

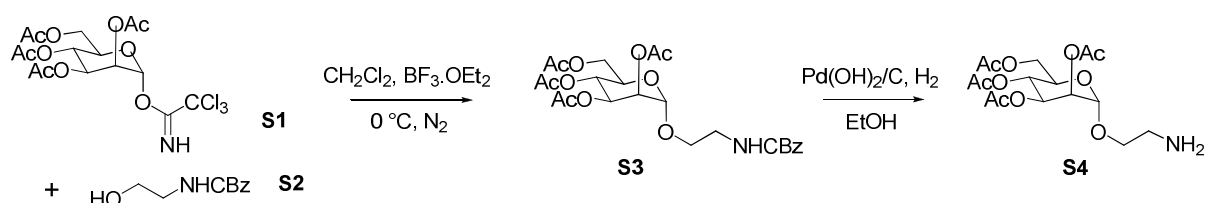
## Assessing the cluster glycoside effect during the binding of concanavalin A to mannosylated artificial lipid rafts

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### Supplementary information

#### S1. Synthesis

##### S1.1 Synthesis of linked mannose compounds



**Scheme S1.** Synthesis of short mannose headgroup **S4**.

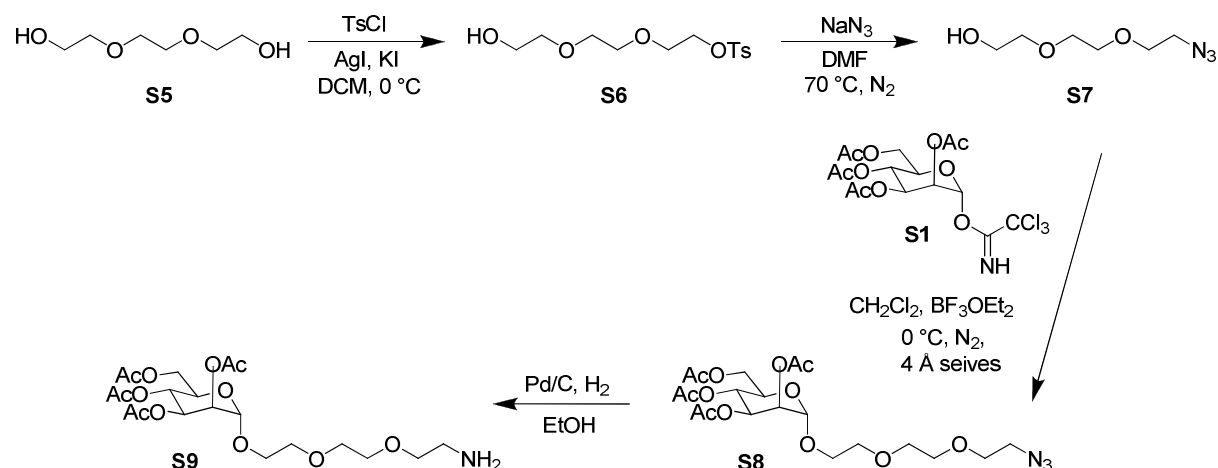
##### 2,3,4,6-Tetra-*O*-acetyl-1-(2-benzyloxycarbonylamino-ethoxy)-mannose (**S3**)

2,3,4,6-Tetra-*O*-acetyl-D-mannopyranosyl trichloroacetimidate (500 mg, 1.02 mmol, 1 eq.) and *N*-Z-ethanolamine (396 mg, 2.03 mmol, 2 eq.) were dissolved in dry THF (10 mL) in a round bottom flask in the presence of activated molecular sieves. The mixture was stirred at  $-30\text{ }^{\circ}\text{C}$  and trimethylsilyl trifluoromethanesulfonate (50  $\mu\text{L}$ , 0.276 mmol, 0.271 eq.) in THF (5 mL) was added slowly. The reaction was allowed to warm to room temperature and stirred for a further 5 hours, at which point mass spectroscopy showed no starting material. The molecular sieves were removed by filtration, and the solvent removed from the filtrate under reduced pressure. The residue was purified using flash column chromatography (3:7 ethyl acetate/40-60  $^{\circ}\text{C}$  petroleum ether slowly increasing to 1:1, on silica). The product was obtained as a clear oil (328 mg, 62%); **TLC**  $R_f$  0.23, (4:6 ethyl acetate/40-60  $^{\circ}\text{C}$  petroleum ether);  **$^1\text{H NMR}$**  (400 MHz,  $\text{CDCl}_3$ , 25  $^{\circ}\text{C}$ ):  $\delta_{\text{H}}$  1.99 (3 H, s, Ac  $\text{CH}_3$ ), 2.03 (3 H, s, Ac  $\text{CH}_3$ ), 2.08 (3 H, s, Ac  $\text{CH}_3$ ), 2.15 (3 H, s, Ac  $\text{CH}_3$ ), 3.41-3.47 (3 H, m,  $\text{CH}_2\text{NHCBz}$ , NH), 3.56 (1 H, ddd,  $^3J = 3.7, 5.6, 10.1$  Hz,  $\text{OCH}_a\text{H}_b$ ), 3.77 (1 H,  $^3J = 3.8, 5.5, 9.7$  Hz,  $\text{OCH}_a\text{H}_b$ ), 3.96 (1 H,  $^3J = 1.7, 5.5, 11.8$  Hz, sugar H-5), 4.07 (1 H, dd,  $^3J = 1.5, 12.1$  Hz, sugar H-6<sub>a</sub>), 4.26 (1 H, dd,  $^3J = 5.6, 12.2$  Hz, sugar H-6<sub>b</sub>), 4.82 (1 H, d,  $^3J = 1.2$  Hz, sugar H-1), 5.11 (2 H, s,  $\text{OCH}_2\text{Ar}$ ), 5.24 (1 H, dd,  $^3J = 1.2, 3.1$  Hz, sugar H-2), 5.27 (1 H, dd,  $^3J = 9.9, 11.5$  Hz, sugar H-4), 5.31 (1 H, dd,  $^3J = 3.2, 9.9$  Hz, sugar H-3), 7.31-7.35 (5 H, m, ArH);  **$^{13}\text{C NMR}$**  (100 MHz,  $\text{CDCl}_3$ , 25  $^{\circ}\text{C}$ ):  $\delta_{\text{C}}$  21.0 ( $2 \times \text{CH}_3$ ), 21.2 ( $\text{CH}_3$ ), 21.4 ( $\text{CH}_3$ ), 41.0 ( $\text{CH}_2$ ), 62.8 ( $\text{CH}_2$ ), 66.4 (CH), 67.2 ( $\text{CH}_2$ ), 68.0 ( $\text{CH}_2$ ), 69.1 (CH), 69.3 (CH), 69.7 (CH), 98.1 (CH), 128.6-128.9 ( $5 \times \text{CH}$ ), 136.8 (C), 156.8 (C=O), 170.0-171.0 ( $4 \times \text{C=O}$ ); **MS** ( $\text{ES}^+$ )  $m/z$  548.6 [ $\text{M}+\text{Na}$ ] $^+$  (100%), 526.6 [ $\text{M}+\text{Na}+\text{H}$ ] $^+$  (92%); **HRMS** ( $\text{ES}^+$ )  $m/z$  calculated for  $\text{C}_{24}\text{H}_{32}\text{NO}_{12}^+$  expected 526.1925, found 526.1912, calculated for  $\text{C}_{24}\text{H}_{31}\text{NO}_{12}\text{Na}^+$  expected 548.1744, found 548.1725.

##### 2,3,4,6-Tetra-*O*-acetyl-1-(2-aminoethoxy)-mannose (**S4**)

2,3,4,6-Tetra-*O*-acetyl-1-(2-benzyloxycarbonylaminoethoxy)-mannose (**S3**, 80 mg) was dissolved in EtOH (5 mL) in a round bottom flask. 10% Palladium hydroxide on carbon (10 mg) was added, the mixture was stirred under  $\text{N}_2$  for 5 minutes, then flushed with hydrogen and stirred under hydrogen for 30 minutes. The hydrogen was removed and the reaction filtered over Celite, and the Celite washed with ethanol. The solvent was then evaporated under reduced pressure to yield a clear oil (45 mg, 75%) that gave satisfactory spectroscopic data<sup>49</sup>; **TLC**  $R_f$  0.1 (4:6 ethyl acetate/40-60  $^{\circ}\text{C}$  petroleum ether);  **$^1\text{H NMR}$**  (400 MHz,  $\text{CDCl}_3$ , 25  $^{\circ}\text{C}$ ):  $\delta_{\text{H}}$  1.99 (3 H, s, Ac  $\text{CH}_3$ ), 2.04 (3 H, s, Ac  $\text{CH}_3$ ), 2.10 (3 H, s, Ac  $\text{CH}_3$ ), 2.16 (3 H, s, Ac  $\text{CH}_3$ ), 2.93 (2 H, bs,  $\text{CH}_2\text{NH}_2$ ), 3.50 (1 H, ddd,  $^3J = 5.0, 5.1, 10.2$  Hz,  $\text{OCH}_a\text{H}_b$ ), 3.74 (1 H, ddd,  $^3J = 5.0, 5.1, 9.9$  Hz,  $\text{OCH}_a\text{H}_b$ ), 4.00 (1 H, H, ddd,  $^3J = 3.0, 5.5, 10.1$  Hz, sugar H-5), 4.11 (1 H, dd,  $^3J = 2.8, 12.2$  Hz, sugar H-6<sub>a</sub>), 4.28 (1 H, dd,  $^3J = 5.3, 12.2$  Hz, sugar H-6<sub>b</sub>), 4.85 (1 H, d,  $^3J = 1.8$  Hz, sugar H-1), 5.25 (1 H, dd,  $^3J = 1.7, 3.6$  Hz, sugar H-2), 5.29 (1 H, dd,  $^3J = 9.6, 9.8$  Hz, sugar H-4), 5.35 (1 H, dd,  $^3J = 3.2,$

10.0 Hz, sugar H-3); **<sup>13</sup>C NMR** (100 MHz, CDCl<sub>3</sub>, 25 °C) : δ<sub>C</sub> 20.6 (2 × CH<sub>3</sub>), 20.7 (CH<sub>3</sub>), 20.9 (CH<sub>3</sub>), 41.4 (CH<sub>2</sub>), 62.5 (CH<sub>2</sub>), 66.2 (CH), 68.6 (CH), 69.1 (CH), 69.6 (CH), 70.6 (CH<sub>2</sub>), 97.8 (CH), 169.7-170.6 (4 × C=O); **MS** (ES<sup>+</sup>) *m/z* 422.1 (63%), 392.4 [M+H]<sup>+</sup> (97%), 170.1 (100%); **HRMS** (ES<sup>+</sup>) *m/z* calculated for C<sub>16</sub>H<sub>26</sub>NO<sub>10</sub><sup>+</sup> expected 392.1556, found 392.1551.



