Synthesis of Per-Glycosylated β-Cyclodextrins Having Enhanced Lectin Binding Affinity

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A cyclomaltooligosaccharide containing seven α-(1→4)-D-glucopyranosyl units (β-cyclodextrins) was transformed into heptakis 6-deoxy-6-iodo (**13**) and heptakis 6-amino-6-deoxy (**25**) derivatives using known procedures. Compound **13** was peracetylated and condensed in one pot with the known peracetylated pseudothiouronium salts of β-D-glucopyranose (**4**), β-D-galactopyranose (**5**), or β-D-*N*-acetylglucopyranosylsamine (**6**) or with α-D-1-deoxy-1-thiomannopyranose (**8**) using cesium carbonate in dimethylformamide. Alternatively, peracetylated 4-aminophenyl-α-D-mannopyranoside (**9**) was transformed into either extended pseudothiouronium **11** following N-chloroacetylation and nucleophilic substitution by thiourea or into 4-isothiocyanatophenyl α-D-mannopyranoside **12** using thiophosgene. Each of the four thiolated sugar derivatives **4**−**6** or **8** were also coupled to heptakis chloroacetamido β-CD **26** obtained from heptakis amine **25** after N-chloroacetylation. Further incorporation of a hexamethylenediamine spacer arm onto heptakis iodo β-CD **13** using thiol derived from mono-Boc derivative **36** and coupling to isothiocyanate **12** after suitable deprotection afforded permannosylated derivative **38**. Zemplén de-O-acetylation of all β-CD derivatives provided water-soluble persubstituted compounds containing D-glucopyranosides (**18**, **30**), D-galactopyranosides (**19**, **31**), D-*N*-acetylglucosaminides (**20**, **32**), and D-mannopyranosides (**22**, **24**, **34**, **39**), respectively. The compounds were then evaluated for their relative binding properties toward natural carbohydrate binding plant lectins using both microtiter plate competitive inhibition experiments, double sandwich assays using horseradish peroxidase labeled lectins and by turbidimetric assays. The plant lectins from *Pisum sativum* (pea), *Arachis hypogea* (peanut), *Canavalia ensiformis* (Concanavalin A), and *Triticum vulgaris* (WGA, wheat germ agglutinin) were used for β-D-glucose, β-D-galactose, α-D-mannose, and β-D-*N*-acetylglucosamine, respectively. All persubstituted β-CDs showed good to excellent inhibitory properties together with abilities to cross-link their analogous plant lectins. The capacity of perglycosylated β-CDs to anchor both microtiter plate-coated lectins and their corresponding peroxidase-labeled derivatives further confirmed the usefulness of these multivalent neoglycoconjugates in bioanalytical assays.

Introduction

Several important biological processes such as infection, immune response, cell differentiation, and neural development are regulated by weak protein−carbohydrate interactions.[1] One area of therapeutic interest in carbohydrate recognition has relied on the role of carbohydrates as cell surface receptors enabling adherence of bacteria, parasites, and viruses in the early stages of infection. Cell-surface oligosaccharides occur as clusters, and the saccharide−receptor interactions are often claimed to be multivalent. For this reason, the so-called cluster effect[2,3] has attracted considerable attention from researchers and promoted the investigation of receptor-binding properties of a variety of multiantennary synthetic saccharide derivatives such as polymers and oligomers,[4−6] dendrimers,[6−13] calix[4]arenes,[14−17] crown ethers,[18,19] surfactant aggregates,[20] and metal complexes.[21]

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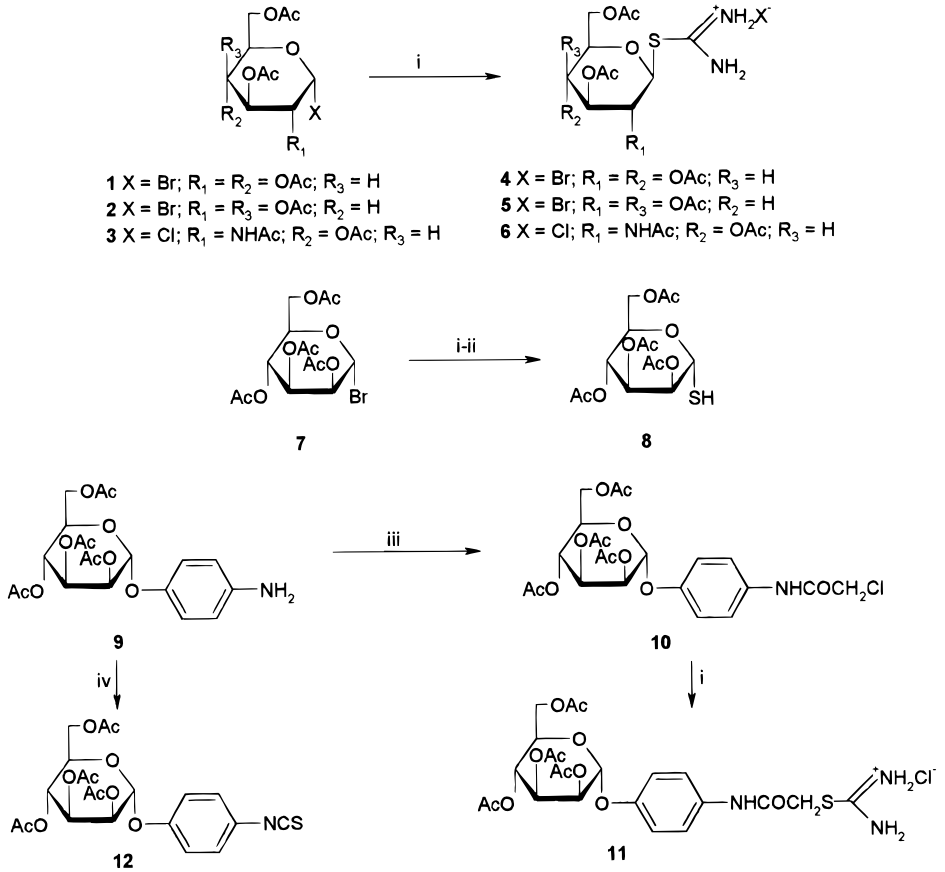
Scheme 1[a]



[a]Key: (i) $(H_2N)_2CS$, acetone; (ii) Na_2SO_3, acetone, H_2O; (iii) $(ClCH_2CO)_2O$; (iv) $CSCl_2$, $CaCO_3$

Cyclodextrins (CDs) are cyclomaltooligosaccharides containing six (α-CD), seven (β-CD) and eight (γ-CD) α-(1→4)-D-glucopyranosyl units, respectively.[22−26] The hydrophobic interior of these molecules has provided the focus of much of the chemistry and applications of CDs. This cavity binds hydrophobic organic molecules of appropriate size (guest molecules), yielding inclusion complexes.[27] The potential utility of this inclusion phenomenon includes solubilization, encapsulation, and nonspecific transport of biologically active molecules by CDs and their derivatives.[28,29] Most drug−CD inclusion complexes exhibit very little site specificity as they lack biologically recognizable sites. To develop systems that can selectively deliver drugs to their sites of action within the organism, the grafting of biorecognizable carbohydrate structures onto CDs has been addressed as a targeting method. The particular structural features of CDs make these compounds adequate candidates to function as scaffolds of multivalent systems of those bioactive molecules. Homogeneous (CDs which have only glucose or maltooligosaccharides as side chains) and heterogeneous branched CDs (CDs having glycosyl moieties as side chains of parent or homogeneous branched CDs) can be obtained by chemical synthesis but in most cases are prepared by enzymatic reactions. However, these reactions generally lead to complex mixtures with fairly low yields that require tedious chromatographic techniques for their purification. The majority of the oligosaccharide-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[30−33]

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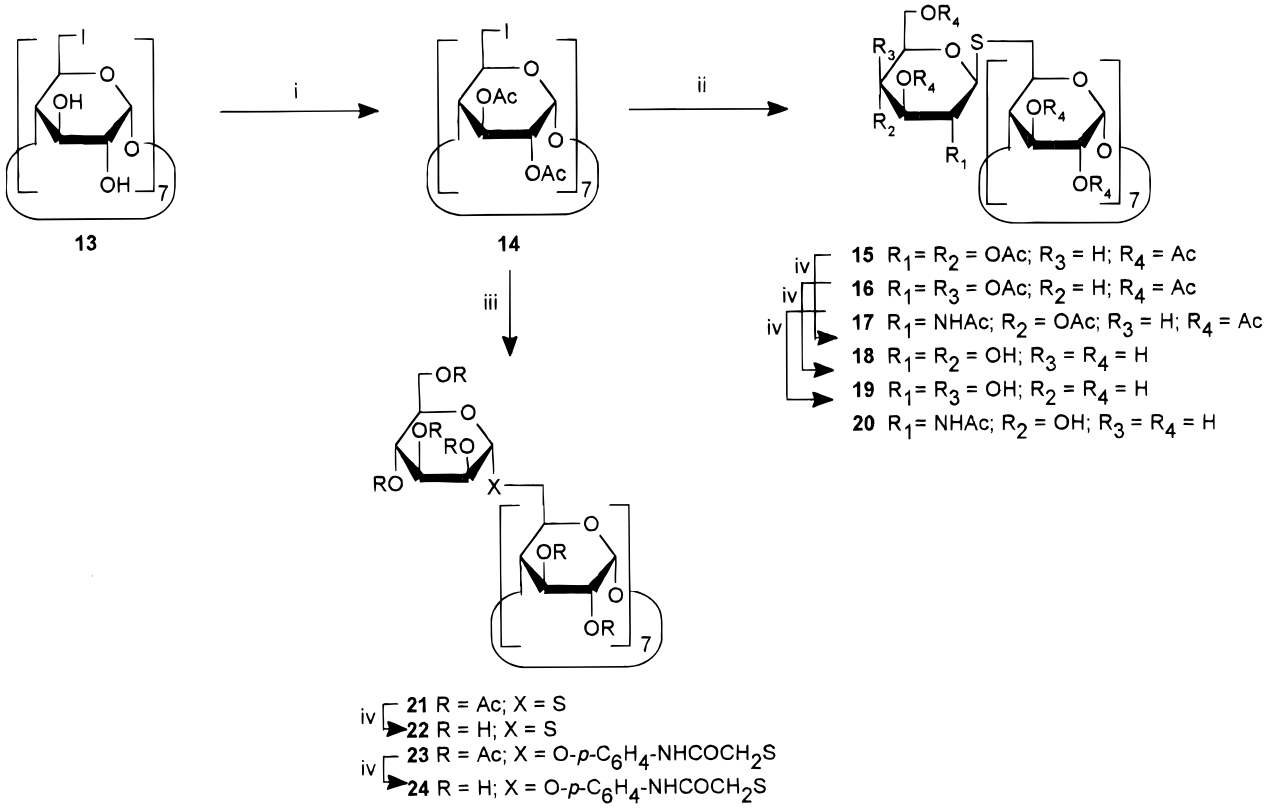
Scheme 2[a]



[a]Key: (i) Ac_2O, Py; (ii) **4-6**, Cs_2CO_3, DMF; (iii) **8** or **11**, Cs_2CO_3, DMF; (iv) NaOMe, MeOH

or via a spacer arm.[32,34−37] Only a few persubstituted branched-monosaccharide CDs have been synthesized. 6-*S*-Glucosyl-β-CD[38] was prepared, and its solubility and inclusion properties were studied. Galactose was either bound to the seven monosacharidic units of β-CD directly[32] or using a spacer arm with nine carbon atoms,[35] both of which were evaluated in vitro for their specific recognition toward the galactose-specific lectin KbCWL. In the present paper, we describe the easy access of a variety of persubstituted branched-monosaccharide β-CDs and the study of their specific lectin binding properties.

