and immunity were evaluated in C57BL/6 mice. Reduction of tumor growth and prolonged survival were observed in a dose-dependent manner. Increase of $CD69^{+}$ cells in the spleen and their appearance inside the tumors, early progressive release of IL-1β, a later production of INFγ and IL-2 concomitant to an increment of $CD4^{+}$ cells were observed. The (β-D-$GlcNAc)_8$-PAMAM dendrimers can stimulate an antitumor immune response including both innate and acquired immunity. Immunohistochemistry and tissue distribution of the fluorescent (fluorescein, rhodamine) labeled (β-D-$GlcNAc)_4$-PAMAM dendrimers were also studied.

Transepithelial and endothelial transport of PAMAM dendrimers has been studied [300] depending on molecular size, molecular geometry and surface chemistry. By optimization of these parameters it is possible to develop dendrimers as oral delivery systems for targeted drug transport. The mechanism of transport must be further elucidated.

A new signal amplification strategy using PAMAM dendrimers has been described [318]. Fluorescent signals generated from antibody–fluorescein (Ab–Fl), antibody–dendrimer–fluorescein (Ab–den–Fl) conjugates and their immunocomplexes with antigens were studied by fluorescent microscopy. By this technique, the immunoreactions between anti-(botulinum toxoid)–dendrimer–Fl bioconjugates and the botulinum toxoid can be imaged and studied in real time. Multiple dye bioconjugates and Abs give more intense signals and higher assay sensitivity. Nonspecific binding was not observed. PAMAM dendrimers can be used for signal amplification for immunoassays and other receptor-based assays.

PAMAM dendrimers are members of a class of polyamine polymers with significant gene delivery ability [113]. CD and fluorescence spectroscopy were used to characterize the assembly of complexes consisting of plasmid DNA bound to a series of PAMAM dendrimers [302]. The behavior is generation dependent and can be divided into two classes: G2 and the larger generations (G4, G7 and G9). The higher generations have activation energies for binding decreasing in the order G4 > G7 > G9. The activation energies for condensation of complexes composed of the same dendrimers decrease in the order G9 > G7 > G4. The authors postulate that a balance between energetically more favorable condensation and a less favorable binding may prove beneficial to the competing needs of cellular uptake and improve gene delivery ability of PAMAM dendrimers.

The same authors [305] studied PAMAM dendrimers G2, G4, G7 and G9 by CD, Fourier-transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC) and ITC in order to find structural properties that correlate with transfection. Despite DNA structural and stability changes detected by CD, FTIR, DSC and

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ITC, which are similar to those seen with other cationic delivery vehicles, clear correlations with transfection activity were not apparent. This can be caused by the heterogeneity of the complexes.

DNA-assembled PAMAM dendrimer clusters were used for cancer-cell-specific targeting [309,319–321]. Instead of conjugation of all the molecules to a single dendrimer, synthetic oligonucleotides were used as a tool to self-assemble two dendrimer particles: one bearing folic acid and the other carrying fluorescein as the imaging agent. This DNA–PAMAM dendrimer allows the delivery of drugs, imaging agents and genetic materials to cancer cells through their folate receptors. The use of DNA as a functional linker of dendrimers is unique because it may allow the combination of different drugs with different targeting and imaging agents. These DNA–PAMAM dendrimers also enable development of combinatorial therapeutics.

Dendrimer syntheses by self-assembly of single-site, ssDNA-functionalized PAMAM dendrons [322] afforded novel core–shell nanostructures representing a new class of precise monodisperse, linear-dendritic architectural copolymers.

Transfection activity of PAMAM dendrimers with hydrophobic AA residues in the periphery was studied [323]. Under weakly acidic conditions, the dendrimer with 64 phenylalanine residues formed a complex with DNA, thereby achieving highly efficient transfection. The leucine PAMAM dendrimer was unable to improve the transfection activity, probably owing to relatively lower hydrophobicity. In comparison with some widely used transfection agents, the phenylalanine-modified PAMAM dendrimer exhibited higher transfection activity and a lower cytotoxicity.

Mannosylated PAMAM dendrimer/α-cyclodextrin conjugates (Man-α-CD conjugates) improve *in vitro* and *in vivo* gene delivery efficiency and act as a novel non-viral vector in a variety of cells [324,325].

Two new types of dendrimer-bound adenine nucleotides were synthesized by coupling ATP and ADP with PAMAM dendrimers [326]. In the first case, native adenine nucleotides were coupled directly with a carboxy-terminated PAMAM dendrimer. The second type used modified nucleotides with an introduced spacer arm containing a carboxylic end group (N^6-R-ATP and N^6-R-ADP), respectively, which were coupled with an amine-terminated PAMAM. The final coupling was in both cases done with WSCI activation. The coenzymatic activities (both relative and specific) of the carboxy-terminated PAMAM-bound adenine nucleotides against glucokinase and acetate kinase (from *Bacillus stearothermophilus*) were much higher than those of the amine-terminated PAMAM-boud adenine nucleotides despite having no spacer arm. The easy preparation and high coenzymatic activity of the carboxy-terminated PAMAM-bound ATP are superior to the amine-terminated PAMAM-bound N^6-R-ATP. The use of carboxy-terminated PAMAM-bound ATP or ADP for development of an efficient enzymatic process using a hollow-fiber membrane reactor in which both the enzymes and the polymer-bound cofactor are confined was discussed.

PAMAM dendrimer delivery systems (with the stress to oral drug delivery) have been described [113,148,306,311,327,328]. The role of lymphoid and nonlymphoid tissue, bioadhesion and molecular encapsulation of drugs via dendrimers were discussed in the context of nanotechnological applications for the oral route. PAMAM dendrimers are probably the most widely investigated from a pharmaceutical perspective.

For synthesis, biological and physico-chemical properties, and applications of PAMAM dendrimers in medicine, including drug targeting, gene delivery, MRI contrast agents, etc. See also [22,27,32,44,45,49,51,53, 56,57,69,76,89,90,108,109,128,149,159–166,174, 200,249,277,288–290,297–311,319,329–333].

Aminobis (Polypropylamine) MAGs

These dendrimers contain glycine and 1,3-diaminopropane building blocks (Figure 8) [210,313]. In comparison with classical PAMAM dendrimers (Figure 12) the amino acid is one carbon shorter and the amine is one carbon longer. Dendritic α-sialosides with valencies 2, 4, 8 and 16 based on 3, 3′-iminobis(propylamine) core [313] were tested as inhibitors of human erythrocyte hemagglutination by influenza viruses.

The term 3, 3′-iminobis(propylamine) dendrimers [34,56,59,89,171,313] is used more often than aminobis (polypropylamine) dendrimers [210].

Poly (Propyleneimine) Dendrimers

Poly (propyleneimine) dendrimers (PPI) (Figure 11(b)) [49,53,56,57,159,210,277,289,290,304], in comparison with their PAMAM counterparts, are used somewhat more rarely to prepare glycodendrimers. While the physicochemical properties of PPI dendrimers are very similar to those of PAMAM, PPI dendrimers are of lower availability.

Low-generation (G2 and G3) PPI dendrimers were studied as potential cellular delivery systems of antisense oligonucleotides targeting the epidermal growth factor receptor in A431 epidermoid carcinoma cells [334]. The data show that PPI dendrimers represent a delivery system capable of delivering antisense oligonucleotides. The gene expression knockdown is comparable with a proprietary lipid-base system, with the advantage of reduced toxicity. Therefore, PPI dendrimers are prospective delivery systems for antisense oligonucleotides both *in vitro* and *in vivo*.

Galactose-coated G5.0 PPI dendrimer (64 residues) [335] was studied as delivery system of primaquine phosphate (an antimalarial drug), a liver schizonticide, directly to liver cells. The formulations were made by

DOI: 10.1002/psc

equilibrium dialysis of dendrimers with the solution of primaquine phosphate. Release rate, hemolytic toxicity, biodistribution and blood level were studied. The galactose coating of PPI dendrimers increases the drug entrapment efficiency by 5–15 times depending upon generations. The release was prolonged up to 5–6 days (with galactose coating) in comparison with 1–2 days for uncoated PPI dendrimers. Blood level studies proved that the galactose coating of PPI dendrimers makes them more effective for targeted delivery of primaquine phosphate to liver. The selective delivery to liver parenchyma cells reduces the toxicity to other organs and at the same time could increase its efficacy for a radical cure from malaria.

Different generations of Gd^{3+} diethylenetriaminepentaacetic acid-terminated PPI dendrimers were studied as contrast agents for magnetic resonance imaging [167]. Higher generations of dendrimers display lower concentration detection limits and are suitable for molecular imaging.

For toxicity and biocompatibility of PPI dendrimers see Ref. [312].

MAGs with Unnatural Aromatic Amino Acids (3,5-di(2-Aminoethoxy)Benzoic Acid; 3,5-Diaminobenzoic Acid; 1,3-Dicarboxy-5-Hydroxybenzene)

Lactose-containing dendrimers with 3,5-di(2-aminoethoxy)benzoic acid in the branches were prepared in generation 1, 2 and 3 (2, 4 and 8 end groups) [117,157]. These lactose dendrimers were tested by fluorescence titration spectroscopy for their ability to bind to the cholera toxin B (CT-B) subunit. The relative binding potency for lactose, 2, 4 and 8-valent dendrimer was 1, 77, 182 and 545, respectively. The relative potency (per 1 lactose) was 1, 38, 46 and 68, respectively. These results point to potential application as a cholera therapeutic.

