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Review

Glycopeptide dendrimers. Part II‡

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Abstract: Glycopeptide dendrimers are regularly branched structures containing both carbohydrates and peptides. Various types of these compounds differing in composition and structure are mentioned, together with their practical use spanning from catalysis, transport vehicles to synthetic vaccines. This Part II (for Part I see Ježek J, *et al.*, J. Pept. Sci. 2008; **14**: 2–43) covers linear oligomers with variable valency (brush dendrimers, comb dendrimers), sequential oligopeptide carriers SOCn-I and SOCn-II, chitosan-based dendrimers, and brush dendrimers. Other types of glycopeptide dendrimers are self-immolative dendrimers (cascade release dendrimers, domino dendrimers), dendrimers containing ω-amino acids (Gly, β-Ala, γ-Abu and ε-aminohexanoic acid), etc. Microwave-assisted synthesis of dendrimers and libraries of glycopeptides and glycopeptide dendrimers are also included. Characterization of dendrimers by electromigration methods, mass spectrometry, and time-resolved and nonlinear optical spectroscopy, etc. plays an important role in purity assessment and structure characterization. Physicochemical properties of dendrimers including chirality are given. Stability of dendrimers, their biocompatibility and toxicity are reviewed. Finally, biomedical applications of dendrimers including imaging agents (contrast agents), site-specific drug delivery systems, artificial viruses, synthetic antibacterial, antiviral, and anticancer vaccines, inhibitors of cell surface protein–carbohydrate interactions, intervention with bacterial adhesion, etc. are given. Glycopeptide dendrimers were used also for studying recognition processes, as diagnostics and mimetics, for complexation of different cations, for therapeutic purposes, as immunodiagnostics, and in drug design. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: artificial virus; cascade-release dendrimers; dendrimers; domino dendrimers; glycobiology; glycocluster; glycoconjugates; glycodendrimers; glycopeptide dendrimers; glycopeptide libraries; glycopeptides; glycotope; lectin; ligation chemistry; multiple antigen glycopeptides (MAGs); review; self-immolative dendrimers; synthetic vaccine

LINEAR POLYMERS (OLIGOMERS) WITH VARIABLE VALENCY (BRUSH DENDRIMERS, COMB DENDRIMERS, ETC)

There are many types of linear glycopeptide dendrimers [1,2], sequential oligopeptide carriers (SOCn-I and SOCn-II), chitosan, tumor-associated carbohydrate antigens, etc. Brush dendrimers (cylindrical polymer brushes) [3,4] have been reviewed. There are generally three methods for the synthesis of dendritic brushes possessing densely grafted side chains covalently bonded to a linear backbone: 'grafting through', 'grafting onto', and 'grafting from'.

Sequential Oligopeptide Carriers SOCn-I and SOCn-II

A new class of helicoids-type SOC for anchoring antigenic epitopes has been modeled [5–8] from the repetitive Lys-Aib-Gly (SOCn-I) and Aib-Lys-Aib-Gly (SOCn-II) sequences in order to develop scaffolds with predetermined 3D structures. These fully synthetic compounds carrying different epitopes have found wide applications in immunology, including synthetic vaccine development. However, till now, no sugar has been bound to these Lys-containing structures.

A new type of glycopeptide with a periodical sequence H-[Glu(OMe)-Ser(β-D-GlcNAc)-Aib]$_n$-OH ($n = 7$ and 8) was synthesized [9] by polymerization of H-[Glu(OMe)-Ser(β-D-GlcNAc(OAc)$_3$)-Aib]-OH with diphenylphosphoryl azide (DPPA) or via ONp active ester. The final product was obtained by hydrazinolytic deprotection of the *O*-acetyl groups. Conformation analysis by semiempirical molecular orbital calculations supported by CD and FT-IR spectroscopy indicates that the free oligopeptide has an α-helical conformation in aqueous solution.

Chitosan-based Dendrimers

Sashiwa *et al.* [10–17] described the syntheses of different chitosan–dendrimer hybrids. In general, two

Abbreviations: Standard abbreviations have been followed throughout this paper (*J. Peptide Sci.*, 2006; **12**: 1–12). Other abbreviations: MAS, magic angle spinning; MRI, magnetic resonance imaging; NKR-P1A, natural killer receptor protein 1, activating; NKR-P1B, natural killer receptor protein 1, inhibitory; PAMAM, poly(amidoamine); RAFT, regioselectively addressable functional template; T_N antigen, GalNAc $\alpha(1 \rightarrow O)$Ser/Thr. When not stated otherwise, amino acids are of L-configuration and carbohydrates are of D-configuration.

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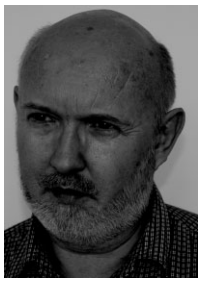
‡ Dedicated to Jan Pospisek (Institute of Organic Chemistry and Biochemistry, Prague) on the occasion of his 75th birthday.

BIOGRAPHY



Jan Ježek, PhD., was born in 1951 in Strážnice, Czechoslovakia. In 1974 he graduated from Charles University, Prague. He joined the Institute of Organic Chemistry and Biochemistry, Czechoslovak (from 1993 Czech) Academy of Sciences, Prague in 1978 for the doctoral degree, and was awarded the PhD degree in 1981(for both synthesis and structure–activity studies in the newly established area of muramyl glycopeptides; tutor Dr M. Zaoral, DSc.). The following studies have been carried out abroad: (i) at the Shemyakin Institute of Bioorganic Chemistry, Moscow, with Prof. V.T. Ivanov and T.M. Andronova, PhD. in 1988 on the synthesis of oligosaccharide muramyl peptides and lipoglycopeptides; (ii) at the Rockefeller University, New York, with Prof. R.B. Merrifield on glucagone analogs and Torrey Pines Institute for Molecular Studies, San Diego, with R.A. Houghten, PhD. on the simultaneous multiple peptide synthesis, T-bag method (1989–1990); (iii) at the Institute for Biochemistry and Biophysics, Friedrich Schiller University, Jena, Germany, with Prof. S. Reissmann on bradykinine analogs with backbone-to-backbone cyclization, synthesis and structure–activity studies (1992–1993). His research areas include SPPS, MAPs, MAGs, peptide and glycopeptide dendrimers, coupling reagents, synthetic peptide, and glycopeptide vaccines. His hobbies include postage stamp collection, body building, and minerals.

Dr Petr Niederhafner was born in 1966 in Mlada Boleslav, Czechoslovakia. In 1988 he graduated from the Institute of Chemical Technology, Prague. He taught at the secondary school (1990–1996). He carried out research in industry from1996 to 2000, and from 2000 has been at the Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, where he is a PhD student (supervisor, Jan Hlavacek, PhD.). His research areas include peptide synthesis on PEG carriers and synthetic vaccines.

BIOGRAPHY

Jaroslav Šebestík PhD. was born in 1977 in Ostrava, Czechoslovakia. In 2000, he obtained the MSc. Degree (Organic and Medicinal Chemistry) from the Institute of Chemical Technology, Prague. In 2005, he was awarded the BSc. degree (Teaching of Chemistry) by the Institute of Chemical Technology, Prague. At the end of 2006, he obtained the PhD. degree (Organic Chemistry), working with Dr Jan Hlaváček and Prof. Ivan Stibor from the Institute of Chemical Technology, Prague. Since 2007, he has been a postdoc with Dr Petr Bour, Department of Molecular Spectroscopy, Institute of Organic Chemistry and Biochemistry, AS CR, Prague.

approaches can be used. In the first one, the corresponding dendrimers bearing aldehyde and spacer are synthesized, and then reacted with chitosan by reductive alkylation. The advantage of this procedure is that no cross-linking takes place during the reaction. However, generation of reactive dendrimer is limited owing to its steric hindrance. The second method uses commercial amino dendrimers such as PAMAM and poly(ethylene imine) dendrimers. Disadvantage of this method is the possibility of cross-linking [10]. The term 'tree-type hybrid' or 'tree-type molecule' is used [10,16] on the basis of the assumption that chitosan is the trunk, the spacer part is the main branch, dendrimer is a subbranch, and the functional sugar is a flower (or leaf). An example of a 'tree-type hybrid' synthesized by the first method is given in Figure 1 [10,11,16]. PAMAM dendrimers of G1 to G3 with tetraethylene glycol spacer were prepared, attached to sialic acid by reductive *N*-alkylation (i.e. two sialic acid residues per one primary amino group), and finally attached to chitosan. The degree of substitution decreased with increasing generation (0.08 for G1, 0.04 for G2, and 0.02 for G3) owing to the steric hindrance of the dendrimer. Other type of chitosan with sialodendrimer composed of gallic acid as a junction point is shown in Figure 2 [10,12]. Trivalent (G1) and nonavalent (G2) dendrons with gallic acid as the branching unit and tri- or tetraethylene glycol as the spacer arm were prepared and attached to sialic acid *p*-phenylisothiocyanate derivative. The aldehyde sialodendrons were convergently attached to the chitosan. These compounds were prepared as model compounds for the study of inhibition of viral pathogens including the influenza virus [10–13,18]. Biodegradation of chitosan–dendrimer hybrids has been studied [10,17,18]. The biodegradation is independent on the water solubility, but depends on the chemical structure. The largest dendritic size showed the lowest biodegradation. The probable reason is that degradation by enzyme is inhibited by steric hindrance. The chemistry of chitin and chitosan, including dendrimers, has been reviewed [10,18].