Results and Discussion

The synthesis of perglycosylated β-CDs in which the glycosidic moiety is scaffolded onto the CD core through a direct binding of the anomeric position to the primary position of β-CD was first carried out. Linkage of D-glucose, D-galactose, D-*N*-acetylglucosamine, and D-mannose to β-CD was achieved by reaction of the pseudothioureas **4**−**6** and the thiol **8**[39] with per-2,3-di-*O*-acetyl-6-deoxy-6-iodo-β-CD **14**. Pseudothioureas **4**−**6** are easily accessible from the corresponding glycopyranosyl halides **1**−**3** as previously reported,[40,41] and their use presents the additional advantage that they are stable solids (Scheme 1). Peracetylated glycoCDs **15**−**17** and **21** were obtained in high yields (85−94%) when the reactions were performed in DMF using Cs_2CO_3 at room temperature and were transformed in the corresponding hydroxyl derivatives **18**−**20** and **22** by standard Zemplén de-O-acetylation (NaOMe, MeOH) in order to evaluate their specific recognition properties (Scheme 2). The syntheses of compounds **18** and **19** have been previously reported by Laine et al.[38] and Robertis et al.,[42] respectively, using the sodium salts of the corresponding 1-thio-β-D-glycopyranoses which were treated with 6-deoxy-6-iodo- or -bromo-β-CD.

Considering the potent inhibitory activity shown by multivalent dendritic aromatic α-D-mannopyranosides,[43] the pseudothiourea salt **11** was next prepared for its grafting to periodo-β-CD derivative **14**. Compound **11** was obtained from 4-aminophenyl-α-D-mannopyranoside **9** using conventional chemistry [$(ClCH_2CO)_2O$, then $(H_2N)_2$-CS, 64%)] (Scheme 1). Nucleophilic displacement of iodide from **14** by the cesium thiolate generated in situ from pseudothiourea **11** using Cs_2CO_3 provided per-O-acetylated glycoCD **23** (98% yield) which was deprotected by

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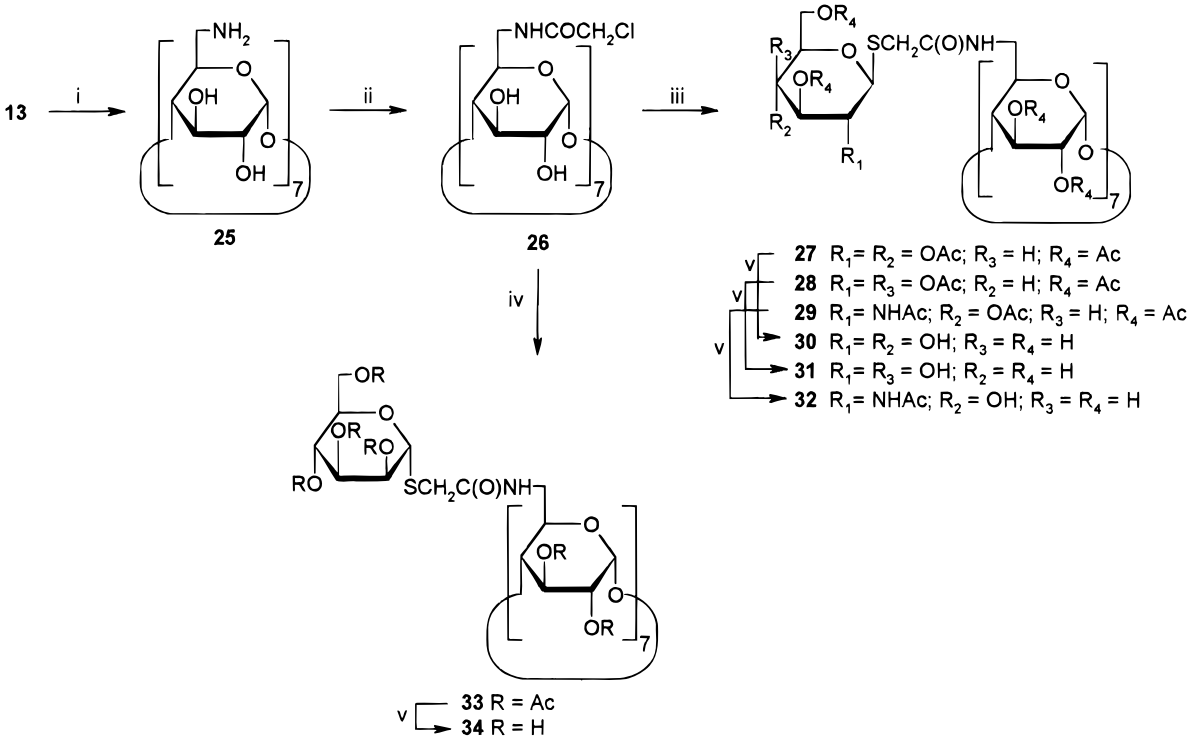
Scheme 3[a]



[a]Key: (i) (a) NaN_3, DMF; (b) Ph_3P, NH_4OH; (ii) $(ClCH_2CO)_2O$, MeOH; (iii) **4**-**6**, Cs_2CO_3, DMF; (iv) **8**, Cs_2CO_3, DMF; (v) NaOMe-MeOH

transesterification with NaOMe−MeOH giving **24** in quantitative yield (Scheme 2).

There are several lines of evidence suggesting that glyco-β-CDs with short spacer arms between the sugar moieties and the core structures might provide sterically congested architectures at the convergent CD's lower rim, thus preventing efficient sugar recognition and accessibility. Recently, Kassab et al.[35] have demonstrated that perglycosylated CDs binding to protein were strongly dependent on the length of the spacer chain between the CD and the sugar headgroup. Considering these facts, it was anticipated that the syntheses of glycoCDs with longer spacer arms incorporating $CH_2C(O)NH$ residues could be readily accomplished. Satisfactory results previously obtained from pseudothioureas as in the case of **14** led us consider the introduction of the chloroacetamido function at the primary position of the β-CD (Scheme 3). Thus, addition of chloroacetic anhydride to a suspension of per-6-amino-6-deoxy-β-CD **25**[44] in methanol allowed the isolation of the desired functionalized β-CD **26** in high yield (98%). This compound was then treated with pseudothioureas **4**−**6** and thiol **8** under the same conditions as described above. The reaction mixtures were subjected to standard acetylation in order to facilitate the isolation and the characterization of the glycoconjugates. GlycoCDs **27**−**29** and **33** were obtained in 84−89% yields which after Zemplén de-O-acetylation provided unprotected glycoCDs **28**−**30** and **34** in essentially quantitative yields.

We also prepared the assembly of D-mannose to the β-CD core through a longer spacer (Scheme 4). The synthetic strategy followed involved using thioacetate **36** which was readily prepared in one pot (90% yield) starting from commercially available Boc-monoprotected hexamethylenediamine **35** by treatment with chloroacetic anhydride followed by subsequent treatment with thioacetic acid and triethylamine. The latent nucleophilic thiol was then prepared by treatment with a saturated methanolic solution of ammonia. Subsequent treatment with per-6-deoxy-6-iodo-β-CD **13** with DBU in DMF followed by standard acetylation of the reaction mixture yielded per-*tert*-butoxycarbonylamino derivative **37** (92% overall yield). Deprotection of the Boc protecting group (50% TFA in CH_2Cl_2) afforded a crude product that was directly reacted with 4-isothiocyanatophenyl β-D-mannopyranoside (**12**) (DIPEA, pyridine). Using this strategy, thiourea-linked glycoCD **38** was obtained in high yield (85%). Finally, Zemplén de-O-acetylation gave water-soluble derivative **39** in almost quantitative yield.

The ^1H NMR spectra of the glycoCDs containing branches of *N*-acetylglucosamines **17** and **29** showed broad and unresolved signals in DMSO-d_6 at room temperature, indicating restricted mobility in the NMR time scale. When the spectra were recorded at 80−100 °C, the signals appeared well resolved and the spectra showed typical signals attributed to both *N*-acetylglucosamine moieties as well as those corresponding to the β-CD. No similar improvement was observed for compound **26** (80 °C), indicating that in this case conformer interconversions were still slow on the NMR time scale, thus supporting the assumption of slow mobility and perhaps steric congestion.

