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Efficient Preparation of Glycoclusters from Silsesquioxanes

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Supporting Information

1. General Procedures
2. Synthesis and Spectroscopic Data
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General Procedures Chemicals were from Aldrich (Milwaukee, WI) and used as received. All photoadditions were performed in borosilicate glass flasks and irradiated at 254 nm with a UV Lamp (115 V, 0.16 Amps, bulb 10x110 mm). Column chromatography was carried out using E. Merck Silica Gel 60 F (230-400 mesh). Analytical thin-layer chromatography (TLC) was performed on aluminum sheets coated with silica gel 60 F₂₅₄ (0.25mm thickness, E. Merck). Detection of the compounds was by quenching of fluorescence and/or charring with 15% H₂SO₄ in ethanol solution followed by heating at 180 °C. The ratios of solvents used for TLC are expressed in vol/vol.

Gel filtration chromatography was performed using either (1) a column packed with Sephadex G-25 (5x200 cm) eluting with 0.1M acetic acid or (2) a column packed with Sephadex G-15 (2x145 cm) eluting with distilled water. Melting points were determined with a Fisher-Johns apparatus and are not corrected. ¹H NMR and ¹³C NMR spectra were recorded with a Varian-400 NMR spectrometer at nominal resonance frequencies of 400 MHz and 100 MHz respectively in CDCl₃ (referenced to internal Me₄Si at δ_{H} 0 ppm, δ_{C} 0 ppm) and D₂O (referenced to internal acetone at δ_{H} 2.225 ppm, δ_{C} 29.8 ppm). The chemical shifts (δ) were in parts per million (ppm). First order *J* values were given in Hz. Fast atom bombardment mass spectra (FAB MS) data were obtained using *m*-nitrobenzyl alcohol as matrix. Matrix-assisted laser desorption ionization time-of-flight mass spectra (MALDI-TOF-MS) were recorded with a MALDI-Compact (Kratos) instrument in the positive mode using 2, 5-dihydroxybenzoic acid (DHB) as matrix unless otherwise indicated and an average of 50 laser shots per sample.

N-Benzyloxycarbonyl 5-aminopentanol **2**¹ and *N*-fluorenylmethoxycarbonyl-6-aminohexanal **9**² were prepared according to the known methods.

5-(Benzyloxycarbonylamino)pentyl 2,3,4, 6-tetra-acetyl- α -D-mannopyranoside (3)³

This compound was prepared by a method similar to that described in ref 3.

Tin(IV) chloride (0.75 ml, 6.4 mmol) was added dropwise over 20 min to a solution of 1,2,3,4, 6-penta-acetyl- α -D-mannopyranoside (2.28 g, 5.95 mmol) and 5-(benzyloxycarbonylamino)pentanol **2**¹ (1.0 g, 3.98 mmol) in dichloromethane (50 ml), and the mixture was stirred for 12 h. It was then poured into ice-cold aqueous sodium bicarbonate (50 ml) with vigorous stirring, and the resulting precipitate was removed by filtration through a pad of on a Celite. The filtrate was extracted with chloroform, and the combined extracts, were dried and concentrated. The residual syrup was purified on silica gel (5:4 Hexanes/EtOAc as eluent) to give **3** as a colorless syrup (1.82 g, 55%).

¹H NMR (400 MHz, CDCl₃, r. t.) δ 5.35 (dd, 1H, J =3.6 and 10.0 Hz, H-3), 5.27 (t, 1H, J =10.0 Hz, H-4), 5.23 (br. d, 1H, J =3.6 Hz, H-2), 5.10 (s, 2H, PhCH₂), 4.79 (d, 1H, J =1.6 Hz, H-1), 4.28 (dd, 1H, J =5.3 Hz and 12.0 Hz, H-6b), 4.10 (dd, 1H, J =2.4 Hz and 12.0 Hz, H-6a), 3.98 (ddd, 1H, J =2.4, 5.3, and 10.0 Hz, H-5), 3.70-3.67 (m, 1H, OCH₂), 3.46-3.44 (m, 1H, OCH₂), 3.24-3.19 (m, 2H, CH₂NHCbz), 2.16, 2.09, 2.05, 1.99 (4s, 12H, CH₃CO), 1.63 (m, 2H), 1.55 (m, 2H), 1.40 (m, 2H).

