Binding Affinity Properties of Dendritic Glycosides Based on a β-Cyclodextrin Core toward Guest Molecules and Concanavalin A

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The inclusion behavior and concanavalin A binding properties of hepta-antennated and newly synthesized tetradeca-antennated C-6-branched mannopyranosyl and glucopyrannosyl cyclomaltoheptaose (β-cyclodextrin) derivatives have been evaluated by isothermal titration microcalorimetry and enzyme-linked lectin assay (ELLA), respectively. The synthesis of three first-order dendrimers based on a β-cyclodextrin core containing 14 1-thio-β-D-glucose, 1-thio-β-mannose, and 1-thio-β-rhamnose residues was performed following a convergent approach and involving (1) preparation of a thiolated bis-branched glycoside building block and (2) attachment of the building block onto heptakis(6-deoxy-6-iodo)-β-cyclodextrin. Calorimetric titrations performed at 25 °C in buffered aqueous solution (pH 7.4) gave the affinity constants and the thermodynamic parameters for the inclusion complex formation of these β-cyclodextrin derivatives with guests sodium 8-anilino-1-naphthalensulfonate (ANS) and 2-naphthalenesulfonate. The host capability of the persubstituted β-cyclodextrins decreased with respect to the native β-CD when sodium 2-naphthalenesulfonate was used as a guest and improved when ANS was used as a guest molecule. Heptavalent mannoclusters based on β-CD cores enhance the lectin binding affinity due to the cluster effect; however, the increase of the valency from 7 to 14 ligands did not contribute to the improvement of the concanavalin A binding affinity. In addition, the synthesized hyperbranched mannoCDs lost completely the capability as a host molecules.

Introduction

Cyclodextrins (CDs) are cyclomaltooligosaccharides formed by six (α-CD), seven (β-CD), and eight (γ-CD) α-(1→4)-D-glucopyranosyl units, respectively.[1,2] These compounds and their derivatives are of great interest for different scientific and industrial fields due to their well-known ability to include hydrophobic molecules forming host/guest complexes. One of their applications is the use of these host/guest complexes as drug delivery systems, although most drug-CDs complexes exhibit very little site specificity as they lack biologically recognizable sites.[3] To develop CD drug carrier systems that can selectively deliver drugs to their sites of action within the organism, the attachment of bio-recognizable saccharides onto CDs has been addressed as a targeting method.[3b,4]

The structural features of CDs make these macrocycles adequate candidates to function as scaffolds of bioactive multivalent systems. It can be argued that this feature would allow the use of the so-called cluster effect[5] as a means to increase the protein-saccharide binding,[6] and therefore, it could improve the effectiveness of the drug delivery system. Most of the saccharide-branched CDs synthesized chemically or chemoenzymatically are monosubstituted derivatives at the primary position of the CDs in which simple sugars as well as disaccharides or oligosaccharides have been bound either directly or via a spacer arm.[4,7] Since the first reports on the syntheses of perthiogalactosylated[4a,d] and perthioglucosylated β-CD[4b] several other synthesis have been reported recently, such as the preparation of per-(C-6)-substituted branched glucosylthioureido-cyclomaltoheptaose derivatives[8] and,

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more recently, per-glycopyranosylated β-CD having the attached glycopyranosides onto the primary face[9] and both the primary and the secondary faces simultaneously.[10] Alternatively, the synthesis of monosubstituted multivalent CDs bearing glycodendrimers has been also reported.[11]

In line with this research, we have described[12] the synthesis of perglycosylated CDs having a variety of monosaccharidic units, mainly as *O*-glycosides, *S*-glycosides and glycopyranosylamines, grafted onto the primary face of a β-CD core by means of different spacer arms. We also have demonstrated the ability of some of these compounds to interact with plant lectins with enhanced affinity due to the multivalent effect.[12a]

Working on the same concept, other research groups have reported the synthesis cluster glycosides based on different cores such as calixarenes[13] and calix[4]resorcarenes.[14] In particular, Aoyama and co-workers[14] have demonstrated that the latter type of compounds can deliver guest molecules to polar solid surfaces such as quartz, but also to biological targets such as lectins.

The use of CDs as a scaffold of cluster glycosides offers several advantages over other macrocyclic compounds: it is more available and affordable, more biocompatible, and has the ability to form inclusion compounds with a large variety of guests in aqueous solution.[3]

In this paper, we evaluate a series of saccharide-clustered CDs for their binding properties toward some representative guest molecules and toward the plant lectin from *Canavalia ensiformis* (concanavalin A, Con A). For these purposes, we performed the synthesis and characterization of first-order manno- and glucodendrimers based on a β-CD core. The inclusion complexation behavior of those glyco CDs was studied by using titration microcalorimetry to obtain information of the consequences of the branching and hyperbranching of β-CD with saccharides upon complexation. Similarly, ELLA assays allowed us obtain information about the biorecognizable properties of the glycoCDs.

Results and Discussion

Synthesis. The synthesis of the hyperbranched CDs was carried out by first constructing the glycodendrons, followed by their coupling to the key template heptakis 6-deoxy-6-iodo-β-CD derivative[15] (**1**) (Schemes 1 and 2). The branching structure was derived from 3,3′-iminobispropylamine, which was converted into the bis(carbobenzyloxy) derivative **2** as reported.[6a] Protection of the secondary amine of **2** with di-*tert*-butyl dicarbonate provided the *N*-Boc derivative **3**, which was then subjected to hydrogenolysis of the *N*-Cbz group, followed by treatment with chloroacetic anhydride and triethylamine to give the *N*-chloroacetylated derivative **4** in 80% yield overall. Coupling of the β-D-glucopyranoside, α-D-mannopyranoside, and α-L-rhamnopyranoside derivatives was performed by the reaction of **4** with the isothiouronium salt **5**,[16a] and the thiols **6**[16b] and **7**,[16c] respectively, in the presence of Cs_2CO_3 in dimethylformamide (DMF) at room temperature.

Under these conditions, the bis-branched glycosides **8**−**10** were obtained in 97%, 87%, and 91% yield, respectively. Removal of the Boc-*N* protecting group in **8**−**10** using TFA in CH_2Cl_2 followed by treatment with chloroacetic anhydride in MeOH afforded **11**−**13** in 87−97% yield. Subsequent treatment of the *N*-chloroacetyl derivatives **11**−**13** with thiourea and sodium sulfite gave the thiols **14**−**16** (52%, 66%, and 70% yield, respectively). The attachment of the dendrons **14**−**16** to the CD core was accomplished by reaction of thiols **14**−**16** with per-6-deoxy-6-iodo-β-CD (**1**) in the presence Cs_2CO_3 at 60 °C in dry DMF (Scheme 2). The products were isolated as the corresponding peracetylated derivatives **17**−**19**, obtained by conventional acetylation (Ac_2O, Py, DMAP), in 70%, 88%, and 83% yield, respectively. Zemplén de-*O*-acetylation of **17**−**19** afforded the free-glycoside-containing 14-mer **20**−**22** in 80−93% yield.

Hyperbranched glycoCDs **17**−**22** were satisfactorily characterized by NMR spectroscopic techniques with COSY, HMQC, and selective TOCSY experiments and MALDI-TOF mass spectrometry. Measurements of the NMR data were performed at 80−100 °C to avoid broadening of the signals and to improve the resolution of the spectra. The NMR spectra show a single signal pattern for all saccharide residues. The ratios of the integrals for the signals of the saccharide residue protons and for the signals of those belonging to the CD core are in accordance with the structures of the products. The

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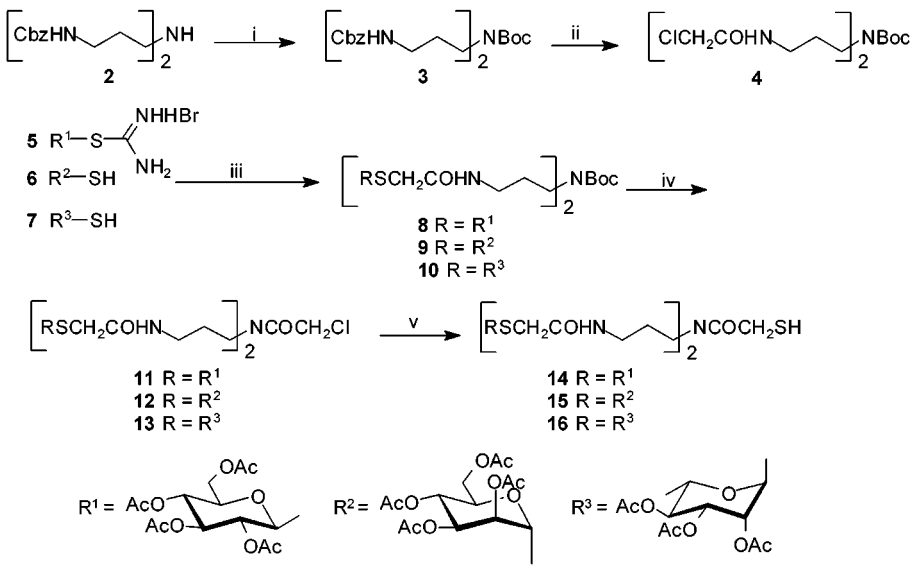
Scheme 1. Synthesis of the Bis-glycosyl Thiols[a]



[a] Key: (i) Et_3N, $((CH_3)_3CO)_2O$, CH_3CN, 12 h, rt; (ii) (a) Pd/C, MeOH, H_2 (2.5 atm), 1 h, (b) Et_3N, $(ClCH_2CO)_2O$, CH_3CN, 24 h, rt; (iii) Cs_2CO_3, DMF, 24 h, rt; (iv) (a) TFA, CH_2Cl_2, 2−3 h, 0 °C, (b) DIPEA, $(ClCH_2CO)_2O$, CH_3CN, 6−7 h, rt; (v) (a) $(NH_2)CS$, $(CH_3)_2CO$, 12 h, rt; (b) Na_2SO_3, H_2O, 30 min, rt.

^{13}C NMR spectra show two anomeric carbon signals at 96.3−102.8 ppm (C-1) and 81.1−85.6 ppm (C-1″) corresponding to the CD core and the sugar residues, respectively.