The same authors [146] studied the same lactose-containing MAGs (G1–G3) as inhibitors of binding of mammalian galectins to glycoproteins, lactose maxiclusters and cell surface glycoconjugates. Galectins are a group of β-galactoside-binding lectins that are widespread throughout all animal kingdoms and even plants and fungi. They are involved in cell–matrix and cell–cell adhesion, cell migration and growth regulation in connection with inflammation and tumor spread, angiogenesis, metastasis, etc. [144]. Therefore, the design of low-molecular-weight inhibitors is highly desirable. Inhibition of the binding of lectins (*Viscum album* L. agglutinin, galectin-3, galectin-1, galectin-7) to immobilized glycoproteins (lactosylated BSA with 28 sugar moieties, laminin, galectin-3, serum amyloid P component, asialofetuin) by MAGs was studied [146]. IC_{50} and relative inhibitory potency are given. Potent inhibition and strong cluster effects were obtained for the homodimeric galectin-1, especially in combination with biantennary *N*-glycans as matrix. The tetravalent MAG reached relative inhibitory potency of 1667 in the inhibition of galectin-1 binding to the serum amyloid P component. In hemagglutination experiments, galectin-3 was sensitive to increased sugar valency with relative potency of 150. The results obtained are important for the design of glycodendrimers wih galectin-selective properties.

Glycodendrimers of the second to sixth generation with 4–64 endgroups were synthesized using 3,5-diaminobenzoic acid as branching points. The branches contained 4-aminomethylbenzoic acid. The end groups were α-D-Gal epitope Gal$\alpha(1 \rightarrow 3)$Gal$\beta(1 \rightarrow 4)$GlcNAc or lactose, both in β-glycosidic form. These small glycodendrimers self-assemble to form noncovalent nanoparticles [180,181]. The ability of these dendrimers to self-assemble to nanoparticles which function as polyvalent ligands *in vitro* and *in vivo* was studied. The divalent MAG with two trisaccharide substituents seems to be too small for efficient core–core interactions. G2 with 4 trisaccharide units was optimal. Higher generations lead to decrease in particle weight. The authors explain this by the increasing number of large end groups per individual dendrimer molecule inducing a more globular shape. This leads to more effective shielding of the core by carbohydrates, which makes intermolecular core–core contact more difficult. The particle size is significantly affected by temperature. There is a dynamic self-assembly process that rapidly reaches thermodynamic equilibrium, because comparable particle weights were determined at identical temperatures for both the heating and the cooling period. Atomic force microscopy was used to investigate the shape and size of the formed nanoparticles in the solid state. The size of the particles decreases with increasing molecular weight of the individual glycodendrimer, with highest diameter for G2 (valency 4). Interestingly, these particles, and not the individual molecules, efficiently inhibit polyvalent interactions such as IgM binding to the Gal$\alpha(1 \rightarrow 3)$Gal$\beta(1 \rightarrow 4)$GlcNAc epitope (α-D-Gal), both *in vitro* and *in vivo*. Anti-α-D-Gal Abs are most abundant natural human Abs. They cause the hyperacute rejection of pig organs transplanted into primates. The IgM class is dominant in the immediate, complement-dependent, hyperacute destruction of the xenograft. Unfortunately, there are no pharmacological agents affecting IgM production, which could block or remove anti-α-D-Gal Abs prior to pig-to-primate xenotransplantation. Two *in vitro* assays, the inhibition of both the anti-α-D-Gal IgM binding to the xenoantigen, and the anti-α-D-Gal antibody-mediated lysis of pig erythrocytes, showed highest potency for G2 and G3 (IC_{50} 0.01 μM for both assays), which form large nanoparticles. Therefore, the activity clearly correlates with the size of the aggregates but not with the size of the individual molecules. For *in vivo* profiling in

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cynomolgus monkeys, the most active G3 dendrimer was elected. Within 5 min after injection, the anti-αGal IgM, detected by ELISA, were reduced to 20% of the initial value. This effect lasted for more than 4 h. The anti-α-D-Gal IgM-mediated hemolytic activity was completely eliminated. These results are of great importance for selective modulation of Ab response in order to suppress unwanted response of the host to the transplanted tissue.

In the framework of a systematic study of biological and physical properties, a series of glycodendrimers (with CONH, without peptide) with different length of branches between the branching points were assembled in a parallel combinatorial manner [239]. Reiterative use of first and second generation building blocks accelerated the process. 1,3-Dicarboxy-5-hydroxybenzene was used both as a core and branching point, and α,ω-diamines with 3–5 carbon atoms as branches. The eight end amino groups of G3 were chloroacetylated and 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-1-thio-β-D-glucopyranose was ligated to the dendrimer. The *O*-Ac groups were split off by NH_3 in DMF. The hydrogelation behavior (gel transition T_{gel}) of these free glycodendrimers was studied. Small structural variations lead to significant differences in their hydrogelation properties. An increase in thermal stability was concomitant with a decrease in the internal tier length. An increase in external tier length also leads to elevation of transition temperatures. The dimensions of the innermost tiers have the most profound influence on the gel transition behavior of these three-tier MAGs.

Dendritic gelators in general have been reviewed [336].

DENDRIMERS WITH CYCLIC CORE STRUCTURES

Cyclodextrin-based Dendrimers

The branches and core can be created also by using different types of cyclodextrins [57,115,119–122,125,126, 138,139,145,147,210,249–251,253–255,337]. The advantage of this approach is the defined spatial conformation given by limited flexibility of the cyclic 'core'. The carbohydrate structure on the surface can be bound to the cyclodextrin core either directly or by peptide or another spacer. CDs are cyclic oligosaccharides typically comprising six, seven or eight (α-, β-, or γ-CD, respectively) α-1,4-linked D-glucopyranosyl residues (Figure 13) [126,252]. They have a long recorded history [255,338] of forming inclusion complexes with hydrophobic guests within their largely hydrophobic cavities. In addition to many 1 : 1 and 1 : 2 host–guest interactions, the CDs also form pseudopolyrotaxanes with linear polymers such as poly (ethylene)glycol (PEG), poly (propylene) glycol and poly (tetrahydropyran). Many CD 'beads' thread spontaneously onto polymer 'strings'. CDs represent another possibility of defined stereoselective structure, which can serve for cluster (multiplication) effect.

Many classes of multi- and monovalent β-CD neoglycoconjugates of β-D-glucopyranose, β-D-galactopyranose, α-D-mannopyranose, β-D-fucopyranose, β-L-fucopyranose, cellobiose, lactose, GM_3 trisaccharide, etc. (bound with thioether, thiourea and amide bonds) were prepared [119,337] by chemical or chemoenzymatic synthesis. Lectin-binding and inclusion ability of monovalent *versus* multivalent polysubstituted CD neoglycoconjugates was tested with many classes of lectins. Multivalent conjugates are generally bound with much higher association constants. In general, the activity depends also on the spacer, being usually higher for neoglycoconjugates having long spacers between the β-CD core and the external saccharide marker. It must be mentioned that the lectin binding results are highly dependent on the evaluation method. This makes reliable comparison of literature data difficult. Use of β-CD neoglycoconjugates with different external saccharide markers for drug targeting and delivery was also discussed.

The dependence of Con A binding on anomeric configuration, linkage type and ligand multiplicity for thiourea-bridged mannopyranosyl-β-cyclodextrin conjugates has been studied [121]. The presence of the thiourea functionality in the CD conjugates results in improved water solubility and decreased hemolytic character as compared to the parent CDs. Con A does not discriminate between α- and β-D-mannopyranosylthiourea ligands. ELLA data show pronounced decrease of activity (binding inhibition of labeled Con A to yeast mannan) for heptasubstituted β-CD in comparison with the monosubstituted one.

Dendritic galactosides based on a β-CD core were used for the construction of site-specific molecular delivery systems [125]. A series of galactosides containing seven 1-thio-β-lactose or β-lactosylamine residues bound to β-CD were synthesized. The first synthesis of three first-order dendrimers based on a β-CD core containing fourteen 1-thio-β-D-galactose, 1-thio-β-lactose and 1-thio-β-melibiose, respectively, was also performed (Figure 14). Isothermal calorimetric titrations in a buffered aqueous solution were used to measure affinity constants and thermodynamic parameters for the complex formation of β-CD dendrimers with lectin from peanut (*Arachis hypogaea*) (PNA) and the guests sodium 8-anilino-1-naphtalene-sulfonate and 2-naphtalenesulphonate. PNA forms cross-linked soluble complexes with β-CD dendrimers containing galactosides and lactosides, but not with derivatives containing galactopyranosylamines or melibiose. Both perbranched and hyperbranched β-CDs form stronger complexes with PNA than the monomeric analogs. Because PNA lectin is able to recognize a cluster lactoside host with a guest molecule inside its cavity, the

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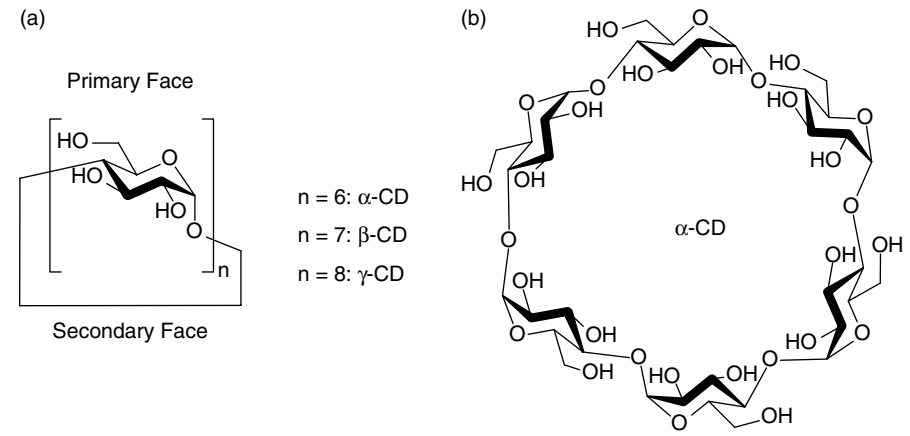

Figure 13 (a) Condensed structural formulas of α-, β-, and γ-cyclodextrin with indicated primary and secondary faces; (b) full structural formula of α-cyclodextrin [126].