¹³C NMR (100 MHz, CDCl₃, r. t.): δ 170.70, 170.13, 169.96, 169.79 (4 CO), 156.43 (PhCH₂OCO), 136.65, 128.52, 128.09, 97.55, 69.69, 69.11, 68.44, 68.27, 66.62, 66.28, 62.57, 60.39, 40.92, 29.77, 28.87, 23.40, 21.05, 20.93, 20.77, 20.72.

5-Aminopentyl α -D-mannopyranoside (4)

The fully protected lactoside **3** (3.75 g, 6.6 mmol) was dissolved in dry methanol (50 ml), and the solution was made basic (pH 11 by moist pH paper) by addition of solid NaOMe (about 50 mg), and the mixture was left at room temperature. When the reaction

was complete (6 h), as shown by TLC, H₂O (20 ml) was added to dissolve the precipitates formed, and the reaction was stirred for a further 2 h, during which time the precipitate dissolved completely. The reaction mixture was neutralized with Dowex-50WX8-400 (H⁺ form) resin and filtered. The filtrate and washings were concentrated to ca. 15 ml, and then acetic acid (10 ml) and Pd/C 10% (600 mg) were added, whereafter the mixture was hydrogenated at atmospheric pressure for 12 h. After completion of (TLC chloroform/methanol, 1:1), the mixture was filtered through a pad of Celite, washed with water (30 ml), concentrated to ~3 ml, and fractionated on a Sephadex G-25 column (5x 200 cm, eluted with 0.1 M HOAc). The appropriate fractions were pooled and lyophilized to give compound **4** (acetate form, 1.85 g, 86%).

¹H NMR (400 MHz, D₂O, r. t.) δ 4.86 (d, 1 H, *J*=1.6 Hz, H-1), 3.93 (dd, *J*=3.2 Hz and 1.6 Hz), 3.87 (m, 1 H), 3.77-3.73 (m, 3 H), 3.64-3.62 (m, 2 H), 3.57 (m, 2 H), 3.0 (t, 2 H, *J*=8.0 Hz), 1.68 (m, 4 H), 1.46 (m, 2H).

¹³C NMR (100 MHz, D₂O, r.t.): δ 99.2, 72.3, 70.2, 69.6, 67.0, 66.3, 60.5, 26.1.

MALDI-TOF-MS: *m/z* 287.1 [M-H+Na]⁺.

2-(*N*-Benzyloxycarbonyl)-aminoethyl (2,3,4,6-tetra-*O*-benzoyl-β-*D*-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl- β-*D*-glucopyranoside (7)

Silver triflate (768 mg, 3.0mmol) was added to a solution of 2-(*N*-benzyloxycarbonyl)-aminoethanol **6** (665 mg, 3.4 mmol) and perbenzoyl lactosyl bromide **5** (2.66 g, 2.3 mmol) in dry CH₂Cl₂ (25 ml) at -15 °C containing 4-Å molecular sieves (5 g). After stirring for 30 min., the mixture was allowed to reach room temperature over 30 min. After 1h, TLC (toluene/acetone, 5:1) indicated that the reaction

was complete, the mixture was diluted with CH_2Cl_2 (60 ml), filtered through Celite, and washed with satd. aq. NaHCO_3 . The aqueous phase was extracted with CH_2Cl_2 (2x30 ml), and the combined organic phases were dried (anhydrous Na_2SO_4), filtered, and concentrated. Flash chromatography of the residue on silica gel (toluene/acetone, 20:1) gave compound **7** (2.28 g, 78%).

^1H NMR (400 MHz, CDCl_3 , r. t.) δ 7.88-8.01 (m, 12H, Aryl-H), 7.71-7.73 (m, 2H, Aryl-H), 7.64-7.12 (m, 26H, Aryl-H), 5.78 (t, 1H, $J=4.4$ Hz, H-3), 5.71 (dd, 1H, $J=10.4$ Hz and 7.8 Hz, H-2'), 5.40 (dd, 1H, $J=9.4$ Hz and 6.0 Hz, H-2), 5.37 (dd, 1H, $J=10.4$ Hz and 3.2 Hz, H-3'), 5.10 (t, 1H), 4.95 (m, 1H), 4.89 (d, 1H, $J=8.0$ Hz, H-1'), 4.66 (d, 1H, $J=8.0$ Hz, H-1), 4.60 (dd, 1H, $J=12.0$ Hz and 1.6 Hz, H-6a), 4.47 (dd, 1H, $J=12.0$ Hz and 4.4 Hz, H-6b), 4.23 (t, 1H, H-4), 3.91, 3.76 (2m, 2H, OCH_2), 3.80 (m, 2H, H-5 and H-6), 3.73-3.66 (m, 2H, H-6a' and H-6b'), 3.30 (m, 2H, CH_2NH).

