Synthesis of Persialylated β-Cyclodextrins

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The synthesis of homogeneous hepta-antennated C-6 branched sialosyl cyclomaltoheptose derivatives (persialylated β-cyclodextrins) has been performed in good to excellent yields, and the compounds have been fully characterized. The thioacetate *N*-acetylneuraminic acid derivative **6** was selectively de-S-acetylated and coupled by nucleophilic displacement in a one-pot reaction to the heptakis(chloroacetamido) β-CDs **2** and **5**, yielding multivalent sialosides **8** and **9**, respectively. The thiourea-linked sialyl-CD **10** was obtained by reaction of the 4-isothiocyanatophenyl *N*-acetylneuraminic acid derivative **7** with the per-*tert*-butoxycarbonylamino β-CD derivative **2** after suitable deprotection of the amino function.

Sialic acids (neuraminic acid derivatives)[1] are common mammalian sugars that usually end oligosaccharide sequences of glycolipids, N- and O-linked glycoproteins, and some proteoglycans. As such, sialic acids are forefront carbohydrate haptens responsible for a wide variety of recognition events.[2] Cell surface sialosides are involved as anchoring motifs for microbial attachment. Various pathogenic agents such as viruses, bacteria, and bacterial toxins can adhere and colonize host tissues after binding to sialosides.[3] It has also been demonstrated that blocking receptor sites with high serum carbohydrate concentrations including sialic acid can prevent bacterial infections[4] and cancer metastasis.[5] As a consequence of these facts, the design of sialoside-based inhibitors is currently a challenge in therapeutics. To achieve medicinal applications of such artificial inhibitors, their construction has to overcome the low binding affinities of sialic acids toward their receptor counterparts as a major drawback.[6] As several cell-surface oligosaccharides and their corresponding receptors are also multivalent, the so-called cluster effect[7] has attracted considerable attention and promoted the rational synthesis of a wide variety of multivalent glycoconjugates that can mimic multiantennary glycoproteins.[8−14]

In this regard, cyclodextrins (CDs)[15] have found widespread applications as scaffolds and templates in supramolecular chemistry.[16] In particular, CDs have been used as cores in the synthesis of medium-sized glycoconjugates, mainly with the aim of developing new vectors for site-specific delivery of therapeutics. Those structures try to combine both the inclusion capabilities of the hydrophobic cavity of CDs[17] and the high biological receptor binding ability of multiple saccharide epitopes in the same molecule. The majority of such CD-based glycoconjugates synthesized chemically or chemoenzymatically are monosubstitued derivatives at a single primary position of the CDs in which monovalent branches of simple sugars as well as disaccharides and oligosaccharides have been bound either by direct means[18] or via a spacer arm.[18a,19] Recently, the monoconjugation of β-mannosylated dendritic branches has been described.[20] Persubstituted CDs are scarce, and only a few of them contain mono-[18c,e,19b,21] or disaccharidic monovalent

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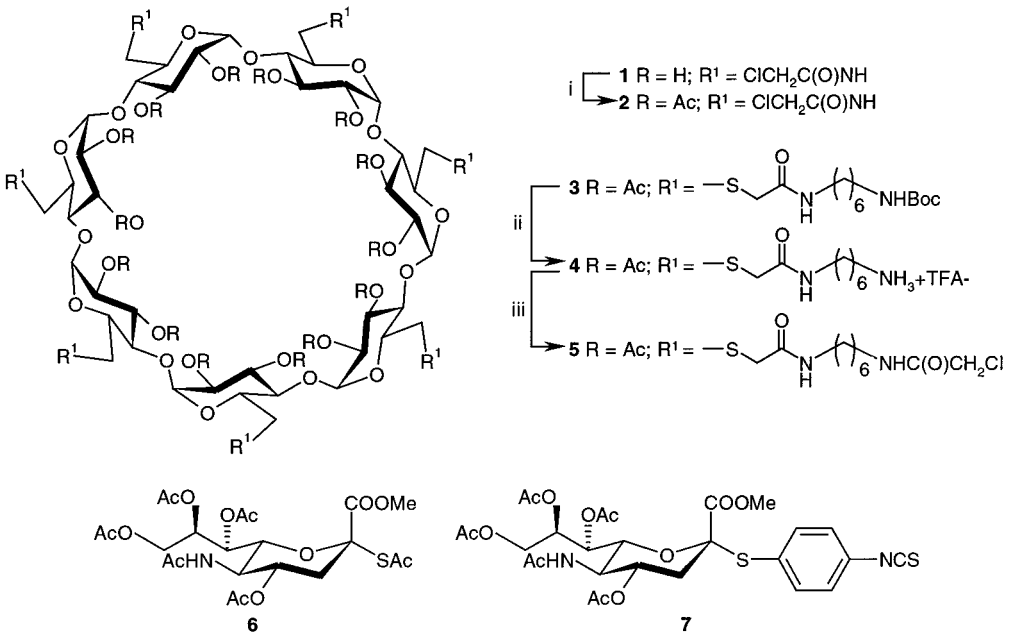
Scheme 1[a]