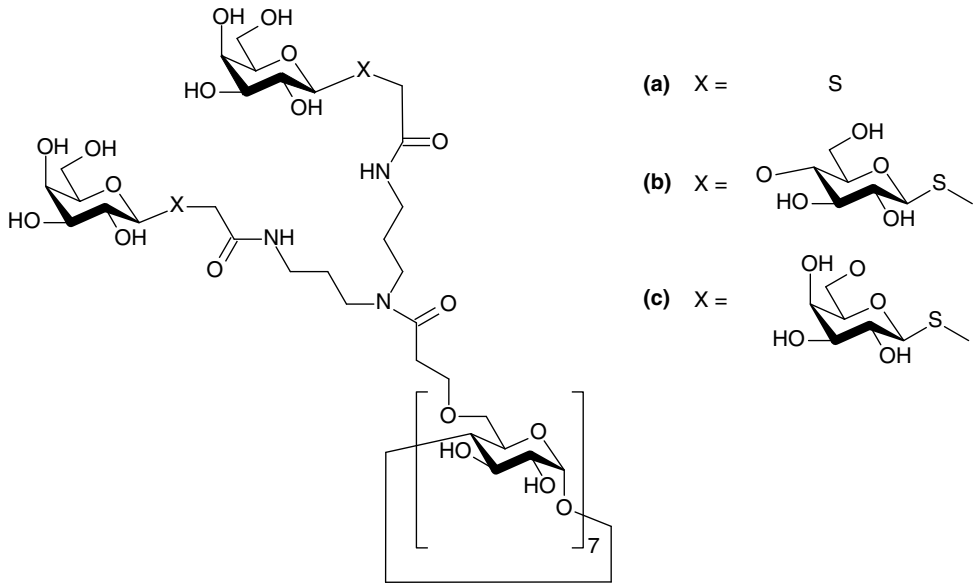

Figure 14 Hyperbranched β-cyclodextrin dendrimers with (a) 1-thio-β-D-galactose (b) 1-thio-β-D-lactose (c) 1-thio-β-D-mellibiose [125].

β-CD lactoside dendrimers could be used as molecular carriers for transportation of guests to specific lactoside receptors such as PNA.

Dendritic derivatives of β-CD bearing multivalent (2, 3, 4, 6) mannosyl ligands have been prepared and assessed for their binding efficiency toward the tetrameric plant lectin Con A and mammalian mannose/fucose-specific receptors from macrophages [138]. The reactivity between isothiocyanate and amine functionalities was exploited in a synthetic strategy for the high-yielding assembly via thioureido links of the various building blocks, including host, spacer, branching, and carbohydrate ligand elements, respectively. Both convergent and divergent approaches have been implemented. A series of β-CD mannose scaffolds differing in the ligand valency and geometry have been used to study the effects of the architecture on the binding efficiency, the influence between the CD core and the glycodendritic moieties on the molecular inclusion and lectin-binding properties, etc. The consequence of inclusion complex formation using the anticancer drug docetaxel (Taxotere) as a target guest on biological recognition has been studied. This new type of tailor-made hexavalent CD dimer host for docetaxel (Figure 15(a)) showed high drug solubilization capability. The results can be used in very efficient design of molecular transporters for docetaxel based on glycodendritic CD dimers. Results of the binding inhibition of horseradish peroxidase-labeled Con A to yeast mannan by mannosylated β-CD-dendrimers were expressed as IC_{50} (μM) values. Compound with one CD core (Figure 15(b)) had the lowest IC_{50} (10 μM) with a relative efficiency of 23 (valency-corrected).

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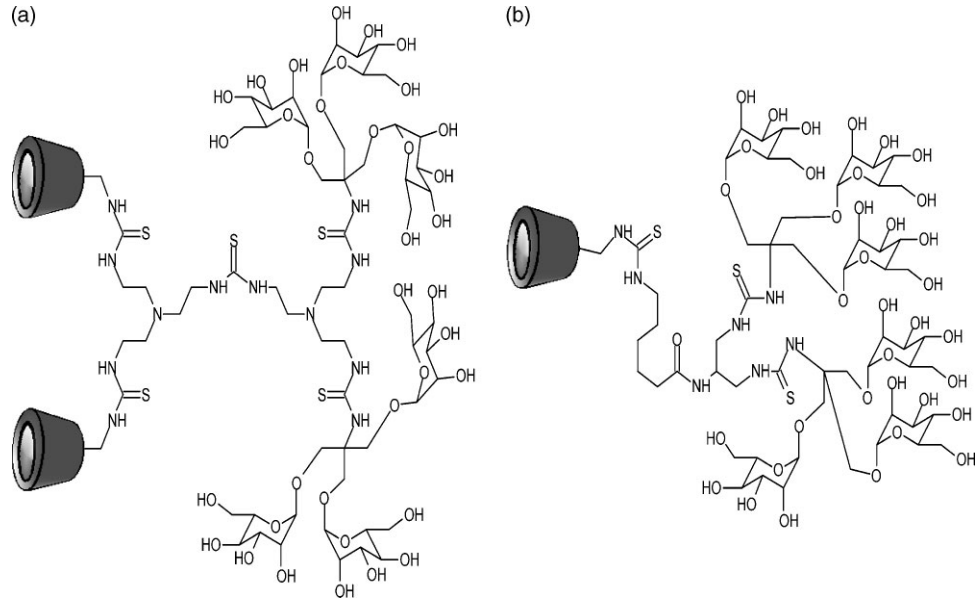

Figure 15 (a) Tailor-made hexavalent β-cyclodextrin dimer host for docetaxel [138] (b) monosubstituted hexavalent β-cyclodextrin conjugate.

In order to study oligosaccharide–protein interactions, synthetic 6-β-CD-aminosuccinyl-trimannosylamine (**2**), 6-β-CD-aminosuccinyl-Tyr-trimannosylamine (**3**), and 6-β-CD-aminosuccinyl-Tyr-Glu-bis(trimannosylamine) (**4**) were prepared [139] starting from hemisuccinate-modified 6-amido-6-deoxy-β-CD. The Con A binding was studied by ELLA. The IC_{50} values for compounds **2, 3** and **4** were 21, >1 and 3.2 μM, respectively. Addition of 1-adamantyl-carboxylate (AC) caused a dramatic increase in Con A-binding affinity for **3** (IC_{50} 22 μM), while that of **2** remained unaffected. For compound **4**, only a slight increase (IC_{50} 1 μM) was observed by increasing the proportion of AC. Addition of a suitable AC scavenger, e.g. a trimeric α,α'-trehalose-based receptor CT3, which perfectly matches the size and symmetry complementarity with AC, leads to 'switching off' of the AC-activated samples. These results indicate that reversible tuning and switching of the binding affinity in a model carbohydrate-lectin system is possible. This are the first examples of allosteric activation/deactivation of binding and of the multivalent effect.

Carbohydrate dendrimers based on β-CD persubstituted with β-D-thioglucosyl or β-D-thiolactosyl residues on either (i) the primary face (C6-OH groups), (ii) the secondary face (C2-OH groups) or (iii) both the primary and secondary faces (C6 and C2-OH groups) of their cyclodextrin tori have been described [250]. Only the most substituted example with 14 lactosyl groups is given (Figure 16). The key step was the photoaddition of thiol groups, positioned at the anomeric centres of the carbohydrate residues, to allyl ether functions on the cyclodextrins. Facile removal of *O*-Ac groups afforded the β-D-thioglucosyl or β-D-thiolactosyl CD dendrimers. These compounds were studied by NMR, MALDI-TOF

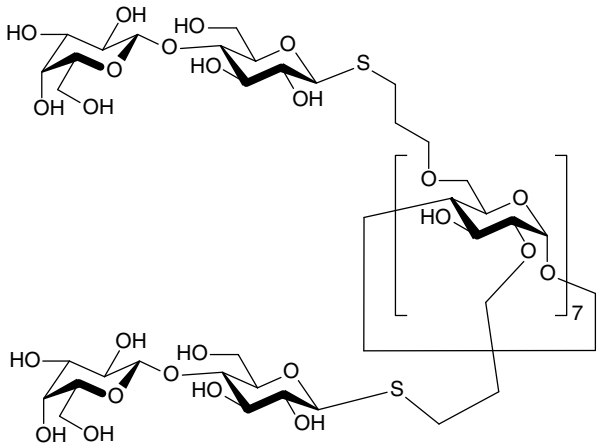

Figure 16 Cyclodextrin cluster substituted on both the primary and secondary faces by lactose [250].

and molecular modeling. The above-mentioned strategy enables the attachment of more complicated oligosaccharides onto CD cores.

Sialic acids are common mammalian sugars that usually end oligosaccharide sequences of glycolipids, *N*- and *O*-linked glycoproteins and some proteoglycans [339]. Cell surface sialosides are involved as anchoring motifs for microbial attachment. Pathogenic agents (viruses, bacteria and bacterial toxins) can adhere and colonize host tissues after binding to cell surface sialosides. High serum carbohydrate concentration including sialic acid can prevent bacterial infections and cancer metastasis. For the design of sialoside-based inhibitors, CDs have been used as scaffolds. These structures combine the inclusion capabilities of the hydrophobic cavity of CDs and the high biological

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receptor-binding ability of multiple saccharide epitopes in the same molecule. The authors [339] synthesized homogeneous, fully characterized hepta-antennated C-6 branched sialosyl cyclomaltoheptose derivatives (persialylated β-cyclodextrins) in good yields.

