Amphiphilic *N*-Glycosyl-thiocarbamoyl Cyclodextrins: Synthesis, Self-Assembly, and Fluorimetry of Recognition by *Lens culinaris* Lectin

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Amphiphilic β-cyclodextrins have been synthesized bearing hexylthio, dodecylthio, and hexadecylthio chains at the 6-positions and glycosylthiocarbamoyl-oligo(ethylene glycol) units at the 2-positions. The glycosyl residues (α-D-mannosyl and β-L-fucosyl) are intended for cell-targeting. Self-assembly of these new amphiphilic glycosylated cyclodextrins in water to form vesicles was investigated by dynamic light scattering and transmission electron microscopy. Selective binding of the hexylthio assemblies to a protein receptor (*Lens culinaris* lectin) was confirmed by fluorescence spectroscopy.

Introduction

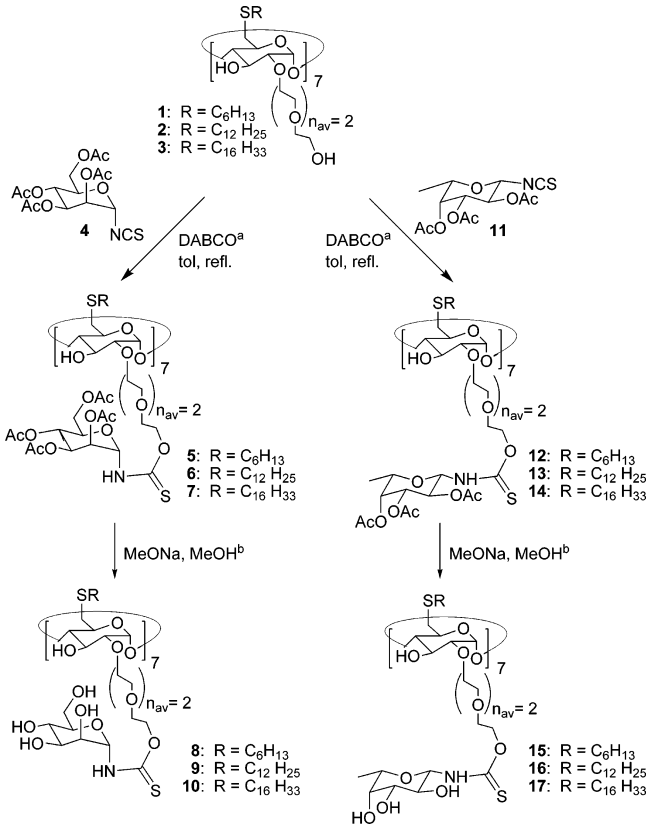
Cyclodextrins (CDs) are macrocyclic oligomers of glucose. Their properties as molecular hosts,[1,2] their multifunctionality,[3−6] and their unusual adaptability as oligosaccharides to selective modification[7] make them unique mesomolecular subjects for chemical biology.

We have demonstrated that specially designed amphiphilic CDs are capable of forming micellar aggregates[8] or vesicles[9,10] of potentially low immunogenicity due to their oligo(ethylene glycol) exterior[11] and are more versatile as drug encapsulators than separate CD molecules.[10,12] These developments have opened new possibilities for the use of cyclodextrins as advanced drug delivery systems,[12] since there is superior guest molecule retention by these aggregates.[10]

Glycosylation of CDs or of amphiphiles is a means of targeting them to saccharide-specific cell receptors. Vesicles formed by amphiphilic cucurbit[6]uril modified with sugar have been prepared, and their interactions with concanavalin A lectin (ConA) have been investigated.[13] Amphiphilic CDs substituted with one or seven biorecognizable sugar residues on the primary face have also been synthesized.[14]

In an earlier paper we described the synthesis of galactosylated amphiphilic CDs[15a] and binding of their nanoaggregates and vesicles to lectins by multivalent interactions (cluster effect). Recently we also reported a spectroscopic investigation of aggregation involving galactose-specific lectin and the amphiphilic CDs[15b] and have demonstrated that this recognition is influenced by the morphology of the nanoaggregate, which, in turn, depends on the balance between hydrophobic and hydrophilic components of the cyclodextrins.[15c]

Scheme 1. Synthesis of Mannosylated and Fucosylated CDs[a,b]



[a] Prior to reaction, the reactants were dissolved in toluene followed by immediate evaporation of the solvent and drying in vacuo. [b] For compounds **6**, **7**, **13**, and **14** DMF was added to complete the reaction.

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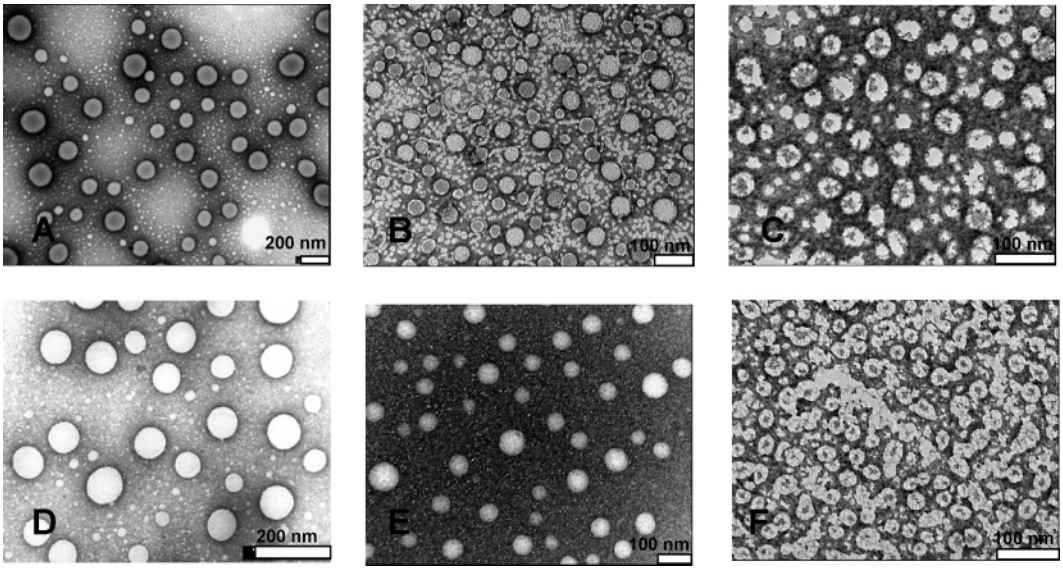

Figure 1. Electron micrographs of assemblies of amphiphilic cyclodextrins: (A) SC6-α-Man-CD, bar = 200 nm; (B) SC12-α-Man-CD, bar = 100 nm; (C) SC16-α-Man-CD, bar = 100 nm**;** (D) SC6-*β*-Fuc-CD; bar = 200 nm; (E) SC12-*β*-Fuc-CD, bar = 100 nm; (F) SC16-*β*-Fuc-CD, bar = 100 nm.

