# Glycodendrimers as Anti-Adhesion Drugs Against Type 1 Fimbriated *E. coli* Uropathogenic Infections

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**Abstract:** Bacterial drug resistance against antimicrobial agents is a prevalent and central worldwide impasse. Infections with resistant organisms lead to adverse clinical outcomes, increased mortality, and are costly to healthcare systems. Several infectious diseases are initiated by the binding of pathogenic lectins to host cells glycoconjugates. The molecular understanding of these adhesion phenomena is crucial and presents promising new alternatives compared to traditional antibiotic therapies. Glycans or glycan mimetics could be used to inhibit the initial recognition events leading to adhesion and colonization of host tissues by pathogens. The bladder and urothelial lining are widely covered by cell surface glycoproteins bearing the required carbohydrate ligands responsible for the adhesion phenomena. However, when these interactions are measured on a per saccharide basis, they are generally too weak (mM) for the design of beneficial inhibition therapies. The interactions between microbial pathogens and host cells are often governed by polyvalent and overall strong avidities. To overcome this drawback, glycobiologists have design a new family of well-defined small macromolecules, called glycodendrimers that can successfully address this issue. This review will provide a brief introduction on glycodendrimers and detailed descriptions of design and applications of mannosylated inhibitors against fimbriated type 1 *E. coli.*

**Key Words:** Glycodendrimers, *E. coli*, uropathogenic, antiadhesins, lectin, type 1 fimbriae (FimH), mannosides.

## 1. INTRODUCTION

*Escherichia coli*, a member of the *Enterobacteriaceae* family, is a common inhabitant of the digestive tracts of humans and animals wherein several types or strains live in harmlessly. Alternatively, it is a bacterium responsible for serious nosocomial infections as it is the most common cause of Gram-negative cystitis and pyelonephritis. It is the major causative agent for urinary tract infections (UTI). Indeed, *Escherichia coli* are the most common aetiological agents responsible for UTI (60.4%), followed by *Klebsiella* spp. (11.2%), and *Pseudomonas aeruginosa* (8.3%). Unexpectedly, *Enterococcus* spp. isolates caused only 2.3% of UTIs [1]. It is estimated that 3-5 million office visits occur each year in the United States alone due to UTIs. Moreover, some strains produce powerful toxins that cause bloody diarrhea and occasionally can cause severe blood problems and kidney failure. Bacterial infections are becoming more problematic worldwide due to increased resistance to antibiotics (Fig. **1**) [2, 3]. The prevalence of *Escherichia coli* resistant strains is steadily increasing and traditional first-line antimicrobial agents such as trimethoprim/sulfamethoxazole (Tmp/Smx), gentamicin (Genta), piperacillin/tazobactam (Pip/tazo), ceftriaxone (Ctx), and ciprofloxacin (Cipro) are becoming almost obsolete (Fig. **2**) [2].

Antibiotics play a major role in modern medicine's battle against bacterial infections. Without antibiotics, millions of people would succumb to complications from serious skin

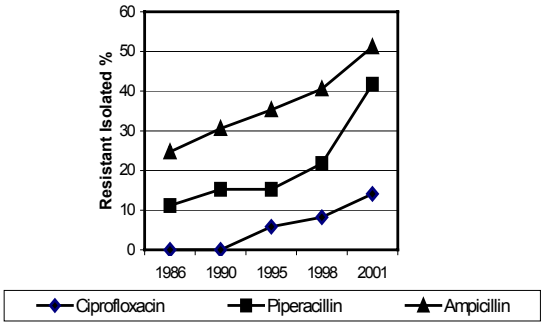


**Fig. (1)**. Increase in resistance to classical antibiotics in Mid-European *E. coli* isolated from 1986 to 2001, data adapted from the Paul-Ehrlich-Gesellschaft (www.p-e.de) and [3].

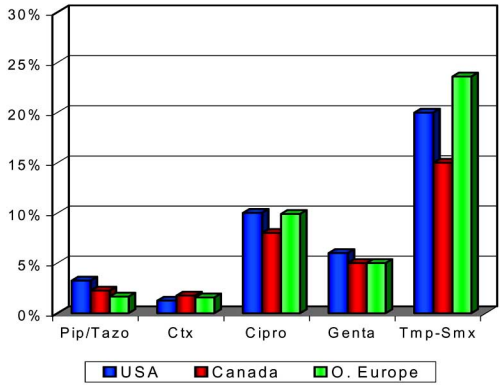


**Fig. (2).** Prevalence of antibiotic-resistant *E. coli* in clinical isolated from patients in the USA, Canada, and Occidental Europe, data adapted from [2, 3].

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and wound infections and diseases like pneumonia, appendicitis, and meningitis. From the mid-20$^{th}$ century onward, antibiotics became modern-day wonder drugs. A dose of penicillin can terminate a streptococcus throat infection in twenty-four hours. Unfortunately, overuse and misuse of antibiotics unintentionally produced new strains of resistant bacteria and increased our vulnerability to untreatable bacterial infections. Most antibiotics destroy bacteria by attacking their metabolic structures. In general, bacteria will be wiped out initially when hit by new therapeutic agents. Sometimes the stronger bacteria survive the treatment and pass on their resistance to future generations. Over time, resistance spreads and the medicine becomes less effective. Doses that are too low or incomplete kill only weaker germs, allowing descendents of stronger survivors to develop even more protection. Widespread and frequent usage increases the opportunity for resistance to expand on a large scale.

Hence, there is an urgent, global need for new antibacterial strategies, among which preventive vaccination against bacterial polysaccharides has been added successfully to the growing arsenal [4]. It is becoming increasingly accepted that early inhibition of bacterial adhesion might also constitute a necessary alternative [5]. Mammalian cell surfaces expose wide arrays of complex glycoconjugates. These carbohydrate structures are playing critical roles in multiple key cellular events [6], several of which have been characterized by weak but multivalent carbohydrate-protein interactions [7-10]. Thus, multiantennary glycans of glycoproteins, polysaccharides, or glycolipids constitute a first line of weak contacts with pathogens bearing the corresponding carbohydrate recognition domain (CRD) on their pilli or lectins [11, 12]. In spite of these ubiquitous phenomena, the basic carbohydrate structures recognized by carbohydrate-binding proteins are surprisingly simple, with at best a tri- or tetrasaccharide moiety being deeply involved in the protein's active sites [13], unless conformational epitopes are involed. Additionally, several proteins or interactive mechanisms utilize a limited set of similar sugars, thus further raising the issues of selectivity. For instance, the innate immune system exploits the structures of mannosides on yeast, fungi, and bacteria through their mannoside binding protein (MBP) as the first defense mechanism against this type of infectious agents [14]. Yet, dendritic cells, macrophages, and hepatocytes also utilize mannosides against pathogens and cellular recognition or activation. Alternatively, bacteria, such as fimbriated *Escherichia coli*, possesses proteins at the tip of their fimbriae (FimH) that also recognize and bind to mannosides of host human tissues as the premise for bacterial infections [15]. Obviously then, the sole carbohydrate structures, taken individually, could not account for the large multivalent interactions that solicit a given glycan. Consequently, valencies, geometries, and topographical saccharide arrangements must be keys for the high selectivities and affinities observed. With these questions in mind, glycodendrimers [9, 10, 16-19] were born to address fundamental aspects in multivalent carbohydrate-protein interactions that could not be entirely resolved with glycopolymers [8, 20-22], or their corresponding artificially glycated proteins that otherwise turn into generally immunogenic materials. Moreover, glycodendrimers can be assembled with an infinite variety of chemical architectures and exposed number of carbohydrate moieties [16-19]. They may thus constitute an arsenal of novel principles for the treatment of infectious agents by the inhibition of attachment of the infecting pathogens to the host's cell surfaces [15, 23-25]. The attachment is often a prerequisite for the later stages of infection, colonization, and invasion of specific human tissues. Since the chemical structure of the adhesion inhibitors most likely would be quite similar to the natural attachment ligands used by the pathogenic agents, it is unlikely that resistance (mutations) would give them the capability to overcome the inhibitory effect of the antiadhesive drug without impairing their own ability to adhere to host cells. Glycodendrimers are therefore suitably design for inhibition of bacterial adhesions to host tissues (Fig. **3**).

