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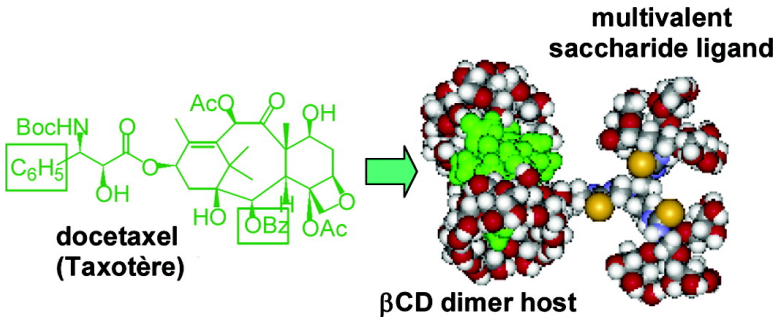

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Optimizing Saccharide-Directed Molecular Delivery to Biological Receptors: Design, Synthesis, and Biological Evaluation of Glycodendrimer−Cyclodextrin Conjugates

Juan M. Benito,[†] Marta Gómez-García,[‡] Carmen Ortiz Mellet,*[,‡] Isabelle Baussanne,[§] Jacques Defaye,*[,§] and José M. García Fernández*[,†]

Contribution from the Instituto de Investigaciones Químicas, CSIC, Américo Vespucio 49, Isla de la Cartuja, E-41092 Sevilla, Spain, Departamento de Química Orgánica, Facultad de Química, Universidad de Sevilla, Aptdo. 553, E-41071 Sevilla, Spain, and CNRS − Université Joseph Fourier-Grenoble 1, Département de Pharmacochimie Moléculaire (UMR 5063, FR 2607 ICMG), BP 138, F-38243 Meylan, France

Received April 14, 2004; E-mail: jogarcia@iiq.csic.es; mellet@us.es; jacques.defaye@ujf-grenoble.fr

Abstract: Dendritic β-cyclodextrin (βCD) derivatives bearing multivalent mannosyl ligands have been prepared and assessed for their binding efficiency toward the tetrameric plant lectin concanavalin A (Con A) and a mammalian mannose/fucose specific cell surface receptor from macrophages. The synthetic strategy exploits the reactivity between isothiocyanate and amine functionalities for the high-yielding assembly via thioureido links of the various building blocks, including host, spacer, branching, and carbohydrate ligand elements. The methodology has been applied to the preparation of a series of βCD-polymannoside scaffolds differing in the ligand valency and geometry. This series allowed us to explore: (i) The effects of the glycodendritic architecture on the binding efficiency; (ii) the mutual influence between the cyclodextrin core and the glycodendritic moieties on the molecular inclusion and lectin-binding properties; and (iii) the consequence of inclusion complex formation, using the anticancer drug docetaxel (Taxotère) as a target guest, on biological recognition. Our results confirm the high drug solubilization capability of this new type of βCD−dendrimer construct and indicate that subtle changes in the architecture of the conjugate may have important consequences on receptor affinity. Interestingly, the host−guest interaction can be monitored to build up supramolecular dynamic glycoclusters with increased lectin affinity. Alternatively, the information obtained from the structure−lectin-binding avidity−inclusion capability studies has been put forward in the design of very efficient molecular transporters for docetaxel based on glycodendritic CD dimers.

Multivalent interactions between carbohydrate-binding proteins (lectins) and the oligosaccharide moieties of glycoprotein and glycolipid components of extracellular matrices and cell surfaces are involved in extensive cellular recognition processes including development, differentiation, morphogenesis, fertilization, the immune response, implantation, cell migration, and cancer metastasis.[1] The high specificity of oligosaccharide−lectin interactions has already been exploited in the development of multivalent sugar-based therapeutic agents[2] designed to interfere with carbohydrate molecular recognition[3] and carbohydrate-based anticancer drugs.[4] The targeted aggregation and clearance of pathogenic species is another promising application of this concept.[5] The synthesis and characterization of compounds that not only possess multivalent carbohydrate recognition sites, but also the potential to act as carriers of drugs, is gaining an increased amount of attention. Glycoligands with

[†] Instituto de Investigaciones Químicas, CSIC.
[‡] Universidad de Sevilla
[§] CNRS and Université Joseph Fourier.

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(2) Multivalency is a prerequisite to attain biologically useful affinities between carbohydrate ligands and their protein receptors. Presentation of the sugar epitopes as multiple copies on an appropriate scaffold (molecular, dendritic, polymeric) creates a multivalent display that can efficiently mimic the nature mode of affinity enhancement, resulting in higher affinities than expected from the addition of the individual interactions. For selected literature on this concept, termed generically the cluster effect, see: (a) Kiessling, L. L.; Pontrello; Schuster, M. C. Synthetic Multivalent Carbohydrate Ligands as Effectors or Inhibitors of Biological Processes. In *Carbohydrate-based Drug Discovery*; Wong, C.-H., Ed.; Wiley-VCH: Weinheim, Germany, 2003; Vol. 2, pp 575−608. (b) Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555−578. (c) Lee, R. T.; Lee, Y. C. *Glycoconjugate J.* **2000**, *17*, 543−551. (d) Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754−2794. (e) Lee, Y. C.; Lee, R. T. *Acc. Chem. Res.* **1995**, *28*, 321−327.

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this added value could act as "intelligent" vectors for site-specific delivery of therapeutics. For this purpose, various components, which are designed for encapsulation, solubilization, stabilization, targeting, reduction of immunogenicity and toxicity, and pharmacological activity, among others, have to be combined in a sophisticated manner. Some of the frequently used methods include conjugation of carbohydrate ligands with proteins or other polymers, polymerization of glycosylated monomers, and formation of liposomes using glycolipids or neoglycolipids.[6] Although these approaches have been successful to some extent, the products thus obtained are generally ambiguous in composition and structure, which may be troublesome regarding accurate reproducibility of the biological results. Moreover, this heterogeneity frequently impairs a systematic study of the consequences of structural modifications of the different components of the system in the final properties.

Grafting biorecognizable saccharide epitopes onto macrocyclic molecular hosts to produce homogeneous compounds with a well-defined structure and a precise number of carbohydrate ligands is an interesting alternative for receptor-mediated glycotargeting.[7] Cyclodextrins (cyclomaltooligosaccharides, CDs) offer unique features toward this goal because they are essentially nonimmunogenic carbohydrates with inherent low pharmacological activity and high biocompatibility, and a variety of selectively functionalized derivatives are accessible through recent methodologies for manipulation of their polyfunctional framework. They are well known to encapsulate various organic molecules of appropriate size within their truncated cone-shaped hydrophobic cavity to afford host−guest supramolecular species in aqueous solution, a property that has already been exploited in pharmaceutical applications.[8] Multivalent cyclodextrin conjugates may, therefore, form ternary lectin−carbohydrate ligand−guest complexes through simultaneous host−guest complexation at the CD cavity and carbohydrate−protein interaction at the carbohydrate ligand moiety (Figure 1).