Inclusion Complexation Capability. We studied the inclusion complexation behavior of these CD-based glycodendrimers **20**−**22** together with the heptakis glycoCDs **23**−**30**[12a,b] (Chart 1) using sodium 8-anilino-1-naphthalensulfonate (ANS) (**31**) and sodium 2-naphthalensulfonate (**32**) as guest molecules by using isothermal titration calorimetry (ITC). The obtained data were assessed by comparison with those obtained for native *β*-CD using the same guest molecules.[17] The thermodynamic data are listed in Table 1. ITC measurements provide direct determination of *n*, the stoichiometry, $\Delta H°$, the enthalpy of binding, and *K*, the binding constant. From measurements of *K*, the free energy of binding, $\Delta G°$, can be calculated and, hence, the entropy of binding, $\Delta S°$, determined. In these experiments, the soluble guest molecule is titrated into a solution-containing CD derivative and the heat released during binding is measured as a function of the guest/host molar ratio (Figure 1). The best fit for the three variables *n*, $\Delta H°$, and *K* was found for 1:1 stoichiometry in accordance with the most commonly claimed stoichiometry ratio for CD complexes.[18] The forces responsible for the formation of the CD complexes involve a number of different contributions from a wide variety of weak interactions,[18] but the van der Waals and the hydrophobic interactions are considered to be the most important.[17a] In previous thermodynamic studies of molecular recognition by mono modified CDs, it was shown the influence of the relative size between the cavity of the CD and the guest molecule, as well as the shape, dipole, charge and functional group of the branch attached to the primary face edge of the CD in determing how the guest molecule fits into the host cavity.[17] In our case, the structure of the branched CD **20**−**30** is the result of the substitution the OH-6 of every single glucose unit by bulky groups separated from the CD cavity edge by spacer arms of different length. The *β*-CD persubstitution may affect the conformation of the oligosaccharide ring and therefore modify the overall shape of the CD cavity. In addition, the spacer arms could influence the steric congestion at the narrower rim depending on their length, but also on the microenviromental hydrophobicity at the primary face, depending on their lipophilic pattern.

From Table 1 and Figure 2, it can be seen that we obtained higher binding constants for the hosts heptakis *β*-GlcNHCOCH$_2$SCD **25**, *β*-GlcNHCOCH$_2$SCH$_2$CONHCD **26**, α-ManNHCOCH$_2$SCD **29**, and α-ManNHCOCH$_2$SCH$_2$CONHCD **30** than for native *β*-CD when ANS (**31**) was used as a guest. However, we did not observe complexation between the guest ANS (**31**) and the hyperbranched glycoCDs **21** and **22**, both bearing 14 glycoside residues on the primary face of the CD.

The binding heat for complexes formed by ANS (**31**) and glycoCDs **25**, **26**, **29**, and **30** is exothermic. However, while for the *β*-GlcNHCOCH$_2$SCD **25** and α-ManNHCOCH$_2$SCD **29**, with a shorter spacer arm, the complexation is enthalpy driven, for *β*-GlcNHCOCH$_2$SCH$_2$CONHCD **26** and α-ManNHCOCH$_2$SCH$_2$CONHCD **30**, with a longer spacer arm, the complexation is entropy driven. When compared with the complexation of guest **31** with native *β*-CD, the persubstitution on the primary face, as occurs for **25** and **29**, led to a slight increase of the enthalpic gain of 0.27 and 1.18 kcal mol^{-1}, respectively, as well as an almost negligible increase of the $T\Delta S°$ value (0.9 and 0.1 kcal mol^{-1}, respectively). These data suggest a deeper penetration of the anilino group[19] into the CD cavity and a concomitant efficient dehydratation of the cavity upon complexation. In the case of hosts **26** and **30**, possessing seven GlyNHCOCH$_2$SCH$_2$NH groups, the higher affinity

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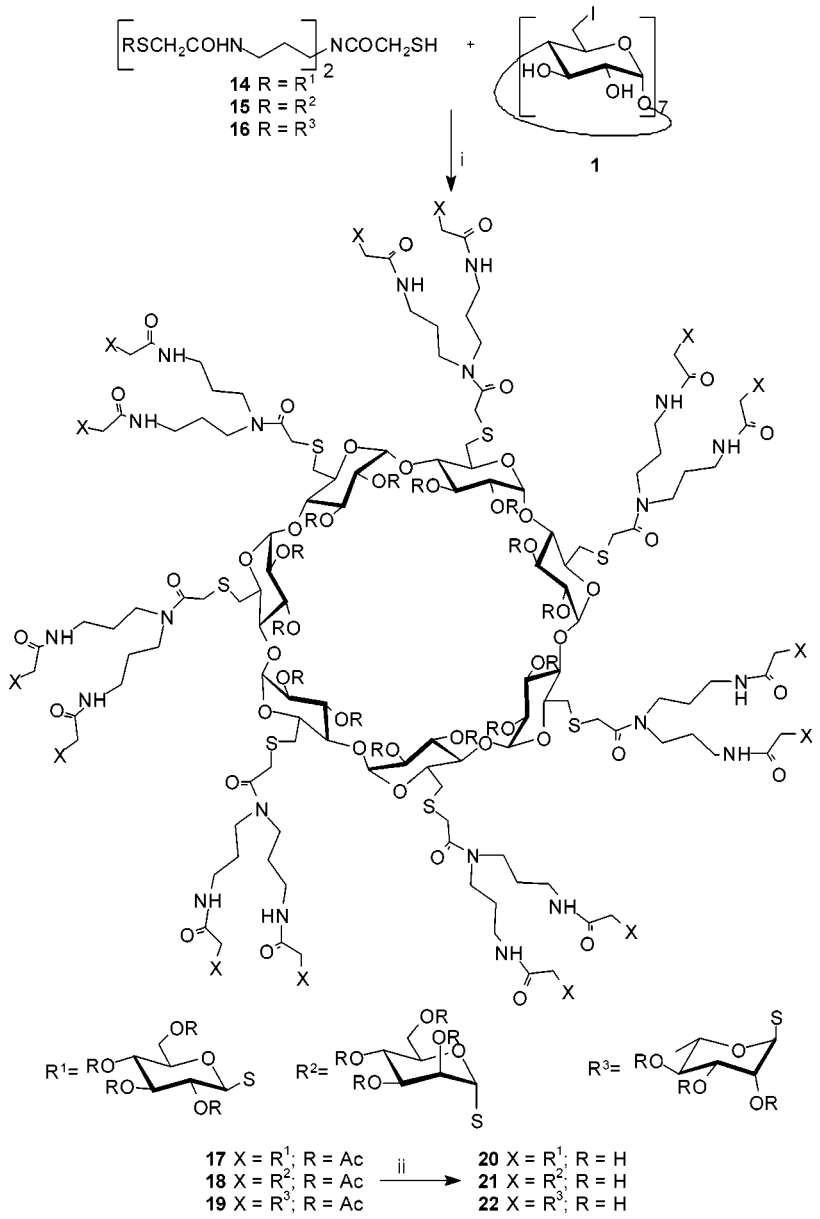
Scheme 2. Synthesis of Tetradeca-antennated C-6 Branched Glycosyl CDs 20−22[a]



[a] Key: (i) (a) Cs_2CO_3, DMF, 7 d, 60 °C, (b) Ac_2O, Py, 48 h, 40 °C; (ii) NaOMe, MeOH, 12 h, rt.

with **31**, when compared with the binding of **31** to the native β-CD, is caused by an increase of the $T\Delta S°$ value of 1.89 and 1.48 kcal mol^{-1}, while the enthalpy change remains almost unchanged but with tendency to be less favorable than that for the native β-CD. This suggests a more extensive desolvation of the hosts due to the presence of $GlyNHCOCH_2SCH_2NH$ groups on C-6 upon penetration of the anilino group into the cavity.

From Table 1 and Figure 3 it can be seen that the inclusion complexation of 2-naphthalenesulfonate (**32**) with native β-CD is very strong. It is an enthalpy-driven complex with minimal entropic gain.[17b] As previously reported by Inoue et al.[17c] for monomodified CDs, heptakis glycoCDs **24**−**30** afforded substantially less stable complexes with the guest **32** than the native β-CD. In addition, we did not detect the binding of **32** to heptakis β-GlcSCD **23** and to the glycoside-containing 14-mer CDs **20**−**22**. Previous studies[17c,20] have shown that monomodified β-CDs with functional groups on the narrower rim can undergo self-inclusion processes preventing guest inclusion. In our case, NMR spectra of these CDs did not provide conclusive information in such respect, although the broadening of the signals observed in some cases when the NMR experiments were performed at room temperature could be indicative of a self-inclusion process. In addition, steric factors on the primary face may induce a narrowing of the secondary side of the torus so preventing guest penetration. All the complexes formed by host **24**−**30** and guest **32** are exothermic and enthalpy-driven with the exception of **30**, which is entropy-driven. When compared with the complexation of **32** and native β-CD, the persubstitution on the primary face of the CD with the glycosyl residues led to a decrease of the enthalpic gain up to 2.00−5.04 kcal mol^{-1} for **24**−**30**, suggesting a lesser penetration of the naphthalene part of **32** into the CD cavity. In contrast, an increase of the

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Chart 1. Hepta-antennated C-6 Branched Glycosyl β-Cyclodextrins 23−30