**Scheme S2.** Synthesis of long mannose headgroup **S9**.

### Synthesis of monoazido triethylene glycol

Synthesis of compounds **S6** and **S7** was achieved by modification of literature procedures.

#### Formation of monotosylated triethylene glycol (**S6**)<sup>S1</sup>

Triethylene glycol (10 g, 66.591 mmol) and tosyl chloride (15 g, 78.7 mmol, 1.2 eq.) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (100 mL). The mixture was cooled to 0 °C and silver oxide (20 g, 86.3 mmol, 1.08 eq.) was added followed by potassium iodide (1.90 g, 11.4 mmol, 0.143 eq.). The reaction was stirred for 30 minutes and filtered through a small plug of silica, washing through with ethyl acetate. The solvent was removed under vacuum and purified using column chromatography (silica/ethyl acetate) to yield a clear viscous oil (10.290 g, 51 %). **TLC** *R<sub>f</sub>* 0.45 (ethyl acetate); **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>, 25 °C) : δ<sub>H</sub> 2.45 (4 H, s, OH + tosyl CH<sub>3</sub>), 3.57 (2 H, t, <sup>3</sup>*J* = 4.8, TEG CH<sub>2</sub>), 3.61 (4 H, s, 2 × TEG CH<sub>2</sub>), 3.71 (4 H, m, 2 × TEG CH<sub>2</sub>), 4.17 (2 H, t, <sup>3</sup>*J* = 4.8, TEG CH<sub>2</sub>), 7.35 (2 H, d, <sup>3</sup>*J* = 8.0, 2 × tosyl Ar-*H* (meta to sulphate)), 7.80 (2 H, d, <sup>3</sup>*J* = 8.3, 2 × tosyl Ar-*H* (ortho to sulphate)); **<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>, 25 °C) : δ<sub>C</sub> 21.6 (CH<sub>3</sub>), 61.6 (CH<sub>2</sub>), 63.5 (CH<sub>2</sub>), 69.3 (CH<sub>2</sub>), 70.4 (CH<sub>2</sub>), 71.0 (CH<sub>2</sub>), 72.4 (CH<sub>2</sub>), 127.8 (CH), 128.0 (CH), 129.8, (CH), 130.0 (CH), 132.7 (C), 145.0 (C); **MS** (ES<sup>+</sup>) *m/z* 305.1 [M+H]<sup>+</sup> (100 %), 306.1 [M+2H]<sup>+</sup> (17 %); **HRMS** (ES<sup>+</sup>) *m/z* calculated for C<sub>13</sub>H<sub>21</sub>O<sub>6</sub>S<sup>+</sup>, expected 305.1053, found 305.1083.

#### Formation of monoazido triethylene glycol (**S7**)<sup>S2</sup>

Monotosylated triethylene glycol (**S6**, 1 g, 3.286 mmol) was dissolved in dry DMF (25 mL). Sodium azide (1.07 g, 16.462 mmol, 5 eq.) was added and the mixture heated at 70 °C overnight under nitrogen. Water (50 mL) was added and the reaction mixture cooled. The product was then extracted using ethyl acetate (3 × 30 mL), which was then washed with water (3 × 25 mL) and dried over anhydrous sodium sulphate. The ethyl acetate was then removed under vacuum and the crude mixture purified using column chromatography (silica gel/ethyl acetate) to yield a clear liquid (280 mg, 58 %). **TLC** *R<sub>f</sub>* 0.41 (ethyl acetate); **<sup>1</sup>H NMR** (300 MHz, CDCl<sub>3</sub>, 25 °C) : δ<sub>H</sub> 3.06 (1 H, s, OH), 3.31 (2 H, t, <sup>3</sup>*J* = 3.8, TEG CH<sub>2</sub>N<sub>3</sub>), 3.51 (2 H, t, <sup>3</sup>*J* = 3.7, TEG CH<sub>2</sub>CH<sub>2</sub>OH), 3.59 (6 H, m, 3 × TEG CH<sub>2</sub>), 3.63 (2 H, t, <sup>3</sup>*J* = 3.2, TEG CH<sub>2</sub>OH); **<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>, 25 °C) : δ<sub>C</sub> 50.5 (CH<sub>2</sub>), 61.5 (CH<sub>2</sub>), 69.9 (CH<sub>2</sub>), 70.3 (CH<sub>2</sub>), 70.5 (CH<sub>2</sub>), 72.5 (CH<sub>2</sub>); **MS** (ES<sup>+</sup>) *m/z* 176.1 [M+H]<sup>+</sup> (100 %), 198.1 [M+Na]<sup>+</sup> (44 %), 217.1 [M+CH<sub>3</sub>CN+H]<sup>+</sup> (93 %), 235.1 [M+CH<sub>3</sub>CN+Na]<sup>+</sup> (51 %); **HRMS** (ES<sup>+</sup>) *m/z* calculated for

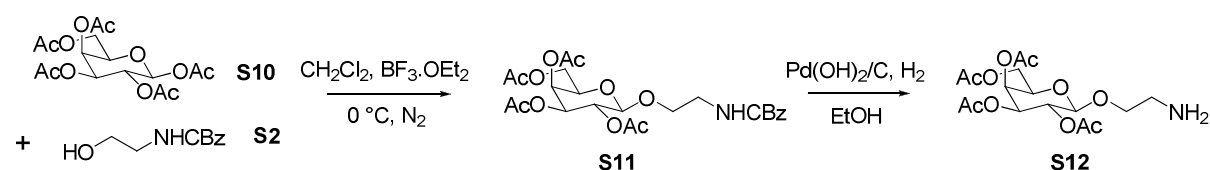
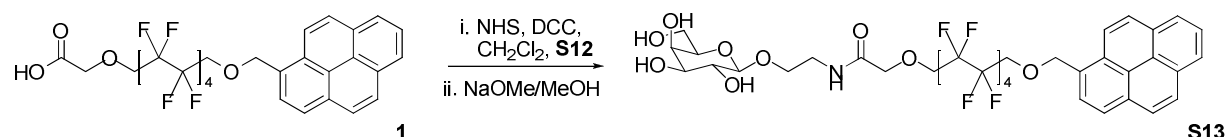
$C_6H_{13}N_3O_3Na^+$ , expected 198.0855, found 198.0849; **IR** :  $cm^{-1}$  3403.8 (broad, OH), 2916.0 (broad, CH), 2874.5 (broad, CH), 2106.6 (sharp,  $N_3$ ).

#### Formation of 2,3,4,6-tetra-*O*-acetyl-1-(9-azido-triethylene glycol)-mannose (**S8**)<sup>S3</sup>

2,3,4,6-Tetra-*O*-acetyl-D-mannopyranosyl trichloroacetimidate<sup>4</sup> (**S1**, 100 mg, 0.203 mmol) and monoazido triethylene glycol (**S7**, 42 mg, 0.240 mmol, 1.2 eq.) were dissolved in dry  $CH_2Cl_2$  (5 mL). 4 Å molecular sieves were added, then the mixture placed under an inert atmosphere and cooled to 0 °C. Boron trifluoride diethyl etherate (15  $\mu$ L, 0.122 mmol, 0.6 eq.) was added dropwise and the reaction was stirred for 1.5 hours at room temperature. Triethylamine was added dropwise until the  $BF_3$  was quenched, the mixture was filtered and the solvent was removed under from the filtrate under reduced pressure. The residue was purified using column chromatography (silica gel/4:6 ethyl acetate/40-60 °C petroleum) to yield a viscous clear oil (74 mg, 72 %). **TLC**  $R_f$  0.43 (1:1 ethyl acetate/40-60 °C petroleum ether); **<sup>1</sup>H NMR** (400 MHz,  $CDCl_3$ , 25 °C) :  $\delta_H$  1.92 (3 H, s,  $OCCH_3$ ), 1.98 (3 H, s,  $OCCH_3$ ), 2.04 (3 H, s,  $OCCH_3$ ), 2.09 (3 H, s,  $OCCH_3$ ), 3.34 (2 H, t,  $^3J = 5.0$ , TEG  $CH_2N_3$ ), 3.62 (9 H, m, 4  $\times$  TEG  $CH_2$  + TEG  $C_{(1)}H_a$ ), 3.75 (1 H, dt,  $^3J = 5.2$ , 8.7, TEG  $C_{(1)}H_b$ ), 4.01 (1 H, m, sugar H-5), 4.04 (1 H, dd,  $^3J = 2.2$ , 12.4, sugar H-6<sub>a</sub>), 4.23 (1 H, dd,  $^3J = 5.2$ , 12.5, sugar H-6<sub>b</sub>), 4.81 (1 H, d,  $^3J = 1.6$ , sugar H-1), 5.19 (1 H, dd,  $^3J = 1.8$ , 3.4, sugar H-2), 5.23 (1 H, dd,  $^3J = 9.9$ , 10.6 sugar H-4), 5.29 (1 H, dd,  $^3J = 3.3$ , 10.1, sugar H-3); **<sup>13</sup>C NMR** (101 MHz,  $CDCl_3$ , 25 °C) :  $\delta_C$  20.7 (2  $\times$   $CH_3$ ), 20.8 ( $CH_3$ ), 20.9 ( $CH_3$ ), 50.6 ( $CH_2$ ), 62.4 ( $CH_2$ ), 66.1 (CH), 67.4 ( $CH_2$ ), 68.4 (CH), 69.1 (CH), 69.5 (CH), 70.0 ( $CH_2$ ), 70.1 ( $CH_2$ ), 70.6 ( $CH_2$ ), 70.7 ( $CH_2$ ), 97.7 (CH), 169.8 (C=O), 170.0 (C=O), 170.1 (C=O), 170.8 (C=O); **MS** ( $ES^+$ )  $m/z$  506.1  $[M+H]^+$  (10 %), 528.1  $[M+Na]^+$  (100%), 529.1  $[M+H+Na]^+$  (23%); **HRMS** ( $ES^+$ )  $m/z$  calculated for  $C_{20}H_{31}N_3O_{12}Na^+$ , expected 528.1800, found 528.1805; **IR** :  $cm^{-1}$  2926 (br, CH), 2106 (sh,  $N_3$ ), 1747 (sh, C=O).