Several examples of oligosaccharide-branched cyclodextrins have been reported in the past few years.[9,10] Three main

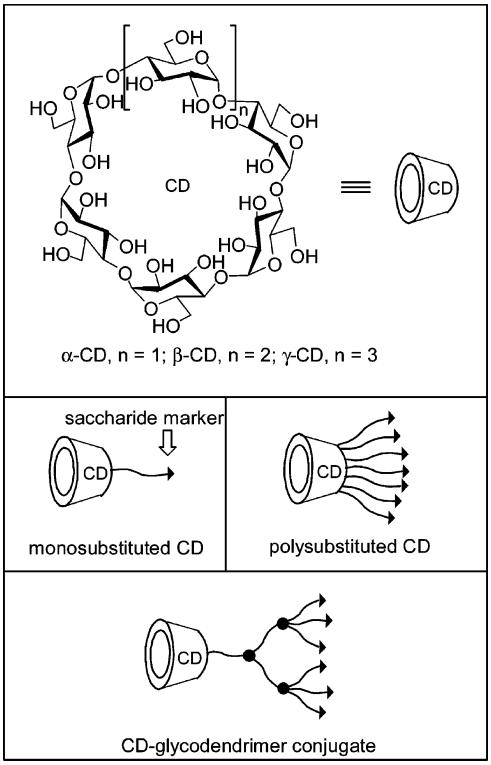

Figure 1. Schematic representation of cyclodextrin−glycodendrimer conjugates considered in this study. Polysubstituted derivatives bearing multiple biorecognizable saccharide ligands show better lectin-binding properties due to the multivalent effect; yet, monosubstituted conjugates exhibit superior inclusion capabilities. CD−glycodendrimer architectures are now proposed to combine both favorable features.

conclusions stem from the literature data: (i) the CD scaffold is particularly well-suited for glycocluster−lectin interaction studies; (ii) polyglycosylated structures exhibit higher affinities toward specific lectins than do monosubstituted structures, as expected from the cluster effect; and (iii) polysubstitution may, however, seriously impair inclusion and complex stabilization of potential guests as a result of limiting access to the hydrophobic cavity, lack of stabilizing interactions of the included guest, and distortion of the cavity due to steric hindrance.[9] Our own results indicated an about 10-fold decrease in the solubilization capability of hydrophobic guests in water for branched βCDs upon (C-6)-heptafunctionalization.[11] Ideal CD carriers for drug targeting should combine both the advantages of monosubstitution with regard to efficient inclusion capabilities and a multivalent display of the required saccharide epitope to comply with the need of high biological receptor binding efficiency. We assumed that this goal could be achieved by conjugating a monosubstituted CD host moiety and a glycodendritic domain[12] (Figure 1). We have now developed an efficient preparation of dendritic wedges suitable for both external carbohydrate coating and covalent attachment to the

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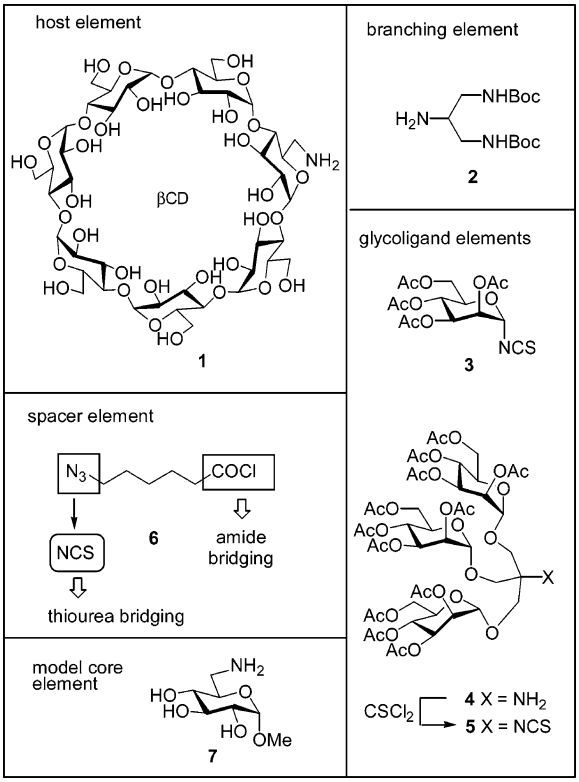
β-cyclodextrin (βCD) core,[13] the most readily available CD representative. A critical advantage of the methodology is that it allows sampling compounds with varied, yet perfectly defined, structural characteristics. In a first series of compounds, we explored the effect of the presence of the CD host moiety on receptor binding as a function of the overall shape of the system and of the density and valency of the receptor-binding elements. The model lectin we studied for this purpose is the tetrameric plant lectin concanavalin A (Con A),[14] that specifically recognizes α-D-mannopyranosyl epitopes. The binding affinity was evaluated by enzyme-linked lectin assay (ELLA),[15] which provides information on the intrinsic lectin−ligand affinity, devoid of aggregation effects.[2b,15a]

For the most efficient ligands in the above study, the influence of CD complex formation on lectin affinity was regarded using docetaxel (**DTX**, Taxotère) − one of the most successful agents currently in use for the chemotherapy of breast and ovarian cancer[16] − as the guest molecule. The drug carrier potential was further investigated using macrophages expressing the well-characterized mannose/fucose specific receptor,[17] a lectin of interest as a therapeutic target, at their surface. The information obtained from structure−lectin-binding affinity−inclusion capability studies, which pointed for guest-induced clusterization effects, may be exploited in the design of tailor-made molecular transporters. As a rationale of this concept, we further report here the extension of the CD−dendrimer approach to the preparation of multitopic glycodendrimer−CD dimer conjugates designed to form very strong sandwich-type complexes with Taxotère while retaining optimal biological receptor recognition properties.

Results and Discussion

Synthetic Strategy. To build the typical tree structure of dendrimers onto the primary hydroxyl rim of the βCD torus, a set of building blocks that can be combined in an iterative and modular manner with a relatively low synthetic cost has been devised. The key templates are 6^I-amino-6^I-deoxy-βCD (**1**), for which an improved synthetic route has been recently elaborated,[13] the selectively protected 1,2,3-triaminopropane branching element **2**,[18] and the isothiocyanate-functionalized α-D-mannopyranosyl derivatives **3**[19] and **5**. The latter was prepared by reaction of the known tris(α-D-mannopyranosyloxymethyl)-methylamine **4**[20] with thiophosgene. Intercalation of a six-carbon spacer, by using 6-azidohexanoyl chloride[21] (**6**) as a bifunctional bridging segment, has also been considered to ensure the accessibility of the grafted bioactive components to molecular recognition events. Alternatively, methyl 6-amino-6-deoxy-α-D-glucopyranoside[22] (**7**), a convenient surrogate for the substituted glucopyranosyl subunit in monofunctionalized cyclodextrin derivatives, was used as the core element instead of **1** for comparative purposes (Chart 1). The coupling methodology exploits the connectivity of isothiocyanate and amine-functionalized monomers to give thiourea adducts,[23] taking advantage of the efficient transformation of the terminal azido group in the spacer element into an isothiocyanate functionality through a tandem Staudinger−aza-Wittig-type reaction with triphenylphosphine and carbon disulfide.[24] The amide bridging reaction was also used to couple the spacer element to the dendritic moiety.

