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Glyconanocavities: Cyclodextrins and Beyond

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Abstract

Cyclodextrins (CDs) represent a unique example of complementarity between nanotechnology and biotechnology. Their molecular nanocavity character and the possibility of selective functionalization offer an excellent opportunity for chemical elaboration of unique nanostructures in a three-dimensional network. Several approaches from our laboratories, aimed at endowing CDs with biorecognition properties by incorporation of saccharide ligands, are discussed. Applications range from site-specific drug delivery systems to more fundamental studies on carbohydrate–protein interactions. Results on the *de novo* synthesis of a new family of glyconanocavities constructed from α,α-trehalose building blocks, namely cyclotrehalans (CTs), and on their complexing properties are also presented.

Introduction

Carbohydrates have only relatively recently formally entered the nanoscience and nanotechnology era, although this is a rather logic evolution when we consider the high level of organization of glycopolymers such as cellulose or lignocelluloses in use for centuries at the technological level. The current research on glycoclusters [1–3], glycodendrimers [4–7], glycopolymers [8–10] glycoliposomes [11–14] or glycoarrays [15–19] as biomimetic multivalent sugar displays has profoundly improved the current notions on the role of carbohydrates as carriers of biological information. Multivalent carbohydrate–protein and carbohydrate–carbohydrate interactions are involved in cell–cell and cell–environment communication, mediating a battery of biological and pathological processes including development, differentiation, morphogenesis, fertilization, bacterial and viral infection, the immune response, implantation, cell migration and cancer metastasis [20–27]. With the recent burst of the *nano* era, it became then apparent that coating preformed nanospheres [28], nanoparticles [29–37], nanotubes [38, 39] or quantum dots [40–42] with biologically relevant carbohydrate ligands could provide new devices for more accurate fundamental studies as well as for biomedical applications in antiadhesive therapies, drug release control, targeting or diagnosis, for instance. In fact, carbohydrate chemists have been dealing with nanometric biomaterials such as polysaccharide–protein aggregates, since already a long time. The critical relationship of the size of such supramolecular architectures with their properties was, however, not always realized. The field is now mature enough to recognize that the merging between glycosciences and nanosciences (*glyconanotechnology*) opens new and exciting opportunities that will bring about important advances within the incoming years.

Cyclomaltooligosaccharides (cyclodextrins, CDs) represent a paradigmatic example of carbohydrate derivatives exhibiting a close relationship between molecular status and supramolecular functional properties. Their ability to form supramolecular complexes with other organic molecules closely relates to the size of their nanometric hydrophobic cavity. Their propensity to auto-associate in the form of organized nanostructures is a further characteristic. Consequently, CDs must be considered as a class of nanobiomaterials, susceptible of further manipulation in order to modulate their topology and recognition features with the environment. Their precise characterization and the development of applications such as active transport, intelligent drug delivery systems and catalysis will greatly benefit from a nanobiotechnological approach. Giving the above step ahead is, in some way, a question of semantics. By

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coining the term glyconanocavities in the title of this contribution, we intend to stress the emergency of CDs, and more generally of sugar-built in cavities, into the growing field of glyconanosciences.

Coating a preformed nanoparticle with carbohydrates is relatively straightforward by making use of the methodologies already developed to attach carbohydrates to a variety of cores and surfaces. Coating inside a nanocavity with sugars or building up a glyconanocavity from carbohydrate building blocks is, however, much less evident. That is the reason why cyclodextrins are the only representatives of this family of nanomaterials that are available at a scale that allow not only for fundamental studies but also for commercial applications [43, 44]. The particular properties of CDs in terms of encapsulation ability, biocompatibility and solubilisation properties for hydrophobic materials have enforced their privileged position in supramolecular chemistry. However, the number of natural CDs currently available (cyclomaltohexaose, -heptaose and -octaose; αCD, βCD and γCD, respectively) is still rather limited and does not satisfy all the requirements that fundamental studies or commercial applications demand. A main strategy to overcome this limitation relies in the chemical modification of native CDs. As an alternative, *de novo* synthesis of glycomacrocycles is another opportunity. In the first part of this article, we intend to summarize the approaches that were developed in our laboratories toward the first goal. In the second part, we will discuss our efforts to develop a new and conceptually different family of unnatural glyconanocavities based on α,α-trehalose building blocks, namely cyclotrehalans (CTs) [45], as well as some preliminary results on their inclusion properties as compared to CDs.

Materials and methods

All materials, synthetic procedures and methods of characterization have been described previously, as have the protocols for carbohydrate–protein binding affinity measurements and association constants determination (see Refs. [46–49], [50–56] and [57–61] in the following discussion).

Results and discussion

Modified cyclodextrins

Chemical modification of CDs was primarily aimed at improving the water solubility of the native (first generation) compounds, especially of βCD, the most interesting representative from the commercial point of view, as well as of their inclusion complexes with pharmacologically active compounds. By taking advantage of the differences in reactivity of the various hydroxyl groups in the cyclooligosaccharide structure, some regioselective transformations were achieved. Thus, the primary C-6 hydroxyl groups are sterically more accessible, while the secondary hydroxyls at C-2 are the more acidic. Currently, three families of chemically modified CDs can be found in the market (Figure 1): the alkylated CDs TRIMEB (mainly per-2,3,6-tri-*O*-methyl-βCD), DIMEB (mainly per-2,6-di-*O*-methyl-βCD) and RAMEB (randomly methylated βCD); the hydroxyalkylated CDs HPCDs (randomly dihydroxypropylated-βCD as mixture of isomers); and the anionic SBCDs (randomly polysulfobutylated-βCD; Captisol™). The advent of these second generation CDs has given rise to new formulations, thus broadening the range of applications of these glyconanocavities in pharmacy [62, 63]. The problems associated with their heterogeneous composition have prevented, however, a wider use. On the other hand, drug transport by either the first or the second generation CDs is essentially unspecific, since CDs themselves do not possess the capability of molecular recognition at the level of biological receptors.