#### Formation of 2,3,4,6-tetra-*O*-acetyl-1-(9-amino-triethylene glycol)-mannose (**S9**)<sup>S5</sup>

2,3,4,6-Tetra-*O*-acetyl-1-(9-azido-triethylene glycol)-mannose (**S8**, 75 mg, 0.149 mmol) was dissolved in ethanol (5 mL). 10 % Palladium on charcoal (2 mg) was added and the mixture was placed under nitrogen. The mixture was evacuated and placed under a hydrogen atmosphere, then stirred for 2 hours at room temperature. The mixture was then filtered through celite and the solvent evaporated to yield a clear oil (63 mg, 89%). **<sup>1</sup>H NMR** (400 MHz,  $CDCl_3$ , 25 °C) :  $\delta_H$  1.93 (3 H, s, Ac  $CH_3$ ), 1.98 (3 H, s, Ac  $CH_3$ ), 2.04 (3 H, s, Ac  $CH_3$ ), 2.09 (3 H, s, Ac  $CH_3$ ), 3.39 (2 H, m,  $CH_2NH_2$ ), 3.49 (2 H, t,  $^3J = 4.85$ , TEG  $CH_2CH_2NH_2$ ), 3.59 (9 H, m, 4  $\times$  TEG  $CH_2$  + TEG  $C_{(1)}H_a$ ), 3.75 (1 H, dt,  $^3J = 4.3$ , 8.4, TEG  $C_{(1)}H_b$ ), 4.00 (1 H, m, sugar H-5), 4.04 (1 H, dd,  $^3J = 3.2$ , 11.1, sugar H-6<sub>a</sub>), 4.22 (1 H, dd,  $^3J = 4.9$ , 12.2, sugar H-6<sub>b</sub>), 4.81 (1 H, d,  $^3J = 1.5$ , sugar H-1), 5.19 (1 H, dd,  $^3J = 1.68$ , 2.94, sugar H-2), 5.22 (1 H, dd,  $^3J = 8.2$ , 8.8, sugar H-4), 5.27 (1 H, dd,  $^3J = 2.8$ , 8.9, sugar H-3); **<sup>13</sup>C NMR** (100 MHz,  $CDCl_3$ , 25 °C) :  $\delta_C$  20.7 (2  $\times$   $CH_3$ ), 20.8 ( $CH_3$ ), 20.9 ( $CH_3$ ), 41.1 ( $CH_2$ ), 62.4 ( $CH_2$ ), 66.1 (CH), 67.4 ( $CH_2$ ), 68.5 (CH), 69.1 (CH), 69.2 (CH), 69.9 ( $CH_2$ ), 70.2 ( $CH_2$ ), 70.6 ( $CH_2$ ), 70.7 ( $CH_2$ ), 97.7 (CH), 169.7 (C=O), 170.0 (C=O), 170.1 (C=O), 170.7 (C=O); **MS** ( $ES^+$ )  $m/z$  480.2  $[M+H]^+$  (100 %); **HRMS** ( $ES^+$ )  $m/z$  calculated for  $C_{20}H_{33}NO_{12}H^+$  480.2081, found 480.2072; **IR** :  $cm^{-1}$  3404 ( $NH_2$ ), 2926 (br, CH), 1747 (sh, C=O).

**S1.2 Synthesis of galactose analogue of 2 (S13)****Scheme S2.** Synthesis of short galactose headgroup **S12**.**Scheme S3.** Synthesis of short galactose lipid **S13**.**2,3,4,6-Tetra-O-acetyl-1-(2-benzyloxycarbonylamino-ethoxy)-galactose (S11)**

Pentaacetyl galactose (**S10**, 500 mg, 1.28 mmol) and *N*-Z-ethanolamine (**S2**, 300 mg, 1.54 mmol, 1.2 eq.) were dissolved in dry dichloromethane (15 mL). The solution was cooled to 0 °C, stirred under N<sub>2</sub> and BF<sub>3</sub>·Et<sub>2</sub>O (0.5 mL, 4.81 mmol, 3.1 eq.) was added dropwise. The reaction was stirred overnight. The reaction was then quenched with Et<sub>3</sub>N (1 mL) and the solvent evaporated. The crude mixture was then acetylated using Ac<sub>2</sub>O (3 mL) and pyridine (9 mL) for 1 hour and the solution was evaporated. The product was purified using column chromatography (4:6 ethyl acetate/40-60 °C petroleum ether on silica) to yield a clear oil (399 mg, 59%); **TLC** *R*<sub>f</sub> 0.53 (4:6 ethyl acetate/40-60 °C petroleum ether); **<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>, 25 °C) : δ<sub>H</sub> 1.90 (3 H, s, Ac CH<sub>3</sub>), 1.93 (3 H, s, Ac CH<sub>3</sub>), 1.95 (3 H, s, Ac CH<sub>3</sub>), 2.07 (3 H, s, Ac CH<sub>3</sub>), 3.32 (2 H, m, CH<sub>2</sub>NHCBz), 3.62 (1 H, ddd, <sup>3</sup>*J* (H,H) = 10.2, 7.1, 3.6 Hz, OCH<sub>a</sub>H<sub>b</sub>CH<sub>2</sub>), 3.80-3.82 (2 H, m, OCH<sub>a</sub>H<sub>b</sub>CH<sub>2</sub> and sugar CH-5), 4.06 (2 H, d, <sup>3</sup>*J* (H,H) = 6.6, sugar CH<sub>2</sub>), 4.38 (1 H, d, <sup>3</sup>*J* (H,H) = 7.9, sugar CH-1), 4.93 (1 H, dd, <sup>3</sup>*J* (H,H) = 3.4, 10.5, sugar CH-3), 5.02 (2 H, s, Ph-CH<sub>2</sub>), 5.10 (1 H, dd, <sup>3</sup>*J* (H,H) = 8.0, 10.4, sugar CH-2), 5.19 (1 H, t, <sup>3</sup>*J* (H,H) = 5.4, NHCBz), 5.31 (1 H, dd, <sup>3</sup>*J* (H,H) = 0.7, 3.4, sugar CH-4), 7.25 (5 H, m, Ar-H) **<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>, 25 °C) : δ<sub>C</sub> 20.5-20.7 (4 × CH<sub>3</sub>), 40.8 (CH<sub>2</sub>), 61.3 (CH<sub>2</sub>), 66.7 (CH<sub>2</sub>), 67.0 (CH), 68.8 (CH), 69.4 (CH<sub>2</sub>), 70.7 (2 × CH), 101.5 (CH), 128.2 - 128.5 (5 × CH), 136.5 (C), 156.3 (C=O), 169.6-170.4 (4 × C=O); **MS** (ES<sup>+</sup>) *m/z* 549.6 [M+Na+H]<sup>2+</sup> (28%), 526.6 [M+H]<sup>+</sup> (100%); **HRMS** (ES<sup>+</sup>) *m/z* calculated for C<sub>24</sub>H<sub>31</sub>NO<sub>12</sub>Na<sup>+</sup> expected 548.1738, found 548.1747.