Chart 1. Building Blocks Used To Build β-Cyclodextrin-Centered Mannosyl-Coated Glycodendrimers



Both convergent and divergent approaches have been implemented for the preparation of this new type of hybrid neogly-

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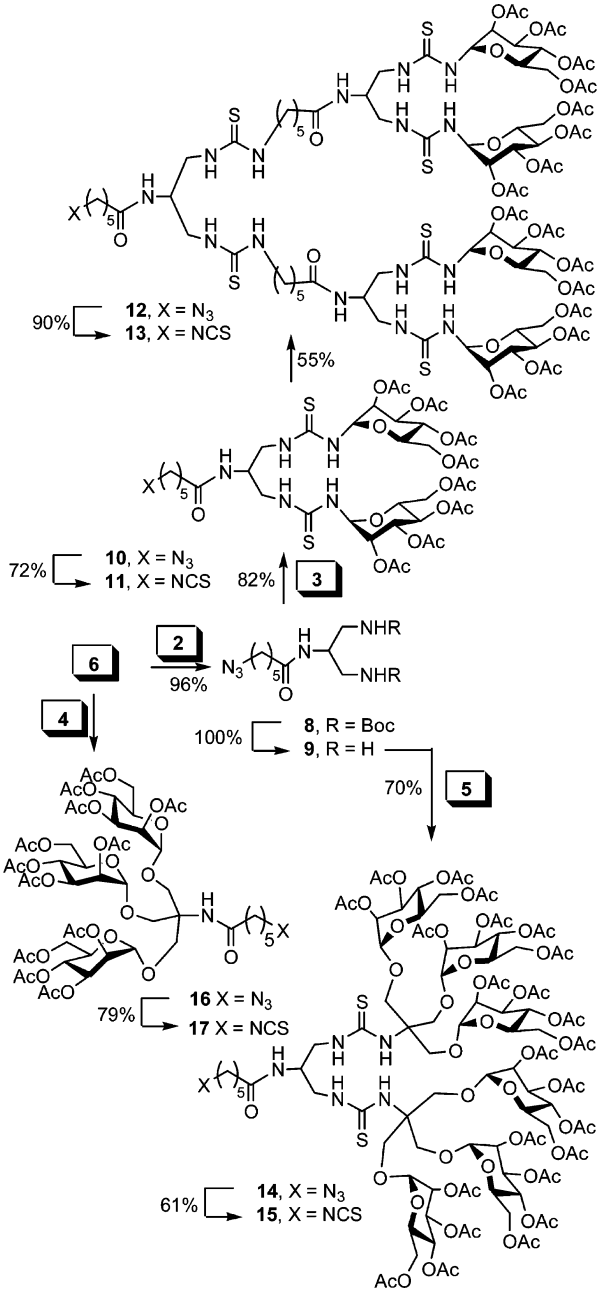
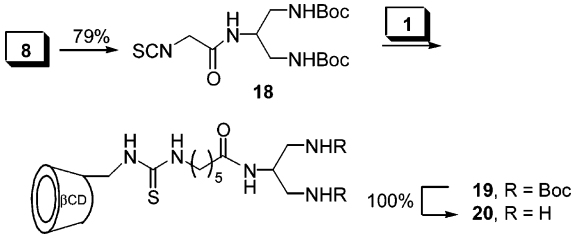
Scheme 1. Synthesis of Isothiocyanate-Armed Polymannoside Dendrons



coconjugates. In the convergent approach, defined glycodendrons bearing a terminal isothiocyanate group (**11**, **13**, **15**, and **17**) were used to generate the multivalent mannosyl displays. Variation in the number and density of the mannose residues was achieved by judicious combination of the different building blocks as indicated in Scheme 1. The divergent approach started from the thiourea bridging reaction between the host element **1** (or model core **7**) and the spacer-armed branching element **18**. Acid-catalyzed hydrolysis of the Boc protecting groups in the corresponding adduct **19** (→**20**) generated two amine functionalities suitable for further nucleophilic addition to isothiocyanate-functionalized glycodendrons (Scheme 2). Either of these two routes led, after the final assembly of the mannosyl ligands and the βCD (or model core) fragments, to the corresponding hemiacetylated conjugates **21**−**26** (Chart 2) in satisfactory overall yield. Albeit a synthesis without protecting groups is

Scheme 2. Synthesis of Precursors for Divergent Preparations of βCD−Glycodendrimer Conjugates



feasible,[25] we encountered that purification, usually the most delicate step in the preparation of such complex molecules, is much more easily performed at this stage. Base-catalyzed removal of the acetyl protecting groups afforded the target fully unprotected compounds **27**−**32** in high purity and virtually quantitative yield.[26] Gel permeation chromatography of the crude product allowed isolation of analytically pure samples that were used in the biological evaluation tests. The monovalent ligand **27** and the model conjugates **33**−**38** served as control compounds in the binding studies (Chart 2).

The structure of the new βCD conjugates was ascertained from their microanalytical, FAB, or MALDI-TOF mass spectrometry and NMR data. The ^{1}H and ^{13}C NMR spectra recorded at room temperature showed the typical line broadening associated with restricted rotation at the pseudoamide NH−(C═S) bonds.[23a] Line broadening was still evident at higher temperatures (50−70 °C) for compounds having multiple thiourea groups. Moreover, the unsymmetrical nature of the monosubstituted βCD moiety and the occurrence of diastereotopism at the carbon-centered branching points resulted in extensive signal overlapping. Resonance attribution was accomplished by combining 1D and 2D NMR experiments and comparison of the spectra with data for the corresponding methyl α-D-glucopyranoside-derived model conjugates. Thus, the ^{13}C NMR resonances at 184−183 ppm ($\delta_{C=S}$) and the high field chemical shifts for the substituted primary hydroxyl carbon atom C-6^I, as well as for the N-linked carbon atoms in the spacer and branching elements, confirmed the presence of the thiourea tethers. The signals for C-4^I and C-5^I exhibited characteristic low-field and high-field shifts, respectively, as compared to the corresponding signals of the unsubstituted sugar units. The resonances for the anomeric carbon atoms ($\delta_{C-1'}$) of the coating monosaccharides were found at 80−82 ppm for mannopyranosylthiourea units and 99−100 ppm for O-linked mannopyranosides (see Figure 2).