To overcome the above limitations, our approach has consisted in the development of new methods for the selective chemical functionalization of cyclodextrins in combination with the incorporation of oligosaccharide ligands that would be specifically recognized by protein receptors (lectins) at the surface of the cell (Figure 2). These molecular shuttles should be able, in principle, to deliver a drug or a probe included in the cyclodextrin nanocavity to a specific cell or tissue. It is obvious that the choice of a particular functionality and the nature of the bridging element between the carrier and the biorecognizable ligand is going to play a decisive role not only in the design of the synthetic strategy, but also in the final properties of the conjugate such as water solubility, toxicity, affinity towards the receptor target or inclusion ability. An important prerequisite is that the tether group must be generated with high efficiency and with total chemoselectivity, avoiding protection-deprotection steps of the hydroxyl groups of the cyclodextrin with eventually painstaking separations, which is probably the main impediment in CDs chemistry. We anticipated also that the incorporation of a functional group having a high tendency to establish hydrogen bonds with water molecules would help to break the internal H-bond network in CDs, which is responsible to a great extent for their tendency to form insoluble aggregates, and would therefore impart higher water solubility to the conjugates. In this context, the thiourea functionality appeared as a very attractive candidate, since it is an excellent H-bond donor and is very well suited to convergent synthetic approaches in which the last step is the coupling reaction between an amine and an isothiocyanate [64]. Moreover, previous results in our groups had indicated a decrease in the hemolytic character of CD derivatives bearing nitrogen functionalities [65].

Preliminary results toward this approach fully confirmed our expectations. So, 6^I-deoxy-6^I-methyl-

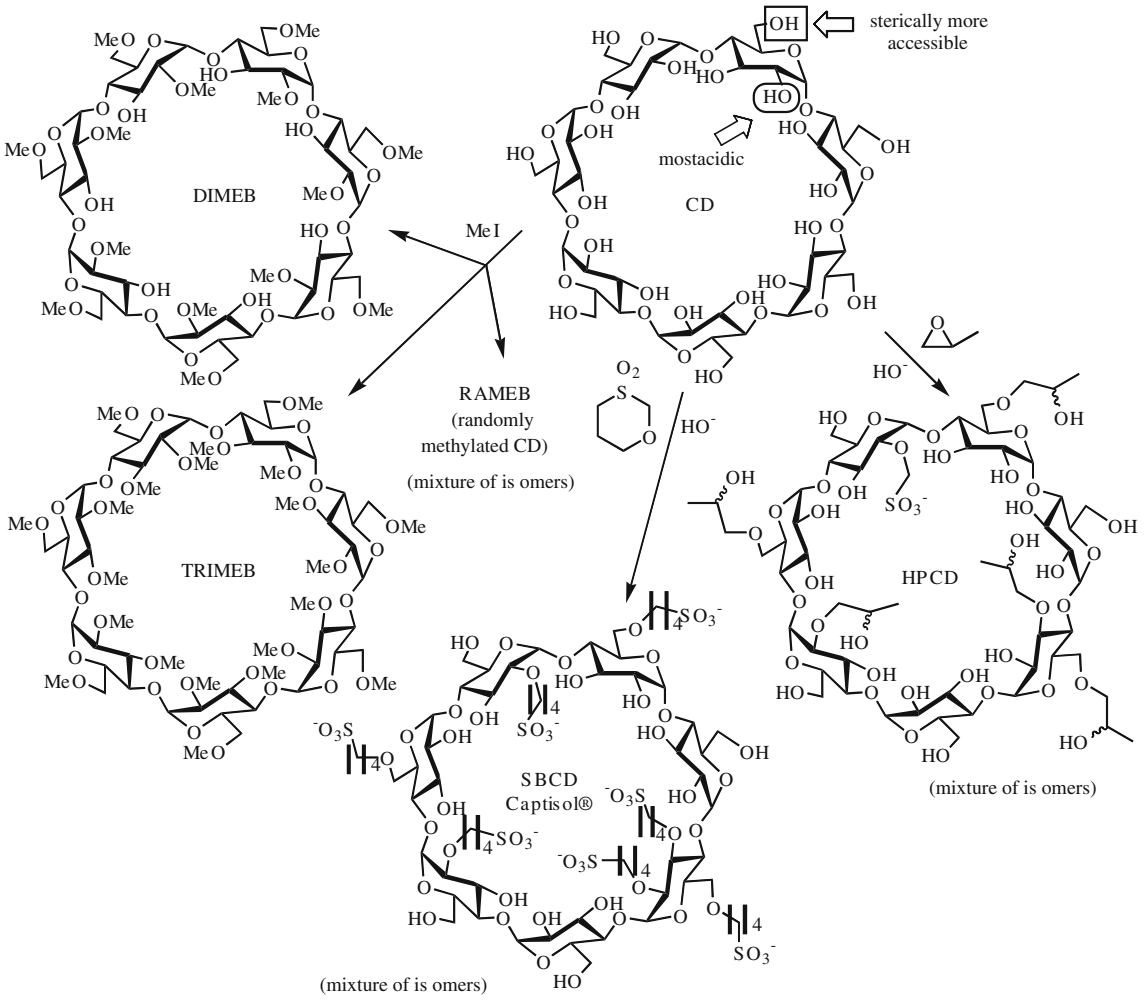


Figure 1. Commercially available second-generation cyclodextrins.

thioureidocyclomaltoheptaose (6-MTU), obtained by coupling the corresponding (C-6)-monoamine with commercial methyl isothiocyanate without need of hydroxyl protection [66], turned out to be 42-fold more soluble in water than the native βCD, which is remarkable for a neutral derivative. When compared with the commercial DIMEB, it was found to be not only more soluble in water but also eightfold less hemolytic, what actually means that it is suited for parenteral administration. This compound has been shown to be a very efficient carrier for the radioactive organometallic tracer ^{99m}Tc-NOET, which is used in heart imaging (Figure 3). The study has already been performed with cardiomyocytes, in isolated hearts and also in vivo in mice and dogs with success [67].