Chitosan glass beads modified with a 1,3-thiazolidine linker have been prepared [19]. A terminal aldehyde group produced by nitrous acid degradation of chitosan was used for coupling with L-Cys linker to glass beads.

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This method could be applied for synthesis of different types of silica materials usable for biological and pharmaceutical applications.

Laminin is involved in the metastasis of tumor cells. A peptide YIGSR, corresponding to a partial sequence of laminin, inhibited angiogenesis and depressed tumor growth. A chitosan conjugate with Ac-YIGSR-β-Ala has been prepared [20,21] with one peptide per 6.3 glucosamine residues. This compound had higher inhibitory activity against experimental lung metastasis of B16BL6 melamoma cells in mice than the parent peptide.

For other laminin-1 peptides conjugated to chitosan membranes and their biological activities see Refs [10,18,22].

Brush Dendrimers

Glycopolymers exhibiting low fluctuations in both size and composition have been generated by cationic polymerization, ring-opening polymerization (ROP), ring-opening metathesis polymerization (ROMP), cyanoxyl- and nitroxide-mediated radical polymerization, as well as transition-metal-catalyzed atom transfer radical polymerization (ATRP).

ROMP [23–26] provides synthetic polymers mimicking natural multivalent ligands in structure and activity. The utility of these materials is demonstrated by the development of a cellular binding assay for L-selectin, a cell-surface protein that plays a role in inflammation [25]. ROMP was used also for an efficient synthesis of a fluorophore-labeled multivalent display of

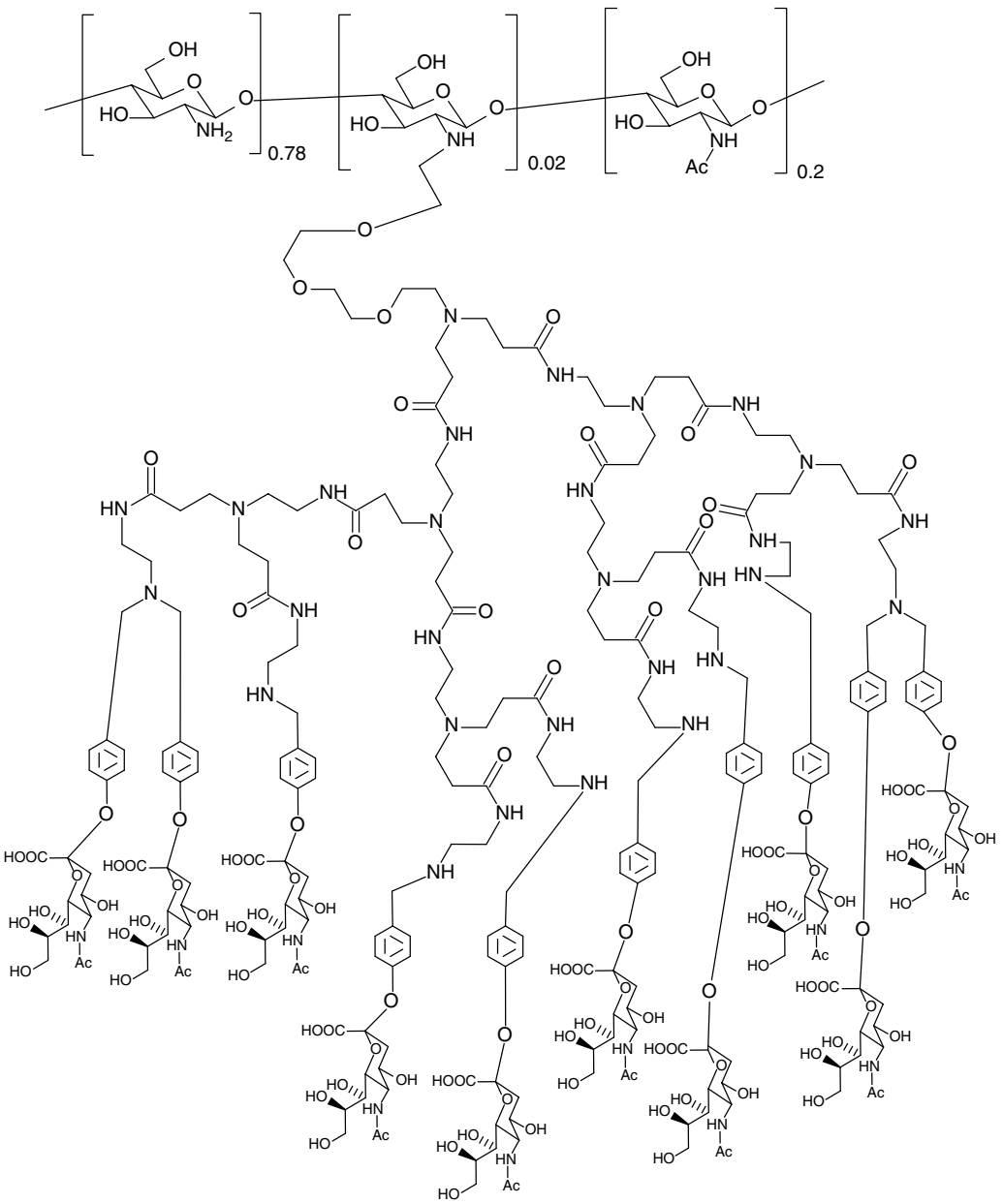

Figure 1 Chemical structure of chitosan–sialodendrimer hybrid [10,11,16].

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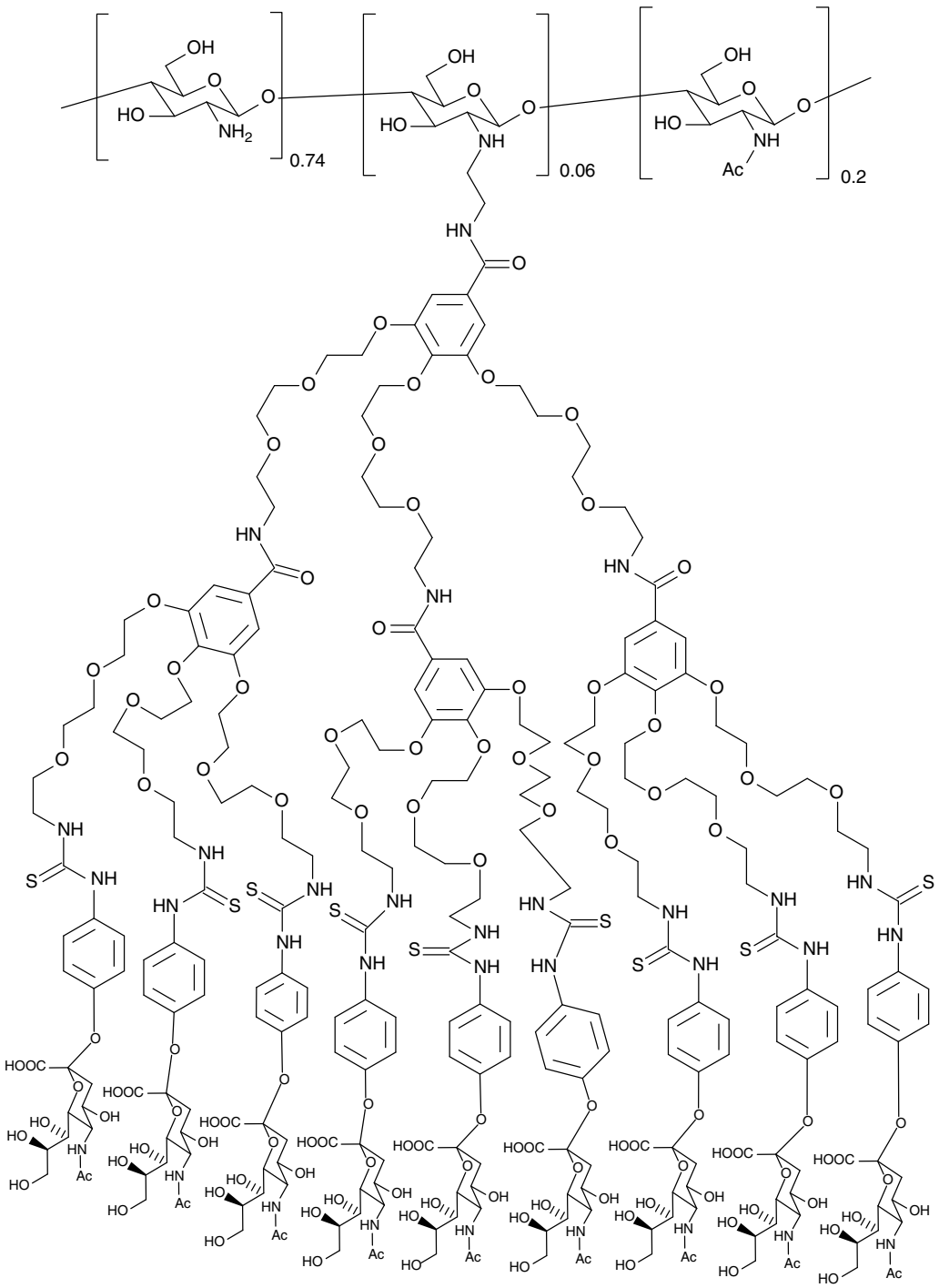

Figure 2 Nonavalent chitosan–sialodendrimer hybrid [12].

a CD22-binding trisaccharide containing NeuNAc [27]. The resulting polymers can serve for visualizing ligand interactions with CD22-producing cells.