Con A Binding Efficiency. The affinity of the prepared βCD conjugates **27**−**32** toward Con A was measured by the enzyme-linked lectin assay (ELLA).[15] In addition, the analogous polymannosides in which the βCD core has been replaced by a single α-D-glucopyranoside subunit **33**−**38** were tested in the binding studies to ascertain the influence of the CD moiety on the protein−ligand recognition event. ELLA measures the ability of a soluble saccharide to inhibit the association between a labeled lectin and a polymeric ligand attached to the microtiter

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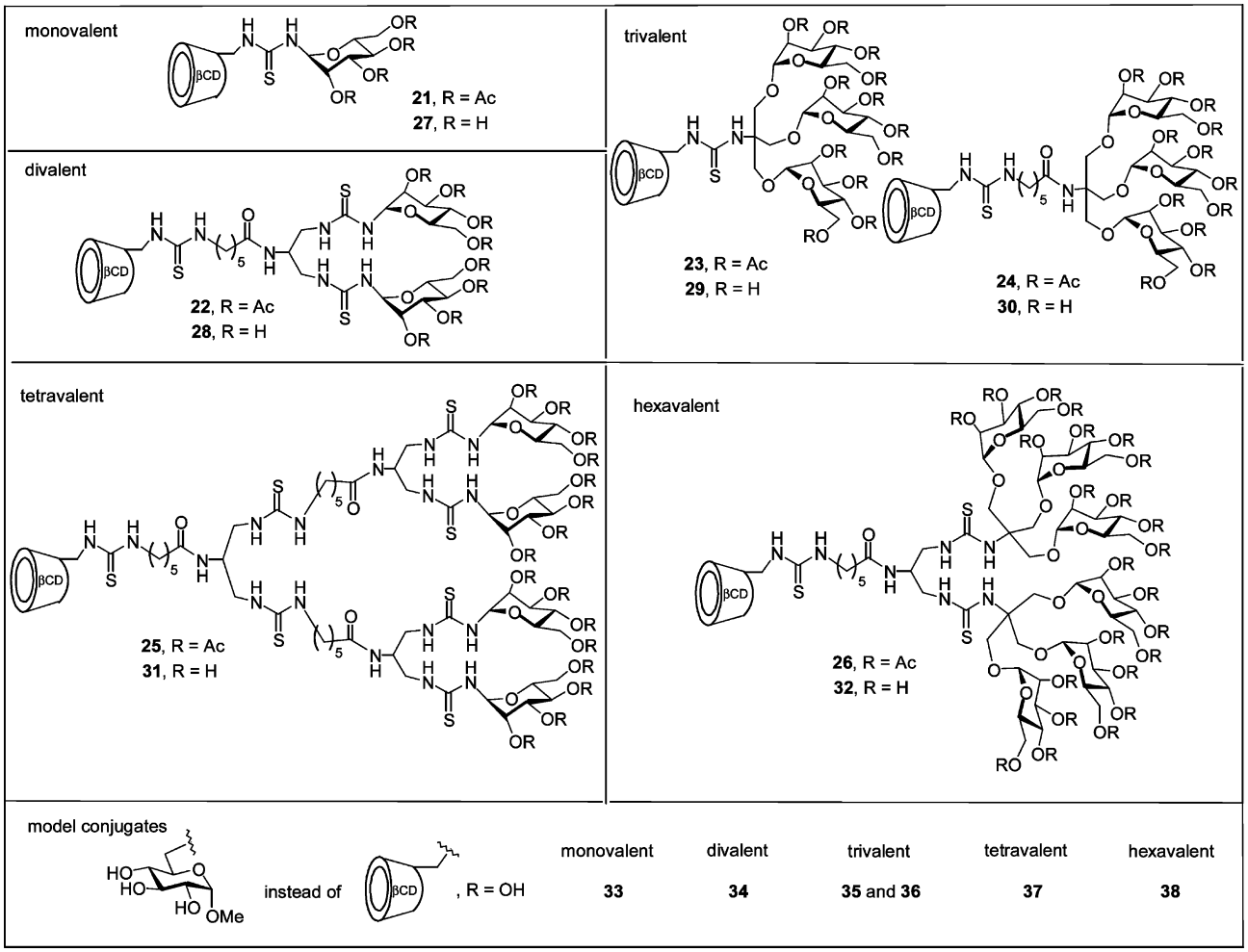
(26) It should be noted that deacetylation must be conducted at 0 °C in the case of conjugates bearing mannopyranosyltioureido substituents (**21**, **22**, **25**, **33**, **34**, and **37**) to avoid undesired anomerization reactions. See: Benito, J. M.; Ortiz Mellet, C.; Sadalapure, K.; Lindhorst, T. K.; Defaye, J.; García Fernández, J. M. *Carbohydr. Res*. **1999**, *320*, 37−48.

Chart 2. Chemical Structures of the βCD−Glycodendrimer Conjugates Prepared in This Study and of the Corresponding Model Compounds in Which the βCD Host Moiety Has Been Replaced by a Methyl α-D-Glucopyranosyl Subunit



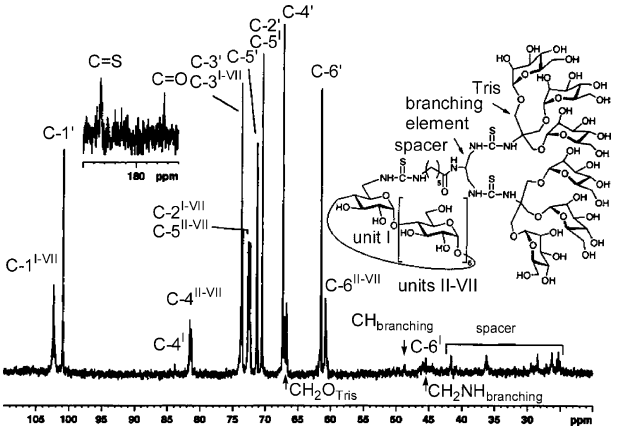


Figure 2. ^{13}C NMR spectrum (125.7 MHz, D_2O, 343 K) of the hexavalent βCD−dendrimer conjugate **32** showing atom notation and diagnostic signals.

Table 1. Results from the Binding Inhibition of Horseradish Peroxidase-Labeled Con A to Yeast Mannan by 6^I-Mannosylated βCD−Dendrimer and Methyl α-D-Glucopyranoside−Dendrimer Conjugates

	valency	IC_{50} (μM)	relative efficiency[a]
27	1	800	1.7 (1.7)
33	1	1360	1.0 (1.0)
28	2	780	1.75 (0.87)
34	2	710	1.9 (0.96)
29	3	91	14.9 (4.98)
30	3	180	7.6 (2.52)
35	3	190	7.2 (2.39)
36	3	186	7.3 (2.44)
31	4	95	14.3 (3.58)
37	4	110	12.4 (3.09)
32	6	10	136 (22.7)
38	6	12	113 (18.8)

[a] Taking the value for methyl 6-deoxy-6-(*N*′-α-D-mannopyranosylthio-ureido)-α-D-glucopyranoside **33** (1360 μM) as the reference. Values in parentheses are valency-corrected.

well (horseradish peroxidase-labeled Con A and yeast mannan, respectively, in the present case). The concentration needed to achieve 50% inhibition (IC_{50}) is then assumed to be inversely proportional to the lectin−saccharide binding free energy. The results are collected in Table 1.