^{13}C NMR (100 MHz, CDCl_3 , r. t.): δ 165.9, 165.6, 165.4, 165.3, 165.22, 164.8 (7 CO), 156.3 (CONH), 136.6, 133.5, 133.4, 133.3, 130.0, 129.8, 129.7, 129.6, 129.5, 129.4, 129.1, 129.0, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.0 (Aryl-C), 101.42, 100.98 (C-1 and C-1'), 76.7, 75.9, 73.1, 72.6, 71.76, 71.78, 71.4, 69.9, 67.5, 66.5, 62.2, 61.1, 40.8.

FAB-MS m/z : 1248 $[\text{M}+\text{H}]^+$; FAB-HRMS: calcd for $[\text{M}+\text{H}]^+$ $\text{C}_{71}\text{H}_{62}\text{O}_{20}\text{N}$, 1248.3864; found, 1248.3880.

2-Aminoethyl β -D-galactopyranosyl-(1 $\rightarrow$ 4)-O- β -D-glucopyranoside (**8**)

The fully protected lactoside **7** (3.5 g, 2.8 mmol) was taken up in dry methanol (60 ml), and the solution was made basic (pH 11 towards moist pH paper) by the addition of

NaOMe, and the mixture was left at room temperature. After TLC indicated completion of the reaction (6 h), H₂O (20 ml) was added to dissolve precipitates, and the reaction was left to stir for a further 2 h. The reaction was neutralized with Dowex-50WX8-400 (H⁺ form) and filtered. The filtrate and washings were concentrated to 15 ml, and then acetic acid (10 ml) and Pd/C 10% (500 mg) were added, whereafter the mixture was hydrogenated at atmosphere pressure for 12 h. When the reaction was complete (by TLC chloroform/methanol, 1:1), the mixture was filtered through a pad of Celite, washed with water (30 ml), concentrated and lyophilized to give compound **8** (acetate form, 1.02 g, 82%).

¹H NMR (400 MHz, D₂O, r. t.) δ 4.54 (d, 1H, H-1, $J=7.6$ Hz), 4.45 (d, 1H, $J=8.0$ Hz, H-1'), 4.12 (m, 1H), 3.96 (m, 2H), 3.92, 3.76 (2m, 2H, OCH₂), 3.84-3.62 (m, 8H), 3.55 (m, 1H), 3.37 (m, 1H), 3.25 (t, 2H, CH₂NCO).

¹³C NMR (100 MHz, D₂O, r. t.): δ 102.5, 101.5 (C-1,1'), 77.80, 75.0, 74.3, 73.8, 72.3, 72.1, 70.5, 68.1, 65.6, 60.6, 59.5, 39.0.

FAB-MS m/z : 386 [M+H]⁺; FAB-HRMS Calcd for C₁₄H₂₈NO₁₁ [M+H]⁺, 386.1660; found, 386.1664.

***N*-(9-Fluorenylmethoxycarbonyl)-aminoethyl (2,3,4,6-tetra-O-benzoyl- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-O-benzoyl- β -D-glucopyranoside (**10**)**

Solid Silver triflate (429 mg, 1.68 mmol) was added to a solution of *N*-(9-fluorenylmethoxycarbonyl)-aminoethyl-1-ol **9** at -15 °C (608 mg, 1.79 mmol) and perbenzoyl lactosyl bromide **5** (1.36 g, 1.2 mmol) in dry CH₂Cl₂ (15 ml) containing 4Å molecular sieves (2.0 g). After stirring for 30 min., the mixture was allowed to warm to

room temperature during 30 min, and 2h thereafter, the mixture was diluted with CH_2Cl_2 (50 ml), filtered through a pad of Celite, and washed with satd. aq. NaHCO_3 (50 ml). The aqueous phase was extracted with CH_2Cl_2 (2x30 ml), and the combined organic phases were dried (anhydrous Na_2SO_4), filtered, and concentrated. Flash column chromatography (toluene/acetone, 20:1) of the residue gave compound **10** (1.23 g, 74%).