The above results prompted us to further extend the thiourea bridging strategy to the preparation of cyclodextrin–glycoligand conjugates for site-specific drug delivery [46–48]. Actually, several groups have been working with the development of CDs incorporating oligosaccharide markers that could be specifically recognized by membrane receptors, the so-called third generation CDs [49, 68–72]. Although the coupling methodologies varies to some extent, most of the reported examples can be classified into two general models: the monosubstituted derivatives at the hydroxymethyl rim and the per(C-6)-substituted derivatives. In both series, the wider rim of the trunconical cavity is free for drug inclusion. In principle, for the persubstituted derivatives one could expect an improved affinity for specific carbohydrate recognition proteins due to the

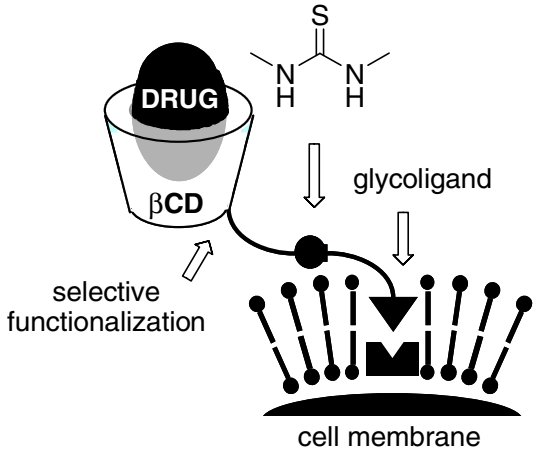


Figure 2. Schematic representation of glycoligand-driven site-specific drug delivery with cyclodextrin conjugates bearing the thiourea functionality.

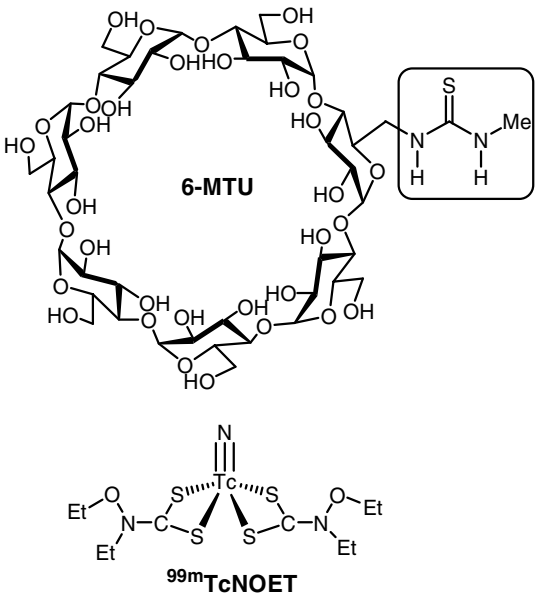


Figure 3. Structure of 6-MTU and ^{99m}TcNOET.

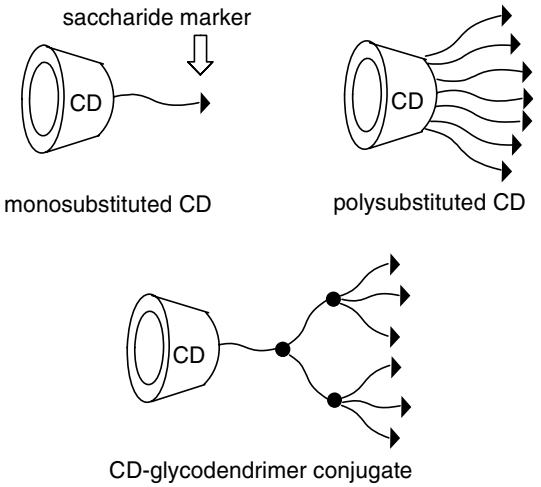


Figure 4. Schematic representation of (C-6)-monosubstituted, (C-6)-persubstituted and monosubstituted dendritic CD scaffolds bearing saccharide ligands (third-generation cyclodextrins).

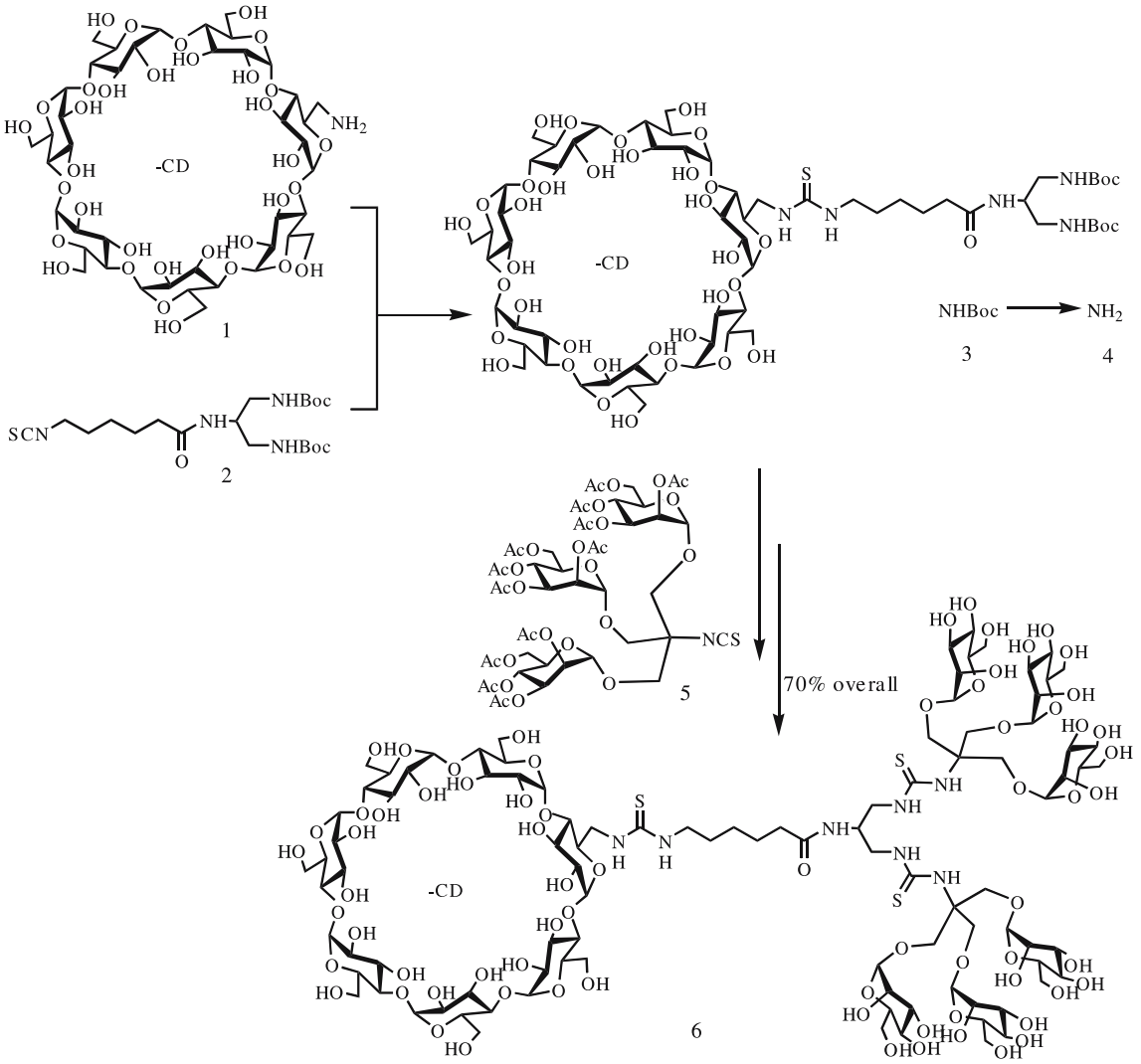
so-called ''multivalency'' or ''cluster effect'' [73]. This effect is of utmost importance in many processes involving transmission of biological information by sugars. It refers to the increase in the binding affinity between a ligand and its receptor when the ligand is presented in multiple copies, and is essential to attain biologically useful associations.