Effect of Valency and Density. The corresponding IC_{50} values for inhibition of Con A−yeast mannan binding (Table 1) reflected the expected amplification of lectin-binding strength for the higher-valent representatives (up to 22-fold in a molar basis for hexavalent as compared to monovalent derivatives), indicative of a strong cluster effect.[2] The Con A binding capacity of the monosubstituted hexavalent βCD conjugate **32** (IC_{50} = 10 μM) actually surpassed that previously encountered for per-(C-6)-substituted βCD heptamannoside derivatives (IC_{50} > 70 μM),[11b] thus illustrating the strong dependence of carbohydrate−lectin recognition processes upon the presentation mode of

receptor-binding epitopes in multivalent ligands.[27] Notwithstanding, virtually no difference in binding efficiency was detected between mono- and divalent or between tri- and tetravalent ligands. In fact, from the present data it may be inferred that the observed affinity increases are mainly associated with incorporation of triads of mannopyranosyl ligands, that is, a density effect. Because, presumably, ELLA provides information on the interaction between the glycoclusters and a single binding site in the lectin, without contributions from chelating or cross-linking effects,[28] it can be concluded that the observed density effect operates at the level of the so-called microcluster effect.[29] While optimizing the macrocluster effect is of great importance when looking for inhibitors or effectors of particular biological functions, optimizing the intrinsic affinity for a biological receptor may be particularly relevant for targeting purposes. This microcluster effect can reach biologically relevant values at a relatively low number of binding motifs, but is very sensitive to structural (orientational, density, geometrical) considerations.[30] Although the mechanism by which it operates is still unclear,[31] our data highlight the importance of flexible, diversity-oriented synthetic strategies for the identification and optimization of good ligand candidates.

Effect of the Host in Lectin Binding. Comparison of ELLA data for βCD (**27**−**32**) and methyl α-D-mannopyranoside conjugates (**33**−**38**) allows the evaluation of the impact of the presence of the host moiety in lectin binding. No significant differences were observed between both series of compounds when the six-carbon spacer element was present in the structure. However, the IC_{50} values for the monovalent and trivalent adducts **27** and **29** (800 and 91 μM, respectively), in which the carrier and the mannosyl ligand are directly linked through a thiourea group, were indicative of binding efficiencies 85−90% higher as compared to the homologous methyl α-D-glucopyranoside derivatives **33** and **35** (IC_{50} values 1360 and 180 μM, respectively). This must be essentially ascribed to an additional stabilizing interaction involving the cyclic heptasaccharide carrier. It is probable that once the mannosyl ligand has been accommodated in the Con A recognition site, the βCD framework is close enough to interact with residues at the protein surface. A similar situation has been found in the complex formed by a βCD−gastrin conjugate and the human CCK-B receptor.[32] Comparison of ELLA data for **29** and **30**, differing exclusively in the presence of the six-carbon spacer, unequivocally shows that this interaction is dependent on the proximity between the CD host and the biorecognizable saccharide epitopes. The contribution of the βCD−Con A interaction to the total free energy of binding can be estimated, from the comparative IC_{50} values,[15b] at about −0.35 kcal mol^{-1} under the particular conditions of the ELLA test. This result may be relevant as the CD−receptor interaction might involve the CD cavity, inducing the release of an included guest just after delivery to the target site.

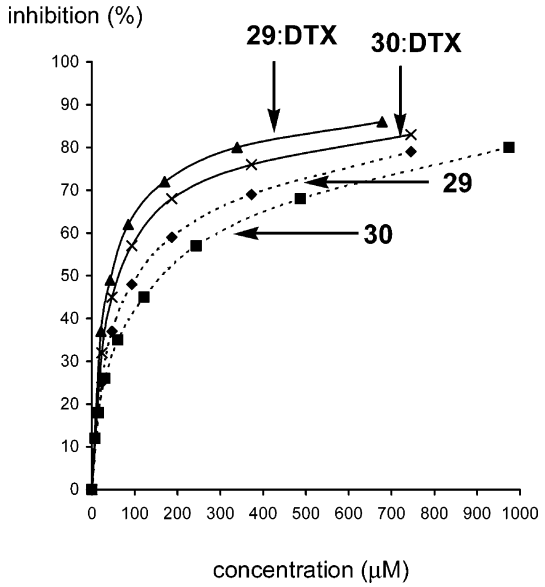


Figure 3. Inhibition of binding of horseradish peroxidase-labeled Con A to yeast mannan by increasing concentrations of the monosubstituted trivalent mannose−βCD conjugates **29** and **30** (dotted lines) and of their corresponding inclusion complexes with docetaxel (solid lines). The βCD conjugate−docetaxel molar ratio was 5:1.

Drug Carrier Capabilities. The prepared cyclodextrin conjugates exhibited extremely high water solubilities, above 20-fold higher as compared to the parent βCD (15 mM). Moreover, docetaxel solubilization experiments in water showed solubility values similar to those obtained previously for monovalent CDs, significantly higher than those reported for per-(C-6)-substituted derivatives.[11b] For instance, up to 4.5 and 4.7 g L^{-1} of Taxotère was solubilized in 25 mM aqueous solutions of **29** and **30** at 25 °C, respectively, that is, above 1000-fold solubility enhancements as compared to the water solubility of the drug (0.004 g L^{-1}).[16a] This already supports the hypothesis depicted in Figure 1 and illustrates the superior potential of dendritic cyclodextrins as drug carriers as compared to monovalent or polysubstituted conjugates.

The ability of the docetaxel:**29** and docetaxel:**30** complexes to bind Con A was evaluated by ELLA using lyophilizates containing 1:5 guest:host molar ratios. It was established that the docetaxel:βCD complex at the same molar ratio did not interact with the lectin. The plots for the inhibition of the Con A−yeast mannan association by the βCD polymannosides **29** and **30** and the corresponding complexes with docetaxel (**DTX**) are shown in Figure 3. No precipitation was observed under the experimental conditions, discarding decomplexation of the

(27) For recent studies on the dependence of Con A−polymannoside binding modes on multivalent ligand architecture and density, see: (a) Gestwicki, J. E.; Cairo, C. W.; Strong, L. E.; Oetjen, K. A.; Kiessling, L. L. *J. Am. Chem. Soc.* **2002**, *124*, 14922−14933. (b) Cairo, C. W.; Gestwicki, J. E.; Kanai, M.; Kiessling, L. L. *J. Am. Chem. Soc.* **2002**, *124*, 1615−1619.

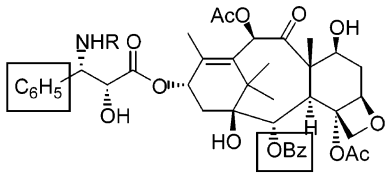
(28) The short distances between the mannopyranosyl binding epitopes in the prepared conjugates cannot expand the 50 Å distance between two binding sites in tetrameric Con A, precluding a chelate effect. On the other hand, the presence of the relatively voluminous HRP protein as a label in the lectin used for ELLA experiments probably prevents cross-linking processes for such short intermannosyl spacers. Actually, no precipitation was observed during the assays, supporting this assumption. For a discussion on the significance of lectin-binding evaluation by ELLA, in comparison with other techniques, see ref 15a.