Synthesis of these amphiphilic cyclodextrins involved bromination of 2-oligo(ethylene glycol) chains for reaction with thiosugars. We have since sought a more direct procedure for grafting glycosyl residues onto the CD core. In this approach glycosyl isothiocyanates of mannose and fucose have been reacted with the oligo(ethylene glycol) chains to give a thiocarbamoyl linkage between glycosyl and CD, thus avoiding the bromination step.

Mannose and fucose were chosen as targeting epitopes since they interact with receptors present on a number of cell types. For example, it has been demonstrated that mannosylated[16] and fucosylated[17] bovine serum albumins are efficiently taken up by liver non-parenchymal cells, mainly composed of sinusoidal endothelial cells and Kupffer cells, after intravenous injection. Similar results have been obtained with mannosylated[18] and fucosylated liposomes.[19]

In this paper we describe the synthesis of thiocarbamoyl-linked mannosylated and fucosylated amphiphilic cyclodextrins. The morphological properties of new glycosylated CD aggregates were investigated by dynamic light scattering and transmission electron microscopy. Interaction of the CDs nanoaggregates with *Lens culinaris* lectin was assessed by fluorescence spectroscopy. Lectins are known as useful receptors[20] for assessing targeting to proteins by glycosylation, and *L. culinaris* is suitable for recognition of mannosylated[21a] and fucosylated[21b] derivatives. Surface plasmon resonance as well as high-throughput methods for the study of recognition phenomena such as lectin chips[22] or carbohydrate microarrays[23] perturb the investigated systems by immobilizing or labeling one of the interacting partners; fluorescence techniques are less invasive.[15b]

Results and Discussion

The amphiphilic CDs **1**−**3** (Scheme 1) were obtained in a three-step synthesis from *β*-CD as previously described.[8a]

The isothiocyanate derivatives of D-mannose **4** and L-fucose **11** (Scheme 1) were synthesized as follows. Peracetylation of the starting sugar, followed by anomeric bromination, provided the corresponding glycosyl bromide. α-D-Mannosyl-bromide[24] was treated with KSCN[25] in the presence of $(C_4H_9)_4N^+Br^-$ to give α-D-mannosyl isothiocyanate **4**.[26] The same procedure was applied to α-L-fucosyl bromide[27] leading to *β*-isothiocyanate fucose derivative **11**.[26]

Derivatives **4** and **11** were reacted with the terminal hydroxyl groups of the amphiphilic CDs' oligo(ethylene glycol) chains to give a thiocarbamoyl linkage (compounds **5**−**7** and **12**−**14**, Scheme 1). The coupling was carried out in toluene using 1,4-diazabicyclo[2.2.2]octane (DABCO) as a basic promoter to give the glycosylated CDs **5**−**7** and **12**−**14** in 65−95% yield. Treatment of the α-mannosylated **5**−**7** and *β*-fucosylated compounds **12**−**14** with methanolic sodium methoxide afforded deprotected α-mannosylated **8**−**10** and *β*-fucosylated **15**−**17** CDs. In the cases of the SC12 and SC16 derivatives, partially deacetylated products precipitated from solution as the reaction progressed, requiring addition of dimethylformamide (DMF) to maintain solution.

These derivatives lend themselves well to analysis by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). The MALDI mass spectra are reported in Figures S1 and S2 (Supporting Information); they represent the molecular weight distributions for the mannosylated CDs (compounds **8**, **9**, and **10**) and the fucosylated CDs (compounds **15**, **16**, and **17**). In these spectra peaks up to mass 5500 D are detectable, corresponding to the $[M + Na]^+$ pseudomolecular ions having mainly $\Delta m/z = 44$ D, as emerges from the degree of polydispersity due to the grafted C_2H_4O (ethylene oxide) units. Both the signal intensity and the overall resolution of the mass spectra obtained reflect the complexity of the compounds synthesized and the high heterogeneity of the samples. For instance, MALDI analyses of SC12 and SC16 derivatives show peaks at lower *m*/*z* due to partial glycosylation, though it should be noted that full glycosylation is not essential for targeting.

Self-assembling properties in water of these new glycosylated CDs were investigated by dynamic light scattering (DLS) and transmission electron microscopy (TEM). Dynamic light scattering indicated the presence of aggregates (Table 1 in the Supporting Information); under the hydration and sonication conditions used here, these range from 50 to 200 nm in size.

The morphologies of the aggregates were imaged using TEM (Figure 1). Spherical aggregates with diameters of 50−200 nm

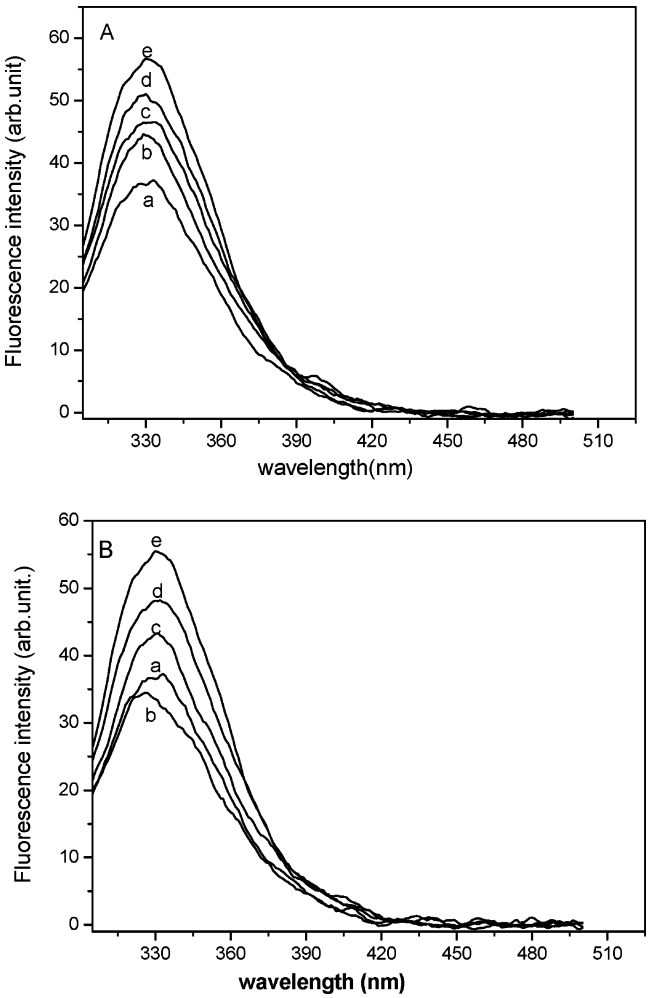

Figure 2. (A) Fluorescence emission[37] (λ_{exc} = 290 nm) of LcH (6 μM) in water (trace a) and in the presence of **8** at 1:0.5, 1:4, 1:5, and 1:6 molar ratios (traces b, c, d, e). (B) Fluorescence emission of LcH (6 μM) in water (trace a), in the presence of unglycosylated SC6CD-OH (trace b), in the presence of **8** (freshly mixed solution, trace e; after 1 h, trace d), and in the presence of **15** at 1:6 lectin/CD molar ratio (trace c).