[a] Key: (i) AcCl−AcOH−Ac_2O, 4 days; (ii) 20% TFA in CH_2Cl_2, 3 h; (iii) $(ClCH_2CO)_2O$, Et_2N, DMF, 2 h.

branches,[18e,22] as well as divalent branches.[22] Grafting of the saccharides to the CDs core has been performed in the majority of cases by nucleophilic displacement[18a−c,21a−c,22] or by means of amide[18b,d,19] or thiourea[18e,20,21b] linkages obtained by reaction of isothiocyanates with amines. To the best of our knowledge, fully sialylated constructs onto CD scaffolds have not yet been previously reported. Only Sakegawa et al.[23] have communicated the grafting of two sialic acids residues to α-cyclodextrin. Continuing our efforts in the design of multivalent glycoforms varying in molecular weights, shapes, valencies and geometries, we decided to prepare persialylated *β*-cyclodextrins.

As a first approach to achieve this objective, we chose the readily available thioacetylated sialic acid derivative **6** (Scheme 1)[24] as an adequate building block suitable for attachment onto *β*-CD templates by nucleophilic displacement reactions. To avoid the construction of congested architectures that will result from the direct attachment of sialic acid at the convergent CD's lower rim and on the basis of our previous experience,[21b] *β*-CD-containing chloroacetamido functions at the primary position appeared to be the template of choice to carry out such substitution reactions. The previously described *N*-chloroacetamide derivative **1**,[21b] obtained by addition of chloroacetic anhydride to a suspension of per-6-amino-6-deoxy-*β*-CD[25] in methanol, was O-acetylated with the acylating mixture AcCl−AcOH−Ac_2O prior to the coupling with **6** to facilitate the isolation and characterization of the resulting coupling product. The 2,3-di-O-acetylated derivative **2** was thus obtained in 77% yield and then treated with **6**, which was selectively de-S-acetylated in situ by treatment with diethylamine, yielding adduct **8** in 57% yield (Scheme 2).

Next, we prepared the assembly of **6** onto the *β*-CD core through a longer spacer using per-*tert*-butoxycarbonylamino derivative **3** previously prepared by us,[21b] which was converted into the corresponding N-chloroacetylated derivative **5** by treatment with chloroacetic anhydride. Removal of the Boc protecting group (20% TFA in CH_2Cl_2) of **3** afforded the crude compound **4**, which was directly treated with chloroacetic anhydride to give **5** in 81% yield. Treatment of this compound with the thioacetate sialic acid derivative **6**, using the same reaction conditions as those described above for the preparation of compound **8**, afforded fully sialylated *β*-CD derivative **9** in 83% yield.

Considering the generally higher inhibitory potency of aromatic haptenic sugars,[26] the 4-isothiocyanatophenyl derivative **7**[27] was used next as a building block to provide thiourea-linked *β*-CD clusters. Direct grafting of **7** onto the primary position of *β*-CD by reaction with a heptaamino CD derivative[25] in DMF failed to afford the corresponding homogeneous persubsituted *β*-CD derivative, and only a complex mixture of compounds was detected by TLC that was not further investigated. However, reaction of **7** with compound **4**, obtained by N-Boc deprotection of compound **3** as indicated above,