**2,3,4,6-Tetra-O-acetyl-1-(2-amino-ethoxy)-galactose (S12)**

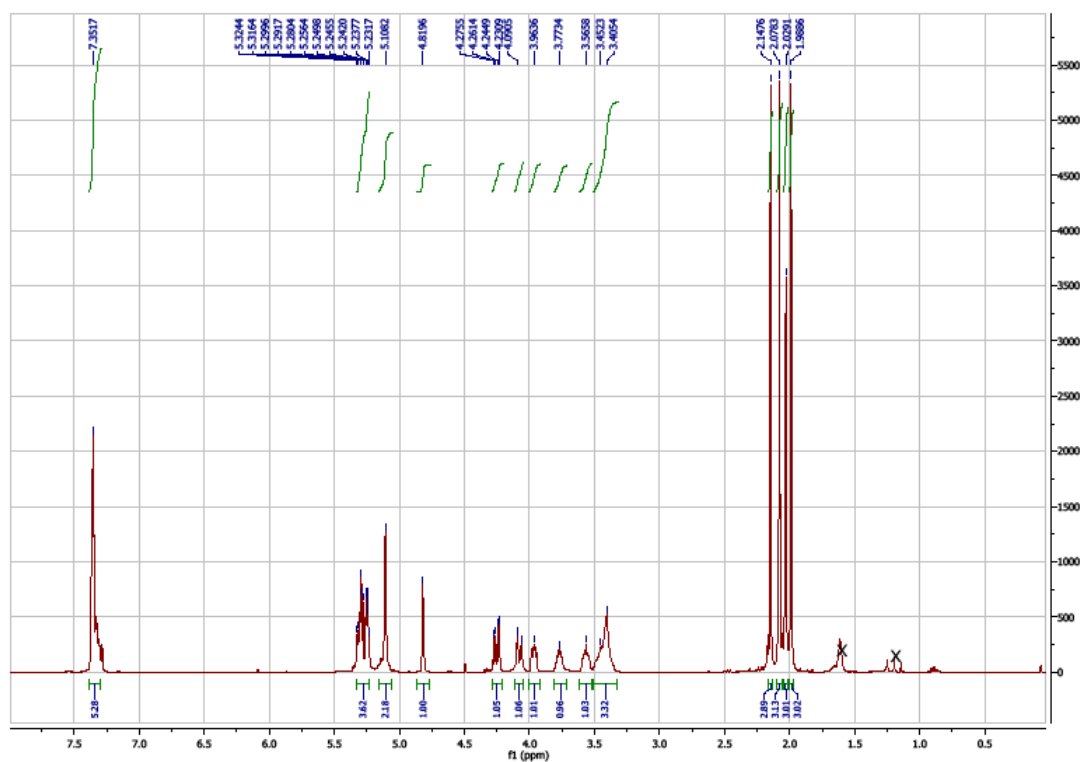
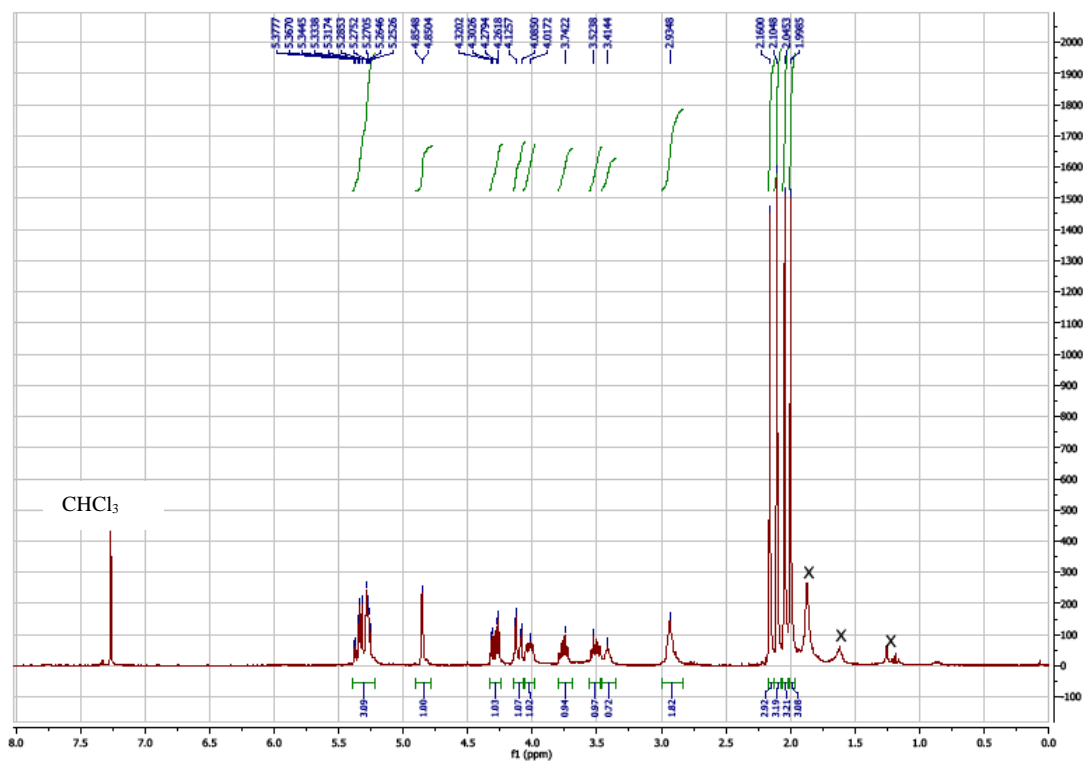
2,3,4,6-Tetraacetyl-2-(2-benzyloxycarbonylamino-ethoxy)-galactose (**S11**, 100 mg, 0.19 mmol) was dissolved in ethanol (5 mL). 10% palladium hydroxide on carbon (10 mg) was added and a rubber stopper was used to plug the flask. The mixture was stirred under N<sub>2</sub> for 5 minutes, then flushed using a balloon of H<sub>2</sub> and stirred under H<sub>2</sub> for 30 minutes. The H<sub>2</sub> balloon was removed and the reaction was filtered over celite, washing with EtOH. The EtOH was then evaporated under vacuum to yield a clear oil (73 mg, 99%). **TLC** *R*<sub>f</sub> 0.1 (4:6 ethyl acetate/40-60 °C petroleum ether); **<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>, 25 °C) : δ<sub>H</sub> 1.96 (3 H, s, OCCH<sub>3</sub>), 2.02 (3 H, s, OCCH<sub>3</sub>), 2.04 (3 H, s, OCCH<sub>3</sub>), 2.13 (3 H, s, OCCH<sub>3</sub>), 2.81 (1 H, m, CH<sub>a</sub>H<sub>b</sub>NH<sub>2</sub>), 2.84-2.91 (1 H, m, CH<sub>a</sub>H<sub>b</sub>NH<sub>2</sub>), 3.14 - 3.17 (2 H, bs, NH<sub>2</sub>), 3.58 (1 H, ddd, <sup>3</sup>*J* (H,H) = 3.8, 7.2, 10.0, OCH<sub>a</sub>H<sub>b</sub>), 3.86-3.91 (2 H, m, OCH<sub>a</sub>H<sub>b</sub> and sugar H-5), 4.08 - 4.17 (2 H, m, sugar CH<sub>2</sub>), 4.48 (1 H, d, <sup>3</sup>*J* (H,H) = 8.0 Hz, sugar H-1), 5.00 (1 H, dd, <sup>3</sup>*J* (H,H) = 3.4, 10.5, sugar H-3), 5.18 (1 H, dd, <sup>3</sup>*J* (H,H) = 8.0, 10.4, sugar CH-2), 5.37 (1 H, dd, <sup>3</sup>*J* (H,H) = 0.7, 3.3, sugar H-4); **<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>, 25 °C) : δ<sub>C</sub> 20.5-20.8 (4 × CH<sub>3</sub>), 41.5 (CH<sub>2</sub>), 61.3 (CH<sub>2</sub>), 67.0 (CH), 68.8 (CH), 70.5 (2 × CH), 72.1 (CH<sub>2</sub>), 101.5 (CH), 169.6-170.4 (4 × C=O); **MS** (ES<sup>+</sup>) *m/z* 392.1 [M+H]<sup>+</sup> (100%); **HRMS** (ES<sup>+</sup>) *m/z* calculated for C<sub>16</sub>H<sub>26</sub>NO<sub>10</sub><sup>+</sup> expected 392.1556, found 392.1567.

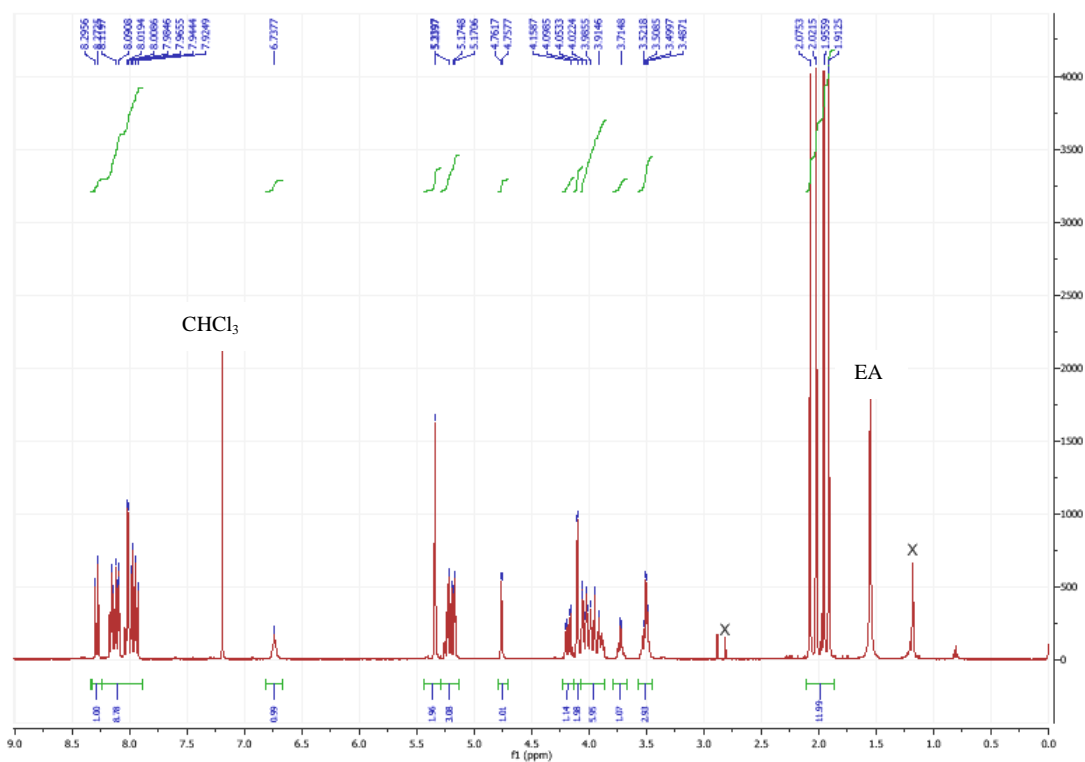
**2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Hexadecafluoro-10-(pyren-1-ethoxy)-decycloxy-N-(2- $\alpha$ -D-(2,3,4,6-tetra-O-acetyl)-galactopyranosyl-ethyl)-acetamide**