Chaikof *et al.* use cyanoxyl (·OC≡N)-mediated free-radical polymerization of acryl-derivatized glycomonomers. This is a convenient tool to produce water soluble glycopolymers from unprotected alkene- and acrylate-derivatized sulfated and unsulfated glycomonomers consisting of a mono- and disaccharide species [28–31]. This polymerization technique is tolerant to a broad range of functional groups (OH, NH_2, COOH, OSO_3^-), can be conducted in aqueous solution, yields low-polydispersity polymers with high saccharide content, and can be applied to the synthesis of block or graft copolymers. One of the substances prepared by this method showed interesting results [32]. Glycopolymer bearing sulfated lactose residues (Figure 3) mediated proliferative responses to fibroblast growth factor 2 (FGF-2). Its proliferative response to FGF-2 is higher than those observed with heparan sulfate and is

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selective only to FGF-2. This compound protects FGF-2 from proteolytic, acid, and heat-induced degradation and effectively replaces heparin and heparan sulfate as a molecular chaperone for FGF-2. The capacity of this heparin mimic to promote an FGF-2 specific proliferative cell response suggests potential applications in areas related to therapeutic angiogenesis.

Thermal stability of new cellulose-based poly(propylene imine) and poly(amido amine) hyperbranched derivatives has been studied [33] using thermogravimetric analysis to study the effect of branching on the thermal decomposition parametres. The activation energy and onset of degradation temperature of the thermal degradation decreased with increasing branching of the cellulose based hyperbranched derivatives.

Unprotected oligosaccharides were used to synthesize di- and tetra-antennary *N*-linked glycopeptides [34] H-GlyGlnGlyAsn(R) Asn(R)-OH and H-GlyGlyGlnAsn(R)Asn(R)Asn(R)Asn(R)-OH with two and four vicinal maltotriose glycans, respectively. To the best of our knowledge [34], this is the first report of the chemical synthesis of a glycopeptide with four vicinal complex oligosaccharides.

Comb-like glycodendrimers bearing mono-, di-, or tri-T_N clusters showed surprising reactivities with rat NKR-P1A and NKR-P1B receptors [35]. Whereas compounds bearing monomers and dimers of T_N antigen reacted equally with both isoforms of NKR-P1 receptor, the compound with trimer of T_N antigen reacted exclusively with the rat NKR-P1B isoform. This selectivity is corroborated by the results obtained in *in vitro* cytotoxicity tests where this compound efficiently inhibited natural killing in both rats and humans. These results are surprising because previous studies have indicated that α anomers of *N*-acetyl hexosamines would have low binding potency towards the NKR-P1 receptor.

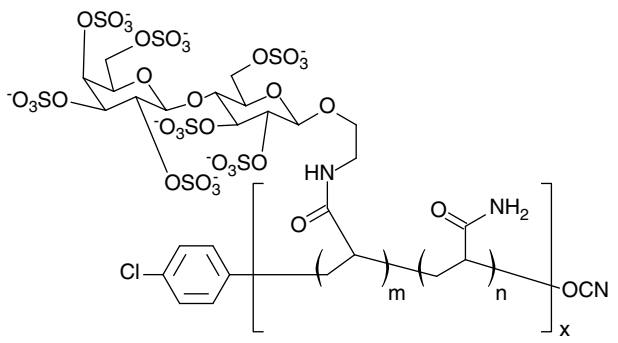

Figure 3 Sulfated lactose containing a polyacrylamide-based polymer [32].

OTHER TYPES OF GLYCOPEPTIDE DENDRIMERS

Self-immolative Dendrimers (Cascade Release Dendrimers, Domino Dendrimers)

Dendrimers can be used for drug release inside diseased cells [36]. Three teams [37–39] have independently explored a new concept: simultaneous release of all the functional groups of a dendrimer by a single chemical trigger. All three teams utilized the fact that the dendrimer skeleton can be built up in such a way that it can be made to disintegrate into known molecular fragments once the disintegration process has been initiated. These types of dendrimers are named 'cascade release dendrimers' [37], 'self-immolative dendrimers' [38,40–42], or 'geometrically disassembled dendrimers' [39]. For these dendrimers, the unifying terms 'domino dendrimers' [43] or 'cleavable dendrimers' [44] are coined. All these systems have the ability to perform a chemical amplification reaction. This principle was applied by de Groot and coworkers [37] for releasing the anticancer drug paclitaxel (Taxol). Besides, they have shown that the dendrimer degradation products are not cytotoxic, except for paclitaxel itself, which kills cancerous cells.

First application of 'self-immolative' dendrimers as a carrier for multi-prodrugs, activated by a single enzymatic cleavage has been described [40]. Doxorubicin and camptothecin were released from homo and heterodendritic carrier with a triggering substrate of catalytic antibody 38C2, which functions as a model enzyme. The bioactivation of the dendritic prodrugs was studied in cell-growth inhibition assay with the Molt-3 leukemia cell line in the presence and absence of Ab 38C2. A remarkable increase in toxicity was observed after activation.

Owing to their well-defined structure, dendrimers allow fine control of shape, size, and composition of the release system. In these dendrimers with many identical units, 'amplification can be achieved as a kind of explosion' [36].

Self-immolative dendrimers with poly(ethylene glycol) have been prepared via click chemistry [45]. Their activation by penicillin-G amidase under physiological conditions released the active prodrug camptothecin.

Another example of self-immolative, receiver-amplifier dendritic molecules with an architecture and signal-conducting activity related to that of neurons were prepared by Shabat *et al.* [42].

Self-immolative dendrimers can serve as novel drug delivery platforms [41,43].

The topic of self-immolative dendrimer synthesis, disassembly, and biomedical applications has been reviewed [41,43,44,46].

Imaging Agents (Contrast Agents)

A new class of 1,4,7,10-tetrakis(carboxymethyl)-1,4,7,10-tetraazacyclododecane (DOTA) monoamide-linked

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glycopeptide dendrimers (Glc, Gal, Lac) of different valencies (mono, di, and tetra) and their Sm^{3+}, Eu^{3+}, and Gd^{3+} complexes were synthesized, characterized, and studied as potential lectin-mediated medical imaging agents [47]. The *in vitro* relaxivity of the Gd^{3+} glycoconjugates was measured by ^1H nuclear magnetic relaxation dispersion and the parameters influencing the relaxivity were determined. The authors hypothesized that the glycoconjugate–lectin association could substantially slow down the rotation and therefore enhance proton relaxavity of the Gd^{3+} glycoconjugates. To prove this concept, the interaction of Gd^{3+} glycoconjugates was studied *in vitro* with the model lectin *Ricinus communis* agglutinin through relaxometric measurements. The known recognition of sugars by lectins makes these DOTA glycoconjugates good candidates for medical imaging agents (MRI and gamma scintigraphy).

Rotational dynamics of of G5, G7, and G9 PAMAM dendrimeric Gd-based MRI contrast agents has been studied [48]. Their proton relaxivity shows an important pH dependency, which is related to the pH-dependent rotational dynamics. This is the first time that such pH dependency is reported for dendrimeric Gd^{3+} complexes. Most probably, it is a general feature of PAMAM-based dendrimers.

The topic of dendrimer-based imaging and contrast agents has been reviewed [49–62]. Development of targeted MRI contrast agents [62,63] directed to specific molecular entities could significantly expand the range of magnetic resonance applications by combining the noninvasiveness and high spatial resolution of MRI with specific localization of molecular targets.

Glycopeptide Dendrimers Containing ω-Amino Acids (Gly, β-Ala, γ-Abu and ε-Aminohexanoic Acid)

It is necessary to consider not only the side chain length, but also the distance between the side chain branching points, to give diversity to the glycoclusters [64–66]. 2-Aminoethyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranoside was used for incorporation of Gal to the dendrimers. Different types of ω-amino acids (Gly, β-Ala, γ-Abu and ε-aminohexanoic acid) were used as spacers of different lengths. At the end of the convergent synthesis, the benzoyl groups were split off by NaOMe-catalyzed transesterification in methanol. Asymmetric 3, 4, 5, 6, 8, 9 and 12 valent glycopeptide dendrimers were obtained in high yield. Some of the dendrimers were fluorescence-labeled (dansylation). The elaborated methodology enables the modulation of not only the length of the side-chain but also the distance between the side-chain branched points. The most complex dendrimer with 12 branches (Figure 4) was termed the 'glycocluster-cluster' [66].

The same authors [67] prepared for immunological studies two types of glycopeptide dendrimers based on peptoids containing β-Ala. Each of them contains four copies of a trisaccharide and one dansyl group as fluorescence label. The trisaccharide is related to a major antigenic epitope in pectic polysaccharides from *Bupleurum falcatum* L. The roots of this plant (Japanese name *Saiko*) have been used in Chinese and Japanese herbal medicine for the treatment of chronic hepatitis, autoimmune diseases, and nephrosis syndrome.

These compounds are a special type of peptoids [68–70], in which besides *N*-substituted Gly also other ω-amino acids are used. For details see Part III (Ježek J. *et al., J. Peptide Sci.,* manuscript under preparation).

For T_N MAGs with γ-Abu insert [71,72] see also Part III, section 'Molecular dynamic'.