Biochemical Assays. The various persubstituted β-CDs were then evaluated for their relative binding and inhibitory properties against plant lectins known to

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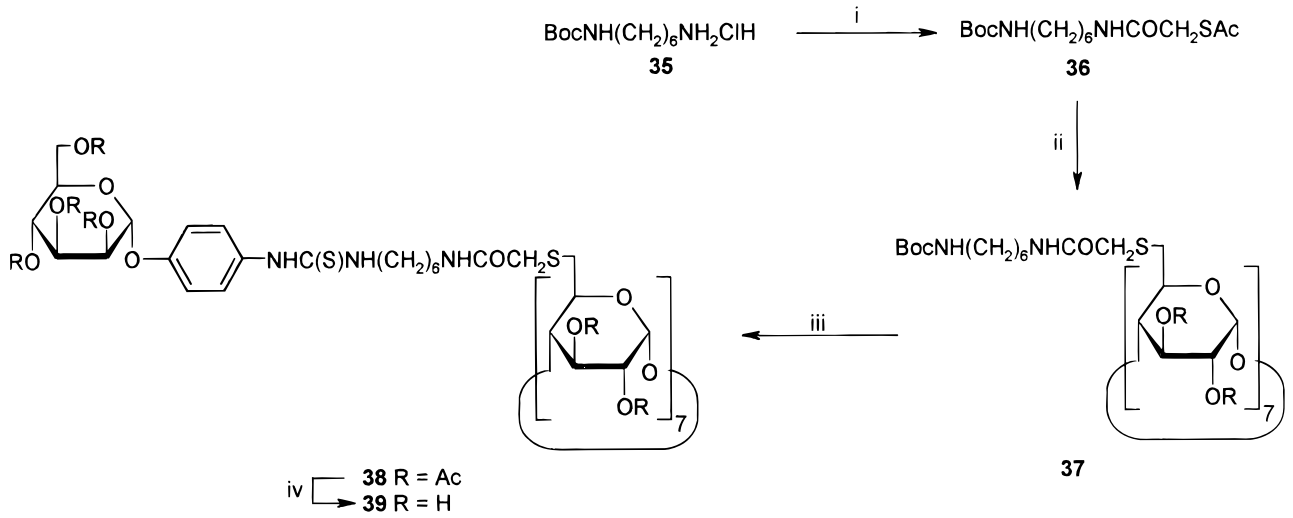
Scheme 4[a]



[a]Key: (i) (a) $(ClCH_2CO)_2O$, Et_3N, CH_2Cl_2; (b) AcSH; (ii) (a) NH_3-MeOH, (b) **13**, DBU, DMF; (c) Ac_2O, Py, DMAP (iii) (a) TFA, CH_2Cl_2; (b) **12**, DIPEA, Py; (iv) NaOMe-MeOH

Table 1. ELLA Inhibition of Binding of Peanut Lectin to Poly(acrylamide-*co*-allyl *β*-D-lactoside)[5,43] by Methyl *β*-D-Galactopyranoside and *β*-D-Galactosylated-*β*-CD

compounds	mol wt	IC_{50} (mM)	rel potency[a]
methyl *β*-D-Gal	194.18	4.46	1.00
19 (*β*-D-Gal-CD)	2380.58	1.49	2.99 (0.43)
31 (*β*-D-Gal-CD)	2779.58	1.16	3.84 (0.55)

[a] Values in parentheses are expressed relative to per carbohydrate residue in each compound.

Table 2. ELLA Inhibition of Binding of Pea Lectin to Poly(acrylamide-*co*-allyl α-D-mannoside)[5,43] by Methyl *β*-D-Glucopyranoside and by *β*-D-Glucosylated-*β*-CD

compounds	mol wt	IC_{50} (mM)	rel potency[a]
methyl *β*-D-Glc	203.19	9.47[b]	1.00
18 (*β*-D-Glc-CD)	2380.58	0.87	10.9 (1.56)
30 (*β*-D-Glc-CD)	2779.73	0.09	105.2 (15.0)

[a] Values in parentheses are expressed relative to per carbohydrate residue in each compound. [b] Extrapolated value.

recognized the individual sugar derivatives.[45] Initially, galactosylated (**19, 31**) and glucosylated (**18, 30**) *β*-CDs were used to inhibit the lectin binding to analogous lactose- or mannose-containing water-soluble copolyacrylamides[5,43] that were used as coating materials in competitive solid-phase microtiter plate assays.[6b] The results were expressed as the concentration necessary to inhibit 50% of the binding (IC_{50}) and compared to those of monosaccharides. Both *β*-CD derivatives with longer spacer arms between the CD core and the haptenic sugar moieties (**31** for Gal and **30** for Glc) (Tables 1 and 2) showed improved inhibitory properties in comparison to derivatives with shorter spacer arms. When expressed on a persugar residue, glucosylated *β*-CD **30** was shown to be 15-fold more potent than the corresponding monosaccharide (methyl *β*-D-Glc). The lectin *Arachis hypogea* (peanut lectin) was used for binding to the galactosyl residues in lactosylated copolyacrylamide, while *Pisum sativum* (pea lectin) was used for binding to an analogous D-mannosylated copolyacrylamide. The lectin is well-known for its capacity to bind both D-glucose and D-mannose derivatives.[45] In the last case, methyl *β*-D-glucopyranoside was used as a reference standard.

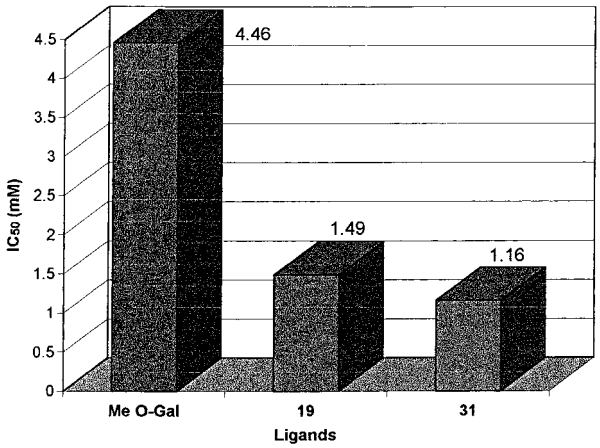


Figure 1. Microtiter plate inhibition of binding of horseradish peroxidase-labeled *A. hypogea* lectin (peanut lectin) to poly(acrylamide-*co*-allyl *β*-D-lactoside) by methyl *β*-D-galactopyranoside (Me O-Gal) and galactosylated *β*-CDs **19** and **31**.

Figures 1 and 2 illustrate the relative inhibitory IC_{50}s for each of the glycoCDs relative to their corresponding monosaccharides.

To further illustrate that more than one saccharide unit is involved in the direct binding with the multivalent lectins, glucosylated *β*-CDs **18** and **30** were used in a sandwich assay. Thus, unlabeled pea lectin was adsorbed onto the surface of the microtiter plates. After appropriate washing of the plate surfaces and blocking with an irrelevant protein (bovine serum albumin, BSA), the heptakis sugar ligands were allowed to bind to the adsorbed pea lectin. The lectin-bound glucosylated *β*-CD, having freely accessible glucoside residues (unbound), was then captured by a second horseradish peroxidase-labeled pea lectin. The adsorbed species were then detected using a peroxidase substrate (ABTS) which gave a chromophore adsorbing at 490 nm upon reaction. Again, glucosylated *β*-CD **30** having a longer spacer arm was shown to be more potent in the assay (Figure 3).

Finally, the capability of the multivalent *β*-CD derivatives to act as cross-linking reagents was further substantiated by turbidimetric assays using a microtiter

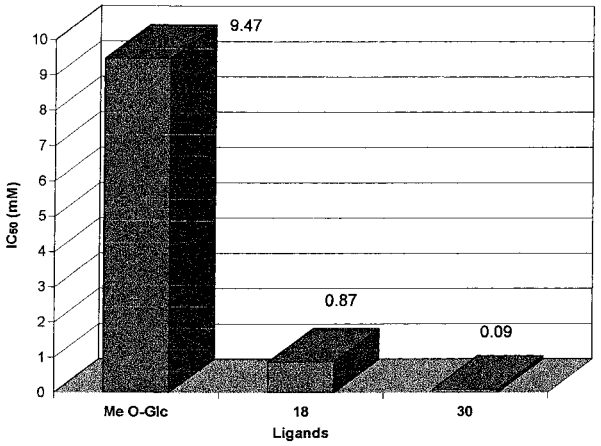


Figure 2. Microtiter plate inhibition of binding of horseradish peroxidase-labeled *P. sativum* lectin (pea lectin) to poly(acrylamide-*co*-allyl α-D-mannopyranoside) by methyl β-D-glucopyranoside (Me O-Glc) and glucosylated β-CDs **18** and **30**.

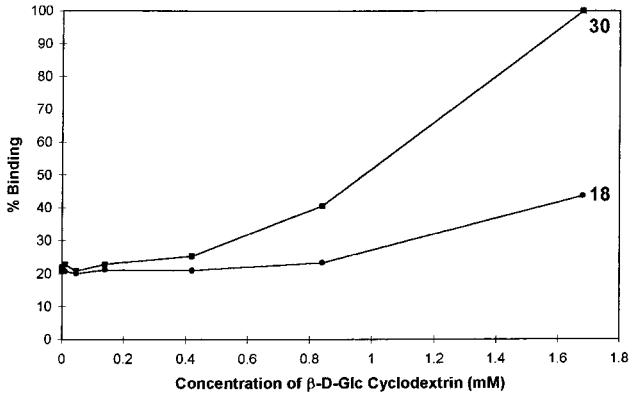


Figure 3. Microtiter plate sandwich assay with *P. sativum* lectin (pea lectin) as coating protein receptor. Glucosylated β-CDs **18** and **30** were then captured by the coated lectin and detected with horseradish peroxidase-labeled pea lectin using ABTS as peroxidase substrate.

plate format.[43] The time course of lectin precipitation by their corresponding sugar haptens is illustrated in Figures 4 and 5. With concanavalin A, aromatic-containing spacer arm mannoside **24** was shown to be very fast in forming an insoluble cross-linked lattice. Interestingly, heptakis mannoside **34**, having a slightly longer spacer arm than **22**, showed almost equivalent but slower cross-linking behavior. N-Acetylglucosaminated β-CDs **20** and **32** also showed their ability to cross-link wheat germ agglutinin. The above set of assays are relevant in demonstrating the relative protein binding properties of the persubstituted β-CDs synthesized herein and further substantiate the usefulness of multivalent neoglycoconjugates in bioassays. In light of the potential use of perglycosylated cyclodextrins as both vectors and drug carriers, the derivatives shown here may offer advantages lacking in partially glycosylated CDs.

Experimental Section

For typical experimental protocols, see ref 49. ^{1}H and ^{13}C spectra were recorded at 300 MHz and when specified at 400 or 500 MHz. ^{1}H and ^{13}C resonances for compounds **15**−**17**, **21**, **27**−**29**, and **38** were assigned by ^{1}H−^{1}H NOESY and ^{13}C−^{1}H HMQC correlation experiments.