^1H NMR (400 MHz, CDCl_3 , r. t.) δ 7.89-8.03 (m, 12H, Aryl-H), 7.77-7.72 (m, 4H, Aryl-H), 7.64-7.11 (m, 27H, Aryl-H), 5.80 (t, 1H, $J=9.4$ Hz, H-3), 5.72 (dd, 1H, $J=10.4$ Hz and 7.8 Hz, H-2'), 5.46 (dd, 1H, $J=10.0$ Hz and 8.0 Hz, H-2), 5.38 (dd, 1H, $J=10.4$ Hz and 3.2 Hz, H-3'), 4.87 (d, 1H, $J=8.0$ Hz, H-1'), 4.67 (d, 1H, $J=8.0$ Hz, H-1), 4.60 (dd, 1H, $J=12.0$ Hz and 1.6 Hz, H-6a), 4.48 (dd, 1H, $J=12.0$ Hz and 4.4 Hz, H-6b), 4.39 (dd, 2H, OCOCH_2), 4.25 (m, 2H, H-4, OCOCH_2CH), 3.86 (m, 2H, H-5' and OCH_2), 3.80 (m, 1H, H-5), 3.70 (dd, 2H, $J=6.4$ Hz and 3.6 Hz, H-6a' and H-6b'), 3.43 (m, 1H), 2.98 (m, 2H, CH_2NHCO), 1.46 (m, 2H), 1.18-1.11 (m, 6H).

^{13}C NMR (100 MHz, CDCl_3 , r. t.): δ 165.9, 165.6, 165.4, 165.2, 165.1, 164.8 (7 CO), 156.3(CONH), 144.0, 141.3, 133.6, 133.4, 133.3, 133.2, 130.0, 129.8, 129.7, 129.6, 129.5, 129.4, 129.0, 128.8, 128.7, 128.64, 128.58, 128.52, 128.4, 128.3, 127.8, 127.0, 125.3, 125.0, 120.0 (Aryl-C), 101.19, 100.98 (C-1 and C-1'), 76.1, 73.0, 72.9, 71.8, 71.4, 70.1, 69.9, 67.5, 66.4, 62.4, 61.1, 47.3, 40.8, 29.6, 29.1, 26.1, 25.4.

FAB-MS: m/z $[\text{M}+\text{H}]^+$ 1392.7.

6-Aminohexyl β -D-galactopyranosyl-(1 $\rightarrow$ 4)-O- β -D-glucopyranoside (**11**)

NaOMe (740 mg) was added to a stirred solution of fully protected lactoside **10** (3.8 g) in dry methanol (50 ml) and the mixture was left at room temperature. After the

reaction was complete (by TLC) (8 h), H₂O (25 ml) was added, and the reaction was stirred for a further 1 h, during which time the precipitate dissolved. The reaction was neutralized with Dowex-50WX8-400 (H⁺ form) and filtered. The solvents were removed *in vacuo* and the resulting residue was taken up with H₂O (50 ml), and extracted with toluene (30 ml) and ethyl acetate (30 ml). The aqueous layer was evaporated to a small volume (5 ml) and applied to a Sephadex G-25 column (5x200 cm, 0.1M HOAc as eluent). Appropriate fractions were pooled, concentrated to a small volume and lyophilized to give the fully deblocked compound **11** (acetate form, 1.14 g, 95%).

¹H NMR (400 MHz, D₂O, r. t.): δ 4.48 (d, 1H, *J*=7.6 Hz, H-1), 4.44 (d, 1H, *J*=8.0 Hz, H-1'), 3.96 (m, 2H), 3.91, 3.76 (2m, 2H, OCH₂), 3.82-3.78 (m, 2H), 3.73-3.63 (m, 6H), , found, 442.2285. 3.60-3.52 (m, 2H), 3.30 (m, 1H), 2.99 (t, 2H, CH₂NCO), 1.65 (m, 4H), 1.41(m, 4H).

¹³C NMR (100 MHz, D₂O, r. t.): δ 102.5, 101.6 (C-1,1'), 78.0, 74.9, 74.3, 74.0, 72.4, 72.1, 70.5, 69.9, 68.1, 60.6, 59.7, 38.9, 28.0, 26.2, 24.8, 24.1.