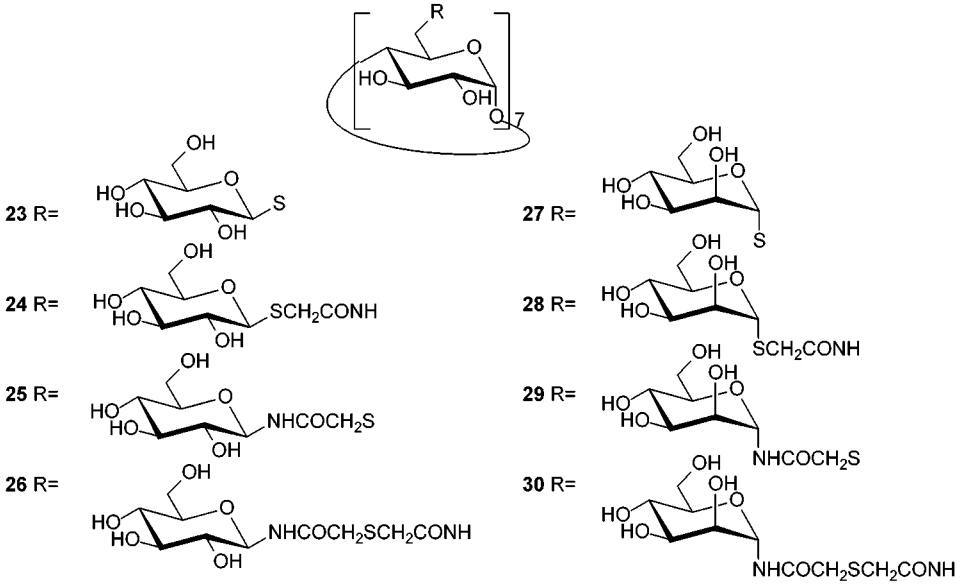

Table 1. Thermodynamics of Binding of Guests 31 and 32 to CDs 20−30 in Buffered Aqueous Solution (pH 7.4) at 25 °C

host	guest	$K \times 10^{-3}$ (M^{-1})	$-\Delta G°$ (kcal mol^{-1})	$-\Delta H°$ (kcal mol^{-1})	$T\Delta S°$ (kcal mol^{-1})
β-CD	**31**	0.11	2.70	1.79	0.93
21	**31**	N.B.[a]			
22	**31**	N.B.			
25	**31**	0.68 ± 0.06	3.86 ± 0.08	2.06 ± 0.10	1.80 ± 0.13
26	**31**	0.90 ± 0.21	4.03 ± 1.85	1.21 ± 0.17	2.82 ± 1.86
29	**31**	0.86 ± 0.21	3.99 ± 1.01	2.97 ± 0.43	1.03 ± 1.10
30	**31**	0.72 ± 0.17	3.90 ± 0.08	1.53 ± 0.20	2.41 ± 0.22
β-CD	**32**	234.42	7.33	7.00	0.31
20	**32**	N.B.			
21	**32**	N.B.			
22	**32**	N.B.			
23	**32**	N.B.			
24	**32**	1.32 ± 0.08	4.26 ± 0.07	2.29 ± 0,07	1.97 ± 0.11
25	**32**	1.86 ± 0.12	4.46 ± 0.10	3.02 ± 0.09	1.44 ± 0.14
26	**32**	0.67 ± 0.04	3.86 ± 0.01	2.84 ± 0.11	1.02 ± 0.11
27	**32**	2.53 ± 0.15	4.64 ± 0.24	5.00 ± 139	−0.35 ± 0.27
28	**32**	0.63 ± 0.02	3.82 ± 0.02	1.96 ± 0.03	1.86 ± 0.04
29	**32**	6.61 ± 0.16	5.21 ± 0.03	3.61 ± 0.04	1.60 ± 0.05
30	**32**	1.96 ± 0.07	4.49 ± 0.10	2.00 ± 0,03	2.50 ± 0.11

[a] N.B. = no binding.

$T\Delta S°$ value up to 0.71−2.19 kcal mol^{-1} for **24**−**26** and **28**−**30** is observed with respect to that value for the binding of **32** to β-CD, indicating a more extensive desolvation of the cavities of the persubstituted CDs. Comparing hosts having the same glycosyl residue, guest **32** forms stronger complexes with hosts having sulfur at C-6. In those cases, such as for β-GlcNHCOCH$_2$SCD **25** and α-ManNHCOCH$_2$SCD **29**, the latter with the highest affinity for **32** of the series ($K = 6.61 \times 10^3$ M^{-1}), higher enthalpic gain ($-\Delta H°$ 3.02 and 3.61 kcal mol^{-1}, respectively) can be observed than that for those having nitrogen at C-6 ($-\Delta H°$ 1.96−2.84 kcal mol^{-1} for **24**, **26**, **28**, and **30**). This may be attributable to the fact that the presence of the sulfur atom at C-6 allows a deeper inclusion of the naphthalene group of **32** than that allowed by the presence of the nitrogen atom at C-6. The opposite complexation behavior of the two heptakis GlySCD **23** and **27** in which the glycosyl residues are bound directly to C-6 through a sulfur atom is noteworthy. As mentioned above, while β-GlcSCD **23** does not form complex with guest **32**, α-ManSCD **27** has the second highest stability constant ($K = 2.5 \times 10^3$ M^{-1}) of the series of glycoCDs. The different configurations of the carbons C-1″ and C-2″ of the glycosyl residues is the only structural difference between both compounds **23** and **27**. Previous reports have shown the less solubilization power of mono *S*-β-glucosylated β-CD when compared with the mono *S*-α-glucosylated β-CD measured for certain guests.[4b] Chemical modifications on the primary face of β-CD could affect to the average interresidue distance of hydrogens[21] H-1 and H-4. For undistorted CDs, H-1 and H-2 of adjacent glucopyranoside units are in close spatial proximity and far away from any inner hydrogens H-3 and H-5. However, rotations around the glycosidic linkages move H-1 away from H-4 of the adjacent unit and bring H-1 closer to H-3 and H-5 of the adjacent glucoside. The equatorial sulfur in β-GlcSCD **23** may contribute to a preferred macrocycle conformation in which an average rotation around the glycosidic linkage leads to a less accessible cavity for the guest molecule, as shown by the absent binding of **32** to the CD cavity. The axial sulfur in α-ManSCD **27** would reduce the extent of that distortion and the wider rim of the CD would be more accessible for the penetration of the guest molecule. In fact, α-ManSCD **27** gives the largest enthalpic gain ($-\Delta H°$ 5.00 kcal mol^{-1}) and therefore, the least decrease of the enthalpic gain (2 kcal mol^{-1}) of the series when compared with the native β-CD. In addition, unlike the rest of the series of glycoCDs, the $T\Delta S°$ value for **27** is slightly negative and decreases with respect to that found with the β-CD. This suggests that the naphthalene group of **32** is included in the **27** cavity deeper than it is in the rest of the series of the glycoCDs. We obtained the intramolecular NOE data for compounds **23** and **27** from two-dimensional NOESY experiments. In both cases, H-1 shows dipolar interactions with H-4 and H-2, but similarly, in both cases, a weak interaction of H-1 with H-3 and H-5 was detected. Unfortunately, the superimposition of signals in both spectra did not allow us to further substantiate more significant conformational differences between **23** and **27**.

Lectin Affinity Evaluation. Table 2 and Figure 4 show the results of the ELLA with the mannoside and

(21) Uccello-Barretta, G.; Balzano, F.; Cuzzola, A.; Menicagli, R.; Salvadori, P. *Eur. J. Org. Chem.* **2000**, 449−453.

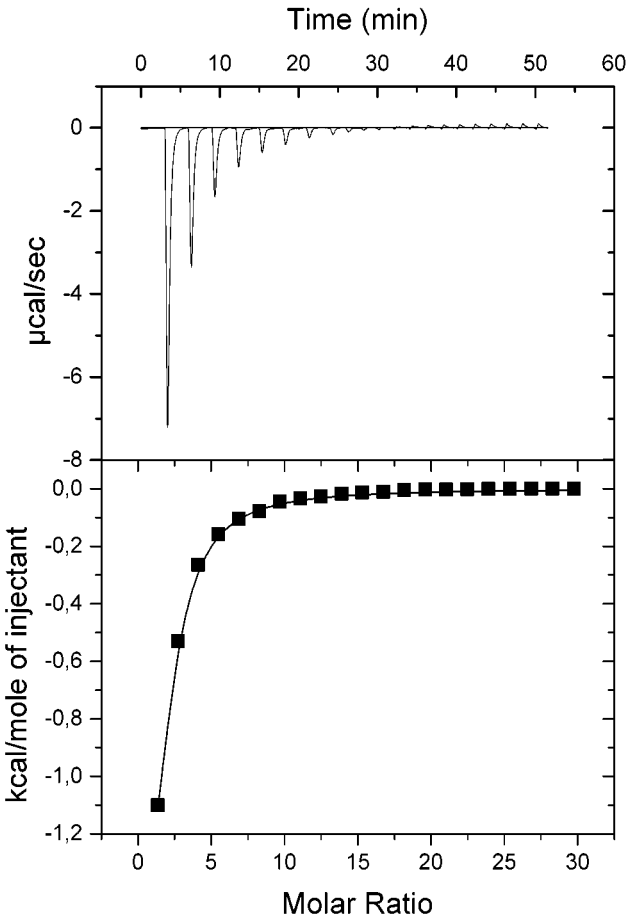

Figure 1. Titration of 0.115 mM of branched CD **29** with 25 aliquots (7 μL each) of sodium 2-naphthalenesulfonate (stock concentration of 43.50 mM) in 10 mM phosphate buffer at pH 7.4 and 25 °C. The top panel shows the raw data, denoting the amount of generated heat (negative exothermic peaks) following each injection of guest. The area under each peak represents the amount of heat released upon the binding of sodium 2-naphthalenesulfonate to CD **29**. Note that, as the titration progresses, the area under the peaks progressively becomes smaller due to an increased occupancy of the CD by guest. The area under each peak was integrated and plotted against the molar ratio of guest to CD **29**. The smooth solid line represents the best fit of the experimental data to a model with 1:1 stoichiometry.

rhamnoside-containing 14-mer CDs **21** and **22**, the heptakis glucosylated CDs **25** and **26** and the heptakis mannosylated CDs **27**−**30** as inhibitors of binding of Con A with yeast mannan. The results were expressed as the concentration necessary to inhibit 50% of binding (IC_{50}) and compared to those of methyl α-D-mannopyranoside. The inhibition activity showed by the rhamnosylated and glycosylaminated CDs **22**, **25**, **26**, **29**, and **30** was nonexistent or below the activity shown by the monosaccharide. Thiomannoside-containing CDs **21**, **27**, and **28** showed lower IC_{50} values than the monosaccharide. When the relative potency per sugar residue was evaluated, less inhibitory potency for the tetradecavalent CD **21** than the monosaccharide was observed. The results obtained from the heptavalent CDs α-ManSCD **27** and α-ManSCH_2CONHCD **28** represented a 2.44- and 2.30-fold increase in potency, respectively, over that of the monosaccharide. Thus, while no multivalency effect is observed for tetradecavalent CD **21**, heptavalent CDs **27** and **28** demonstrate cluster glycoside effect. Interestingly,