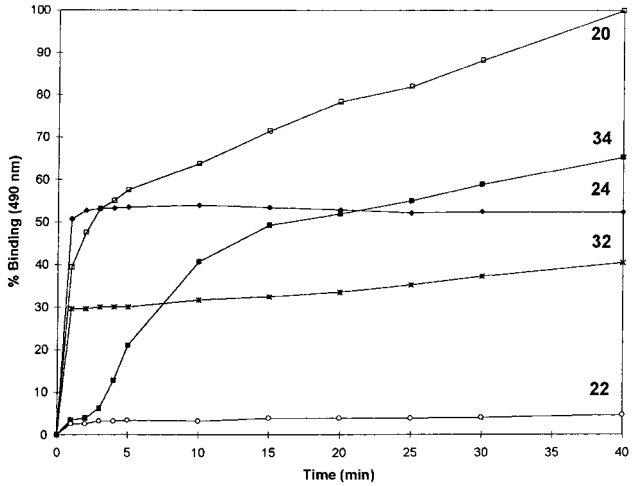


Figure 4. Time course of microtiter plate turbidimetric assay showing the cross-linking properties of the glycosylated β-CDs. Mannosylated β-CDs **22**, **24**, and **34** were used to precipitate concanavalin A (*C. ensiformis*) while *N*-acetylglucosamine-containing β-CDs **20** and **32** were used to precipitate wheat germ agglutinin (WGA from *T. vulgaris*).

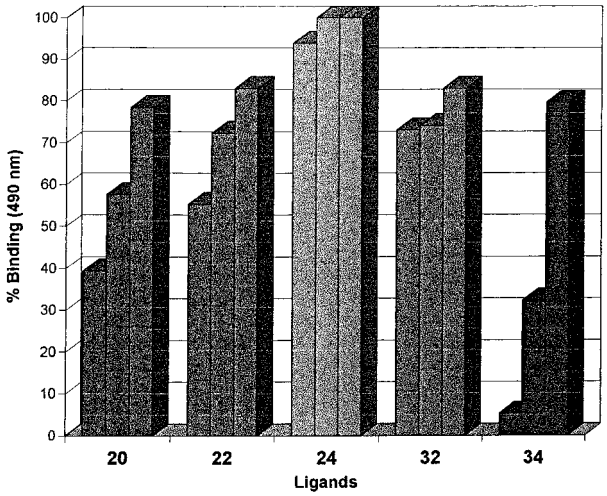


Figure 5. Comparative results from the time course cross-linking (precipitation) (1, 5, and 20 min) of concanavalin A (Con A) by mannosylated β-CDs **22**, **24**, **34**, and wheat germ agglutinin (WGA) by *N*-acetylglucosaminated β-CDs **20** and **32**.

Starting Materials. The pseudothioureas derivatives **4**−**6** were obtained from the corresponding halides **1**−**3** according to the procedures described by Horton et al.[40,41] Acetylated 1-thio-α-D-mannose **8** was obtained from acetobromomannose **7** following the procedure described by Matta et al.[39] Compounds **9** and **12** were obtained following the method described by Monsigny et al.[46] Heptakis(6-deoxy-6-iodo)cyclomaltoheptaose **13** was obtained following the method described by Gadelle et al.[47] and the modifications introduced by Ashton et al.[44] Heptakis(2,3-di-*O*-acetyl-6-deoxy-6-iodo)cyclomaltoheptaose **14** was obtained from compound **13** according to the procedure described by Baer et al.[48] Heptakis(6-amino-6-deoxy)cyclomaltoheptaose **25** was obtained from compound **13**

(46) Monsigny, M.; Roche, A.-C.; Midoux, P. *Biol. Cell* **1984**, *51*, 187.
(47) Gadelle, A.; Defaye, J. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 78.
(48) Baer, H. H.; Vargas Berenguel, A.; Shu, Y. Y.; Defaye, J.; Gadelle, A.; Santoyo González, F. *Carbohydr. Res.* **1992**, *228*, 307.
(49) Santoyo-González, F.; Calvo-Flores, F. G.; García-Mendoza, P.; Hernández-Mateo, F.; Isac-García, J.; Robles-Díaz, R. *J. Org. Chem.* **1993**, *58*, 6122.

following the method described by Ashton et al.[44] Chloroacetic anhydride (technical grade 90%) and *N-tert*-butoxycarbonyl-1,6-hexamethylenediamine hydrochloride (**35**) were purchased from Aldrich. The lectins from *Canavalia ensiformis* (concanavalin A, Con A) and *Triticum vulgaris* (wheat germ, WGA) were purchased from Sigma (cat. nos. C2631 and L9640, respectively). The lectin from *Pisum sativum* (pea lectin) and horseradish peroxidase labeled pea lectin were purchased from EY Laboratories (cat. nos. L-2701-10 and H-2701-1). Linbro microtiter plates (Titertek) were purchased from ICN (Costa Mesa, CA). Xenobind microtiter plates were purchased from Xenopore (lot no. A52751, Hawthorne, NJ).

4-(Chloromethylcarbonylamino)phenyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranoside (10). To a solution of **9** (1.5 g, 3.41 mmol) in dry CH_2Cl_2 (25 mL) were added Et_3N (0.953 mL, 6.82 mmol) and chloroacetic anhydride (0.97 g, 5.10 mmol). After 1 h TLC (ether, double irrigation) showed completed disappearance of the starting material. CH_2Cl_2 (50 mL) was added, and the solution was washed with water (3 × 50 mL). The organic solution was dried (Na_2SO_4), filtered, and concentrated to give a crude product that was crystallized from ether−hexane 1:1 (50 mL) to give **10** (1.14 g). The mother liquors were purified by column chromatography (EtOAc−hexane 1:1) yielding additional **10** (0.33 g) (86.5% overall yield): mp 181−183 °C dec; $[\alpha]_D$ +68° (*c* 1, chloroform); IR (Nujol) 3300, 1776, 1749, 1676, 1579, and 1508 cm^{-1}; ^1H RMN ($CDCl_3$) δ 8.21 (bs, 1 H), 7,47 (d, 2 H, J = 9.0 Hz), 7.09 (d, 2 H, J = 9.0 Hz), 5.54 (dd, 1 H, J = 10.0 and 3.5 Hz), 5.49 (d, 1 H, J = 1.8 Hz), 5.43 (dd, 1 H, J = 3.5 and 1.8 Hz), 5.36 (dd, 1 H, J 10.1 and 10.0 Hz), 4.28 (dd, 1 H, J = 12.2 and 5.2 Hz), 4.18 (s, 2 H), 4.11−4.04 (m, 2 H), 2.20, 2.05, 2.04, 2.03 (4 s, 12 H); ^{13}C NMR ($CDCl_3$) δ 170.6, 170.0, 169.8, 163.8, 152.9, 132.0, 121.9, 117.2, 96.1, 69.4, 69.3, 68.9, 62.2, 42.9, 20.9, 20.7; HRMS (FAB) calcd for $C_{22}H_{26}ClNO_{11}$ + Na 538.1092 $(M + Na)^+$, found 538.1092. Anal. Calcd for $C_{22}H_{26}ClNO_{11}$: C, 51.22; H, 5.08; N, 2.71. Found: C, 51.33; H, 5.20; N, 2.75.

4-[(2′-Isothiouronium)methylcarbonylamino]phenyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranoside Hydrochloride (11). To a solution of **10** (0.79 g, 1.53 mmol) in dry acetone (10 mL) was added thiourea (0.24 g, 3.16 mmol). The reaction mixture was kept at room temperature for 72 h. Apparition of a white solid was observed, which was filtered off to yield **11** (0.67 g, 73%): mp 212−214 °C dec; $[\alpha]_D$ +61° (*c* 1, methanol); IR (Nujol) 3340, 1747, 1715, 1660, 1615, 1552, 1508 cm^{-1}; ^1H NMR (DMSO-d_6) 10.70, 9.30, 9.15 (3 bs, 3 H), 3.55 (d, 2 H, J = 8.9 Hz), 7.12 (d, 2 H, J = 8.9 Hz), 5.66 (d, 1 H, J = 1.1 Hz), 5.32 (m, 1 H), 5.31 (dd, 1 H, J = 7.8 and 3.6 Hz), 5.16 (dd, 1 H, J = 9.9 and 7.9 Hz), 4.21 (bs, 2 H), 4.14 (dd, 1 H, J = 11.9 and 5.4 Hz), 4.05 (ddd, 1 H, J = 9.9, 5.4 and 2.1 Hz), 3.95 (dd, 1 H, J = 11.9 and 2.1 Hz), 2.13, 2.03, 1.96, 1.91 (4 s, 12 H);^{13}C NMR ($CDCl_3$) δ 169.8, 169.7, 169.6, 169.5, 164.9, 151.1, 133.7, 120.6, 117.5, 95.5, 68.6, 68.4, 68.3, 65.2, 61.6, 34.6, 20.6, 20.4; HRMS (FAB) calcd for $C_{23}H_{30}ClN_3O_{11}S$ + Na − ClH 578.1420 $(M + Na- ClH)^+$, found 578.1421.

General Procedure for the Synthesis of GlycoCDs 15−17, 21, and 23. To a solution of the pseudothiourea derivatives **4**−**6** and **11** or the thiol **8** (1.3 mmol) and **14** (0.095 mmol) in dry DMF (10 mL) was added Cs_2CO_3 (2 mmol). The reaction mixture was stirred at room temperature under an argon atmosphere (72 h for **4, 5**; 96 h for **8**, **11**; and 144 h for **6**). When the thiol **8** was used the reaction mixture was treated with Ac_2O−Py (10:6 mL) for 24 h at 40 °C. Aqueous 5% HCl (100 mL) was then added, and the aqueous layer was extracted with EtOAc (2 × 200 mL). The combined organic phase was washed with aqueous 5% HCl (100 mL) and water (2 × 100 mL). The organic solution was dried (Na_2SO_4), filtered, and evaporated to give a crude product that was purified by column chromatography.