(2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Hexadecafluoro-10-(pyren-1-ylmethoxy)-decycloxy)-acetic acid (**1**, 50 mg, 0.068 mmol) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (2 mL). The solution was stirred under nitrogen and dicyclohexylcarbodiimide (DCC) (18 mg, 0.087 mmol, 1.29 eq) was added followed by *N*-hydroxysuccinimide (10 mg, 0.0879 mmol, 1.27 eq.). The solution was left to stir for 3 h at room temperature, after which time a milky white precipitate had formed. The reaction mixture was filtered through cotton wool to remove the urea precipitate and 2,3,4,6-tetra-*O*-acetyl-1-(2-aminoethoxy)-galactose (**S12**, 37 mg, 0.095 mmol, 1.40 eq.) was added in dry CH<sub>2</sub>Cl<sub>2</sub> (0.25 mL). The mixture was stirred under dry nitrogen overnight. The crude mixture was washed with NaHCO<sub>3</sub> and water, dried over MgSO<sub>4</sub> and the CH<sub>2</sub>Cl<sub>2</sub> evaporated under reduced pressure. The crude mixture was then purified using flash column chromatography (3:2:1 ethyl acetate/chloroform/cyclohexane on silica) to yield a light-yellow solid (52 mg, 78%). **TLC** *R*<sub>f</sub> 0.25 (3:2:1 ethyl acetate/chloroform/cyclohexane); **<sup>1</sup>H NMR** (300 MHz, CDCl<sub>3</sub>, 25 °C) :  $\delta_{\text{H}}$  1.98 (3 H, s, Ac CH<sub>3</sub>), 2.03 (3 H, s, Ac CH<sub>3</sub>), 2.04 (3 H, s, Ac CH<sub>3</sub>), 2.13 (3 H, s, Ac CH<sub>3</sub>), 3.47-3.50 (2 H, m, CH<sub>2</sub>NH), 3.69 (1 H, ddd, <sup>3</sup>*J* (H,H) = 3.6, 7.1, 10.6, OCH<sub>a</sub>H<sub>b</sub>), 3.86-3.91 (2 H, m, OCH<sub>a</sub>H<sub>b</sub> and sugar H-5), 4.01 (2 H, t, <sup>3</sup>*J* (H,F) = 14.0, CH<sub>2</sub>CF<sub>2</sub>), 4.06 (2 H, t, <sup>3</sup>*J* (H,F) = 14.2, CH<sub>2</sub>CF<sub>2</sub>), 4.09 – 4.15 (4 H, m, CH<sub>2</sub>CONH, sugar CH<sub>2</sub>), 4.47 (1 H, d, <sup>3</sup>*J* (H,H) = 7.9, sugar CH-1), 5.00 (1 H, dd, <sup>3</sup>*J* (H,H) = 3.4, 10.5, sugar CH-3), 5.19 (1 H, dd, <sup>3</sup>*J* (H,H) = 7.9, 10.5, sugar CH-2), 5.39 (3 H, m, Ar-CH<sub>2</sub>, sugar CH-4), 6.75 (1 H, t, <sup>3</sup>*J* (H,H) = 5.6, amide NH), 7.97 - 8.34 (9 H, m, pyrene CH); **<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>, 25 °C) :  $\delta_{\text{C}}$  20.8-20.9 (4 × CH<sub>3</sub>), 39.1 (CH<sub>2</sub>), 61.6 (CH<sub>2</sub>), 66.8 (t, <sup>3</sup>*J* (C,F) = 25.8, CH<sub>2</sub>), 67.3 (CH), 68.5 (t, <sup>3</sup>*J* (C,F) = 25.3, CH<sub>2</sub>), 68.6 (CH<sub>2</sub>), 69.1 (CH), 71.1 (CH), 71.2 (CH), 72.3 (CH<sub>2</sub>), 73.4 (CH<sub>2</sub>), 101.5 (CH), 123.3 (CH), 124.8 (CH), 124.9 (C), 125.3 (C), 125.8 (2 × CH), 126.4 (CH), 127.5 (CH), 127.6 (CH), 128.2 (CH), 128.5 (CH), 129.4 (C), 129.9 (C), 131.1 (C), 131.5 (C), 132.1 (C), 168.4 (C=O), 169.8 – 170.4 (4 × C=O); **MS** (ES<sup>+</sup>) *m/z* 1130.1 [M+Na]<sup>+</sup> (12%), 1108.4 [M+H]<sup>+</sup> (8%), 916.0 (30%), 894.0 (14%), 471.3 (12%), 449.3 (10%), 331.0 (41%), 266.2 (67%), 215.2 [pyreneCH<sub>2</sub>]<sup>+</sup> (100%); **HRMS** (ES<sup>+</sup>) *m/z* calculated for C<sub>45</sub>H<sub>41</sub>F<sub>16</sub>NO<sub>13</sub>Na<sup>+</sup> expected 1130.2215, found 1130.2249.

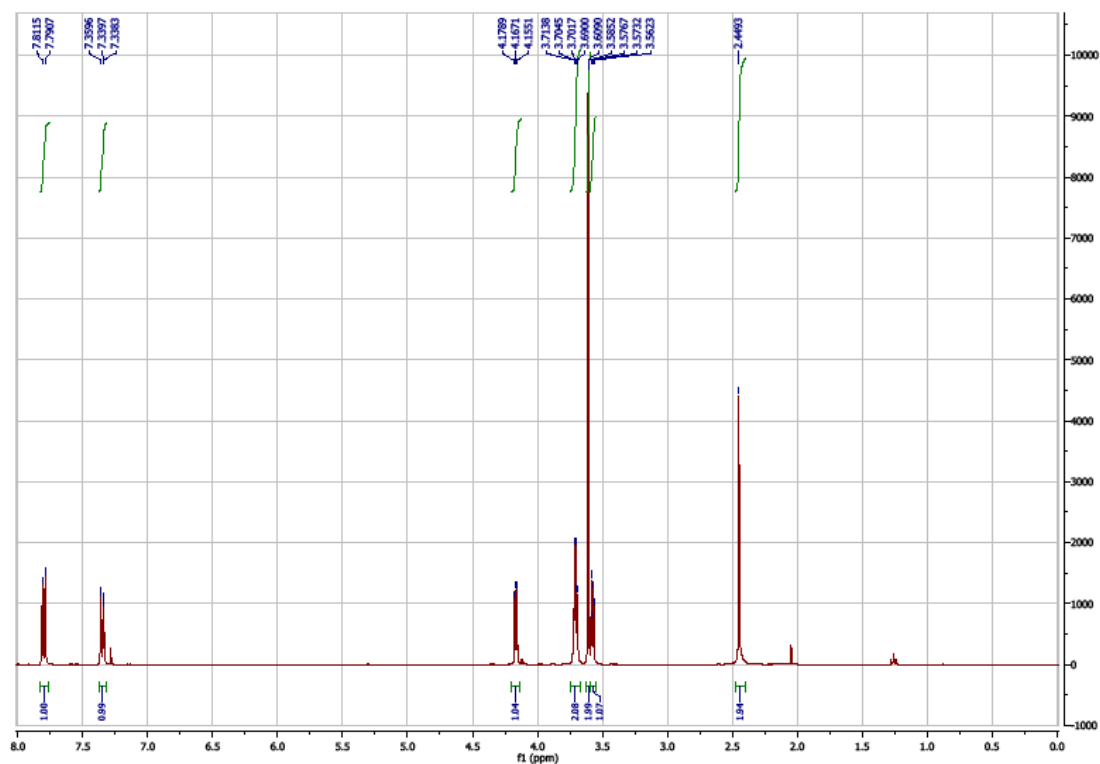
**2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Hexadecafluoro-10-(pyren-1-ethoxy)-decycloxy-N-(2- $\alpha$ -D-galactopyranosyl-ethyl)-acetamide (S13)**