Miscellaneous

Iodoacetyl-Tyr was bound to a triantennary undecasaccharide *N*-glycan with galactose at the nonreducing end. Ligation with CWC(Acm)K_{15}C(Acm)K led to the partially protected glycopeptide. Subsequent removal of the Acm-protecting groups on internal cysteine residues by silver tetrafluoroborate led to the desired triantennary undecasaccharide-peptide [73] (Figure 5). Analogously, the pegylated undecasaccharide-CW$(CK_3)_4$CK was prepared. The glycopeptides were found to bind to plasmid DNA and undergo disulfide cross-linking resulting in stable DNA condensates. The binding affinity, particle size, and ξ-potential analysis of DNA condensates have been studied. These compounds with potential utility for *in vivo* gene delivery have been investigated as low-molecular-weight DNA carrier molecules [73,74].

Cu^{2+} self-assembling bipyridyl-glycoclusters and dendrimers bearing the T_N-antigen cancer marker have been synthesized [75] and their lectin binding properties studied. Building blocks containing bipyridyl dimers with either a short or a long amino acid spacer arm, together with the tetramer built from the short spacer derivative were prepared by convergent strategy using 2,2′-bipyridine-4,4′-dicarboxylic acid chloride and the aminated sugar derivatives, respectively. Cu^{2+}-nucleated T_N derivatives with four and eight T_N antigens were obtained. These glycodendrimers were tested for relative inhibitory potencies against monomeric allyl α-D-GalNAc using solid-phase competition assay 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 (IC_{50} 7.14, 1.82, 4.09 μM, respectively) 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. On a per-saccharide basis, however, the tetravalent Cu^{2+} derivative with the longest inter-sugar distances showed the highest affinity (IC_{50} 0.63 μM).

The synthesis of nonavalent cluster mannosides was accomplished starting from a number of trivalent,

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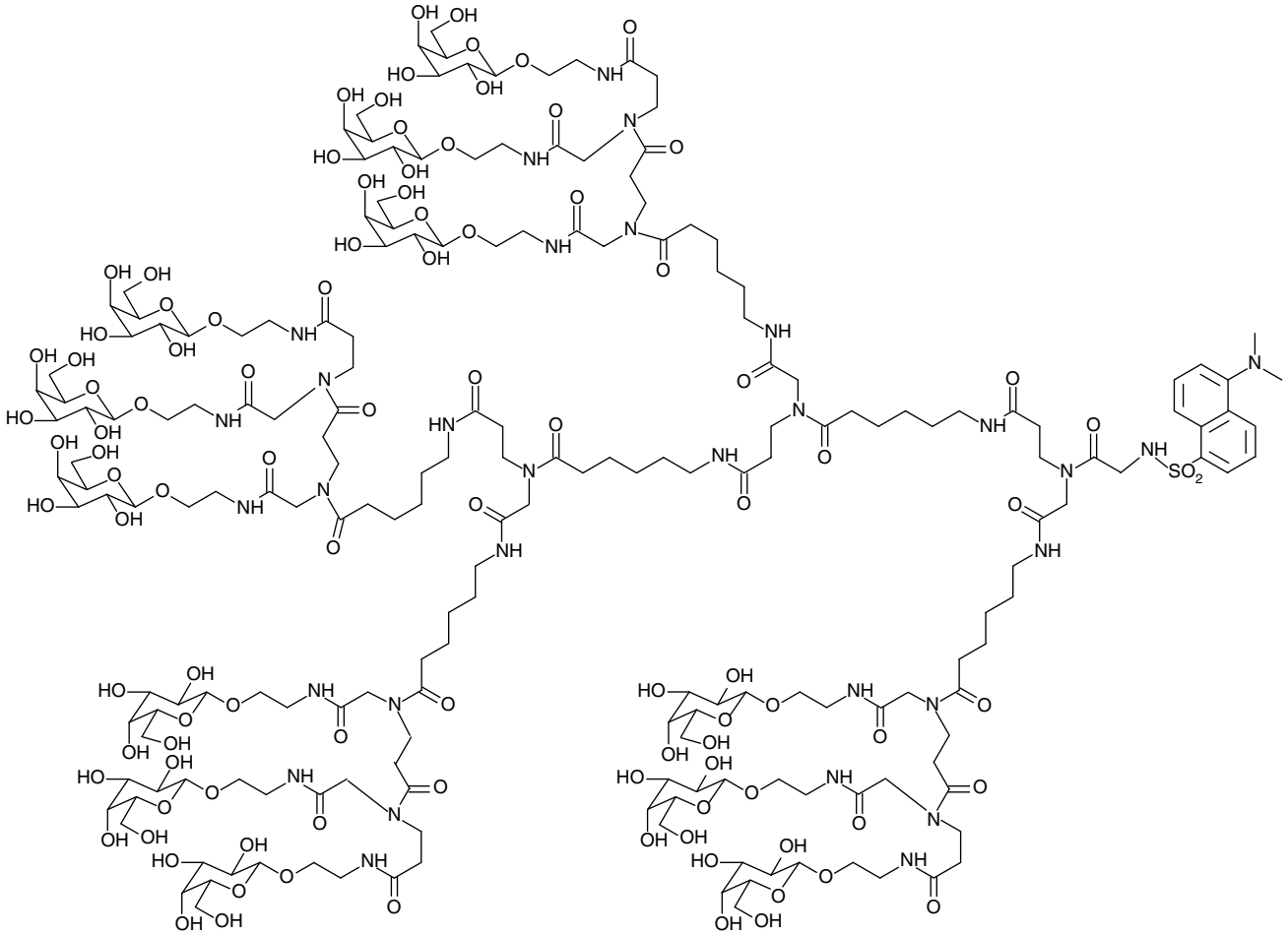

Figure 4 Dendrimer with 12 branches ('glycocluster-cluster') [66].

branched molecular wedges. The chemical characteristics of their spacer moieties and spacer lengths were varied [76]. The trivalent dendrons were connected to the target nonavalent structures by a peptide bond (HATU). Some of the prepared compounds are shown in Figure 6. The synthetic nonavalent glycopeptide dendrimers containing methyl α-D-mannopyranose were tested as inhibitors of the type 1 fimbriae-mediated adhesion using ELISA. Unfortunately, only very poor or no inhibitory activities were found. The reasons for these facts are discussed.

The 'glycocalyx' is a thick layer on the surface of all eukaryotic cells composed of complex carbohydrates. This layer plays a key role in molecular recognition and interactions of protein receptors, such as lectins, selectins, and their carbohydrate ligands. To throw more light to these interactions, the same group [77] (Figure 7) used analogous trivalent MAGs containing L-fucose and D-mannose, respectively, coupled to thio-functionalized alkane and alkane-oligoethylene glycol spacers in order to study the formation of self-assembled monolayers on gold. These MAGs can be assembled on gold wafers to serve as glycocalyx mimetics.

Selenylsulfide proteins are rarely present in nature. Glycosyl selenylsulfides represent a new class of glycosylating reagents. They can be used not only for the glycoconjugation of simple thiols and peptides (EtSH and dipeptides), but also of proteins [78]. The exquisite selectivity of S–Se chemistry obviates the need for protecting groups during glycoconjugation. Whereas most site-specific glycoconjugation methods take advantage of the nucleophilic character of Cys thiols, the S–Se chemistry uses a poweful electrophilic glycoconjugation mechanism. This approach enables the synthesis of fully deprotected glycoconjugates and glycoproteins. The authors demonstrated multiple site-selective glycoconjugation, which provides access to polyvalent neoglycoproteins.

Glycodendrimers and carbohydrate-oligonucleotide conjugates have been synthesized utilizing a DNA synthesizer [79]. The solid-phase synthesis allows the preparation of complex carbohydrate–oligonucleotide conjugates within hours as opposed to classical approaches requiring weeks to months. Tetravalent α-mannosylated conjugate with CAAGCCATGTCTGA-GACTTTG was prepared on standard guanosine support. The formation of duplexes with complementary

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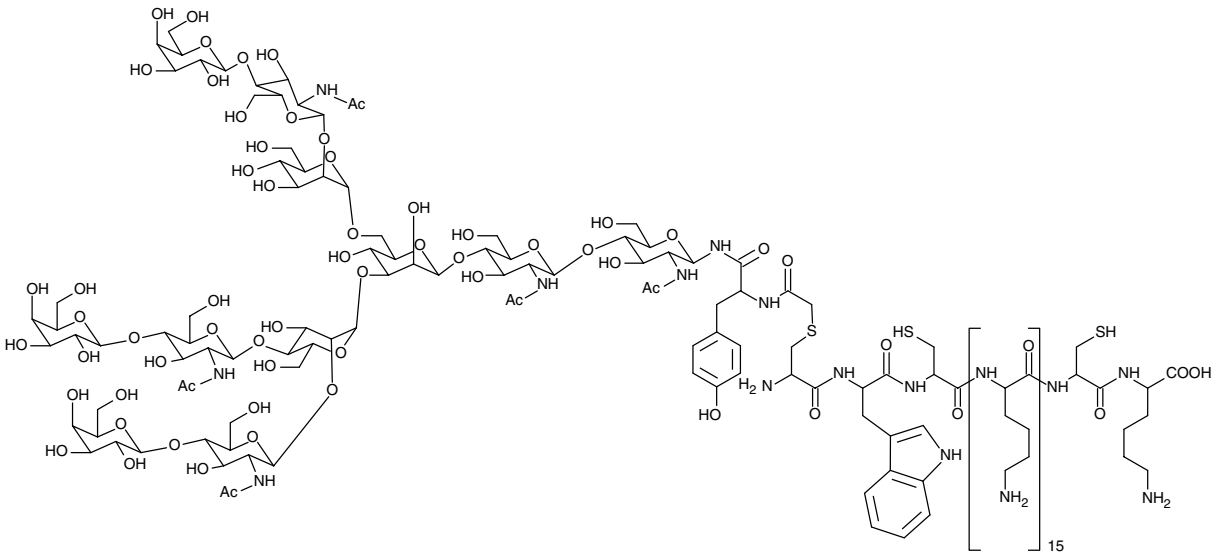

Figure 5 Triantennary-CWCK$_{15}$-CK glycopeptide [73].

