Supramolecular Chemistry of Carbohydrate Clusters with Cores having Guest Binding Abilities

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Abstract: This review concentrates on both the protein receptor and guest binding abilities of carbohydrate clusters based on a cyclodextrin core. The combination of both complexation abilities is the basis of one of the pursued approaches for developing site-specific drug delivery systems. Influence on the molecular recognition properties of the number of appended saccharides, the type of carbohydrate clustering and the type of the spacer arms, among other factors, are discussed.

Keywords: Cyclodextrins, neoglycoconjugates, glycoclusters, glycodendrimers, drug delivery, multivalent effect.

I. INTRODUCTION

Clustered carbohydrates on simple scaffolds, surfaces, linear polymers or attached on the terminal groups of dendrimers, have been the focus of attention of many researchers, from synthesis to biological and pharmaceutical applications, for more than ten years [1,2]. This interest on glycoside clusters and dendrimers is based on the originally observed glycoside cluster effect by Lee *et al.* [3], currently, commonly referred to as glycoside multivalent effect.

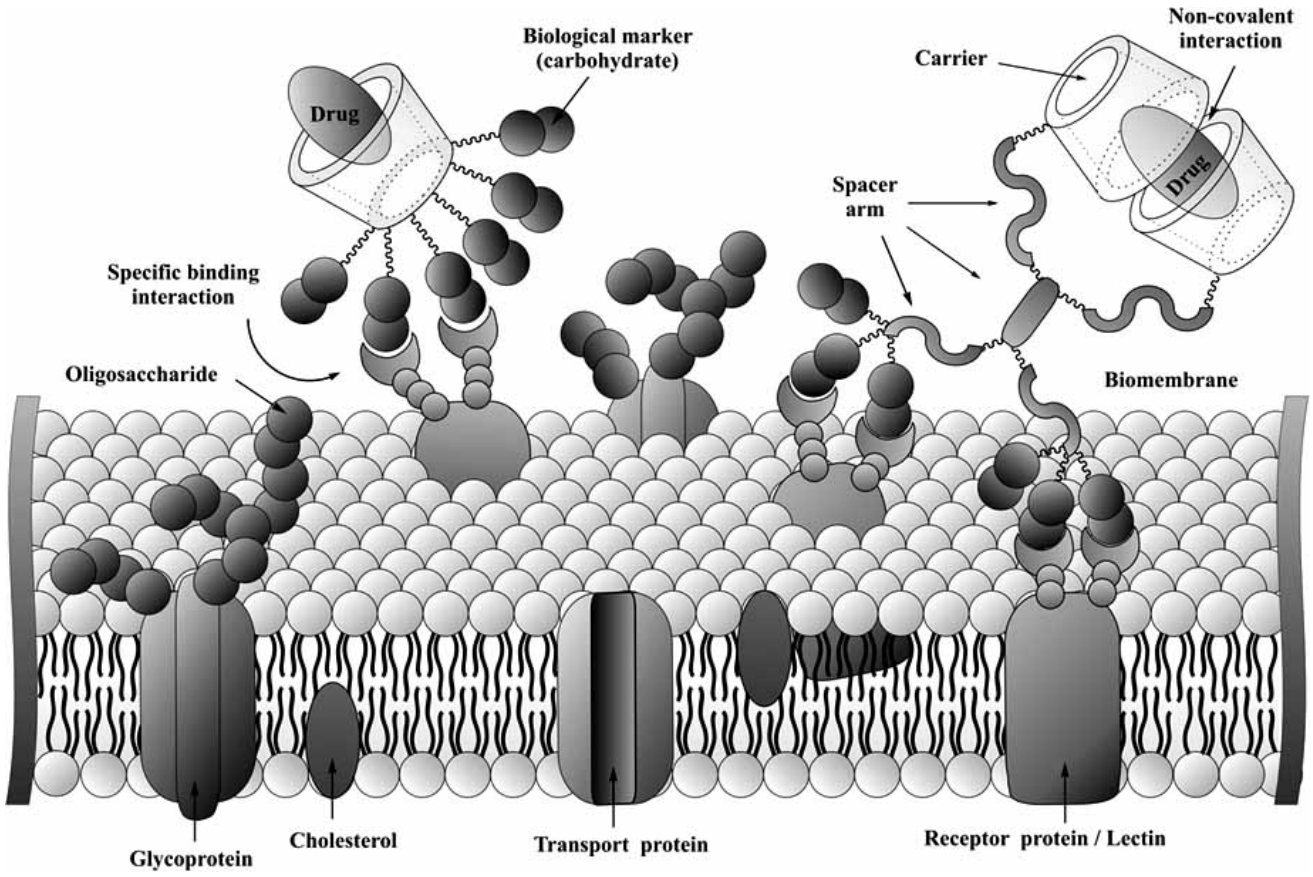

Fig. (1). Host-guest complexes for site-specific drug delivery.

Accordingly, clustered copies of carbohydrate ligands -multivalent ligands- interact with their protein receptors in a stronger and more selective fashion than the analogous single –monovalent- ligands. This phenomenon is

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ubiquitous in nature and involved in a wide range of important biological functions. The understanding of this phenomenon and particularly the development of therapeutic applications have promoted most of the work in this area. If the glycocluster has the ability to encapsulate guest molecules, and particularly drugs, it could be possible to design a molecular delivery system with the potential of carrying drugs in a noncovalent way toward a specific biological receptor (Fig. **1**) [4,5]. The selectivity of the drug carrier for the biological target would be dictated by the enhanced binding affinity of the glycocluster for that receptor as a result of the multivalent effect.

One of the strategies followed by the different authors has been the use of macrocycles with known host-guest properties as scaffolds for the construction of glycoclusters and glycodendrimers as well as the attachment of glycodendrimers onto the macrocycle. Previous reviews in this field have been published by Fulton *et al.* [4] and Ortiz Mellet *et al.* [5]. β-Cyclodextrin (CD) is the most used macrocycle for this purpose. The use of CDs as a scaffold of cluster glycosides offers several advantages over other macrocyclic compounds: it is more readily available and affordable, more biocompatible, and has the ability to form inclusion compounds with a large variety of guests in aqueous solution [6].

In this review we will focus on recent reports of multivalent glycocyclodextrins with demonstrated ability to form complexes with carbohydrate receptors and/or guest molecules.

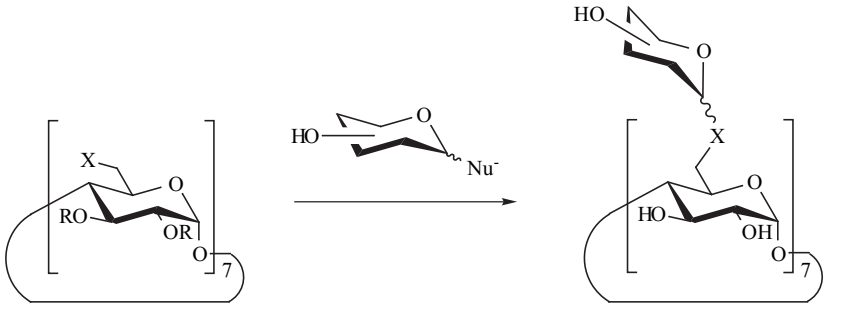
II. PER-SUBSTITUTED CYCLODEXTRINS ON A FACE

Per-substitution of the CD at C-6 has been one of the most convenient ways to create a glycocluster onto the CD ring. One of the most used strategies has been based on nucleophilic substitution reactions as a means to attach the carbohydrate residues at the CD C-6. As illustrated in Scheme **1**, normally the nucleophilic substitution reaction of per-6-bromo-6-deoxy-cyclomaltoheptaose **1** and per-6-deoxy-6-iodo-cyclomaltoheptaose **2a** (or the peracetylated derivatives **2b**) with the sodium salt derivatives of thio-α- and thio-β-glycopyranoside gives the per-6-*S*-glycosil-thiocyclomaltoheptaose derivatives **3** (Scheme **1**) [7].

Thus, de Robertis *et al.* [7a] used the sodium salt of 1-thio-β-, 1-thio-α- and 1,2-ethanedithio-α-galactopyranoside as nucleophiles in the presence of *N*,*N*'-dimethylpropyl-eneurea (DMPU) to give per-6-galactosyl-thio-CDs **3a-c** in 50-80% yield. In order to study the biorecognizable properties of these per-substituted CDs, a biological test was carried out using the galactose specific lectin *Kluyveromyces bulgaricus*. The flocculation assay showed that all the galactosyl CDs **3a-c** had inhibitory abilities. First, it was shown that per-substitution of the CD at C-6 with galactoside units led to better inhibition properties than the monosubstituted CDs. The hepta-α-galactoside CD derivative **3b** was twice more efficient than the hepta-β-galactoside CD **3a**. In addition, the presence of the spacer between the CD core and the pendant galactoside afforded a galacto CD derivative **3c** with better inhibition properties, being similar to those shown by *p*-nitrophenyl α-D-galactopyranoside.

Lainé *et al.* [8] synthesised the per-6-*S*-β-glucosylthio-(**3d**) and per-6-*S* -α-glucosylthio-cyclomaltoheptaose (**3e**). The complexation ability of the per-glucosyl-CD **3d** with two types of guest was studied by NMR: i) *p*-nitrophenol, which forms a complex with β-CD by inclusion into the cavity through the narrower primary hydroxy groups side, did not form any complex with the heptavalent glucosyl-CD **3d**; ii) prednisolone, which forms a inclusion complex with β-CD (K_a 2 x 10^3 M^{-1}) by penetration through the secondary side cavity, showed a K_a value of 0.2 x 10^3 M^{-1}. The authors attributed this important decrease of the complex stability to the absent of the 6-hydroxyl groups, that in the case of β-CD, contribute to the stabilization of the complex by formation of hydrogen bonding with the carbonyl group (C-3) of prednisolone.