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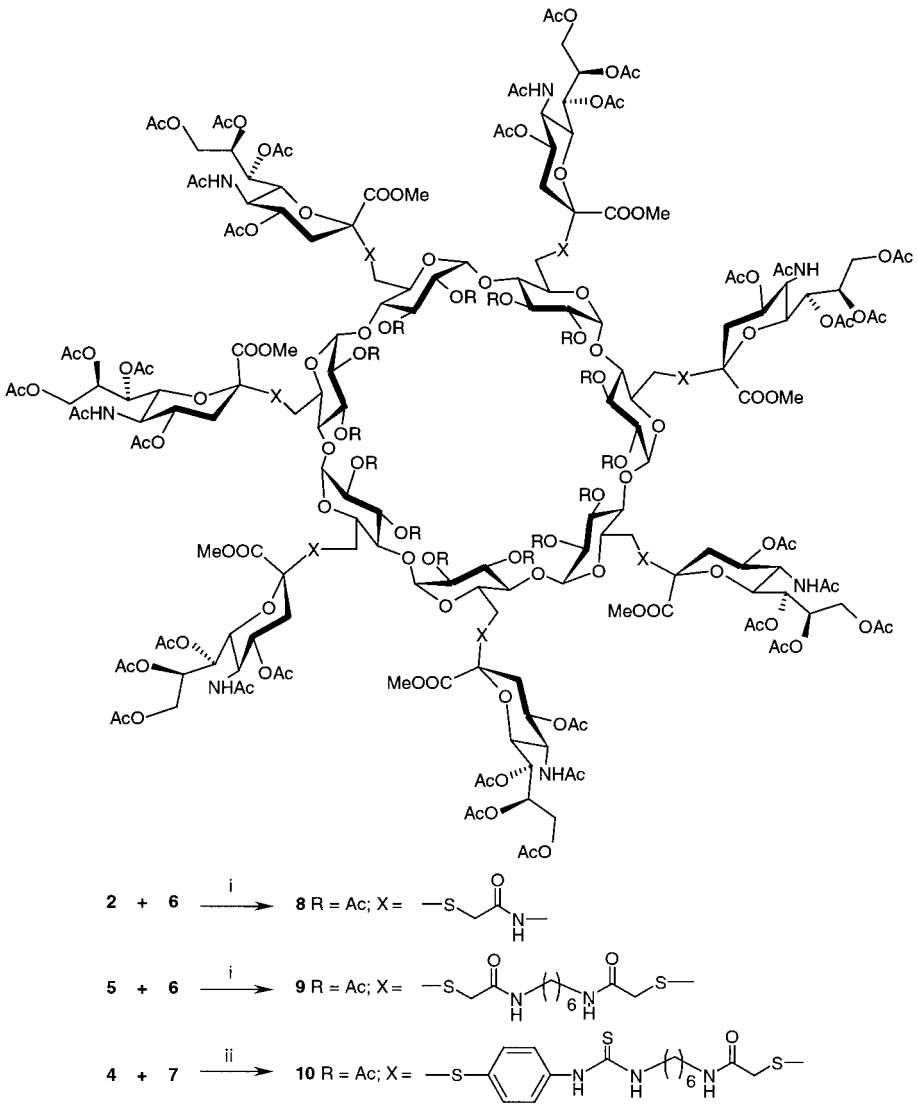
Scheme 2[a]



[a] Key: (i) Et_2NH, room temperature, 3 days; (ii) DIPEA, DMF, room temperature, 4 days.

using dry DMF as solvent and DIPEA as base under a nitrogen atmosphere, yielded the persialylated β-CD derivative **10** in excellent yield (94%).

Per-sialylated β-CDs **8**−**10** were characterized by NMR spectroscopic data. The room-temperature ^1H NMR spectra showed a considerable broadening of the signals that indicated restricted mobility on the NMR time scale in accordance with previous observation in other persubstituted β-CDs.[21b,c] When measurements of the NMR data were performed at 50−70 °C, the resolution of the spectra was considerably improved, allowing the assignments of the signals which were made on the basis of $^1H-^1H$ NOESY and $^{13}C-^1H$ HMQC correlation experiments. The success of the assembly became evident from the 7-fold symmetry of the products, as deduced from NMR experiments.

In conclusion, the synthesis of homogeneous hepta-antennated C-6 substituted sialosyl cyclomaltoheptose derivatives (persialylated β-cyclodextrins) have been performed in good to excellent yields and the compounds fully characterized. The preparation of such multivalent sialosides with potential biorecognizable properties contributes to expand the already existing array of such glycoforms.