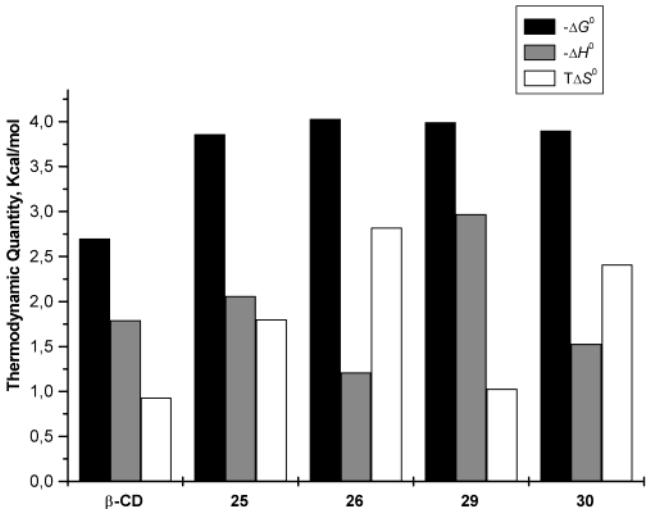

Figure 2. Free energy (−ΔG°), enthalpy (−ΔH°), and entropy changes (TΔS°) for the inclusion complexation of sodium 8-anilino-1-naphthalensulfonate **31** with β-cyclodextrins and derivatives **25**, **26** and **29**, **30** in a buffered aqueous solution (pH 7.4) at 25 °C.

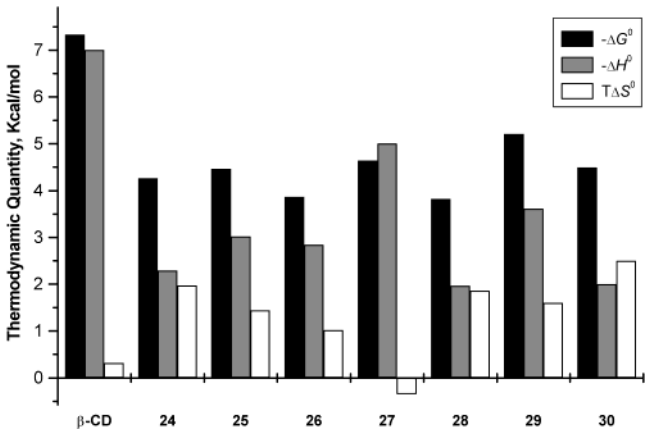

Figure 3. Free energy (−ΔG°), enthalpy (−ΔH°), and entropy changes (TΔS°) for the inclusion complexation of sodium 2-naphthalensulfonate **32** with β-cyclodextrins and derivatives **24**−**30** in a buffered aqueous solution (pH 7.4) at 25 °C.

Table 2. ELLA Inhibition of Binding of ConA Lectin to Mannan by Methyl α-D-Mannopyranoside, α-D-Mannosylated β-CDs 21 and 27−30, α-L-Rhamnosylated β-CD 22, and β-D-Glucosylated β-CDs 25−26

compd	mol wt	IC_{50} (μM)	rel potency[a]
methyl α-D-Man	194.2	1.144	1
21	5753.5	0.392	2.91 (0.21)
22	5529.6	N.I.	
25	2781.8	N.I.	
26	3181.1	N.I.	
27	2382.4	0.067	17.07 (2.44)
28	2781.8	0.071	16.11 (2.30)
29	2781.8	N.I.	
30	3181.1	N.I.	

[a] Values in parentheses are expressed relative to per carbohydrate residue in each compound. N.I. = no inhibition.

27 having, a shorter spacer arm than **28**, showed slightly higher relative inhibitory potency than **28**.

Conclusion

In light of our results, β-CD can be transformed in a biorecognizable molecule without totally losing its capa-

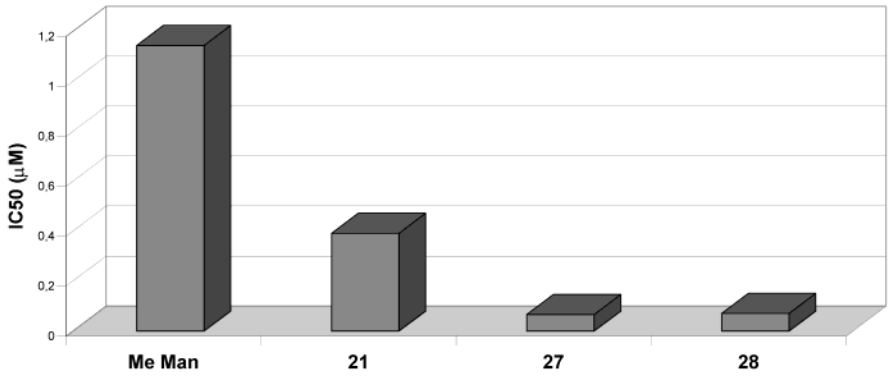

Figure 4. Microtiter plate inhibition of binding of peroxidase-labeled ConA to mannan by methyl α-D-mannopyranoside and mannosylated-β-CD **21**, **27**, and **28**.

bility for inclusion guest molecules. Heptavalent mannoclusters based on β-CD cores enhance the lectin binding affinity due to the cluster effect. The host capability of the persubstituted β-CDs can decrease with respect to the native β-CD due to steric effects, although they can be improved depending on the guest molecule. In our case, the increase of the valency from seven to fourteen ligands did not contribute to the improvement of the Con A binding affinity. In addition, the synthesized hyperbranched mannoCDs lost completely the host properties. Contrary to initial expectations, a persubstituted CD, such as α-ManSCD **27**, in which the mannoside residues are bound directly to the CD core without spacer arm gave the highest Con A binding affinity as well as the second highest affinity constant of the series for the guest molecule used.

Experimental Section

General Methods. TLC was performed on silica gel 60 F_{254} aluminum sheets with detection by charring with sulfuric acid and by UV light when applicable. Flash column chromatography was performed on silica gel (230−400 mesh). Melting points are uncorrected. Optical rotations were recorded at room temperature. ^{1}H and ^{13}C NMR spectra were recorded on a 300 and 75.5 MHz spectrometer. Chemical shifts are given in ppm and referenced to internal $SiMe_4$ (δ_H, δ_C 0.00). *J* values are given in Hz. ^{1}H and ^{13}C resonances were assigned by COSY, one-dimensional TOCSY, ^{1}H-^{1}H NOESY and ^{13}C−^{1}H HMQC experiments. MALDI-TOF mass spectra were recorded using 2,5-dihydroxybenzoic acid matrix. The synthesis of the glycoCDs **23**-**30** has been previously reported.[12a,b] Sodium 8-anilino-1-naphthalensulfonate (ANS) (**31**), 2-naphthalensulfonate (**32**) and methyl α-D-mannopyranoside were purchased from Aldrich, Fluka, and Sigma, respectively. The peroxidase-labeled Concanavalin A (Con A) lectin and *Saccharomyces cerevisiae* mannan were purchased from Sigma.

Calorimetry. Isothermal titration calorimetry experiments were performed using an MCS isothermal titration calorimeter (ITC) from Microcal, Inc. (Northampton, MA). A complete description of its predecessor instrument, OMEGA-ITC, experimental strategies, and data analyses are given by Wiseman et al.[22] The calorimeter was calibrated by known heat pulses as recommended by the manufacturer. During titration, the reference cell was filled with Milli Q water. Prior to the titration experiments, guests and CD derivatives were degassed for 10 min with gentle stirring under vacuum. The sample cell was filled either with 1.8 mL (effective volume: 1.38 mL) of buffer (for control experiment) or with an appropriately host solution. Solutions were prepared in 0.1 M sodium phosphate buffer (pH 7.4). During the titration, the reaction mixture was continuously stirred at 400 rpm. The background titration profiles, under identical experimental conditions, were obtained by injecting the guest into appropriate buffer solutions. The observed heat effects were concentration-independent and were identical to the heat signals detected after the saturation is reached. The raw experimental data were presented as the amount of heat produced per second following each injection of guest into the CD derivative solution (corrected for the ligand heats of dilution) as a function of time. The amount of heat produced per injection was calculated by integration of the area under individual peaks by the Origin software provided with the instrument. The errors are provided by software from the best fit of the experimental data to the model of equal and independent sites and correspond to the standard deviation in the fitting of the curves.

Enzyme-Linked Lectin Assay (ELLA). Microtitration plates were coated with *S. cerevisiae* mannan at 100 μL/well of a solution of 10 μg/mL in 10mM phosphate buffer (PBS, pH 7.4) for 2 h at 37 °C. The wells were then washed twice with 10 mM phosphate buffer containing 1% (v/v) Tween 20 (PBST) and once with PBS. This washing procedure was repeated after each incubation period. Wells were then blocked with 300 μL/well of BSA/PBS (1% w/v) for 2 h at 37 °C. Serial dilutions of the CDs or methyl α-D-mannopyranoside were made up in PBS and 440 μL of peroxidase-labeled ConA (5 μg/mL in PBS, pH = 6.8, containing 0.1 mM Ca^{2+} and 0.1 mM Mn^{2+}) were added. A 100 μL/well portion of the mixture of CDs or methyl α-D-mannopyranoside and the peroxidase-labeled lectin were added, and the plates were incubated for 2 h at 37 °C. After that, 50 μL/well of a solution of *o*-phenylenediamine dihydrochloride (20 mg/50 mL) in citrate-phosphate buffer (pH 5.0 with 0.4% H_2O_2) was added. The plates were incubated for 30 min at 37 °C, and the absorbance was measured at 492 nm.