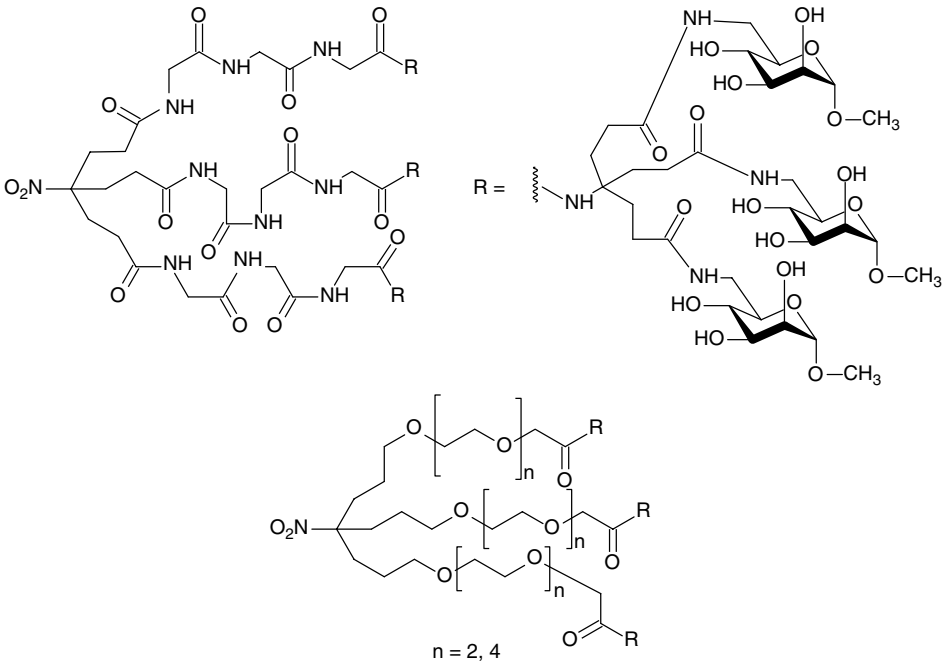

Figure 6 Nonavalent glycopeptide dendrimers containing methyl α-D-mannopyranose [76].

oligonucleotide strands was not hindered. The large glycoconjugates are well soluble in water and present potential high-affinity ligands for carbohydrate-binding proteins. For other oligonucleotide derivatives, see also Defrancq [80].

A very straightforward and short synthesis of structurally diverse aminoglycoside mimics has been developed [81] on the basis of the Ugi reaction. By this method, different amino sugar units can be coupled in a very much defined way. The synthesis of neoglycoconjugates with 2,6-diamino-2,6-dideoxy glucose by the Ugi multicomponent approach leading to di- and trivalent carbohydrate clusters is described.

Economical incorporation of up to 27 unprotected fucose, mannose, and lactose residues, respectively, in reproducible high yields (up to 92%) under catalysis of Cu^{+} has been achieved by 'click' chemistry in combination with ultrafiltration [82]. This method has proved to be an invaluable tool for quick, efficient, and reliable conjugation of unprotected alkyne-derived carbohydrates to dendritic systems incorporating terminal azides. This approach is better than the lengthier process of employing protected glycosides or introducing the click functionality on each generation of previously synthesized dendrimers. The same principles were used to prepare PEGylated MAGs [83].

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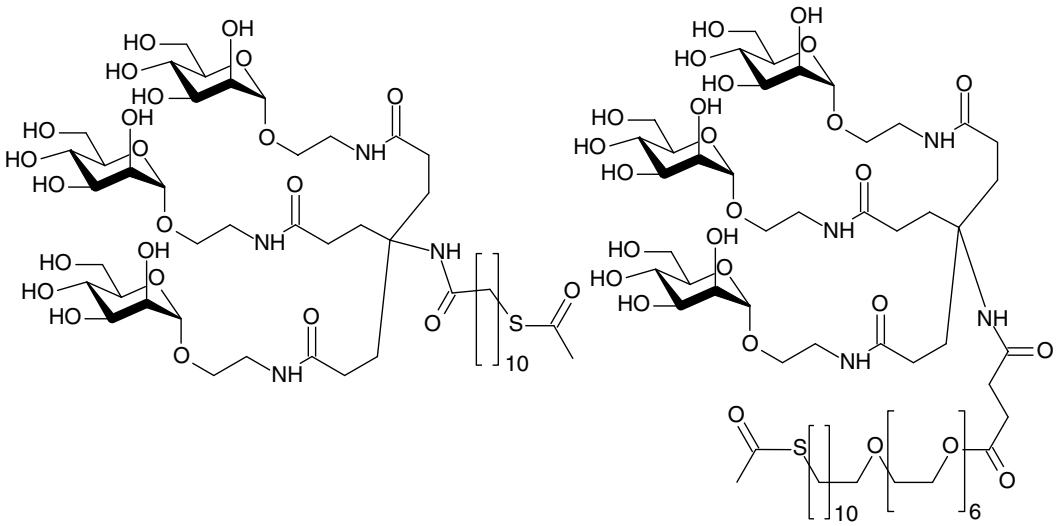

Figure 7 Trivalent MAG containing D-mannose [77].

MICROWAVE-ASSISTED SYNTHESIS OF DENDRIMERS

Multivalent dendrimeric peptides have been synthesized via a microwave-assisted Huisgen 1,3-dipolar cycloaddition ('click chemistry') between azido peptides and dendrimeric alkynes in yields ranging from 46 to 96% [84]. The azido peptides were derived from the α-amino group, ε-amino group of lysine, or an ω-aminohexanoyl spacer. Dendrimers with valency 2, 4, 8, and 16 have been obtained. For Huisgen 1,3-cycloaddition reaction, see also Ref. 85.

A straightforward high-yielding microwave-assisted synthesis of triazole-linked glycodendrimers by Cu^{+}-catalyzed [3 + 2] cycloaddition has been described [86]. The microwave-assisted syntheses can be applied also for the preparation of other types of dendrimers.

For other examples of Cu^{+}-catalyzed [1,3]-dipolar cycloadditions ('click chemistry') see Refs 45,82–84,87, 88. The 'click chemistry' in dendrimer, polymer, and materials science, including carbohydrates and peptides has been reviewed [89].

LIBRARIES OF GLYCOPEPTIDES AND GLYCOPEPTIDE DENDRIMERS

In general, low affinity is obtained for interactions between carbohydrate receptors and modified oligosaccharides designed as mimetics of natural carbohydrate ligands. Therefore, glycopeptides have been explored as alternative mimics. Glycopeptides in general have proven to be superior ligands with higher affinity for a receptor than the natural carbohydrate ligand. Glycopeptide and oligosaccharide libraries have been reviewed [90–98] with different glycotopes (T_N, TF, mannose, GlcNAc, fucose, etc.) and resins (TentaGel, PEGA, POEPOP, SPOCC, etc.). The effect of the solid support to the outcome of the screening results needs to be thoroughly investigated. The libraries can be prepared by synthesis in solution, on the solid phase, and also by chemoenzymatic syntheses. This powerful technique can be used generally for the identification and analysis of complex interactions between carbohydrates and their receptors.

MUC1-derived glycopeptide libraries with improved major histocompatibility complex anchors have been developed [99]. They are strong antigens and prime mouse T cells for proliferative responses to lysates of human breast cancer tissue.

For a library of proline-based biodendrimers for use as delivery agents, see Ref. 100.

Temporary attachment of carbohydrates to cyclopeptide templates was used as a new strategy for single-bead analysis of multivalent neoglycopeptides [101]. With this strategy, the application of multivalent cyclic neoglycopeptides in split-mix libraries with a subsequent screening process has become possible for the first time.

TentaGel S NH_2 bound *C*-linked glycopeptide libraries have been used for identification of α-D-galactosyl epitope mimetics [102]. As a *C*-glycoside galactose building block, 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl-(2-acetic acid) was used. The portion-mixing method ('one-bead-one-compound' library), screening with human anti-Gal Abs (IgG, IgM, and IgA), and final MALDI sequencing identified the mimetics of α-D-Gal epitopes. In comparison with the inhibition activities of known Gal$\alpha(1 \rightarrow 3)$Gal peptide mimics, the found glycopeptides show better activities.

A 15 625-membered glycopeptide dendrimer combinatorial library [103] with α-*C*-fucosyl residues at the *N*-termini was prepared on Tentagel and screened for binding to fucose-specific lectins. Glycopeptide dendrimer (Fuc-α-CH_2-CO-Lys-Pro-Leu)$_4$-(Lys-Phe-Lys-Ile)$_2$-Lys-His-Ile-NH_2 was identified as a potent ligand

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against *Ulex europaeus* lectin UEA-I (IC_{50} = 11 μM) and *Pseudomonas aeruginosa* lectin PA-IIL (IC_{50} = 0.14 μM). Further optimization of the structure with the aim to obtain inhibitors of biofilm formation in the *P. aeruginosa* pathogen is in progress.