Experimental Section

For typical experimental protocols, see ref 21b. ^1H and ^{13}C spectra were recorded at 400 MHz for compound **10** and 500 MHz for compounds **2**, **5**, **8**, and **9**. ^1H and ^{13}C resonances were assigned by $^1H-^1H$ NOESY and $^{13}C-^1H$ HMQC correlation experiments.

Synthesis of Compound 2. Compound **1**[21b] (1 g, 0.6 mmol) was suspended in a mixture of AcCl−AcOH−Ac_2O (2:2:1; 5, 5, and 2.5 mL). The reaction mixture was maintained at room temperature under strong magnetic stirring. The course of the reaction was monitored by TLC (chloroform−methanol 5:1). After 4 days the reaction was estimated to be complete. Ether−hexane (1:1; 250 mL) was then added, giving **2** (1.04 g, 77%) as a solid that was filtered: mp 227−230 °C; $[\alpha]_D$ +86° (*c* 1, chloroform); IR (KBr) 3455, 1751, 1655, 1246 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.15 (br s, 7 H, NH), 5.15 (t, 7 H, J = 9.0 Hz, H-3), 5.04 (d, 7 H, J = 3.2 Hz, H-1), 4.76 (dd, 7 H, J = 10.0 and 3.2 Hz, H-2), 4.13 (AB system, 14 H, J = 13.3 Hz, Δδ = 14.4 Hz, CH_2NH), 3.98 (m, 7 H, H-5), 3.75 (t, 7 H, J = 8.8 Hz, H-4), 3.60 (m, 14 H, H-6,6′), 2.00, 1.98 (2 s, 42 H, 2 MeCO); ^{13}C NMR (DMSO-d_6) δ 169.9, 169.2, 167.7 (CO), 96.5 (C-1), 77.5 (C-4), 70.0, 69.8 (C-2,3,5), 42.6 (CH_2Cl), 39.6 (C-6), 20.9, 20.5 (MeCO); HRMS (FAB) *m*/*z* calcd for $C_{84}H_{112}Cl_7N_7O_{49}$ + Na 2274.418 $(M + Na)^+$, found 2274.470.

Synthesis of Compound 5. A solution of trifluoroacetic acid in methylene chloride (20% v/v, 20 mL) was added to

compound **3**[21b] (0.6 g, 0.16 mmol). After 3 h at room temperature TLC (chloroform−methanol 8:1) indicated complete disappearance of the starting material. Removal of the solvent and the excess trifluoroacetic acid was effected by evaporation under vacuum, giving crude compound **4** that was immediately used without purification by dissolving in anhydrous DMF (3.0 mL). Then chloroacetic anhydride (0.45 g, 2.3 mmol) and triethylamine (1.0 mL) were added. The reaction mixture was left at room temperature for 2 h. Evaporation and coevaporation with toluene (2 × 50 mL) gave a crude product that was purified by column chromatography (chloroform−methanol 9:1) to give **5** (0.466 g, 81%) as a syrup: ^{1}H NMR (DMSO-d_6) δ 8.14 (br s, 7 H, NH), 7.98 (br s, 7 H, NH), 5.18 (t, 7 H, J = 9.4 Hz, H-3), 5.03 (dd, 7 H, J = 2.6 Hz, H-1), 4.68 (dd, 7 H, J = 9.4 and 2.6 Hz, H-2), 4.06 (m, 7 H, H-5), 4.01 (s, 14 H, CH_2-Cl), 3.89 (t, 7 H, J = 9.0 Hz, H-4), 3.24 (m, 7 H, H-6), 3.07−3.00 (m, 35 H, H-6 and 2 CH_2NH), 2.00, 1.99 (2 s, 42 H, 2 MeCO), 1.38 and 1.24 (2 m, 56 H, $(CH_2)_4$); ^{1}H NMR (Cl_3CD) δ 7.04 (br s, 7 H, NH), 6.30 (br s, 7 H, NH), 5.20 (t, 7 H, J = 9.0 Hz, H-3), 5.03 (d, 7 H, J = 3.4 Hz, H-1), 4.70 (dd, 7 H, J = 9.9 and 3.4 Hz, H-2), 4.10 (m, 7 H, H-5), 4.00 (s, 14 H, CH_2Cl), 3.68 (t, 7 H, J = 8.0 Hz, H-4), 3.40−2.90 (m, 56 H, H-6,6′, CH_2S, 2 NHCH_2), 2.02, 1.98 (2 s, 42 H, 6 MeCO), 1.51, 1.31 (2 m, 56 H, $(CH_2)_4$); ^{13}C NMR (DMSO-d_6) δ 170.1, 169.2, 168.9, 168.4 (CO), 96.2 (C-1), 71.9 (C-4), 71.2 (C-2), 70.2 (C-3,5), 42.6 (CH_2Cl), 38.9, 38.8 (CH_2NH), 36.6 (C-6), 35.1 (SCH_2CO), 28.9, 28.8, 26.2, 26.1 (4 $(CH_2)_4$), 20.6 (*Me*CO); ^{13}C NMR (Cl_3CD) δ 170.6, 169.6, 166.3 (CO), 96.6 (C-1), 79.2 (C-4), 71.3, 70.5, 70.5 (C-2,3,4), 42.6 (CH_2Cl), 40.0 (C-6), 39.6 (CH_2NH), 29.1, 29.0, 26.3, 26.2 ($(CH_2)_4$), 20.7, 20.6 (MeCO); HRMS (FAB) *m/z* calcd for $C_{140}H_{210}Cl_7N_{14}O_{56}S_7$ (M+ Na)$^+$ 3480.86, found 3480.00.