(29) The terms mini cluster (microcluster) and macrocluster effect have been previously used in the literature to refer to the observed increases on lectin-binding affinities for small glycoclusters and large glycopolymers, respectively. See: (a) Quesenberry, M. S.; Lee, R. T.; Lee, Y. C. *Biochemistry* **1997**, *36*, 2724−2732. (b) Fan, J.-Q.; Quesenberry, M. S.; Takegawa, K.; Iwahara, S.; Kondo, A.; Kato, I.; Lee, Y. C. *J. Biol. Chem.* **1995**, *270*, 17730−17735.

(30) For recent evidence on the existence of an aggregation-independent microcluster effect, see: Köhn, M.; Benito, J. M.; Ortiz Mellet, C.; Lindhorst, T. K.; García Fernández, J. M. *ChemBioChem* **2004**, *5*, 771−777.

(31) A sliding mechanism or the existence of extending binding sites in the protein has been postulated previously to account for enhanced binding affinities between lectins and small glycoclusters that do not fit either to the chelate or to the aggregation model. See cf. ref 1a.

(32) Schaschke, N.; Fiori, S.; Wehyher, E.; Escrient, C.; Fourmy, D.; Müller, G.; Moroder, L. *J. Am. Chem. Soc.* **1998**, *120*, 7030−7038.



docetaxel (**DTX**, Taxotère®), R = Boc
paclitaxel (**PTX**, taxol), R = Bz

Figure 4. Chemical structure of docetaxel (**DTX**) and of the related drug paclitaxel (taxol, **PTX**).

insoluble drug upon lectin binding. The results were indicative of 2-fold higher Con A affinities in the case of the complexes (IC_{50} values 43 and 51 μM) as compared to the individual hosts (91 and 190 μM, respectively). An identical effect was observed for the hexavalent derivative **32** (IC_{50} = 6 μM for **32**:**DTX** complex as compared to 10 μM for **32**).

The existence of additional interactions between the included drug and the receptor protein might be at the origin of the above effect. Notwithstanding, this observation seems to be independent of the distance between the receptor-binding cluster and the carrier moiety, suggesting a different mechanism. Probably, the existence of two functional moieties in the docetaxel molecule for inclusion in the βCD cavity (i.e., the two aromatic rings; see Figure 4) results in the formation of species with a 1:2 guest−host stoichiometry, therefore with a formal hexavalent character. The possibility of exploiting guest-promoted clusterization of multivalent carriers, a biomimetic mechanism, may actually represent a new approach in active drug targeting. On the other hand, this observation suggests that CD dimer derivatives, capable of interacting simultaneously with both aromatic rings in docetaxel to form 1:1 sandwich-type complexes, could be still more efficient carriers for this particular drug. A demonstration that the disclosed modular synthetic strategy is flexible enough to respond to such design requirements is given hereinafter.

Tailor-Made Dual-Cavity Hosts for Docetaxel. Recently, the formation of sandwich-type complexes of the related drug paclitaxel (**PTX**, taxol; Figure 4) with cyclodextrin dimers has been reported.[33] Interestingly, such dual-cavity hosts can either fully sequester the drug, therefore acting as detoxifying agents, or not affecting the cytotoxic activity at all, depending on the CD dimer structure. The above commented results suggest a similar ability to interact simultaneously with two βCD cavities for docetaxel. The preparation of tailor-made multitopic hosts able to satisfy the requirements for sandwich-type complexation and biological receptor recognition was, therefore, appealing (Figure 5). In fact, the possibility of generating molecular diversity at the host end of the CD−glycodendritic architecture to optimize host−guest interaction was already purposely

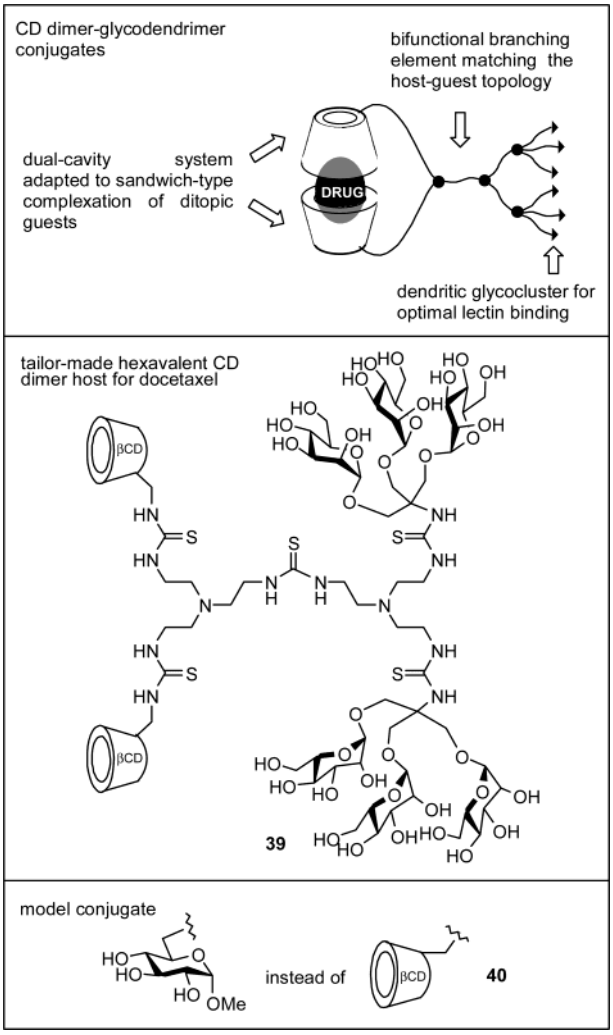


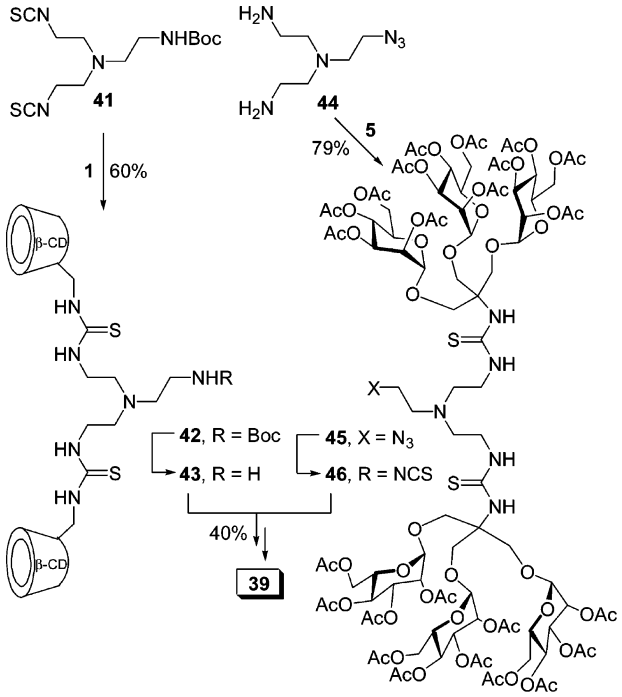
Figure 5. General architecture of CD dimer−glycodendrimer conjugates and the chemical structure of compound **39**, proposed in this study as docetaxel carrier (the corresponding model compound **40** was also prepared for control experiments).

implicit in the modular strategy developed. Taking advantage of our previous experience on the preparation of CD conjugates through the thiourea bridging reaction, a convergent synthesis of the glycodendritic host **39**, the first example of a CD dimer derivative endowed with the capability of biological receptor recognition, has been devised.