Glycodendrimers are an interesting class of synthetic well define biomacromolecules that have been initially designed to address the issue of low affinity carbohydrate-protein interactions encountered in so many biologically relevant situations [8-10, 16-22]. They were initially thought to expand our understanding of the « glycoside cluster effect » that exemplifies an affinity enhancement well above

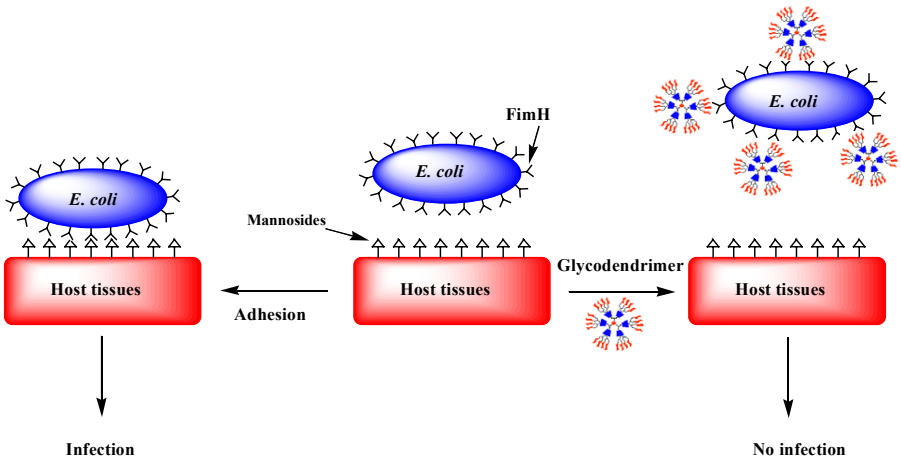


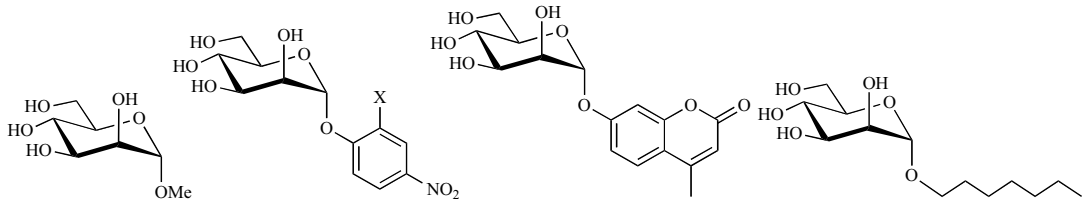

**Fig. (3).** Glycodendrimers are potentially useful agents to block bacterial adhesion.

linearity [26]. In its widespread version, it is usually assumed that the glycoside cluster effect has its source in the enhanced avidity of a given multivalent glycoside toward a protein also possessing multiple CRDs by fully occupying *one active site* at a time. The phenomenon is now widely accepted of having it basis on stabilization by macroscopic cross-linking effects.

Glycodendrimers, just like any other typical tree-like organic dendrimers, may take several structural architectures as they can be prepared as dendrons (wedge structures), spherical or globular dendrimers, and even polymer-dendrimer hybrids called dendronized polymers [25, 27-29]. Glycodendrimers can be synthesized by divergent, convergent, or double stage convergent strategies using a hypercore molecule as central elements. Moreover, for sake of simplicity and diminished cost, the inner scaffold portion of the molecules can be synthesized by a one-pot procedure using hyperbranched polymer methodologies. This can be achieved based on $AB_2$ monomer systems such as those found in commercially available hyberbranched polyglycerols, hyperbranched polyethyleneimine (PEI), and Boltorn® dendrimers of low dispersities. Should commercial applications of glycodendrimers arise; the latter strategy will allow low cost production, albeit at the expense of structural homogeneity.

## 2. ESCHERICHIA COLI FimH

As mention above, the critical step in host tissue colonization and biofilm formation is achieved through bacterial adhesion commonly mediated by carbohydrate-binding lectin-like proteins expressed on or shed from bacterial surfaces. Type 1 fimbriae are the most common type of adhesive appendages in *E. coli* and several other enterobacteria and mediate mannose-specific adhesion via the 30 kDa lectin-like subunit FimH [11, 12]. The natural receptor for *E. coli* is believed to be uroplakin, a highly mannosylated glycoprotein present on the surface of the endothelial lining of urinary tissues [30]. The crystal structure of the FimH from uropathogenic *E. coli*, primarily causing pyelonephritis, has been solved together with methyl α-D-mannopyranoside (**1**) [31] as well as with the more hydrophobic butyl α-D-mannopyranoside [32]. The strongest monovalent inhibitor known to date for the FimH is heptyl α-D-mannopyranoside (**5**, $K_D$ 5 nM) which is eight times better than the general standard *p*-nitrophenyl α-D-mannopyranoside (PNPαMan, **2**, $K_D$ 44 nM) and four times better than methyl umbelliferyl α-D-Man (**4**, $K_D$ 20 nM) [32]. Table **1** shows the relative affinities, $K_D$, and ΔG° of a series of synthetic mannoside derivatives (Fig. **4**) against the FimH of *E. coli* K12 as measured by surface plasmon resonance (SPR) [32].




**1** (1,0) **2** X = H (69,70) **3** X = Cl (717, 470) **4** (600, 1015) **5**

**Fig. (4).** Mannoside derivatives and their relative inhibitory properties against fimbriated *E. coli* O25 or O128 in agglutination of yeasts or adherence to epithelial cells (number in parentheses refer to O25 or o128 strains).

**Table 1. Relative Affinity of Mannosides Against Isolated FimH from *Escherichia coli* K-12 as Measured by Surface Plasmon Resonance (SPR) [Adapted from ref. 32]**

| Ligand | $K_d$ (nM) | ΔG° (Kcal $mol^{-1}$) | Relative Affinity |
|---|---|---|---|
| Mannose | 2.3 x $10^3$ | -7.6 | 0.96 |
| MeαMan (**1**) | 2.2 x $10^3$ | -7.7 | 1.00 |
| EthylαMan | 1.2 x $10^3$ | -8.1 | 1.8 |
| PropylαMan | 300 | -8.9 | 7.3 |
| ButylαMan | 151 | -9.3 | 15 |
| PentylαMan | 25 | -10.4 | 88 |
| HexylαMan | 10 | -10.9 | 220 |
| HeptylαMan (**5**) | 5 | -11.3 | 440 |
| OctylαMan | 22 | -10.4 | 100 |
| *p*NPαMan (**2**) | 44 | -10.0 | 50 |
| MeUmbαMan (**4**) | 20 | -10.5 | 110 |

Amongst the series of alkyl mannosides tested from methyl to octyl, there were a thousand fold increases from ethyl to higher homologues and a steady increase up to the heptyl mannoside (**5**) after which, the $K_D$ started to increase. It is also noticeable that the hydrophobic aglycones of the best candidates are flanked by two tyrosine residues (Tyr48 and Tyr 137) in the active site (Fig. **5**) that may provide the rationale for the observed, more than two decades ago, of the high affinities of *o*-chloro-*p*-nitrophenyl α-D-mannoside (**3**) against other strains of *E. coli* (346 (025) and 128) [33]. Indeed, in inhibition of yeast aggregation by the bacteria, compound (**3**) showed relative inhibitory behavior that was 717 and 470 times better than the reference compound (**1**), respectively (Fig. **4**). It is our opinion that the most potent glycodendrimers will be those constructed using the best monosaccharide structures [34] mounted on dendritic/polymeric structures having optimized scaffolds, valencies, and linker distances. These factors should necessarily vary from one receptor to another and will further confer specificities [35].

## 3. GLYCODENDRIMERS FOR *E. COLI* TYPE 1 FIMBRIAE INHIBITION

### 3.1. Lysine-Based Glycodendrimers

The first glycodendrimers to appear in the literature in 1993 were built using divergent solid-phase peptide chemistry and L-lysine as repeating assemblies [36]. They were bearing exposed sialic acid residues and were constructed for the inhibition of flu virus attachment by competing with sialoside ligands present on respiratory tracts against the hemagglutinin of the virus particles. Using dendrons having eight sialosides on the surface, it was demonstrated that each saccharide residues were thousand fold better, on a per saccharide basis, than the corresponding monomer [37]. When the same poly-L-lysine scaffold was utilized with mannoside residues bearing an arylated aglycone (partly optimized monomer), the resulting 8-mer glycondendron (Fig. **6**) happened to show 100,000 fold increase inhibitory potency against fimbriated *E. coli* K12 on a per mannoside basis [38].