FAB-MS *m/z*: 442 [M+H]⁺; HRMS (FAB) Calcd for C₁₈H₃₆NO₁₁ [M+H]⁺, 442.2288.

Synthesis of Mercaptans **12**, **13**, **14** from the Corresponding Primary Amines.

General Procedures⁴. The RNH₂ (2 mmol), DTT (5 mmol) and thiobutyrolactone (12 mmol) were added to a mixture of 0.5 M sodium bicarbonate (12ml) and ethanol (12ml). A stream of N₂ was bubbled through the solution for 15 min. Then the mixture was heated at 50 °C for 8--10 h until TLC indicated the reaction was complete. The mixture was cooled to room temperature and then adjusted to pH 7 with 1 M HCl. The solvents and volatile side products were removed *in vacuo*, and the residue was subjected to

chromatography on silica gel.

5-(3'-Mercaptopropionylamido)pentyl α -D- mannopyranoside (12) from (4): Reflux for 8 h, eluent, ethyl acetate/methanol 3:1; yield, 90%.

^1H NMR (400 MHz, D_2O , r. t.) δ 4.85 (s, 1H, H-1), 3.92 (m, 1H), 3.89-3.86 (m, 1H), 3.79-3.76 (m, 2H), 3.74-3.71 (m, 1H), 3.64-3.62 (m, 2H), 3.53 (m, 1H), 3.19 (t, 2H, CH_2NH), 2.54 (t, 2H, CH_2SH), 2.35 (t, 2H, COCH_2), 1.88 (quintet, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{SH}$), 1.62 (m, 2H), 1.53 (m, 2H), 1.38 (m, 2H).

^{13}C NMR (100MHz, D_2O , r. t.): δ 175.34, 99.20, 72.29, 70.20, 69.64, 67.18, 66.30, 60.47, 38.72, 33.99, 29.11, 27.72, 27.64, 22.64, 22.40.

MALDI-TOF MS: m/z 366.2 $[\text{M-H}]^-$.

2-(3'-Mercaptopropionylamido)ethyl β -D-galactopyranosyl-(1 $\rightarrow$ 4)-O- β -D-glucopyranoside (13) from (8): Reflux for 10 h, eluent, ethyl acetate/methanol 2:1; yield, 86%.

^1H NMR (400 MHz, D_2O , r. t.) δ 4.49 (d, 1H, $J=7.6$ Hz), 4.45 (d, 1H, $J=8.0$ Hz), 3.99-3.97 (m, 2H), 3.92 (d, 1H, $J=3.2$ Hz), 3.83-3.73 (m, 4H), 3.68-3.64 (m, 3H), 3.60 (m, 1H), 3.47-3.42 (m, 2H), 2.55 (t, 2H, CH_2SH), 2.38 (t, 2H, COCH_2), 1.89 (quintet, 2H, $\text{CH}_2\text{CH}_2\text{SH}$).

^{13}C NMR (100MHz, D_2O , r. t.): δ 175.84 (CONH), 102.48, 101.73 (C-1,1'), 77.93, 74.89, 74.30, 73.83, 72.35, 72.04, 70.48, 68.08, 67.97, 60.56, 59.63, 38.80, 33.93, 28.96, 22.61.

MALDI-TOF-MS: m/z 510.2 $[\text{M}+\text{Na}]^+$.

6-(3'-Mercaptopropionylamido)hexyl- β -D-galactopyranosyl-(1 $\rightarrow$ 4)-O- β -D-

glucopyranoside (14) from (11): Reflux for 10 h, eluent, ethyl acetate/methanol 2:1;

yield, 82%.

^1H NMR (400 MHz, D_2O , r. t.) δ 4.48 (d, 1H, $J=8.0\text{Hz}$, H-1'), 4.45 (d, 1H, $J=8.0\text{ Hz}$, H-1), 3.99-3.90 (m, 3H), 3.82-3.76 (m, 3 H), 3.73-3.70 (m, 1H), 3.68-3.63 (m, 4H), 3.60-3.52 (m, 2H), 3.30 (m, 1H), 3.18 (t, 2H, CH_2NH), 2.54 (t, 2H, CH_2SH), 2.35 (t, 2H, COCH_2), 1.88 (quintet, 2H, $\text{COCH}_2\text{CH}_2\text{CH}_2\text{SH}$) 1.63, 1.51, 1.34 (3 m, 8H).