have previously been observed for the unglycosylated and galactosylated alkylthio oligo(ethylene glycol) cyclodextrins.[9a] We have confirmed by carrying out encapsulation studies that such aggregates for SC12 (dodecylthio) and SC16 amphiphiles are vesicular in nature.[9a] The SC6 compound however is micellar or nanoparticulate.[8b,15c] Electron microscopy usually shows annular images for the collapsed vesicles of SC12 and SC16 amphiphiles but may also show them as apparent solid spheres, depending on experimental conditions.[9b,15a,28]

The annular images are more common with the amphiphiles having the longer lipophilic chains. Here this trend is also evident for the glycosylated amphiphiles; the particles formed by the SC6 and SC12 derivatives appear spherical, while the micrographs for the SC16 derivatives show central hollows due to the collapsed aqueous core. The grafting-on of glycosyl groups would increase the hydrophilic portion of all molecules; thus the appearance of the SC12 mannosylated and fucosylated derivatives is changed from that of the more clearly vesicular unglycosylated precursor.[8a,28]

Tryptophan fluorescence in LcH lectin was used as a measure of binding of glycosylated CDs to the protein. This lectin from the common lentil *L. culinaris* has been characterized[29] as two distinct hemagglutinins (LcH-A and LcH-B). The tetramer, composed of a repeated dimer, contains five tryptophans and seven tyrosines. It binds terminal α-D-mannosyl and α-D-glucosyl residues as well as fucose.[21]

Resolved in the presence of calcium and manganese ions (2LAL, Protein Data Bank),[30−32] glucose,[33] and sucrose (1LES, Protein Data Bank),[34] the structure showed Trp 128, Trp 152, Tyr 124, and Tyr 179 in proximity to the binding site. To the best of our knowledge, no crystalline structure of LcH mannose or LcH fucose complex has been obtained. On excitation at 290 nm the contribution of the Trp components is maximized and is not obscured by the Tyr emission.[35,36]

Fluorescence spectra of SC6 derivatives in the presence of *L. culinaris* lectin are illustrated in Figure 2.[37]

Figure 2A shows the fluorescence spectra of LcH (6 μM) and of LcH with **8** immediately after mixing at different molar ratios. The maximum of tryptophan emission (332 nm) in the spectrum of free LcH increases regularly with the addition of increasing amounts of mannosylated CD. In Figure 2B, fluorescence of the lectin alone (trace a) is compared with its emission intensity 1 h after mixing with mannosylated CD (trace d). The latter shows a decrease of about 10% compared with the fresh solution (trace e), which is probably due to particle aggregation over time due to the protein. At the same molar ratio, the fluorescence spectrum of LcH in the presence of the untargeted SC6CD-OH (Figure 2B, trace b) shows a lower intensity than the spectrum for the lectin with mannosylated CD. This aspecific interaction could be tentatively assigned to interaction of the tryptophans with the more polar hydrophilic regions of assembled amphiphiles.

A solution of lectin with fucosylated CD **15** at a 1:6 lectin/CD molar ratio (Figure 2B, trace c) also shows an increase of fluorescence emission. These results demonstrate a significant interaction between the glycosylated CDs and LcH lectin in contrast to unglycosylated CD.[38] The fluorescence increase is consistent with a less polar environment for the tryptophans in proximity to the binding site due probably to less exposure to water on binding the targeted CD.

Conclusions

The versatility of amphiphilic cyclodextrins has been previously shown by investigating the supramolecular binding of anionic photosensitizers in cationic amphiphilic nanoaggregates, cellular internalization, and cellular damage upon irradiation.[12a,c] Cationic amphiphilic CDs are also being developed as vectors for gene therapy.[6] Taken with previous results, this more efficient approach to the synthesis of targeted cyclodextrin assemblies is another example of their convergent synthesis using activated carbohydrates to give multiple linked recognition groups. Comparison of aggregates formed by the glycosylated CD derivatives with those formed by their unglycosylated precursors indicates that attachment of glycosyl groups does not interfere with their self-assembly. Selective binding to LcH lectin of mannosylated or fucosylated nanoaggregates confirms the potential of these self-assembling CDs for targeted drug delivery.

Experimental Section

General Conditions. Reagents and LcH lectin from *L. culinaris* were obtained from Sigma-Aldrich unless otherwise indicated; DMF, toluene, and methanol were anhydrous, from sealed bottles under N_2. Thin-layer chromatography (TLC) was carried out on Merck Kieselgel 60 analytical plates with the specified solvent system; cyclodextrin and saccharide derivatives were detected by UV light or by dipping in 5%

sulfuric acid−ethanol and heating. Lipophilic Sephadex LH-20 (25−100 μm) and silica gel (Kieselgel 60, 0.040−0.063 mm) from Merck were used for chromatography. NMR analyses, including ^{1}H−^{1}H COSY and ^{1}H−^{13}C HSQC, were performed on 300 and 500 MHz Varian Unity spectrometers in the indicated solvents. MALDI-TOF analyses were performed on a Perseptive (Framingham, MA) Voyager STR instrument equipped with delayed extraction technology. Ions were formed by a pulsed UV laser beam (nitrogen laser, λ = 337 nm) and accelerated through 24 kV. Samples were diluted in $CHCl_3$ and mixed 1:1 v/v with the matrix solution obtained by dissolving 2,5-dihydroxybenzoic acid (DHB) in CH_3OH/0.1% trifluoroacetic acid/CH_3CN (1:1:1 by volume) at a concentration of 30 mg/mL. Exactly 1 μL of this mixture was deposited onto a stainless steel 100 sample MALDI plate and allowed to dry at room temperature before running the spectra in the positive polarity.