Bis(3-benzyloxycarbonylaminopropyl)-*N*-*tert*-butoxycarbonylamine (3). To a solution of compound **2**[6a] (1.97 g, 4.93 mmol) in CH_3CN (20 mL) were added di-*tert*-butyl dicarbonate (1.24 g, 5.67 mmol) and triethylamine (498 mg, 4.93 mmol). The reaction mixture was stirred overnight, and then the solvent was evaporated and the crude product chromatographed on silica gel (EtOAc/hexane 1:1→2:1) to give **3** (2.45 g, 99%) as a syrup: IR (KBr) 3333, 2983, 1694, 1524, 1250, 1149, 1026 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40−7.30 (m, 10H, Ph), 5.80 (br s, 2H, NH), 5.10 (s, 4H, CH_2O), 3.18 (br s, 8H, CH_2NH, CH_2N), 1.66 (br s, 4H, $CH_2CH_2CH_2$), 1.45 (s, 9H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 156.4−155.9 (CO), 136.6−127.9 (Ph), 80.0 (CH_3C), 66.4 (CH_2CO), 44.0−43.2 (CH_2N), 38.2−37.6 (CH_2NH), 28.7−27.8 ($CH_2CH_2CH_2$), 28.2 (CH_3); HRMS (FAB) *m*/*z* calcd for $C_{27}H_{37}N_3O_6$ $(M + Na)^+$ 522.2580, found 522.2589.

Bis(3-chloroacetamidopropyl)-*N*-*tert*-butoxycarbonylamine (4). A suspension of compound **3** (8.05 g, 16.11 mmol) and Pd/C (822 mg) in MeOH (100 mL) was stirred under H_2 atmosphere (2.5 atm) for 1 h at room temperature. The mixture was filtered and the Pd/C washed twice with MeOH. The combined filtrates were dried and the solvent evaporated. The

(22) Wiseman, T., Williston, S., Brandts, J. F.; Lin, L.-N. *Anal. Biochem.* **1989**, *17*, 131−137.

obtained crude product was dissolved in CH_3CN (50 mL) and chloroacetic anhydride (6.61 g, 38.67 mmol) and triethylamine (3.26 g, 32.22 mmol) were added to the solution. The reaction mixture was stirred for 24 h at room temperature. Then the solvent was evaporated, and the crude product cromatographed on silica gel (EtOAc/hexane 5:1) to give **3** (5.03 g, 81%) as a solid: mp 96.6−98.8 °C; IR (KBr) 3327, 3260, 2970, 2948, 1690, 1664, 1419, 1173, 1142 cm^{-1}; ^1H NMR (300 MHz, $(CDCl_3)$ δ 7.61 (br s, 1H, NH), 6.71 (br s, 1H, NH), 4.04 (s, 4H, CH_2Cl), 3.28 (br s, 8H, C*H*$_2$NH,CH_2N), 1.71 (br s, 4H, $CH_2CH_2CH_2$), 1.46 (s, 9H, CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 166.2−156.2 (CO), 80.3 (CH_3C), 44.5−43.3 (CH_2N), 42.6 (CH_2Cl), 37.5−36.2 (CH_2NH), 28.6−27.5 ($CH_2CH_2CH_2$), 28.4 (CH_3); HRMS (FAB) *m*/*z* calcd for $C_{15}H_{27}N_3O_4Cl_2$ (M + Na)$^+$ 406.1276, found 406.1279.

General Procedure for the Synthesis of Thioglycoside 8−10. A mixture of **4** (1.16 mmol for reaction with **5**,[16a] 0.52 mmol for reaction with **6**,[16b] 0.50 mmol for reaction with **7**[16c]), Cs_2CO_3 (5 equiv), and the compound **5** (3 equiv), **6**, or **7** (4 equiv) in anhydrous DMF (6−10 mL) was kept under Ar for 24 h at room temperature. After this time, the precipitated material was filtered. Aqueous HCl (5%, 100 mL) was added and the aqueous layer extracted with CH_2Cl_2 (2 × 150 mL). The combined organic phases were washed successively with aqueous HCl (5%, 100 mL), H_2O (100 mL), saturated aqueous $NaHCO_3$ (2 × 100 mL), and H_2O (2 × 100 mL). The organic solution was dried (Na_2SO_4), filtered, and evaporated, giving a residue that was subjected to column chromatography.

Bis[3-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl-1-thiomethylcarbonylamino)propyl]-*N*-*tert*-butoxycarbonylamine (8). Column chromatography (EtOAc/MeOH 60:1 → 40:1) gave **8** (1.17 g, 97%) as a solid: mp 48.4−50.2 °C; $[\alpha]_D$ −21° (*c* 0. 5, chloroform); IR (KBr) 3475, 2945, 1756, 1663, 1368, 1227, 1038 cm^{-1}; ^1H NMR (300 MHz, $(CD_3)_2SO$, 80 °C) δ 7.75 (br s, 2H, NH), 5.35 (t, 2H, *J* = 9.2 Hz, H-3), 5.06 (t, 2H, *J* = 9.4 Hz, H-4), 5.02 (d, 2H, *J* = 9.0 Hz, H-1), 4.98 (t, 2H, *J* = 9.2 Hz, H-2), 4.26 (dd, 2H, *J* = 12.3, 5.2 Hz, H-6), 4.16 (dd, 2H, *J* = 12.3, 2.6 Hz, H-6′), 4.06 (ddd, 2H, *J* = 5.2, 2.6 Hz, H-5), 3.44 (d, 2H, *J* = 14.2 Hz, CHS), 3.34 (d, 2H, *J* = 14.2 Hz, CHS), 3.26 (t, 4H, *J* = 7.1 Hz, CH_2N), 3.17 (dd, 4H, *J* = 13.3, 6.5 Hz, C*H*$_2$NH), 2.12, 2.10, 2.09, 2.05 (4s, 24H, 8 CH_3CO), 1.74 (m, 4H, $CH_2CH_2CH_2$), 1.51 (s, 9H, CH_3C); ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$, 80 °C) δ 169.3, 168.7, 168.6, 168.4, 167.4, 154.3 (CO), 81.4 (C-1), 78.1 (CH_3C), 74.4 (C-5), 72.9 (C-3), 69.7 (C-2), 68.1 (C-4), 61.6 (C-6), 44.2 (CH_2N), 36.5 (CH_2NH), 32.4 (CH_2S), 27.8 ($CH_2CH_2CH_2$), 27.7 (CH_3C), 19.8, 19.7, 19.6, 19.5 (CH_3CO); HRMS (FAB) *m*/*z* calcd for $C_{43}H_{65}N_3O_{22}S_2$ (M + H)$^+$ 1040.3580, found 1040.3559.

Bis[3-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-1-thiomethylcarbonylamino)propyl]-*N*-*tert*-butoxycarbonylamine (9). Column chromatography (Cl_3CH/MeOH 60:1) gave **9** (468 mg, 87%) as a solid: mp 53.6−56.9 °C; $[\alpha]_D$ +113° (*c* 0. 5, chloroform); IR (KBr) 3438, 2938, 1750, 1667, 1371, 1227, 1051 cm^{-1}; ^1H NMR (300 MHz, $(CD_3)_2SO$, 80 °C) δ 7.77 (br s, 2H, NH), 5.58 (d, 2H, *J* = 1.6 Hz, H-1), 5.35 (dd, 2H, *J* = 3.1, 1.7 Hz, H-2), 5.28 (t, 2H, *J* = 9.9 Hz, H-4), 5.25 (dd, 2H, *J* = 10.2, 2.7 Hz, H-3), 4.39 (m, 2H, H-5), 4.29 (dd, 2H, *J* = 12.1, 4.8 Hz, H-6), 4.21 (dd, 2H, *J* = 12.2, 3.1 Hz, H-6′), 3.42 (d, 2H, *J* = 14.2 Hz, CH_2S), 3.35 (d, 2H, *J* = 14.5 Hz, CH_2S), 3.26 (t, 4H, *J* = 7.3 Hz, CH_2N), 3.19 (dd, 4H, *J* = 12.9, 7.0 Hz, C*H*$_2$-NH), 2.21, 2.13, 2.05 (3s, 24H, 8 CH_3CO), 1.76 (m, 4H, $CH_2CH_2CH_2$), 1.52 (s, 9H, CH_3C); ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$, 80 °C) δ 169.1, 168.7, 168.7, 168.6, 167.1, 154.3 (CO), 81.1 (C-1), 78.0 (CH_3C), 69.7 (C-2), 68.7 (C-3,5), 65.7 (C-4), 61.5 (C-6), 44.2 (CH_2N), 36.5 (CH_2NH), 32.8 (CH_2S), 27.8 ($CH_2CH_2CH_2$), 27.6 (CH_3C), 19.7, 19.6, 19.5, 19.4 (CH_3CO); HRMS (FAB) *m*/*z* calcd for $C_{43}H_{65}N_3O_{22}S_2$ (M + Na)$^+$ 1040.3580, found 1040.3581.