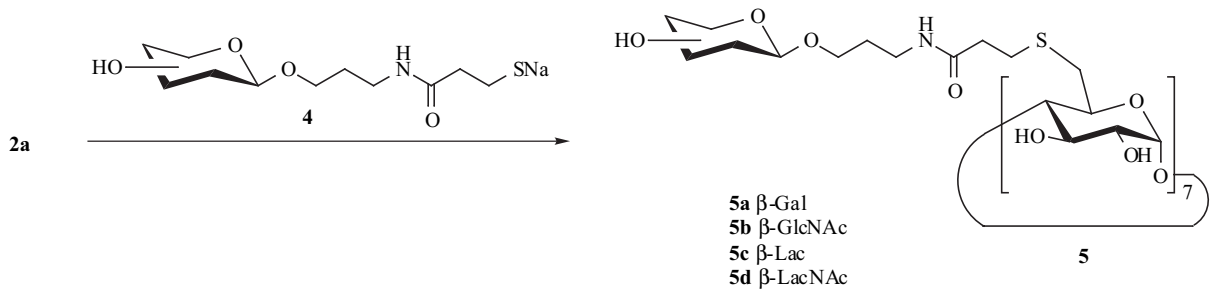
The nucleophilic displacement strategy has also been applied for the synthesis of a heptavalent persubstituted CDs in which the carbohydrate ligands are bound to C-6 through diverse spacer arms of different length and nature. Furuike *et al.* [9] synthesised per-substituted glycoclusters **5** with galactose, *N*-acetylglucosamine, lactose and *N*-acetyllactos-amine by reaction of the sodium thiolate derived from 3-(3-thioacetyl propionamido)propyl glycosides **4** with per-6-



1 X= Br; R= H
2a X= I; R= H
2b X= I; R= Ac

3a X= S, β-Gal
3b X= S, α-Gal
3c X= SCH_2CH_2S, α-Gal
3d X= S, β-Glc
3e X= S, α-Glc

Scheme 1.



Scheme 2.

deoxy-6-iodo-cyclomaltoheptaose **2a** in high yields (Scheme **2**). Lectin binding studies with glycoCDs **5** were performed by haemagglutination inhibition test of the wheat germ (*Triticum vulgaris*) agglutinin (WGA) and *Erythrina corallodendron* lectin (ECorL) which are known as *N*-acetylglucosamine and *N*-acetyllactosamine specific lectins, respectively. The minimum inhibitory concentrations for glycoCDs **5d** and **5b** were 4 and 40-fold higher, respectively, than the *N*-acetyllactosamine and *N*-acetyl-glucosamine monomeric analogues used as control. The influence of factors such as the carbohydrate appendage density and topology on the lectin binding abilities has been studied by measurements of the inhibition activities of glycoCDs **5** against galactoside-specific agglutinin from *Viscum album* L, as a plant lectin, and galectin-1, 3 and 7 and lactoside-binding immunoglobulin G, as mammalian lectins [10]. The inhibitory capacities of glycoCDs **5** were determined by solid-phase assays, haemagglutination as well as cell binding assays. It was found that heptavalent glycoCDs showed notable inhibitory potencies per galactosyl residue as compared with free lactose. Also, glycoCD **5** showed capacity to discriminate between galectins.

Later work by Furuike *et al.* [11] showed that a chemoenzimatic synthesis of glycoCDs having seven sialyl Lewis X units (Fig. **2**) can be performed by an efficient and convenient method. The nucleophilic substitution reaction was used for the chemical synthesis of glycoCD **5 b** containing seven residues of *N*-acetyl-D-glucosamine. Subsequent enzymatic reactions of **5b** by using three glycosyltransferases in the presence of the corresponding sugar donor substrates afforded the per-(sialyl-Lewis X)-CD **6** in high overall yield (Fig. **2**). The inhibitory properties of **6** on the interaction between E-selectin and neoglycoprotein containing multiple SLeX residues (SLeXn-BSA) was investigated by surface plasmon resonance (SPR). Compound **6** showed a highly enhanced inhibitory effect (IC_{50} of 1.5 mM) toward the binding of E-selectin with immobilized SLeXn-BSA chip, while SLeX did not show significant inhibition under the same condition.

The synthesis of CD-based glycoclusters and glycodendrimers of the type **8** and **9** was also carried out by application of the nucleophilic displacement strategy (Scheme **3**) [12]. Per-6-deoxy-6-iodo-β-CD **2b** and per-6-chloroacetamido-6-deoxy-β-CD **7** were used as electrophilic compounds in the reaction with isothiouronium salt or the thiol derivatives of the monosaccharide, the disaccharide and the divalent glycodendron in the presence of Cs_2CO_3 to obtain the CD **8a-d** and **9a-f** having seven and fourteen appended carbohydrate units in high yields.

Calorimetric titration of glycoCDs **8** and **9** gave the affinity constants and the thermodynamic parameters for the inclusion complex formation of these compounds with sodium 8-anilino-1-naphthalensulfonate (ANS) and 2-naphthalensulfonate (NS) as guest models. The persubstitution of the primary face of the β-CD with the appended carbohydrates led to a slight increase of the binding for the inclusion complexation with ANS as compared with β-CD. However, the increase of the steric congestion on the primary face caused a decrease of the binding ability when NS was used as guest. These studies also have shown that the spacer arms that link the appended saccharide units and the CD core were not passive elements and had an influence on the binding ability according to their chemical nature. The increase of the valency of the

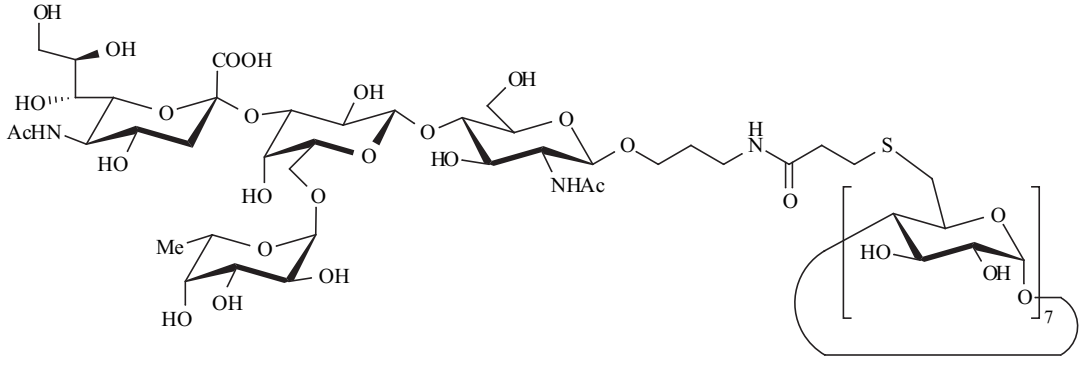
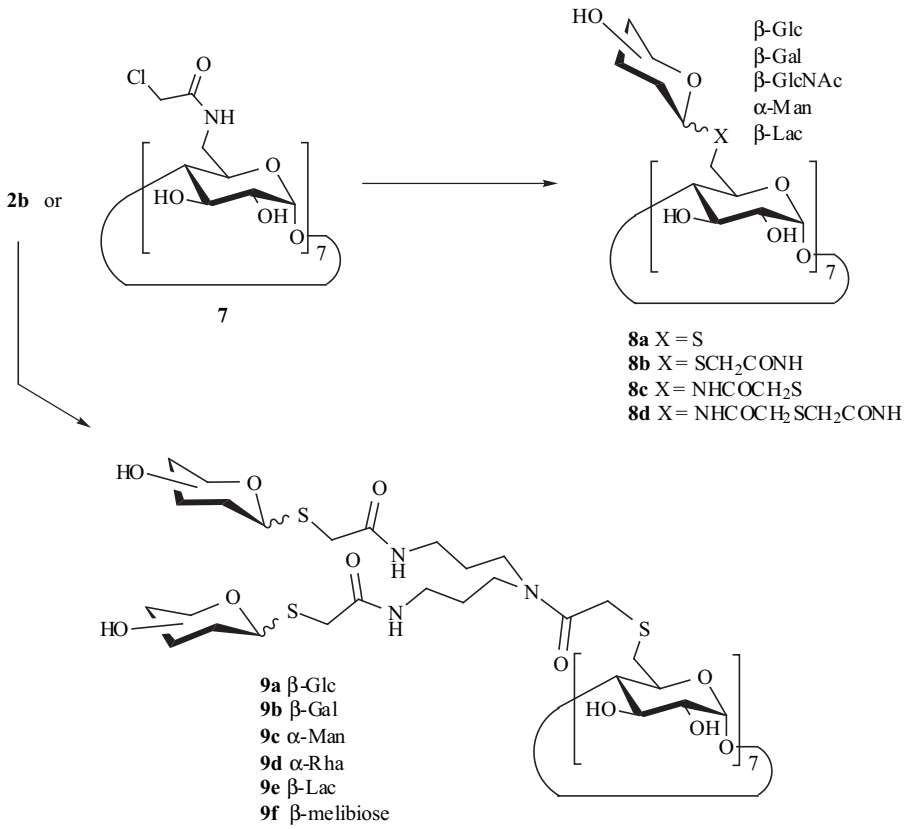
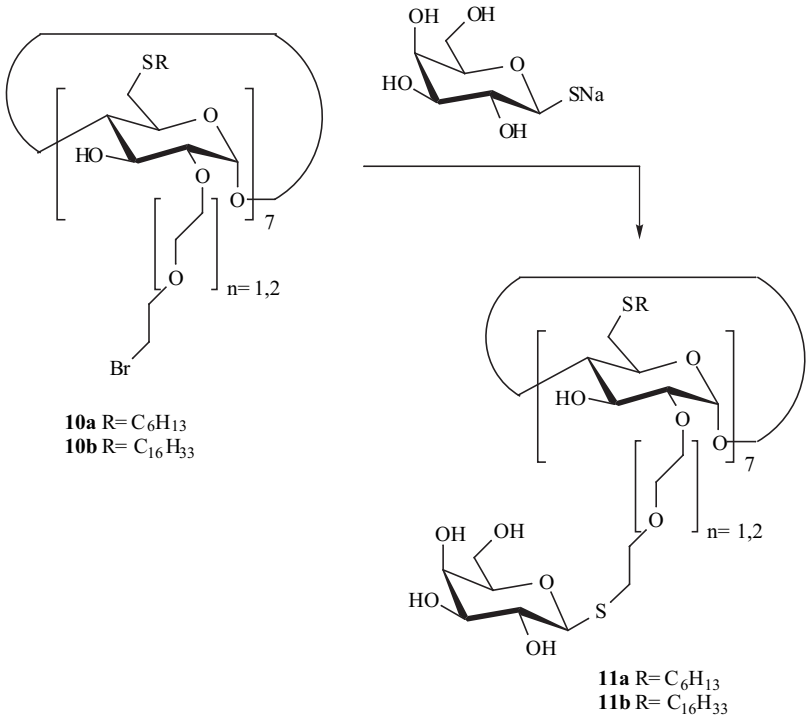


Fig. (2). Per-sialyl Lewis X-cyclomaltoheptaose.