Examination of the receptor binding and drug inclusion properties of a series of (C-6)-modified CD conjugates led to two somehow conflicting results: whereas monosubstituted derivatives provided better complexing abilities, the persubstituted derivatives proved to be superior for targeting purposes provided that a suitable spacer would be inserted between the cavity and the saccharide ligand. In view of these observations, we decided to modify the general design, trying to combine the advantages of both models, that is, monosubstitution and multivalency at the same time. The highly ordered structure of dendrimers, with synthetic strategies that can either converge or diverge from a single point, seems particularly appealing for this purpose, being moreover compatible with the external coating by a variety of biorecognizable saccharide markers (Figure 4).

To build up the typical tree structure of dendrimers onto the narrower rim of β-CD, we selected a set of building blocks which could be combined in a very flexible and modular manner, looking for a synthetic scheme that could allow an easy tuning of the geometrical requirements for both optimal binding affinity by a specific lectin and efficient inclusion capabilities with a relatively low synthetic cost. The molecular kit includes a six-carbon spacer, namely 6-azidohexanoic acid, to warrant accessibility of the dendritic wedges to recognition events, a branching element derived from 1,2,3-triaminopropane, and the saccharide marker, which in this particular study consisted in α-D-mannopyranosyl ligands. The various elements could be connected through peptidic or thiourea linkages that were generated in high yield and total chemoselectivity [50, 51].

Noteworthy the (C-6)-monoamine derivative of βCD is a crucial building block for this approach. The synthesis of the corresponding (C-6)-monotosylate, which is the universal precursor for accessing (C-6)-monosubstituted βCD derivatives, typically proceeds in moderate yields and represents a real bottleneck within this type of approach. We have recently improved significantly the yield and the reproducibility of the synthesis by using a sandwich-type complex with copper (II) as the key intermediate. In this organometallic complex, the presence of the metal cation hinders the secondary hydroxyl rim, thereby preventing tosylation at position C-2, which is the main problem with other methodologies [52].

With all the necessary elements in hand, we started the preparation of a series of multivalent monosubstituted βCD-dendrimer conjugates. As an example, the divergent preparation of an hexavalent derivative is shown in Scheme 1. In a first step, the Boc-protected spacer and branching element **2** was coupled with the (C-6)-monoamino-βCD **1** and, after hydrolysis of the carbamate groups in the adduct **3** ($\rightarrow$**4**), the external mannosyl markers (**5**) were incorporated. The coupling yields are high (75–85%) and purification, which is a critical point when dealing with this type of macromolecular compounds, can by easily accomplished by simple column chromatography at the hemi-acetylated stage. After deacetylation, the dendritic conjugate **6** was obtained in pure form and >95% yield. Typically, the final compounds were subjected to gel permeation chromatography to obtain analytically pure samples that were used for the biological evaluation studies. We have also explored the convergent approach by first preparing the isothiocyanate-armed glycodendrons and performing the coupling reaction with the C-6-monoamino-βCD as the final step. The overall yields are practically identical for both approaches.



Scheme 1. An example of divergent synthesis of a monosubstituted hexavalent mannosyl-βCD conjugate **6**.

The dendritic presentation of saccharide ligands was found to be extremely efficient in lectin binding studies using the tetrameric plant lectin concanavalin A (Con A), a mannose specific lectin used as a model receptor. For instance, the above hexavalent conjugate **6** exhibited a Con A binding efficiency 80-fold higher than the corresponding hepta-(C-6)-(α-D-mannopyranosylthioureido)-βCD conjugate in enzyme-linked lectin affinity (ELLA) studies. It was also an excellent encapsulating agent for the anticancer drug docetaxel (Taxotere®, **7**), used extensively and efficiently in the treatment of breast cancer among others, solubilizing up to 4 gL^{-1} of drug in water at 25 mM concentration. This means an increase of more than 1000-fold as compared with the solubility of the drug in the absence of any solubilizing agent. We also confirmed, by using peritoneal macrophages from mouse, that the complex could be specifically delivered to the mannose/fucose specific receptor at the surface of these cells [51].

In the course of the above experiments, we observed a certain tendency for the formation of 2:1 βCD dendrimer–docetaxel complexes, which could be expected considering that the host molecule contains two aromatic rings that are susceptible of inclusion in the CD nanocavity. We anticipated that properly designed dimeric βCD conjugates that could interact simultaneously with these two functional subunits, would be still more efficient as taxane carriers. The new objective was to prepare dual-cavity derivatives which could bear the multivalent ligand, building up the whole architecture from relatively simple building blocks that would be linked together through thiourea bridges (Figure 5). After some molecular modeling, a derivative of tris(2-aminoethylamine) (TREN) was selected as bifunctional branching element, since it provides the right length to expand the distance between the two key aromatic rings in the guest. As a matter of fact, an extremely high association constant ($>10^6$ M^{-1}) was inferred from solubility experiments for this new type of dimeric βCD conjugates (e.g. **8**). The whole system, incorporating the mannosyl dendrons, was very efficiently recognized by specific mannose receptors, including those on macrophage cells [51, 53].