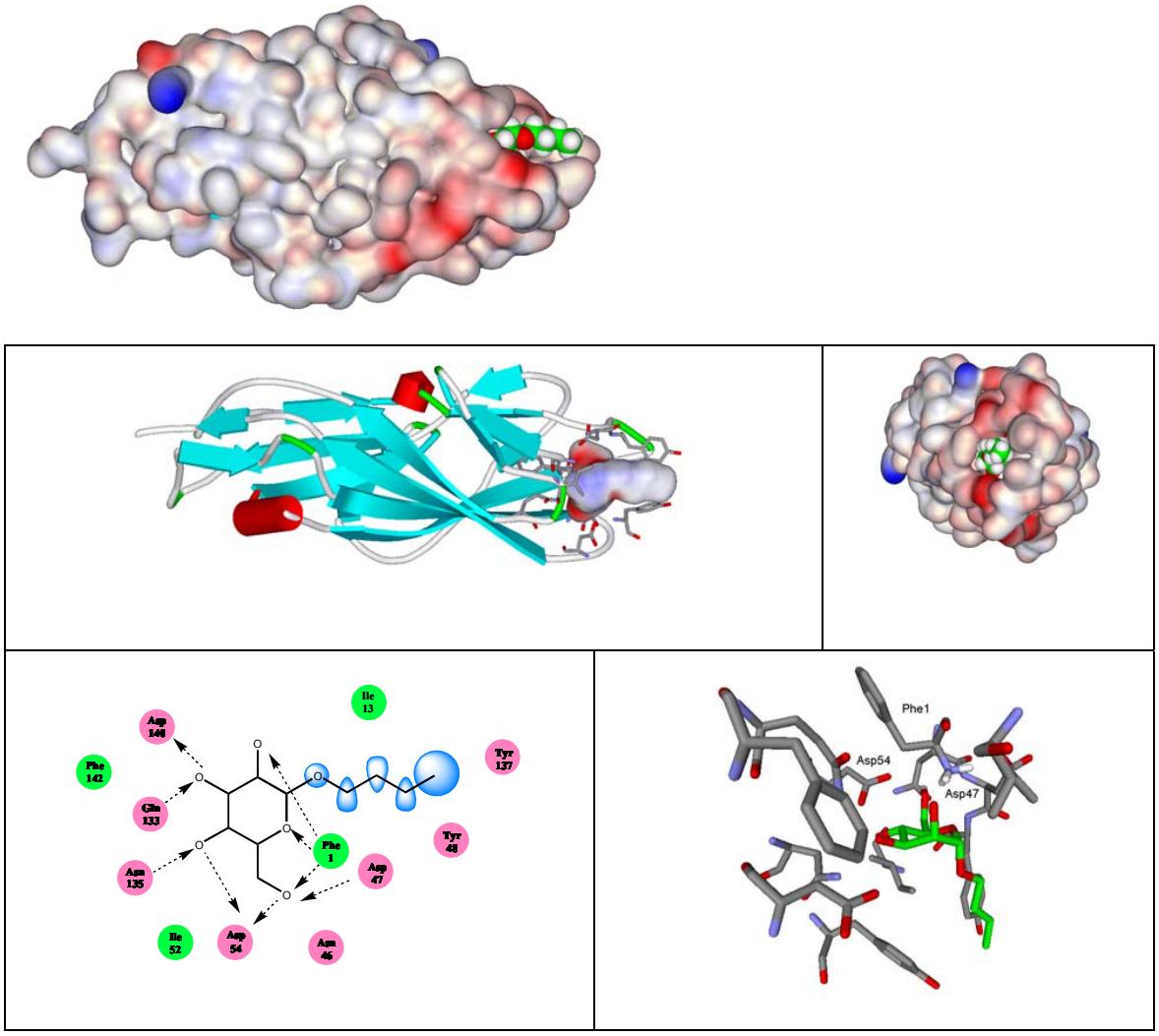



**Fig. (5).** Crystal structure and active site of *E. coli* K12 FimH incorporating the potent inhibitor butylαMan (adapted from PDB 1UWF).

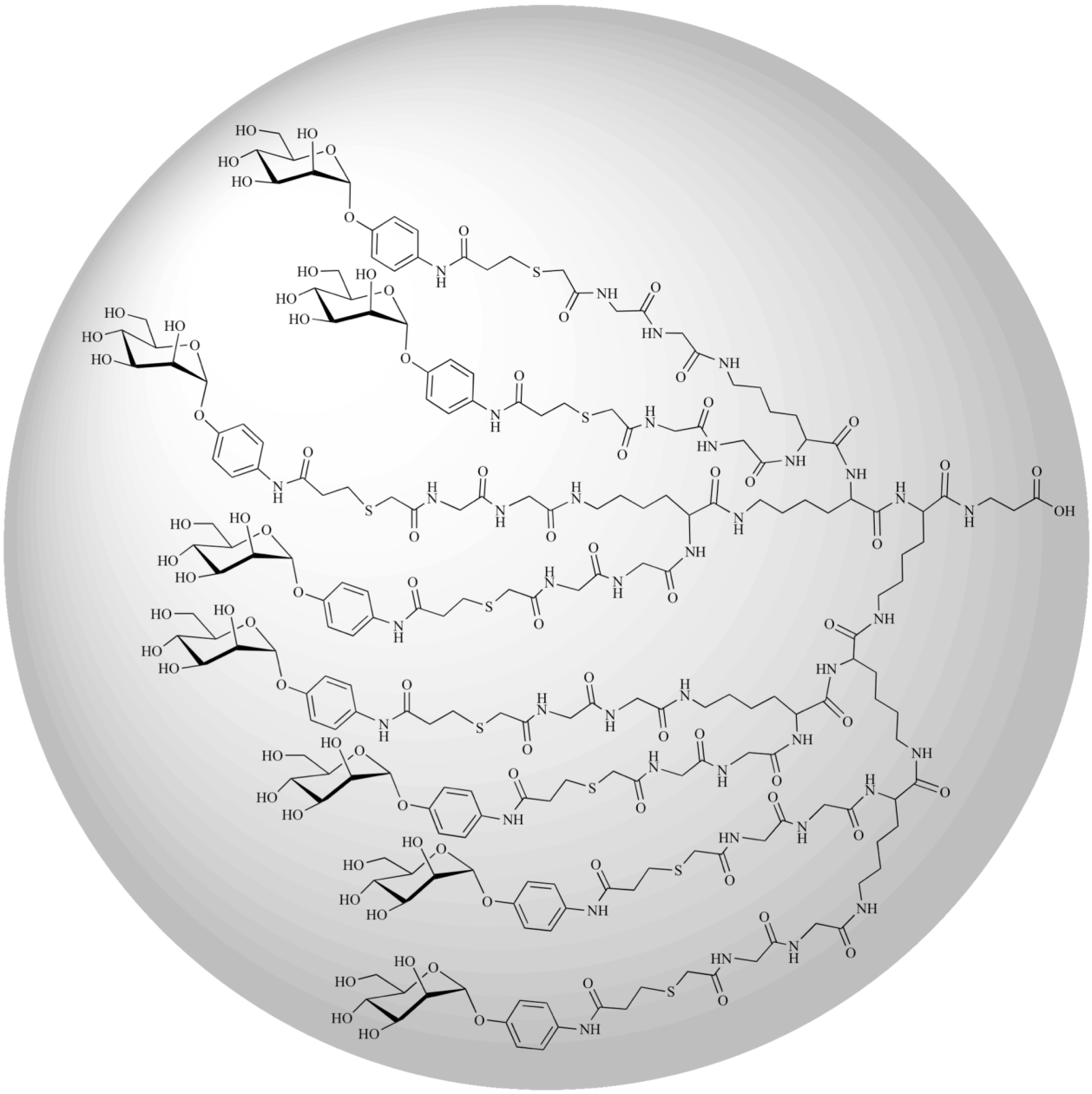


**Fig. (6).** Arylated octa-mannoside based on polylysine core showing an $IC_{50}$ of 14 μM against *E. coli* K12 [38].

Dendronized lysines represent one of the most widely used core structures in glycodendrimers. A review by Niederhafner *et al*. describes the synthesis of peptide dendrimers and their application [39]. Glycosylated lysine dendrimers have been prepared both on solid supports [40] and in solution [41]. Reaction of the peripheral amino groups with a variety of electrophiles carrying pendant sugar residues lead to glycodendrimers [16].

### 3.2. PAMAM-Based Glycodendrimers

Polyamidoamine (PAMAM) dendrimers [42], because of their commercial availability, have also attracted considerable interest toward the design of glycodendrimers. These dendrimers are built in a divergent manner using polyamidoamine scaffold ending with amine functionalities. The sugars are then usually attached via amide and thiourea linkages. Several groups [43-45] have used such strategy for the anchoring of protected and unprotected mannoside moieties. For instance, Lindhorst *et al.* [43] prepared mannosylated PAMAM dendrimers (**6-12**) (Figs. **7**, **8**) to investigate their relative potencies in the inhibition of hemagglutination of guinea pig erythrocytes by type 1 fimbriated *E. coli* HB 101 (pPK14), which expresses only type 1 fimbriae on their surfaces [46]. With the divalent and trivalent clusters, an increase inhibitory potency of over 70-fold was achieved; however this inhibitory behavior was surpassed by hexa- and