Synthesis of Compound 8. To a degassed solution of **2** (0.150 g, 0.066 mmol) and **6** (0.467, 0.93 mmol) in DMF (3 mL) was added diethylamine (0.150 mL, 2.05 mmol) under a nitrogen atmosphere. The reaction mixture was kept at room temperature for 3 days. After this time, the solution was evaporated under vacuum. The resulting mixture was processed by taking it up in EtOAc (200 mL) and washing the solution several times with 5% HCl solution (30 mL) and water (2 × 30 mL). Evaporation of the dried (Na_2SO_4) solution and coevaporation with toluene (2 × 30 mL) gave a crude product that was dissolved in the minimum amount of EtOAc. Ether was added, giving **8** as a white solid that was filtered (0.210 g, 57%): mp 164−167 °C; $[\alpha]_D$ +60° (*c* 1, chloroform); IR (KBr) 3443, 1744, 1670, 1236 cm^{-1}; ^{1}H NMR (Cl_3CD) δ 6.88 (m, 14 H, 2 NH), 5.41 (m, 7 H, H-8′), 5.37−5.31 (m, 14 H, H-3,7′), 5.29 (d, 7 H, J = 3.8 Hz, H-1), 4.87 (m, 7 H, H-4′), 4.85 (dd, 7 H, J = 10.0 and 3.7 Hz, H-2), 4.27 (dd, 7 H, J = 11.2 and 2.3 Hz, H-9′), 4.14−3.64 (m, 49 H, H-4,5,6,6,5′,6′,9′), 3.81 (s, 21 H, MeO), 3.65, 3.50 (2 d, AB system, 14 H, J = 15.4 Hz, SCH_2), 2.74 (m, 7 H, H-3′), 2.14, 2.11, 2.02, 2.01, 2.00, 1.99, 1.85 (7 s, 147 H, 7 MeCO), 1.92 (m, 7 H, H-3′); ^{1}H NMR (DMSO-d_6, 70 °C) δ 7.50 (d, 7 H, J = 8.2 Hz; N*H*Ac), 7.39 (br s, 7 H, NH), 5.26 (m, 7 H, H-8′), 5.22−5.13 (m, 2 H, H-3,7′), 5.10 (dd, 7 H, J = 2.9 Hz, H-1), 4.77 (dd, 7 H, J = 9.5 and 2.8 Hz, H-2), 4.73 (m, 7 H, H-4′), 4.23 (dd, 7 H, J = 12.0 and 2.6 Hz, H-9′), 4.04 (dd, 7 H, J = 12.0 and 6.0 Hz, H-9′), 4.00, 3.88−3.57, 3.46−3.25 (3 m, 70 H, H-4,5,6,6,5′,6′, 2 CH_2CO), 3.76 (s, 21 H, MeCO), 2.67 (dd, 7 H, J = 11.2 and 3.0 Hz, H-3′), 2.06, 2.00, 1.99, 1.95, 1.95, 1.91, 1.65 (7 s, 147 H, 7 MeCO), 1.79 (br t, 7 H, J = 12.3 Hz, H-3′); ^{13}C NMR (Cl_3CD): δ 171.0, 170.7, 170.6, 170.4, 170.0, 169.4, 168.6, 168.4 (CO), 96.7 (C-1), 82.5 (C-1′), 73.9, 70.8, 70.5, 70.3, 69.9, 69.4, 68.3, 67.03 (C-2,3,4,5,4′,6′,7′,8′), 62.1 (C-9), 53.5 (MeO), 49.4 (C-5), 30.8 (C-6), 37.4 (C-3), 32.5 (SCH_2CO), 21.3, 20.8, 20.5 (*Me*CO); ^{13}C NMR (DMSO-d_6, 70 °C) δ 169.6, 169.3, 169.2, 168.9, 168,7, 168.0, 167.4, 96.0, 82.3, 75.81, 73.5, 70.3, 69.7, 69.5, 69.3, 69.0, 67.1, 61.6, 52.7. 47.8, 39.0, 37.2, 31.8, 22.2, 20.5, 20.4, 20.2, 20.1; HRMS (FAB) *m/z* calcd for $C_{224}H_{308}N_{14}O_{133}S_7$ + Na 5571.58 (M + Na)$^+$, found 5571.59.