Bis[3-(2,3,4-tri-*O*-acetyl-6-deoxy-α-L-mannopyranosyl-1-thiomethylcarbonylamino)propyl]-*N*-*tert*-butoxycarbonylamine (10). Column chromatography (Cl_3CH/MeOH 60:1) gave **10** (418 mg, 91%) as a solid: mp 70.8−72.5 °C; $[\alpha]_D$ −117° (*c* 0. 5, chloroform); IR (KBr) 3434, 2930, 1749, 1652, 1371, 1224, 1053 cm^{-1}; ^1H NMR (300 MHz, $(CD_3)_2SO$, 80 °C) δ 7.76 (br s, 2H, NH), 5.50 (d, 2H, *J* = 1.5 Hz, H-1), 5.35 (dd, 2H, *J* = 3.6, 1.5 Hz, H-2), 5.21 (dd, 2H, *J* = 10.0, 3.5 Hz, H-3), 5.04 (t, 2H, *J* = 9.8 Hz, H-4), 4.25 (m, 2H, H-5), 3.40 (d, 2H, *J* = 14.2 Hz, CH_2S), 3.34 (d, 2H, *J* = 14.5 Hz, CH_2S), 3.26 (t, 4H, *J* = 7.1 Hz, CH_2N), 3.18 (dd, 4H, *J* = 13.4, 6.5 Hz, C*H*$_2$-NH), 2.21, 2.14, 2.04 (3s, 18H, 6 CH_3CO), 1.76 (m, 4H, $CH_2CH_2CH_2$), 1.52 (s, 9H, CH_3C), 1.27 (d, 6H, *J* = 6.2 Hz, H-6); ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$, 80 °C) δ 168.8, 168.7, 167.2, 154.3 (CO), 81.3 (C-1), 78.0 (CH_3C), 70.3 (C-4), 70.1 (C-2), 68.6 (C-3), 66.6 (C-5), 44.2 (CH_2N), 36.5 (CH_2NH), 33.1 (CH_2S), 27.8 ($CH_2CH_2CH_2$), 27.6 (CH_3C), 19.7, 19.7, 19.5 (CH_3CO), 16.5 (C-6); HRMS (FAB) *m*/*z* calcd for $C_{39}H_{61}N_3O_{18}S_2$ (M + H)$^+$ 924.3471, found 924.3468.

General Procedure for the Synthesis of *N*-Chloroacetyl Compound 11−13. A solution of **8** (1.40 mmol), **9** (0.39 mmol), or **10** (0.86 mmol) in 40 mL of 22% the trifluoroacetic acid in CH_2Cl_2 was stirred for 2−3 h at 0 °C (ice bath). The solution was concentrated and dried under vacuum. The obtained residue was suspended in CH_3CN (20 mL). Diisopropylethylamine was added until basic moist pH, and then chloroacetic anhydride (2 equiv) was added. The reaction mixture was stirred at room temperature for 6−7 h. The solution was concentrated at 30 °C, aqueous HCl (5%, 100 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 (2 × 150 mL). The combined organic phases were washed successively with aqueous HCl (5%, 100 mL), H_2O (100 mL), saturated aqueous $NaHCO_3$ (2 × 100 mL), and H_2O (2 × 100 mL). The organic solution was dried (Na_2SO_4), filtered, and evaporated and the crude product chromatographed on silica gel.

Bis[3-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl-1-thiomethylcarbonylamino)propyl]-*N*-chloracetylamine (11). Column chromatography (EtOAc/MeOH 30:1 → 20:1) gave **11** (1.23 g, 87%) as a solid: mp 70.3−73.0 °C; $[\alpha]_D$ −22° (*c* 0. 5, chloroform); IR (KBr) 3482, 2946, 1754, 1649, 1376, 1227, 1038 cm^{-1}; ^1H NMR (300 MHz, $(CD_3)_2SO$, 80 °C) δ 7.76 (br s, 2H, NH), 5.35 (t, 2H, *J* = 9.0 Hz, H-3), 5.06 (t, 2H, *J* = 8.5 Hz, H-4), 5.05 (d, 2H, *J* = 9.9 Hz, H-1), 4.98 (t, 2H, *J* = 9.3 Hz, H-2), 4.36 (s, 2H, CH_2Cl), 4.26 (dd, 2H, *J* = 12.4, 5.2 Hz H-6), 4.17 (dd, 2H, *J* = 12.4, 2.6 Hz, H-6′), 4.06 (ddd, 2H, *J* = 5.2, 2.6 Hz, H-5), 3.45 (d, 2H, *J* = 14.0 Hz, CH_2S), 3.40 (t, 4H, *J* = 7.9 Hz, CH_2N), 3.35 (d, 2H, *J* = 14.4 Hz, CH_2S), 3.20 (dd, 4H, *J* = 12.7, 6.8 Hz, C*H*$_2$NH), 2.12, 2.11, 2.09, 2.05 (4s, 24H, 8 CH_3CO), 1.80 (m, 4H, $CH_2CH_2CH_2$); ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$, 80 °C) δ 169.3, 168.8, 168.6, 168.5, 167.6, 165.4 (CO), 81.4 (C-1), 74.4 (C-5), 72.9 (C-3), 69.7 (C-2), 68.1 (C-4), 61.6 (C-6), 41.2 (CH_2Cl), 36.2 (CH_2NH), 32.5 (CH_2S), 19.8, 19.7, 19.6, 19.5 (CH_3CO); HRMS (FAB) *m*/*z* calcd for $C_{40}H_{58}N_3O_{21}S_2$-Cl (M + H)$^+$ 1016.2772, found 1016.2779.

Bis[3-(2,3,4,6-tetra-*O*-acetyl-α-D-manopyranosyl-1-thiomethylcarbonylamino)propyl]-*N*-chloracetylamine (12). Column chromatography ($CHCl_3$/MeOH 30:1) gave **12** (342 mg, 91%) as a solid: mp 70.8−72.4 °C; $[\alpha]_D$ +97° (*c* 0. 5, chloroform); IR (KBr) 3481, 2945, 1749, 1655, 1371, 1227, 1051 cm^{-1}; ^1H NMR (300 MHz, $(CD_3)_2SO$, 80 °C) δ 7.94 (br s, 2H, NH), 5.60 (d, 2H, *J* = 1.2 Hz, H-1), 5.35 (dd, 2H, *J* = 3.2, 1.7 Hz, H-2), 5.29 (t, 2H, *J* = 9.5 Hz, H-4), 5.23 (dd, 2H, *J* = 10.0, 3.3 Hz, H-3), 4.40 (m, 2H, H-5), 4.36 (s, 2H, CH_2Cl), 4.30 (dd, 2H, *J* = 12.3, 4.8 Hz, H-6), 4.19 (dd, 2H, *J* = 12.2, 2.9 Hz, H-6′), 3.42 (d, 2H, *J* = 14.4 Hz, CH_2S), 3.40 (t, 4H, *J* = 5.2 Hz, CH_2N), 3.36 (d, 2H, *J* = 14.7 Hz, CH_2S), 3.10 (dd, 4H, *J* = 12.1, 6.0 Hz, C*H*$_2$NH), 2.22, 2.13, 2.05 (3s, 24H, 8 CH_3CO), 1.80 (m, 4H, $CH_2CH_2CH_2$); ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$, 80 °C) δ 169.4, 169.0, 168.9, 168.8, 167.4, 165.4 (CO), 81.2 (C-1), 69.7 (C-2), 68.7 (C-3,5), 65.6 (C-4), 61.5 (C-6), 41.3 (CH_2Cl), 36.3 (CH_2-NH), 32.8 (CH_2S), 19.9 19.8 19.7 (CH_3CO); HRMS (FAB) *m*/*z* calcd for $C_{40}H_{58}N_3O_{21}S_2Cl$ (M + H)$^+$ 1016.2772, found 1016.2773.

Bis[3-(2,3,4-tri-*O*-acetyl-6-deoxy-α-L-mannopyranosyl-1-thiomethylcarbonylamino)propyl]-*N*-chloracetylamine (13). Column chromatography ($CHCl_3$/MeOH 30:1) gave **13** (755 mg, 97%) as a solid: mp 67.0−69.4 °C; $[\alpha]_D$ −146° (c 0. 5, chloroform); IR (KBr) 3481, 2941, 1747, 1649, 1373, 1225, 1053 cm^{-1}; ^1H NMR (300 MHz, $(CD_3)_2SO$, 80 °C) δ 7.93 (br s, 2H, NH), 5.51 (d, 2H, *J* = 1.5 Hz, H-1), 5.34 (dd, 2H, *J* = 3.4, 1.6 Hz, H-2), 5.18 (dd, 2H, *J* = 10.1, 3.6 Hz, H-3), 5.04 (t, 2H, *J* = 9.9 Hz, H-4), 4.36 (s, 2H, CH_2Cl), 4.20 (m, 2H, H-5), 3.40 (d, 2H, *J* = 14.2 Hz, CH_2S), 3.40 (t, 4H, *J* = 6.1 Hz, CH_2N),

3.34 (d, 2H, J = 14.5 Hz, CH_2S), 3.20 (dd, 4H, J = 12.1, 6.7 Hz, CH_2NH), 2.21, 2.14, 2.04 (3s, 18H, 6 CH_3CO), 1.79 (br s, 4H, $CH_2CH_2CH_2$), 1.26 (d, 6H, J = 6.2 Hz, H-6); ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$, 80 °C) δ 169.1, 169.0, 167.5, 154.4 (CO), 81.3 (C-1), 70.2 (C-4), 70.1 (C-2), 68.6 (C-3), 66.7 (C-5), 41.3 (CH_2-Cl), 36.3 (CH_2NH), 33.1 (CH_2S), 20.0, 19.9, 19.8 (*C*H_3CO), 16.7 (C-6); HRMS (FAB) m/z calcd for $C_{36}H_{54}N_3O_{17}S_2Cl$ $(M + H)^+$ 900.2662, found 900.2665.

General Procedure for Synthesis of Thiols 14−16. To a solution of **11** (1.07 mmol), **12** (0.17 mmol), or **13** (1.07 mmol) in anhydrous acetone (5−25 mL) was added thiourea (10 equiv). The reaction mixture was stirred at room temperature for 24 h. The solution was concentrated approximately up to half volume without heating. Then a solution of Na_2SO_3 (3 equiv) in H_2O (5−20 mL) was added, and the reaction was stirred for 30 min. Aqueous HCl (5%, 5 mL) and H_2O (100 mL) were added, and the aqueous layer was extracted with CH_2-Cl_2 (2 × 150 mL). The combined organic phases were washed with H_2O (100 mL). The organic layer was dried (Na_2SO_4), filtered, and evaporated and the crude product chromatographed on silica gel.