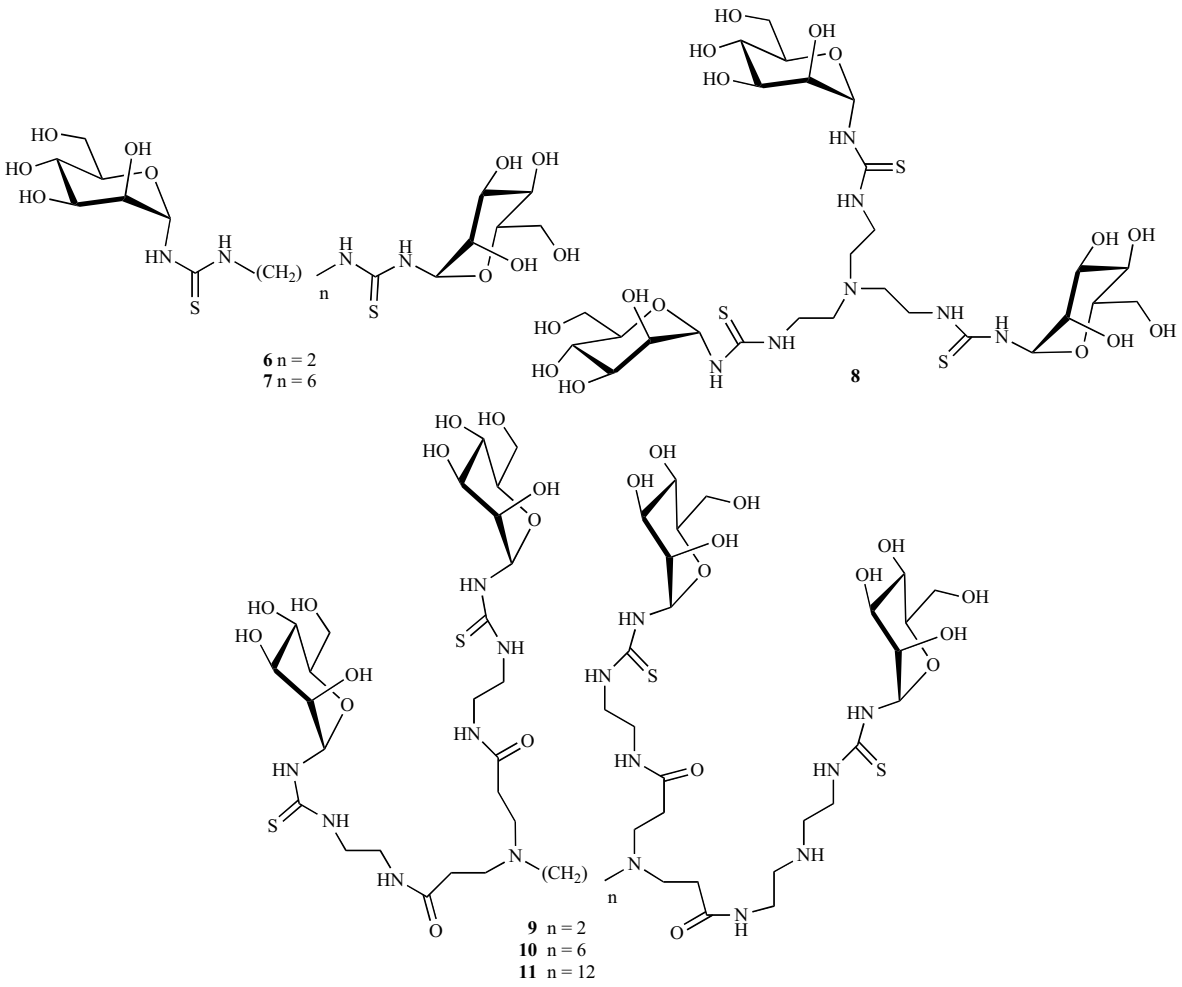



**Fig. (7).** Di-, tri-, and tetra-valent clusters obtained using unprotected mannopyranosyl thioisocyanate.

octa-mers. It was argued that non-specific interactions such as hydrophobic and π-stacking effects have to be considered for the interpretation of the results. Based on the thiourea bond formation, Lindhorst and co-workers have also descri bed the synthesis of C-6-linked triantennary cluster mannosides [47] (**13**, **14**) (Fig. **9**). This approach is however debatable since it is clear that the *E. coli* FimH binding pocket accommodates the mannoside residues from their non-reducing end, the aglycone of which protruding outside (Fig. **5**).

Cluster mannosides having methyl or *p*-nitrophenyl aglycones were tested as inhibitors of hemagglutination of guinea pig erythrocytes by *E. coli* HB 101 (pPKl4). Enzyme-linked immunosorbent assay (ELISA) yielded $IC_{50}$-values for the inhibition of *E. coli* adhesion to yeast mannan. Introduction of hydrophobic phenyl moieties in the case of cluster (**14**) (relative $IC_{50}$ ($RIC_{50}$) = 8.1), instead of the methyl aglycones in cluster (**13**) ($RIC_{50}$ = 0.6), decreased the $IC_{50}$-values over 100-fold, the inhibitory potency of *p*NPMan (**2**) ($RIC_{50}$ = 49) was not reached.

Pieters and co-workers have described the preparation and evaluation of multivalent mannosides, including small divalent systems, glycodendrimers, and glycopolymers, as inhibitors of type 1 fimbriated uropathogenic *E. coli*. The mannosylated surface groups were obtained by an amide bond formation between 3-aminopropyl α-*D*-mannopyranoside that was pretreated with diglycolic anhydride. Typical BOP ((Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate) or TBTU ((*O*-(Benzotriazol-1-yl)-*N, N,N′,N′*-tetramethyluronium tetrafluoroborate) peptide coupling reagents were used to furnish the desired PAMAM-dendrimers (**15**) (Fig. **10**) [48].

A novel and easy bioassay was set for this purpose. The mannosylated dendrimers to be tested were used in an ELISA-based assay for their ability to inhibit the binding of mannose binding type I fimbriated *E. coli* (FimH) to a monolayer of T24 cell lines derived from human urinary bladder epithelium. The PAMAM mannosylated dendrimers displayed the highest affinity towards the target, although their relative potencies, when expressed on a per mannoside residue, was rather low. Glycopolymers having 3 to 21 mannoside residues on the backbones showed enhanced activity with increasing mannoside substitution up to an $IC_{50}$ as low as 12 μM. The relative potency of the polymer series on a

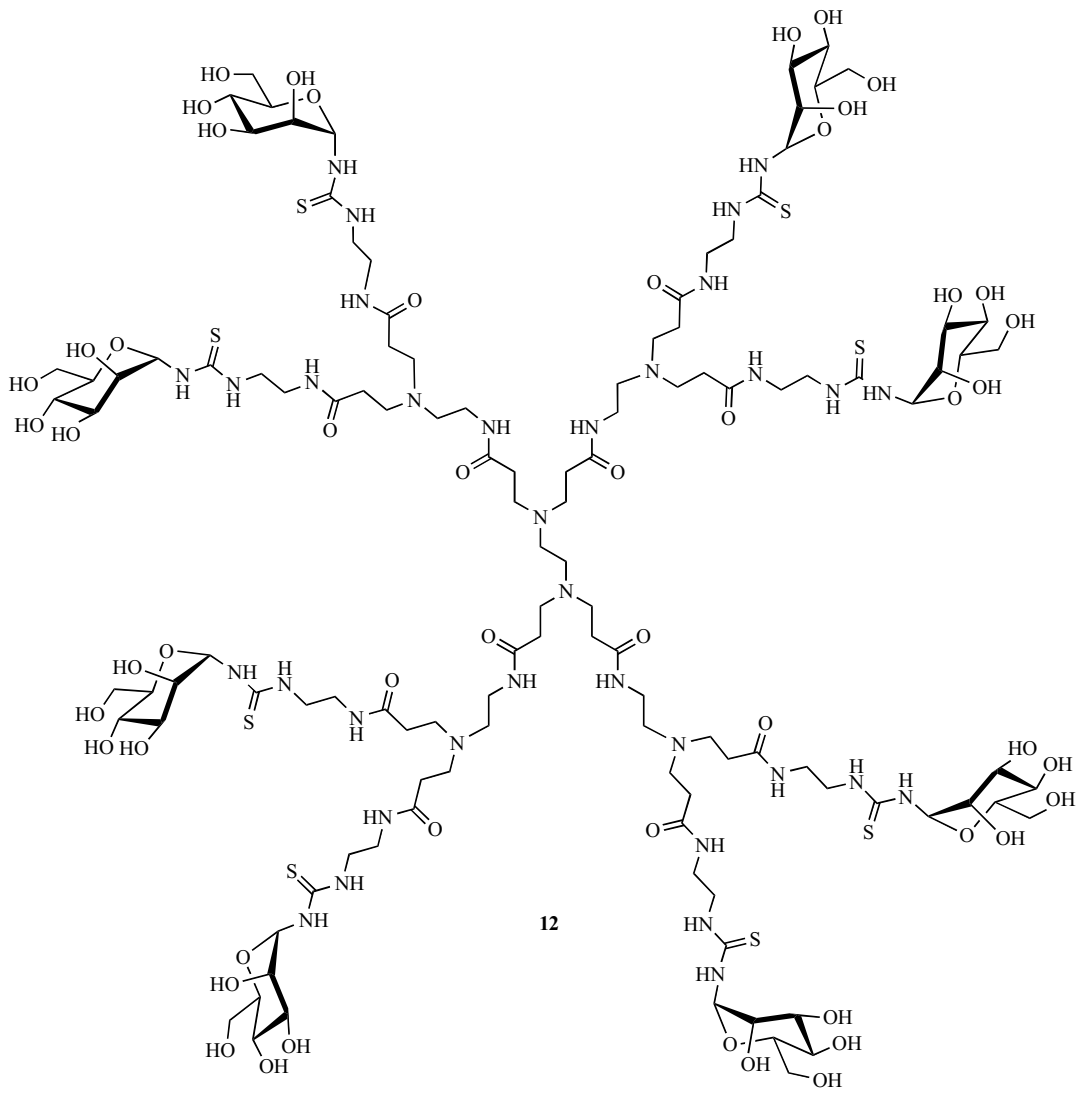



**Fig. (8).** Other thiourea-bridged PAMAM-based dendrimer (**12**) obtained using unprotected mannopyranosyl thioisocyanate.