Heptakis[2,3-di-*O*-acetyl-6-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-6-thio)]cyclomaltoheptaose (15). Column chromatography (EtOAc) of the crude product gave **15** (94%) as a solid: mp 147 °C dec; $[\alpha]_D$ +17° (*c* 1, chloroform); IR (KBr) 1752, 1227, 1038 cm^{-1}; ^1H NMR (Cl_3CD) δ 5.27 (t, 7 H, J = 9.5 Hz, H-3′), 5.24 (t, 7 H, J = 9.4 Hz, H-3), 5.16 (d, 7 H J = 3.0 Hz, H-1), 5.13 (t, 7 H, J = 9.5 Hz, H-4′), 5.01 (t, 7 H, J = 9.5 Hz, H-2′), 4.80 (dd, 7 H, J = 9.4 and 3.0 Hz, H-2), 4.63 (d, 7 H, J = 9.5 Hz, H-1′), 4.33 (m, 7 H, H-5), 4.18 (m, 14 H, H-6′,6′), 3.96 (t, 7 H, J = 9.4 Hz, H-4), 3.91 (m, 7 H, H-5′), 3.28 (m, 7 H, H-6), 3.16 (m, 7 H, H-6), 2.13 (s, 21 H, 7 Ac), 2.09 (s, 42 H, 14 Ac), 2.04 (s, 21 H, 7 Ac), 2.02 (s, 21 H, 7 Ac), 2.00 (s, 21 H, 7 Ac); ^{13}C NMR (Cl_3CD) δ 170.7−169.4 (6 peaks, CO), 97.6 (C-1), 83.2 (C-1′), 78.7 (C-4), 76.0 (C-5′), 73.9 (C-3′), 70.8 (C-3), 70.3 (C-2,2′), 70.1 (C-5), 68.6 (C-4′), 62.4 (C-6′), 31.5 (C-6), 20.9−20.6 (6 peaks, CH_3CO); MS (FAB) *m*/*z* 4168 for $[M + Na]^+$, calcd for $C_{168}H_{224}O_{105}S_7$ M 4145.

Heptakis[2,3-di-*O*-acetyl-6-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-6-thio)]cyclomaltoheptaose (16). Column chromatography (EtOAc) of the crude product gave **16** (94%) as a solid: mp 174 °C; $[\alpha]_D$ +47° (*c* 1, chloroform); IR (KBr) 1753, 1224, 1048 cm^{-1}; ^1H NMR (Cl_3CD) δ 5.44 (bs, 7 H, H-4′), 5.23 (dd, 7 H, J = 8.9 and 8.9 Hz, H-3), 5.11 (m, 21 H, H-1,2′,3′), 4.75 (dd, 7 H, J = 9.4 and 3.6 Hz, H-2), 4.66 (d, 7 H, J = 9.7 Hz, H-1′), 4.09 (m, 35 H, H-4,5,5′,6′,6′), 3.27 (bd, 7 H, J = 11.7 Hz, H-6), 3.09 (bd, 7 H, J = 11.7 Hz, H-6), 2.16 (s, 21 H, 7 Ac), 2.09 (s, 42 H, 14 Ac), 2.07 (s, 21 H, 7 Ac), 2.03 (s, 21 H, 7 Ac), 2.02 (s, 21 H, 7 Ac), 1.95 (s, 21 H, 7 Ac); ^{13}C NMR (Cl_3CD) 170.5−169.5 (6 peaks, CO), 97.7 (C-1), 85.3 (C-1′), 78.5 (C-4), 74.2 (C-5′ or C-5), 71.7 (C-3′ or C-2′), 70.7 (C-5 or C-5′), 70.5 (C-3), 70.2 (C-2), 68.2 (C-2′ or C-3′), 67.1 (C-4′), 60.7 (C-6′), 33.52 (C-6), 20.8−20.6 (6, CH_3CO); MS (FAB) *m*/*z* 4168 for $[M + Na]^+$, calcd for $C_{168}H_{224}O_{105}S_7$ M 4145.

Heptakis[2,3-di-*O*-acetyl-6-*S*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-6-thio)]cyclomaltoheptaose (17). Column chromatography (methanol−chloroform, 1:20) of the crude product gave **17** (85%) as a solid: mp 159 °C dec; $[\alpha]_D$ +43° (*c* 1, chloroform); IR (KBr) 3586, 3366, 1748, 1667, 1543, 1240, 1044 cm^{-1}; ^1H NMR (DMSO, 80 °C) δ 7.91 (d, 7 H, J = 8.8 Hz, NH), 5.14 (t, 7 H, J = 9.7 Hz, H-3′), 5.12 (t, 7 H, J = 9.5 Hz, H-3), 5.08 (d, 7 H, J = 3.4 Hz, H-1), 4.81 (t, 7 H, J = 9.7 Hz, H-4′), 4.81 (d, 7 H, J = 9.7 Hz, H-1′), 4.71 (dd, 7 H, J = 9.5 Hz, H-2), 4.22 (dd, 7 H, J = 12.5 and 4.04 Hz, H-6′), 4.09 (m, 7 H, H-5), 3.99 (bd, 7 H, J = 10.1 Hz, H-6′), 3.94 (dd, 7 H, J = 9.5 Hz, H-4), 3.79 (m, 7 H, H-5′), 3.62 (q, 7 H, J = 9.7 Hz, H-2′), 3.16 (m, 7 H, H-6), 3.30−3.10 (m, 7 H, H-6), 2.12 (s, 21 H, 7 Ac), 2.10 (s, 42 H, 14 Ac), 2.07 (s, 21 H, 7 Ac), 2.03 (s, 21 H, 7 Ac), 1.92 (s, 21 H, 7 Ac); ^{13}C NMR (DMSO, 80 °C) δ 169.3−168.6 (6 peaks, CO), 97.3 (C-1), 84.1 (C-1′), 77.9 (C-4), 74.5 (C-5′), 73.0 (C-3′), 69.9 (C-3,5), 69.4 (C-2), 68.4 (C-4′), 61.6 (C-6′), 53.3 (C-2′), 31.9 (C-6), 22.1 (CH_3-CONH), 20.0−19.8 (5 peaks, CH_3CO); MS (FAB) *m*/*z* 4161 for $[M + Na]^+$, calcd for $C_{168}H_{231}N_7O_{98}S_7$ M 4138.

Heptakis[2,3-di-*O*-acetyl-6-*S*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-6-thio)]cyclomaltoheptaose (21). Column chromatography (EtOAc → methanol−EtOAc, 1:30) of the crude product gave **21** (88%) as a solid: mp 161 °C; $[\alpha]_D$ +186° (*c* 1, chloroform); IR (KBr) 1750, 1228, 1048 cm^{-1}; ^1H NMR (Cl_3CD) δ 5.43 (dd, 7 H, J = 3.0 and 1.2 Hz, H-2′), 5.38 (d, 7 H, J = 1.2 Hz, H-1′), 5.33 (dd, 7 H, J = 10.2 and 9.2 Hz, H-4′), 5.27 (dd, 7 H, J = 10.2 and 3.0 Hz, H-3′), 5.21 (dd, 7 H, J = 9.5 and 8.4 Hz, H-3), 5.04 (d, 7 H, J = 3.8 Hz, H-1), 4.88 (dd, 7 H, J = 9.5 and 3.8 Hz, H-2), 4.36 (dd, 7 H, J = 12.4 and 3.8 Hz, H-6′), 4.20−4.12 (m, 14 H, H-5,5′), 4.07 (dd, 7 H, J = 12.4 and 2.4 Hz, H-6′), 3.94 (t, 7 H, J = 8.4 Hz, H-4), 3.08 (AB system, 14 H, J_{AB} = 12.2 Hz, $\Delta\delta$ = 25.0 Hz, H-6,6), 2.18 (s, 21 H, 7 Ac), 2.09 (s, 21 H, 7 Ac), 2.08 (s, 21 H, 7 Ac), 2.05 (s, 21 H, 7 Ac), 2.03 (s, 21 H, 7 Ac), 1.97 (s, 21 H, 7 Ac);^{13}C NMR (Cl_3CD) δ 170.5−169.7 (6 peaks, CO), 97.1 (C-1), 83.8 (C-1′), 77.9 (C-4), 71.3 (C-2′), 71.0 (C-5 or C-5′), 70.9 (C-3), 70.2 (C-5 or C-5′), 70.0 (C-2), 69.2, 66.1 (C-3′,4′), 62.3 (C-6′), 32.7 (C-6), 21.0−20.5 (6 peaks, CH_3CO); MS (FAB) *m*/*z* 4168 for $[M + Na]^+$, calcd for $C_{168}H_{224}O_{105}S_7$ M 4145.

Heptakis{2,3-di-*O*-acetyl-6-*S*-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)phenylene-*p*-aminocarbonylmethyl]-6-thio}cyclomaltoheptaose (23). Silica gel column chromatography (EtOAc) of the crude product gave **23** (98%) as a solid: mp 104−106 °C dec; $[\alpha]_D$ +81° (*c* 1, chloroform); IR (KBr) 3366, 1751, 1595, 1508, 1224, 1043 cm^{-1}; ^1H NMR (Cl_3CD) δ 9.03 (bs, 7 H), 7.52 (d, 14 H, J = 8.8 Hz), 7.02 (d, 14 H, J = 8.8 Hz), 5.49 (dd, 7 H, J = 10.2 and 3.3 Hz), 5.47 (d, 7 H, J = 1.2 Hz), 5.41 (dd, 7 H, J = 3.3 and 1.2 Hz), 5.35 (dd, 7 H,

J = 9.9 and 9.9 Hz), 5.23 (dd, 7 H, J = 9.0 and 9.0 Hz), 4.99 (d, 7 H, J = 3.2 Hz), 4.69 (dd, 7 H, J = 9.0 and 3.2 Hz), 4.28−3.96 (m, 28 H), 3.62 (dd, 7 H, J = 9.0 and 9.0 Hz), 3.47−3.06 (m, 28 H), 2.04, 2.01 (2 s, 126 H);^{13}C NMR (Cl_3CD) δ 170.6, 170.5, 170.0, 169.8, 169.6, 168.4, 162.6, 152.4, 133.3, 121.6, 117.2, 96.7, 96.2, 79.4, 71.5, 70.6, 69.4, 69.2, 69.0, 65.9, 62.1, 38.6, 35.2, 20.9, 20.7. Anal. Calcd for $C_{224}H_{273}N_7O_{119}S_7 \cdot 5H_2O$: C, 50.94; H, 5.40; N, 1.86; S, 4.25. Found: C, 50.99; H, 5.62; N, 1.85; S, 4.56.

General Procedure for the Zemplén De-O-acetylation of GlycoCDs 15−17, 21, and 23. A solution of compounds **15−17, 21**, and **23 (**0.072 mmol) in dry methanol (10 mL) was made alkaline to pH 8−9 (indicator paper) with an NaOMe solution. After 12 h, diethyl ether was added and the resulting solid was filtered and washed with ether, yielding **18 (**93%), **19** (95%), **20** (94%), **22** (100%), and **24** (100%), respectively.