L-, P- and S-selectins are transmembrane glycoproteins responsible for the adhesion of leukocytes to the vascular endothelium cells in the early cascade of the inflammation process. The tethering and rolling of leukocytes on endothelial cells of blood vessels are the initial stage in the recruitment of leukocytes to the inflamed tissue. The minimal carbohydrate structural motif expressed on leukocytes and on endothelial cells required for the initial recognition by L-selectin are sialylated and fucosylated oligosaccharides related to the sLe^x tetrasaccharide Neu5Ac$\alpha(2 \rightarrow 3)$-D-Gal$\beta(1 \rightarrow 4)$-(L-Fuc$\alpha(1 \rightarrow 3)$-D-GlcNAcβ1-OR. SLe^x related analogs are potential anti-inflammatory agents. However, the binding affinity of low-molecular-weight sLe^x derivatives is weak. Therefore the authors [147] used the cluster effect via β-CD. Heptabranched glycocluster $(sLe^x)_7$-CD that exhibited highly enhanced inhibitory effect (IC_{50} = 1.5 mM as normalized concentration) toward the tight binding of E-selectin with immobilized sLe^{x}n–BSA chip was synthesized. The sLe^{x}n–BSA complex has 10 sLe^x moieties per single BSA. The results clearly demonstrate that the enhanced inhibitory effect by $(sLe^x)_7$–CD was due to clustered sLe^x arrays displayed on β-CD.

Neoglycoconjugates based on cyclodextrins and calixarenes have been reviewed [126]. An efficient method was developed for the construction of dendrons, substituted with mannosyl residues on their peripheries. The reaction of primary amino group in position 6 of the cyclodextrin with isothiocyanates leads smoothly to thioureas (Figure 17). A series of compounds with 1–4 or 6 mannosyl residues were prepared [126,140] and their abilities to interact with Con A were measured by ELLA. IC_{50} values for inhibition of Con A yeast mannan reflected the expected amplification of lectin-binding strength for the higher-valent representatives. These mannosyl-coated β-CD dendrimers have also an ability to solubilize the anticancer drug Taxotére.

Lactose-bound α- and β-CD derivatives have been synthesized and threaded to hydrophobic polymers in aqueous solution. The formed dynamic multivalent lactosides were designed for binding to lectins [252]. The threading process, i.e. the formation of pseudopolyrotaxanes from lactose-bearing CDs threaded onto hydrophobic polymers such as poly (tetrahydropyran) and poly (propylene) glycol, proceeds quickly and can be observed by one- and two-dimensional NMR. These pseudopolyrotaxanes were developed as dynamic multivalent glycoconjugates for binding lectins. The individual CD beads should be able to slide along and to rotate around the polymer axes, allowing the lactose ligands to adopt the most favorable conformation for interaction with their protein receptors. No biological data were given.

Multivalent glycoclusters can provide fundamental insights into the impact of two spatial factors of binding, i.e. topologies of ligand (cluster presentation, branching mode) and carbohydrate recognition domains in lectins [122,145]. Persubstituted β-CDs were obtained by nucleophilic substitution of iodine from heptakis 6-deoxy-6-iodo-β-CD by the unprotected sodium thiolate of 3-(3-thioacetyl propionamido)propyl glycosides (galactose, lactose, *N*-acetyllactosamine). These glycoclusters were tested as competitive inhibitors in solid-phase assays. A plant toxin from mistletoe and an IgG fraction from human serum were markedly sensitive. The inhibitory potency of each galactose moiety in the heptavalent β-CD was increased 400-fold relative to free lactose (217-fold relative to free galactose). These glycoclusters can therefore interfere with xenoantigen-dependent hyperacute rejection. Among the tested galectins selected from these adhesion- and growth-regulatory endogenous lectins, the heptavalent CD-based glycoclusters acted as sensors, which were able to delineate topological differencies between the two dimeric prototype proteins. The reactivity with chimera-type galectin-3 (a mediator of tumor growth and metastasis) was relatively strong, showing selectivity for glycocluster binding among galectins. The CD-based glycoclusters can thus be used for the design of galectin- and ligand-type selective compounds.

Supramolecular chemistry of carbohydrate clusters with cyclodextrin core has been reviewed [254,255], including site-specific drug delivery systems.

De novo synthesis of a new family of glyconanocavities constructed from α,α-trehalose building blocks, namely cyclotrehalans [337], and their complexing properties have been described.

Cyclotriveratrylene-based Dendrimers

In the framework of a program aimed at designing molecules that can block the flow of nutrients through bacterial general porins, amino acid glycoconjugates based on cyclotriveratrylene scaffold were prepared (Figure 18) [256]. Such compounds may thus serve as a novel class of antibiotics. The trivalent 'stopper' ('corks') constructs could have a multivalent interaction with the trimeric general porins. As a trivalent scaffold, cyclotriveratrylene was used. This scaffold has many advantages: (i) it is readilly accessible, (ii) its derivatization is easy, and (iii) its well-defined geometry ensures parallel orientation of all three arms. Oligoethylene glycol moieties were used as spacers between the scaffold and the interaction sites. They are commercially available in different lengths, are water soluble and can be easily derivatized. To the end of each spacer, a glyco amino acid was attached as a 'bait'. All parts of the molecule can be varied: diethyleneglycol to tetraethyleneglycol, different amino

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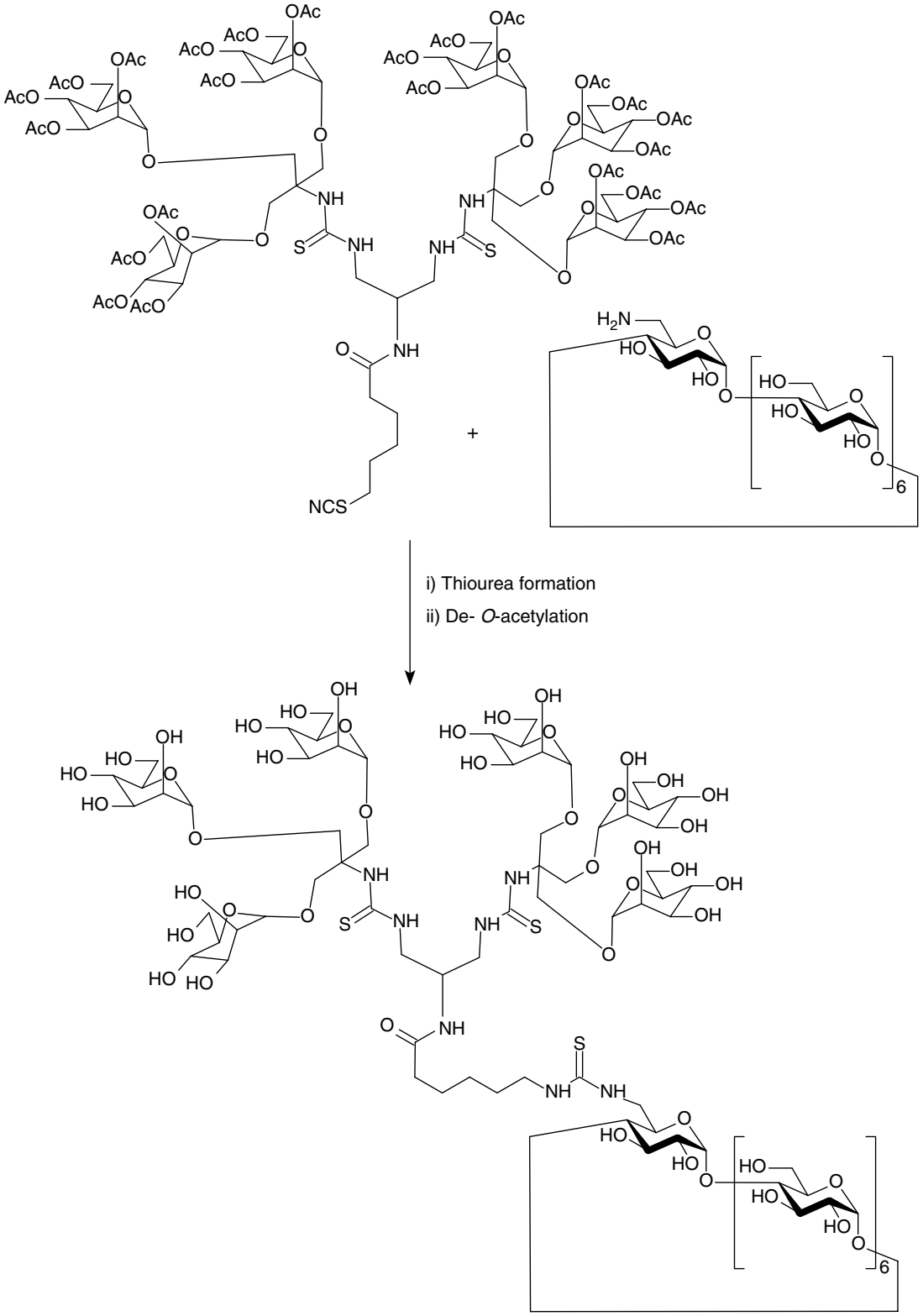

Figure 17 Attachment of a dendritic wedge containing six mannosyl residues to the periphery of a β-CD core [126].

acids (e.g. Ala, Glu, Lys) and carbohydrate residues (e.g. glucose, galactose, lactose). This enables the preparation of a combinatorial library of trivalent amino acid glycoconjugate dendrimers. The HPLC of deprotected compounds afforded two peaks. After separation, both of them gave the same mass spectrum. This is caused by the chirality of the cyclotriveratrylene core. Therefore, enantiopure glyco amino acids coupled to a racemic scaffold (core) afforded diastereomers in ratios 1 : 3 up to 1 : 7. The most problematic step

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in this synthesis was the coupling of an *N*-linked glyco amino acid building block to an alcohol via *p*-nitrophenylcarbonate (Figure 18). The best results were obtained with sodium bicarbonate as a base in THF. After deprotection of the Bzl and Z groups by hydrogenolysis and transesterification of *O*-Ac groups by sodium methoxide, free glycoconjugates ('sugar corks') were obtained. No biological activity was given.