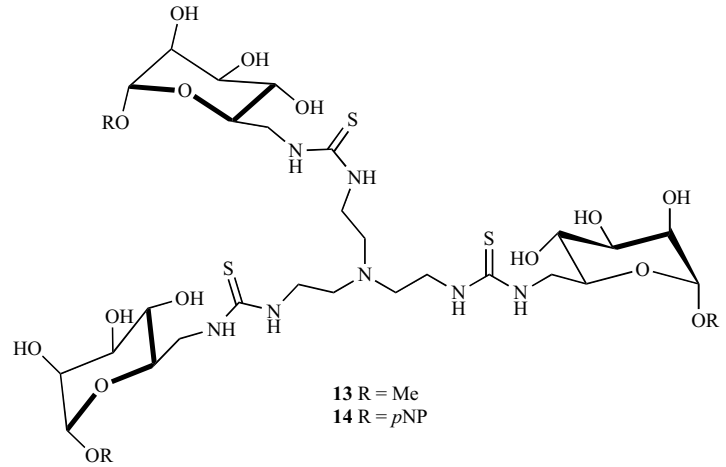
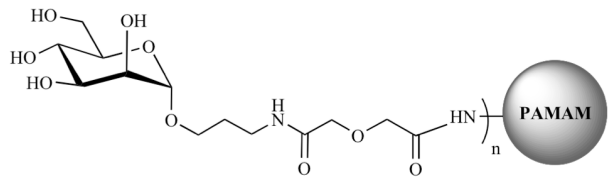



**Fig. (9)**. Trivalent mannosides attached through position C-6 of the sugar residue.




**15** n = 8, 16

**Fig. (10).** PAMAM glycodendrimers (**15**) obtained from 3-aminopropyl α-*D*-mannopyranoside [48].

per mannoside basis was relatively constant at around 30-40-fold better than that of methyl mannoside.

### 3.3. Boltorn-Based Glycodendrimers

In another application of hyper-branched Boltorn® polymers, our group applied the versatile "click chemistry" conditions to an azido-functionalized Boltorn® dendrimer (**17**) (BH20) (Touaibia and Roy, unpublished data). Dendrimer (**17**) was obtained by treatment of hydroxylated BH20 core with azidoacetic anhydride (Fig. **11**). Infrared (IR) analysis was used to demonstrate complete hydroxyl group transformation and azide function introduction. The "clicked" hyper-branched dendrimer (**18**) was obtained under typical reaction conditions ($CuSO_4$ and sodium ascorbate) using non-protected propargyl α-*D*-mannopyranoside (**16**). The relative inhibitory potency of the clicked dendrimers in the inhibition of agglutination of *E. coli* by yeast mannan was approximately 400 times higher than that of the respective methyl α-*D*-mannopyranoside (Roy *et al*., unpublished data).

Other workers have also used cycloaddition reaction toward the syntheses of mannosylated dendrimers [49, 50]. Riguera and co-workers have described a quick, efficient, and reliable multivalent conjugation of unprotected alkyne-derived carbohydrates (mannose, fucose and lactose) to three generations of azido-terminated gallic acid-triethylene glycol dendrimers [49]. Hawker and co-workers have described a fashion-controlled strategy toward glycodendrimers in which two distinct moieties (targeting and detection probes) were placed at the chain ends. Click chemistry was used as the key step, thus allowing simple and original buildup of these bi-functional dendrimers [50].

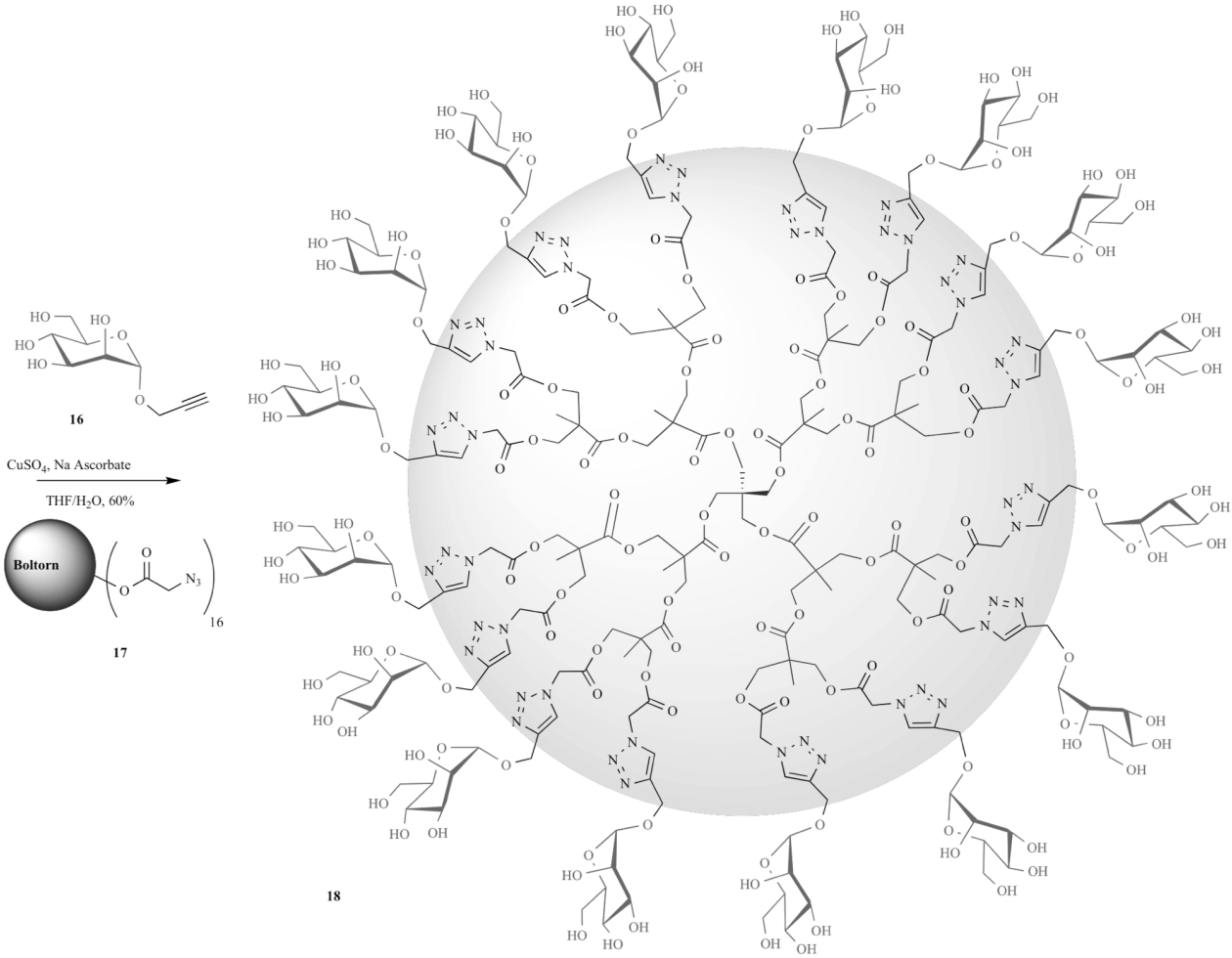



**Fig. (11).** Boltorn® H20 mannodendrimer (**18**) obtained by click chemistry (Roy *et al*., unpublished data).

### 3.4. Aromatic Scaffolds-Based Glycodendrimers

Using an aromatic core, the group of Pieters described the synthesis of multivalent mannosides as inhibitors of type 1 fimbriated uropathogenic *E. coli* [48]. A 3,5-di-(2-aminoethoxy)-benzoic acid scaffold (**18**) was used as repeating unit and a carboxylic acid moiety was introduced onto the carbohydrate (**19**), thus enabling peptide couplings with mono-, bis- and multivalent amino functionalized aromatic scaffolds to give dendrons such as (**20**, **21**) (Fig. **12**).

These mannosylated dendrimers were tested for their relative inhibitory potencies using a developed ELISA test discussed in the PAMAM section above. As expected, mannose itself was a poor inhibitor of binding of *E. coli* to urinary cell lines with an $IC_{50}$ of only 7.6 mM. Increasing the number of mannoside residues from two to sixteen, greatly improved the affinities, both in absolute value ($IC_{50}$ 51μM for the tetramer **21**) and in relative terms when expressed on a per mannoside basis. Somewhat surprisingly, a simple divalent compound, (**20**) having an elongated spacer, showed the highest relative potency on per sugar basis with a 141-fold enhancement ($IC_{50}$ 27 μM). Unexpectedly, mannosylated polymers of analogous structures were not as good as the dimer above.