Heptakis[6-*S*-β-D-glucopyranosyl-6-thio]cyclomaltoheptaose (18): mp 229 °C dec (lit.[38] mp 248 °C dec); $[\alpha]_D$ +28° (*c* 1, H_2O) [lit.[38] +23° (*c* 1, H_2O)]; IR (cm^{-1}, KBr) 3382, 1636, 1419, 1154, 1067, 1040; the ^{1}H and ^{13}C NMR data are in agreement with those reported by Laine et al.;[38] MS (FAB) *m*/*z* 2404 for $[M + Na + H]^+$ calcd for $C_{84}H_{140}O_{63}S_7$ M 2380.

Heptakis[6-*S*-β-D-galactopyranosyl-6-thio]cyclomaltoheptaose (19): mp 243 °C dec; $[\alpha]_D$ +52° (*c* 1, H_2O) [lit.[42] +62.7 ° (*c* 0.5, H_2O)]; ^{13}C NMR data are in agreement with those reported in ref 42; MS (FAB) *m*/*z* 2404 for $[M + Na + H]^+$ calcd for $C_{84}H_{140}O_{63}S_7$ M 2380.

Heptakis[6-*S*-β-D-2-acetamido-2-deoxyglucopyranosyl-6-thio]cyclomaltoheptaose (20): mp 230−238 °C dec; $[\alpha]_D$ +58° (*c* 1, H_2O); ^{13}C NMR (D_2O) δ 174.3, 102.5, 85.8, 83.0, 80.3, 75.3, 72.9, 72.3, 70.4, 70.2, 61.5, 55.2, 32.6, 22.5; MS (FAB) *m*/*z* 2691 for $[M + Na + H]^+$, calcd for $C_{98}H_{161}N_7O_{63}S_7$ M 2667.

Heptakis[6-*S*-α-D-mannopyranosyl-6-thio]cyclomaltoheptaose (22): mp 196−198 °C dec; $[\alpha]_D$ +170° (*c* 1, H_2O); ^{13}C NMR (D_2O) δ 102.26, 85.7, 84.8, 73.7, 73.2, 72.5, 72.1, 71.5, 70.8, 67.2, 61.2, 32.7; MS (FAB) *m*/*z* 2405 for $[M + Na + 2H]^+$, calcd for $C_{84}H_{140}O_{63}S_7$ M 2380.

Heptakis{6-*S*-(α-D-mannopyranosyloxy)phenylene-*p*-aminocarbonylmethyl-6-thio}cyclomaltoheptaose (24): mp >210 °C dec; $[\alpha]_D$ +78° (*c* 0.5, DMSO); ^{13}C NMR (D_2O, 125 MHz) δ 169.1, 152.3, 131.5, 121.9, 116.9, 101.6, 98.1, 84.0, 72.8, 71.4, 70.0, 69.5, 65.9, 60.1, 36.8, 33.5. Anal. Calcd for $C_{140}H_{189}N_7O_{77}S_7 \cdot 4H_2O$: C, 44.71; H, 5.95; N, 2.67; S, 6.10. Found: C, 46.11; H, 5.88; N, 2.72; S, 5.75.

Synthesis of Heptakis(6-chloroacetamido-6-deoxy)-cyclomaltoheptaose (26). To a suspension of **25** (1.12 g., 7 mmol) in methanol (25 mL) was added chloroacetic anhydride (3.59 g., 21 mmol), and the reaction mixture was stirred for 22 h at room temperature. Removal of the solvent under reduced pressure and addition of EtOAc (100 mL) yielded a solid that was filtered and characterized as **26** (1.549 g, 98%) as a solid: mp 202−204 °C dec; $[\alpha]_D$ + 108° (*c* 0.25, methanol); IR (KBr) 3345, 1660, 1536, 1157, 1045, 1010 cm^{-1}; ^{1}H NMR spectra showed very broad signals in DMSO at 25 °C as well as at 80 °C;^{13}C NMR (DMSO) δ 166.9, 102.2, 83.3, 72.6, 72.2, 70.1, 42.6.

General Procedure for the Synthesis of GlycoCDs 27−29 and 33. A mixture of **26** (0.14 mmol), Cs_2CO_3 (4.9 mmol), and the monosaccharide derivative **4**−**6** or **8** (2.42 mmol) in anhydrous DMF (7 mL) was kept under Ar for 96 h at 60 °C. After this time, the reaction mixture was allowed to reach room temperature and then Ac_2O−Py (15:10 mL) and DMAP (catalytic amount) were added. The mixture was stirred for 30 min at room temperature and then 24 h at 40 °C. After cooling, the salt was filtered and the filtrate was poured on ice−water. Aqueous 5% HCl (150 mL) was added and the aqueous layer extracted with Cl_2CH_2 (2 × 150 mL). The combined organic phase was washed with aqueous 5% HCl (100 mL), saturated aqueous $NaHCO_3$ (2 × 150 mL), and H_2O (2 × 100 mL). The organic solution was dried (Na_2SO_4), filtered, and evaporated, giving a crude product that was purified by column chromatography.

Heptakis[2,3-di-*O*-acetyl-6-amino-6-deoxy-6-*N*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-1-thiomethylcarbonyl)]cyclomaltoheptaose (27). Silica gel column chromatography (EtOAc → methanol−EtOAc 1:40) of the crude product gave **27** (89%): mp 125 °C dec; $[\alpha]_D$ +21° (*c* 1, chloroform); IR (KBr) 3382, 1754, 1668, 1224, 1041 cm^{-1}; ^{1}H NMR (Cl_3CD) δ 7.62 (bs,7H, NH), 5.30 (t, 7 H, J = 9.3 Hz, H-3′), 5.17 (t, 7 H, J = 9.0 Hz, H-3), 5.14 (t, 7 H, J = 9.3 Hz, H-4′), 5.06 (d, 7 H, J = 4.0 Hz, H-1), 5.02 (t, 7 H, J = 9.3 Hz, H-2′), 4.86 (d, 7 H, J = 9.3 Hz, H-1′), 4.84 (dd, 7 H J = 4.0 and 9.0 Hz, H-2), 4.27 (dd, 7 H, J = 12.3 and 4.0 Hz, H-6′), 4.17 (dd, 7 H, J = 12.3 and 2.1 Hz, H-6′), 4.04 (m, 7 H, H-5), 3.96 (m, 7 H, H-6), 3.89 (m, 7 H, H-5′), 3.48 (m, 14 H, H-4,6), 3.42 (bs, 14 H, C*H*$_2$), 2.09 (bs, 42 H, 2 Ac), 2.08 (s, 21 H, Ac), 2.04 (s, 21 H, Ac), 2.03 (s, 21 H, Ac), 2.00 (s, 21 H, Ac); ^{13}C NMR (Cl_3CD) δ 170.7−169.5 (7 peaks, CO), 97.4 (C-1), 83.7 (C-1′), 79.2 (C-4), 75.7 (C-5′), 73.7(C-3′), 70.7 (C-3), 70.6 (C-5), 70.0 (C-2′), 69.7 (C-2), 68.4 (C-4′), 62.1 (C-6′), 40.4 (C-6), 34.1 (CH_2S) 20.8−20.6 (6 peaks, *C*H_3CO); MS (FAB) *m*/*z* 4587 for $[M + 2Na - 3H]^+$, 4571 for $[M + Na + 4H]^+$, calcd for $C_{182}H_{245}N_7O_{112}S_7$ M 4544.

Heptakis[2,3-di-*O*-acetyl-6-amino-6-deoxy-6-*N*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-1-thiomethylcarbonyl)]cyclomaltoheptaose (28). Silica gel column chromatography (EtOAc → methanol−EtOAc 1:40) of the crude product gave **28** (84%) as a solid: mp 156 °C dec; $[\alpha]_D$ +39° (*c* 1, chloroform); IR (KBr) 3374, 1751, 1661, 1225, 1051 cm^{-1}; ^{1}H NMR (Cl_3CD) δ 7.65 (bs, 7 H, NH), 5.47 (d, 7 H, J = 2.4 Hz, H-4′), 5.18 (m, 21 H, Hz, H-2′,3,3′), 5.08 (d, 7 H, J = 3.7 Hz, H-1), 4.85 (m, 14 H, H-1′,2), 4.12 (m, 21 H, H-5′,6′,6′), 4.07 (m, 7 H, H-5), 3.95 (m, 7 H, H-6), 3.52 (m, 14 H, H-4,6), 3.41 (AB system, 14 H, J = 15.5 Hz, Δν = 15.5 Hz, CH_2), 2.16 (s, 21 H, Ac), 2.09 (s, 21 H, Ac), 2.08 (s, 21 H, Ac), 2.04 (s, 21 H, Ac), 2.03 (s, 21 H, Ac), 2.00 (s, 21 H, Ac); ^{13}C NMR (Cl_3CD) δ 170.6−169.5 (7 peaks, CO), 97.6 (C-1), 84.5 (C-1′), 79.2 (C-4), 74.2 (C-5′), 71.7 (C-2′), 70.7 (C-3′or 3,5), 69.7 (C-2), 67.4 (C-3 or 3′), 67.2 (C-4′), 60.8 (C-6′), 40.5 (C-6), 34.4 (CH_2S), 20.8−20.6 (6 peaks, *C*H_3CO); MS (FAB) *m*/*z* 4569 for $[M + Na + 2H]^+$, calcd for $C_{182}H_{245}N_7O_{112}S_7$ M 4544.