Bis[3-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl-1-thiomethylcarbonylamino)propyl]-*N*-mercaptoacetylamine (14). Column chromatography (EtOAc/MeOH 20:1) gave **14** (736 mg, 66%) as a solid: mp 71.6−73.0 °C; $[\alpha]_D$ −26° (*c* 0.5, chloroform); IR (KBr) 3482, 2943, 1754, 1653, 1375, 1227, 1038 cm^{-1}; ^{1}H NMR (300 MHz, $(CD_3)_2SO$, 80 °C) δ 7.76 (br s, 2H, NH), 5.36 (t, 2H, J = 9.2 Hz, H-3), 5.06 (t, 2H, J = 9.0 Hz, H-4), 5.05 (d, 2H, J = 9.9 Hz, H-1), 4.98 (t, 2H, J = 9.3 Hz, H-2), 4.26 (dd, 2H, J = 12.2, 5.1 Hz H-6), 4.17 (dd, 2H, J = 12.3, 2.6 Hz, H-6′), 4.06 (ddd, 2H, J = 5.1, 2.6 Hz, H-5), 3.48−3.33 (m, 10H, CH_2SH, CH_2S, CH_2N), 3.20 (dd, 4H, J = 12.6, 6.4 Hz, CH_2NH), 2.66 (br s, 1H, SH), 2.12, 2.11, 2.09, 2.05 (4s, 24H, 8 CH_3CO), 1.79 (m, 4H, $CH_2CH_2CH_2$); ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$, 80 °C) δ 169.3, 168.8, 168.6, 168.5, 167.5 (CO), 81.4 (C-1), 74.4 (C-5), 72.9 (C-3), 69.7 (C-2), 68.1 (C-4), 61.6 (C-6), 42.4 (CH_2N), 36.3 (CH_2NH), 32.5 (CH_2S), 27.3 ($CH_2CH_2CH_2$), 25.1 (CH_2SH), 19.8, 19.7, 19.6, 19.5 (*C*H_3CO); HRMS (FAB) m/z calcd for $C_{40}H_{59}N_3O_{21}S_3$ $(M + H)^+$ 1014.2882, found 1014.2869.

Bis[3-(2,3,4,6-tetra-*O*-acetyl-α-D-manopyranosyl-1-thiomethylcarbonylamino)propyl]-*N*-mercaptoacetylamine (15). Column chromatography ($CHCl_3$/MeOH 20:1) to give **15** (141 mg, 83%) as a solid: mp 70.0−72.4 °C; $[\alpha]_D$ +152° (*c* 0.25, chloroform); IR (KBr) 3447, 2938, 1748, 1648, 1371, 1228, 1052 cm^{-1}; ^{1}H NMR (300 MHz, $(CD_3)_2SO$, 80 °C) δ 7.93 (br s, 2H, NH), 5.59 (d, 2H, J = 1.4 Hz, H-1), 5.34 (dd, 2H, J = 3.0, 1.7 Hz, H-2), 5.28 (t, 2H, J = 9.5 Hz, H-4), 5.22 (dd, 2H, J = 9.3, 3.2 Hz, H-3), 4.39 (m, 2H, H-5), 4.29 (dd, 2H, J = 12.2, 4.7 Hz, H-6), 4.19 (dd, 2H, J = 12.3, 3.4 Hz, H-6′), 3.46 (d, 2H, J = 7.1 Hz, CH_2SH), 3.41 (d, 2H, J = 14.5 Hz, CH_2S), 3.38 (m, 4H, CH_2N), 3.36 (d, 2H, J = 14.5 Hz, CH_2S), 3.20 (m, 4H, CH_2NH), 2.66 (t, 1H, J = 7.1 Hz, SH), 2.22, 2.13, 2.05 (3s, 24H, 8 CH_3CO), 1.79 (br s, 4H, $CH_2CH_2CH_2$); ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$, 80 °C) δ 169.4, 169.0, 168.9, 168.8, 168.7 (CO), 81.2 (C-1), 69.7 (C-2), 68.7, 68.6 (C-3,5), 65.6 (C-4), 61.5 (C-6), 36.3 (CH_2NH), 32.8 (CH_2S), 25.1 (CH_2SH), 19.9, 19.8, 19.7, 19.6 (*C*H_3CO); HRMS (FAB) m/z calcd for $C_{40}H_{59}N_3O_{21}S_3$ $(M + H)^+$ 1014.2882, found 1014.2881.

Bis[3-(2,3,4,6-tetra-*O*-acetyl-6-deoxy-α-L-mannopyranosyl-1-thiomethylcarbonylamino)propyl]-*N*-mercaptoacetylamine (16). Column chromatography ($CHCl_3$/MeOH 30:1) gave **16** (675 mg, 70%) as a solid: mp 73.0−75.8 °C; $[\alpha]_D$ −121° (*c* 0.5, chloroform); IR (KBr) 3435, 2934, 1747, 1647, 1372, 1227, 1053 cm^{-1}; ^{1}H NMR (300 MHz, $(CD_3)_2SO$, 80 °C) δ 7.91 (br s, 2H, NH), 5.50 (br s, 2H, H-1), 5.35 (dd, 2H, J = 3.2, 1.4 Hz, H-2), 5.19 (dd, 2H, J = 10.2, 3.4 Hz, H-3), 5.04 (t, 2H, J = 9.8 Hz, H-4), 4.25 (m, 2H, H-5), 3.47 (d, 2H, J = 7.1 Hz, CH_2SH), 3.41 (d, 2H, J = 14.2 Hz, CH_2S), 3.39 (t, 4H, J = 6.8 Hz, CH_2N), 3.34 (d, 2H, J = 14.2 Hz, CH_2S), 3. (m, 4H, CH_2NH), 2.67 (t, 1H, J = 6.9 Hz, SH), 2.21, 2.14, 2.04 (3s, 18H, 6 CH_3CO), 1.78 (br s, 4H, $CH_2CH_2CH_2$), 1.26 (d, 6H, J = 6.2 Hz, H-6); ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$, 80 °C) δ 168.9, 168.8, 167.5 (CO), 81.3 (C-1), 70.2 (C-4), 70.1 (C-2), 68.7 (C-3), 66.7 (C-5), 36.3 (CH_2NH), 33.1 (CH_2S), 25.1 (CH_2SH), 19.9, 19.7 (*C*H_3CO), 16.7 (C-6); HRMS (FAB) m/z calcd for $C_{36}H_{55}N_3O_{17}S_3$ $(M + H)^+$ 898.2773, found 898.2766.

General Procedure for the Synthesis of CDs 17−19. A mixture of **1** (0.04 mmol for reaction with **14**, 0.05 mmol for reaction with **15**, and 0.05 mmol for reaction with **16**), Cs_2CO_3 (2.5 equiv), and the thiol **14**, **15**, or **16** (2 equiv) in anhydrous DMF (9−20 mL) was kept under Ar for 7 days at 60 °C. After this time, Ac_2O (4 mL), pyridine (2 mL), and DMAP (catalytic amount) were added, and the reaction mixture was stirred for 48 h at 40 °C. Then the precipitated material was filtered, and the filtrate was poured over ice/H_2O. Aqueous HCl (5%, 100 mL) was added and the aqueous layer extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phases were washed successively with aqueous HCl (5%, 100 mL), H_2O (100 mL), saturated $NaHCO_3$ (2 × 150 mL), and H_2O (2 × 100 mL). The organic solution was dried (Na_2SO_4), filtered, and evaporated to give a residue that was subjected to column chromatography.

Heptakis[2,3-di-*O*-acetyl-6-*S*-[N-bis[3′-(2″,3″,4″,6″-tetra-*O*-acetyl-*β*-D-glucopyranosyl-1″-thiomethylcarbonylamino)propyl]aminocarbonylmethyl]-6-thio]cyclomaltoheptaose (17). Column chromatography (EtOAc/MeOH 1:0 → 10:1) The isolated solid was dissolved in CH_2Cl_2, and ether was added until precipitation. The resulting precipitate was filtered and gave **17** (273 mg, 73%) as a solid: mp 116.0 °C dec; $[\alpha]_D$ −70° (*c* 0.5, chloroform); IR (KBr) 3480, 2942, 1752, 1656, 1373, 1228, 1041 cm^{-1}; ^{1}H NMR (300 MHz, $(CD_3)_2SO$, 80 °C) δ 7.73 (br s, 14H, NH), 5.35 (t, 14H, J = 9.0 Hz, H-3″), 5.32 (br t, 7H, J = 11.1 Hz H-3), 5.18 (br d, 7H, J = 3.0 Hz, H-1), 5.06 (t, 14H, J = 9.9 Hz, H-4″), 5.04 (d, 14H, J = 11.1 Hz, H-1″), 4.98 (t, 14H, J = 9.2 Hz, H-2″), 4.83 (br dd, 7H, J = 10.0, 3.0 Hz, H-2), 4.27 (dd, 14H, J = 12.5, 5.5 Hz, H-6″), 4.24 (m, 7H, H-5), 4.17 (dd, 14H, J = 12.2, 2.6 Hz, H-6″), 4.17 (m, 7H, H-4), 4.05 (ddd, 14H, J = 5.5, 2.6 Hz, H-5″), 3.75 (br d, 7H, J = 14.1 Hz, H-6), 3.57 (br d, 7H, J = 15.3 Hz, H-6), 3.49−3.36 (m, 70H, CH_2S, CH_2N),3.21 (br s, 28H, CH_2NH), 2.13, 2.12, 2.11, 2.09, 2.05 (5s, 210H, 70 CH_3CO), 1.80 (br s, 28H, $CH_2CH_2CH_2$); ^{13}C NMR (75 MHZ, $(CD_3)_2SO$, 80 °C) δ 169.5, 169.4, 168.9, 168.7, 168.6, 168.5, 168.0, 167.6 (CO), 96.4 (C-1), 81.5 (C-1″), 77.6 (C-4), 74.4 (C-5″), 72.9 (C-3″), 71.3 (C-5), 69.9, 69.8 (C-2,3), 67.6 (C-2″), 68.0 (C-4″), 61.6 (C-6″), 45.3, 43.1 (CH_2N), 36.5 (CH_2NH), 35.1 (C-6), 32.5 (CH_2S), 28.3, 27.1 ($CH_2CH_2CH_2$), 20.0, 19.9, 19.8, 19.7, 19.6, 19.5 (*C*H_3CO); MALDI-TOF-MS m/z calcd for $C_{350}H_{497}N_{21}O_{189}S_{21}$ $(M + Na - H)^+$ 8718.20, found 8718.27.