Noteworthy, in both the CD- and the dimeric CD-glycodendrimer conjugates, the native CD is chemically

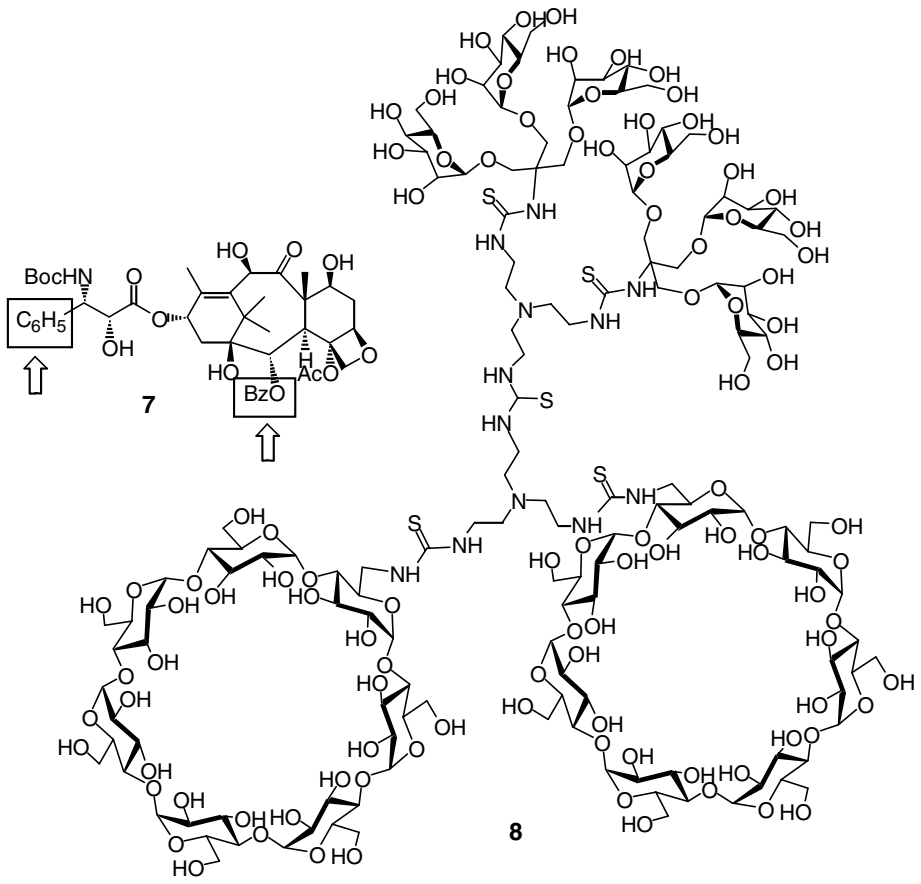
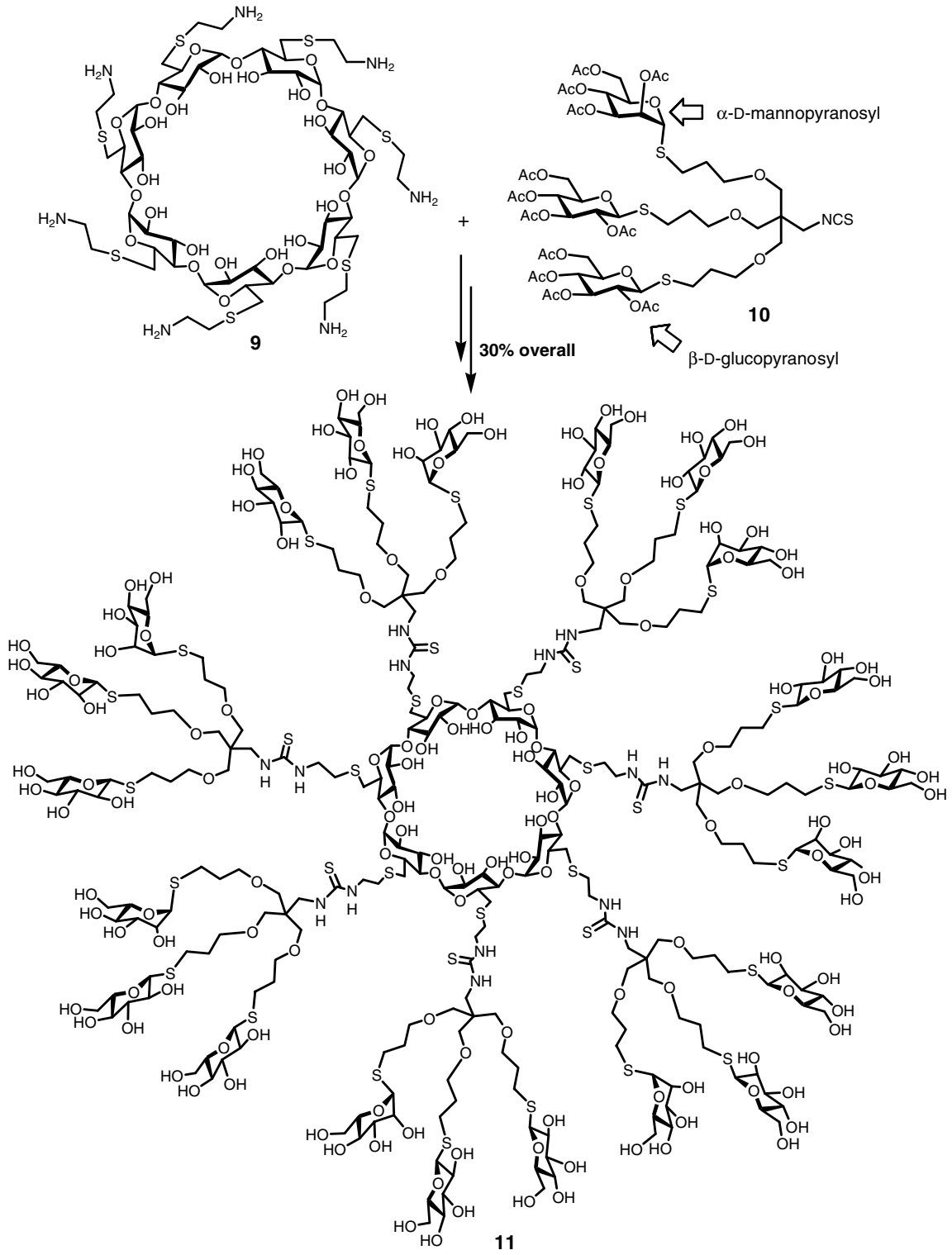