In the above molecular design, we have kept the elements that were already identified as optimal for lectin binding (i.e., a hexavalent dendritic presentation of the biorecognizable mannosyl epitopes grouped in triads to account for the favorable density effect) and inclusion capabilities (i.e., C-6 monosubstituted βCD elements). The synthetic strategy is depicted in Scheme 3. Coupling reaction of the βCD monoamine **1** with the selectively functionalized tris(2-aminoethyl)amine (TREN)-derived branching element **41** provided the CD dimer moiety **42**, which was activated for a further coupling process by trifluoroacetic acid-catalyzed hydrolysis of the *N*-Boc protecting group (→**43**). The reaction of diamine **44** and isothiocyanate **5** afforded the hexavalent bis(thiourea) **45** that was subsequently armed by direct isothiocyanation of the terminal azido group (→**46**).[24] Conjugation of **43** and **46** followed by deacetylation yielded the target TREN-based first-generation bidirectional dendritic architecture **39**. Similarly, by replacing the βCD

(33) (a) Liu, Y.; Chem, G.-S.; Li, L.; Zhang, H.-Y.; Cao, D.-X.; Yuan, Y.-J. *J. Med. Chem.* **2003**, *46*, 4634−4637. (b) Moser, J. G.; Rose, I.; Wagner, B.; Wieneke, T.; Vervoorts, A. *J. Inclusion Phenom. Macrocycl. Chem.* **2001**, *39*, 13−18. For additional examples on the synthesis and complexing properties of cyclodextrin dimers, see: (c) Breslow, R.; Belvedere, S.; Gershell, L.; Leung, D. *Pure Appl. Chem.* **2000**, *72*, 333−342. (d) de Jong, M. R.; Engbersen, J. F. J.; Huskens, J.; Reinhoudt, D. N. *Chem.-Eur. J.* **2000**, *6*, 4034−4040. (e) Baugh, S. D. P.; Yang, Z.; Leung, D. K.; Wilson, D. M.; Breslow, R. *J. Am. Chem. Soc.* **2001**, *123*, 12488−12494. (f) Liu, Y.; Chen, Y.; Wada, T.; Inoue, Y. *Chem.-Eur. J.* **2001**, *7*, 2528−2535. (g) Jiang, T.; Sukumaran, D. K.; Soni, S.-D. Lawrence, D. S. *J. Org. Chem.* **1994**, *59*, 5149−5155. (h) *Modified Cyclodextrins: Scaffolds and Templates for Supramolecular Chemistry*; Easton, C. J., Lincoln, S. F., Eds.; Imperial College Press: London, 1999; pp 191−226.

Scheme 3. Synthesis of the Hexavalent βCD Dimer−Polymannoside Conjugate **39** and Its Precursors



building block **1** by the methyl α-D-glucopyranosyl-derived core element **7** in the synthetic scheme, the model conjugate **40**, lacking the dual CD cavity system, was prepared as a reference compound for control experiments.

The choice of nitrogen-centered TREN-derived branching elements in the above synthetic design responds to two important considerations: (i) according to molecular modeling calculations, the length of the corresponding bis(thiourea) linker between the CD units in **39** matches the distance between the aromatic rings in **DTX**, allowing the appropriate orientation of the two cavities for an optimal cooperative complexation; (ii) the use of nitrogen-centered dendrons prevents the occurrence of diasterotopism in the NMR spectra of the final conjugates, thus facilitating NMR analysis.

Comparison of Docetaxel Carrier Capabilities and Lectin-Binding Properties for CD Mono and Dimer Conjugates. Both the monomeric and the dimeric hosts **32** and **39** formed supramolecular complexes with docetaxel and dramatically increased its solubility in water. Association constants (K_c) of 4000 and 1.5×10^5 M^{-1}, respectively, were estimated from the slope of the corresponding phase solubility diagrams, assuming a 1:1 host:guest stoichiometry.[34] The 37.5-fold increase in association constant for the dimeric with respect to the monomeric host is in agreement with the simultaneous interaction of the drug with both CD cavities in a sandwich-type complex (see Figure 6).[35] Both conjugates exhibited strong binding affinities toward Con A as determined by ELLA, with similar IC_{50} values (10 and 13 μM for **32** and **39**, respectively).

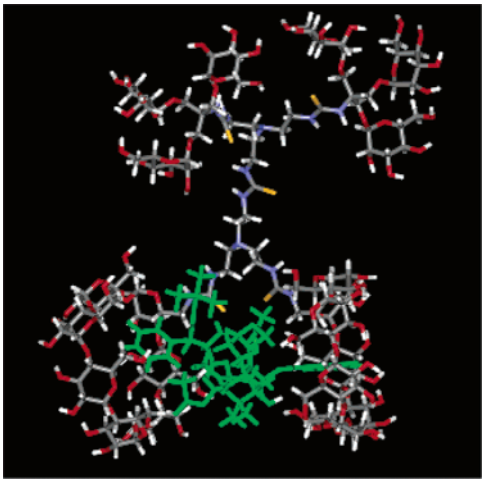


Figure 6. Molecular model of the sandwich-type complex between the hexavalent βCD dimer conjugate **39** (light gray, dark red, white, light blue, and yellow represent carbon, hydrogen, oxygen, nitrogen, and sulfur atoms, respectively) and docetaxel (in green). According to docking experiments (MACROMODEL 6.0, MM2*), the benzoyl ring (right side) would be deeply included in one of the βCD cavities, while the benzyl group (left side) would be only shallowly included in the second CD cavity due to the steric hindrance imposed by the neighboring Boc group.

Actually, the dual-cavity system did not affect glycodendrimer−lectin recognition at all as indicated by control experiments using the model conjugate **40** (IC_{50} 12 μM). Comparative evaluation of the lectin-binding properties for the corresponding complexes evidenced, however, significant differences. Thus, in the case of the CD dimer derivative **39**, the interaction with the protein was not affected upon complex formation, in sharp contrast with the 2-fold increase in Con A affinity observed for the monomeric CD conjugate **32**:**DTX** complex as compared to the free host **32**. This result supports our hypothesis of a docetaxel-promoted clusterization of the glycodendritic system in the case of **32**, a mechanism that is inhibited in the case of **39** due to the sandwich-type supramolecular organization. Moreover, these data indicate that both host−glycodendrimer architectures, not only as such but also as the corresponding docetaxel complexes, are efficiently recognized by the lectin and that the sugar ligand-lectin affinity can be allosterically modulated, to some extent, by acting at the host end.