Compounds **1**−**3** were prepared as previously described.[8a]

Heptakis[6-hexylthio-2-(ω-(*N*-tetra-*O*-acetyl-α-D-mannopyranosyl-thiocarbamoyl)-oligo(ethylene glycol))]-β-cyclodextrin (5). Compounds **1** (217 mg, 0.088 mmol), **4**[26] (265 mg, 0.691 mmol), and DABCO (208 mg, 1.86 mmol) were dissolved in anhydrous toluene (10 mL) under nitrogen. The toluene was evaporated using a rotary evaporator, and the residue was dried in vacuo for 18 h. Anhydrous toluene (10 mL) was then added, and the reaction mixture was stirred at room temperature. TLC (9:1 $CHCl_3$/MeOH) was used to monitor the reaction, and full conversion to product was observed after 7 days. Purification was performed by size exclusion chromatography (LH 20 Sephadex, eluent MeOH), and the resulting solid was dried in vacuo (415 mg, 93%). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 5.88 (br, 7 H, H-1′), 5.25 (br, 21 H, H-2′, H-3′, H-4′), 4.97 (br, 7 H, H-1), 4.56 (br, 14 H, CH_2−D), 4.30−3.60 (m, 77 H, CH_2−A, CH_2−B, CH_2−C, H-3, H-5, H-5′, H-6′a, H-6′b), 3.34 − 3.25 (br, 14 H, H-4, H-2), 2.95−2.73 (m, 14 H, H-6a, H-6b), 2.53 (m, 14 H, SCH_2), 2.09−1.84 (m, 84 H, (C═O)CH_3), 1.50 (m, 14 H, CH_2), 1.19 (m, 42 H, $(CH_2)_3$), 0.82 (m, 21H, CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ 191.8 (C═S), 170.3 (C═O × 4), 101.5 (C-1), 86.0−81.2 (C-1′, C-4, C-2), 73.7−67.0 (C-3, C-5, C-2′, C-5′, C-3′, C-4′, C−A, C−B, C−C), 62.5 (C−D, C-6′), 34.1 (C-6, SCH_2), 31.9 (CH_2), 30.2 (CH_2), 29.0 (CH_2), 23.0 (CH_2), 21.1 ((C═O)CH_3), 14.4 (CH_3) ppm. IR (cm^{-1}): 1534 (CSNH−). Anal. calcd for $C_{235}H_{379}N_7O_{114}S_{14}$ (23 EO): C, 50.63; H, 6.85; N, 1.76; S, 8.05. Found: C, 50.10; H, 6.76; N, 1.92; S, 8.50.

Heptakis [6-dodecylthio-2-(ω-(*N*-tetra-*O*-acetyl-α-D-mannopyranosyl-thiocarbamoyl)-oligo(ethylene glycol))]-β-cyclodextrin (6). Reaction of **2** (300 mg, 0.10 mmol) with **4** (295 mg, 0.76 mmol) and DABCO (232 mg, 2.07 mmol) in anhydrous toluene (10 mL) was performed as described for **5**. Purification was performed by gradient flash chromatography ($CHCl_3$ to 9:1 $CHCl_3$/MeOH), and the resulting solid was dried in vacuo (410 mg, 72%). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 5.97 (br, 7 H, H-1′), 5.59−5.21 (br, 21 H, H-2′, H-3′, H-4′), 5.05 (br, 7 H, H-1), 4.64 (br, 14 H, CH_2−D), 4.38 (br, 7 H, H-6′a), 4.15 (br, 7 H, H-6′b), 4.05−3.32 (m, 77 H, H-2, H-3, H-4, H-5, CH_2−A, CH_2−B, CH_2−C, H-5′), 3.00 (m, 14 H, H-6a, H-6b), 2.60 (m, 14 H, SCH_2), 2.18−2.08 (s, 84 H, (C═O)CH_3), 1.59 (m, 14 H, CH_2), 1.45−1.28 (m, 126 H, $(CH_2)_9$), 0.90 (m, 21 H, CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ 191.9 (C═S), 171.0−169.8 (C═O × 4), 101.3 (C-1), 85.7 (C-4), 81.0 (C-1′, C-2), 74.3−68.4 (C-3, C-5, C-5′, C-6′, C−A, C−B, C−C), 66.7−66.6 (C-2′, C-3′, C-4′), 62.3−61.6 (C−D, C-6′), 34.4−34.0 (C-6, SCH_2), 32.2 (CH_2), 30.6−29.4 $(CH_2)_9$), 23.0 (CH_2), 21.0 ((C═O)CH_3), 14.3 (CH_3) ppm. Anal. calcd for $C_{259}H_{427}N_7O_{105}S_{14}$ (14 EO): C, 53.93; H, 7.46; N, 1.70; S, 7.78. Found: C, 53.07; H, 7.05; N, 1.78; S, 9.26.

Heptakis[6-hexadecylthio-2-(ω-(*N*-tetra-*O*-acetyl-α-D-mannopyranosyl-thiocarbamoyl)-oligo(ethylene glycol))]-β-cyclodextrin (7). Reaction of **3** (300 mg, 0.087 mmol) with **4** (261 mg, 0.652 mmol) and DABCO (205 mg, 1.83 mmol) in anhydrous toluene (10 mL) was performed as described for **5**. Purification was performed by gradient flash chromatography ($CHCl_3$ to 9:1 $CHCl_3$/MeOH), and the resulting solid was dried in vacuo (350 mg, 65%). ^{1}H NMR (500 MHz, DMSO-d_6, 80 °C): δ 5.80 (br, 7 H, H-1′), 5.68 (m, 7 H, H-3′), 5.16−5.06 (br, 21 H, H-2′, H-4′, H-1), 4.53 (br, 14 H, CH_2−D), 4.18 (br, 7 H, H-6′a), 4.11 (br, 7 H, H-6′b), 4.06−3.38 (m, 70 H, H-3, H-5, CH_2−A, CH_2−B, CH_2−C, C-4, C-2), 3.05 (m, 14 H, H-6a, H-6b), 2.63 (m, 14 H, SCH_2), 2.09−1.95 (m, 84 H, (C═O)CH_3), 1.54 (m, 14 H, CH_2), 1.25 (m, 182 H, $(CH_2)_{13}$), 0.85 (m, 21 H, CH_3) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 80 °C): δ 191.6 (C═S), 170.4−169.8 (C═O × 4), 101.2 (C-1), 85.6 (C-4), 81.1 (C-1′, C-2), 71.6−67.3 (C-3, C-5, C-2′, C-3′, C-4′, C-5′, C−A, C−B, C−C), 62.8 (C-6′), 60.1 (C−D), 33.6 (C-6, SCH_2), 31.9 (CH_2), 30.2 (CH_2), 29.7 ($(CH_2)_{11}$), 29.3 (CH_2), 21.0 ((C═O)CH_3), 14.2 (CH_3) ppm. Anal. calcd for $C_{287}H_{483}N_7O_{105}S_{14}$ (14 EO): C, 55.95; H, 7.90; N, 1.59; S, 7.29. Found: C, 53.15; H, 6.92; N, 1.78; S, 7.56.