Scheme 3.

glycoCDs from 7 to 14 ligands reduced drastically their host capability. The authors suggest that a narrowing of the secondary side of the torus may be induced by steric factors on the primary face preventing guest penetration. The binding properties of glycoCDs **8** and **9** towards plants lectins were evaluated using inhibition experiments (Enzyme-Linked Lectin Assays, ELLA), turbidimetric assays [12c] and Isothermal Titration Calorimetry (ITC) [12c,d]. *S*-



Scheme 4.

GlycoCDs, but no *N*-monosaccharide-CDs, showed good to excellent inhibition properties with lectins *Pisum sativum* (pea lectin), *Arachis hypogaea* (peanut lectin) and *Canavalia ensiformis* (Concanavalin A). ITC experiments with peanut lectin (PNA) showed the formation of soluble cross-linked complexes with Gal_7-CD **8b**, Lac_7-CD **8a-d**, and Lac_{14}-CD **9e** but not with the galactosylamine Gal_7-CD **8c** and **8d**. These Gal_7-CDs form stronger complexes with PNA than the monomeric analogues due to the cluster effect. For both Con A and PNA, glycoCDs having 14 carbohydrate ligands form stronger complexes than the monomeric analogues. However, the use of 14-valent Glyco-CDs did not contribute to the improvement of the complex stability relative to the seven-valent ones.

Mazzaglia *et al.* [13] synthesised amphiphilic β-CDs **11a,b** having alkythio chains at the primary face and galactosylthio-oligo-(ethyleneglycol) at the secondary face by nucleophilic displacement of the ω-bromo-oligo(ethylene glycol)-CD derivatives **10a,b** with sodium salt of 1-thio-β-D-galactose (Scheme **4**).

The amphiphilic CDs **11a**,**b** formed nanoparticles and vesicles, respectively, showing multivalent effect in their binding to a lectin. The binding of the amphiphilic CD colloids to galactose specific lectins from *Pseudomonas aeruginosa* and to PNA was investigated by MALDI-MS and fluorescence and by SPR, respectively. CD **11b**-based vesicle showed better binding abilities than CD **11a**-based nanoparticle. In addition, it has been suggested that a binding optimisation effect based on a dynamic and reversible self-assembly process might occur upon the binding interaction between the aggregates and the lectin.

Yasuda *et al.* [14] have reported the synthesis of per-6-galactosyl-glucono-amide-CD **12** and its monovalent analogue **13** (Fig. **3**) by nucleophilic displacement of halogen-β-CD. SPR measurements showed that **12** and **13** form complexes with PNA and the anticancer drug doxorubicin. The association constants of the complex formation between compounds **12** and **13** with PNA were 1.3×10^5 and 8.1×10^3 M^{-1}, respectively, while, association constants for the immobilized doxorubicin (DXR) were 6.2×10^4 and 3.1×10^3 M^{-1}, respectively. Interestingly, the per-substituted CD showed better K_a than the monovalent CD. The authors attributed this behaviour to the formation of an inclusion complex in the cavity formed by the carbohydrate appendages based on a kind of induced-fit phenomena as illustrated in Scheme **5**.

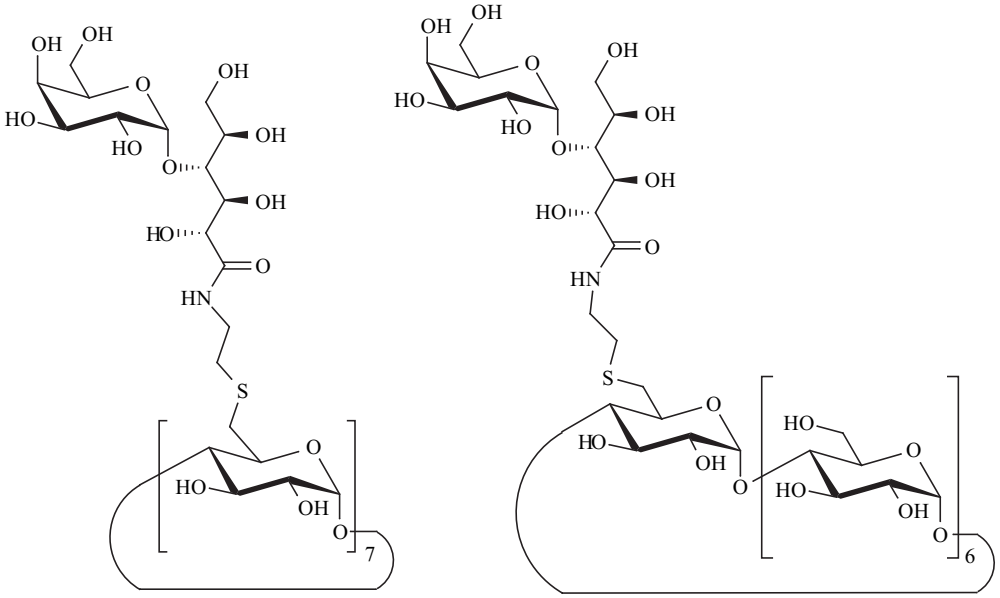
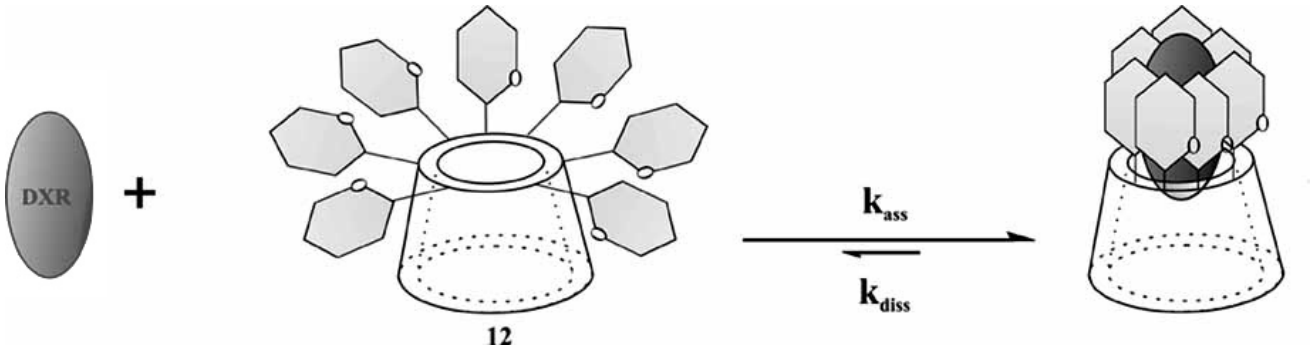


Fig. (3). Per- and mono-6-galactosyl-glucono-amide-cyclomaltoheptaose **12** and **13**.



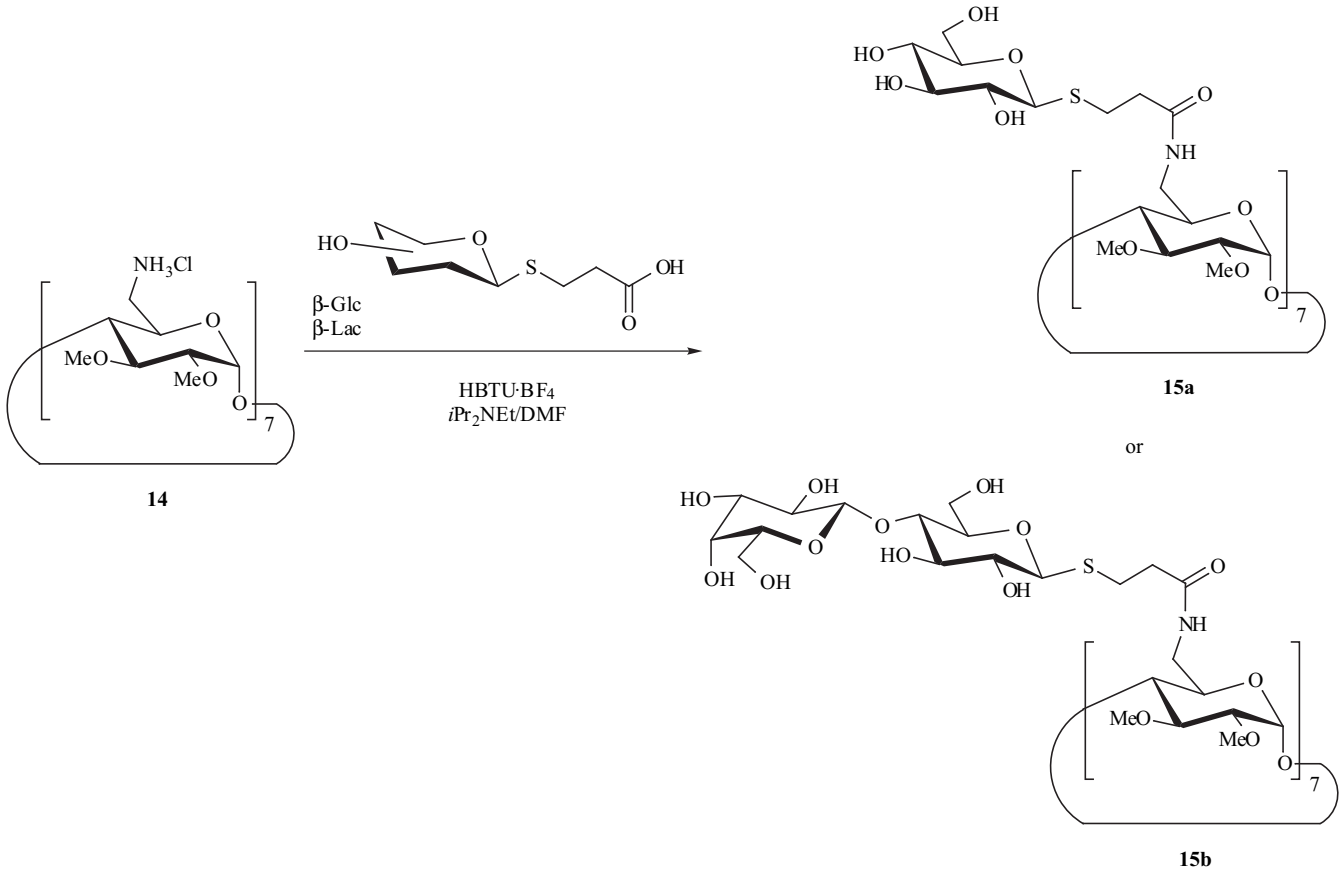
Scheme 5.

