Reviews

Dendritic polymers in glycobiology*

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Approaches to the synthesis of glycodendrimers, monodisperse and polyvalent models used to study molecular recognition processes, and their biological properties are considered.

Key words: glycodendrimers, conjugates, carbohydrates, polylysine, blocking agents, polyaminoamides, polypropylenimine (PPI).

Introduction

Cell recognition processes often involve cluster structures. Examples of such contacts are the interaction of macrophages with bacteria or other pathogens, binding of polyvalent immunoglobulins to cell surfaces and virus pathogens, resulting in the formation, on the membrane surface, of aggregates, immune complexes, and other structures that trigger cascades of physiological reactions.[1] The understanding of the mechanisms of reactions involved in the recognition, binding, and transport processes opens up the way for controlling these processes and for directed design of new drugs. Studies of these mechanisms are impossible without employment of so-called synthetic molecular probes, which reproduce, to some extent, properties of natural clusters formed on the membrane surface.

Glycoconjugates based on linear polymer carriers, which enable effective binding to complementary protein receptors, have found application as synthetic models in bioanalytical studies of physiological processes. Carbochain polymers are most available. The polyvalence of such conjugates substantially strengthens the binding compared to the monovalent ligand—receptor interaction. This effect is often more pronounced than it would be expected taking into account only the valence of conjugates. This increase in the affinity has been called the "cluster effect."[2,3]

The increase in the efficiency of the glycoconjugate binding by optimization of their structures requires controlling the local concentration, accessibility, and spatial arrangement of carbohydrate residues. However, when glycoconjugates based on linear polymers are used as molecular probes, it always remains unknown what is the mutual arrangement of the ligands, how many and what particular ligands are recognized by the corresponding receptors, and what of these are shielded by the polymer

* Dedicated to Academician Nikolai Konstantinovich Kochetkov, the founder of the Russian school of carbohydrates on the occasion of his 90th birthday.

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chain or neighboring ligands and are thus inaccessible for interaction. In some cases, this not only hampers correct interpretation of the results of biological experiments but also restricts the scope for further optimization of the glycoconjugate structure for the design of more active compounds. It should also be noted that such glycoconjugates based on linear polymer carriers are markedly cytotoxic;[4] they are slowly excreted from the body due to the lack of enzymes that decompose carbochain polymers. For the therapeutic use, the materials should possess low acute and chronic toxicities and low inherent immunogenicity (allergenicity) and also have a time of circulation in the blood sufficient for the therapeutic effect to be manifested.

In view of the above requirements, dendrimers representing a new class of highly branched polymers have been intensively studied in recent years as polymeric carriers for the design of bioconjugates, including glycoconjugates. Unlike natural proteins or synthetic linear polymers, the dendrimer molecules have a nearly spherical shape, a regular structure, low proper immunogenicity, and moderate toxicity. The location of functional groups strictly at the chain ends and their outward orientation open up extensive opportunities for the controlled variation of the structures of dendrimer matrices and conjugates based on them.

Dendrimer types. Recently considerable attention of researchers engaged in organic, polymer, and coordination chemistry has been attracted by highly branched polymeric and oligomeric molecules, both symmetrical and nonsymmetrical. The modern concept of branched three-dimensional molecules appeared in the early 1940s when the notions of a branched structural unit and a combination of these units linked by chemical bonds were defined.[5,6]

It has been suggested[5] that a highly branched polymer rather than a cross-linked or a polycyclic polymer can be prepared in a highly functional system. Subsequently,[7] a statistical model of these structures that simplifies the earlier approach and a graphical dendrite model (Fig. 1) have been developed. The dendrite theory was combined with the mathematical cascade theory and used subsequently to describe high-molecular branched structures.

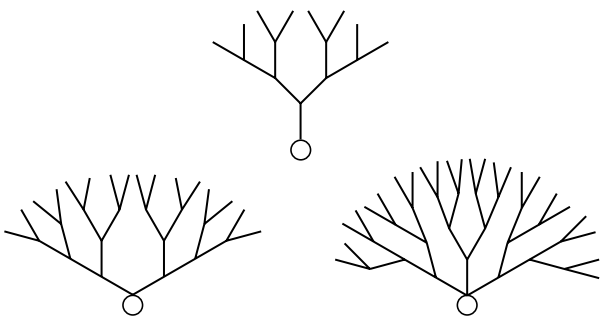


Fig. 1. Graphical views of dendrimers of various generations.

The existing types of dendrimers can be divided into two classes, those with a symmetrical (with identical structural fragments attached to the branching point) and an nonsymmetrical monomer unit. Although nonsymmetrical dendrimers (polylysine) were the first to be prepared due to ease of the synthesis, dendrimers with a symmetrical unit are much more interesting. Only these can form molecules with a dense outer surface coated by functional groups and a shape resembling most of all the shape of natural globular glycoproteins.[8,9] For steric reasons, the defect-free growth of this symmetrical dendrimer is not infinite and reaches a limit at a definite length of the monomer unit, the number of branching points, and the size of the terminal group.[10]

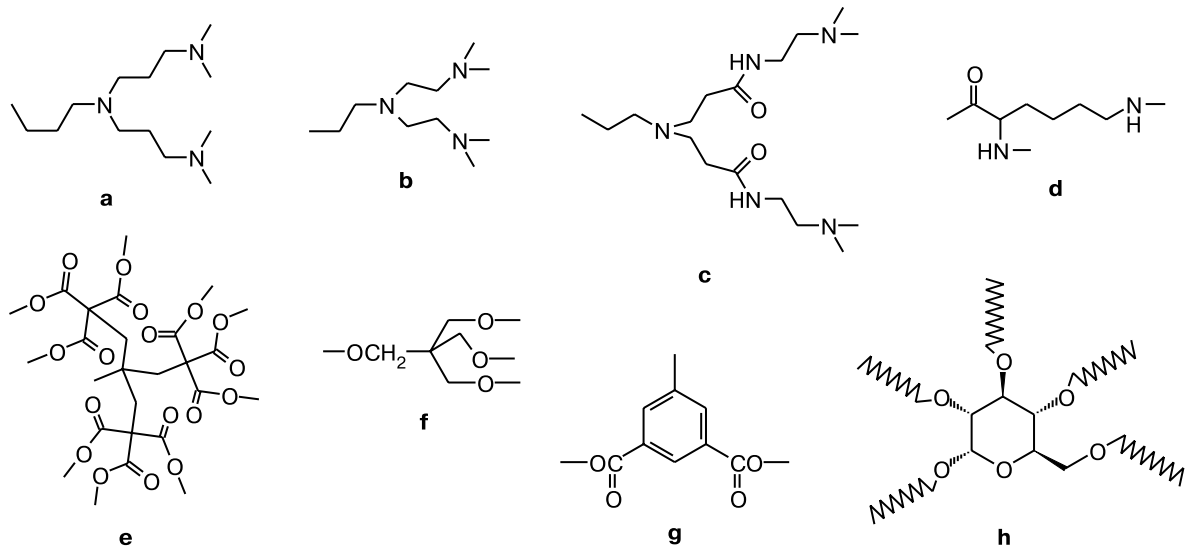
Regular dendrimers are synthesized using divergent and convergent strategies. Although the differences between these approaches are arbitrary, the vast majority of dendrimers are prepared using the divergent approach.

Synthesis of dendrimers requires high selectivity in each stage, high degree of conversion of the terminal groups, and efficient methods for the separation of products from the initial reactants and side products. The size, the topology, and the geometry of dendrimers depend on several parameters, which are purely molecular characteristics of the monomer they are built of. Thus, the macroscopic parameters of the dendrimer are controlled at the molecular level during its synthesis.

A diversity of structural fragments with branching sites at the nitrogen or carbon atom or at the aromatic ring are used as the monomer units of dendrimers. The following classes are encountered: polypropyleneimines (**a**),[11—14] polyethyleneimines (**b**),[15] and polyaminoamides (**c**)[16—18] with N-branching points; polyamides with C-branching points (**d**),[19—25] polyesters and polyethers with C-branching points (**e**, **f**),[26] and polyamides with aryl- (**g**)[27,28] or carbohydrate-branching points (**h**).[29,30]

The term glycodendrimers is used to mean carbohydrate-containing dendrimers either completely built of carbohydrate residues (**h**) or containing carbohydrates only attached to the terminal groups of the matrix dendrimer. In this review, exactly the latter type of dendrimers "coated" by carbohydrate residues is called glycodendrimers.

Of the dendrimers listed above, polylysines, polyaminoamides, and polypropyleneimines are the most readily available, well-studied, and defect-less polymers. Due to the extensive opportunities for the modification of their terminal functional groups and low toxicity, these compounds can be considered to be promising candidates for the synthesis of conjugates and their investigation as multivalent inhibitors.



Synthesis of glycoconjugates based on dentritic matrices and their activities in model experiments

The development of the chemistry of dendritic molecules allowed the transition from studying their properties and methods of synthesis to the study of their use in related branches of science. Specific properties of dendrimers are successfully used in the design of biomimetics, immunochemistry, genetic engineering, and other biomedical fields. The types of dendrimers used are determined, first of all, by their availability, reactivity, solubility, and biocompatibility.

Glycoconjugates based on polylysine dendrimers. Studies of glycoconjugates with the dendritic polylysine were preceded by the synthesis of conjugates with peptide ligands, which were called multiple antigenic peptides (MAP).[31] The synthesis of these compounds did not require development of special chemical methods. The branching site was represented by L-lysine with two Boc-protected amino groups. Its activated *p*-nitrophenyl ester fitted well into the standard protocol of automated peptide synthesis, which resulted in dendritic polylysines; then the peptide antigens were synthesized attached to the terminal amino groups.