Figure 5. Structures of docetaxel (Taxotère®, **7**) and of the hexavalent mannosyl-CD dimmer conjugate **8**.

modified endowing it with biorecognition capabilities and tuning its inclusion properties to fit to specific guests. The overall topology of the CD glyconanocavity remains, however, unchanged. The possibility of effecting face-selective functionalization offers also a unique opportunity for using cyclodextrins as nanometric scaffolds to build new jelly-fish-like nanometric architectures. The grafting of hyperbranched glycotentacles to the narrower rim will create a new, more flexible cavity at the top of the truncated cone that might host an eventual guest or, eventually, favor or disfavor the entrance of a given guest in the rigid cavity. Moreover, this type of systems would constitute excellent nanometric mimics of the glycocalix cell membrane region where specific sugar epitopes are overexpressed, allowing the study of recognition phenomena involving highly dense, space-oriented glycoclusters.

To access hyperbranched CDs through the thiourea-forming approach, we first tried the use of the per(C-6)amine derivative, which is accessible in three steps from βCD through per(C-6)halogenation, as the heptanucleophile. However, we found that the reaction with isothiocyanate-armed glycodendrons was incomplete, resulting in a complex mixture of undersubstituted compounds. To avoid these steric problems, we decided to introduce a cysteaminyl spacer. The resulting per(C-6)-2′-aminoethylthio-βCD **9** can be prepared in just two steps from βCD, with no need for chromatographic purification, by nucleophilic displacement on a per(C-6)halogeno derivative using commercial cysteamine hydrochloride. Moreover, this compound proved to be an excellent heptanucleophile [54].

Following the above concept, we have prepared a variety of homogeneous as well as heterogeneous hyperbranched CDs by reaction of the heptacysteaminyl βCD **9** and isothiocyanate-armed glycodendrons (e.g. **10**, Scheme 2). The relative proportions of the different sugar epitopes (α-D-mannopyranosyl, β-D-glucopyranosyl, β-lactosyl) could be controlled by judicious design of the glycodendron structure. Noteworthy, the mixed hyperbranched conjugates constitute the first model of a heterogeneous region of the glycocalix. By examining the binding affinity toward the protein receptors of a series of compounds of this type, we discovered a new biological effect that involves synergetic interactions between different sugars. Thus, comparison of the IC_{50} value for the inhibition of the association between yeast mannan and Con A by a hyperbranched βCD bearing seven α-D-mannopyranosyl residues and 14 β-D-glucopyranosyl substituents with the corresponding value for an isosteric hepta-(α-D-mannopyranosyl) derivative, indicated an about sixfold affinity enhancement on a mannose molar basis. A similar situation was encountered when comparing a 14-valent α-D-mannopyranosyl-βCD and a mixed hyperbranched derivative bearing, in addition, seven β-D-glucopyranosyl substituents, a



Scheme 2. Synthesis of a hyperbranched βCD derivative (**11**) incorporating 7 α-D-mannopyranosyl and 14 β-D-glucopyranosyl thiourea linked substituents.

striking feature taking into account that β-D-glucopyranosides are not ligands for Con A. The origin and biological significance of this heterocluster effect is still unclear. Nevertheless, isothermal titration microcalorimetry experiments indicated a partial enthalpy–entropy compensation considering the mannose homoclusters and heteroclusters with increasing glucose proportions, which is compatible with a sliding mechanism involving a higher mobility of the mixed hypervalent derivatives over the lectin binding sites [55].

Trehalose-based glyconanocavities

In our search for new third-generation CDs, we considered the possibility of modification of the size and structure of the cavity of the cyclodextrin core. In fact,

several chemical transformations of CDs resulting in modifications of the shape and size of the cavity, by epimerization of some hydroxyl groups, incorporation of new functionalities or inversion of the chair conformation of the glucosyl subunits, for instance, have been reported [56, 74]. The taylor-made, de novo synthesis of glyconanocavities is, however, a far more complicated challenge [75].

The basic structure of cyclomaltooligosaccharides involves the disaccharide maltose. Consequently, the interior of the convex cavity exposes the α-face of the monosaccharide constituents, that is, the H-3 and H-5 methine protons, thus providing hydrophobicity to the internal wall. After formation of a supramolecular complex, we conventionally obtain informations on the interaction of the included guest with the α-face of the monosaccharide constituents. The β-face remains on the dark screen, in contact with the bulk solvent. All the reported attempts to synthesize glyconanocavities follow this general scheme. However, the β-face of the monosaccharide constituents is also hydrophobic, exposing the H-1, H-2 and H-4 methine protons. In principle, it would be conceivable to prepare hydrophobic glyconanocavities with this face directed to the inside of a convex cavity (''reverse cyclodextrins'') for instance by closing the ring through the primary positions.

In order to be able to confirm the above hypothesis, some prior considerations are important. At first, the interglycosidic linkages must be rigid enough to prevent rotation, in order to preserve the convex-type cavity structure. Secondly, the resulting glyconanocavities should keep a high degree of symmetry to facilitate both the synthetic scheme and further inclusion studies. On such basis, we focused on α,α-trehalose (**16**) as building block, since it is a C_2 symmetric disaccharide having a very rigid structure which is anchored by dual anomeric as well as exoanomeric effects. If we connect α,α-trehalose units through their primary positions, we should generate symmetric convex glyconanocavities (cyclotrehalans, CTs) [45] with the β-face of the constituent α-D-glucopyranosyl directed toward the inside, provided that the rigid structure of the disaccharide segments is conserved.