Calixarene-based Dendrimers

The name calixarene is derived from a type of Greek vase called a calix, because they can exist in a conformation reminiscent of a basket or a vase. Calixarenes are macrocyclic compounds commonly derived from the base-induced reaction of phenols with formaldehyde [126]. Although phenol-derived calixarenes have [1_n]metacyclophane constitutions (Figure 19(a), (b)), and are, in general, composed of four, six, or eight arene units, compounds with other numbers of arene units are also known. In this review compounds with four arenes per macrocycle, broadly referred to as calix[4]arenes are described. For calix[8]arene-based glycoconjugates see Ref. [340]. The term 'upper rim' is used for location of substituents on the 'wider rim' of calixarene. Those on the 'narrow rim' are said to be situated on the 'lower rim' of the macrocycle. The effect of guest inclusion

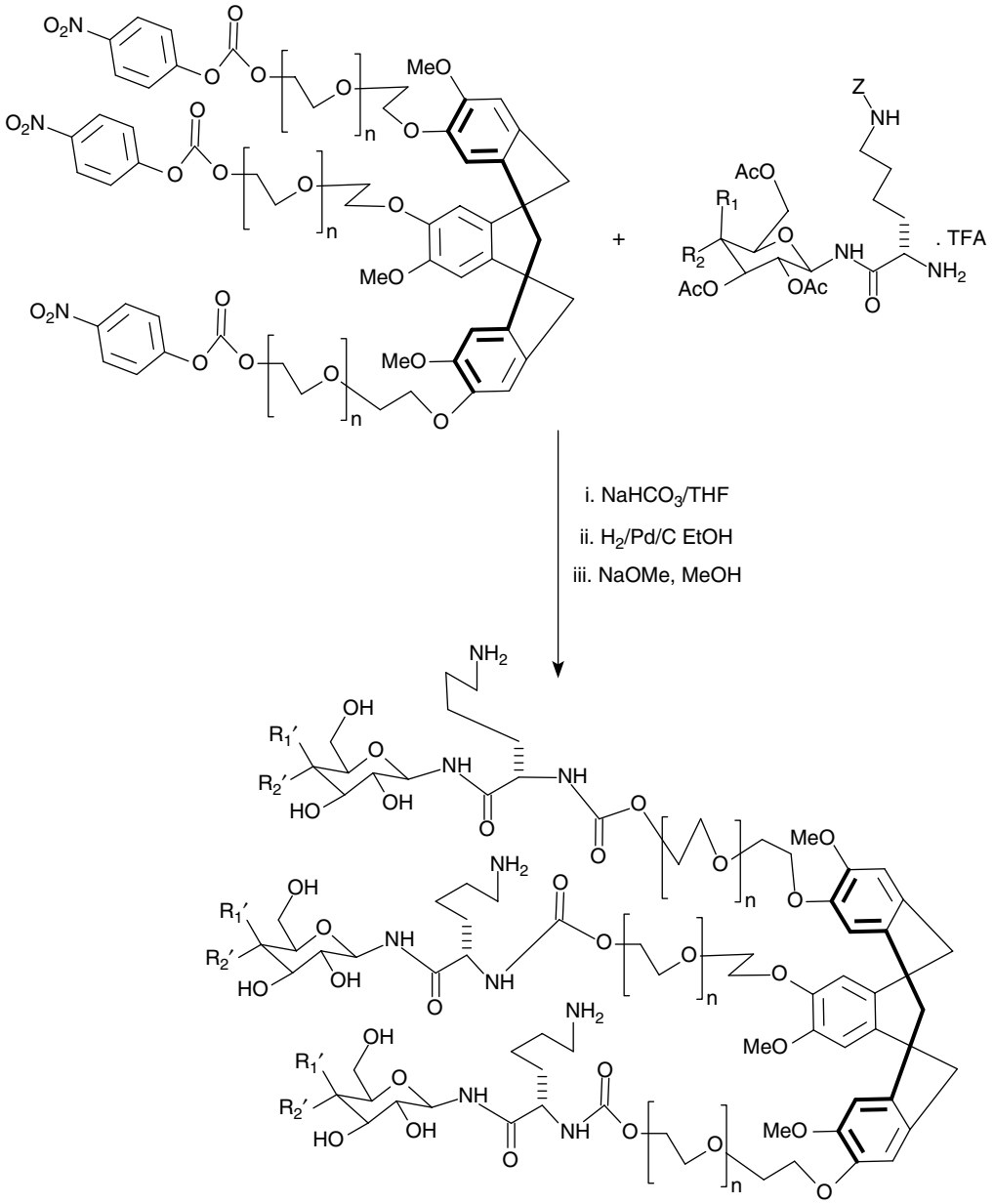

Figure 18 Synthesis of glycoconjugates based on cyclotriveratrylene scaffold [256]. R_1 = H, OAc; R_2 = OAc, H; R'_1 = H, OH; R'_2 = OH, H; n = 1, 2, 3.

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on the crystal packing of *p-tert*-butylcalix[4]arenes [258], peptido- and glycocalixarenes have been reviewed [115,249,259,260]. Glycocalix[4]arenes show the phenomenon of multivalency in their binding to specific lectins.

A semiconvergent approach was used to synthesize calixarenes with 4, 8 and 16 valencies carrying α-D-GalNAc [126,127]. The relative lectin-binding properties were measured with the lectin *Viccila villosa* agglutinin (VVA), which is known to bind α-D-GalNAc derivatives. In a solid-phase, competitive inhibition experiment, the hexadecavalent dendrimer (Figure 20) was found to be a very effective inhibitor ($IC_{50} = 13.4 \times 10^{-6}$ M) of the binding of VVA with asialoglycophorin (a natural human blood group serotype).

A divalent cholera toxin (CT) glycocalix[4]arene ligand **5** having higher affinity than natural GM1 oligosaccharide was synthesized [158]. The CT is a pentavalent sugar-binding protein belonging to the class of AB_5 toxins. The B pentamer has five identical sugar-binding sites on a single face and is responsible for cell-surface binding. Ganglioside GM1 [Galβ(1 → 3)GalNAcβ(1 → 4)NeuAcα(2 → 3)Galβ(1 → 4)Glcβ(1 → 1)Cer] is a cell-surface ligand of CT. The GM1 oligosaccharide (GM1os) interacts with the toxin via the terminal galactose and sialic acid residues. Calix[4]arene fixed in the *cone* conformation, allowing the introduction, on the *upper rim*, of sugar units projected in the same portion of space, was used to prepare the divalent ligand **5** (Figure 21). Fluorescence spectroscopy was used to study the interaction of **5** with CT. Values of concentration of the ligand at 50% saturation of 48 nM for **5** and of 219 nM for GM1os were calculated from the changes in the fluorescence emission intensity. This finding was confirmed by ELISA tests using GM1-coated plates. At low inhibitor concentration (<200 μM), the efficiency of the divalent compound **5** is superior to that shown by GM1os. For example, at 20 μM, **5** gives 54% inhibition *versus* 20% with GM1os. The inhibition power of compound **5** above 200 μM is saturated at *ca* 40%. Roughly 4000-fold (2000-fold per sugar mimic) affinity enhancement for the divalent ligand **5,** relative to the monovalent one, was estimated by fluorescence spectroscopy. This is exceptionally high in comparison with that normally measured for a divalent ligand interacting with a polyvalent receptor.

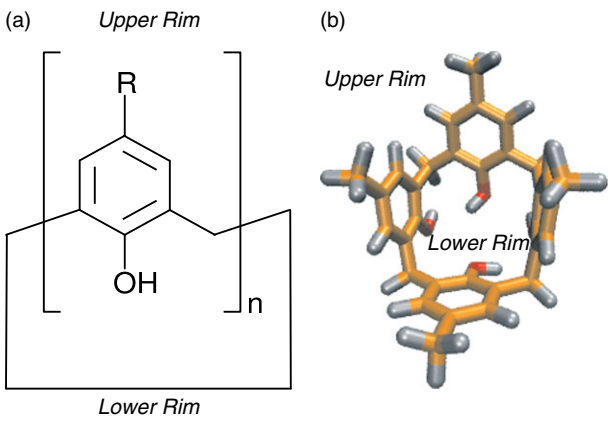

Figure 19 (a) Condensed structural formulas for the calix[*n*]-arenes with upper and lower rims (b) The complete structural representation of a calix[4]arene [126].

Thiourea linked upper rim tetrapropoxycalix[4]arene glycoconjugates with exposed two or four glucose, galactose and lactose units have been synthesized for the first time [142] and their conformations and binding properties studied. The thiourea groups act not only as linkers but also as binding units for anionic substrates as evidenced by solution ^{1}H NMR and ESI-MS experiments. Turbidimetric analysis indicates that the tetraglucosyl and tetragalactosyl derivatives specifically bind to Con A and PNA, respectively. The binding is through multivalent interactions, which for the first time evidenced upper rim calix[4]arene glycoclusters. The above data show that the calixarene glycodendrimers could also be used as site-specific molecular delivery systems.