Synthesis of Compound 9. To a degassed solution of **5** (0.198 g, 0.057 mmol) and **6** (0.402, 0.8 mmol) in DMF (3 mL) was added diethylamine (0.125 mL, 1.19 mmol) under a nitrogen atmosphere. The reaction mixture was kept at room temperature for 3 days. After this time the solution was evaporated under vacuum. The resulting mixture was processed by taking it up in EtOAc (200 mL) and washing the solution several times with 5% HCl solution (30 mL) and water (2 × 30 mL). Evaporation of the dried (Na_2SO_4) solution and coevaporation with toluene (2 × 30 mL) gave a crude product that was dissolved in the minimum amount of EtOAc. Ether was added, giving **9** as a white solid that was filtered (0.310 gr, 83%): mp 136−138 °C; $[\alpha]_D$ +58° (*c* 1, chloroform); IR (Nujol) 3295, 1742, 1654, 1541, 1134 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 7.74 (br t, 7 H, J = 5.8 Hz, NH), 7.10 (br t, 7 H, J = 5.4 Hz, NH), 6.96 (br d, 7 H, J = 9.8 Hz, MeCON*H*), 5.41 (m, 7 H, H-8′), 5.34 (t, 7 H, J = 10.0 Hz, H-3), 5.33 (dd, 7 H, J = 8.8 and 2.0 Hz, H-7′), 5.18 (d, 7 H, J = 3.4 Hz, H-1), 4.85 (dt, 7 H, J = 11.4 and 4.7 Hz, H-4′), 4.78 (dd, 7 H, J = 10.0 and 3.6 Hz, H-2), 4.26 (dd, 7 H, J = 12.23 and 2.9 Hz, H-9′), 4.20 (m, 7 H, H-5), 4.07 (m, 14 H, H-5′,9′), 3.93 (m, 14 H, H-4,6′), 3.79 (s, 21 H, MeO), 3.49−3.19 (m. 56 H, CH_2CONHCH_2, CH_2NHCOCH_2S), 2.77 (dd, 7 H, J = 13.2 and 4.3 Hz, H-3′), 2.16, 2.07, 2.04, 2.04, 1.99, 1.94, 1.77 (7 s, 147 H, 7 MeCO), 1.86 (br t, 7 H, J = 12.5 Hz, H-3′), 1.55 and 1.40 (2 m, 56 H, $(CH_2)_4$); ^{13}C NMR (acetone-d_6) δ 171.2, 171.0, 170.9, 170.5, 170.3, 170.2, 170.1, 169.5, 168.5 (CO), 97.5 (C-1), 83.3 (C-2′), 79.5 (C-4), 75.0 (C-6′), 72.5 (C-5), 71.6, 71.4 (C-2,3), 70.5 (C-4′), 69.0, 68.2 (C-7′,8′), 63.1 (C-9′), 53.6 (MeO), 49.4 (C-5′), 40.3, 38.1, 35.1, 33.3 (2 CH_2CO, 2 $NHCH_2$), 38.6 (C-3′), 30.3, 27.4 ($(CH_2)_4$), 23.0, 21.6, 21.0, 20.8 (MeCO); HRMS (FAB) *m/z* calcd for $C_{280}H_{413}N_{21}O_{140}S_{14}$ + Na 6785.2 (M + Na)$^+$, found 6785.60.