Heptakis[2,3-di-*O*-acetyl-6-amino-6-deoxy-6-*N*-(2-acetamido-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-1-thiomethylcarbonyl)]cyclomaltoheptaose (29). Silica gel column chromatography (methanol−EtOAc 1:20 → 1:7) of the crude product gave **29** (84%) as a solid: mp 185 °C; $[\alpha]_D$ +20° (*c* 0.25, chloroform); IR (KBr) 3300, 1750, 1663, 1544, 1242, 1045 cm^{-1}; ^{1}H NMR (DMSO, 100 °C) δ 7.74 (d, 7 H, J = 9.65 Hz, NH), 7.62 (bs, NH), 5.3 (dd, 7 H, J = 9.6 Hz, H-3′), 5.3 (t, 7 H, J = 9.5 and 8.0 Hz, H-3), 5.2 (d, 7 H, J = 3.7 Hz, H-1), 5.0 (t, 7 H, J = 9.6 Hz, H-4′), 5.0 (d, 7 H, J = 10.0 Hz, H-1′), 4.90 (dd, 7 H, J = 9.5 and 3.7 Hz, H-2), 4.30 (dd, 7 H, J = 12.1 and 4.75 Hz, H-6′), 4.20 (dd, 7 H, J = 12.1 and 2.7 Hz, H-6′), 4.13 (m, 7 H, H-5), 4.0 (q, 7 H, J = 9.6 Hz, H-2′), 3.94 (m, 7 H, H-5′), 3.83 (t, 7 H, J = 9.5 Hz, H-4), 3.81−3.60 (m, 14 H, H-6,6′), 2.12 (s, 42 H, 14 Ac), 2.10 (s, 21 H, 7 Ac), 2.07 (s, 21 H, 7 Ac), 2.03 (s, 21 H, 7 Ac), 1.93 (s, 21 H, 7 Ac); ^{13}C NMR (DMSO, 80 °C) δ 169.5.-168.6 (6 peaks, CO), 96.2 (C-1), 83.0 (C-1′), 76.9 (C-4), 74.5 (C-5′), 73.3 (C-3′), 70.1 (C-3), 69.7 (C-5), 69.5 (C-2), 68.6 (C-4′), 61.7 (C-6′), 52.3 (C-2′), 39.2 (C-6), 32.9 (CH_2), 22.1 (*C*H_3CONH), 20.0−19.7 (5 peaks, *C*H_3CO); MS (FAB) *m*/*z* 4565 for $[M + Na + 5H]^+$, calcd for $C_{182}H_{245}N_7O_{112}S_7$ M 4537.

Heptakis[2,3-di-*O*-acetyl-6-amino-6-deoxy-6-*N*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-1-thiomethylcarbonyl)]cyclomaltoheptaose (33). Silica gel column chromatography (EtOAc → methanol−EtOAc 1:40) of the crude product gave **33** (85%) as a solid: $[\alpha]_D$ +90° (*c* 1, chloroform); IR (KBr) 1751, 1654, 1224, 1050 cm^{-1}; ^{1}H NMR (Cl_3CD) δ 7.55 (bs, 7 H, NH), 5.42 (d, 7 H, J = 1.0 Hz, H-1′), 5.34 (dd, 7 H, J = 9.3 and 9.3 Hz, H-4′), 5.32 (dd, 7 H, J = 3.3 and 1.0 Hz, H-2′), 5.22 (dd, 7 H, J = 10.0 and 3.3 Hz, H-3′), 5.15 (dd, 7 H, J = 8.0 and 8.0 Hz, H-3), 5.10 (d, 7 H, J = 3.9 Hz, H-1), 4.85 (dd, 7 H, J = 8.8 and 3.9 Hz H-2), 4.32 (m, 14 H, H-5′,6′), 4.14 (dd, 7 H, J = 14.0 and 4.0 Hz, H-6′), 4.05 (m, 7 H, H-5), 3.89 (m, 7 H, H-6), 3,64 (m, 7 H, H-6), 3.48 (dd, 7 H, J = 10.0 and 7.0 Hz, H-4), 3.33 (AB system, 2H, J = 15.0 Hz, Δν = 28.8 Hz, CH_2), 2.15 (s, 21 H, Ac), 2.09 (s, 21 H, 7 × Ac), 2.06 (s, 21 H, 7 × Ac), 2.04 (s, 42 H, 14 × Ac), 2.00 (s, 21 H, 7 × Ac); ^{13}C NMR (Cl_3CD) δ 170.8−169.6 (7 peaks, CO), 97.1 (C-1), 82.1 (C-1′), 78.7 (C-4), 70.8 (C-3), 70.6 (C-2′ or C-4′ and C-5), 70.4 (C-3′), 69.6, 69.3 (C-2,5′), 66.0 (C-4′ or C-2′), 62.2 (C-6′), 40.2

(C-6), 33.3 (CH_2S), 20.9−20.7 (6 peaks, CH_3CO); MS (FAB) *m/z* 4567 for $[M + Na]^+$, calcd for $C_{182}H_{245}N_7O_{112}S_7$ M 4544.

General Procedure for the Zemplén De-O-acetylation of GlycoCDs 27−29 and 33. A solution of compounds **27**−**29** and **33** (0.072 mmol) in dry methanol (10 mL) was made alkaline to pH 8−9 (indicator paper) with NaOMe solution. After 12 h, diethyl ether was added and the resulting solid was filtered and washed with ether, yielding **30** (98%), **31** (99%), **32** (99%), and **33** (98%), respectively.

Heptakis[6-amino-6-deoxy-6-*N*-(β-D-glucopyranosyl-1-thiomethylcarbonyl)]cyclomaltoheptaose (30): mp 166 °C dec; $[\alpha]_D$ +24° (*c* 0.25, H_2O); IR (KBr) 3366, 1646, 1560 cm^{-1}; ^{13}C NMR (D_2O) δ 172.4, 101.9, 85.2, 82.7, 79.9, 77.1, 72.8, 72.2, 71.9, 70.3, 69.3, 60.9, 40.1, 33.3; MS (FAB) *m/z* 2803 for $[M + Na + H]^+$, calcd for $C_{98}H_{161}N_7O_{70}S_7$ M 2779.

Heptakis[6-amino-6-deoxy-6-*N*-(β-D-galactopyranosyl-1-thiomethylcarbonyl)]cyclomaltoheptaose (31): mp 204−206 °C dec; $[\alpha]_D$ +40° (*c* 0.25, H_2O); IR (KBr) 3377, 1646, 1552 cm^{-1}; ^{13}C NMR (D_2O) δ 172.5, 102.3, 85.8, 83.0, 79.2, 74.1, 73.2, 72.4, 70.6, 69.9, 69.0, 61.3, 40.2, 32.7; MS (FAB) *m/z* 2803 for $[M + Na + H]^+$, calcd for $C_{98}H_{161}N_7O_{70}S_7$ M 2779.

Heptakis[6-amino-6-deoxy-6-*N*-(2-acetamido-β-D-glucopyranosyl-1-thiomethylcarbonyl)]cyclomaltoheptaose (32): mp 204−206 °C dec; $[\alpha]_D$ +34° (*c* 1, H_2O); IR (KBr) 3376, 1657, 1552 cm^{-1}; ^{13}C NMR δ (D_2O) 174.2, 172.2, 102.0, 84.2, 83.0, 79.9, 75.0, 72.7, 72.2, 70.1, 69.8, 61.0, 54.7, 40.3, 33.3; MS (FAB) *m/z* 3066 for $[M+Na]^+$, calcd for $C_{112}H_{182}N_{14}O_{70}S_7$ M 3066.

Heptakis[6-amino-6-deoxy-6-*N*-(α-D-mannopyranosyl-1-thiomethylcarbonyl)]cyclomaltoheptaose (34): mp 196 °C dec; $[\alpha]_D$ +320° (*c* 0.5, H_2O); IR (KBr) 3368, 1653, 1559 cm^{-1}; ^{13}C NMR (D_2O) δ 171.9, 102.2, 84.5, 83.5, 73.5, 73.1, 72.3, 71.4, 71.3, 70.3, 66.9, 60.8, 40.5, 32.7 (C-6); MS (FAB) *m/z* 2803 for $[M + Na + H]^+$, calcd for $C_{98}H_{161}N_7O_{70}S_7$ M 2779.

Synthesis of *N*-*tert*-butoxycarbonyl-*N*′-acetylthiomethylcarbonyl-1,6-hexamethylenediamine (36). To a solution of **35** (1.5 g, 5.9 mmol) in dry CH_2Cl_2 (50 mL) were added Et_3N (3 mL) and chloroacetic anhydride (1.24 g), and the solution was kept at room temperature for 3 h. After this time, Et_3N (3 mL) and thioacetic acid (0.46 mL) were added and the reaction mixture was treated for an additional 12 h. CH_2Cl_2 (200 mL) was added, and the solution was washed with aqueous 5% HCl (50 mL), aqueous saturated $NaCO_3H$ (2 × 50 mL), and water (50 mL). The organic phase was dried (Na_2SO_4) and evaporated, giving a residue that was purified by column chromatography (EtOAc) to give **36** (1.57 g, 90%) as a solid: mp 79−80 °C; IR (Nujol) 3352, 3319, 1690, 1673, 1652, 1519 cm^{-1}; ^1H NMR (Cl_3CD) δ 6.3 (bs, 1 H), 4.5 (bs, 1 H), 3.48 (s, 2 H), 3.25−3.00 (m, 4 H), 2.37 (s, 3 H), 1.40 (s, 9 H), 1.50−1.20 (m, 8 h); ^{13}C NMR (Cl_3CD) δ 168.1, 156.1, 79.1, 40.4, 39.7, 33.1, 30.3, 30.0, 29.2, 28.5, 26.4, 26.3; HRMS (FAB) *m/z* 355.1668 for $[M + Na]^+$, calcd for $C_{15}H_{28}N_2O_4SNa$ M 355.1667.

Synthesis of Heptakis[6-*S*-(10-*tert*-butoxycarbonyl-2-oxo-3,10-diazaundecan-1-yl)-6-thio]cyclomaltoheptaose (37). Compound **36** (0.56 g, 2.94 mmol) was dissolved in a saturated ammonia solution in methanol (25 mL). After 1 h at room temperature, the solution was evaporated under reduced pressure and the residue dissolved in dry DMF (10 mL). Compound **13** (0.56 g, 0.29 mmol) and DBU (0.44 mL, 2.94 mmol) were then added, and the mixture was allowed to remain at room temperature for 24 h. Ac_2O−Py (5:3 mL) and a catalytic quantity of DMAP were then added, and the reaction was kept for additional 24 h. Methanol (50 mL) was added and the solution evaporated and coevaporated with toluene (4 × 50 mL). The mixture was processed by taking it up in EtOAc (200 mL) and washing the solution several times with 5% HCl (100 mL) followed by aqueous $NaCO_3H$ (100 mL) and water (100 mL). Evaporation of the dried (Na_2SO_4) solution gave a crude product that was purified by column chromatography using acetone−toluene 1:3 → 1:1, giving **37** (0.97 g, 92%) as a syrup that crystallized on standing: mp 70−72 °C; $[\alpha]_D$ +77° (*c* 1, chloroform); IR (Nujol) 3314, 1752, 1700, 1654, 1523 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 7.87 (bs, 7 H, NH), 6.67 (bs, 7 H, NH), 5.18 (dd, 7 H, *J* = 9.0 and 9.0 Hz, H-3), 5.03 (bs, 7 H, H-1), 4.65 (dd, 7 H, *J* = 9.9 and 2.4 Hz, H-2), 4.08 (bs, 7 H, H-5), 3.89 (dd, 7 H, *J* = 8.3 and 8.3 Hz, H-4), 3.31−2.84 (several m, 56 H, SCH_2CO, H-6,6′, 2 × CH_2-NH), 2.09, 1.99 (2 s, 42 H, 14 Ac), 1.35 (s, 63 H, 7 × Me_3CO), 1.35, 1.22 (2 m, 56 H, 7 × $(CH_2)_4$); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.0, 169.3, 169.0, 155.5 (CO), 96.2 (C-1), 78.0 (C-4), 77.3 (CMe_3), 71.2 (C-5), 70.2−70.1 (C-2,3), 39.8, 38.9 (2 × CH_2N), 36.7, 34.0 (2 × CH_2S), 29.5, 29.1, 26.2, 26.1 ($(CH_2)_4$), 28.3 (CMe_3), 20.6 ($MeCO$); HRMS (FAB) *m/z* 3650.598 for $[M + Na]^+$, calcd for $C_{161}H_{266}N_{14}O_{63}S_7Na$ M 3650.598.