Another often followed strategy for the synthesis of per-glycoCDs has been the attachment of carbohydrate residues by amide bond formation. Ichikawa *et al.* [15] reported the synthesis of several β-CD derivatives conjugated with carbohydrates via aminohexyl linkages. β-CD was oxidized to give its per-functionalised carboxylate derivative at C-6. The amidation reaction did not provide the full attachment of carbohydrate residues onto C-6 of the CD and afforded multivalent CD with an average number of carbohydrate ligands of 4 to 5. The lectin binding abilities of these CD-conjugates were studied by turbidimetric assays with the galactose-binding protein *Griffonia simplicifolia* I and the *N*-acetyl glucosamine-binding protein WGA.

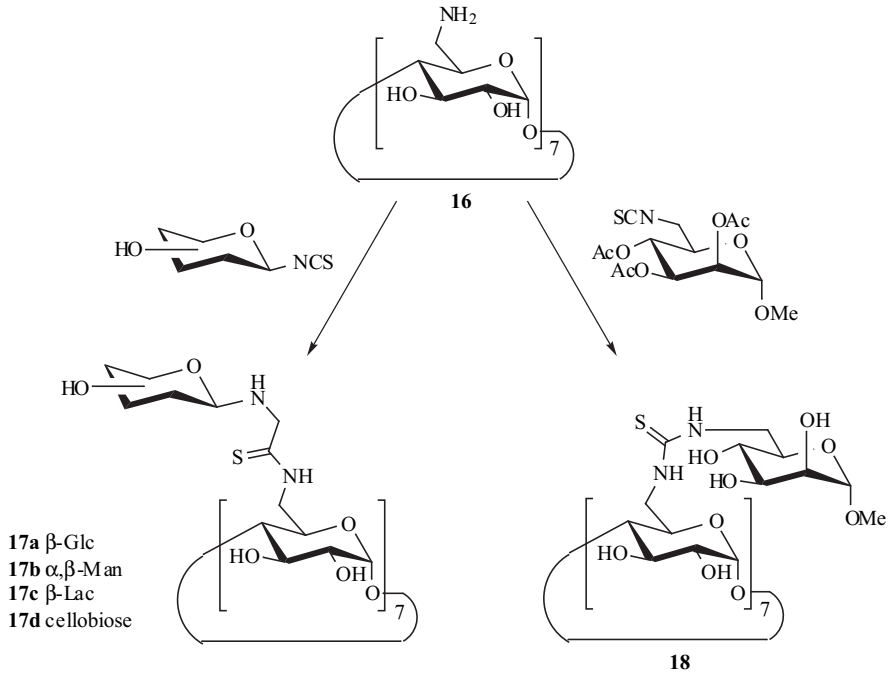
Fulton *et al.* [16] described the synthesis of β-CDs derivatives persubstituted with seven thio-β-D-glucosyl and lactosyl units. In this case, the authors performed the reaction between the per-6-amino-2,3-di-*O*-methyl-β-CD (**14**) as its HCl salt and glycopyranosyl carboxylic acid in presence of HBTU·BF_4 and *i*PrNEt in DMF giving the perfunctionalized-β-CD **15a**,**b** (Scheme **6**). The complexation ability of β-CD, per-2,3-di-*O*-methyl-β-CD and hepta lactose cluster **15b** with the antinflammatory drug naproxen was determined by UV-vis spectrophotometric titration. The association constants were 374, 351 and 165 M^{-1}, respectively. Therefore, persubstitution on the primary face of the CD decreased the naproxen binding ability.

Another successful strategy for the efficient attachment of carbohydrates on CD is based on the formation of thiourea bridges [17]. Following this strategy, the synthesis of per-glycosyl-thioureido-β-CDs **17a-d** and **18** has been performed in high yields by reaction of the per-6-amino-6-deoxi-β-CD **16** with glycosyl isothiocyanates in water/acetone at pH 8 (Scheme **7**). The resulting thioureido-β-CD derivatives **17a-d** exhibited water solubility several times higher than native β-CD. In addition, anticancer drug docetaxel solubilization experiments in water showed solubility values higher than those obtained for the native β-CD but lower than those for the monovalent glycoCD analogues. The binding ability of per-mannosyl-thioureido-β-CD **17b** and **18** towards Con A was evaluated by using ELLA assays. For these cases, in which the mannopyranosyl ligands are bound to the β-CD C-6 through a thiourea function, the heptavalent persubstituted glycoCDs **17b** and **18** exhibit a much lower binding ability to Con A than the monovalent CD analogues (IC_{50} 3200 and 2900 μM, respectively).

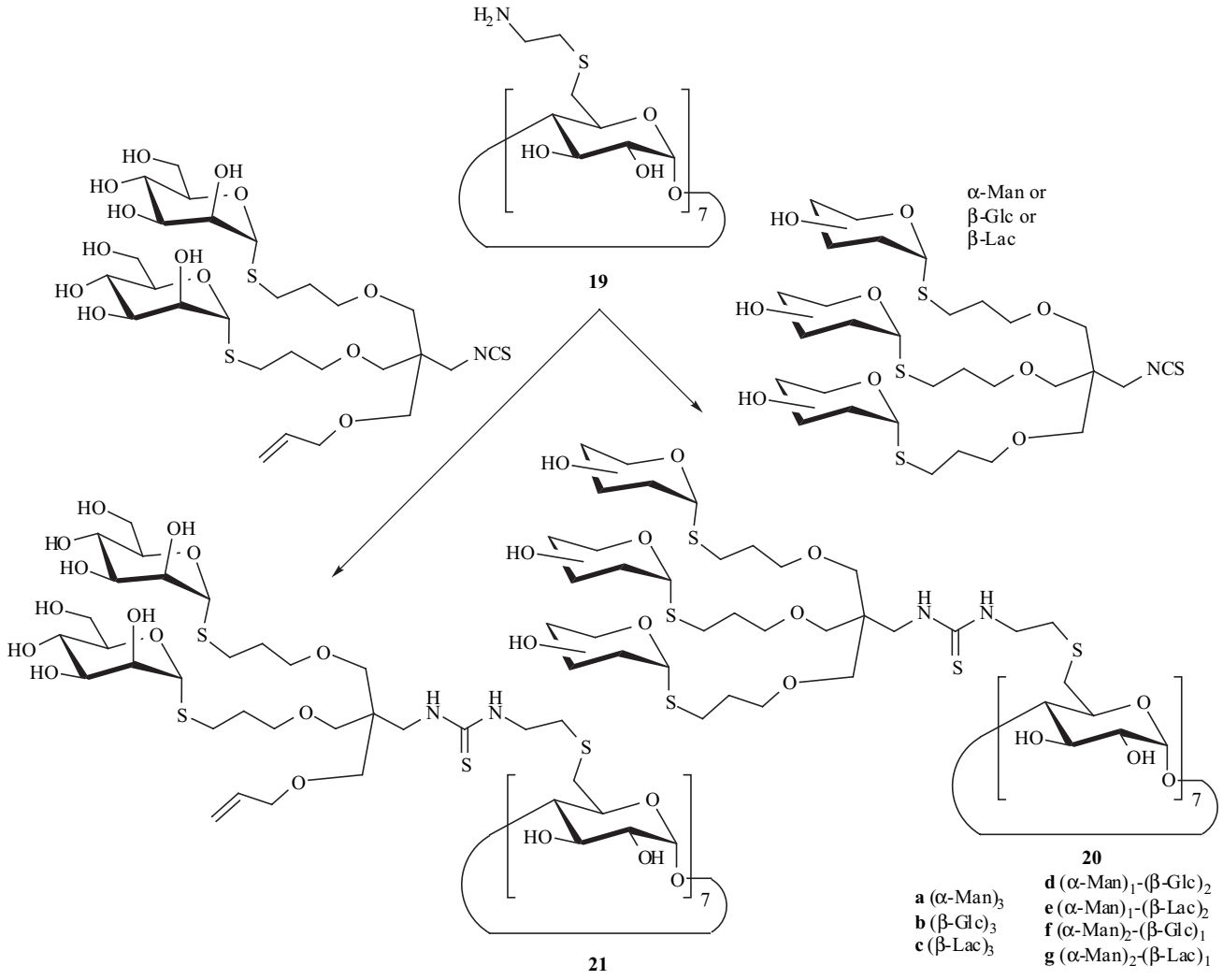
Recently [18], the synthesis of highly dense CD-centered homo and heteroglycoclusters has been reported. These compounds were used as heterogeneous cell-surface model system to investigate secondary carbohydrate-proteins interactions. The thiourea bridge strategy was applied to attach triantennary glycodendrimers onto the per-6-cysteaminyl-β-CD derivative **19** giving rise to 21-antennary homo and heteroglycoclusters **20** and **21** (Scheme **8**). The synthesis of the homo and heteroglycodendron was performed by first, radical anti-Markovnikov addition of thiosugars to tri-*O*-allylated pentaerythritol derivatives. This reaction affords mono-, di-, or trivalent glycodendron derivatives in a controlled manner. The mono- or divalent derivatives were further reacted with another thiosugar to



Scheme 6.



Scheme 7.



Scheme 8.

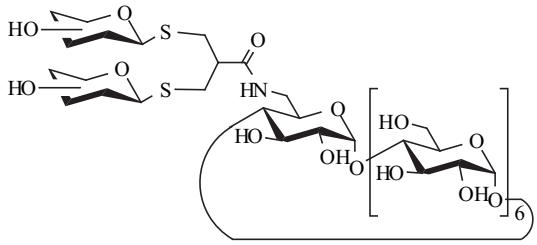
produce the bifunctional ligands. Finally, the isothiocyanate-containing glycodendrons obtained in three steps from the dendron primary hydroxy group were coupled onto **19**.