^{13}C NMR (100MHz, D_2O , r. t.) δ 175.40 (CONH), 102.47, 101.58 (C-1,1'), 77.92, 74.90, 74.30, 74.01, 72.40, 72.07, 70.50, 70.12, 68.10, 60.58, 59.65, 38.78, 34.01, 29.13, 28.18, 27.69, 25.29, 24.22, 22.62.

MALDI-TOF MS: m/z 565.3 $[\text{M-H}+\text{Na}]^-$.

Glycocluster (15)

A solution of thiol **12** (528 mg, 1.45 mmol) in H_2O (10 ml) was mixed with a solution of octavinyl-POSS (26 mg, 0.041 mmol) in THF (10 ml) was added. A stream of nitrogen was bubbled through the mixture for 30 min. AIBN (48 mg, 0.29 mmol) was added. The suspension, kept under N_2 , was irradiated at 254 nm and stirred for 24 h. ^1H NMR indicated completion of the reaction. Following the removal of the solvents, the residue was purified by gel filtration chromatography (Sephadex G-15, 2x145 cm, H_2O as eluent) to afford the product **15** as a white foam (103 mg, 70%).

^1H NMR (400 MHz, D_2O , r. t.) δ 4.85 (s, 8H, H-1), 3.91 (m, 8H), 3.89-3.85 (m, 8H), 3.80-3.66 (m, 32H), 3.64-3.61 (m, 8H), 3.52 (m, 8H), 3.20 (bs, 16H), 2.70 (bs, 16H), 2.36 (bs, 16H), 1.90 (bs, 16H), 1.63 (bs, 16H), 1.55 (bs, 16H), 1.41 (bs, 16H), 1.09 (bs, 16H, SiCH_2).

^{13}C NMR (100 MHz, D_2O , r. t.) δ 173.98 (8 CONH), 99.34 (C-1), 72.32, 70.35, 69.82, 67.04, 66.25, 60.48, 38.91, 34.50, 28.15, 25.07, 22.78, 12.13.

MALDI-TOF MS (DHB-HIQ matrix): 3608.30 $[\text{M}+\text{K}]^+$

Glycocluster (16)

Reaction of thiol **13** (385 mg, 0.79 mmol) with octavinyl-POSS (16 mg, 0.025 mmol) and AIBN (28 mg, 0.17 mmol) in a mixture of H_2O :THF (1:1, 12 ml) for 48 h as described for (**15**) afforded **16** as a white foam (75 mg, 66 %).

^1H NMR (400 MHz, D_2O , r. t.) δ 4.49 (d, 8H, $J=8.0$ Hz, H-1'), 4.45 (d, 8 H, $J=7.6$ Hz, H-1), 3.99-3.92 (m, 24H), 3.83-3.72 (m, 40H), 3.68-3.65 (m, 24H), 3.62-3.53 (m, 16H), 3.46 (m, 16H), 3.35 (bt, 8 H), 2.70 (bs, 16H), 2.60 (bs, 16H), 2.38 (bs, 16H), 1.90 (bs, 16 H), 1.06 (bs, 16H, SiCH_2).

^{13}C NMR (100 MHz, D_2O , r. t.) δ 175.30 (8 CONH), 102.55, 101.85 (8 C-1 and 8 C-1'), 78.00, 74.93, 74.36, 73.89, 72.38, 72.12, 70.52, 68.11, 60.60, 59.71, 38.89, 34.34, 24.76, 12.51.

MALDI-TOF MS (DHB-HIQ matrix): 4568.50 $[\text{M}+\text{K}]^+$

Glycocluster (17)

Reaction of thiol **11** (588 mg, 1.08 mmol) and octavinyl-POSS (22 mg, 0.035 mmol) with AIBN (36 mg, 0.22 mmol) in H_2O /THF (1:1, 16 ml) for 36 h as described for **15** gave the product **17** as a foam (128 mg, 73%).