'One-bead-two-compound' libraries for detecting chemical and biochemical conversions have been developed [104].

Solid-phase glycosylation of peptide templates and on-bead MAS-NMR analysis of glycopeptide libraries have been developed [105]. A novel photolabile linker was introduced to facilitate analysis by both MALDI-TOF MS and nanoprobe MAS-NMR spectroscopy. Product analysis of glycopeptides obtained by glycosylation with a variety of glycosyl trichloroacetimidate donors, done with MAS-NMR, LC-MS, and MALDI-TOF MS showed that the reactivity order of the OH side-chain functions was Tyr > Ser > Thr. The high glycosylation yields and the efficient on-bead product analysis by nanoprobe MAS-NMR enable a truly solid-phase approach both for the synthesis and analysis of glycopeptide libraries.

New, important types of libraries are dynamic combinatorial libraries (DCL) [96]. The main reason for the synthesis of combinatorial libraries is the discovery of new drug leads. The production of DCL [106–110] in the presence of a target should produce a library of compounds skewed towards compounds that interact favorably with that target. The reactions involved in such library formation must be reversible. DCL can be therefore viewed 'as a transient collection of compounds that are reversible assemblies of a collection of building blocks. The final composition of such a mixture is equilibrium driven with any thermodynamically more stable product amplified in concentration' [96]. The equilibrium can be shifted by the introduction of a template, to which the library members can bind. Tight binders favor those with weak binding or no binding ability. Structures identified from such a templated library should be good hosts or guests for the introduced template, and therefore potential drug leads.

A facile route to dynamic glycopeptide libraries based on gentle air oxidation of a slightly basic aqueous solution of 1-thiosugar and cysteine-rich oligopeptide building blocks has been described [111]. The building components of disulfide members are exchangeable with each other in the presence of dithiothreitol as an initiator leading to dynamic equilibration. The reactivities of the glycosidic and peptide (Cys) SH groups are comparable to give a balance in peptide–peptide, sugar–sugar, and sugar–peptide S–S bond formation, giving rise to a rich disulfide library.

Many building blocks, both natural and unnatural, have been prepared to increase the diversity of libraries. Sugar amino acids [94,112–119] (glycosamino acids) represent an important class of such polyfunctional scaffolds, in which the carboxyl, amino, and hydroxyl termini provide an excellent opportunity to create structural diversities akin to nature's molecular arsenal. Kessler [114] defines sugar (carbohydrate) amino acid (SAA) as a compound with immediate linkages of both amino and carboxy functionalities to a carbohydrate frame. SAAs are widely distributed in nature, with sialic acid and *N*-acetylmuramic acid being the most prominent examples.

CHIRALITY OF DENDRIMERS

Chiral dendrimers in general and their applications including chiral catalysis in asymmetric syntheses and chiral chromatography have been reviewed [120,121]. Dendrimers can have (i) a chiral core, (ii) chiral branches, (iii) chiral peripheral units and their combinations. Another possibility is a rigidly chiral conformation without possessing any stereogenic centres or chiral moieties.

Gel forming properties of lysine dendrimers have been studied by CD [122]. The chirality of the dendritic building blocks plays a crucial role. When using dendrons based on D-lysine, the resultant materials have identical thermal properties (e.g. T_{gel} values), but equal and opposite CD spectra, indicating the formation of identical fibrous assemblies but with opposite helicities. Small admixture of the 'wrong' enantiomer in the gel led to rapid destruction of the nanoscale chiral organization and reduction of the thermal stability of the macroscopic gel. Cryo-scanning electron microscopy revealed that the racemic gel had new nanoscale morphology – flattened woven ribbons. Investigation of dendrons with different chirality indicates that the effective organization of the chiral centers in the gel is an important prerequisite for anisotropic self-assembly. Molecular-scale features have a direct influence on both nanoscale self-assembly and macroscopic behavior.

Preservation of stereochemistry during the convergent and divergent synthesis of lysine MAPs has been monitored using polarimetry, NMR, and HPLC [123]. The chiral purity in the dendritic peptide was dependent on the method of synthesis. Divergent methodology affords lower racemization. Stereochemical considerations will be of key importance in the development of bioactive dendritic systems, for which the existence of the dendritic molecule as a single enantiomer will be desirable (pharmaceutical chemistry, enantioselective catalysis).

For chiral dendrimers see also Ref. 124 in section 'MAGs based on α,α-disubstituted β-alanine' and the section 'Cyclotriveratrylene-based dendrimers' [125] in Part I.

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CHARACTERIZATION OF DENDRIMERS

Characterization of dendrimers by different types of mass spectrometry, NMR, IR, Raman, UV–vis spectrometry, fluorescence, optical rotation, CD, X-ray difraction, small-angle X-ray scattering, small-angle neutron scattering, laser light scattering, different chromatographic and electromigration methods (electrophoresis, capillary electrophoresis), electron paramagnetic resonance, dielectric spectroscopy, differential scanning calorimetry, etc. have been reviewed [126,127]. Therefore, only some methods will be mentioned.

Electromigration Methods

Besides HPLC, capillary electrophoresis, capillary electrochromatography, and other electromigration methods [126–134] are indispensable both for purity assessment and for purification of peptides, glycopeptides, and glycopeptide dendrimers. These methods are 'orthogonal' to the most used RP-HPLC, i.e. they use other physical and physicochemical principles. It is important to use methods that differ in the separation principle and check their preparative (separative) efficiency by other analytical techniques: for example, purity by RP-HPLC and CE, preparative separation by HPLC and purity control by both HPLC and CE, or purification by free-flow electrophoresis and purity control by both HPLC and CE. Unfortunately, many authors check the final purity after separation by the same technique. It is a must to use also an 'orthogonal' method. Even by these steps it may be not possible to remove all by-products differing by only a single modification or deletion.

Some of the above-mentioned methods [133] use dendrimers as the pseudostationary phase in capillary electrochromatography. Dendrimers have shown better performance than conventional micelles. Therefore, dendrimers have a dual role, both as subject and object of separation, because they can serve as tools to analyze dendrimers.

Mass Spectrometry

The most important methodology for structure elucidation and determination of glycopeptides and glycopeptide dendrimers is mass spectrometry (FAB-MS, ES-MS, MALDI-TOF MS, ESI-MS, MS/MS tandem MS, QqTOF quadrupole-quadrupole time-of-flight, etc.) [126,135–141]. MS has been utilized to characterize the structure of peptide and glycopeptide mimics, MAPs, glyco- and glycopeptide dendrimers, and other constructs in the design of synthetic immunogens. This methodology determines not only the molecular weight (mass) of the product but shows also its purity. Mass spectrometry is not only an indispensable tool for determination of molecular identity but can also be used for studies of conformation and dynamics of biomolecules including glycopeptides [142].

Novel ESI-MS-based methods have been used for the determination of K_a values of anion complexation by glycocluster thioureamethyl calix [4]resorcarenes [143]. To the best of our knowledge, this is the first case where ESI-MS has been used to study anion complexation *quantitatively*. The linear relationship found between the square root of the intensity and the concentration of the formed host–guest complex allows direct determination of the K_a value by means of a titration experiment. Other methods based on competition experiments have been elaborated as well. Therefore it is an excellent method for rapid and quantitative determination of the complex behavior of a host toward a variety of guests.

It sometimes happens that it is not possible to obtain the correct MALDI-TOF MS spectra; see Part I for examples of MAGs [144,145].

Mass spectrometry is generally considered to be one of the very few methods, if not the only reliable one, to investigate dendrimers [140,146,147]. Comparison of ESI and MALDI mass spectra of different dendrimers showed 'fake defects' in the ESI and MALDI mass spectra. In the first case, ESI MS of poly(propyleneimine) dendrimers indicated a high abundance of new type of defects, which were found in neither ^{1}H or ^{13}C NMR spectra nor MALDI MS. As the second example, dendrimers with sulfonamide groups in their perifery were studied. Their ESI MS show high sample purity, while MALDI MS produces signals for defects that seem to be generated during synthesis. The true reasons are thermal reactions during ionization within the matrix and not synthetic problems. Therefore, mass spectral data on dendrimer purity must be evaluated and interpreted with care, keeping in mind that sometimes false negative data can be obtained.

Standard MALDI-TOF-MS has been used for molecular wieght determination of ultra-high mass compounds [148] such as PAMAM dendrimer generation 10 and immunoglobulin M.

Capillary electrophoresis-mass spectrometry has been used for glycoscreening in biomedical research [129]. Application of mass spectrometry in supramolecular chemistry, in particular molecular recognition and self-assembly including different types of dendrimers, has been reviewed [149]. Many examples for the use of mass spectrometry as a (mere) analytical tool for the determination of molecular weight data for noncovalently bound supramolecular aggregates have been given.

Time-resolved and Nonlinear Optical Spectroscopy

Time-resolved and nonlinear optical spectroscopy of organic dendrimers and branched chromophores

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[150,151] has been reviewed. The nonlinear optical and excited-state dynamics of different dendrimers and other branching chromophore structures have been studied. Methods of time-resolved fluorescence, two-photon absorption, transient absorption, and three-pulse photon echo peak shift were used to study the degree of intramolecular coupling in dendrimers. The aspects of intramolecular interactions in dendrimers and other branched systems were discussed in connection with enhanced nonlinear optical effects and their use in modern optical devices. These methods can be applied also to glycopeptide dendrimers.