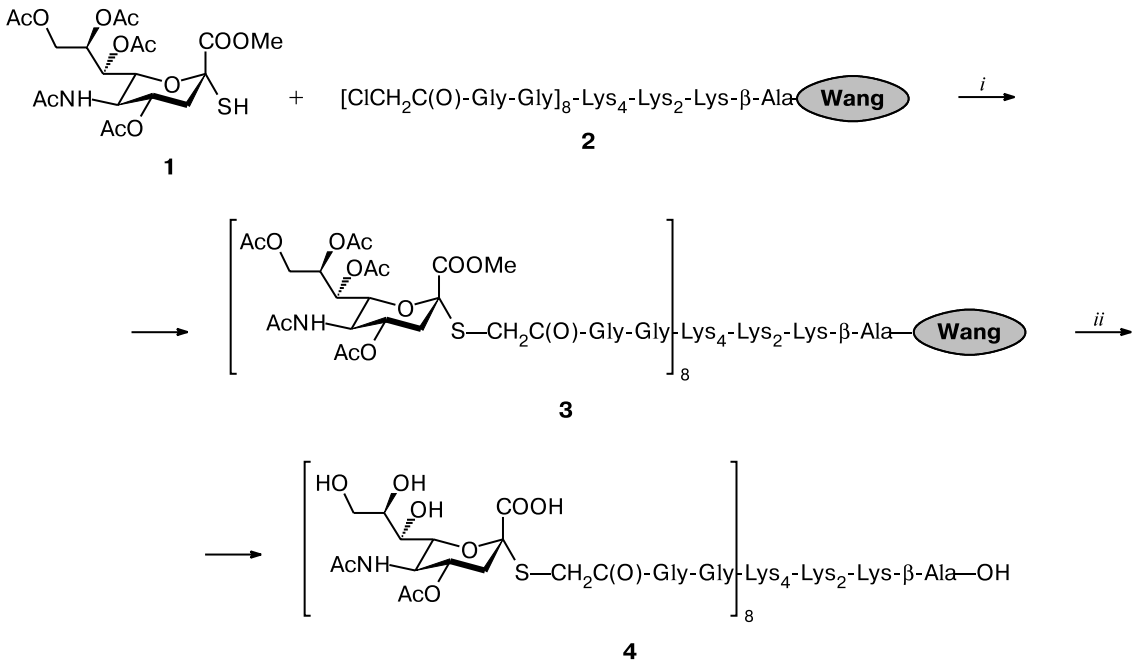
Study of their biological properties has shown that immunization of laboratory animals with MAP stimulates the production of specific antibodies, which is unusual for low-molecular-weight antigens and, hence, the MAP samples can be considered as prototypes of artificial vaccines. Subsequently, the interest in the MAP has not faded away, but the development of methods for their preparation is limited by the possibilities of the solid-phase peptide synthesis. However, the studies of MAP have stimulated the research into similar conjugates with nonpeptide ligands.

Syntheses of various glycopolylysine dendrimers bearing β-glucosamine,[32] β-lactosamine,[33] α-mannose,[34,35] sialic (*N*-acetylneuraminic) acid,[36,37] and a more complex carbohydrate ligand, the SLe^x tetrasaccharide,[38] have been reported. These studies do not present data on the biological activity of the obtained compounds; for some of them, only the ability of dendritic glycopolylysines to mimic natural glycoconjugates was demonstrated. For instance, branched glycoconjugates containing 8 and 16 terminal α-mannose residues bind to concanavalin A and lectin from *Pisum sativum* in 4- to 86-fold lower concentrations[34] than mannose.

N-Acetylneuraminic acid plays an important role in various inflammatory processes and in the pathogenesis of influenza.[39–41] These natural processes are simulated by appropriate glycoconjugates, which react with either purified carbohydrate-binding lectins or natural pathogens.

Glycoconjugates based on dendritic polylysine and sialic acid (sialopolylysines) were synthesized as molecular probes to study the topology of hemagglutinin (HA)-mediated binding of the influenza virus to carbohydrate ligands. A series of 2-, 4-, 8-, and 16-dentate glycodendrimers were prepared to investigate the inhibitory activity of sialopolylysines.[42] The polylysine matrix was obtained by the solid-phase peptide synthesis with fluorenylmethoxycarbonyl (Fmoc) protective groups on the Wang carrier. Fully protected thiol **1** was conjugated with chloroacetylated dendritic polylysyl polymer **2** (Scheme 1). The protected glycoconjugate was cleaved from the glycopeptidyl polymer **3** by treatment with 95% TFA and deprotected by treatment with 0.05 *M* NaOH. The data from NMR spectroscopy and FAB mass spectrometry were reported only for the bidentate sialodendrimer.

Scheme 1



Reagents and conditions: *i*. DMF, 1% Et_3N, 25 °C, 16 h; *ii*. (1) TFA (95%), (2) 0.05 *M* NaOH.

The activity of the conjugates of the type **4** was studied by enzyme-linked lectin assay (ELLA, which is a method of analysis similar in the technique to the enzyme-linked immunosorbent assay (ELISA) but with labeled carbohydrate-binding proteins or lectins instead of immunoglobulins) using the peroxidase-labeled wheat-germ agglutinin specific to neuraminic acid.[37] All sialodendrimers were much more active than the monomeric α-thiosialoside and inhibited the interaction of lectin with the immobilized polyacrylamide conjugate of α-thiosialoside in a ~10^6 times lower concentration. The IC_{50} values somewhat decreased with an increase in the thiosialodendrimer denticity; for the 2- and 16-dentate analog of **4**, the IC_{50} values were 40 and 10 nmol L^{-1}, respectively. A more pronounced dependence of the glycodendrimer activity on the denticity was revealed in experiments on the inhibition of erythrocyte hemagglutination by the X-31 strain of the influenza A virus. In this test, the IC_{50} values for bi- and hexadecadentate analog of **4** were 625 and 91 μmol L^{-1}, respectively, while that for monomeric thiosialoside is 3 mmol L^{-1}.

It is evident that the reaction of glycoconjugates with several centers of the HA trimer or several trimers simultaneously should entail an increase in the binding energy and a decrease in the corresponding IC_{50} values. However, the maximum possible distances between the carbohydrate residues of the 2—16-dentate α-thiosialodendrimer molecules of the type **4** do not ensure an efficient interaction with several HA subunits. Therefore, one can assume that their high inhibitory activity is due to other reasons. Most probably, the change in the activity is due only to an increase in the effective concentrations of the ligand near the binding site. In addition, due to the enhancement of the amphiphilic properties of the lysine-α-thiosialodendrimer molecules with an increase in the denticity, aggregates and micellar solutions may form in which the distances between the *N*-acetylneuraminic acid (Neu5Ac) ligands are greater than in α-thiosialodendrimer molecules.

Glycodendrimers based on polyaminoamide and polypropyleneimine matrices. A readily available class of dendrimers includes those dendrimers based on polypropyleneimine (PPI)[13,43] and polyaminoamide (PAMAM).[15] Highly dentate samples of these dendrimers can be prepared in gram quantities and in recent years they have become commercially available.

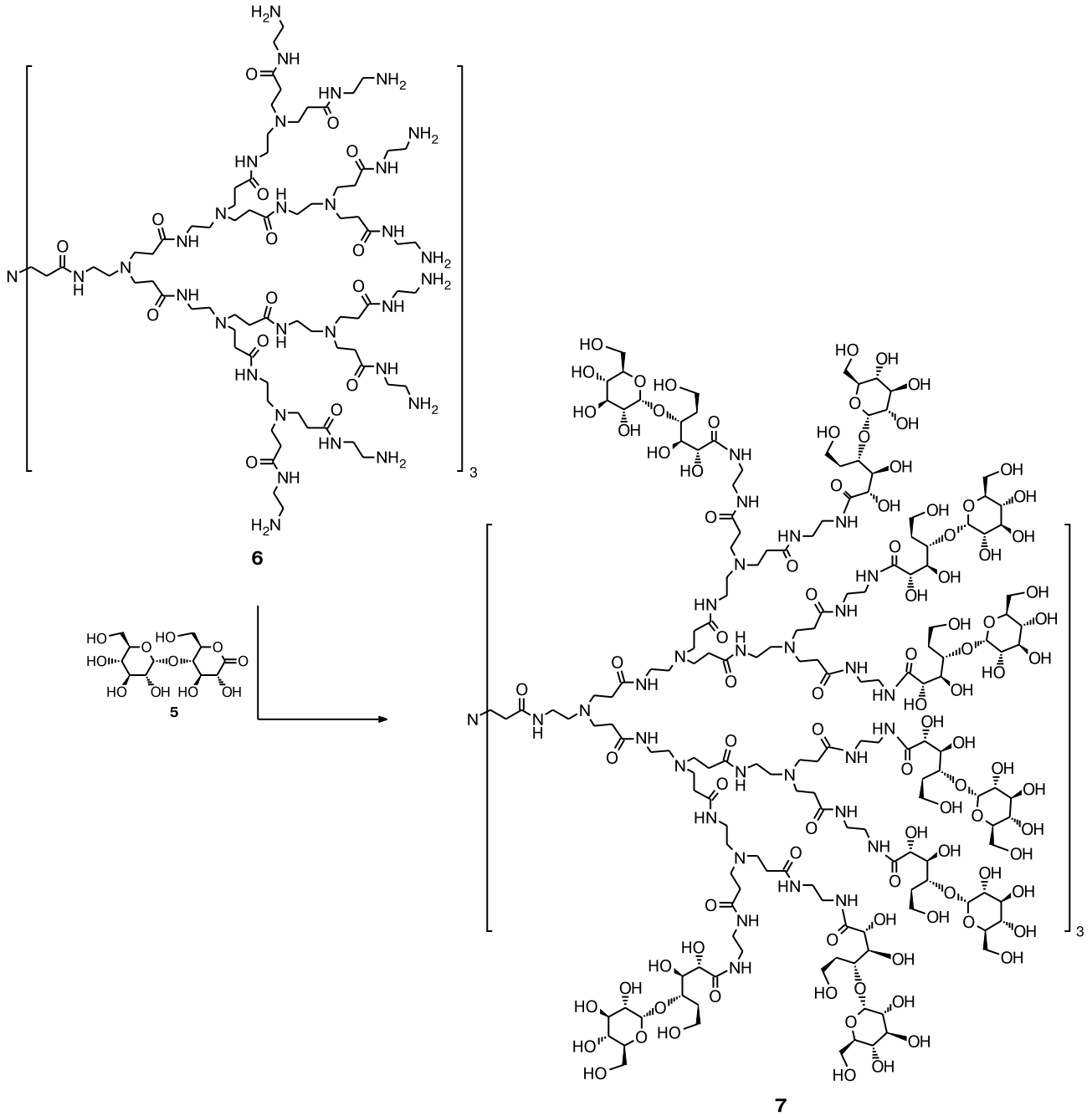
Synthesis of glycodendrimers based on PAMAM has been studied by several research groups. For example, a number of PAMAM-based clusters with a denticity of two to eight bearing α-D-mannose, β-D-galactose, β-cellobiose, and β-lactose residues have been prepared.[44] These compounds were characterized by ^1H and ^{13}C NMR data. The conjugation with the PAMAM amino groups was carried out by the isothiocyanate method. An advantage of this method is the possibility of using synthetic amino-spacered oligosaccharides for the attachment to amino-terminated dendrimers, while the use of toxic thiophosgene during the isothiocyanate synthesis is an obvi-

ous drawback. The activity of the resulting conjugates was not indicated.