Heptakis[2,3-di-*O*-acetyl-6-*S*-[*N*-bis[3′-(2″,3″,4″,6″-tetra-*O*-acetyl-α-D-mannopyranosyl-1″-thiomethylcarbonylamino)propyl]aminocarbonylmethyl]-6-thio]cyclomaltoheptaose (18). Column chromatography (EtOAc/MeOH 1:0 → 10:1). The isolated solid was dissolved in CH_2Cl_2, and ether was added until precipitation. The resulting precipitated was filtered to give **18** (344 mg, 88%) as a solid: mp 98.0 °C dec; $[\alpha]_D$ −206° (*c* 0.25, chloroform); IR (KBr) 3441, 2940, 1748, 1651, 1371, 1228, 1049 cm^{-1}; ^{1}H NMR (300 MHz, $(CD_3)_2SO$, 80 °C) δ 7.80 (br s, 14H, NH), 5.59 (s, 14H, H-1″), 5.35 (br s, 14H, H-2″), 5.32 (overlapped signal, 7H, H-3), 5.29 (t, 14H, J = 10.0 Hz, H-4″), 5.24 (dd, 14H, J = 10.0, 3.0 Hz, H-3″), 5.20 (br d, 7H, J = 2.4 Hz, H-1), 4.85 (br dd, 7H, J = 10.6, 3.0 Hz, H-2), 4.39 (m, 14H, H-5″), 4.33−4.13 (m, 14H, H-4,5), 4.30 (dd, 14H, J = 12.3, 4.6 Hz, H-6″), 4.20 (dd, 14H, J = 12.2, 2.7 Hz, H-6″), 3.76 (d, 7H, J = 13.5 Hz, H-6), 3.58 (d, 7H, J = 14.2 Hz, H-6), 3.46−3.35 (m, 70H, CH_2S, CH_2N), 3.23 (br d, 28H, CH_2NH), 2.21, 2.13, 2.05 (3s, 210H, 70 CH_3CO), 1.83 (br s, 28H, $CH_2CH_2CH_2$); ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$, 80 °C) δ 169.3, 169.2, 168.8, 168.7, 168.6, 168.0, 167.3 (CO), 96.3 (C-1), 81.1 (C-1″), 77.7 (C-4), 71.3 (C-5), 69.9 (C-2,3), 69.7 (C-2″), 68.7, 68.6 (C-3″,5″), 65.7 (C-4″), 61.5 (C-6″), 45.2, 43.2 (CH_2N), 36.6 (CH_2NH), 35.0 (C-6), 32.8 (CH_2S), 28.2, 27.2 ($CH_2CH_2CH_2$), 19.9, 19.8, 19.7, 19.6, 19.5 (*C*H_3CO); MALDI-TOF-MS m/z calcd for $C_{350}H_{497}N_{21}O_{189}S_{21}$ $(M + Na - H)^+$ 8718.20, found 8718.00.

Heptakis[2,3-di-*O*-acetyl-6-*S*-[*N*-bis[3′-(2″,3″,4″-tri-*O*-acetyl-6-deoxy-α-L-mannopyranosyl-1″-thiomethylcarbonylamino)propyl]aminocarbonylmethyl]-6-thio]cyclomaltoheptaose (19). Column chromatography (EtOAc/MeOH

1:0→10:1). The isolated solid was dissolved in CH_2Cl_2, and ether was added until precipitation. The resulting precipitated was filtered to give **19** (353 mg, 83%) as a solid: mp 110.0 °C dec; $[\alpha]_D$ −70° (c 0.5, chloroform); IR (KBr) 3418, 2937, 1747, 1649, 1371, 1225, 1052 cm^{-1}; ^1H NMR (300 MHz, $(CD_3)_2SO$, 80 °C) δ 7.84 (br s, 14H, NH), 5.50 (br s, 14H, H-1″), 5.35 (dd, 14H, J = 3.4, 1.4 Hz, H-2″), 5.32 (br t, 7H, J = 9.7 Hz, H-3), 5.10 (m, 7H, H-1), 5.19 (dd, 14H, J = 10.1, 3.5 Hz, H-3″), 5.04 (t, 14H, J = 9.9 Hz, H-4″), 4.84 (br dd, 7H, J = 9.8, 3.2 Hz, H-2), 4.29−4.20 (m, 21H, H-5,5″), 4.14 (br t, 7H, J = 8.0 Hz, H-4), 3.74 (br d, 7H, J = 14.8 Hz, H-6), 3.58 (br d, 7H, J = 14.9 Hz, H-6), 3.45−3.34 (m, 70H, CH_2S, CH_2N), 3.22 (m, 28H, CH_2NH), 2.21, 2.13, 2.11, 2.04, (4s, 168H, 56 CH_3CO), 1.82 (m, 28H, $CH_2CH_2CH_2$), 1.26 (d, 42H, J = 6.2 Hz, H-6); ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$, 80 °C) δ 169.4, 168.9, 168.8, 168.7, 168.1, 167.5 (CO), 96.3 (C-1), 81.3 (C-1″), 77.7 (C-4), 71.3 (C-5), 70.2 (C-4″), 70.1 (C-2″), 69.8 (C-2,3), 68.7 (C-3″), 66.7 (C-5″), 45.3, 43.2 (CH_2N), 36.6 (CH_2NH), 35.0 (C-6), 33.1 (CH_2S), 28.2, 27.0 ($CH_2CH_2CH_2$), 19.9, 19.8, 19.7 (CH_3CO), 16.6 (C-6″); MALDI-TOF-MS m/z calcd for $C_{322}H_{469}N_{21}O_{161}S_{21}$ $(M + Na)^+$ 7906.69, found 7906.62.

General Procedure for the Zemplen De-O-acetylation of CDs 20−22. A solution of compounds **17** (158 mg, 0.02 mmol), **18** (297 mg, 0.03 mmol), and **19** (302 mg, 0.04 mmol) in dry MeOH (6−8 mL) was made alkaline to pH 9 (indicator paper) with a methanolic solution of NaOMe (1 M). The reaction mixture was stirred overnight at room temperature, and the precipitated material was filtered, washed with MeOH, and dissolved in H_2O (6 mL). The solution was concentrated by lyophilization and gave a solid.

Heptakis[6-*S*-[*N*-bis[3′-(β-D-glucopyranosyl-1″-thiomethylcarbonylamino)propyl]aminocarbonylmethyl]-6-thio]cyclomaltoheptaose (20): yield 99 mg (93%); mp 180.8 °C dec; $[\alpha]_D$ +26° (*c* 0.25, water); IR (KBr) 3399, 2923, 1636, 1043 cm^{-1}; ^1H NMR (300 MHz, D_2O, 80 °C, selected signals) δ 5.54 (br s, 7H, H-1), 5.04 (d, 14H, H-1″); ^{13}C NMR (75.5 MHz, D_2O, 80 °C) δ 172.5, 171.6 (CO), 102.8 (C-1), 85.6 (C-1″), 85.3 (C-4), 80.7(C-5″), 80.5 (C-5), 77.9 (C-3″), 73.9, 73.4 (C-2,3), 73.0 (C-2″), 70.3 (C-4″), 61.9 (C-6″), 46.9, 44.4 (CH_2N), 37.9 (CH_2NH), 35.0 (C-6), 33.9, 33.3 (CH_2S), 28.9, 27.5 ($CH_2CH_2CH_2$); MALDI-TOF-MS m/z calcd for $C_{210}H_{357}N_{21}O_{119}S_{21}$ $(M + Na - 2H)^+$ 5774.56, found 5774.75.

Heptakis[6-*S*-[*N*-bis[3′-(α-D-manopyranosyl-1″-thiomethylcarbonylamino)propyl]aminocarbonylmethyl]-6-thio]cyclomaltoheptaose (21): yield 179 mg (91%); mp 180.8 °C dec; $[\alpha]_D$ +166° (*c* 0.25, water); IR (KBr) 3400, 2929, 1637, 1070 cm^{-1}; ^1H NMR (300 MHz, D_2O, 80 °C, selected signals) δ 5.20 (br s, 14H, H-1), 4.98 (br s, 7H, H-1″); ^{13}C NMR (75.5 MHZ, D_2O) δ 171.6, 170.9 (CO), 102.4 (C-1), 85.4 (C-4), 84.7 (C-1″), 73.4 (C-2″), 72.8, 72.3 (C,2,3,5), 71.2, 70.9 (C-3″,5″), 66.8 (C-4″), 60. 7 (C-6″), 46.1, 43.8 (CH_2N), 37.2 (CH_2NH), 35.0 (C-6), 33.1 (CH_2S), 27.9, 26.6 ($CH_2CH_2CH_2$); MALDI-TOF-MS m/z calcd for $C_{210}H_{357}N_{21}O_{119}S_{21}$ $(M + Na - 2H)^+$ 5774.56, found 5774.50.

Heptakis[6-*S*-[*N*-bis[3′-(6-deoxy-α-L-mannopyranosyl-1″-thiomethylcarbonylamino)propyl]aminocarbonylmethyl]-6-thio]cyclomaltoheptaose (22): yield 169 mg (80%); mp 162.0 °C dec; $[\alpha]_D$ −110° (*c* 0.25, water); IR (KBr) 3403, 2932, 1636, 1066 cm^{-1}; ^1H NMR (300 MHz, D_2O, 80 °C, selected signals) δ 5.14 (br s, 14H, H-1), 4.98 (br s, 7H, H-1″); ^{13}C NMR (75.5 MHZ, D_2O) δ 171.6, 170.8 (CO), 102.3 (C-1), 85.2 (C-1″,4), 72.7 (C-2,3,5), 72.2 (C-4″), 71.3 (C-2″), 70.6 (C-3″), 69.3 (C-5″), 46.0, 43.8 (CH_2N), 37.2 (CH_2NH), 33.7 (C-6, CH_2S), 27.9, 26.6 ($CH_2CH_2CH_2$), 16.7, 16.6 (C-6″); MALDI-TOF-MS m/z calcd for $C_{210}H_{357}N_{21}O_{105}S_{21}$ $(M + Na)^+$ 5552.59, found 5552.30.

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