Our first target molecule was a pseudocyclotetrasaccharide formed from two α,α-trehalose subunits linked through their primary positions through thiourea bridges (**12**; Figure 6). The retrosynthetic scheme is rather simple and would involve the coupling reaction of a (C-6)-diisothiocyanate (**13**) and a (C-6)-diamine (**15**). Introducing such functionalities onto the primary carbons of the disaccharide is relatively straightforward *via* the corresponding (C-6)-diiodo and (C-6)-diazido derivatives [57]. Although the fully unprotected diisothiocyanate **13** was stable in the absence of base, it underwent base-catalysed intramolecular cyclization in the presence of an amine to give an intramolecular bis(carbamate). Nevertheless, after hydroxyl protection (e.g. **14)** the macrocyclization reaction took place in

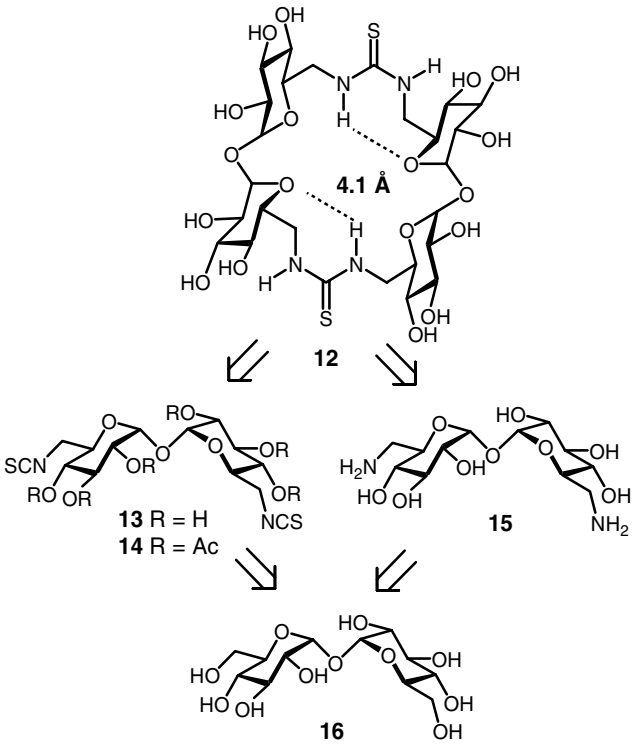


Figure 6. Retrosynthetic scheme for the dimeric cyclotrehalan CT_2 (**12**). The dimensions of the cavity, as determined by molecular modeling, as well as the intramolecular H-bonds, are indicated.

reasonable yield to give the target dimeric cyclotrehalan **12** (CT_2) [58].

Unfortunately, the cavity of CT_2 (4.1 Å) was too small to allow the formation of inclusion complexes with other organic molecules. In fact, the cavity was collapsed by the existence of two intramolecular hydrogen bonds involving two alternate, inside-directed thiourea NH groups. Consistently, the O-peracetylated CT_2 was shown to form hydrogen-bond complexes with carboxylate anions in apolar solvents using exclusively the outside-directed NH protons [59]. We could just observe the formation of weak complexes with mono- and divalent cations by thin layer ligand-exchange chromatography. The only cations that formed strong complexes were the thiophilic cations copper (II) and silver (I), that probably are not included inside the cavity [58].

It became evident from the above results that probing the convex glyconanocavity concept would imply the synthesis of higher CT homologues. Yet, going from the dimer (CT_2) to the trimer (CT_3) means that, at a moment in the synthetic scheme, it will be necessary to break the symmetry of the trehalose molecule, placing a potential amino group onto one of the primary positions and a potential isothiocyanate group at the other one. Preliminary attempts, involving statistic protection of a single amino group in the (C-6)-diamine **15** as the corresponding mono-Boc derivative, required several steps with rather low overall yields. To overcome this problem, the auto-condensation reaction of isothiocyanates to give symmetric thioureas was used. This is a very efficient reaction that allows generation of an intersac-

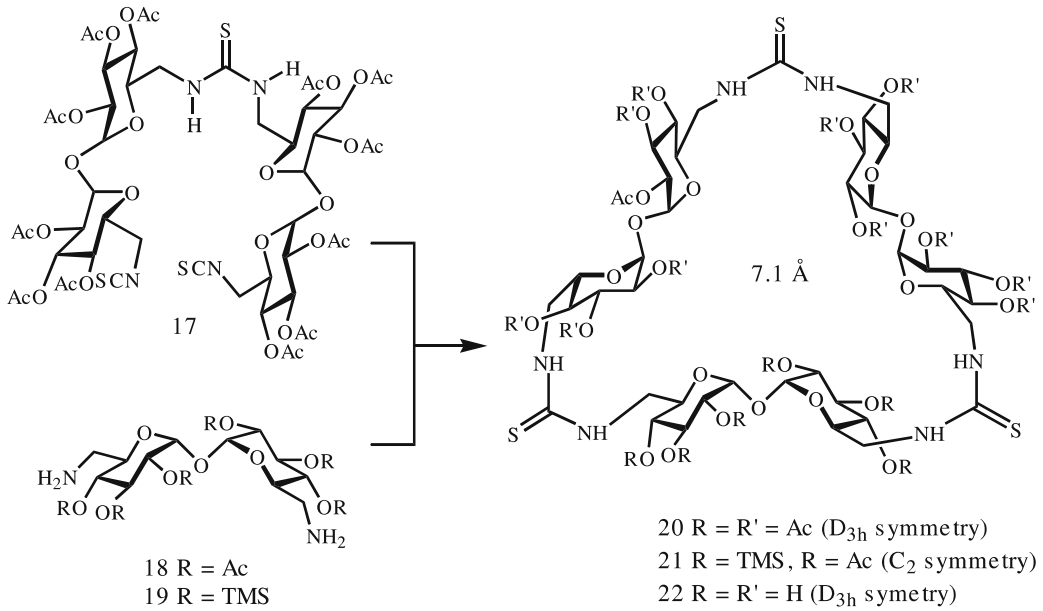
charide thiourea bridge without participation of any even transient amine nucleophile [60] avoiding, for instance, side problems such as migration of protecting groups. When it was applied to the peracetylated (C-6)-diisothiocyanate **14**, the O-peracetylated derivative of the dimeric cyclotrehalan **12** was obtained in a single step (35% yield). But most importantly, the reaction could be stopped at the asymmetric linear tetrasaccharide **17** (45% yield), which is just the precursor needed for the preparation of higher homologues. In this compound, the two α,α-trehalose subunits have been already desymmetrized [61].