Bis- and tetra- *O*- and *C*-glycosyl calix[4]arenes ('calixsugars') have been synthesized [257]. Stereoselective multiple glycosylation of upper rim calix[4]arene polyols afforded *gluco*, *galacto*, and *manno* calix-*O*-glycosides, respectively. A multiple Wittig olefination of upper rim calix[4]arene-derived polyaldehydes by the use of sugar phosphoranes followed by reduction of the alkene double bonds leads to calix-*C*-glycosides. Their structure and conformation were studied by NMR and MD calculations.

Calix(4)Resorcarene-based Dendrimers

Macrocyclic glycocluster compounds $\mathbf{2}_n$ ($n = 2$–7) (Figure 22(a)) containing four undecyl chains and eight oligosaccharide moieties on the opposite sides of the calix[4] resorcarene molecule were synthesized from the corresponding octaamine derivative by reaction with maltooligosaccharide lactones [219,261]. Self-aggregation and phosphate-induced agglutination behaviors of calix[4]resorcarenes was studied by dynamic light scattering (DLS), gel permeation chromatography and transmission electron microscopy (TEM). In water, they form small micelle-like nanoparticles ($d \cong 3$ nm). In the presence of Na_2HPO_4/NaH_2PO_4, the nanoparticles are agglutinated with phosphate ions as a glue to grow in size up to 60–100 nm, as revealed by DLS as well as TEM and atomic force microscopy (Figure 22(b)) [219,261]. The agglutination processes induced by phosphate can be followed by surface plasmon resonance (SPR). The immobilizations of amphiphile $\mathbf{2}_n$ on a hydrophobized sensor chip of SPR and phosphate-mediated multilayer formation were also studied [219]. The phosphate-mediated inter(saccharide) interactions (rate, affinity) are markedly dependent on the oligosaccharide chain

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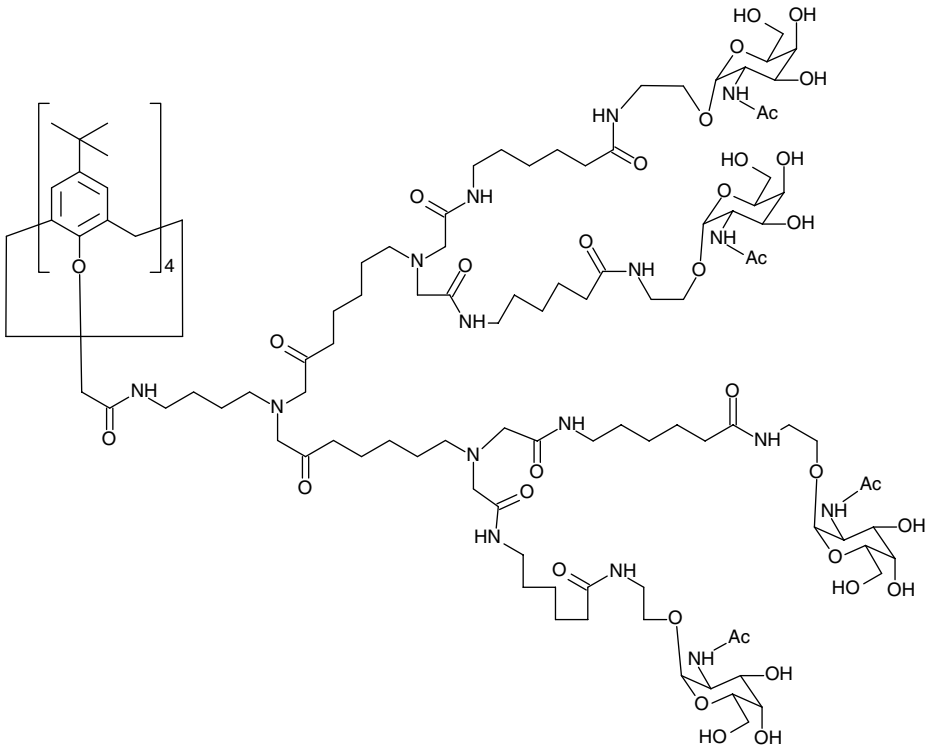

Figure 20 Calix[4]arene-based hexadecavalent α-D-GalNAc containing dendrimer [126].

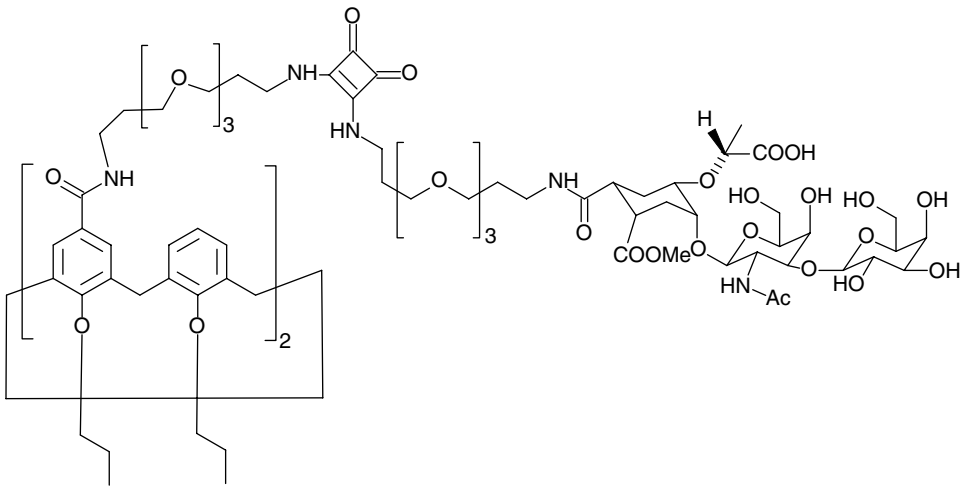

Figure 21 Divalent cholera toxin calix[4]arene ligand **5** [158].

length (n). They grow with increasing chain length. The aggregation and agglutination behaviors observed are discussed in terms of immobilizable and irreversible micelles. This work shows how fruitfully this type of macrocyclic glycoclusters can be used in the study of the cluster (multivalency) effects of saccharides in complexation, recognition and adhesion.

Cone-shaped, quadruple-chain, glycocluster-forming micelle-like aggregates in water were prepared [175–178] and named *micellar glycocluster nanoparticles* (GNPs). These GNPs based on derivatized calix[4]resorcarene were made in order to construct novel artificial glycoviral vectors named 'artificial virus'. The term 'artificial virus' means cationic vectors mimicking some characteristic aspects of viral vectors. 'Artificial viruses' should be mononuclear, stoichiometric, of a viral size $\leq$100 nm and capable of transfection. In analogy with natural viruses, the authors coated the gene (7040 base pairs plasmid pCMVluc) with glycocluster nanoparticles which are adhesive on DNA but were not adhesive with each other. The used calix[4]resorcarene was derivatized with eight or five saccharide moieties with terminal α-D-glucose, β-D-glucose or β-D-galactose residues, respectively, and

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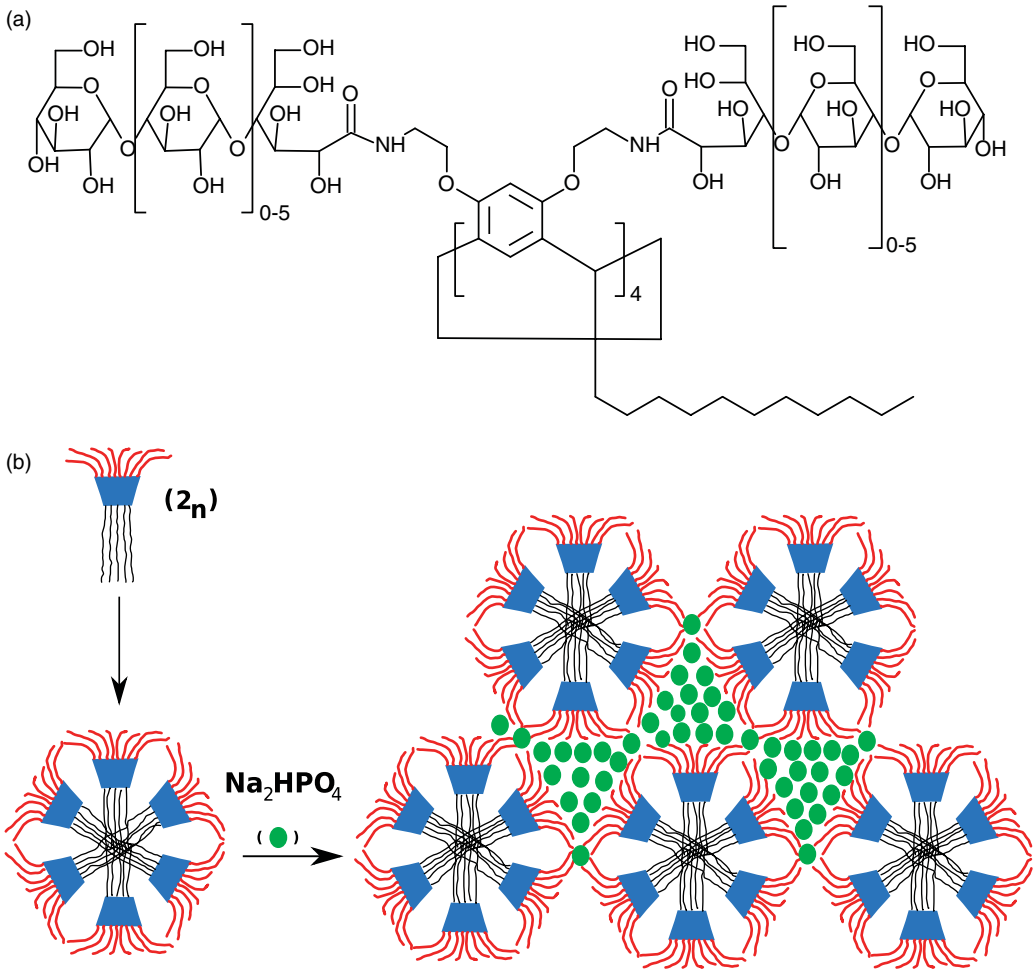

Figure 22 (a) Calix[4]resorcarene-based macrocyclic glycocluster $\mathbf{2}_n$ (b) Self-aggregation and phosphate-induced agglutination of glycocluster $\mathbf{2}_n$. Adapted from [219].

four undecyl chains on the opposite sides of the calix[4]resorcarene framework. The authors studied self-aggregation of glycoviruses. Each aggregation differed depending on the type of the saccharide used for derivatization of the calix[4]resorcarene scaffold. The results showed that β-D-Glc viruses are mostly monomeric, α-D-Glc viruses are highly aggregated and β-D-Gal viruses exhibit an intermediate oligomeric behavior. The resulting viruses are compactly packed, well charge-shielded and transfect cell cultures (HeLa and HepG2) via a nonspecific but highly size-regulated endocytic pathway, where only monomeric viruses possess substantial transfection activities.