The deprotection of 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Hexadecafluoro-10-(pyren-1-ethoxy)-decycloxy-N-(2- $\alpha$ -D-(2,3,4,6-tetra-*O*-acetyl)-galactopyranosyl-ethyl)-acetamide (12 mg) was undertaken in the same fashion as for the mannose compound **2**, to yield a milky yellow solid (9 mg, 75%). **TLC** *R*<sub>f</sub> 0.2 (9:1 chloroform/methanol); **<sup>1</sup>H NMR** (400 MHz, CD<sub>3</sub>OD, 25 °C) :  $\delta_{\text{H}}$  3.43-3.91 (11 H, m, CH<sub>2</sub>CH<sub>2</sub>NH, CH<sub>2</sub>CH<sub>2</sub>NH, sugar OH-2,3,4,6, CH-5,6<sub>a</sub>,6<sub>b</sub>), 4.07-4.28 (5 H, m, sugar CH-1,2,3,4, CH<sub>2</sub>CONH), 4.37 (2 H, t, <sup>3</sup>*J* (H,F) = 14.7 Hz, CH<sub>2</sub>CF<sub>2</sub>), 4.40 (2 H, t, <sup>3</sup>*J* (H,F) = 14.3 Hz, CH<sub>2</sub>CF<sub>2</sub>), 5.55 (2H, s, CH<sub>2</sub>-Ar), 7.45 (1H, t, <sup>3</sup>*J* = 5.8 Hz, NH), 8.11-8.50 (9 H, m, pyrene CH); **<sup>13</sup>C NMR** (75 MHz, CDCl<sub>3</sub>, 25 °C) :  $\delta_{\text{C}}$  40.4 (CH<sub>2</sub>), 63.1 (CH<sub>2</sub>), 68.2 (t, <sup>3</sup>*J* (C,F) = 25.6, CH<sub>2</sub>), 69.5 (t, <sup>3</sup>*J* (C,F) = 25.8, CH<sub>2</sub>), 69.8 (CH<sub>2</sub>), 73.2 (CH<sub>2</sub>), 74.1 (CH<sub>2</sub>), 74.1 (CH), 74.7 (CH), 75.1 (CH), 75.4 (CH), 105.1 (CH), 125.0 (CH), 126.1 (C), 126.2 (CH), 126.3 (C), 127.0 (CH), 127.1 (CH), 127.9 (CH), 129.0 (CH), 129.1 (CH), 129.3 (CH), 129.5 (CH), 131.1 (C), 131.9 (C), 132.5 (C), 132.9 (C), 133.3 (C), 167.9 (C=O); **MS** (ES<sup>+</sup>) *m/z* 962.0 [M+Na]<sup>+</sup> (13%), 963.0 [M+Na+H]<sup>2+</sup> (8%), 940.1 [M+H]<sup>+</sup> (5%), 748.0 [PFEGal-pyreneCH<sub>2</sub>+Na]<sup>+</sup> (32%), 726 [PFEGal-pyreneCH<sub>2</sub>+H]<sup>+</sup> (10%), 563.9 (37%), 266.2 (76%), 215.0 [pyreneCH<sub>2</sub>]<sup>+</sup> (100%); **HRMS** (ES<sup>+</sup>) *m/z* calculated for C<sub>37</sub>H<sub>33</sub>F<sub>16</sub>NO<sub>9</sub>Na<sup>+</sup> expected 962.1798, found 962.1803.

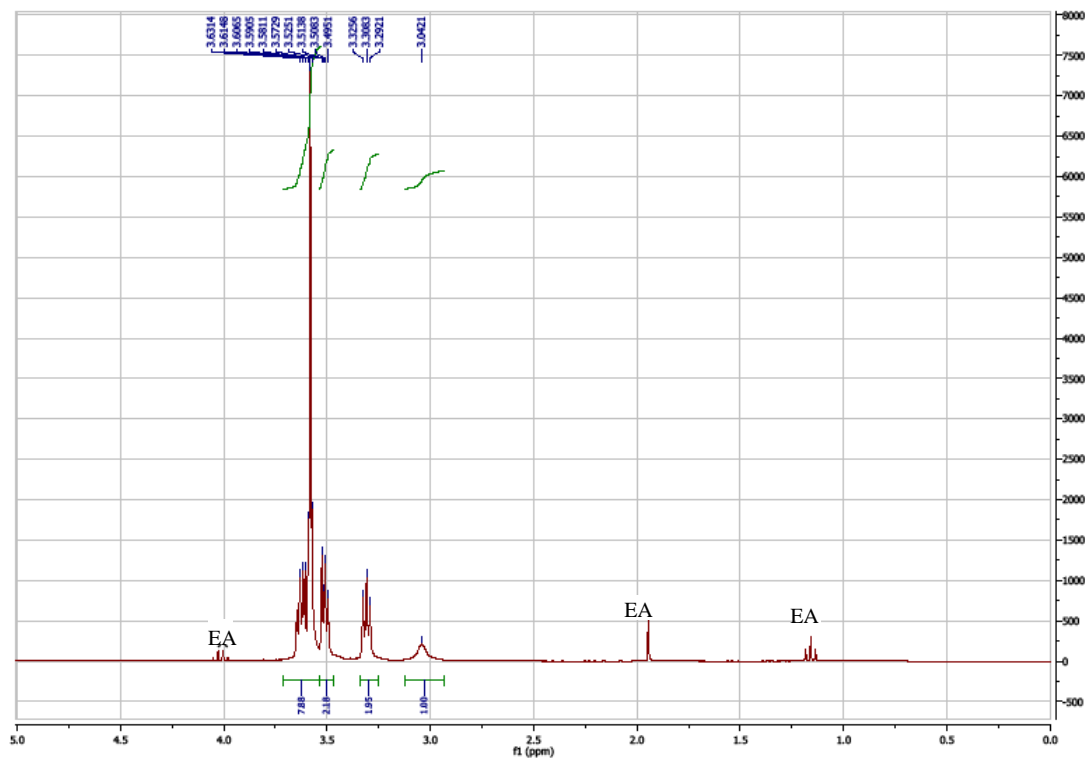
**S2. <sup>1</sup>H NMR spectra****2,3,4,6-Tetra-O-acetyl-1-(2-benzyloxycarbonylamino-ethoxy)-mannose****2,3,4,6-Tetra-O-acetyl-1-(2-amino-ethoxy)-mannose**

**2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Hexadecafluoro-10-(pyren-1-ethoxy)-decycloxy-N-(2- $\alpha$ -D-(2,3,4,6-tetra-O-acetyl)-mannopyranosyl-ethyl)-acetamide**

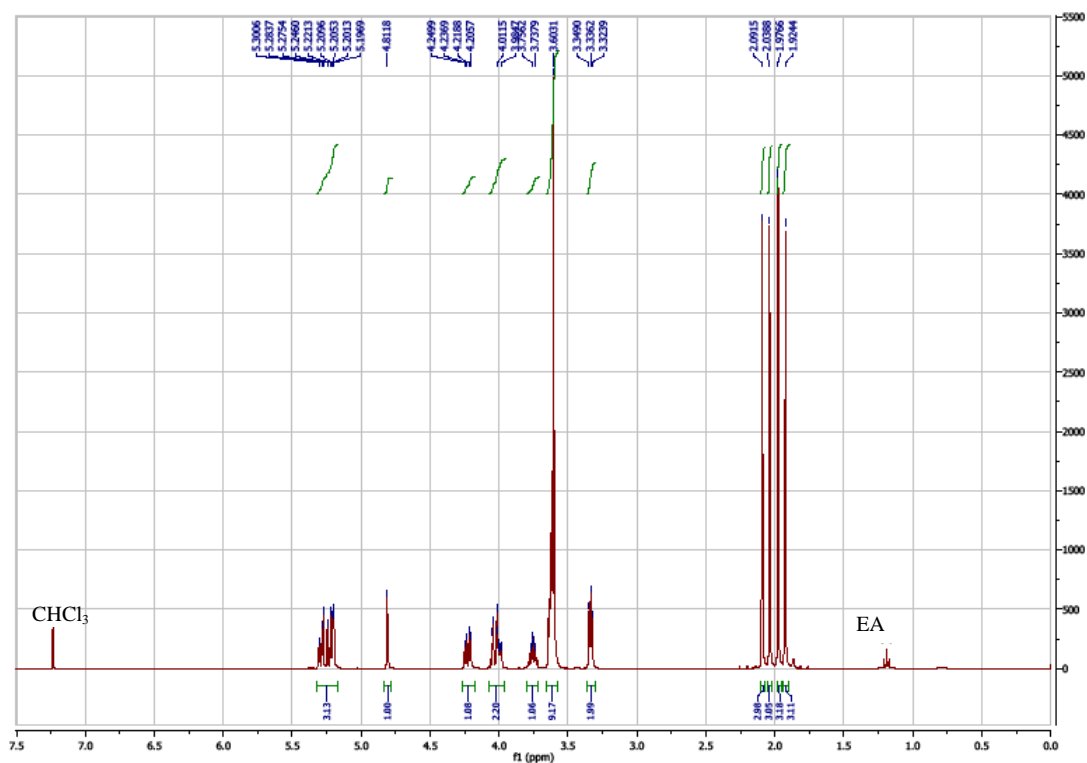
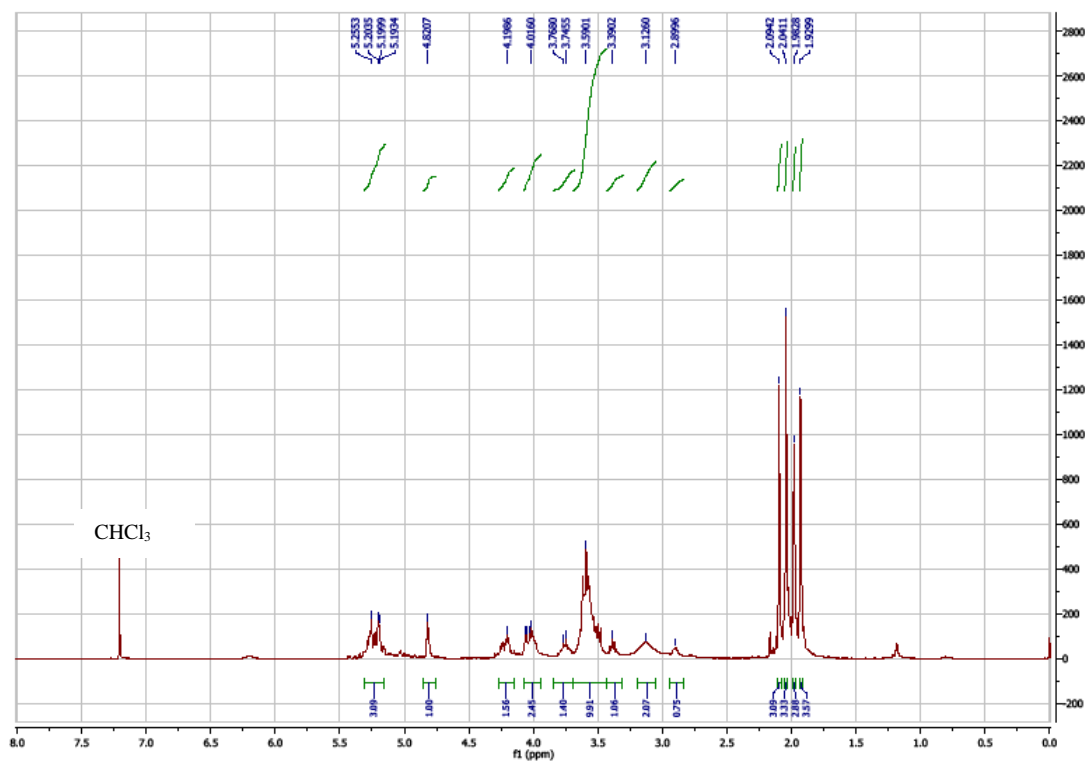
Monotosyl triethylene glycol (S2)