The binding affinity to Con A was evaluated first by ELLA assay. The results showed a higher affinity for the homogeneous α-Man **20a** meanwhile β-Glc **20b** and β-Lac **20c** were not recognized by Con A. The heteroglycoclusters **20d-g** presented 8-fold higher affinities to Con A than the homoglycocluster **20a** with the same number of α-mannosyl residues. Isothermal titration microcalorimetry measurements were also carried out and compared with the ELLA data. The result showed that the stoichiometry of the complex between horseradish peroxidase labeled ConA and **20a**,**d**,**f** were 1:1, which is in agreement with the absence of precipitation. The active role of the α-D-glucose ligand was confirmed by the dramatic decrease in the binding free energy for compound **21** ($-\Delta G° = 21.1 \pm 1.4$ kJ mol^{-1}) as compared with that for **20f** having identical mannose valency.

III. CYCLODEXTRIN-GLYCODENDRIMER CONJUGATES

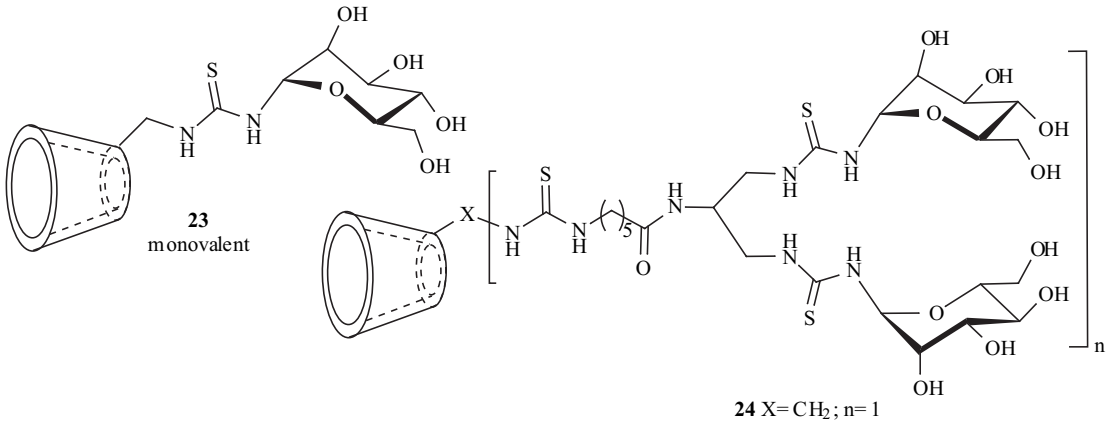
It has been shown in most cases that persubstituion at C-6 produces much stronger lectin binding affinities depending on the length and nature of the linker. However, the inclusion ability of this β-CD-based glycoconjugates has a different behaviour and, in general, by going from mono- to per-substituted β-CDs a notable decrease of the inclusion complex stability occurs. Based on those observations and in order to optimise the dual molecular recognition properties, some groups have addressed this issue by combining the advantages of monosubstituted CDs and those of the multivalent carbohydrate structures through the synthesis of glycodendrimer-cyclodextrins conjugates. First report [7] of this type of conjugates consisted in the coupling of biantennary structures to mono-6-deoxi-6-amino-β-CD obtaining the divalent monosubstituted CD **22a**,**b** (Fig. **4**). The synthesis involved first the coupling of 1-thiosugars to 3-bromo-2-(bromomethyl)-propionic acid followed by amidation reaction with mono-amino-β-CD. Both glycoCDs **22a**,**b** were recognized by lectins from *Ricinus communis* and Con A, respectively. In addition, the lectin binding inhibition assay showed that the minimum inhibition concentration of Gal_2-CD **22a** to inhibit flocculation of *Kluyveromyces bulgaricus* cells in the presence of the lectin were 2.0 mM, a higher value than that obtained for the persubstituted CD **3a**-**c**, and therefore a worse level of lectin recognition (Scheme **1**) [7].

The thiourea approach [19] has been applied for the development of an efficient methodology for the preparation of dendritic wedges for carbohydrate coating and covalent attachment to the β-CD core through the reactivity of isothiocyanate derivatives and amine functionalized monomers. The elements for the synthetic strategy are: mono-6-deoxi-6-amino-β-CD (host element), 1,2,3-triaminopropane (branching element), isothiocyanates-functionalized α-D-mannopyranosyl derivatives (glycoligand elements) and 6-azidohexanoyl chloride (spacer element). A convergent and divergent approach had been used for the preparation of a series of neoglycoconjugates-CD **23**-**28** (Figs. **5** and **6**).



22a β-Gal
22b α-Man

Fig. (4). Biantennary glycosyl-6-deoxi-6-amino-cyclomaltoheptaoses.



23
monovalent

24 X= CH_2 ; n= 1
25 X= $CH_2NH(CS)NH(CH_2)_5CONHCH(CH_2)_2$; n= 2
divalent and tetravalent

Fig. (5). Glycodendrimer-cyclomaltoheptaose conjugates **23**-**25**.

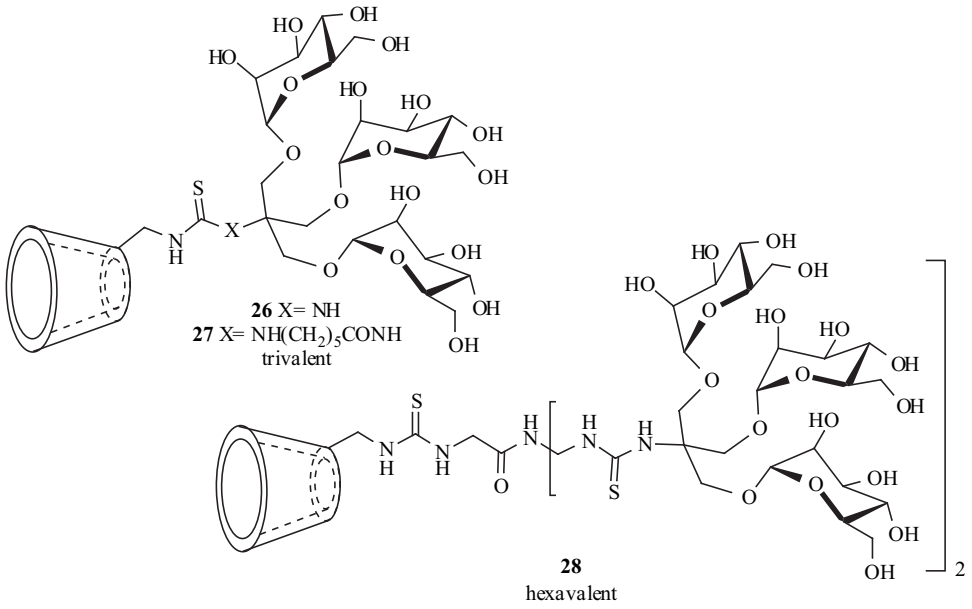


Fig. (6). Glycodendrimer-cyclomaltoheptaose conjugates **26**-**28**.

The binding affinity of these β-CD conjugates **23**-**28** and the polymannoside analogues in which the β-CD core has been replaced by a α-methyl-D-glucopyranoside was measured by ELLA assay. The corresponding IC_{50} values showed the expected amplification of lectin-binding strength for the higher-valent CD (up to 22-fold for hexavalent to monovalent derivatives), due to the cluster effect. Comparison of ELLA data for the β-CD conjugate and methyl-α-D-glucopyranoside indicated that β-CD core produced an additional stabilizing interaction, probably due to the interaction between β-CD residues with some residues at the protein surface. The length of the spacer in the case of **26** and **27** seems to have an influence on the interaction (IC_{50} values of 91 and 180 μM, respectively), which support

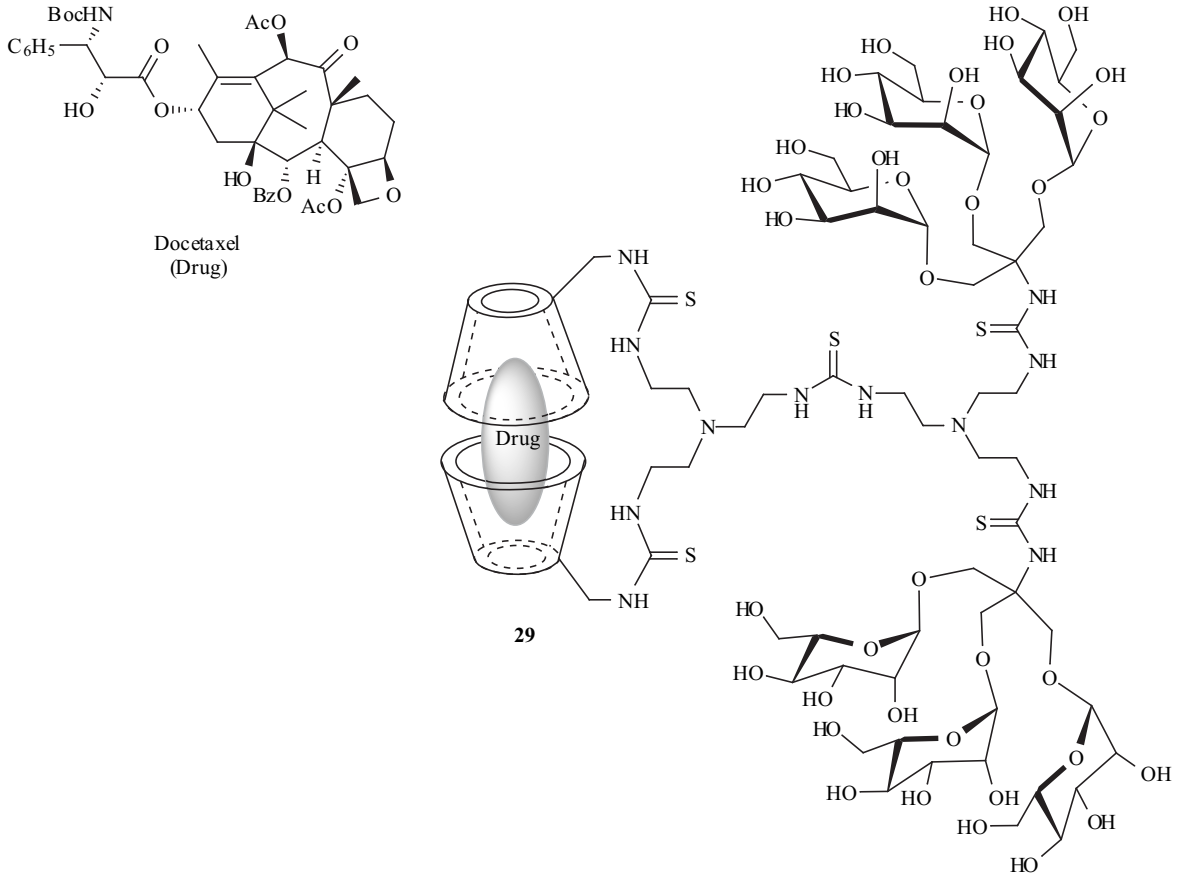


Fig. (7). *Bis*-Cyclodextrin-Glycodendrimer·docetaxel complex.