Heptakis [6-hexylthio-2-(ω-(*N*-α-D-mannopyranosyl-thiocarbamoyl)-oligo(ethylene glycol))]-β-cyclodextrin (8). Compound **5** (395 mg, 0.076 mmol) was dissolved in anhydrous MeOH (5 mL) under N_2. NaOMe (160 μL, 1 M methanolic solution) was added dropwise to this solution. TLC (9:1 $CHCl_3$/MeOH) was used to monitor the reaction, and full conversion to the deacetylated product was observed after 4 h. The solution was neutralized by the addition of IR-120 Amberlite resin (H^+ form) and stirred for 30 min. After filtration the solvent was evaporated under reduced pressure, and the resulting solid was dried in vacuo (320 mg, quantitative). ^{1}H NMR (500 MHz, DMSO-d_6, 80 °C): δ 9.45 (br, 7 H, NH), 5.67 (br, 7 H, OH), 5.08 (m, 14 H, H-1′, H-1), 4.52 (br, 14 H, CH_2−D), 3.94−3.72 (m, 35 H, H-2′, H-3′, H-5′, CH_2−C), 3.61−3.55 (m, 42 H, H-3, CH_2−B, H-4′, H-6′a, H-6′b), 3.46−3.35 (m, 35 H, H-2, H-4, H-5, CH_2−A), 3.06 (m, 14 H, H-6a, H-6b), 2.49 (br, 14 H, SCH_2), 1.48 (m, 14 H, CH_2), 1.28−1.13 (m, 56 H, $(CH_2)_4$), 0.79 (m, 21 H, CH_3) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 80 °C): δ 191.8 (C═S), 101.5 (C-1), 88.4−79.8 (C-2, C-4, C-1′), 77.5−71.4 (C-3, C-5, C−A, C−B, C−C, C-2′, C-3′, C-4′, C-5′), 61.5 (C−D, C-6′), 33.9 (C-6), 31.8 (SCH_2), 30.1 (CH_2), 28.9 (CH_2), 22.8 (CH_2), 18.7 ($(CH_2)_2$), 14.3 (CH_3) ppm. Anal. calcd for $C_{179}H_{323}N_7O_{86}S_{14}$ (23 EO): C, 48.88; H, 7.40; N, 2.22; S, 10.20. Found: C, 47.41; H, 7.14; N, 1.87; S, 10.15. MALDI-TOF MS *m*/*z*: 4025.2 $[M_{14EO,7Man} + Na]^+$, 4334.8 $[M_{21EO,7Man} + Na]^+$, 4643.9 $[M_{28EO,7Man} + Na]^+$.

Heptakis[6-dodecylthio-2-(ω-(*N*-α-D-mannopyranosyl-thiocarbamoyl)-oligo(ethylene glycol))]-β-cyclodextrin (9). Compound **6** (400 mg, 0.069 mmol) was suspended in anhydrous MeOH (10 mL). Anhydrous DMF (4 mL) was added to dissolve **6**. NaOMe (145 μL, 1 M in MeOH) was added dropwise to this solution. DMF was continually added throughout the reaction to maintain a homogeneous solution. TLC (9:1 $CHCl_3$/MeOH) was used to monitor the reaction, and full conversion to the deacetylated product was observed after 6 h. The solution was neutralized by the addition of IR-120 Amberlite resin (H^+ form) and stirred for 30 min. After filtration the solvent was evaporated under reduced pressure, and the resulting solid was dried in vacuo at 50 °C (220 mg, 69%). ^{1}H NMR (500 MHz, DMSO-d_6, 80 °C): δ 5.59 (br, 7 H, OH), 5.09 (m, 14 H, H-1, H-1′), 4.63−4.54 (m, 14 H, CH_2−D), 3.95−3.74 (m, 35 H, H-2′, H-3′, H-5′, CH_2−B), 3.62−3.56 (m, 42 H, H-3, CH_2−C, H-4′, H-6′a, H-6′b), 3.48−3.43 (m, 35 H, H-2, H-4, H-5, CH_2−A), 3.01 (m, 14 H, H-6a, H-6b), 2.57 (m, 14 H, SCH_2), 1.55 (m, 14 H, CH_2), 1.36 (m, 14 H, CH_2), 1.26 (m, 112H, $(CH_2)_8$), 0.86 (m, 21H, CH_3) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 80 °C): δ 191.8 (C═S), 100.9 (C-1), 85.7 (C-4), 79.5 (C-1′, C-2), 75.8 (C-5), 70.1−66.1 (C-3, C−A, C−B, C−C, C-2′, C-3′, C-4′, C-5′), 60.0 (C−D, C-6′), 33.3 (C-6), 32.6 (SCH_2), 30.8−28.0 $((CH_2)_8)$, 21.5 $(CH_2)_2$, 13.2 (CH_3) ppm. Anal. calcd for $C_{203}H_{371}N_7O_{77}S_{14}$ (14 EO): C, 53.11; H, 8.15; N, 2.14; S, 9.77. Found: C, 52.10; H, 8.35; N, 1.95; S, 10.00. MALDI-TOF MS *m*/*z*: 4615.5 $[M_{14EO,7Man} + Na]^+$, 4925.4 $[M_{21EO,7Man} + Na]^+$.

Heptakis [6-hexadecylthio-2-(ω-(*N*-α-D-mannopyranosyl-thiocarbamoyl)-oligo(ethylene glycol))]-β-cyclodextrin (10). Compound **7** (350 mg, 0.069 mmol) was suspended in anhydrous MeOH (10 mL). Anhydrous DMF (5 mL) was added to dissolve **7** fully. NaOMe (119 μL, 1 M methanolic solution) was added dropwise to this solution. DMF was continually added throughout the reaction to maintain