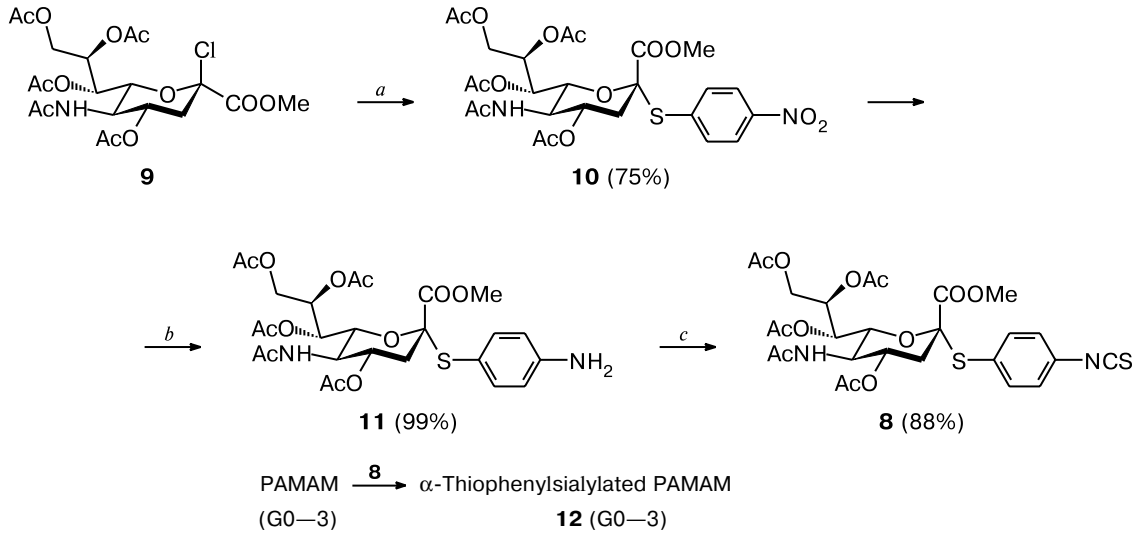
Synthesis of larger glycodendrimers based on PAMAM bearing each 12, 24, and 48 glucose or galactose residues has been described.[45] The conjugation to the terminal NH_2 groups of the matrices was accomplished by opening aldonolactones of the type **5** prepared by oxidation of lactose and maltose. Scheme 2 presents an example of the preparation of 24-dentate glucosyl dendrimer **7** from the appropriate PAMAM **6** and 4-*O*-α-D-glucopyranosyl-glucono-1,5-lactone **5**. Evidently, this conjugation technique is applicable only to readily available carbohydrate ligands.

The difference between binding of concanavalin A by glucose- and galactose-containing glycodendrimers (type **7**) bearing each 24 carbohydrate ligands was demonstrated by turbidimetry. The high affinity of binding was confirmed by competitive inhibition. The reaction of

Scheme 2



Scheme 3



Reagents and conditions: *a*) $HSC_6H_4NO_2$, EtOAc, 1 *M* Na_2CO_3, NBu_4HSO_4, 45 min; *b*) EtOH, $SnCl_2$, Δ; *c*) CH_2Cl_2, $C(S)Cl_2$.

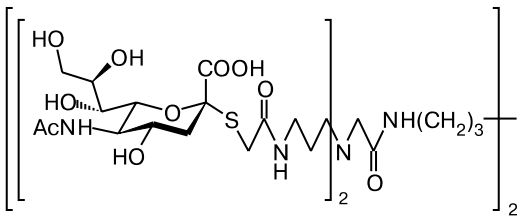
24-dentate glucosyl dendrimer **7** with lectin in the presence of a 24-fold excess of D-glucose was found to be decreased only slightly, the reaction of **7** with lectin being fuly terminated only when the molar excess of glucose increases to 1200-fold.

Attempts to prepare a molecule with an enhanced density of carbohydrate ligands on the glycodendrimer surface by PAMAM dendrimer-initiated polymerization of glycosyl-α-amino acid *N*-carboxyanhydrides have been undertaken.[46] Polymerization was carried out at room temperature for 24—80 h. Block copolymers with an average degree of polymerization of 3—4 and polydispersity of ~1.1, comparable with that for usual linear poly(amino acids) were synthesized. The biological activity of the resulting copolymers has not been studied.

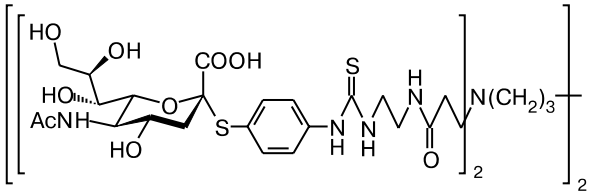
Yet another group of sialylated conjugates based on PAMAMC has been synthesized.[47] Isothiocyanatophenylthioglycoside **8** was used as the ligand. It was prepared by the reaction of peracetylneuraminosyl chloride **9** with 4-nitrothiophenol under phase transfer catalysis conditions (Scheme 3) followed by the reduction of the nitro group to the amino group (**10** → **11**) and final treatment with thiophosgene, resulting in isothiocyanate **8**.[48]

The conjugation of ligand **8** with commercial 4-, 8-, 16-, and 32-dentate PAMAM of generations 0—3 (G0—3) was carried out in CH_2Cl_2 or DMF for 18 h. The alkaline *O*-deacetylation yielded sialylated glycodendrimers **12**, which were characterized by NMR.

α-Thiophenylsialylated PAMAM **12** (G0—3) formed precipitates with lectin from *Limax flavus*. The inhibition of binding with orosomucoid-sensitized multiwell plates was studied by ELLA using peroxidase-labeled lectin. It was found that the specific inhibitory activity increases in the series of sialylated PAMAM with an increase in the denticity. The activity level differs from that of a structurally related glycodendrimer based on 3,3′-di(aminopropyl)iminoacetic acid. A comparison with the activity of the previously described[49] sialylated tetramer **13** (IC_{50} = 149 nmol L^{-1}) shows a lower molar activity for tetradentate sialylated PAMAM **14** (IC_{50} = 277 nmol L^{-1}). Structures **13** and **14** differ in the length of the spacer groups separating the branching point (nitrogen atom) and the ligand and in the nature of the spacer chain (compound **14** contains rigid and hydrophobic aromatic groups). The enhanced activity of conjugate **14** might be related to nonspecific interactions with lectin or to the formation of polymolecular structures (micelles) under the assay conditions.



13



14

Among the sialylated PAMAM under consideration, the highest activity (in the reaction with lectin)[44] was found for the 32-dentate glycodendrimer **12** (G3) (IC_{50} = 36 nmol L^{-1}) homologous to **14**; a sharp change in the activity in the sialodendrimer series takes place on passing from the 8-dentate glycodendrimer **12** (IC_{50} = 172 nmol L^{-1}) to the 16-dentate one (IC_{50} = 46 nmol L^{-1}). It has been suggested[44] that the increase in the specific inhibitory activity is due to the presence of two receptor sites in the lectin, which can interact simultaneously with several neuraminic acid residues within the same glycodendrimer molecule.

Syntheses of 4-, 8-, 16-, 32-, 64-, and 128-dentate glycodendrimers bearing lactose residues have been reported.[50] Lactose ligands were attached to PAMAM through a 4-aminophenoxy spacer, which was obtained from nitrophenylacetyllactoside **15** (Scheme 4).

The conjugates **19** (G0—5) were synthesized by the reaction of 4-isothiocyanophenyl β-lactoside **18** with commercial amino-terminated PAMAM in a ~80% average yield, the target compounds were purified by dialysis, and the completeness of attachment of the carbohydrate ligands was confirmed by MALDI-TOF mass spectrometry. The structure of the 16-dentate conjugate **20** belonging to this series is shown below.

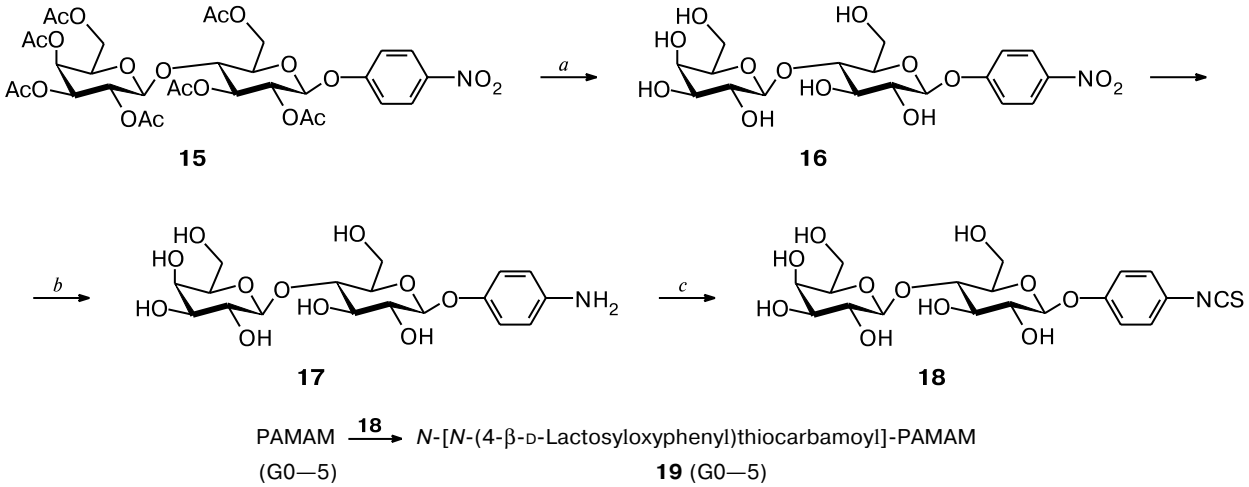
Although the study cited[50] does not present experimental details, it is still of interest due to the description of the biological properties of the obtained glycoconjugates. The researchers carried out a comparative study of the interaction of β-lactosyl dendrimers **19** with several receptors of different nature: a lactose-specific fraction of immunoglobulin G (IgG), a homodimeric galectin-1, monomeric galectin-3, and the plant galactose-specific tetrameric lectin from *Viscum album*. A bovine serum albumin conjugate with lactose derivative **18** ($Lac_{28}BSA$) was used as the immobilized substrate, which was made to interact with biotinylated receptors (in the presence or in the absence of various inhibitors).