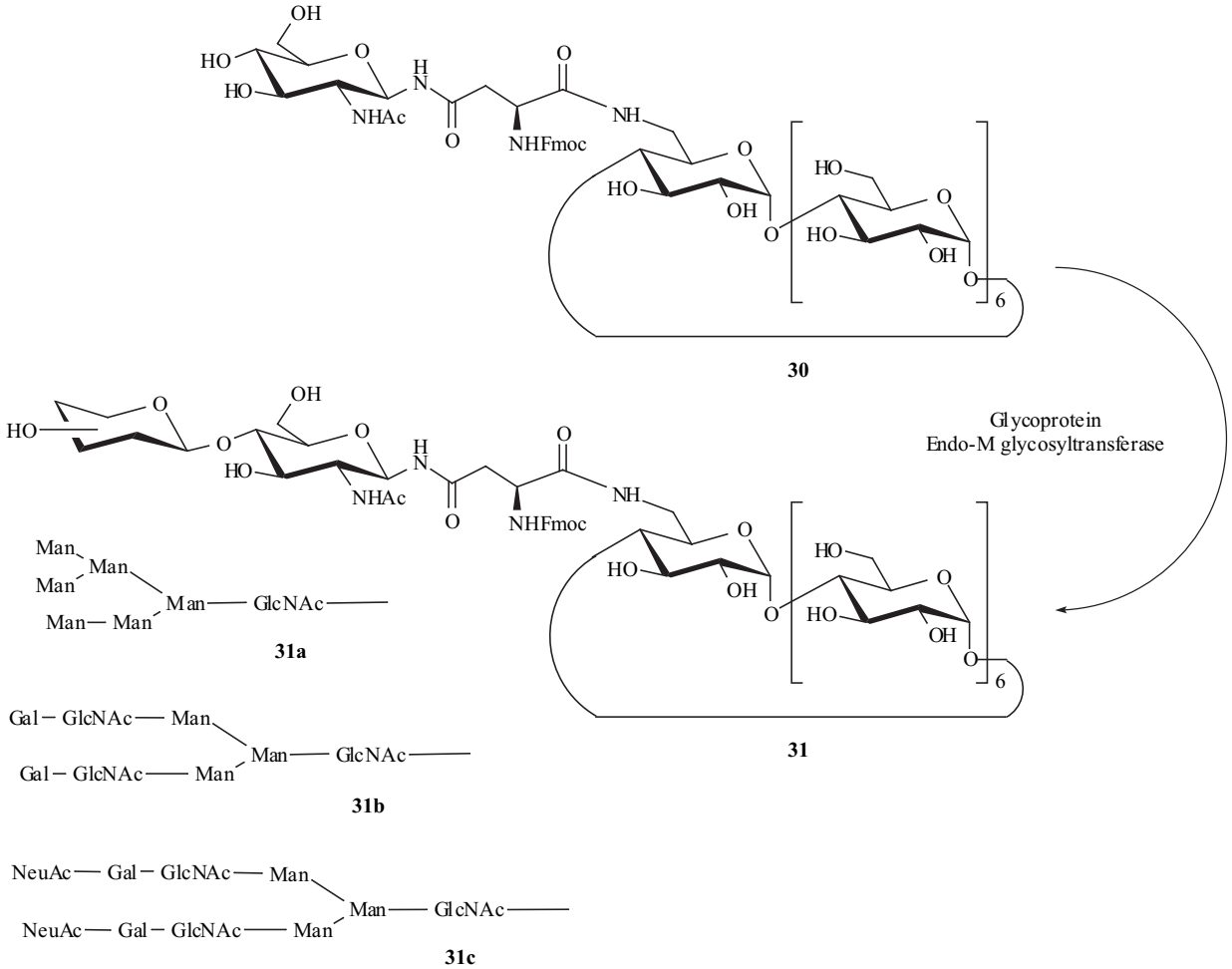
the idea that proximity of the CD core contributes to the stabilization of the lectin-neoglycoconjugate complex.

These β-CD-conjugates presented very high water solubilities (20-fold higher than β-CD). The solubilization experiments in water with docetaxel showed similar values to those obtained previously for monovalent CDs and higher than those for per-6-substituted CDs [17]. The ability of the conjugate-CD-docetaxel complexes to bind Con A also was evaluated by ELLA assay. The results showed that the Con A affinity for the **28**-docetaxel complex is 2-fold higher than that for the free host **28**. This increase in Con A affinity as compared with the free host has been attributed to likely formation of a **28**-docetaxel complex with a stoichiometry 1:2 (guest:host), and therefore the binding interaction could occur between Con A and a hexavalent complex. These results led the authors to synthesize the *bis*-CD-glyco-conjugate **29** (Fig. **7**) by applying the same methodology. The association constants (K_c) for glycoconjugate **28** and *bis*-CD-glycoconjugate **29** were 4000 and 1.5 x 10^5 M^{-1}, respectively. Association constant values were estimated from solubility diagrams assuming a 1:1 complex stoichiometry. The binding affinity of **29** towards Con A had a similar IC_{50} values than for **28** (13 and 10 μM, respectively) as determined by ELLA assay. In the case of **29**, the interaction with the protein was not affected by the complex formation. These results supported the hypothesis that docetaxel induces clusterization of the glycoconjugate **28** due to the formation of a sandwich-type complex.

The potential of conjugates **24** and **29** in drug targeting was tested with a mammalian mannose/fucose specific cell surface receptor from macrophages obtaining parallel results to those shown for their association and their corresponding docetaxel complexes to Con A.

A chemo-enzymatic synthetic strategy was employed for the synthesis of branched oligosaccharide-β-CD conjugates (Scheme **9**) [20]. The endo-β-*N*-acetylglucosaminidase of *Mucor hiemalis* (Endo-M) was found to transfer the asparagine-linked asialo-, sialo complex-type, and high-mannose type oligosaccharides from ovalbumin or human transferrin, to mono-6-Fmoc-Asn-(GlcNAc)-amino-β-cyclodextrin **30** to give β-CD monosubstited with branched oligosaccharides **31a-c** in 6-12 % yield. In order to improve these results, a new strategy was developed [20b] consisting in the use of H-Asn-($GlcNAc_2Man_6$)-OH as a transglycosilation substance of Endo-M. After protection of asparagine residue with Fmoc, the protected compound was coupled with mono-6-amino-β-CD by amidation reaction obtaining the desired oligosaccharide-CD conjugates **31a** in good yields (74%).

The lectin recognition properties of the high-mannose-CD conjugates **31a-c** were studied using immobilized Con



Scheme 9.

A by SPR. The measured rates of association (k_a) and dissociation (k_d) for high-mannose-CD conjugate **31a** were 2.36 x 10^4 $M^{-1}s^{-1}$ and 1.50 x 10^{-3} $M^{-1}s^{-1}$, respectively. The association constant (K_a) for **31a** was determined obtaining a value of 1.57 x 10^7 M^{-1}. The K_a value was 1800 times higher than that for the glucosyl-glucono-amide-β-CD [21]. A high dependence of the association constant values on the saccharide structure of the high-mannose-CD conjugate (hexa-mannosyl > mono-glucosylgluconoamide > monomaltosyl > mono-glucosyl) was observed.

IV. MISCELLANEOUS

As an alternative to the persubstituted glycoCDs and glycodendrimer-CD conjugates, the synthesis of *bis*-branched-CDs has been reported [22] (Fig. **8**). In particular, the synthesis of a series of $6^A,6^D$-diantennary-galactose-β-CD **32a-f** having different spacer arm lengths. The synthesis of **32a-f** involved first, the preparation of galactostyl-glucono-amide-capronic acid derivative that was reacted with 6-aminocapric acid methyl ester and then the methyl group removed. This procedure was repeated several times to obtain the series of galactosyl-glucono-amide acids with different arm lengths between the carbohydrate moiety and the terminal carboxylic function. Condensation of the acid function with $6^A,6^D$-diamino-β-CD with DCC and *N*-hydroxysuccinic imide (HONSu) gave *bis*-Gal-CD derivatives **32a-f** in 23-86% yield. The dual association of these compounds with PNA lectin and the anticancer drug doxorubicin was quantitatively evaluated by SPR and

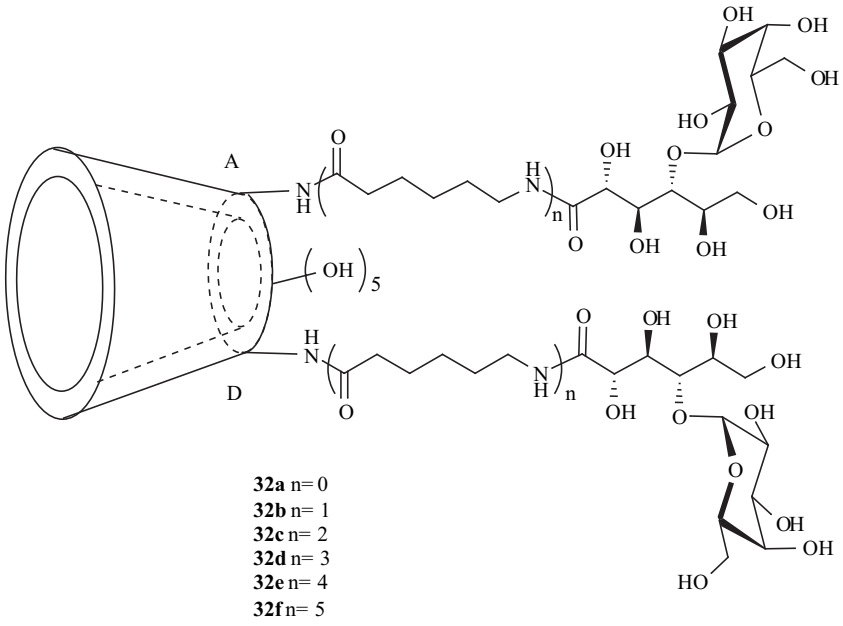


Fig. (8). *bis*-Galactose-branched cyclomaltoheptaoses **32**.