solution. TLC (9:1 $CHCl_3$/MeOH) was used to monitor the reaction, and full conversion to the deacetylated product was observed after 6 h. The solution was neutralized by the addition of IR-120 Amberlite resin (H^+ form) and stirred for 30 min. The solution was decanted, and the solvent was evaporated under reduced pressure. MeOH was added, and the precipitated solid was collected by centrifugation and dried in vacuo (170 mg, 67%). ^{1}H NMR (500 MHz, DMSO-d_6, 80 °C): δ 5.64 (br, 7 H, OH), 5.06 (m, 14 H, H-1, H-1′), 4.68−4.51 (m, 14 H, CH_2−D), 3.93−3.72 (m, 35 H, H-2′, H-3′, H-5′, CH_2−B), 3.61−3.58 (m, 42 H, H-3, H-4′, CH_2−C, H-6′a, H-6′b), 3.45−3.38 (m, 35 H, H-2, H-4, H-5, CH_2−A), 3.06 (m, 14 H, H-6a, H-6b), 2.56 (br, 14 H, SCH_2), 1.54 (br, 14 H, CH_2), 1.35−1.25 (br, 112 H, $(CH_2)_{13}$), 0.85 (m, 21 H, CH_3) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 80 °C): δ 191.3 (C═S), 100.7 (C-1), 85.4 (C-4), 78.7 (C-1′, C-2), 75.6 (C-5), 70.5−66.6 (C-3, C−A, C−B, C−C, C-2′, C-3′, C-4′, C-5′), 60.2 (C−D, C-6′), 32.6 (C-6, SCH_2), 30.8 (CH_2), 29.2 (CH_2), 28.7 ($(CH_2)_{11}$), 21.5 (CH_2), 13.1 (CH_3) ppm. Anal. calcd for $C_{231}H_{427}N_7O_{77}S_{14}$ (14 EO): C, 55.67; H, 8.64; N, 1.97; S, 9.01. Found: C, 53.73; H, 8.64; N, 2.13; S, 8.18. MALDI-TOF MS *m*/*z*: 5143.5 $[M_{17EO,7Man} + Na]^+$, 5185.56 $[M_{18EO,7Man} + Na]^+$, 5231.1 $[M_{19EO,7Man} + Na]^+$.

Heptakis[6-hexylthio-2-(ω-(*N*-tri-*O*-acetyl-β-L-fucopyranosyl-thiocarbamoyl)-oligo(ethylene glycol))]-β-cyclodextrin (12). Reaction of **1** (220 mg, 0.089 mmol) with **11** (230 mg, 0.691 mmol) and DABCO (211 mg, 1.89 mmol) in anhydrous toluene (8 mL) was performed as described for **5**. Purification was performed by size exclusion chromatography (LH-20 Sephadex, eluent MeOH), and the resulting solid was dried in vacuo (350 mg, 82%). ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): δ 5.58 (br, 7 H, H-1′), 5.29 (br, 7 H, H-3′), 5.16−5.05 (m, 21 H, H-2′, H-4′, H-1), 4.61 (br, 14 H, CH_2−D), 3.94−3.40 (m, 77 H, CH_2−A, CH_2−B, CH_2−C, H-2, H-3, H-4, H-5, H-5′), 2.92 (m, 14 H, H-6a, H-6b), 2.60 (br, 14 H, SCH_2), 2.17−2.00 (m, 63 H, (C═O)-CH_3), 1.77 (m, 14 H, CH_2), 1.58 (m, 14 H, CH_2), 1.37−1.20 (m, 49 H, $(CH_2)_2$, H-6′) 0.89 (m, 21 H, CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): δ 191.0 (C═S), 171.0−169.7 (C═O × 3), 101.2 (C-1), 86.1 (C-4), 83.7 (C-1′), 83.1−80.0 (C-2, C-3, C-5), 76.8−70.2 (C-2′, C-4′, C-5′, C−A, C−B, C−C), 61.6 (C−D), 34.0 (C-6), 31.8 (SCH_2), 29.9 (CH_2), 28.9 (CH_2), 22.8 ($(CH_2)_2$), 21.0 ((C═O)CH_3), 16.3 (C-6′), 14.3 (CH_3) ppm. IR (cm^{-1}): 1533 (CSNH−). Anal. calcd for $C_{212}H_{361}N_7O_{99}S_{14}$ (22 EO): C, 50.51; H, 7.21; N, 1.94; S, 8.90. Found: C, 50.37; H, 6.83; N, 2.00; S, 8.73.

Heptakis[6-dodecylthio-2-(ω-(*N*-tri-*O*-acetyl-β-L-fucopyranosyl-thiocarbamoyl)-oligo(ethylene glycol))]-β-cyclodextrin (13). Reaction of **2** (141 mg, 0.046 mmol) with **11** (118 mg, 0.36 mmol) and DABCO (109 mg, 0.97 mmol) in anhydrous toluene (7 mL) was performed as described for **5**. Purification was performed by gradient flash chromatography ($CHCl_3$ to 9:1 $CHCl_3$/MeOH), and the resulting solid was dried in vacuo (240 mg, 96%). ^{1}H NMR (500 MHz, DMSO-d_6, 80 °C): δ 9.52 (m, 7 H, NH), 5.64 (br, 7 H, H-1′), 5.18−5.08 (m, 28 H, H-2′, H-3′, H-4′, H-1), 4.53 (m, 14 H, CH_2−D), 4.06 (m, 7 H, H-5′), 3.95−3.71 (m, 14 H, H-3, H-5), 3.57−3.19 (m, 56 H, CH_2-A, CH_2−B, CH_2−C, H-2, H-4), 3.05 (m, 14 H, H-6a, H-6b), 2.57 (m, 14 H, SCH_2), 2.12−1.91 (m, 63 H, (C═O)CH_3), 1.54 (m, 14 H, CH_2), 1.36−1.26 (m, 126 H, $(CH_2)_9$), 1.08 (m, 21 H, H-6′), 0.87 (m, 21 H, CH_3) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 80 °C): δ 191.0 (C═S), 170.6−169.8 (C═O × 3), 100.1 (C-1), 84.0 (C-4), 83.9 (C-1′), 81.7 (C-2), 74.6 (C-3, C-5), 72.3−69.5 (C-2′, C-3′, C-4′, C-5′, C−A, C−B, C−C), 61.08 (C−D), 33.6 (C-6), 31.9 (SCH_2), 30.2−20.9 ($(CH_2)_{10}$), 20.8 ((C═O)CH_3), 16.4 (C-6′), 14.2 (CH_3) ppm. Anal. calcd for $C_{245}H_{413}N_7O_{91}S_{14}$ (14 EO): C, 54.88; H, 7.76; N, 1.83; S, 8.37. Found: C, 54.57; H, 7.42; N, 1.77; S, 8.32.