Synthesis of Compound 10. Compound **4** was prepared from compound **3** (0.175 g, 0.048 mmol) as indicated above for the synthesis of compound **5**. Crude compound **4** was immediately used without purification by dissolving it in anhydrous DMF (3.0 mL). Compound **7** (0.522, 0.83 mmol) and DIPEA (0.37 mL, 3.5 mmol) were then added under a nitrogen atmosphere. The reaction mixture was kept at room temperature for 3.5 days; after this time the solution was evaporated under vacuum. The resulting mixture was processed by taking it up in EtOAc (200 mL) and washing the solution several times with 5% HCl solution (30 mL) and water (2 × 30 mL). Evaporation of the dried (Na_2SO_4) solution and coevaporation with toluene (2 × 30 mL) gave a crude product that was dissolved in the minimum amount of EtOAc. Ether was added, giving **10** as a white solid that was filtered (0.326 g, 94%): mp 148−151 °C; $[\alpha]_D$ +19° (*c* 1, chloroform); IR (KBr) 3362, 1744, 1662, 1541, 1230 cm^{-1}; ^{1}H NMR (DMSO-d_6, 50 °C) δ 9.5 (br s, 7 H, NH), 7.79 (br s, 14 H, NH), 7.56 (d, 14 H, J = 8.4 Hz, C_6H_4), 7.75 (d, 7 H, J = 9.8 Hz, N*H*Ac), 7.38 (d, 14 H, J = 8.4 H, C_6H_4), 5.21−5−17 (m, 14 H, H-3,8′), 5.13 (dd, 7 H, J = 7.0 and 1.9 Hz, H-7′), 5.05 (br s, 7 H, H-1), 4.70 (dt, 7 H, J = 11.3 and 4.6 Hz, H-4′), 4.70 (m, 7 H, H-2), 4.29 (dd, 7 H, J = 12.2 and 3.0 Hz, H-9′), 4.12 (m, 7 H, H-5), 4.08 (dd, 7 H, J = 12.6 and 6.2 Hz, H-9′), 3.91 (m, 7 H, H-4), 3.87 (dd, 7 H, J = 10.5 and 1.8 Hz, H-6′), 3.76 (q, 7 H, J = 10.0 Hz, H-5′), 3.60 (m, 14 H, H-6,6), 3.55 (s, 21 H, MeO), 3.46 (m, 14 H, CH_2NH), 3.26 (br s, 14 H, HCH_2S), 3.08 (m, 14 H, CH_2NH), 2.67 (dd, 7 H, J = 12.7 and 4.7 Hz, H-3′), 2.02, 1.99, 1.99, 1.90, 1.65 (5 s, 147 H, 7 MeCO), 1.76 (br t, 7 H, J = 12.0 Hz, H-3′), 1.53 (m, 14 H, CH_2CH$_2$NH), 1.43 (m, 14 H, CH_2CH $_2$NH), 1.30 (m, 56 H, $(CH_2)_4$); ^{13}C NMR (DMSO-d_6, 50 °C) δ 180.1 (CS), 169.8, 169.3, 169.1, 169.0, 168.9, 168.8, 168.7, 168.5, 167.6 (CO), 141.3, 136.3, 136.1, 121.6 (C_6H_4), 96.2 (C-1), 87.0 (C-2′), 77.9 (C-4), 73.9 (C-6′), 71.0, 70.0 (C-2,3,5), 69.4 (C-4′), 68.9 (C-8′), 67.4 (C-7′), 61.5 (C-9′), 52.4 (MeO), 47.6 (C-5′), 43.5 (*C*H$_2$NH), 39.5 (CH_2NH), 37.6 (C-3′), 36.6 (C-6), 29.0 (*C*H$_2$CH$_2$NH, $(CH_2)_4$), 27.9 (*C*H$_2$CH$_2$NH), 26.0 ($(CH_2)_4$), 22.31(*Me*CONH), 20.75, 20.50, 20.29 (*Me*CO); FABMS *m/z* 6878 for [M+ Na]$^+$, calcd for $C_{285}H_{416}N_{22}O_{139}S_{35}$ 6855.52.

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