It was shown that IC_{50} for the series of lactosyl-PAMAM in the reaction with the lectin from *Viscum album* decreased as the denticity of the inhibiting glycodendrimer grows to 64, but then it somewhat increased for the 128-dentate conjugate **19** (G5). The highest values were attained for the 32- and 64-dentate conjugates **19** (G3 and G4); for the latter, the specific inhibitory activity was ~3500 times as high as that for lactose

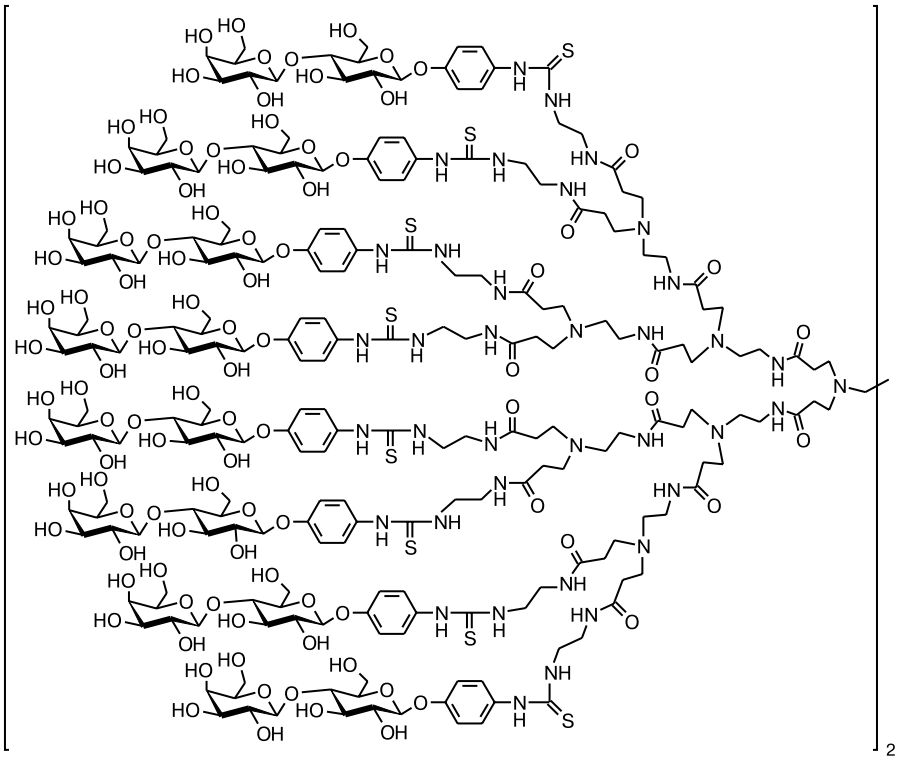
The activity of the β-lactosyl dendrimers with respect to monomeric galectin-3 is comparable with that found for lactose. The inhibition of homodimeric galectin-1 by various glycodendrimers also shows a dependence of IC_{50} on the amount of the lactose attached, although it is less pronounced than that for the plant lectin. The highest IC_{50} values were obtained for 32- and 64-dentate inhibitors **19** (G3 and G4), the latter being 108 times as active as the monomeric ligand. The inhibitory activity of the 128-dentate glycodendrimer **19** (G5) somewhat decreased relative to the 64-dentate homolog for all four galactosyl-binding proteins.

In the case of the reaction of β-lactosyl-PAMAM **19** with lactose-specific immunoglobulins G, the observed IC_{50} is nearly the same for any generation and is 10—20 times smaller than that for lactose. A similar relatively small increase in the inhibitory activity of β-lactosyl-PAMAM in the interaction with IgG can be attributed[50] to the rigidity of the matrices and the spacer group, which restricts the conformational mobility of the lactose residues and prevents them from successful interaction with the receptor active sites.

Scheme 4



Reagents: *a*) MeONa/MeOH; *b*) $HCOONH_4$/Pd; *c*) $S{=}CCl_2$.



20

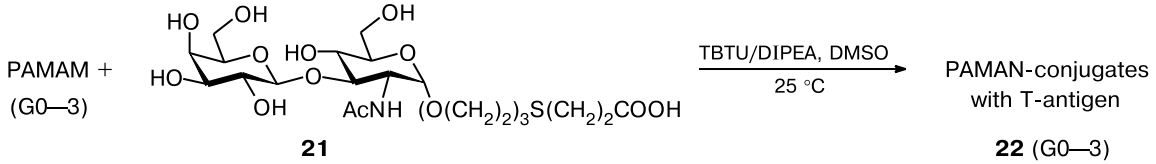
A study of the activity of related glycoconjugates has been reported.[51] 4-, 8-, 16-, and 32-dentate glycosyl-PAMAM containing 3-*O*-(β-D-galactopyranosyl)-2-acetamido-2-deoxy-α-D-galactopyranoside **21** (T-antigen) have been synthesized (yields 73—99%) (Scheme 5). The completeness of acylation was confirmed by the ninhydrin test and ^{1}H NMR data.

Binding of generation G0—3 glycoconjugates **22** with a plant lectin from *Arachis hypogaea* was confirmed by turbidimetry and studied by ELLA. The 32-dentate dendrimer **22** (G3) was bound 18 times more efficiently than the tetradentate dendrimer **22** (G0). The binding with IgG was studied by competitive enzyme-linked immunosorbent assay (ELISA), which showed that G0, G1, G2, and G3 glycodendrimers **22** are 460, 960, 1700, and 3800 times, respectively, more active than the monomeric T-antigen. However, the highest inhibitory activity per carbohydrate residue was found for the tetradentate glycoconjugate and was only 20 times as high as that of the T-antigen.

Among other PAMAM-based glycoconjugates, tetra- and octadentate conjugates with *N*-acylgalactosamine as the ligand have been prepared; their binding to adhesin from *Entamoeba histolytica* has been studied.[52] What is noteworthy is the unusual type of the ligand, which was conjugated through the amino group in position 2 of the sugar residue, the natural configuration of the reducing center remaining intact. The activated ligand **23** was synthesized by the reaction of galactosamine **24** with an excess of di-*N*-hydroxysuccinimide adipate **25** (Scheme 6). The resulting activated ester was conjugated with G0 and G1 PAMAM.

Experiments with rat liver membranes to study the biological activities of type **26** (G0) and **26** (G1) dendrimers have shown a substantial decrease in IC_{50} on passing from the tetradentate to the octadentate glycoconjugate,

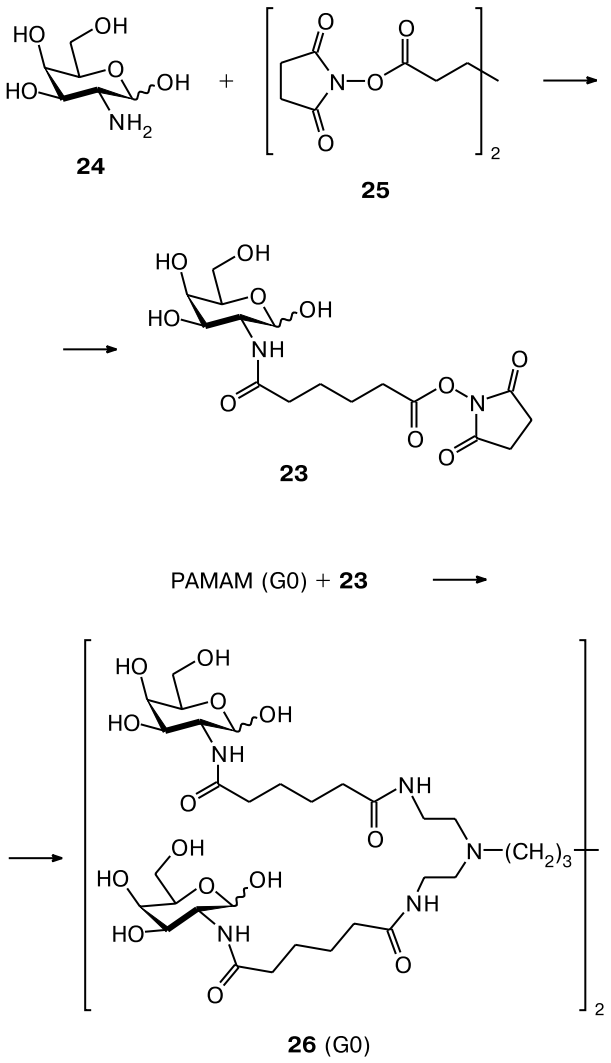
Scheme 5



21 **22** (G0—3)

TBTU is 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, DIPEA is diisopropylethylamine

Scheme 6



whose specific inhibitory activity is 75 and 1550 times as high as that of *N*-acetylgalactosamine. Note that experiments with bacterial membranes show different inhibitory activities of glycoclusters: the specific activity of the octamer proved to be 40% lower than that of *N*-acetylgalactosamine, whereas the tetramer is approximately 3 times as active as *N*-acetylgalactosamine.

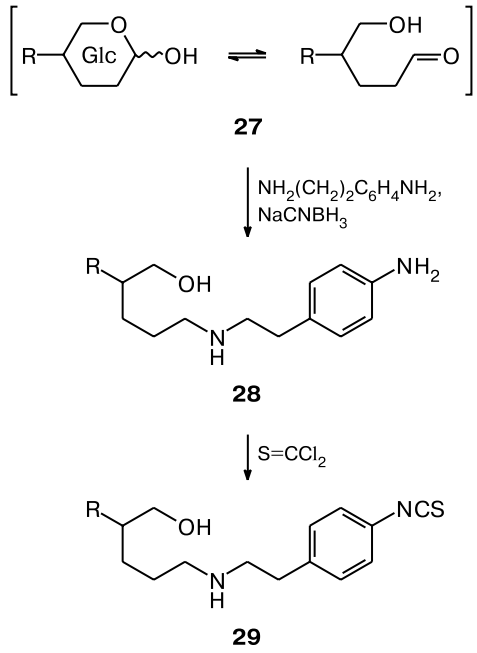
Yet another class of dendritic polymers, PPI, is used somewhat more rarely to prepare glycodendrimers. An obvious reason is the relatively lower availability of PPI, while their physicochemical properties are very similar to those of PAMAM and do not offer any obvious advantages.

A number of oligovalent clusters bearing the pentasaccharide Galβ1-3GalNAcβ1-4[Siaα2—3]Galβ1-4Glc **27**, which was prepared from the natural ganglioside GM_1, have been synthesized.[53] Prior to conjugation, the pentasaccharide **27** was subjected to reductive amination with 2-(4-aminophenyl)ethylamine (Scheme 7) and the resulting amino-terminated product **28** was converted into isothiocyanate **29** ready for conjugation upon treatment with thiophosgene.