PHYSICOCHEMICAL PROPERTIES OF DENDRIMERS

Linear chains in solution exist as flexible coils. In contrast, dendrimers form a tightly packed ball. This fact has a great impact on their rheological properties [49,152–156]. Dendrimer solutions have significantly lower viscosities than linear polymers. With increasing molecular mass, the intrinsic viscosity of dendrimers increases to a maximum at the fourth generation and then begins to decline. This differs from classical polymers, the intrinsic viscosity of which increases continuously with molecular mass. In general, dendrimers can be prepared to about G10, with maximum diameters of 10 nm. The exponentially increasing mass of the higher generations of dendrimers cannot fit with their linearly expanding spherical diameter and the process of growth stops [157].

Comparison of thermosensitive properties of PAMAM dendrimers with peripheral *N*-isopropylamide groups and linear polymers with the same groups has been studied [158]. Marked differences exist in transition entalphy, hydrophobicity, and sensitivity to urea. These properties, together with the globular shape of the thermosensitive dendrimers, could find future use as intelligent nanocapsules for drug delivery and catalysis.

The molecular weight of common dendrimers (e.g. G6-PAMAM) is about 50% of that of a protein with comparable molecular size, e.g. ovalbumin. This phenomenon has two main reasons: The tree-shaped molecular structure of dendrimer has a lower molecular density compared to a native protein. Proteins have higher molecular density owing to the ability to tightly fold the linear polypeptide chain to a three-dimensional structure. This folding is caused by disulfide cross-linking, ion-pairing, as well as hydrogen and hydrophobic bonding. Dendrimers are more compact molecules with a smaller hydrodynamic volume compared to linear polymers. Physicochemical properties of dendrimers in comparison to various biological entities have been reviewed [159,160].

Various types of dendritic systems that form liquid crystalline mesophases have been reviewed [161] including the way they are organized within supramolecular structures. The tuning of the mesomorphic structure by a suitable molecular design enables the development of new liquid crystalline materials to be used in nanotechnology.

Different types of dendrons and dendrimers (bearing the catalytic units on the perifery) immobilized on solid supports were used in the field of heterogeneous catalysis [162]. They have found application in hydroformylation, Heck reaction, olefin epoxidation, Paulson–Khand reaction, and enantioselective reactions. Positive dendritic effects have been observed in the majority of the aforementioned reactions.

Homogeneous catalysis with catalytic groups either at the core or at the periphery of a dendritic support [163] leads to unique properties that affect rates of reaction, substrate specificity, selectivity, activation, etc. When advantageous, these properties are termed 'positive dendrimer effect'.

For solubility of dendrimers and their use in drug delivery, see section 'Biomedical applications of dendrimers'.

STABILITY OF DENDRIMERS

In order to achieve the desired activity it is important to know and respect the needed glycotopes, sterical demands, chemical composition and structure of branches (core), etc. This affords a molecule of predetermined conformation, polarity, charge, solubility, and other physicochemical properties. The fulfilling of all these demands can still lead to very disappointing results, owing to too high (or too low) biological and chemical stability. It is necessary to distinguish the stability of the whole molecule and the stability of the epitope or other biologically active compound bound to the dendrimer. When the dendrimer serves as a transport and delivery system, [41,50,53,56,59,60,74,97,164–173,] it is preferable that the stability of the bond between the active principal and the dendrimer is relatively low, so that the drug is released in reasonable time, but sufficiently high that the drug will be released at the target place and not during the transport process. In the case of the cluster effect, the stability should be high because the released monomers are a few orders of magnitude less active than the parent dendrimer. It is therefore necessary to study and understand the following stabilities: biochemical, enzymatic, and chemical stability of the active principal (both free and dendrimer-bound), bond between the drug (epitope) and dendrimer, stability of branches and core, polarity, solubility, penetration through membranes, and others. In many cases, these demands are contradictory. It is difficult to make some general guidelines because the stability depends on the structure of the whole molecule, the given model, and the methods used, and unfortunately in most cases it is not possible to transfer the data obtained to other

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molecules, models, and activities (see the reviews cited above).

The nature of the bond between peptide and carrier molecule determines the immunogenicity of the construct. As types of bonds, thioester, disulfide, amide, and thioether bonds were investigated [174]. As carrier molecules, a peptide, an *N*-palmitoylated peptide, or a C_{16}–hydrocarbon chain was used. The biostability of the peptide–carrier bond is in the order thioether > amide > disulfide > thioester. However, the immunogenic potency of the constructs used was found to be in the order thioester > disulfide > amide > thioether. Therefore the more labile bond affords higher immunogenicity and vice versa.

The use of dendrimers as drug carriers requires quantitative understanding of the structure–stability relationships of dendrimer-peptide in order to improve drug delivery. Enzymatic barriers represent a major problem that limits the medical and pharmaceutical applications of peptides. The *in vitro* enzymatic stability of different PAMAM-peptide dendrimers has been studied [175] with α-chymotrypsin in pH 7.4 PBS buffer containing 5% DMF. The enzymatic stability of dendritic peptides was in the order peptide-PAMAM-PEG > peptide-PAMAM > free peptide > peptide-PEG-PAMAM. Changing the coupling sequence of peptide, PEG, and the PAMAM dendrimer can vary the enzymatic stability of peptide dendrimers. The ratio of PEG/peptide and coupling sequence of peptide, PEG, and dendrimer can be optimized for maximizing peptide loading and maintaining the enzymatic stability of the peptides. PEG dendrimers have minimal toxicity, reduced immunogenicity, and excellent solubility in both aqueous and most organic solvents. The stability considerations (including experimental verification) should be done before the synthesis of any peptide or glycopeptide dendrimer.

Characterization and stability of PAMAM-dendrimer prodrugs with naproxene have been studied [176]. Hydrolysis of the conjugates was measured at 37 °C in hydrochloric acid buffer (pH 1.2), phosphate buffer (pH 7.4), borate buffer (pH 8.5), and 80% human plasma. Naproxene conjugate with G0 dendrimer by direct amide linkage showed high chemical and enzymatic stability and therefore was not suitable for the development of prodrugs. When lactic acid ester linkage was used to attach naproxene to G0 PAMAM, naproxene was slowly released from the conjugate. This system is promising for drug targeting. Diethylene glycol linker proved to be of high chemical stability in buffers, but readily released naproxene in plasma.

Owing to the inherent propensity for chemical and enzymatic degradation of *O*- or *N*-linked glycoconjugates, carbon-linked conjugates have received great attention. Different *C*-glycoconjugates (mannose, galactose, glucose) with derivatives of Asp, Cys, Lys, Ser, Thr, Glu, Tyr, Phe, Trp, β-amino acids and with unnatural spacing between the carbohydrate and peptide moieties have been reviewed [177]. Application of these *C*-glycoconjugates in dendrimeric form would lead to metabolically and chemically stable compounds.

DENDRIMER BIOCOMPATIBILITY AND TOXICITY

Biocompatibility (or toxicity) of PAMAM dendrimers has been reviewed [49,51,59,157,165,178–183]. Dendrimers have to exhibit low toxicity and be nonimmunogenic in order to be widely used in biomedical applications. It is difficult to choose the right word. The term 'toxicity' is used in pharmaceutical industry to describe unwanted side effects to cells, organs, or the patient. The term 'biocompatibility' is applied in the area of biomedical materials and their applications as a measure of their compatibility with the given organ or organism. More compatibility equals less toxicity and vice versa. At a consensus conference of the European Society for Biomaterials in 1986, biocompatibility was defined as 'the ability of a material to perform with an appropriate host response in a specific application' [178,184]. Therefore, the need to define a material 'biocompatibility' only in the precise context of its use is stressed. When dendrimer is used as a component of a biomedical material, the biocompatibility of the material must be defined. It is not possible to define any dendrimer chemistry as nontoxic or biocompatible without detailed knowledge of the intended precise use. The so-far-limited clinical experience using dendrimers makes it impossible to designate any particular chemistry intrinsically 'safe' or 'toxic' [178].

PAMAM and poly(propylene imine) dendrimers that form cationic groups at low pH are generally hemolytic and cytotoxic. Their toxicity is generation dependent and increases with the number of surface groups. Anionic dendrimers bearing a carboxylate surface are not cytotoxic over a broad concentration range [49,50]. PAMAM dendrimers of equivalent surface functionality are slightly less toxic than poly(propyleneimine) dendrimers with the same number of surface groups. Biodistribution studies of PAMAM dendrimers *in vivo* have shown that cationic dendrimers were cleared rapidly from the circulation after i.v. and i.p. administration. The circulation times of anionic PAMAM dendrimers were longer and generation dependent, and the lower generations circulated longer.

The reported data for PAMAM dendrimers are contradictory [180]. Low acute toxicity of PAMAM in experiments with rats has been found, but this class of compounds is used in cell culture transfection. This implies that PAMAM change the structure of cell membranes and can induce their damage. In a detailed study [178,185], all generations of the NH_2-terminated PAMAM showed cytotoxicity in concentration of 50–300 μg/ml, depending on the denticity. Low-dentate dendrimers circulate in the blood flow

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longer than high-dentate ones, which are retained in the liver. The distribution of PAMAM modified by biotin in mice has been studied [186]. Tetravalent dendrimers are excreted almost entirely with urine; higher generation of PAMAM dendrimers were excreted more slowly.