The topic of artificial viruses was reviewed [112,261].

Carbopeptide Dendrimers, Octopus Dendrimers

Another class of MAGs in a broader sense is carbopeptides [262,264]. In this case the peptides are outside and the carbohydrate inside. They contain a carbohydrate core (template) with exactly defined orientation of the hydroxyl groups (in this case D-galactose), to which β-Ala as a spacer is bound. Then the peptides are bound to the spacer (Figure 23). The β-hemiacetal group was protected as 4-methylenebenzoylamide (MBA-NH_2). The incorporated peptide was Ala-Leu-Ala-Lys-Leu-Gly.

Carbohydrate-centered glycopeptide dendrimers are sometimes called 'octopus glycosides' [49,54,153,249,341].

The same peptide sequence was incorporated to the corresponding methyl 2,3,4,6-*O*-(aminooxyacetyl)-α-D-galactopyranoside using peptide aldehyde ligation [240,264]. The oxime causes *syn–anti* isomerism.

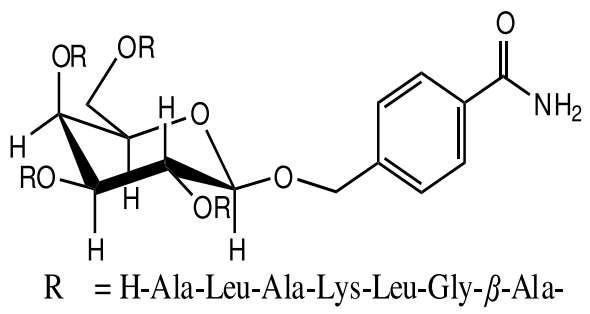

Figure 23 MAP with D-galactose core [262,264].

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For structure elucidation of carbopeptides and carboproteins, the novel technique, synchrotron radiation-based circular dichroism (SR-CD) has been used. This method, applied to metmyoglobin and a 4-α-helix bundle carboprotein, enables CD measurements in the vacuum ultraviolet down to 168 nm in D_2O and 160 nm in TFE [263].

The synthesis of a 64-amino-acid 'carboprotein' by chemoselective ligation of a *C*-terminal hexadecapeptide aldehyde to a tetra-aminooxy functionalized methyl α-D-galactopyranoside template was described. CD spectroscopy and NMR amide H–D exchange experiments indicated that the four-stranded carboprotein forms 4-α-helix bundle structure [241]. The presence of 4 oxime bonds allows many combinations of *syn–anti* isomers. The NMR data show an approximate 1 : 1 ratio of *Z*- and *E*-oxime isomers. This explains why analytical HPLC of the purified products showed somewhat broad peaks. The same authors [159] synthesized three 8.1 kDa 4-α-helix bundle structures by oxime ligation of tetra-aminooxyacetyl functionalized *galacto*-, *gluco*-, and *altro*-pyranoside templates with an amphiphilic *C*-terminal hexadecapeptide aldehyde. The effect of the template on physicochemical properties was studied by CD. The *altro*-based carboprotein was found to be more α-helical than the *galacto*- and *gluco*-carboproteins.

Glucuronic acid derivatives were used as branching units for the synthesis of glycopeptide mimetics [342]. Dendronized saccharides were synthesized to provide glycosyl amino acids having a branching element for the preparation of dendrimeric glycopeptide mimetics. Optimized Staudinger-type reaction was used to prepare complex glycopeptide with three mannose moieties connected to the branched glucuronyl core.

A synthetic route to functionalized glycopeptide dendrimers based on the *scyllo*-inositol scaffold, in which the directionality as well as the number and density of the terminal α-D-Man can be controlled, was described [227]. To the amino groups of the peptide dendrimer α-D-Man was bound via 2,3,4,6-tetra-*O*-acetyl-α-D-mannosyl isothiocyanate. Depending on the generation, glycopeptide dendrimers with valency 6 and 12 on *scyllo*-inositol scaffold were prepared for glycobiological applications. No biological data was given.

Carbopeptides represent the use of carbohydrates as potential templates for *de novo* design of protein models. It is possible to say that carbopeptides are TASPs on carbohydrate templates. The term carboproteins is used for protein models on carbohydrate templates. For reviews on carbopeptides see Refs [253,264.]

Trehalose-based octopus glycosides were used for the synthesis of carbohydrate-centered PAMAM dendrimers and thiourea-bridged glycoclusters [341]. An octa-amino-functionalized trehalose served as a core. The glycoclusters contained α-D-mannose. No biological data was given.

Tethered cluster mannosides with glucose as a core and 6-aminohexane aglycone as an anchor were prepared [209,265]. These compounds can be used to study carbohydrate–protein interactions and for affinity chromatography.

The same authors [154] elaborated a synthesis of dodecavalent octopus neoglycoconjugate using squaric acid diester-mediated coupling and tris(2-aminoethyl)amine as a core. The obtained dendrimer with 12 α-D-mannose units was tested for anti-adhesive properties in an ELISA which allows detection of the adhesion of type 1 fimbriated *E. coli* to a mannan-coated polystyrene surface. The relative inhibitory potency (RIP) of this dendrimer was 190 in comparison with methyl α-D-mannoside (RIP = 1). See also [153].

Carbohydrate-centered glycoclusters were evaluated as ligands for Con A (a mannose specific lectin) by a competitive ELLA. Immobilized yast mannan served as the reference ligand. As a core, α- and β-D-glucopyranose was used [133]. The activity depended on the spacer between the mannose units and the core. Dendrimers with 1-thiomannose were active, but replacement of 1-thiomannose with α-D-mannopyranosylthioureido units virtually abolished any multivalent or statistical effects, with a dramatic decrease of binding affinity. The diastereomeric 1-thiomannose-coated α- and β-D-glucopyranose was a potent ligand for Con A. The effect of glycocluster valency was also studied.

First, second and third generation of glycopeptide dendrons built on a 6-amino-6-deoxy-D-glucose core (Figure 24, only third generation shown), containing 2, 6 and 14 sugar units, respectively, were synthesized [343] in order to study carbohydrate–protein interactions. The use of peptide chemistry in the linking step avoids glycosylation techniques for each coupling step. Stability against glycosidases was tested. According to HPLC analysis, no degradation with β-D-glucosidase from almonds was observed over several hours. See also [265].

Huisgen 1,3-cycloaddition reaction of methyl-2,3,4,6-tetra-*O*-propargyl-β-D-galactopyranoside with 2-azidoethyl glycosides of lactose and *N*-acetyllactosamine was used to prepare water soluble and lectin-recognizable carbohydrate-centered glycoclusters [344]. Capillary affinity electrophoresis using fluorescence-labeled asialoglycans has shown that the binding of a plant lectin RCA_{120} was inhibited by the glycoclusters 400-fold more strongly than with free lactose. For microwave-assisted Huisgen 1,3-cycloaddition reactions see also Part II (Microwave-assisted synthesis of dendrimers, Jezek *et al.*, *J. Peptide Sci.*, manuscript under preparation).

Cluster mannosides with pentaerythritol derivatives as scaffolds were prepared and tested as inhibitors of type 1 fimbriae-mediated adhesion of *E. coli* [155]. Their capacity to block the binding of *E. coli* to yeast

DOI: 10.1002/psc

mannan *in vitro* was more than 200 times higher than with methyl α-D-mannoside. For other trivalent α-D-mannoside clusters and their biotinylated analogs see Ref. [156].

Analogous tri-, tetra- and hexavalent mannoside dendrimers with pentaerythritol or dipentaerythritol core were synthesized by Roy *et al.* [152] using Cu^{+}-catalyzed [1,3]-dipolar cycloadditions to the corresponding core bearing either alkyne or azide functionalities. These mannodendrimers were approximately 100 times more efficient in the inhibition of agglutination of *E. coli* by Baker's yast than the monomer mannose.

Five novel sialyloligosaccharide ligands of sialoadhesin have been synthesized by chemoenzymatic methods [143] to investigate the influence of valency and ligand architecture on multivalent attachment and increased affinity. First, precursor lactose-based monomers, clusters and pentaerythritol dendrons were prepared chemically by photoaddition and reductive amination, and then sialic acid was attached enzymatically to these lactose precursor compounds using sialyltransferases. The sialyltransferases form specifically α-D-Neu5Ac-(2 $\rightarrow$ 3)-β-D-Gal linkages. The resulting sialyloligosaccharides were characterized by MALDI-TOF MS, ^{1}H and ^{13}C NMR spectroscopy. Competitive ELISA was used to measure how much the synthetic sialyloligosaccharides could inhibit the biotinylated probe from binding to sialoadhesin. The most potent compound was the divalent sialoside cellobiosyl-based structure having nearly sevenfold increased inhibitor potency (IC_{50} = 0.2 mM) in comparison with the monovalent sialoside. In contrast, the tetravalent sialoside pentaerythritol dendrimer (IC_{50} = 2.4 mM) was less potent than the sialoside monomer (IC_{50} = 1.4 mM).