The conjugation of isothiocyanate **29** with PPI and PAMAM matrices has given several tetra- and octadentate glycodendrimers, which were characterized by high-performance TLC, IR spectroscopy, and carbohydrate analysis. Unexpectedly, the reactivities of the terminal amino groups of the octadentate PPI and PAMAM proved to be different. Thus, exhaustive acylation of octadentate PPI was attained in 15 h using a 10% molar excess of isothiocyanate **29**, whereas in the case of octadentate PAMAM, a 2.5-fold excess was required and the reaction was completed after 30 h. According to the carbohydrate analysis data, the PPI glycodendrimer contained seven sialic acid residues, while the PAMAM glycodendrimer, only six residues (apparently, owing to ligand destruction during conjugation).

The ability of the obtained tetra- and octadentate PAMAM and PPI glycoconjugates to inhibit binding of the ganglioside GM_1 to cholera toxin B-subunits and the thermolabile enterotoxin from *E. coli* has been studied.[53] The IC_{50} values found for inhibition of the reaction of the cholera toxin B-subunits with immobilized GM_1 with PPI-based octa- and tetradentate glycodendrimers and PAMAM-based octadentate glucoconjugates are similar (~7—8 nmol L^{-1}), whereas the activity of nonconjugated ligand **29** is several μmol L^{-1}. The inhibition of the reaction with the enterotoxin has been studied only for

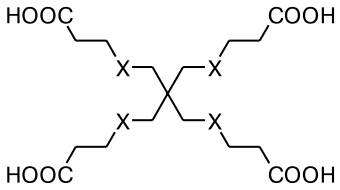
Scheme 7



R = Galβ1-3GalNAcβ1-4[Siaα2—3]Galβ1—

octadentate PPI glycodendrimer. In this case, IC_{50} is equal to ~6 nmol L^{-1}. Similar experiments were carried out with transformed mouse fibroblasts carrying the ganglioside GM_1 on the surface, which demonstrated high inhibitory activity of octadentate PPI glycodendrimer.[54]

Glycoclusters and convergent glycodendrimers. The systematic synthesis of molecular probes reproducing to one or another extent the properties of natural surface clusters gives rise to the simplest glycoconjugates containing three or four carbohydrate ligands. A simple method for the preparation of oligodentate carbohydrate clusters is the addition of ligands to molecules with several activated groups. Pentaerythritol derivatives **30**, 1,3,5-benzenetricarboxylic acid **31**, and tris(2-carboxyethyl)nitromethane **32** are used most often for this purpose



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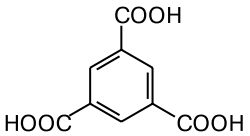

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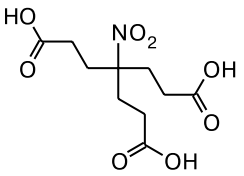
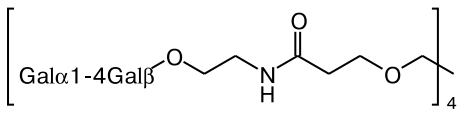


32

X = O, S

Synthesis and properties of a series of oligovalent conjugates bearing galabioside residues has been described.[55] The aminoethylated disaccharide Galα1-4Galβ (**33**) was attached to polycarboxyl compounds in the presence of *N*-hydroxysuccinimide (HOSu, —OSu is the *N*-hydroxysuccinimide residue) as the catalyst and 1-ethyl-3-(dimethylaminopropyl)carbodiimide (EDC) as the dehydrating agent. The ability of the resulting bi-, tri-, and tetradentate oligodisaccharide clusters to inhibit hemagglutination by the bacteria *Streptococcus suis* has been studied. The galabioside tetramer **34** proved to be the most active substance in this series, its activity (3 nmol L^{-1}) being 600 times higher than that of **33**.



34

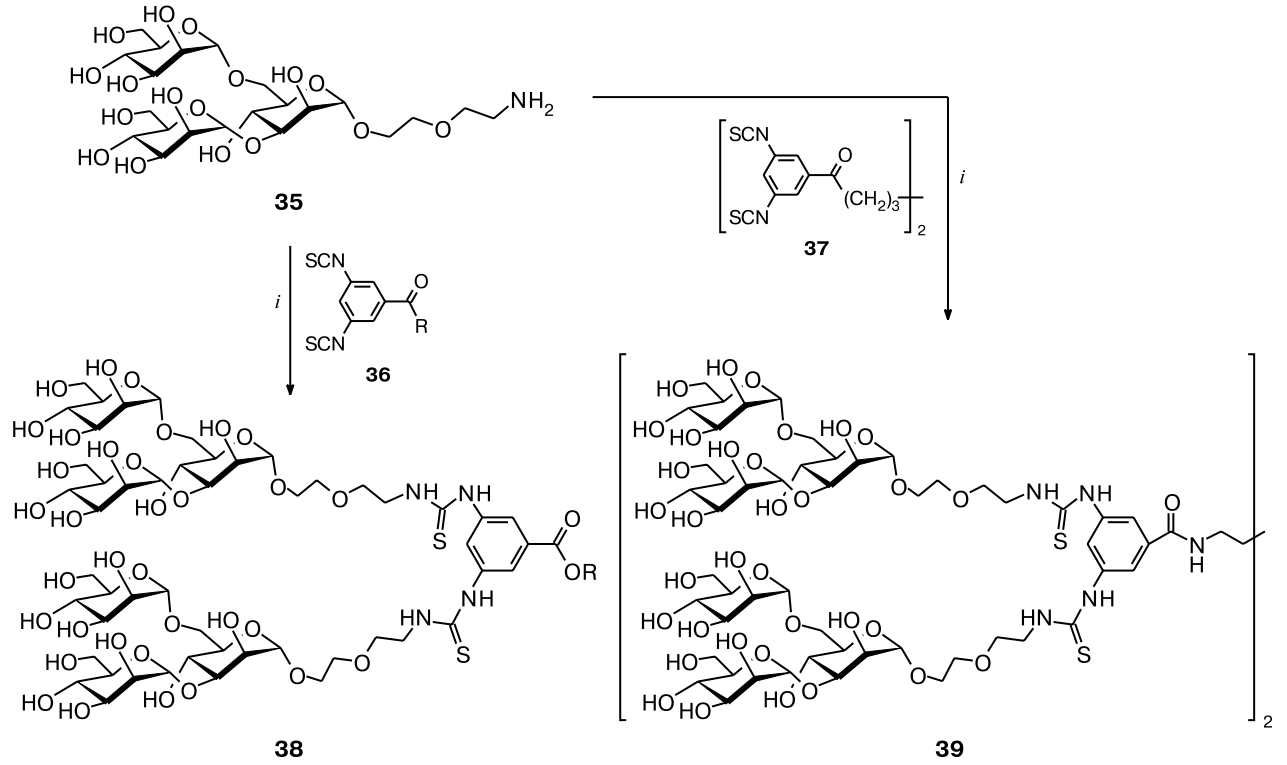
Oligovalent clusters have also been studied.[56] A series of bi-, tri-, and tetradentate clusters with branching points in the aromatic ring and with various spacer groups were synthesized. The amino-spacered mannoside and the trisaccharide Manα(1—6)[Manα(1—3)]Man (**35**) were used as the ligands. The unprotected amino-spacered ligands (Scheme 8 shows only trimannoside **35**) were conjugated with methyl 3,5-diisothiocyanatobenzoate **36**, 1,3,5-triisothiocyanatobenzene, tetraisothiocyanate **37**, and other compounds (only the most active glycoclusters **38** and **39** are shown in the Scheme).

The inhibitory activity of oligovalent clusters was studied by ELLA using immobilized yeast mannan and peroxidase-labeled concanavalin A (tetradentate lectin) and the bidentate lectin from *Pisum sativum*. The inhibition of concanavalin A by bidentate cluster **38** and tetradentate cluster **39** was found to be 257 and 289 times, respectively, more efficient (IC_{50} = 1.8 and 0.8 μmol L^{-1}) than by trimannoside **35**. Trisaccharide ligand-bearing oligodentate clusters are 4—5 times more active than the clusters with attached mannose residues instead of the trisaccharide. In the binding tests of the bidentate lectin from *Pisum sativum*, the inhibition by mannose-containing glycoclusters is 10—20 times weaker, while the bidentate cluster **38** is most active (IC_{50} = 73.5 mmol L^{-1}).

The ligand clusterization has been studied[57] in relation to one more class of glycodendrimers (Scheme 9, compound **42**) constructed from 3,5-bis(2-aminoethoxy)benzoic acid residues **41** and bearing up to eight lactose residues. The conjugation of lactose derivative **40** with amino-terminated matrices was accomplished by the reaction of the isothiocyanate groups with free NH_2 groups. Complete removal of the protective groups gave lactose-containing clusters homologous to the bidentate compound **42** (see Scheme 9). Unfortunately, no detailed description of the synthesis or the physicochemical properties of the glycoclusters is given.

The ability of glycoclusters to interact with several galactose-binding proteins: galectins-1, -3, and -7, the tetrameric plant toxin from *Viscum album*, and lactose-specific human IgG has been studied by competitive inhibition.[57] It was found that an increase in the denticity of analogs **42** results in an increase in the activity per carbohydrate residue of glycoconjugates. However, glycodendrimers interact most strongly with the plant toxin (immobilized on $Lac_{28}BSA$ as a support, Lac is the lactose residue). The tetra- and octadentate analogs **42** were more active; their activity was 357 and 83 times higher than that for lactose, respectively. These results are similar to those reported previously[50] for glycoconjugates **19** of lactose with PAMAM. Note that no clear-cut influence of the nature of the glycodendrimer matrix on the activity can be observed from the reported data[50,57] (although this should be expected for low-dentate glycoclusters). Indeed, the activity levels reduced to a carbohydrate residue are similar in both series, while the activity variations with the change in the denticity do not coincide. An exception is provided by experiments on the

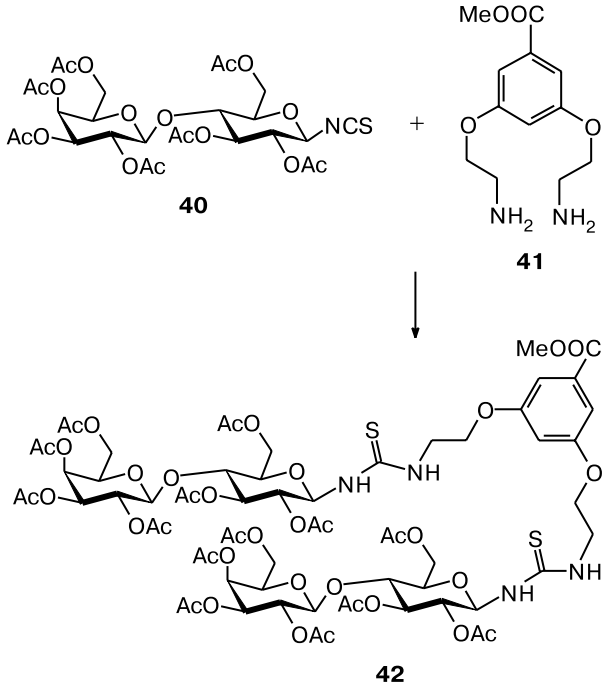
Scheme 8



R = H, OMe

i. DMSO, DIPEA, 30 min.