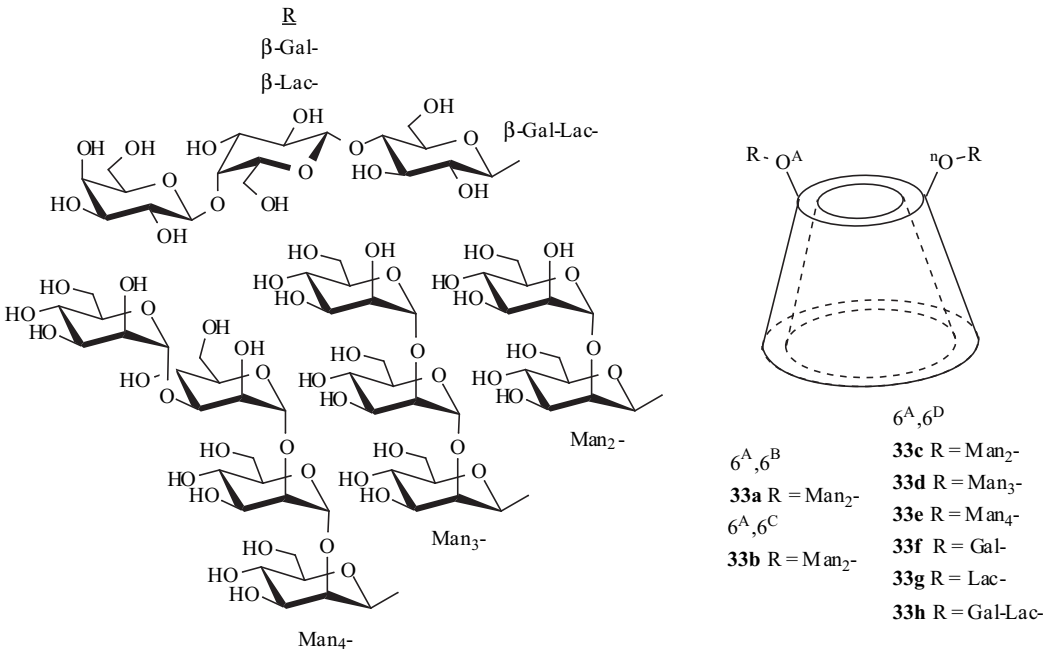


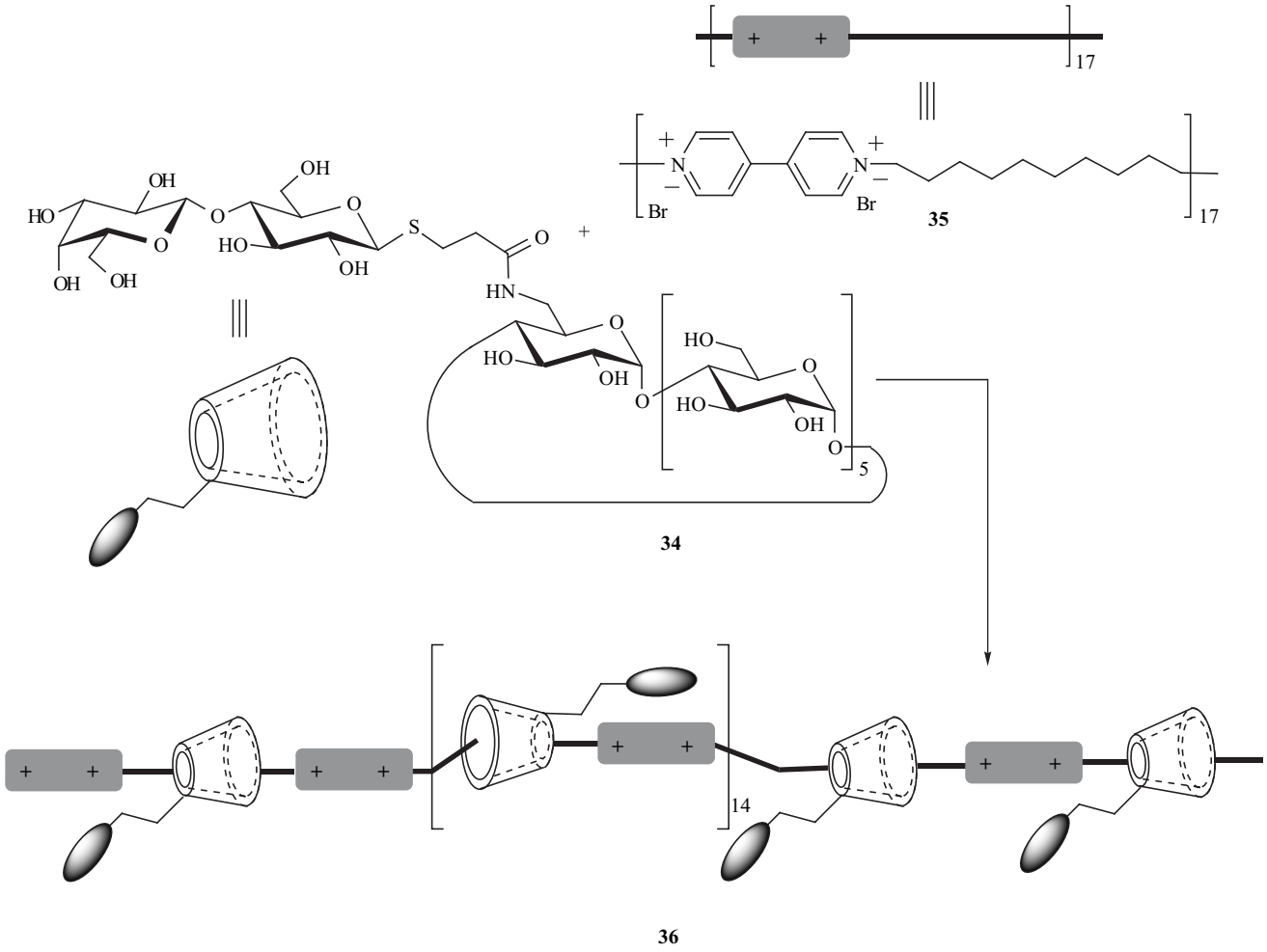
Fig. (9). *Bis*-branched cyclomaltoheptaoses **33**.

atomic force microscopy (AFM). These *bis*-Gal-CDs **32a-f** were found to have the optimum length for association with PNA lectin [K_a value for **32e** (n = 4) 4.6 x 10^6 M^{-1}]. In addition, it was found that the *bis*-Gal-CD·drug complex stability gradually increases along with the spacer lengths [K_a value for **32f** (n = 5) 5.1 x 10^4 M^{-1}].

Tanimoto *et al.* [23] described the synthesis and the binding affinity of *bis*-branched β-CDs having mannose [23a] and galactose [23b] residues at the no-reducing ends of their side chains (Fig. **9**). The synthesis was carried out by glycosylation using the trichloroacetimidate method and the 6^A,6^n-de-*O*-acetylated peracetylated β-CD derivatives (n = B,C,D) as glycosyl acceptors. The specific interactions between these compounds and mannose-binding lectins (Con A and *Pisum sativum* agglutinin) or galactose-binding lectin PNA was investigated using SPR and haemagglutination-based inhibition assays. The results showed that all *bis*-branched CDs **33a-h** interacted with the corresponding lectins. The comparison of the lectin binding affinities on the basis of the spacer arm length showed the following relationship for *K* values: 6^A,6^D: Man_3-CD **33d** >> Man_2-CD **33c** > Man_4-CD **33e** and Gal-Lac-CD **33h** > Lac-CD **33g** > Gal-CD **33f**. When comparing the chain attachment positions at the C-6 of the CD, the *K* values increased as follows: 6^A,6^C >> 6^A,6^D > 6^A,6^B.

Nelson *et al.* [24] reported a self-assembled pseudopolyrotaxanes consisting of lactosides displayed on CD threaded onto a linear polyviologen string (Scheme **10**). The lactose-bearing α-CD **34** was obtained by coupling the lactosyl propionic acid derivative with the mono-6-amino-α-CD using HBTU-BF_4 to activate the carboxylic acid [24a]. The pseudopolyrotaxane **36** was formed by adding the polyviologen **35** to a solution of the CD **34** in water.

This pseudopolyrotaxane **36** was investigated for its ability to inhibit galectin-1 (Gal-1) mediated T-cell agglutination. Gal-1 is a soluble 14 kDa dimeric galactoside-binding lectin. The pseudopolyrotaxane **36**, three trivalent cluster lactosides and a chitosan-derived polymer bearing lactose epitopes were evaluated qualitatively using T-cell agglutination assay. The trivalent glycoclusters showed no improvement over native lactose as inhibitor of Gal-1-induced T-cell agglutination. The rigid chitosan polymer only showed a slight enhancement over native lactose (1.7-fold over native lactose after valency-corrected). Meanwhile pseudopolyrotaxane **36** exhibited a 10-fold enhancement. An explanation for this dramatic difference on the lectin affinities between the lactoside systems is that **36** contains mobile ligands bound by noncovalent interactions with the ability to rotate and translate along the polymer string which can adjust to the relative stereochemistries of the lectin binding sites. CD-based polyrotaxanes **37** displaying



Scheme 10.