Biological properties (cytotoxicity, haemolysis, genotoxic effects, and interactions with model lipid membranes) of low-molecular-mass lysine-based peptide dendrimers with antibacterial activity have been studied [187,188]. The most toxic in all tests were dendrimers with protected ε-amino groups. Derivatives with the α-amino group protected were much less toxic. The steric distribution and type of hydrophobic groups and cationic centres are important components of dendrimeric structure and influence both toxicity and antimicrobial potency.

It has been reported [189–191] that lysine dendrimer G6 showed no significant toxicity to cultured cells compared to PAMAM dendrimer, has no acute hepatic toxicity, and i.v. administered lysine dendrimer G6 was rapidly eliminated from the circulation (mice) within a few minutes.

With some simplification, the toxicity of dendritic structures depends on the polarity and charge of the surface [60,192]. Most toxic, both *in vivo* and *in vitro*, are positively charged dendrimers. On the other hand, anionic dendrimers and noncharged polar dendrimers (PEG-dendrimers) are in most cases not toxic. PEGylated dendrimers [193] have two main advantages: (i) solubility increase and (ii) lowering toxicity by shielding the peripheral amino groups. Other advantages are altering biodistribution and pharmacokinetics, improved utilization as drug delivery systems, etc. Special cases are dendrimers with noncharged apolar (lipidated) surface, which show *in vitro* toxicity, but the *in vivo* toxicity is low. Their advantage is good biopermeability.

The particle size is important also from the point of view of biodistribution, clearance, and toxicity. Nanoparticles in general, including dendrimers [194] and their toxicity with respect to particle size have been reviewed.

BIOMEDICAL APPLICATIONS OF DENDRIMERS

Many biomedical applications of dendrimers have been described: drug delivery systems [41,50,51,56,59,60,164,165,167–170,172,173,182,183,192–202], gene delivery [74,166,172,197,203–205], imaging and contrast agents [31,48,51,53–63,181], antivirals [206–209], microbicides [206,207,210–214], intervention with bacterial adhesion (antiadhesion strategy) [210,212], synthetic vaccines [215–218], artificial enzymes [97,202,219,220], artificial viruses, and synthetic nonviral vectors [59,203,221–224]. Dendrimers can serve as biomimetic artificial proteins [51,97,225], nanoscale containers [51,152,167], gene transfection agents [51,59,182,224,226–228], etc.

PAMAM dendrimers can be used for targeted delivery of an apoptotic sensor to cancer cells [229] and for imaging tumor angiogenesis [62].

Glycodendrimers are also powerful inhibitors of bacterial adhesion [210]. Very significantly, lectin-inhibitory glycodendrimers have been shown to protect mice, rabbits, calves, and monkeys against experimental infection by lectin-carrying bacteria.

Defined architecture and a high ratio of multivalent surface moieties to molecular volume make dendrimers highly interesting for the development of synthetic (nonviral) vectors for therapeutic nucleic acids. The role of dendrimers in gene delivery has been discussed [74,166,204,205,230] including gene therapy, biological properties *in vitro* and *in vivo*, dendrimers as synthetic vectors, experimental therapy and *in vivo* gene expression, etc.

The topic of dendrimer–drug interactions has been reviewed [50,59,60,164,165,168–173,182,183,197] including the impact on drug delivery.

Nonsteroidal antiinflammatory drugs (NSAIDs) are among the most frequently used drugs in the world. The ability of PAMAM dendrimers to facilitate transdermal delivery of NSAIDs was studied [231], using Ketoprofen and Diflunisal as model drugs. Blood drug level studies revealed that the bioavailability was 2.7 times higher for the Ketoprofen–PAMAM dendrimer complex and 2.5 times higher for the Diflunisal–PAMAM dendrimer complex than the pure drug suspensions. This can open the way to new transdermal drug formulations with dendrimers as the skin penetration mediator.

Dendrimers play important role in solubility enhancement [156,183,193,196,201,232]. The solubility is a limiting factor of drug delivery. Dendrimers were used to increase the solubility of Indomethacin, Paclitaxel, Methotrexate, Adriamycin, Diclofenac, Ibuprofen, Naproxen, Ketoprofen, Flurbiprofen, Niclosamide, 5-fluorouracil, Paclitaxel, Adriamycin, methotrexate, and many others.

Comparison of the aqueous solubilization of practically insoluble niclosamide by PAMAM dendrimers and cyclodextrins has been studied [233]. Niclosamide ((5-chloro-*N*-2-chloro-4-nitrophenyl)-2-hydroxybenzamide) is an anthelmintic drug that is active against most tapeworms, including the beef tapeworm, the dwarf tapeworm, and the dog tapeworm. However, problems are associated with the preparation of successful niclosamide formulations; the most severe is that this drug is practically insoluble in water. In binary mixtures, the addition of PAMAM G3 increased the aqueous solubility the most (6178 times), followed by PAMAM G2 (1945 times), PAMAM G1 (1354 times), PAMAM G0 (372 times), and hydroxypropyl-β-cyclodextrin (10 times). The solubility increased exponentially with the generation size from G0 to G3.

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COMPARISON OF DIFFERENT MODEL SYSTEMS

Different model systems with high valency, i.e. glycoclusters, cyclodextrins, calixarenes, dendrimers, neoglycoproteins, soluble polymers, immobilized polymers, hybrid bilayers on glass or gold, self-assembled monolayers, etc., have been studied and compared from the point of view of valency, stability, homogeneity, synthetic effort, and ability to be tested by enzyme-linked lecitin assay, hemagglutination inhibition assay/isothermal titration calorimetry/afinity capillary electrophoresis, and surface plasmon resonance [234].

It is difficult to compare the advantages and disadvantages of the given structures because they differ in spatial arrangement, polarity, structure, composition, distances between the branches, saccharides used, valency, animals and models used for testing the given activity, etc. No studies with only one to two different parameters have been done.

Recommended Literature on Dendrimers in General

A number of excellent reviews have been published on dendrimers in general [126,152–154,159,160,226, 234–238], MAPs [225,239–243], MAGs and glycodendrimers [110,180,244,245], carbopeptides [119,246], and their biomedical applications [51,74,173,181,192, 200,202,247].

CONCLUSIONS

Important conclusions for glycopeptide dendrimer design and biological activities are as follows: (i) optimal biological activities are usually achieved with dendrimers of moderate valency (4, 8, etc.) rather than with high-generation dendrimers that have reached their 'starburst limit' (sterical reasons); (ii) small structural changes in the core or branches can strongly affect the binding affinities; (iii) the geometry and size of the binding site must be respected and its knowledge is important; (iv) no universal optimal valency exists.

In many cases the prepared dendrimeric structures are more a result of an intelectual game to prepare some unusual compounds with new cores, branches, etc., than an exact approach based on the knowledge of size, shape, polarity, and other parameters that must be fulfilled to satisfy the strict demands of the given receptor. Paradoxically, this approach sometimes gives better results than the exact one. Owing to the unlimited variation of structures and biological applications, it is very difficult to make some general conclusions. In some cases, 4-valent dendrimers give the best results, in others the 32-valent ones are the best. The question of valency could be partly deciphered by the knowledge of the tertiary structure and polarity of the active site or receptor. The other one is a 'blind man in a labyrinth' approach. The synthetic chemist prepares dendrimers of the same type with different valencies and the tests give the answer about the best valency. Both approaches are possible, the second one more often. The structural diversity leads to an unlimited number of different dendrimers that are used for many applications. For a really valid comparison, only one parameter can be changed. Keep in mind that the biological activity tested and the methods used for its evaluation are also parameters. It would be great when scientists worldwide could partly coordinate their effort and choose some easily reproducible and internationally respected tests for the given biological activities and compare, e.g., one defined epitop on a carrier of the same valency (e.g. tetravalent MAP, RAFT, SOC, etc.), and test it by the same method. Only then the results will be of real information value.

As it can be seen in the tables in Part I and Part III, most papers were devoted to lectins and their interactions with saccharides, where glycopeptides served as inhibitors. These studies are important for better understanding how viruses and bacteria adhere to cells starting the infection process. Drug delivery systems and imaging agents (contrast agents) represent a second group of dendrimeric compounds with direct impact in medicine. A third, most-frequented topic is tumor-associated carbohydrate antigens and their use in cancer diagnostics and development of antitumor vaccines and vaccines against HIV and influenza (see Part III).

The most often used dendrimers were PAMAM and MAGs. The most often used methodology of glycopeptide dendrimer synthesis is SPPS or ligation strategies, which are carried with freely soluble, prepurified fragments in solution. Therefore, the purification and characterization of the ligated products are much easier than earlier. All these developments lead to defined, pure products and therefore unambiguous results can be achieved. MAGs are promising compounds in human medicine, including treatment of malignant diseases, protection against microbial and viral infection, synthetic vaccine development, transfer and delivery systems, and many others.

We propose the term 'dendrimerology' as an interdisciplinary science about all types of dendrimers, including their synthesis, physicochemical properties, purification, structure elucidation, and application in physics, chemistry, and especially biology and medicine.

Till now, more than 10 000 papers have appeared in the dendrimer field; currently about 1000 papers and 150 patents are published annually. Dendrimers are successfully expanding to all areas of bioorganic chemistry, biological and medical applications, nanotechnology, and many other fields. We can say that the area of dendrimers is fastly dendrimering.

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