Synthesis of Heptakis{2,3-di-*O*-acetyl-6-*S*-[12-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy-4-phenyl)-3,10,12-triaza-2-oxo-11-thioxododecano-1-yl]-6-thio}cyclomaltoheptaose (38). Compounds **37** (0.25 g, 0.068 mmol) were treated with a 50% solution of trifluoroacetic acid in CH_2Cl_2 (4 mL) for 1 h at room temperature. Evaporation of the solvent was followed by addition of ether (25 mL) and Et_3N until pH 8−9 (indicator paper). Evaporation of the solvent gave a residue that was dissolved in Py (4 mL). Phenyl isothiocyanate **12** (0.33 g, 0.68 mmol) and DIPEA (0.37 mL) were added, and the reaction was allowed to remain at room temperature for 96 h. After this time, EtOAc (200 mL) was added and the solution was washed with 5% HCl (2 × 15 mL), aqueous $NaCO_3H$ (30 mL), and water (30 mL). Evaporation of the dried (Na_2SO_4) solution and coevaporation with toluene (2 × 50 mL) gave a residue that was dissolved in the minimum quantity of EtOAc. Ether was added, giving **38** as a white solid that was filtered (0.36 g, 85%): mp 126−129 °C; $[\alpha]_D$ +77° (*c* 1, chloroform); IR (Nujol) 3318, 1751, 1649, 1540 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 8.87, 7.79, 7.30 (3 bs, 21 H, 21 × NH), 7.41, 7.13 (2 d, 28 H, *J* = 8.7 Hz, 7 × C_6H_4), 5.61 (bs, 7 H, H-1′), 5.45 (dd, 7 H, *J* = 9.7 and 3.5 Hz, H-3′), 5.43 (m, 7 H, H-2′), 5.33 (dd, 7 H, *J* = 9.7 and 9.7 Hz, H-3), 5.30 (t, 7 H, *J* = 9.9 Hz, H-4′), 5.16 (d, 7 H, *J* = 3.4 Hz, H-1), 4.77 (dd, 7 H, *J* = 9.9 and 3.5 Hz, H-2), 4.22 (dd, 7 H, *J* = 12.0 and 5.8 Hz, H-6′), 4.22 (m, 7 H, H-5), 4.15 (ddd, 7 H, *J* = 9.9, 5.8 and 2.0 Hz, H-5′), 4.06 (dd, 7 H, *J* = 12.0 and 2.0 Hz, H-6′), 3.90 (t, 7 H, *J* = 9.0 Hz, H-4), 3.58 (m, 14 H, 7 × CH_2N), 3.45 (AB system, 14 H, *J* = 15.3 Hz, Δν = 17.4 Hz, SCH_2CO), 3.34 (bd, 7 H, *J* = 13.0 Hz, H-6), 3.28 (m, 14 H, CH_sN), 3.20 (dd, 7 H, *J* = 0 13.7 and 5.8 Hz, H-6), 2.05, 2.04, 2.04, 2.03, 1.97, 1.95 (6 s, 126 H, 42 Ac), 1.62, 1.56, 1.39 (3 m, 56 H, 7 × $(CH_2)_4$); ^{13}C NMR (125 MHz, acetone-d_6) δ 182.4 (CS), 171.0, 170.8, 170.7, 170.4, 170.3, 170.2, 170.1 (CO), 153.9, 135.1, 126.7, 118.2 (C_6H_4), 97.6 (C-1), 97.2 (C-1′), 79.7 (C-4), 72.6 (C-5), 71.6 (C-2), 71.4 (C-3), 70.2, 69.9, 69.8 (C-2′,3′,5′), 66.6 (C-4′), 62.9 (C-6′), 45.2, 40.4 (2 × CH_2N), 38.2 (SCH_2CO), 35.5 (C-6), 30.2, 29.7, 27.4, 27.3 ($(CH_2)_4$), 21.1, 21.0, 20.7, 20.7, 20.7, 20.6 ($MeCO$); MS (FAB) *m/z* 6322.7 for $[M + Na]^+$, calcd for $C_{273}H_{371}O_{119}N_{21}S_{14}Na$ M 6322.

Synthesis of Heptakis{6-*S*-[12-(α-D-mannopyranosyloxy-4-phenyl)-3,10,12-triaza-2-oxo-11-thioxododecano-1-yl]-6-thio}cyclomaltoheptaose (39). A solution of compounds **38** (0.2 g) in dry methanol−CH_2Cl_2 (5:5 mL) was made alkaline to pH 8−9 (indicator paper) with an NaOMe solution. After 12 h ether was added to increase the amount of precipitate which was then isolated by filtration and washed with ether to give **39** (0.14 g, 98%): mp 195−198 °C (dec); $[\alpha]_D$ +68.8° (*c* 0.75, DMSO); ^1H NMR (400 MHz, DMSO-d_6) (selected signals) δ 9.40 (bs), 7.9 (bs), 7.26 (d, 14 H, *J* = 7.4 Hz), 6.97 (d, 14 H, *J* = 7.4 Hz), 5.27 (s, 7 H), 4.83 (bs, 7 H); ^{13}C NMR (75 MHz, DMSO-d_6) (selected signals) δ 180.6, 169.0, 153.3, 133.6, 125.2, 116.9, 101.9, 99.3, 84.4, 74.8, 72.5, 72.2, 71.8, 70.7, 70.1, 66.7, 61.0, 43.8, 38.9, 36.4, 33.4, 29.0, 28.6, 26.2, 26,2.

Competitive Inhibition by Enzyme-Linked Lectin Assay (ELLA). Xenobind microtitration plates were coated with poly(acrylamide-*co*-allyl α-D-mannoside) at 100 μL/well of a stock solution of 10 μg/mL in 0.01 M phosphate buffer (PBS, pH 7.3) for 2 h at 37 °C. The wells were then washed three times with 300 μL/well of 0.01 M phosphate buffer (pH 7.3) containing 0.05% (v/v) Tween 20 (PBST). This washing procedure was repeated after each incubation period. Wells were then blocked with 250 μL/well of BSA/PBS for 1 h at 37 °C. Methyl β-D-galactopyranoside and methyl β-D-glucopyranoside were used as reference inhibitors together with the synthetic

glyco-β-CDs. These ligands were used as stock solutions of 6.7 mmol/mL of PBS. Each inhibitor was added in serial 2- to 10-fold dilutions (60 μL/well) in PBS with the appropriate lectin−peroxidase conjugates (60 μL/well of 150-fold dilution of a 1 mg/mL stock solution of pea lectin in PBS) on Xenobind microtiter plates. The inhibitor solutions (100 μL) were then transferred to the antigen-coated plates and incubated for another hour at 37 °C. The plates were washed with PBS, and 50 μL/well of the peroxidase substrate (2,2′-azinobis(3-ethylbenzothiazolin-6-sulfonic acid) diammonium salt, ABTS, 1 mg/4 mL) in citrate−phosphate buffer (0.2 M, pH 4.0 with 0.015% H_2O_2) was added. The reactions were stopped after 30 min. by adding 50 μL/well of 1 M H_2SO_4, and the optical density was measured at 410 nm relative to 570 nm. The percent inhibition was calculated as follows:

$$\% \text{ inhibition} = (A_{\text{no inhibitor}} - A_{\text{with inhibitor}})/A_{\text{no inhibitor}} \times 100$$

IC_{50} values were reported as the concentration required for 50% inhibition of the coating antigen. Each test was performed in duplicate.

Two-Site ELLA (Sandwich Assay). Xenobind microtitration plates were coated with pea lectin at 100 μL/well of a stock solution of 5 μg/mL in 0.01 M phosphate buffer (PBS, pH 7.3) for 2 h at 37 °C. The synthesized multivalent cyclodextrin ligands containing β-D-Glc **18** and **30** and β-cyclodextrin as a negative control were used as a stock solution of 3.4 mmol/mL of PBS. The ligands were added in serial 2- to 10-fold dilutions (50 μL/well) in PBS and incubated at 37 °C. After 1 h, horseradish peroxidase-labeled pea lectin (50 μL/well of 200-fold dilution of a 1 mg/mL stock solution in PBS) was added to the microtiter plates which were incubated for another hour at 37 °C. The plates were washed with PBS, and 50 μL/well of ABTS (1 mg/4 mL) in citrate−phosphate buffer (0.2 M, pH 4.0 with 0.015% H_2O_2) was added. The reactions were stopped after 30 min by adding 50 mL/well of 1 M H_2SO_4, and the optical density was measured at 410 nm relative to 570 nm.

Turbidimetric Assay. Turbidimetry experiments were performed in Linbro (Titertek) microtitration plates where 90 μL/well of stock lectin solution prepared from Con A (1 mg/mL PBS) was mixed with 10 μL of stock solutions of inhibitors containing mannosides **22, 24,** or **34** (0.42 μmol/mL PBS) to obtain a final volume of 100 μL per well. For the *N*-acetylglucosamine-containing cyclodextrin inhibitors (**20, 32**), 90 μL/well of a stock lectin solution of WGA (1 mg/mL PBS) was mixed with 10 μL of stock solutions of inhibitors **20** and **32** (0.42 μmol/mL PBS). The solutions were then incubated at room temperature for 2−3 h. The turbidity of the solutions was monitored by reading the optical density (O.D.) at 490 nm at regular time intervals until no noticeable change could be observed. Each test was done in duplicate.

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