When the linear peracetylated diisothiocyanate **17** was coupled to the O-peracetylated (C-6)-diamine **18**, a cyclohexasaccharide having D_3 symmetry (**20**) was obtained. The macrocyclization yield was somehow diminished due to O → N acetyl migration products in the diamine precursor (25% yield). The macrocyclic compound possesses a C_3 axis of symmetry perpendicular to the main plane of the nanocavity and three C_2 axes in the plane, making all the six glucose subunits equivalents. Consequently, the NMR spectra showed a single spin system. When a diamine having trimethylsilyl O-protecting groups (**19**) was employed, the symmetry of the resulting macrocycle (**21**), obtained in 70% yield, decreased to a C_2 axis. Three different spin systems were now identified. NOE experiments fully confirmed that the α,α-trehalose moieties kept the conformation dictated by the exoanomeric effect and that, in agreement with our initial hypothesis, we are in the presence of a true convex glyconanocavity. After complete deprotection (→**22**), the six monosaccharide subunits became again equivalent, similarly to the case of αCD (Scheme 3) [61].

The overall shape of CT_3 exhibits remarkable similarities with that of α, β and γCD. Thus, the geometry also corresponds to a truncated cone structure. But the situation is like if we turn out completely a cyclodextrin as a glove finger, so that the outside becomes the inside. Now, H-3 and H-5 protons are pointing outside while H-1, H-2 and H-4 point to the inside. The hydroxyl groups are limiting the two rims of the cavity while the inside is comparatively apolar. In addition, to the six equivalent glucopyranosyl subunits, the macrocycle incorporates three thiourea groups that can adopt *Z,Z* or *Z,E* rotameric arrangements, thus imparting a restricted flexibility to the structure while, however, inducing no substantial modification of the overall shape and size of the nanocavity. Both the NH and the CS groups could, eventually, provide additional interactions with an included guest. The dimensions of the cavity (7.1 Å mean internal diameter) are intermediate between that of α- (5.1 Å) and β-cyclodextrin (7.8 Å). In principle, one could expect that hydrophobic guest molecules that fit in the α or βCD cavity should also fit in the cyclotrehalan trimer cavity.

A preliminary docking study with benzoic acid as a guest molecule showed that it fits, in principle, in the cavity of CT_3. According to these calculations, the carboxylate group should stay solvated pointing to the wider rim of the cone. We have carried out titration experiments that have confirmed the formation of a 1:1 complex with an association constant K_{as} 8–9 M^{-1}, which is of the same order of magnitude as reported for α and βCDs (K_{as} 10–11 M^{-1}). The ROESY spectra showed intermolecular cross peaks between the aromatic protons and the H-1, H-2 and H-4 protons of the α,α-trehalose subunits (Figure 7) which is in agreement with the complex structure predicted from calculations and fully confirms the convex nature of the cavity. Therefore, we are really obtaining information on the "dark side" of cyclodextrins with this particular cyclotrehalan.

We have further undertaken a systematic study of the supramolecular properties of this new family of



Scheme 3. Synthesis of trimeric cyclotrehalans (CT_3).

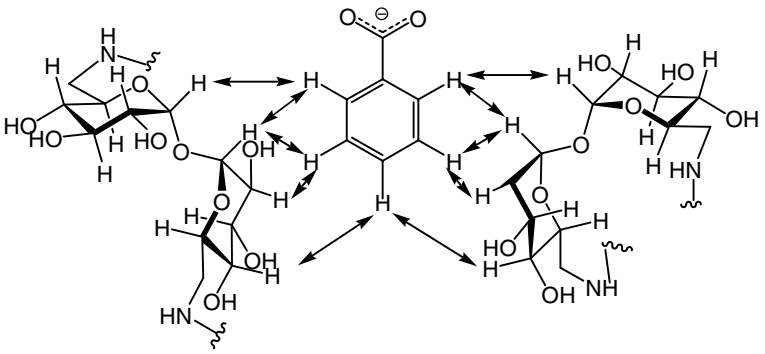


Figure 7. Diagnostic ROESY contacts between CT_3 (**22**) and benzoate in D_2O. The thiourea segments and one of α,α-trehalose subunits are omitted for sake of clarity.

cyclooligosaccharide receptors in comparison with classical CDs. A much higher association constant has been measured for the 1:1 CT_3:naphthalene-2-sulfonate complex (K_{as} 235 ± 15 M^{-1}), probably due to a deeper inclusion of the guest into the cavity in the longitudinal direction. The measured K_{as} value (235 ± 15 M^{-1}) seemed to indicate a much closer analogy with αCD (K_{as} 363 ± 15 M^{-1}) as compared to βCD (K_{as} 2.3×10^5 M^{-1} for the corresponding 1:1 complex with the same guest). In sharp contrast, an extremely efficient complexation was observed for adamantane 1-carboxylate, with a K_{as} value (4.6×10^4 M^{-1}) much closer to that reported for βCD (K_{as} 3.9×10^4 M^{-1}) than for αCD (K_{as} 141 ± 15 M^{-1}). This result is probably the consequence of a perfect size-matching as well as a ternary symmetrical complementarity, according to computational calculations [61].

Conclusions and perspectives

Carbohydrates are, by far, the biomolecules with the highest encoding capacity, much higher as compared with proteins and nucleic acids. Nature already makes use of this biological information storage potential to regulate cell differentiation, cell state and cell communication. Organizing sugar-encoded information in nanometer-scale molecular or supramolecular devices represents a biomimetic approach that is already producing a leap forward in the glycosciences. Among nanometric glycoarchitectures, glyconanocavities provide, probably, the highest degree of organization. In addition to the possibility of incorporation of space-oriented substituents through rim-selective functionalization as well as regioselective transformation of hydroxyl groups, they provide an intrinsic differentiation between the inner and the outer space. The important body of work already accumulated with first-, second- and third-generation cyclodextrins sufficiently illustrates the possibilities of these macrocyclic oligosaccharides both in fundamental studies as well as for commercial uses. Fourth-generation CDs, incorporating the capacity of self-assembling to form larger nanostructures, are already under development, with promising applications in drug delivery and gene transfection, among others. The current investigations in the synthesis of tailor-made glyconanocavities, such as cyclotrehalans, should broaden the range of possible applications in such fields like artificial enzymes and catalysis [76], up to now somehow hampered by the straight-jacket format imposed by the available CD cavity sizes.

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