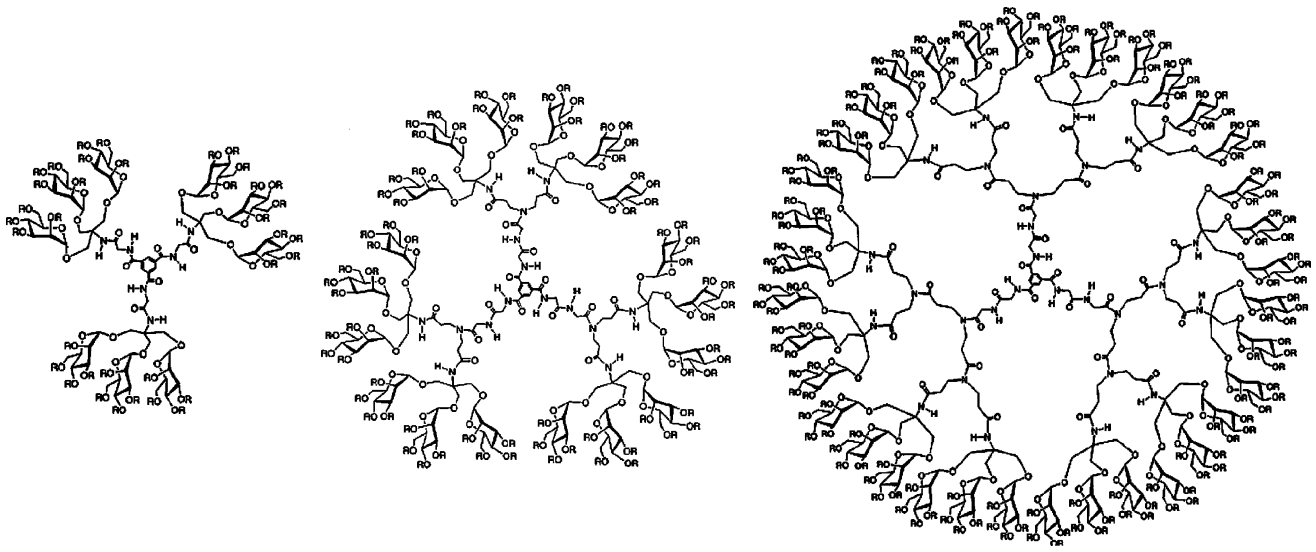
Scheme 9



inhibition of galectin-3 binding to $Lac_{28}BSA$ where PAMAM glycodendrimers **19** were inactive, while analogs **42** demonstrated a 2—6-fold increase in the activity per carbohydrate residue.

Similar competitive inhibition experiments have been carried out[53] for five immobilized glyco- and neoglycoproteins. The results of binding of analogs **42** to galactose-binding proteins were found to be strongly dependent on the nature of the protein immobilized on the support. This dependence is manifested in not only IC_{50} values but also in the ratios of IC_{50} for glycodendrimers of different denticity.

In the dendrimer chemistry, as in organic synthesis, one can distinguish two approaches to the assembly of large molecules, namely (1) consecutive connection of the small fragments of the target molecule; (2) connection of two or several fragments assembled from the small ones. The convergent glycodendrimers are synthesized by attaching several branches bearing carbohydrate ligands to the dendrimer core. Therefore, the convergent glycodendrimers can be regarded as being related to the above-mentioned glycoclusters. The difference is in the size of the branches and in the theoretical possibility of divergent, *i.e.*, layer-by-layer, assembly of a glycodendrimer.



43a,b **44a,b** **45a,b**

R = Ac (**a**), H (**b**)

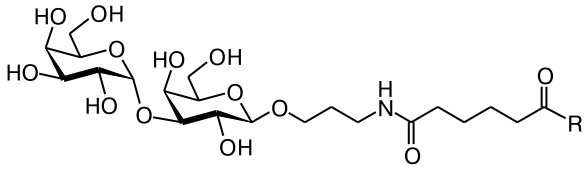
In the case of dendrimer assembly on various cores, the convergent approach is more efficient.[24,58,59] However, this type of synthesis decreases the reactivity of functional groups of the core due to spatial restrictions; therefore, it offers no significant advantages over the synthesis of glycoclusters or glyconjugates based on traditional PAMAM, PPI, or dendrimers of other classes. The extensive research[27,28,60—62] into the synthesis of convergent glycodendrimers made it possible to attain their yields of ~50—60%.

A series of mannose-containing 9- (**43**), 18- (**44**), and 36-dentate dendrimers (**45**) with branching points provided by 3,3´-iminodipropionic acid, 1,3,5-benzenetricarboxylic acid, and a tridentate cluster based on Tris have been described.[28] A study of the inhibition of binding of concanavalin A to yeast mannan by compounds **43a—45a** (R = H, obtained by deacetylation of precursors **43b—45b**) showed that the nonadentate dendrimer **43a** (IC_{50} = 0.65 μmol L^{-1}) is the most active inhibitor; it is 4 times as active as methyl α-D-mannoside. On dilution, the activity of dendrimer solutions varies nonmonotonically, which suggests the possibility of rheological or other processes affecting the local concentrations near the receptors of concanavalin A. Additional studies carried out[28] with sera of Crohn´s disease patients did not reveal activity of mannose-containing glycodendrimers.

Prospects for the use of dendritic polymers in biology and medicine

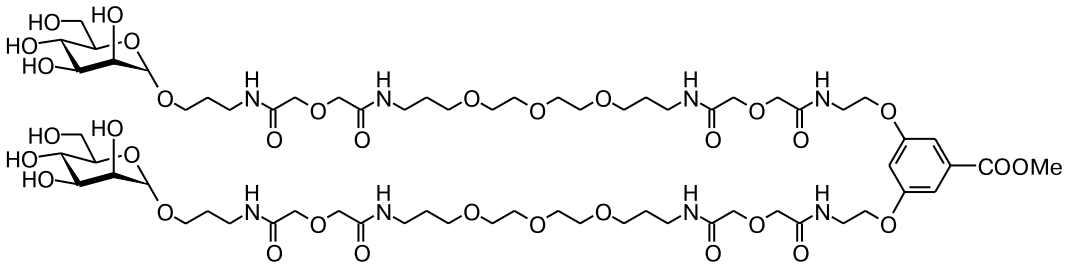
Dendritic blockers of cell interaction. Successful use of dendritic blockers in mouse melanoma has been described.[63,64] Introduction of octadentate PAMAM bearing eight GlcNAc residues into mice increases twofold the survival rate of the animals relative to the control group. In addition, the lack of immunogenicity or toxicity of $GlcNAc_8$PAMAM in the concentrations under study was noted.

The PAMAM glycoconjugates with a denticity of 2—64 were synthesized[65,66] by acylation of the B-disaccharide (B_{di}), Galα1-3Galβ, the antigen determinant responsible for the rejection of pig´s organs transplanted into a human (xenotransplantation), with the activated derivative **46**.[67] A series of PAMAM-glycodendrimers obtained in this way has been studied as natural anti-B_{di}-immunoglobulin blockers in human sera by ELISA and depression of the cytotoxicity of human sera in the RK-15 cell culture.



46: R = ONp
47: R = OH (B_{di})

Glycoconjugates with a denticity of 2—8 inhibited binding of IgG xenoantibodies to the immobilized glycoconjugate of the B-dissaccharide (B_{di}-PAA) with polyacrylamide, similarly to the monomeric **47**. The efficiency of 16—64-dentate glycoconjugates is higher, while that for the 64-dentate glycodendrimer approaches the value found for the conjugate with polyacrylamide. This test also makes it possible to diagnose the inhibition of binding of class M immunoglobulins. However, of the compounds studied, only 32- and 64-dentate glycodendrimers inhibit IgM



47a

binding to immobilized B_{di}-PAA in lower concentrations than monomer **47**.

In the cytotoxicity inhibition tests, the highest activity was also found for 32- and 64-dentate B_{di}-dendrimers, which have the highest valence and the greatest distances between the carbohydrate groups similar to the distance between the Fab-domains of the antibodies. However, the gain in activity of these conjugates with respect to the monovalent B_{di} observed in both experimental models was not so great as could be expected for two-center binding of glycodendrimer molecules to the antibodies.

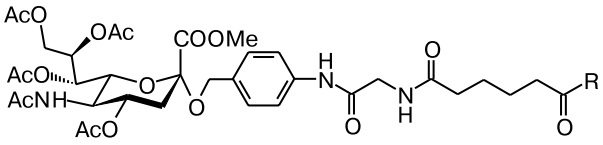
Synthesis of a series of polyvalent mannosides has been described and inhibition of binding of uropathogenic *E. coli* to the urinary bladder epithelium has been studied.[68] Although 8- and 16-dentate PAMAM glycoconjugates containing mannose residues demonstrated high activities (IC_{50} 37 and 19 μmol L^{-1}), the highest specific activity was found for bidentate compound **47a**.

Presumably, the enhanced activity of **47a** is due to the presence of hydrophilic spacer groups, which ensure sufficient distances and the optimal orientation of the mannose residues for binding to two receptors.

Dendrite blockers of viral interactions. The design of synthetic blockers of the adhesion of influenza virions to target cells is an example of using glycodendrimers in virology. Presumably,[69] it is possible to synthesize drugs that would block the vital activity of the virus and the development of the disease through competitive interaction with the carbohydrate-binding HA trimers located on the surface of the virus particle. This hypothesis was confirmed by experiments on the inhibition of the cell adhesion of the influenza virus by glycoconjugates of carbochain polymers bearing sialic acid derivatives or sialooligosaccharides.[70,71]

Further development of this approach and study of the dendritic HA blockers have been described.[2,72] 32- and 64-Dentate sialodendrimers based on PAMAM matrices and a polydentate dendritic polymer with a molecular mass of ~400 kDa were synthesized.[72] The activated *N*-acetylneuraminic acid derivative **48** was attached to the primary amino groups of PAMAM and elaborated into the ligand.