Targeting of βCD−Polymannoside Dendrimers to Macrophages. The above results illustrate the concept that the CD−glycodendrimer architecture, in combination with a versatile diversity-oriented synthetic methodology, allows the systematic study of the different factors influencing sugar−lectin and host−guest interactions. From these results, information can thus be gained for the design of new systems for saccharide-directed molecular delivery of specific guests to a biological receptor. Because lectins having identical sugar binding motifs may differ significantly in their binding-site, quaternary structure, and preferred binding mechanisms, the conclusions obtained for a particular receptor, for example, the plant lectin Con A in the present study, must be taken with care when

(34) The corresponding solubility diagrams for the systems **DTX**:**32** and **DTX**:**39**, in the range of host concentrations investigated (10−25 mM; see Supporting Information), provided quasi-linear plots with slopes of less than unity. Although this situation doest not necessarily mean that only a 1:1 complex is formed (actually, the lectin-binding experiments pointed to the presence of a 1:2 complex in the case of **32**), it is consistent with these species being the predominant ones in the solutions. See: Connors, K. A. *Binding Constants, The Measurement of Molecular Complex Stability*; John Wiley & Sons: New York, 1987; pp 261−281.

(35) For the generation of the three-dimensional model of the **DTX**:**39** complex, the thiourea groups in the host were set in the *Z*,*Z*-conformation, the dihedral angle about the C-5^{I}−C-6^{I} bond was set in the gauche-trans conformation (i.e., the C-4^{I} and the thiourea substituents in the anti disposition), the carbon segments were set in the all-trans conformation, and the carbamate bond in the guest was set in the *Z*-conformation. The host and the guest structures were extensively minimized using the GSB continuum solvent model for water, and the minimized structures were used in the docking experiments.

changing the protein target. To test the potential of these systems in drug targeting, assaying a mammalian lectin was, therefore, of interest.

Among the receptors present at the surface of macrophages, the well-characterized mannosyl/fucosyl receptor is attractive for targeting purposes.[6e,36] Previous results using mannosylated liposomes evidenced a parallelism between binding affinity toward Con A and binding affinity toward the macrophage surface.[37] In an in vitro experiment, the interaction of the hexavalent β-cyclodextrin−dendrimer conjugates **32** and **39** with resident peritoneal macrophages (mice), which present such a lectin at their surface, was studied. Binding of the targeted conjugates and the control (native β-cyclodextrin) to cells was quantified by a fluorimetric method adapted from that reported by Muller and Schuber[37] for mannosylated liposomes, using 6-*p*-toluidino-2-naphthalenesulfonic acid (TNS) as the fluorescent dye. TNS forms a strong 1:1 inclusion complex with βCD,[38] and it was also strongly complexed by **32** and **39**.[39]

To define the specificity of the host interaction with the surface of the macrophage, we have performed competition experiments at 4 °C, a temperature that precludes phagocytosis. The cells were incubated at this temperature with three concentrations of the 1:1 TNS:**32** or TNS:**39** complexes (0.1−80 μmol/2 mL Dulbecco's modified Eagle's medium), and the amount of cell-associated complex was determined fluorimetrically. As compared to the nontargeted TNS:βCD complex, an above 20-fold preferential association of **32** and **39** was observed at a concentration of 650 nM. An inhibition of this binding was observed in the presence of increasing concentrations of the uncomplexed hosts. IC_{50} values of 45 and 48 $\pm$ 5 μM, respectively, were obtained from classical displacement curves for the mono and dimeric hosts (concentration of TNS:host = 40 μM). These results are consistent with a reversible binding of the mannosylated βCD derivatives **32** and **39** to the cell surface under these experimental conditions, both the uncomplexed conjugates and the corresponding TNS inclusion complexes competing for the same binding site. In contrast, when β-cyclodextrin was used, no direct competition was observed for the binding of the TNS:**32** or TNS:**39** complexes.

Interestingly, when the **DTX**:**32** complex (lyophilizate containing 1:5 guest:host molar ratio) was used as competing agent, a value of IC_{50} = 30 $\pm$ 5 μM (referred to **32**) was obtained under the same conditions, indicating a higher affinity toward the macrophage surface as compared to uncomplexed **32**. No inhibition effect was observed, however, for the corresponding complex of docetaxel with β-cyclodextrin, discarding a contribution of the included drug to macrophage surface binding. These results parallel those discussed above for the association of multivalent βCD conjugates and the corresponding docetaxel complexes to Con A, supporting a guest-promoted clusterization mechanism that results in higher-valent species with increased mannose receptor affinity. In the case of **39**, the competition experiment did not show any difference between the uncomplexed host and the corresponding docetaxel complex, in agreement with the proposed sandwich-type structure of the later.

Conclusions

Targeting involving sugar binding to biological receptors has generally relied on the generation of large multivalent displays of the putative epitopes on a macromolecular scaffold. Coupling such high molecular weight ligands to single-molecule molecular containers (e.g., cyclodextrins) would be, however, unpractical. Our results show that it is possible to attain biologically useful affinities by using relatively small glycoclusters of appropriate geometry, even though cross-linking of different carbohydrate-binding domains is unlikely to occur for short intersaccharide bridges. Synthetic methods that allow not only the efficient coupling of the glycocluster and the host moieties but also tailor the ligand−lectin and host−guest interactions should facilitate exploration of the potential of the microcluster effect in vectorized molecular delivery. In this context, the significance of the present study is three-fold. First, it demonstrates an efficient and very flexible strategy for the preparation of tailor-made CD dendrimers bearing biorecognizable markers or reporting groups. Second, the present system suggests a potential utility in sugar-directed delivery of particular drugs or probes to specific saccharide-recepting biological surfaces. Third, the possibility of exploiting guest-promoted clusterization of multivalent carriers may actually represent a new approach in active drug targeting.

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Supporting Information Available: Experimental details, purification and characterization data for the prepared compounds, and experimental procedures for the determination of Con A and macrophage binding efficiency. This material is available free of charge via the Internet at http://pubs.acs.org.

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(36) Düffels, A.; Green, L. G.; Ley, S. V.; Miller, A. D. *Chem.-Eur. J.* **2000**, *6*, 1416−1430.
(37) Muller, C. D.; Schuber, F. *Biochim. Biophys. Acta* **1989**, *986*, 97−105.
(38) Rekharsky, M. V.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875−1917.
(39) Association constants (K_{11}) of 1.8 × 10^4, 1.2 × 10^4, and 9.5 × 10^5 M^{-1} for the corresponding 1:1 TNS:βCD, TNS:**32**, and TNS:**39** complexes were determined by fluorescence titration experiments. Note that this implies that less than 1% of the complex is dissociated in solution, discarding any significant influence of the dissociation equilibrium in the lectin-binding evaluation experiments.