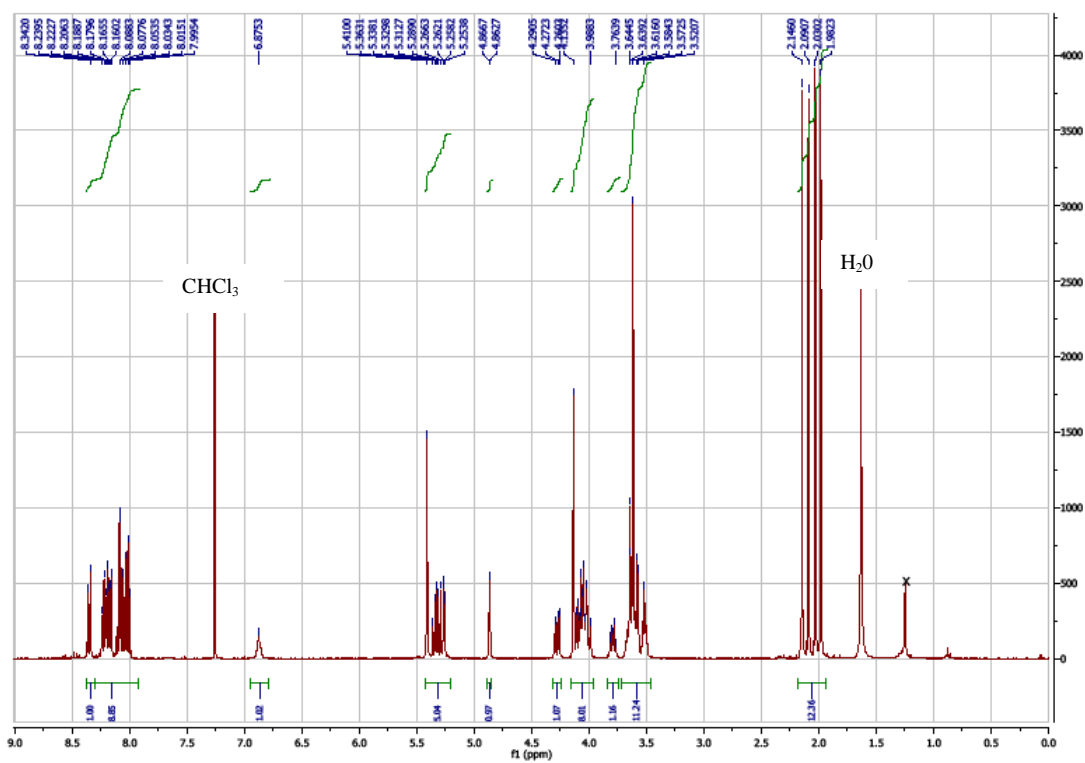
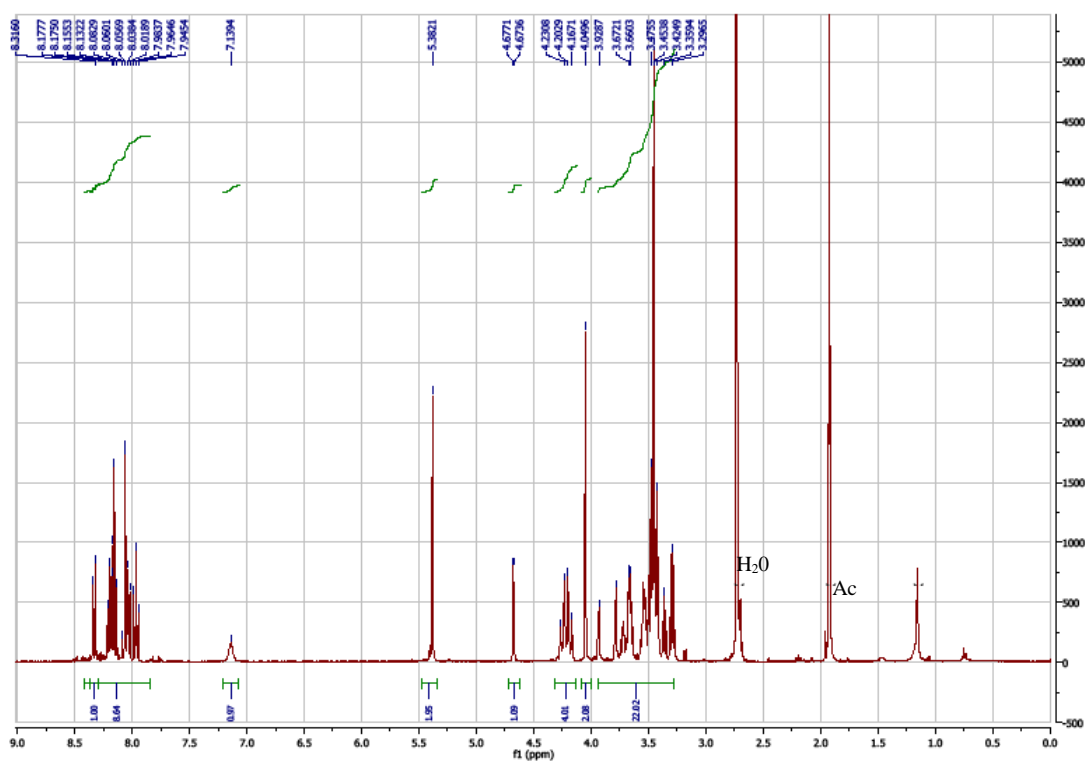