### 3.5. Pentaerythritol-Based Glycodendrimers

Pentaerythritol is a simple five carbon tetraol used in the fabrication of resins, alkylated resins, varnishes, PVC stabilizers, tall oil esters, and olefin antioxidants. Pentaerythritol is an interesting compound that allows for the attachment of four (similar or different) groups at the apex of a tetrahedral scaffold that has been used for the construction of highly branched structures. Accordingly, this compound has received considerable interest as an orthogonally protected handle useful for the generation of combinatorial libraries and as building blocks towards oligonucleotides and peptides, thus providing additional functionalities.

For the inhibition of mannose-specific bacterial adhesion, Lindhorst and co-workers designed a pentaerythritol-based cluster-mannoside (**27**) as shown in Fig. **13**, in which pentaerythritol itself, as well as the included C3 spacers, were used as structural elements of the mannopyranoside moieties [51].

In the first route, a hydroxy linker was introduced within the aglycone moiety (**24**) and a Williamson ether synthesis with commercially available pentaerythritol tetrabromide (**22**) led to a mixture of mono-, di-, tri-, and tetradentate (**26**) products, even when an eight-fold excess of alcohol and

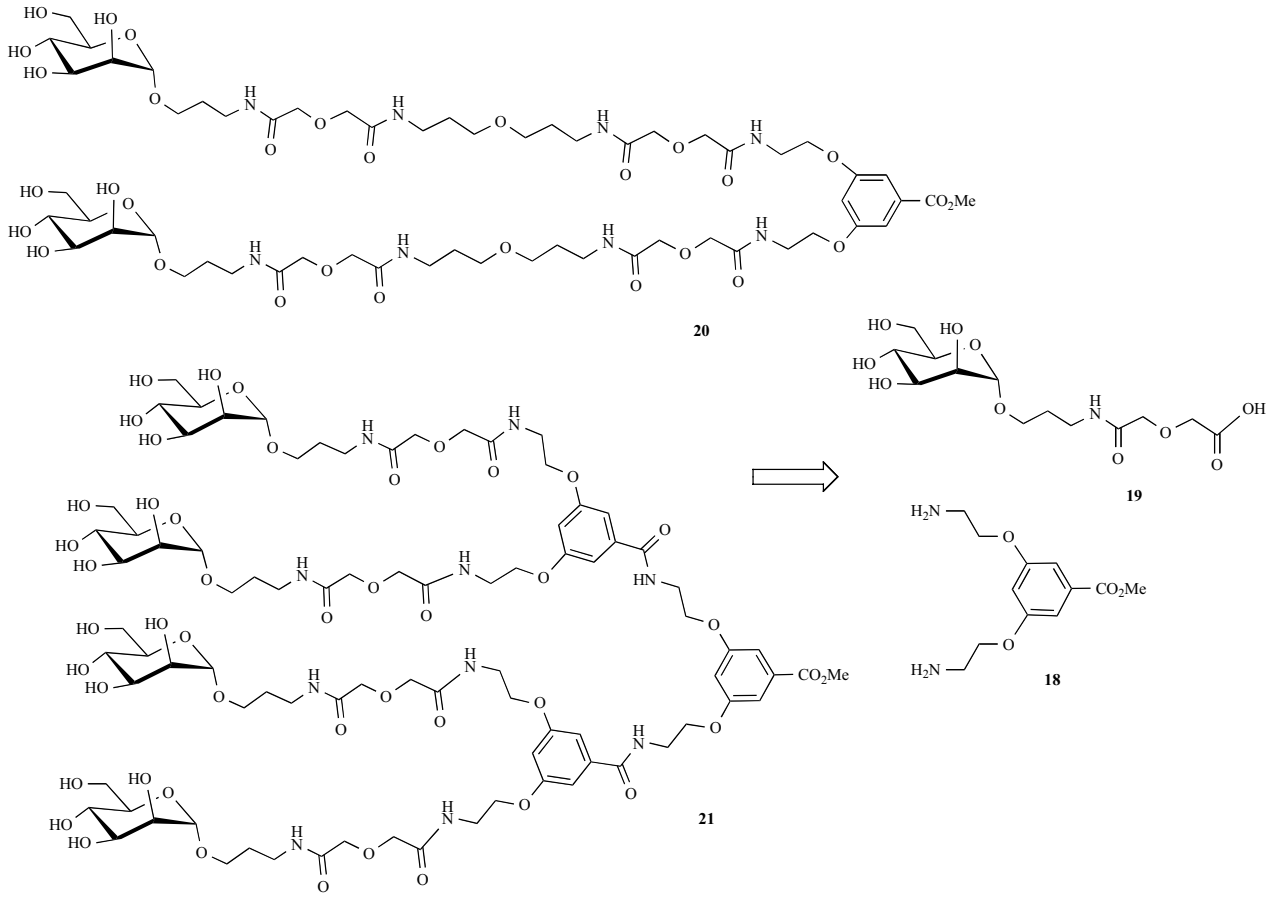



**Fig. (12).** Aromatic core as scaffold for multivalent mannoside clusters used in the inhibition of binding of *E. coli* to human urothelial cell lines [48].

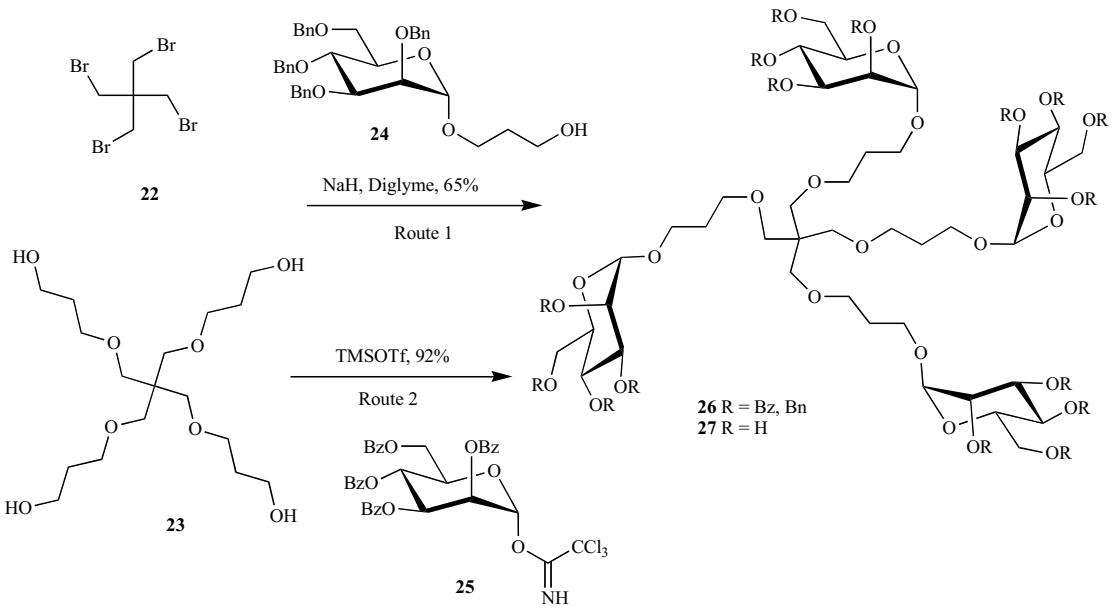



**Fig. (13).** Pentaerythritol-based mannocluster.

forcing reaction conditions were used (Fig. **13**). In the best case, the tetravalent cluster was isolated in 65% yield. An alternative route for the targeting of tetrameric cluster was investigated, in which pentaerythritol was modified to serve as a spacer-equipped tetrafunctional core molecule (**23**) for the subsequent glycosylation step. To this end, pentaerythritol was initially perallylated and the extended tetraol (**23**) was obtained by oxidative ozonolysis (9-BBN, NaOH, $H_2O_2$). Then, perbenzoylated mannosyl trichloroacetimidate (**25**) was used as the glycosyl donor. Protected tetramannoside (**26**) was isolated in excellent yield. Trivalent mannosylated clusters were also prepared and compared to the above tetravalent structures [52] (Fig. **14**).

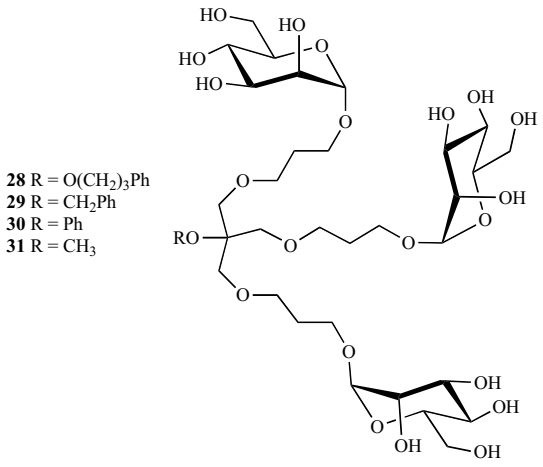



**Fig. (14).** Pentaerythritol-based mannoclusters.

Tri- and tetra-valent mannoclusters (**27-31**) were tested for their capacity to inhibit mannose-specific adhesion of *E. coli* HB101 (pPK14). Except for compound (**31**), the relative inhibitory potencies of the trimeric structures (**28-31**) ($RIC_{50}$ = 22, 33, 33, 67) were about half to that of tetravalent cluster (**27**) ($RIC_{50}$ = 64).