^1H NMR (400 MHz, D_2O , r. t.): δ 4.47 (bs, 8H, H-1'), 4.45 (bs, 8H, H-1), 3.98-3.89 (m, 24H), 3.84-3.76 (m, 24H), 3.74-3.71 (m, 8H), 3.69-3.62 (m, 32H), 3.58-3.54 (m, 16H), 3.32 (bt, 8H), 3.19 (bs, 16H), 2.69 (bs, 16H), 2.59 (bs, 16H), 2.36 (bs, 16H), 1.89 (bs,

16H), 1.64 (bs, 16H), 1.53 (bs, 16H), 1.38 (bs, 32H), 1.08 (bs, 16H, SiCH₂).

¹³C NMR (100 MHz, D₂O, r. t.) δ 173.99 (CONH), 102.56, 101.82 (C-1 and C-1'), 78.04, 74.93, 74.33, 74.10, 72.45, 72.16, 70.53, 69.95, 68.12, 60.60, 59.77, 38.96, 34.53, 28.57, 24.63, 12.15.

MALDI-TOF MS (DHB-HIQ matrix): 5016.80 [M+K]⁺

Inhibitory Effect of Glycoclusters on the Binding between RCA120 and Asialooligosaccharides Derived from Human α 1-acid Glycoprotein

We examined the binding of **16** or **17** by RCA120 which is specific for β -linked galactose at the non-reducing termini of oligosaccharides. The studies were performed by measuring the inhibitory activity of **16** or **17** on the binding of asialooligosaccharides derived from human α 1-acid Glycoprotein (AGP) by RCA120 using capillary affinity electrophoresis (CAE)⁵

Procedures for capillary affinity electrophoresis (CAE) CAE was performed using a P/ACE MDQ glycoprotein system (Beckman Coulter, Fullerton, CA, USA) equipped with a eCAP N-CHO capillary (20 cm effective length, 30 cm total length, 50 μ m i.d., Beckman Coulter) using an argon-laser induced fluorescence detector as described previously⁵. Detection was performed by installing a 520 nm filter for emission with a 488 nm argon-laser for excitation. Tris-acetate buffer (100 mM, pH 7.4) was used as electrolyte throughout the work. The sample solution was introduced to the capillary by pressure method (0.5 psi, 5 sec). Separation was performed at 25 °C at the applied potential of 18 kV, and data were collected and analyzed with a standard 32 Karat software (Version 4.0, Beckman Coulter) on Microsoft Windows 2000. Briefly, prior to CAE, a mixture of 1-aminopyrene-3, 6, 8-trisulfonate (APTS)-labeled

oligosaccharides was electrophoresed in the absence of lectin. Then, the same electrolyte containing a lectin at the specified concentration was filled in the capillary, and the same mixture of fluorescent oligosaccharides was analyzed.

An example using **16** as an inhibitor is shown in **Fig. 1**

Fig.1 Inhibitory effect of **16 on the binding between RCA120 and APTS-labeled asialooligosaccharides from human α 1-acid glycoprotein.**

(a) Separation of APTS-labeled asialooligosaccharides from human AGP; (b) Separation of the same mixture in the presence of RCA120 at 0.25 μ M; (c) **16** was added to the mixture at 100 nM ; (d) **16** was added to the mixture at 1 μ M; (e) **16** was added to the mixture at 10 μ M. Analytical conditions and fluorescent labeling of oligosaccharides were described in ref. 5.

AGP contains di-, tri- and tetra-antennary carbohydrate chains, and the asialooligosaccharides thereof have common Gal β 1-4GlcNAc branches and show high affinity toward RCA120. In the absence of **16**, all oligosaccharides showed obvious retardation and peak broadening due to the binding reaction when analyzed by capillary electrophoresis in the electrolyte containing RCA120 even at 0.25 μ M concentration (**Fig. 1, a and b**). Addition of **16** to the electrolyte clearly resulted inhibition of the binding. At 10 μ M of **16**, the electropherogram showed almost the same migrations with that observed in the absence of **16**, and indicated complete inhibition of the binding (**Fig.1, e**).

We also examined binding effect of **17** and found similar affinity toward RCA120. In contrast, the binding was not fully inhibited even at 20 mM lactose in the electrolyte (data not shown). These data indicated that **16** and **17** showed 200 times or higher inhibitory effect than lactose, and such high affinity is probably due to clustering effect. As predicted by binding specificity of RCA120, **15**, which contains only mannose residues did not show any binding at the concentration of 10 μ M, where **16** and **17** showed complete inhibition of the binding toward asialooligosaccharides. The detailed binding characteristics will be reported elsewhere (manuscript in preparation).

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