Heptakis[6-hexadecylthio-2-(ω-(*N*-tri-*O*-acetyl-β-L-fucopyranosyl-thiocarbamoyl)-oligo(ethylene glycol))]-β-cyclodextrin (14). Reaction of **3** (300 mg, 0.087 mmol) with **11** (220 mg, 0.67 mmol) and DABCO (206 mg, 0.97 mmol) in anhydrous toluene (10 mL) was performed as described for **4**. Purification was performed by gradient flash chromatography ($CHCl_3$ to 9:1 $CHCl_3$/MeOH), and the resulting solid was dried in vacuo (340 mg, 68%). ^{1}H NMR (500 MHz, DMSO-d_6, 80 °C): δ 9.29 (br, 7 H, NH), 5.64 (br, 7 H, H-1′), 5.19−5.00 (m, 28 H, H-2′, H-3′, H-4′, H-1), 4.52 (br, 14 H, CH_2−D), 4.06 (m, 7 H, H-5′), 3.96−3.42 (m, 70 H, H-2, H-3, H-4, H-5, CH_2−A, CH_2−B, CH_2−C), 3.06 (m, 14 H, H-6a, H-6b), 2.63 (br, 14 H, SCH_2), 2.12−1.94 (s, 63H, (C═O)CH_3), 1.54 (m, 14 H, CH_2), 1.53−1.26 (m, 182 H, $(CH_2)_{13}$), 1.08 (m, 21 H, H-6′), 0.89 (m, 21 H, CH_3) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 80 °C): δ 191.6 (C═S), 169.5−168.7 (C═O × 3), 100.2 (C-1), 91.8 (C-4), 84.9 (C-1′), 82.8 (C-2), 73.1 − 70.1 (C-3, C-5), 69.9−66.1 (C-2′, C-3′, C-4′, C-5′, C−A, C−B, C−C), 60.1 (C−D), 33.1 (C-6), 32.6 (SCH_2), 30.9 (CH_2), 29.2−28.1 ($(CH_2)_{12}$), 21.6 (CH_2), 19.9 ((C═O)CH_3), 15.4 (C-6′), 13.6 (CH_3) ppm. Anal. calcd for $C_{273}H_{469}N_7O_{91}S_{14}$ (14 EO): C, 56.98; H, 8.21; N, 1.70; S, 7.79. Found: C, 56.90; H, 8.23; N, 1.50; S, 8.08.

Heptakis[6-hexylthio-2-(ω-(*N*-β-L-fucopyranosyl-thiocarbamoyl)-oligo(ethylene glycol))]-β-cyclodextrin (15). Compound **12** (350 mg, 0.073 mmol) was treated with NaOMe (154 μL, 1 M in MeOH) as described for **8**, and the resulting solid was dried in vacuo (305 mg, quantitative). ^{1}H NMR (500 MHz, DMSO-d_6, 80 °C): δ 5.03 (m, 14 H, H-1′, H-1), 4.50 (m, 14 H, CH_2−D), 4.09−3.40 (m, 77 H, H-2, H-3, H-4, H-5, CH_2−A, CH_2−B, CH_2−C, H-5′), 3.05 (br, 14 H, H-6a, H-6b), 2.53 (br, 14 H, SCH_2), 1.51 (m, 14 H, CH_2), 1.37−1.29 (m, 42 H, $(CH_2)_3$), 1.13 (d, 21 H, J = 5.9 Hz, H-6′), 0.83 (m, 21 H, CH_3). ^{13}C NMR (125 MHz, DMSO-d_6, 80 °C): δ 192.1 (C═S), 101.2 (C-1), 86.1 (C-4), 83.7 (C-1′), 82.9−80.1 (C-2, C-3, C-5), 75.9−68.5 (C-2′, C-3′, C-4′, C-5′, C−A, C−B, C−C), 61.6 (C−D), 34.2 (C-6), 32.1 (SCH_2), 30.0 ($(CH_2)_2$), 29.9 (CH_2), 23.5 (CH_2), 17.1 (C-6′), 14.4 (CH_3) ppm. Anal. calcd for $C_{177}H_{319}N_7O_{78}S_{14}$ (22 EO): C, 50.11; H, 7.58; N, 2.31; S, 10.58. Found: C, 47.09; H, 7.07; N, 2.28; S, 6.28. MALDI-TOF MS *m*/*z*: 3911.7 $[M_{14EO,7Fuc} + Na]^+$, 4217.9 $[M_{21EO,7Fuc} + Na]^+$, 4526.5 $[M_{28EO,7Fuc} + Na]^+$, 4834.5 $[M_{35EO,7Fuc} + Na]^+$.

Heptakis[6-dodecylthio-2-(ω-(*N*-β-L-fucopyranosyl-thiocarbamoyl)-oligo(ethylene glycol))]-β-cyclodextrin (16). Compound **13** (220 mg, 0.041 mmol) was dissolved in an 8:3 MeOH/DMF solution (11 mL). NaOMe (86 μL, 1 M methanolic solution) was added dropwise to this solution. TLC (9:1 $CHCl_3$/MeOH) was used to monitor the reaction, and full conversion to the deacetylated product was observed after 24 h. The solution was neutralized by the addition of IR-120 Amberlite resin (H^+ form) and stirred for 30 min. After filtration the solvent was evaporated under reduced pressure, and the resulting solid was dried in vacuo (175 mg, 97%). ^{1}H NMR (500 MHz, DMSO-d_6, 80 °C): δ 9.09 (br, 7 H, NH), 5.04 (m, 14 H, H-1, H-1′), 4.51 (m, 14 H, CH_2−D), 4.03−3.40 (m, 84 H, H-2′, H-3′, H-4′, H-5′, H-3, H-5, CH_2−A, CH_2−B, CH_2−C), 3.05 (m, 14 H, H-6a, H-6b), 2.56 (br, 14 H, SCH_2), 1.56 (m, 14 H, CH_2), 1.35 (m, 14 H, CH_2), 1.26 (m, 112 H, $(CH_2)_8$), 1.14 (m, 21 H, H-6′), 0.86 (m, 21 H, CH_3) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 80 °C): δ 191.5 (C═S), 101.0 (C-1), 86.3 (C-1′), 85.3 (C-4), 80.2 (C-2), 76.1−68.0 (C-2′, C-3′, C-4′, C-5′, C-3, C-5, C−A, C−B, C−C), 60.3 (C−D), 33.3 (C-6), 32.6 (SCH_2), 30.8 (CH_2), 29.1−28.0 ($(CH_2)_8$), 21.6 (CH_2), 16.1 (C-6′), 13.2 (CH_3) ppm. Anal. calcd for $C_{203}H_{371}N_7O_{70}S_{14}$ (14 EO): C, 54.44; H, 8.35; N, 2.19; S, 10.02. Found: C, 52.56; H, 8.24; N, 2.03; S, 10.55. MALDI-TOF MS *m*/*z*: 4503.2 $[M_{14EO,7Fuc} + Na]^+$, 4811.8 $[M_{21EO,7Fuc} + Na]^+$.