A series of trisaccharide mimetics to serve as ligands for the type-1 fimbrial adhesion was similarly prepared [53]. These glycoclusters were either obtained through an 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ)-assisted peptide-coupling reaction with triacid (**34**) and mannosides (**33**) or (**37**), without further protection to provide trivalent mannosides (**35**, **36**, **38**) (Fig. **15**). This procedure was successfully used to obtain clusters bearing methyl (**35**) and *p*-nitrophenyl (**36**) aglycones after branching through the C-6 amino position. The latter is of interest as aromatic moieties can increase the affinity of a given carbohydrate ligand to its lectin receptor by hydrophobic interactions. Cluster (**32**) was obtained by a classical glycosidation flowed by deprotection. Clusters (**35**) (R = $CH_3$) and (**32**) have surpassed the inhibitory potency of the cluster containing *p*NPMan (**2**). Only the spacer-modified mannosides (**38**) revealed interesting successful inhibition of type-1 fimbriae-mediated adhesion of *E. coli* to high mannose-type surfaces. However, none of these compounds reached the inhibitory potency of (**35**) (R = $CH_3$) and (**32**) (Table **2**). These results were however questionable given the fact that fimbriated *E. coli* (FimH) prefers to bind mannosides from the non-reducing end (see Fig. **5**), thus providing no room for the clusters to enter in the active site by their reducing end.

Only very poor inhibitory potencies of the corresponding nonavalent analogs (**43**, **44**) (Fig. **16**) were obtained [54]. Aagain, theses results supports previous observations indicating that *E. coli* FimH prefers to bind mannosides from the non-reducing end (see Fig. **5**).

### 3.6. Other Glycodendrimers

Glycerol has also been used as scaffold for glycodendrimer syntheses. A report describes the synthesis of up to

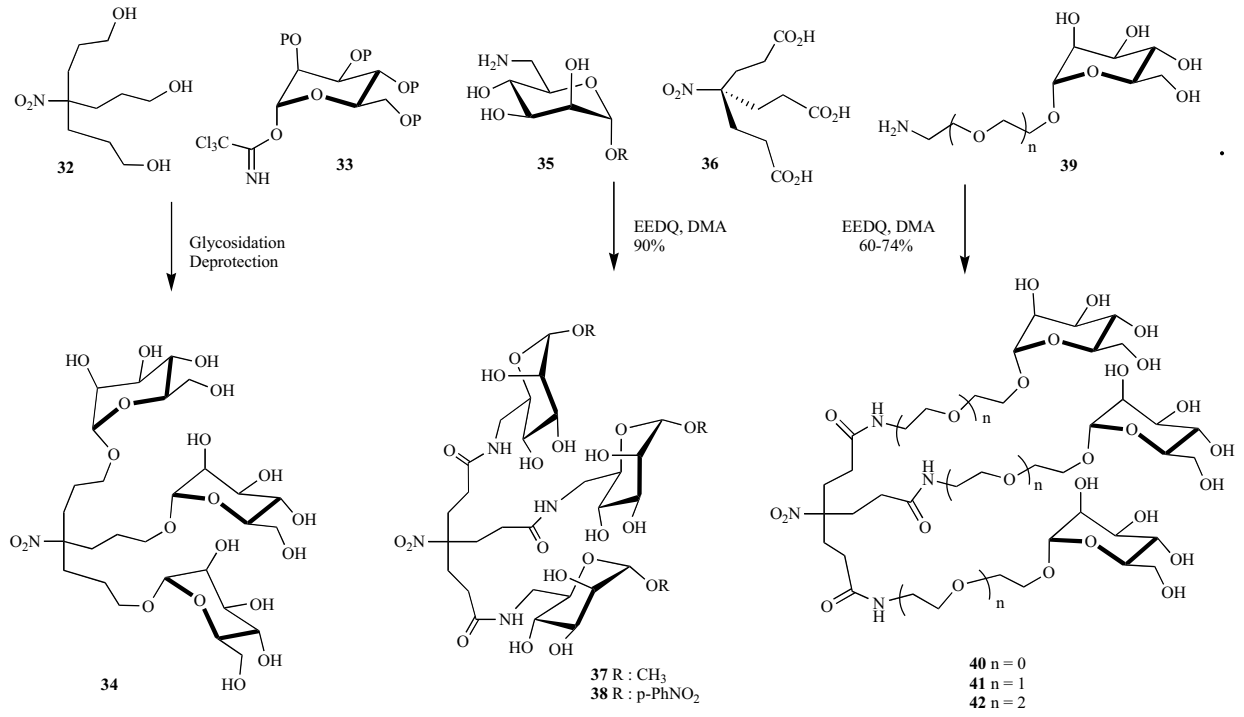



**Fig. (15).** Trivalent clusters used in binding studies against *E. coli*.

**Table 2. Antiadhesive Properties of the Trivalent Cluster Mannosides for *Escherichia coli* HB 101 (pPK14) Measured by a Sandwich-type ELISA Assay**

| $IC_{50}$ (μM) | MeMan | *P*NPMan (2) | 34 | 37 | 40 | 41 | 42 |
|---|---|---|---|---|---|---|---|
| $IC_{50}$ | 4250 | 43 | 17 | 4 | 238 | 222 | 134 |
| $RIC_{50}$ | 1 | 99 | 250 | 1063 | 18 | 19 | 32 |

four mannose-containing residues (**45**, **46**, and **47**) (Fig. **17**) [55]. These compounds were evaluated for their capacity to inhibit mannose-specific adhesion of *E. coli* HB 101 (pPK14). Unfortunately, none of the tested cluster (**45-47**) ($RIC_{50}$ = 6, 10, 13) showed better inhibitor than *p*NPMan (**2**) ($RIC_{50}$ = 50).

The synthesis of α-mannosylated azamacrocycles (Fig. **18**) and the evaluation of their ability to inhibit type I fimbriae-mediated adhesion of *E. coli.* to guinea pig erythrocytes was reported [56]. Using efficient thiourea-bridging strategy, tetravalent clusters were synthesized in good yields using commercially available azamacrocycles 1,4,7,10-tetraaza-cyclododecane (cyclen, **48** n=0), 1,4,8,11-tetraaza-cyclotetradecane (cyclam, **49** n=1) as scaffold and peracetylated α-*D*-mannopyranosyl isothiocyanate (**50**).

The binding potencies of the compounds were determined by inhibiting the hemagglutination of guinea pig erythrocytes by *E. coli.* This test yields half quantitative inhibitory potencies, determined as inhibition titres (IT: the lowest sugar concentration that inhibits hemagglutination). The azamacrocyle clusters show significantly better binding to the type 1 fimbrial lectin in the inhibition agglutination test than *p*NPMan (**2**). Compared with MeMan (**1**) and based on one mannose residue, the unsymmetrical macrocycle (**52** n = 1, RIT = 780) performed even better than the symmetrical analogue (**51** n = 0, RIT = 195) and *p*NPMan (**2**) (RIT = 94). This might be explained by a better conformational availability of the mannosyl residues provided by greater flexibility of the macrocyclic scaffold.

In a recent study from this laboratory, small libraries of mannosides and mannoside clusters were constructed for the establishment of a Quantitative structure activity relationship (QSAR) model toward *E. coli* FimH [34]. The library included several C and O-linked mannopyranoside analogs. As shown in Fig. **19**, click chemistry on a pentaerythritol or bis-pentaerythritol core was used. This family of clusters indicated that they were approximately hundred times more efficient in the inhibition of agglutination of *E. coli* x7122 [57] by baker's yeast than the monomer D-mannose. Indeed, inhibition titers of 370 mM and 3.96 mM were obtained for D-mannose and compound (**53**), respectively. Surface Plasmon Resonance measurements [32] designated a tetramannoside cluster (**53**) as a potent binder at concentrations in the nanomolar range. A tetracluster (**53**) (RKd = 102) was found to be three times more potent than the *p*NPMan (**2**) (RKd = 32).