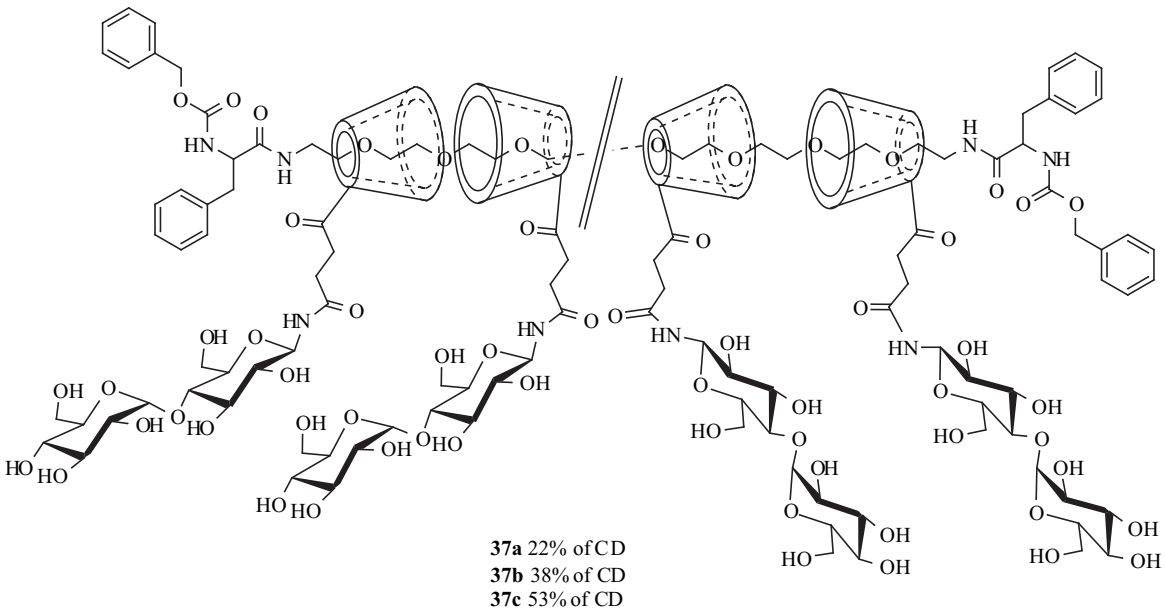


Fig. (10). Cyclodextrin-based polyrotaxanes displaying maltose units.

maltose units have been prepared by Ooya *et al.* [25] (Fig. **10**). The synthesis of **37** was achieved by condensation of β-maltosylamine and carboxyethyl ester-polyrotaxanes in which the functionalized α-CD units are threaded onto a poly(ethylene glycol) chain capped with benzyloxycarbonyl-L-tyrosine. A series of maltosyl-polyrotaxenes **37a-c** were obtained with different threading percentages of α-CD (22, 38 and 53%, respectively) and maltose units (230, 244 and 240, respectively).

The influence of the ligand mobility along the poly(ethylene glycol) chain on the multivalent interaction with Con A was investigated. The association constants (K_a) for the binding affinity of the maltose-polyrotaxane conjugates **37a-c** and Con A were determined by fluorescent assay using fluorescein-labeled Con A. The K_a values of **37a-c** were 5.7 x 10^4, 1.1 x10^6 and 5.3 x 10^5 M^{-1}, respectively. These K_a values were 52, 1000 and 480 times higher than maltose, respectively. Interestingly, the largest K_a value corresponded to polyrotaxane **37b** having the highest mobile motion of the maltose-conjugate α-CD. Since **37a-c** contains a similar number of maltose units, it was concluded that mobile motion due to the mechanically locking structure plays a key role for inducing rapid multivalent interaction.

V. CONCLUSIONS

The first reported cases of carbohydrates grafted onto the β-cyclodextrin macrocycle were prompted by attempts to improve the water solubility of β-cyclodextrin and the drug solubilization of cyclodextrins. However, it soon became obvious that CD was a very convenient scaffold for the construction of multivalent carbohydrates involved in biorecognition processes. This feature, in conjunction with the CD guest inclusion ability, led to an extensive research on the development of site-specific drug delivery systems and the field is now developed enough to pursue biomedical applications. Nevertheless, research in this field is still providing new suggestive progress such as the formation of nanoparticles, vesicles, and dynamic carbohydrate ligand presentations. These new systems provide new approaches for the study of protein-carbohydrate interactions and new biomaterials that can be exploited in different applications as biosensors, drug targeting molecules and scaffold material for tissue regeneration among others.

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REFERENCES

[1] Choi, S. K. *Synthetic Multivalent Molecules*, Wiley-Interscience: Hoboken, **2004**.
[2] Roy, R. *Trends Glycosci. Glycotechnol.* **2003**, *15*, 291.
[3] a) Lee, R. T.; Lee, Y. C. *Glycoconjug. J.* **2000**, *17*, 543; b) Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555.
[4] Fulton, D. A.; Stoddart, J. F. *Bioconjug. Chem.* **2001**, *12*, 655.
[5] Ortiz Mellet, C.; Defaye, J.; García Fernández, J. M. *Chem. Eur. J.* **2002**, *8*, 1982.
[6] Szejtli, J. *Pure Appl. Chem.* **2004**, *76*, 1825.
[7] a) de Robertis, L.; Lancelon-Pin, C.; Driguez, H. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1127; b) Lancelon-Pin, C.; Driguez, H.; *Tetrahedron Lett.* **1992**, *33*, 3125.
[8] Lainé, V.; Coste-Sarguet, A.; Gadelle, A.; Defaye, J.; Perly, B.; Djedaïni-Pilard, F. *J. Chem Soc. Perkin. Trans. 2* **1995**, 1479.
[9] a) Furuike, T.; Aiba, S. *Chem. Lett.* **1999**, 69; b) Furuike, T.; Aiba, S.; Nishimura, S. I. *Tetrahedron* **2000**, *56*, 9909.
[10] André, S.; Kaltner, H.; Furuike, T.; Nishimura, S. –I.; Gabius, H. –J. *Bioconjug. Chem.* **2004**, *15*, 87.
[11] Furuike, T.; Sadamoto, R.; Niikura, K.; Monde, K.; Sakairi, Nishimura, S. I. *Tetrahedron* **2005**, *61*, 1737.
[12] a) García-López, J. J.; Hernández-Mateo, F.; Isac-García, J.; Kim, J.; Roy, R.; Santoyo-González, F.; Vargas-Berenguel, A. *J. Org. Chem.* **1998**, *64*, 522; b) García-López, J. J.; Santoyo-González, F.; Vargas-Berenguel, A.; Giménez-Martínez, J. J. *Chem. Eur. J.* **1999**, *5*, 1775; c) Ortega-Caballero, F.; Giménez-

Martínez, J. J.; García-Fuentes, L.; Ortiz-Salmerón, E.; Santoyo-González, F.; Vargas-Berenguel, A. *J. Org. Chem.* **2001**, *66*, 7785; d) Vargas-Berenguel, A.; Ortega-Caballero, F.; Santoyo-González, F.; Giménez-Martínez, J. J.; García-Fuentes, L.; Ortiz-Salmerón, E. *Chem. Eur. J.* **2002**, *8*, 812.

[13] Mazzaglia, A.; Forde, D.; Garozzo, D.; Malvagna, P.; Ravoo, B. J.; Darcy, R. *Org. Biomol. Chem.* **2004**, *2*, 957.

[14] Yasuda, N.; Aoki, N.; Abe, H.; Hattori, K. *Chem. Lett.* **2000**, 706.

[15] Ichikawa, M.; Woods, A. S.; Mo, H.; Goldstein, I.; Ichikawa, Y. *Tetrahedron Asymmetry* **2000**, *11*, 389.

[16] Fulton, D. A.; Pease, A. R.; Stoddart, J. F. *Isr. J. Chem.* **2000**, *40*, 325.

[17] a) Ortiz Mellet, C.; Benito, J. M.; García Fernández, J. M.; Law, H.; Chmurski, K.; Defaye, J.; O'Sullivan, M. L.; Caro, H. N. *Chem. Eur. J.* **1998**, *4*, 2523; b) Baussane, I.; Benito, J. M.; Ortiz Mellet, C.; García Fernández, J. M.; Defaye, J. *Chembiochem* **2001**, *10*, 777.

[18] Gómez-García, M.; Benito, J. M.; Rodríguez-Lucena, D.; Yu, J. X.; Chmurski, K.; Ortiz Mellet, C.; Gutiérrez Gallego, R.; Maestre, A.; Defaye, J.; García Fernández, J. M. *J. Am. Chem. Soc.* **2005**, *127*, 7970.

[19] a) Baussanne, I.; Law, H.; Defaye, J.; Benito, J. M.; Ortiz Mellet, C.; García Fernández, J. M. *Chem. Commun.* **2000**, 1489; b) Benito, J. M.; Gómez-García, M.; Ortiz Mellet, C.; Baussanne, I.; Defaye, J.; García Fernández, J. M. *J. Am. Chem. Soc.* **2004**, *126*, 10350.

[20] a) Matsuda, K.; Inazu, T.; Haneda, K.; Mizuno, M.; Yamanoi, T.; Hattori, K.; Yamamoto, K.; Kumagai, H. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2353; b) Inazu, T.; Yamanoi, T.; Haneda, K.; Mizuno, M.; Matsuda, K.; Yamazaki, T.; Takeuchi, M.; Tsurui, H.; Hattori, K. In *Proceeding of the 9th International Symposium on Cyclodextrins*, J. J. Torres Lavandeiras and J. L. Vila-Jato (Eds.), Santiago de Compostela (Spain), **1998**, pp. 117-120.

[21] a) Hattori, K.; Imata, H.; Kubota, K.; Matsuda, K.; Aoyagi, M.; Yamamoto, K.; Jindoh, C.; Yamanoi, T.; Inazu, T. *J. Inclusion Phenom. Macrocyclic Chem.* **1996**, *25*, 69; b) Imata, H.; Kubota, K.; Hattori, K.; Aoyagi, M.; Jindoh, C. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 109.

[22] Abe, H.; Kenmoku, A.; Yamaguch, N.; Hattori, K. *J. Inclusion Phenom. Macrocyclic Chem.* **2002**, *44*, 39.

[23] a) Tanimoto, T.; Kishimoto, Y.; Ikuta, A.; Nishi, Y.; Miyake, K. *J. Inclusion Phenom. Macrocyclic Chem.* **2002**, *44*, 297; b) Ikuta, A.; Mizuta, N.; Kitahata, S.; Murata, T.; Usui, T.; Koizumi, K.; Tanimoto, T. *Chem. Pharm. Bull.* **2004**, *52*, 51.

[24] a) Nelson, A.; Stoddart, J. F. *Org. Lett.* **2003**, *5*, 3783. b) Nelson, A.; Belitsky, J. M.; Vidal, S.; Joiner, C. S.; Baum, L. G.; Stoddart, J. F. *J. Am. Chem. Soc.* **2004**, *126*, 11914.

[25] a) Ooya, T.; Eguchi, M.; Yui, N. *J. Am. Chem. Soc.* **2003**, *125*, 13016; b) Ooya, T.; Utsunomiya, H.; Eguchi, M.; Yui, N. *Bioconjug. Chem.* **2005**, *16*, 62.

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