The activity of the synthesized sialodendrimers has been studied by competitive inhibition of the adhesion of



48: R = ONp
49: R = OH

influenza virions to fetuin immobilized on a polystyrene surface. It was found that the inhibitory activity of sialodendrimers depends appreciably on pH. In neutral buffer solutions, the inhibition by 32- and 64-dentate sialodendrimers is comparable with that of monomeric Neu5Acα-OBzl, and a decrease in the pH increases the activity by one to two orders of magnitude. The polydentate sialodendrimer proved to be more active inhibitor than the monomeric ligand **49** (the dissociation constant of the virus—sialoside complexes is <0.001 μmol L^{-1}).

Numerous publications are devoted to the synthesis of glycodendrimers and detailed study of the nature of the dendrite matrix on the biological activity of the corresponding glycodendrimers. Dendritic glycopolylysines have already been mentioned above.[42] Other compounds studied as matrices for glycoconjugate synthesis include dendritic 3,3´-iminodipropylaminoacetamides,[73,74] dendritic derivatives of gallic acid,[75] phosphotriesters,[76] classical PAMAM,[47,50] and mixed-nature oligovalent matrices.[56]

N,*N*-Bis(3-aminopropyl)glycine **50** has been used[73] to prepare two series of α-thiosialodendrimers. In this type of matrix, branching of the dendrimer chain occurs symmetrically to give spherical molecules with a "dense" surface for higher generations. The fact that the symmetrical structure of the dendrimer fragments makes the NMR spectra simpler and their interpretation easier provides an additional advantage. The use of amino acid **50** allows one to prepare dendrimers with a reduced number of defects (compared to that in the classical PAMAM), as the base-catalyzed decomposition reverse to the Michael addition is impossible in this case.[73] Owing to the presence of the carboxy and amino groups in the monomer unit, classical approaches developed for peptides can be used in the synthesis. With tris(2-aminoethyl)amine **51** as the core, solution synthesis of the 6-dentate amino-ter-

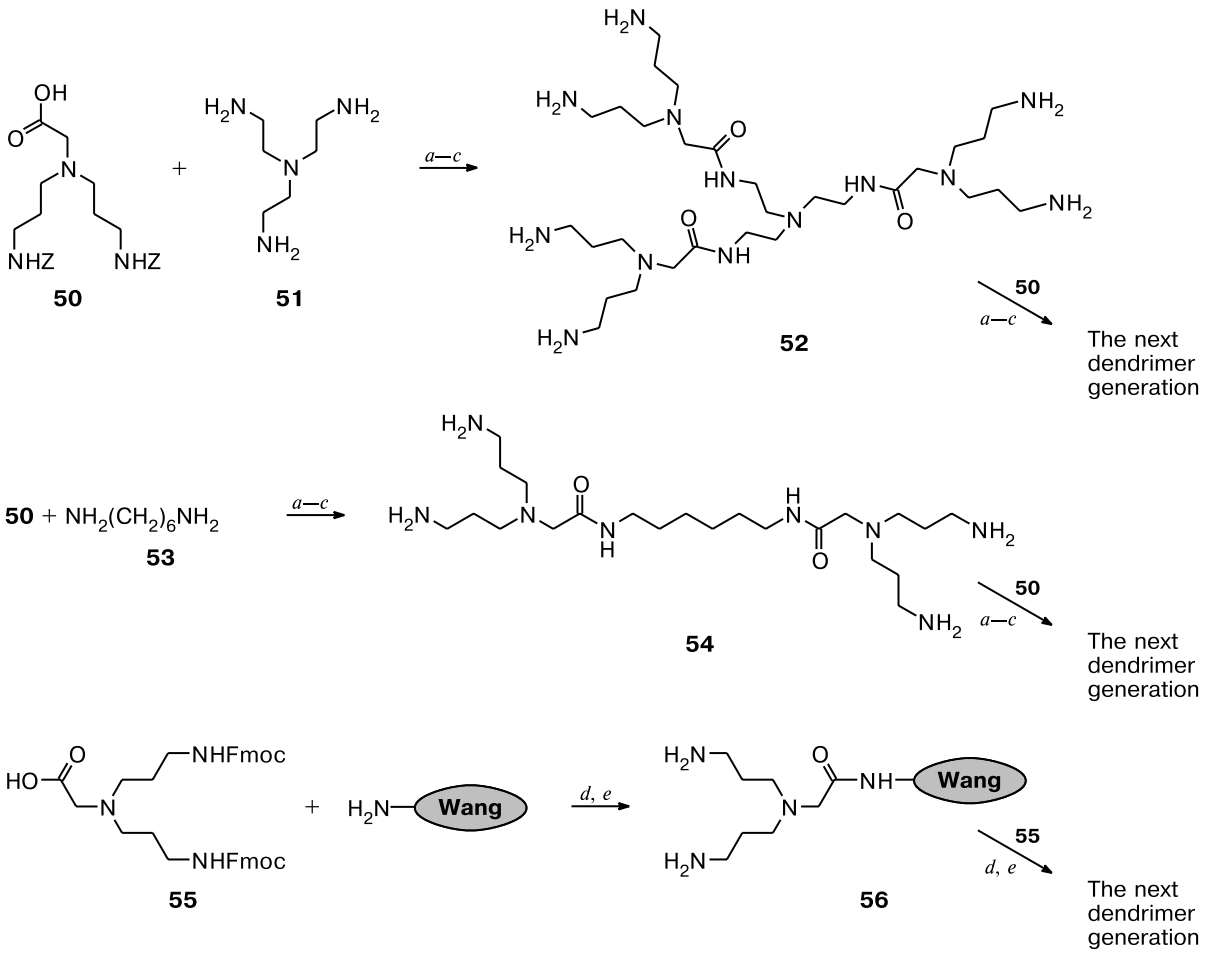
minated dendrimer **52** (Scheme 10) and its 12-dentate homolog was carried out. In addition, using hexamethylenediamine **53** as the core, the tetradentate matrix **54** and the corresponding 4-, 8-, and 16-dentate homologs of the second series were obtained in solution. In addition to the above-mentioned compounds, a series of 2-, 4-, and 8-dentate dendrimers were prepared in a similar way on the Wang polystyrene[49] using the Fmoc-derivative of *N*,*N*-bis(3-aminopropyl)glycine **55**.

The ligand immobilization was carried out by the procedure[74] used in the synthesis of polylysine-based dendritic α-thiosialosides (see Scheme 1). To this end, the amino groups of the resulting dendrite matrices were acylated with chloroacetic anhydride, and conjugated with ligand **1** with subsequent deprotection of the target α-thiosialodendrimers.

The interaction of the α-thiosialodendrimers obtained in this way with lectin from *Limax flavus* was confirmed by turbidimetry. In addition, the inhibition, by glycodendrimers, of binding of this lectin to orosomucoid was studied by the ELLA technique. It was found that the inhibitory activity with respect to the Neu5Ac residue increases 3.5—182-fold in the series of homologous α-thiosialoconjugates **51**, **52** (from 3- to 12-dentate ones). Their activities are comparable with those of sialopolylysines of the type **4**,[36] but in the case of α-thiosialodendrimers based on *N*,*N*-bis(3-aminopropyl)glycine matrices, no monotonic increase in the activity with an increase in the denticity was observed. The 4- (**54**) and 12-dentate homologs of glycoconjugate **52** possessed the highest activity, which exceeded that of the monomeric α-thiosialoside 127- and 182-fold, respectively. Dendrimers of other denticities are much less active.

An extensive series of sialo-containing conjugates has been studied.[4] Glycodendrimers were designed on the basis of both the usual spheroid PAMAM G0—5 (Fig. 2,

Scheme 10



Z is benzyloxycarbonyl

Reagents: (*a*) (Diisopropylcarbodiimide (DIC) + *N*-hydroxybenzotriazole (HOBt) + DIPEA)/MeCN; (*b*) anion exchanger (OH^-); (*c*) H_2/Pd; (*d*) (dicyclohexylcarbodiimide (DCC) + HOBt)/DMF; (*e*) piperidine.

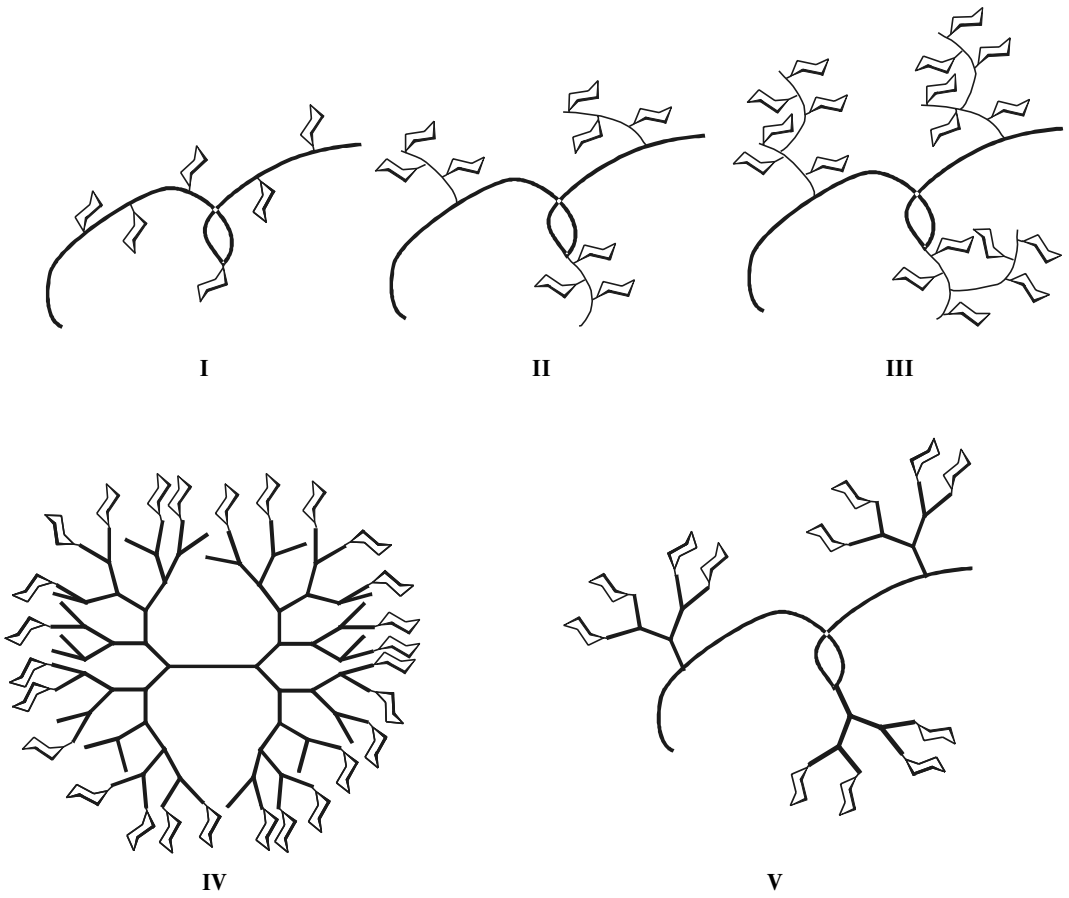


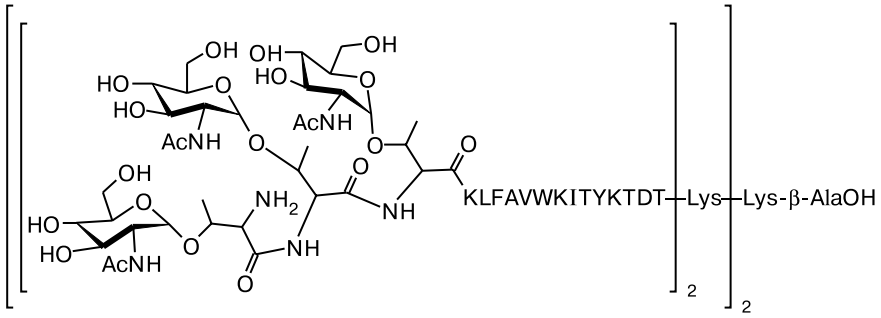
Fig. 2. Synthetic polysialosides, blockers of the pathogenesis of influenza virus:[4] linear (**I**), combined branched (**II**), graft copolymer (**III**), classical spheroid dendrimer (**IV**), linear copolymer with grafted dendrons (**V**).