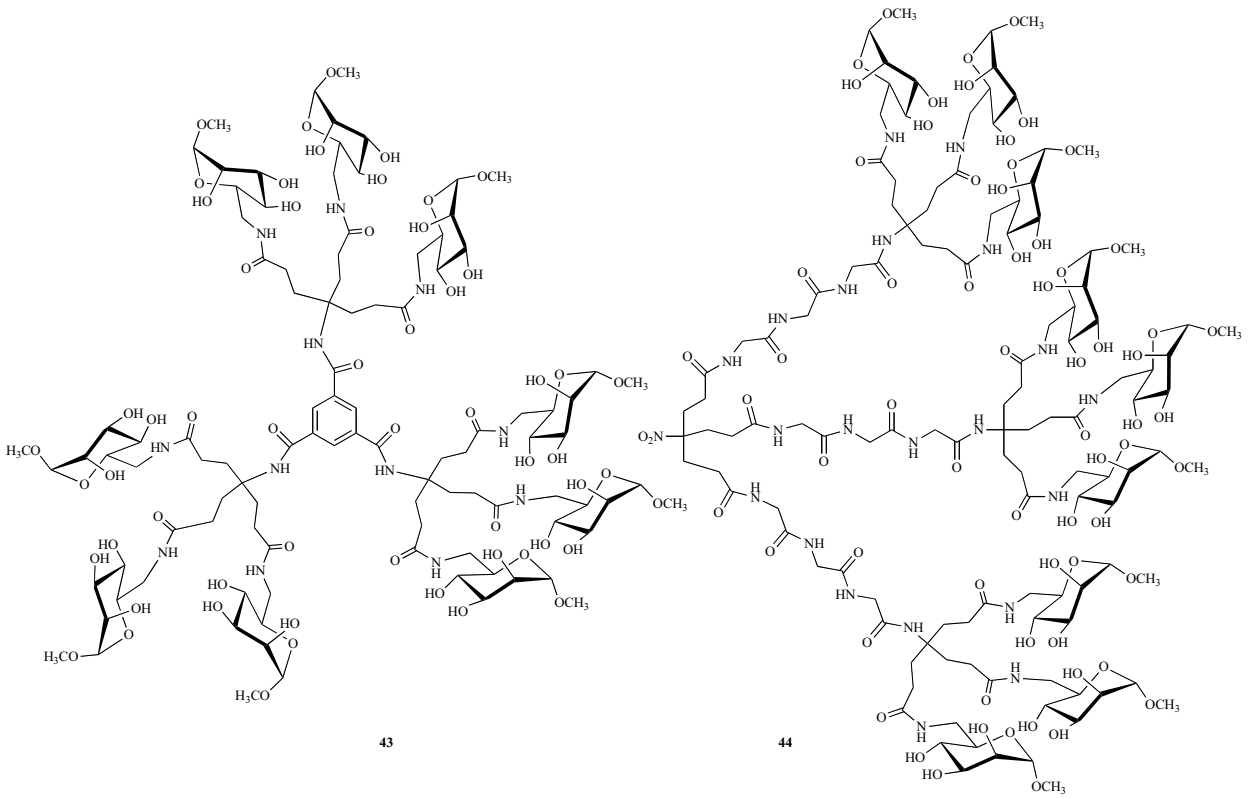


**Fig. (16).** Nonavalent C6-linked mannoclusters.

These novel clusters are effectively useful as lead structures for the synthesis of more potent inhibitors of *E. coli* adhesion.

## CONCLUSIONS

The enormous creativity that has been injected in the field of glycodendrimer syntheses has allowed the prepara-

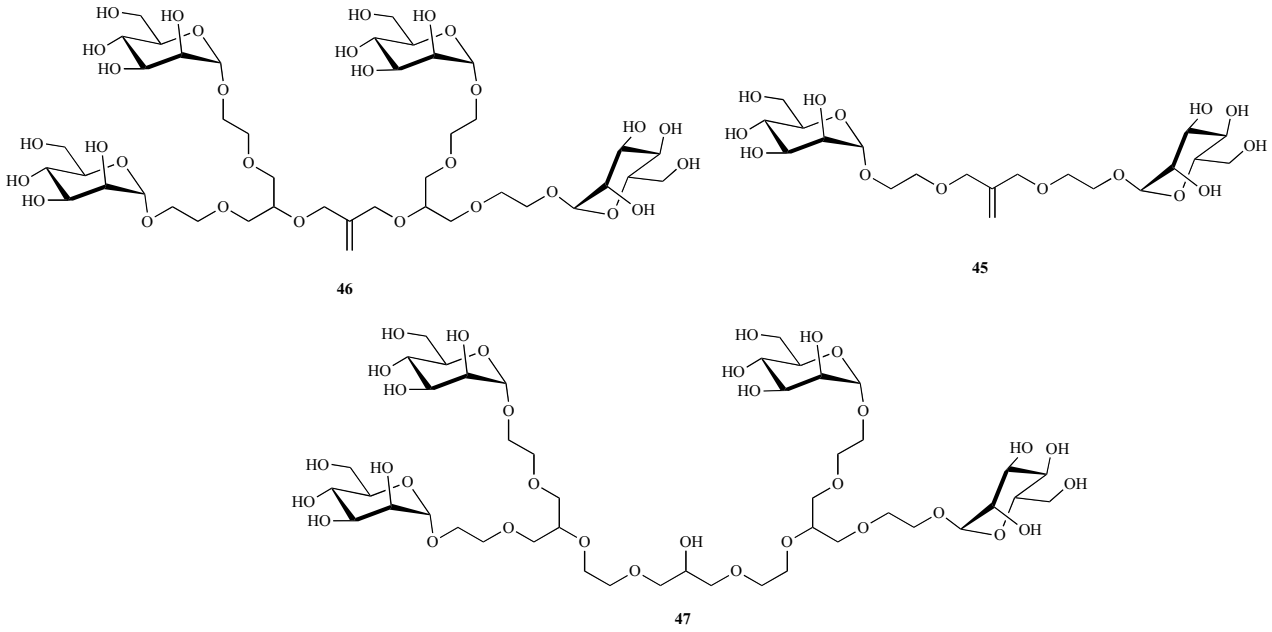


**Fig. (17).** Glycerol-based mannoclusters.

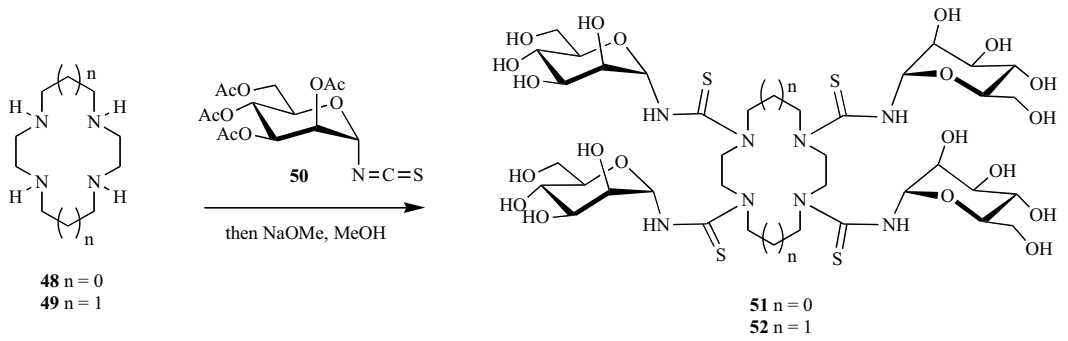



**Fig. (18).** Anomeric and 6-linked trimannoclusters used in binding studies against *E. coli.*

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

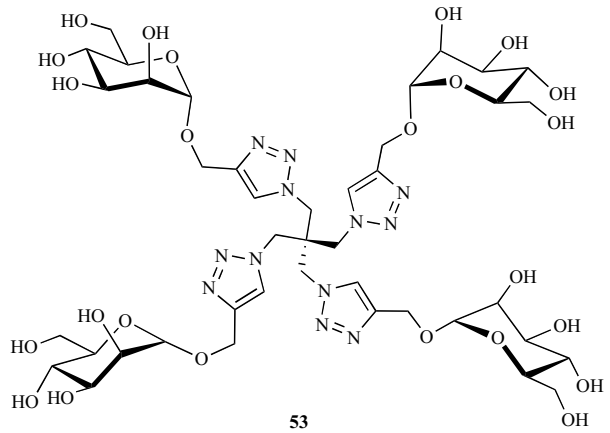



**Fig. (19).** Tetramannoside cluster obtained by click chemistry on a tetrazido pentaerythritol core and propargyl mannoside [57].

The general information at hand tends to indicate that glycodendrimers are better than glycopolymers for binding to FimH on fimbriated *E. coli*. Additionally, longer and rigid linkers have brought some noticeable improvements. It is also clear, that for any given situations, optimization of the key monovalent sugars will have to be investigated, prior to or in parallel to making multivalent clusters. Such task is being undertaken in our laboratory and preliminary data provided monosaccharides that bind in nanomolar concentration to FimH from *E. coli*.

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

With the vast access and information now available regarding new glycodendrimers, more thorough biological investigations will have to be designed. Cellular toxicity is certainly an area behind schedule for glycodendrimers. Cell permeability with varying structural motifs and sugar composition also remains to be further investigated. With the merging of several glycoscientists working together, the future looks very bright for glycodendrimers and, as stated by N. Sharon and coworkers [33] more than two decades ago, the realm of using "sugars" as bacterial anti-adhesion molecules is finally reachable.

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