Novel glycodendrimers based on glycerol and glycerol glycol polyether scaffolds were prepared [151]. As the carbohydrate component, isopropylidene-protected hydroxyethyl-α-mannoside was chosen. The synthesis of the dendrimer proceeded by a convergent approach including iterative Williamson etherification and ozonolysis/hydride reduction steps. For structures with up to four mannose residues, the glycerol glycodendrimers are accessible on multigram scale. The synthesis of octavalent glycodendrimers is more complicated. This is an important distinction between this method and other glycodendrimer syntheses. Free glycodendrimers were tested for their ability to inhibit mannose-specific adhesion of *E. coli* (recombinant strain HB 101) expressing only type 1 fimbriae on its surface. ELISA with microtiter plates coated with yeast mannan was employed. The IC_{50} values obtained in the ELISA reflect the inhibitor concentration causing 50% inhibition of bacterial binding to yeast mannan. Methyl α-D-mannoside (MeMan) inhibited the adhesion of *E. coli* HB 101 at milimolar concentrations, whereas *p*-nitrophenyl

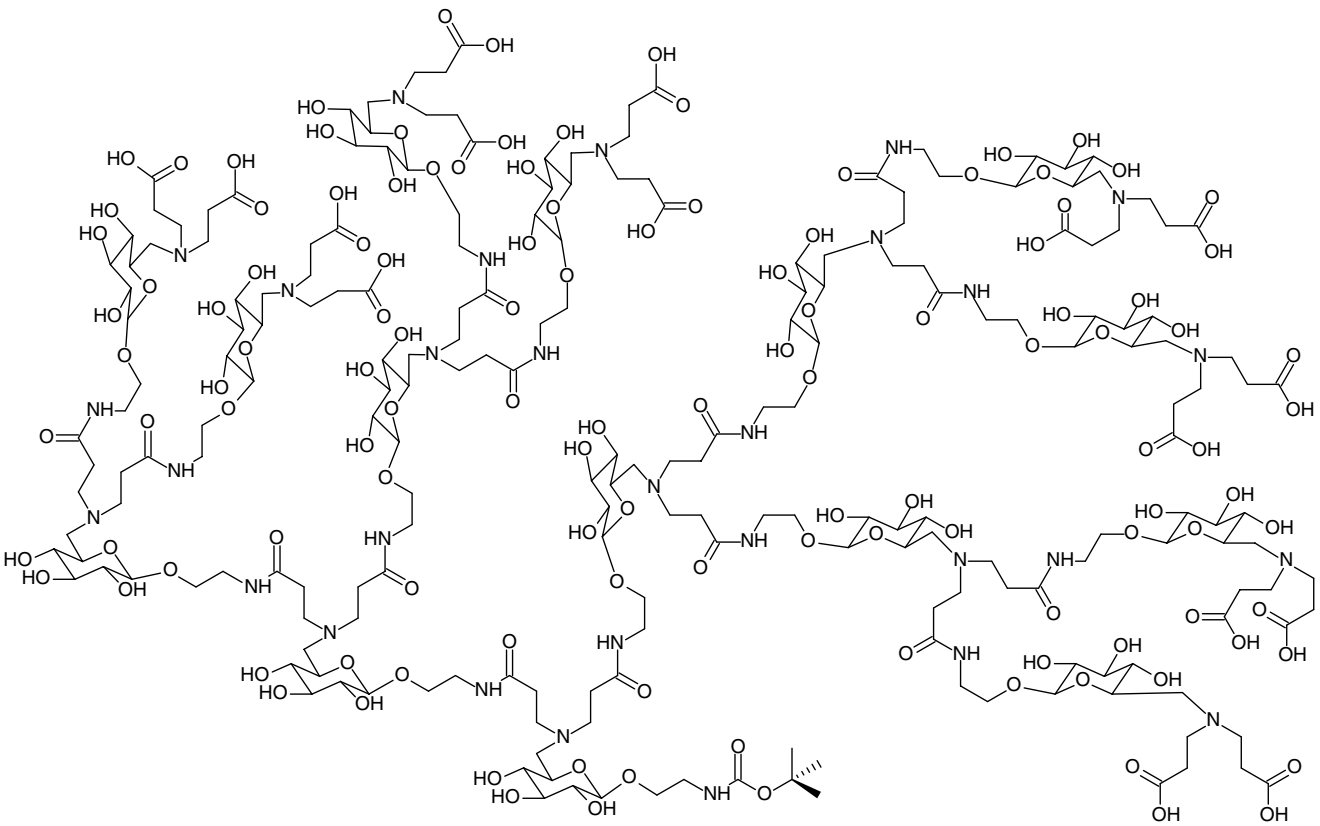

Figure 24 Third generation of glycopeptide dendrons built on a 6-amino-6-deoxy-D-glucose core [343].

DOI: 10.1002/psc

α-D-mannoside (*p*NPMan), owing to its aromatic aglycon, has an inhibitory potency approximately 2 orders of magnitude higher. The di- and tetravalent glycodendrimers had IC_{50} values approximately 10 times lower than MeMan. Their RIPs (relative IC_{50} based on MeMan = 1) were 6–13. These glycodendrimers can be used as potential inhibitors of mannose-binding lectins.

Oligosaccharide mimetics of a high-mannose type were synthesized by mannosylation of spacered D-glucose and oligosaccharide (α,α-trehalose, β-D-melibiose, raffinose) cores [153]. These carbohydrate-centered (octopus) cluster mannosides were tested by ELISA as inhibitors of mannose-specific bacterial adhesion mediated by type 1 fimbriae (*E. coli*). Their IC_{50} and relative inhibitory potencies (RIP, for methyl α-D-mannoside = 1) were compared with a series of mannobiosides and finally with the polysaccharide mannan. The RIP values suggest a new interpretation of the mechanism of bacterial adhesion according to a macromolecular rather than multivalency effect.

RAFT and Analogous Cyclic Structures

A structural prototype of RAFTs has been synthesized and crystallized [267]. Crystal structure of the aromatically substituted cyclodecapeptide was determined by X-ray diffraction. The three-dimensional structure in solution was examined by NMR spectroscopy. For a typical RAFT structure see Figure 5 [188].

Tetravalent template-assembled glycopeptides were prepared by the chemoselective assembly of aminooxy carbohydrates to a cyclic decapeptide template [132] presenting aldehydes, namely a regioselectively addressable functionalized template (RAFT). Derivatives with four β-D-Glc, β-D-Gal, β-D-GalNAc, β-D-Lac, α-D-Glc, α-D-Gal, α-D-GalNAc and α-D-Man, respectively, were isolated in almost 80% yield after semipreparative HPLC. Recognition assays of the tetramannosyl-RAFT with Con A using fluorescence anisotropy method afforded IC_{50} 62 μM in comparison with methyl α-D-mannopyranoside (IC_{50} 1.2 mM).

A new strategy to synthesize glycopeptide–oligonucleotide conjugates was developed. The strategy utilizes a cyclodecapeptide scaffold (RAFT) containing 5 Lys residues to anchor the lactose cluster and the oligonucleotide through sequential oxime ligation [141]. The stability of the duplex formed by the oligonucleotide glycocluster with the complementary sequence was investigated by thermal denaturation experiments and CD spectra. The binding interaction of the oligonucleotide glycocluster to specific lectins obtained from *Arachis hypogaea* (peanut) was efficient. The conjugate does not bind to the nonspecific lectins (Con A from *Canavalia ensiformis* (Jack bean)).

For other multidentate ligands see Refs [41,260,345, 346].

Acknowledgements

This work was supported by grant No. QF3115/2003 of the Ministry of Agriculture, grants No. 203/03/1362 and 203/06/1272 of the Grant Agency of the Czech Republic, Grant Agency of the Czech Academy of Sciences KAN200520703 and Research Project Z40550506. Our thanks are due to Dr Matyáš Turský for a critical reading of the manuscript.

Amp	*cis*-4-Amino-L-proline
CD	Cyclodextrin or circular dichroism
Con A	Plant lectin concanavalin A
CT	Cholera toxin
DOTA	1,4,7,10-tetrakis(Carboxymethyl)-1,4,7,10-tetraazacyclododecane
ELLA	Enzyme-linked lectin assay
FL	Fluorescein
HSV	Herpes simplex virus
Imd	Imidazolidine-2-carboxylic acid
ITC	Isothermal titration calorimetry
MAb	Monoclonal antibody
MEFs	Mouse embryonic fibroblasts
MRI	Magnetic resonance imaging
NKR-P1A	Natural killer receptor protein 1; activating
NKR-P1B	Natural killer receptor protein 1; inhibitory
PAMAM dendrimers	Poly (amidoamine) dendrimers
PNA	Lectin from peanut (*Arachis hypogaea*)
PPI dendrimers	Poly (propyleneimine) dendrimers
RAFT	Regioselectively addressable functional template
sialyl-T_N	NeuNAcα(2 → 6)GalNAcα(1 → O) Ser/Thr
sLe^x	Neolactoseries antigens sialyl-Lewis x
SPR	Surface plasmon resonance
TACAs	Tumor-associated carbohydrate antigens
T_N antigen	GalNAcα(1 → O)Ser/Thr
VVA	*Viccila villosa* agglutinin

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DOI: 10.1002/psc