Heptakis[6-hexadecylthio-2-(ω-(*N*-β-L-fucopyranosyl-thiocarbamoyl)-oligo(ethylene glycol))]-β-cyclodextrin (17). Compound **14** (340 mg, 0.059 mmol) was suspended in a 10:1 DMF/MeOH (11 mL) solution. The reaction mixture was stirred at 25 °C, and NaOMe (123 μL, 1 M methanolic solution) was added dropwise. DMF was continually added throughout the reaction to maintain solution. TLC (9:1 $CHCl_3$/MeOH) was used to monitor the reaction, and full conversion to the deacetylated product was observed after 7 h. The solution was neutralized by the addition of IR-120 Amberlite resin (H^+ form) and stirred for 30 min. The solution was decanted, and the solvent was evaporated under reduced pressure. MeOH was added, and the precipitated solid was collected by centrifugation and dried in vacuo (190 mg, 66%). ^{1}H NMR (500 MHz, DMSO-d_6, 80 °C): δ 5.04 (m, 14 H, H-1, H-1′), 4.52 (m, 14 H, CH_2−D), 4.09−3.93 (m, 21 H, H-2′, H-3′, H-4′), 3.90−3.29 (m, 70 H, H-2, H-3, H-4, H-5, CH_2−A, CH_2−

B, CH_2−C), 3.07 (m, 14 H, H-6a, H-6b), 2.54 (br, 14 H, SCH_2), 1.53 (m, 14 H, CH_2), 1.34 (m, 14 H, CH_2), 1.23 (m, 168 H, $(CH_2)_{12}$), 1.14 (m, 21 H, H-6′), 0.83 (m, 21 H, CH_3) ppm. ^{13}C NMR (125 MHz, DMSO-d_6, 80 °C): δ 191.3 (C═S), 100.1 (C-1), 85.3 (C-1′, C-4), 82.4−80.3 (C-2, C-3, C-5), 74.0−67.6 (C-2′, C-3′, C-4′, C-5′, C−A, C−B, C−C), 60.14 (C−D), 33.0 (C-6), 32.6 (SCH_2), 30.9 (CH_2), 28.7 ($(CH_2)_{12}$), 21.5 (CH_2), 16.1 (C-6′), 13.0 (CH_3) ppm. Anal. calcd for $C_{231}H_{427}N_7O_{70}S_{14}$ (14 EO): C, 56.95; H, 8.83; N, 2.01; S, 9.21. Found: C, 56.87; H, 8.89; N, 1.59; S, 9.33. MALDI-TOF MS *m*/*z*: 4270.82 $[M_{14EO,4Fuc} + Na]^+$, 4475 $[M_{14EO,5Fuc} + Na]^+$, 4681.5 $[M_{14EO,6Fuc} + Na]^+$.

Dynamic Light Scattering. Stock solutions (1 mg/mL) of compounds **8**−**10** and **15**−**17** were made by dissolving 1 mg of sample in 1 mL of $CHCl_3$. These solutions were then sealed and stored at 4 °C. To form the vesicles, 50 μL of the stock solution was transferred to a sample vial and evaporated under reduced pressure to form a thin film. Deionized water (5 mL) was then added to the vial, thus making the concentration of the vesicle solution 0.01 mg/mL. Each solution was maintained at 40 °C for 1 h, then sonicated in a sonication bath at 60 °C for 1.5 h. After being cooled, 2 mL of the vesicle suspension was filtered through a 0.45 μm microfilter and investigated using a Malvern Autosizer 4700 instrument with an Innova 70 Laser (instrument settings: temperature = 25 °C; water viscosity = 0.89 cP; refractive index = 1.333; laser wavelength = 488 nm; voltage = 200 mV; aperture = 100 μm, scattering angle = 90°). The analysis was performed by using the Cumulant program as provided by Malvern Instruments.

Electron Microscopy. For TEM, samples of the amphiphilic CDs were formulated in water as described above at a concentration of 0.1 mg/mL, and applied to 200 mesh copper grids coated with Formvar and a thin carbon layer. A drop of the colloid solution was placed on the grid and allowed to stand for 2 min, then mostly blotted away. The specimen was stained with a drop of 2% w/w uranyl acetate solution, which after standing for 2 min was also blotted away. The specimens were examined with a JEOL 2000 electron microscope operated at 80 kV. Electron micrographs were taken at a magnification of 100 000−200 000.

UV−vis absorption spectra were recorded on a Hewlett-Packard HP 8453 diode-array spectrophotometer in a quartz cell of path length 0.1 cm; and fluorescence measurements on a Jasco model FP-750 spectrofluorimeter with a cuvette path length 3 mm, in the λ range 300−500 nm. All experiments were run at least three times.

Fluorescence Spectroscopy. Lectin from *L. culinaris* (LcH) stock solutions (40 μM for M_w 25598, repetitive dimer) were prepared in microfiltered water, sonicated for a few minutes, and stored at 4 °C overnight. CD colloids were prepared, as described conventionally for liposomes,[39] from stock solutions (500 μM) of **8** and **15**. These CDs were dissolved in $CHCl_3$, slowly evaporated overnight to form thin films, hydrated, and sonicated for 20 min at 50 °C. The precursor of **8** (unglycosylated SC6CD-OH) was formulated from stock solution (1 mM).

The mixed solutions of lectin and CD were prepared in microfiltered water (pH 7) by adding the solution of CD to solutions of protein. The samples were stirred by vortexing (Heidolph) and analyzed by UV and steady-state fluorescence. The investigated colloidal systems were studied at 1:0.5, 1:4, 1:5, and 1:6 lectin/CD molar ratios ([LcH] = 6μM)] both as freshly mixed solutions and after 1 h of incubation (T = 25 °C). Solutions of glycosylated CDs (SC6CD-Man and SC6CDβ-Fuc) alone at the same concentrations as in the lectin and CD mixed solutions were also prepared and analyzed by UV spectroscopy.

The measured absorption spectrum of a mixed sample is the sum of two components, due to absorption and scattering, of both the lectin and lectin−CD complex.[40] To compare the fluorescence emission intensities of the mixed samples with that of protein alone, the emission from each mixed sample was corrected by UV analysis according to the relation

$$I_{Fs} = I'_{Fs}10^{((\Delta A_s(ex)+\Delta A_s(em))/2)} \quad (1)$$

where I_{Fs} is the fluorescence intensity corrected for the measured absorbance of the mixed sample s and I'_{Fs} the measured fluorescence. ΔA_s(ex) represents the lectin/CD sample absorbance corrected for lectin absorbance at the excitation wavelength (290 nm), and ΔA_s(em) is the difference between the lectin−CD mixed sample and the lectin absorbance, respectively, in the emission range. The factor of $^1/_2$ in eq 1 takes into account the experimental geometry: average half optical path length each for excitation and emission. In most of the samples it is possible to neglect ΔA_s(em) (absorption by CD in the emission range).

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Supporting Information Available. MALDI spectra and DLS results for compounds **8**, **9**, **10**, **15**, **16**, and **17**, UV spectra of LcH lectin in the presence of compounds **8** and **15**. This material is available free of charge via the Internet at http://pubs.acs.org

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