structure **IV**) and a number of more complex graft copolymers, for example, polyethyleneimine with grafted PAMAM dendrons G0—2 (Fig. 2, structure **V**). For comparison, were studied branched and graft polyethyleneimines (Fig. 2, structures **II** and **III**) and linear polyacrylamide (PAA) and polyethyleneimine (Fig. 2, structure **I**) related to dendrimers. The activities of polysialosides in systems similar to natural ones have been studied, *i.e.*, hen erythrocyte agglutination under the action of virus particles and development of the influenza A virus in a cell culture.

It was found that PAMAM glycodendrimers of the classical spheroidal type **IV** (see Fig. 2) do not inhibit agglutination in the concentration range studied. The graft sialopolymers **II** and **III** with an average content of sialic acid of 40—50% with respect to the amino groups present are the most active (~40000 with respect to Neu5Ac) inhibitors. Dendrimers **V** (see Fig. 2) are less potent agglutination inhibitors, the activity being observed only for samples with amino groups fully modified with sialic acid. Thus, depending on the structure, the specific inhibitory activity of the tested glycodendrimers differed by four orders of magnitude. It is noteworthy that the specific inhibitory activities of different virus strains are also different.

An additional cytotoxicity measurement in the MDCK cell culture has shown that the cytotoxicity of sialodendrimer **V** (see Fig. 2) is much lower than that of the PAA-based glycoconjugate. The cytotoxicity of the latter manifests itself in concentrations three orders of magnitude lower than the IC_{50} concentration. The sialodendrimer cytotoxicity depends on the content of Neu5Ac and is totally missing for 100% modification of the amino groups with sialic acid. Additionally, the ability of sialodendrimers to reduce of the influenza virus strains H2N2, H3N3 (X-31) in culture was studied *in vivo*. The highest inhibitory activities were found for polymer **V** containing 100% of sialic acid-modified terminal groups and polymers **II** and **III** with a ~50% modification. Note that the results obtained in the test of inhibition of hemagglutination for these sialodendrimers are strictly opposite.

The use of dendrimers in the development of vaccines. The low immunogenicity of small molecules is now a well-established fact; the immunogenicity is usually increased upon polymerization of low-molecular-mass antigens or conjugation with polymeric carriers (proteins,



Tn-antigen

KLFAVWKITYKDT is the amino acid sequence stimulating the T-cellular response (T-epitope)

polysaccharides, liposomes, or synthetic polymers).[77] The use of dendrimers as such carriers is highly promising, as this enables the synthesis of conjugates with a definite and well-reproducible structure. Indeed, the use of peptide antigens immobilized on a dendritic polylysine[31,78,79] was beneficial for the stimulation of the immune response. These works resulted in the development of an antimalarial MAP-vaccine, which is currently under clinical trials.[80]

Dendritic polylysine has been successfully used to prepare glycoimmunogens containing *N*-acetyl-α-D-galactosamine residues (Tn-antigens);[81] the most efficient design is shown below.

Unlike glycoconjugates with polylysine, Tn-antigens conjugated with PAMAM dendrimers (generation 5) did not possess immunogenicity.[82—83] Evidently, mere multiplication of carbohydrate antigens on the dendrimer does not enhance the induced immune response due to the high uniformity of the surface properties of the glycoconjugate molecules.

The change in the hydrophilic-lipophilic balance of PAMAM allows one to use them as adjuvants, as has been shown in relation to the immunization by glycolipids or ovalbumin within multicomponent noncovalent complexes with PAMAM of generation 5.[85,86]

Toxicity and biocompatibility of the most readily available dendrimers. The toxicity of glycodendrimers has been little studied; the toxicological properties were studied only for the most readily available dendritic polymers, PAMAM and PPI. The reported data for PAMAM are contradictory. Low acute toxicity of PAMAM in experiments with rats has been noted;[87] meanwhile, this class is widely used in cell culture transfection,[88,89] which implies that PAMAM change the structure and permeability of cell membranes and can induce their destruction. In another detailed study,[90] it was shown that all generations of the NH_2-terminated PAMAM exhibit cytotoxicity in concentrations of 50—300 μg mL^{-1} (72-h incubation) depending on the denticity, while the carboxy-terminated PAMAM exhibit no cytotoxicity in the concentration range studied. It was found that amino-terminated PAMAM are rapidly removed from the blood flow. One hour after the intravenous administration of labeled 16- and 32-dentate dendrimers in rats, <2% of the radioactive label was detected in the blood flow, whereas in the case of COOH-terminated PAMAM, the concentration of the label observed under the same conditions was 10—20 times higher. Low-dentate dendrimers circulate in the blood flow for longer periods, while high-dentate ones (>32) are retained in liver to 30—90%. The retention of PAMAM in the blood flow may be attributed to binding with the serum albumin, which is confirmed by data[91] on the fluorescence quenching of the tryptophan residue of BSA after the addition of PAMAM.

The distribution of PAMAM exhaustively modified by biotin derivatives in the organism of mice has been studied.[92] During 4 h, tetradentate dendrimers are excreted almost entirely with urine, 8- and 16-dentate derivatives are excreted much more slowly, the highest concentration being observed in the renal tissue and in the liver.

Data on PPI are more scarce. According to Aldrich Material Safety Data Sheets, the LD_{50} for the oral introduction of PPI in rats equals 1400 mg kg^{-1},[93] which is more than twice lower than this value for random polyethyleneimine (3300 mg kg^{-1})[94] and is close to LD_{50} for ethylenediamine (for comparison, LD_{50} for lactose is >10 g kg^{-1}). However, comparison with PAMAM is difficult as the data sheet contains no relevant LD_{50} values.

A study of the interaction of 16-dentate dimethyldecyl-modified PPI with the cell membranes of gram-positive and gram-negative bacteria has shown[95] that polycationic dendrimers replace Ca^{2+} ions on the membrane surface and change the membrane permeability. The bacteriostatic action of dendritic polycations is attributable to the fact that at high concentrations of these species, the sign of the membrane charge can be reversed, which results in the loss of K^+ ions by bacterial cells. The bactericide effect is due to the destruction of cell membranes under the action of the polycationic dendrimer.

The change in the balance and the sign of the charge of dendrimer molecules can change the toxic properties. Thus a study of the 32-dentate PAMAM modified by

disodium salts of 3,6-disulfonaphthyl isothiocyanate or 3,6-dicarboxyphenyl isothiocyanate[96] has shown the lack of toxicity for the cell culture in concentrations of up to 250 μg mL^{-1}. In addition, concentrations markedly below the toxic level (0.3 and 0.1 μg mL^{-1}) depressed the vital activity of the HIV-I virus in MT-4 and CEM cultures.

An interesting example of biological activity for a series of PAMAM and PPI of different generations has been found.[97] Polyamines remove the prion protein (the ethiological cow madness agent) from a cell culture of brain homogenates of infected animals. The activity of polyamine increases with an increase in the generation number, 64-dentate dendrimers being most active. In the case of brain homogenates and prion particles, dendrite polyamines are an order of magnitude more active than the linear compounds with comparable molecular masses. The IC_{50} values observed for PAMAM (~5 ng mL^{-1}) are much lower than the corresponding toxic levels. This opens up the way for removing the scrapie ethiological agent by mild sterilization. This is highly topical, because tool sterilization and removal of prions from the skin or clothes are attained currently by long-term autoclaving or treatment with hard denaturants (1 *M* NaOH, 6 *M* guanidinium thiocyanate).

These data demonstrate that the toxicity (as well as the biological activity) of PAMAM and PPI is determined to a substantial extent by the terminal functional groups located on the surface of molecules, whereas the nature of the internal part of the dendrimer molecule influences the biological properties to a much lesser extent.

Conclusion

Dendrimers with terminal carbohydrate residues represent a new class of biopolymers. Glycobiology studies have at disposal dendritic molecules of diverse structures and shapes with different numbers of carbohydrate residues with a previously unattainable high degree of homogeneity and reproducibility of properties typical of monomeric compounds. These molecules can be easily analyzed by usual NMR and mass-spectrometry methods.

The key lines of research include the antiadhesion therapy and, perhaps in the future, targeted transport involving the receptor interaction. A combination of the cluster effect, resulting in the highly specific binding and the ability of dendrimers to incorporate[98] and transport small molecules,[99] can underlie the development of new highly specific drugs.

Although it has been already assumed that the cluster effect in the glycoconjugate inhibition of the receptor—ligand interaction[1] or the multiplicative increase in the immunogenicity of monomeric agents[100] are due to a variety of factors, the use of dendrite conjugates with a definite structure to study biological systems confirmed once again the inaccuracy of the established views on cooperative processes.

The appearance of the new class of compounds that allows closer simulation of the processes occurring in the living nature is expected to promote both the development of chemical research aimed at improving the methods of dendrimer synthesis and modification and further studies of the ligand—receptor interactions, vaccinating compositions, and drug transport.

Only the simples approaches to the design and study of glycodendrimers and to the comprehension and control of the carbohydrate—protein interactions have been investigated to date; many concealed advantages of this class of biopolymers are waiting for implementation.

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