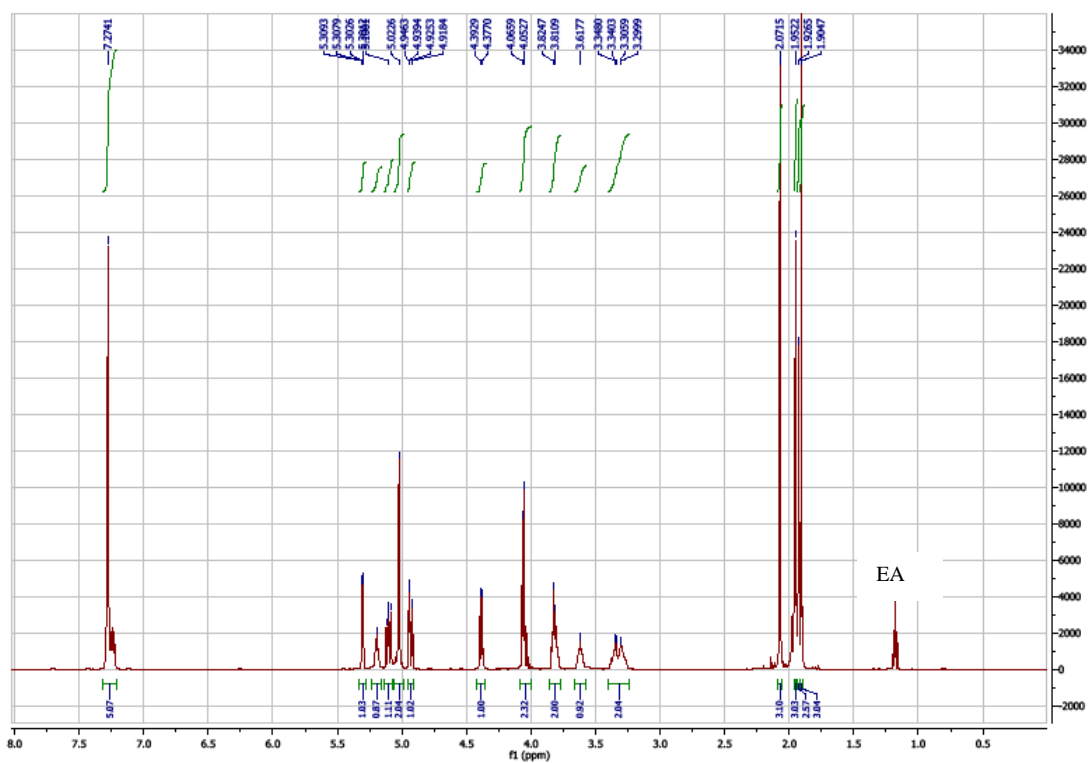
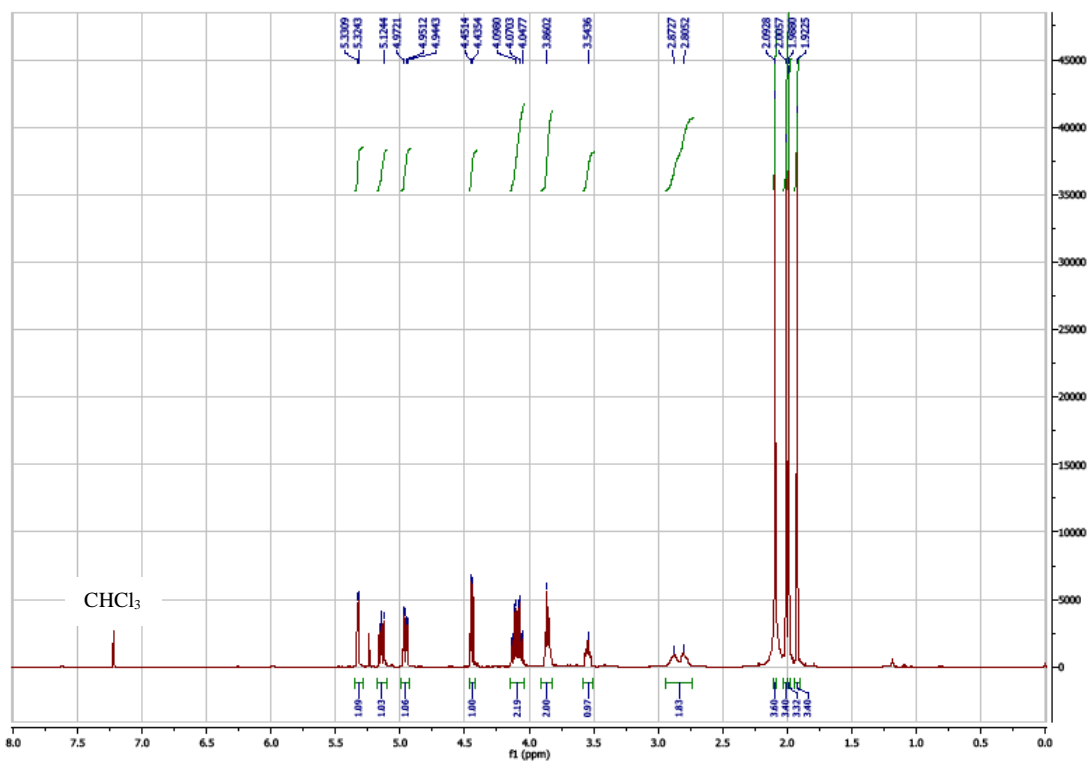
Monoazido triethylene glycol (S3)

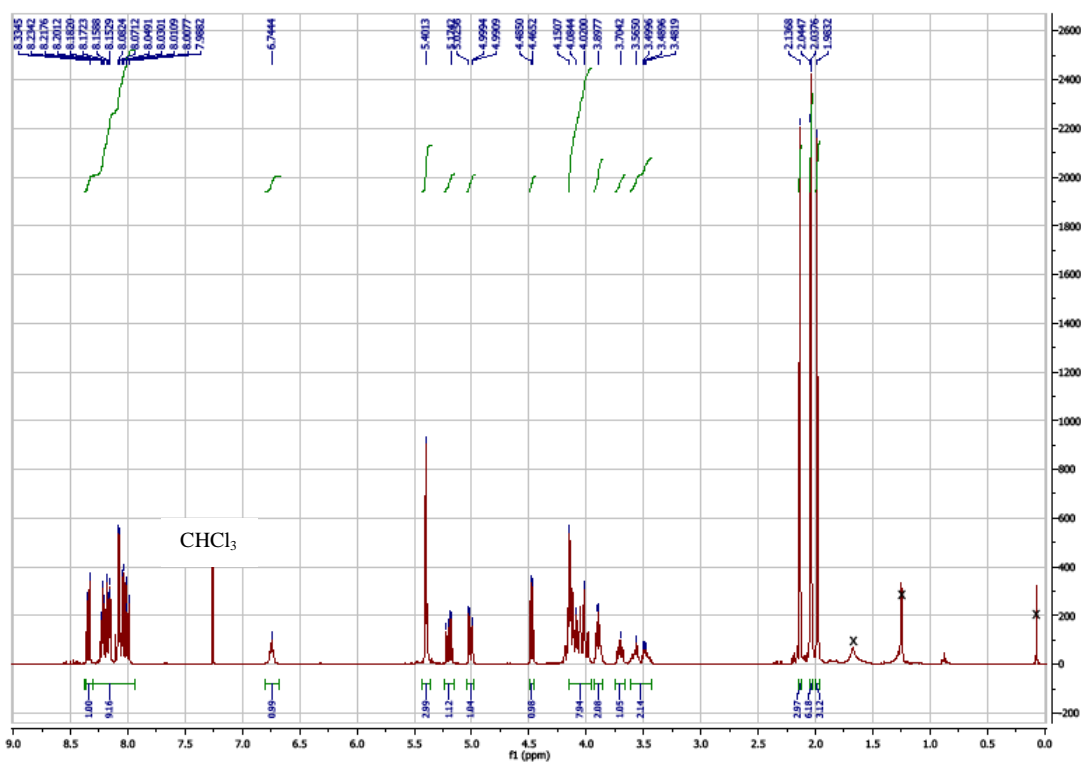
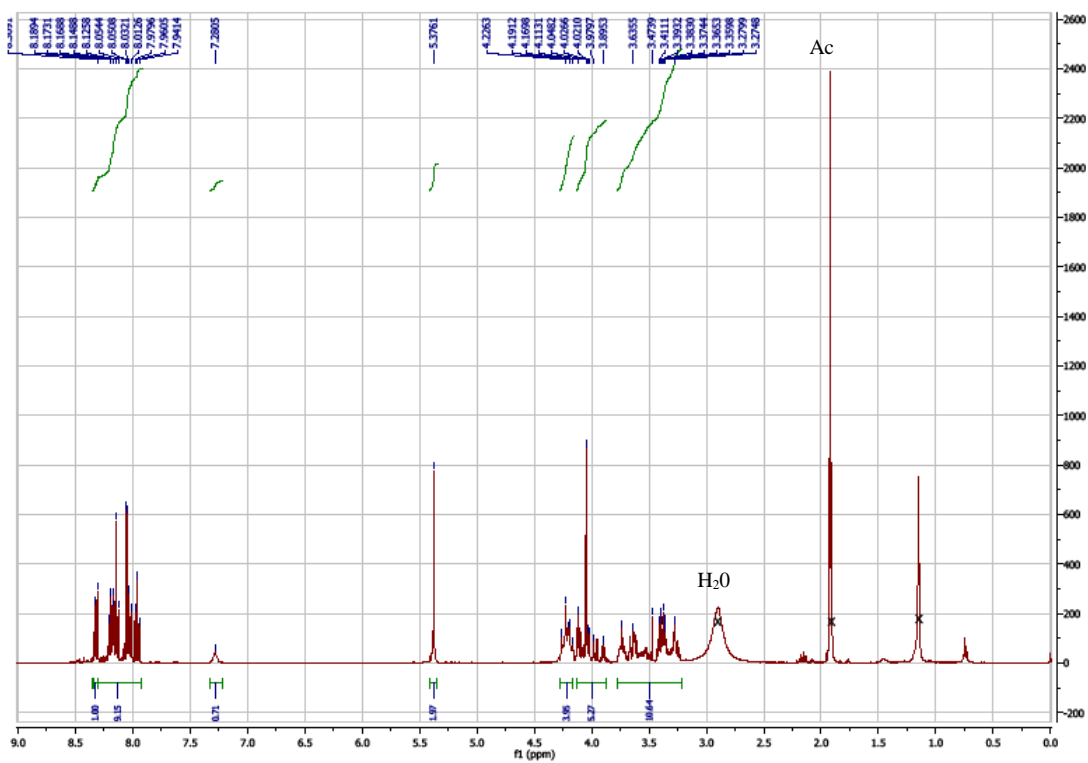


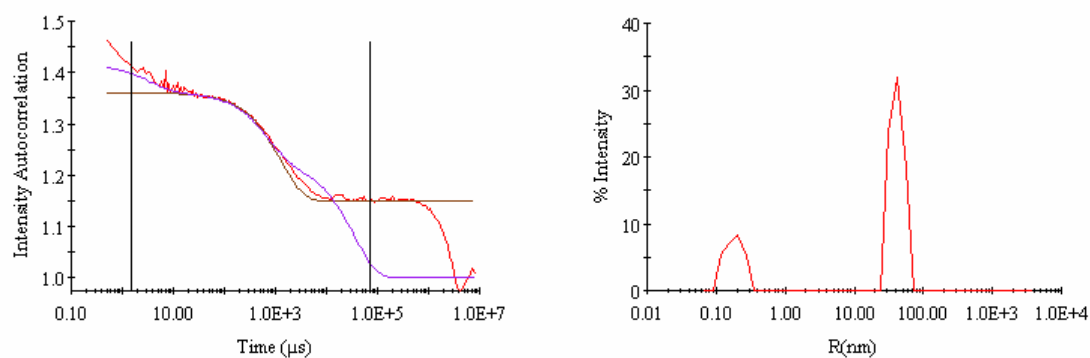


2,3,4,6-tetra-*O*-acetyl-1-(9-azido-triethylene glycol)-mannose (S5)2,3,4,6-tetra-*O*-acetyl-1-(9-amino-triethylene glycol)-mannose (S6)

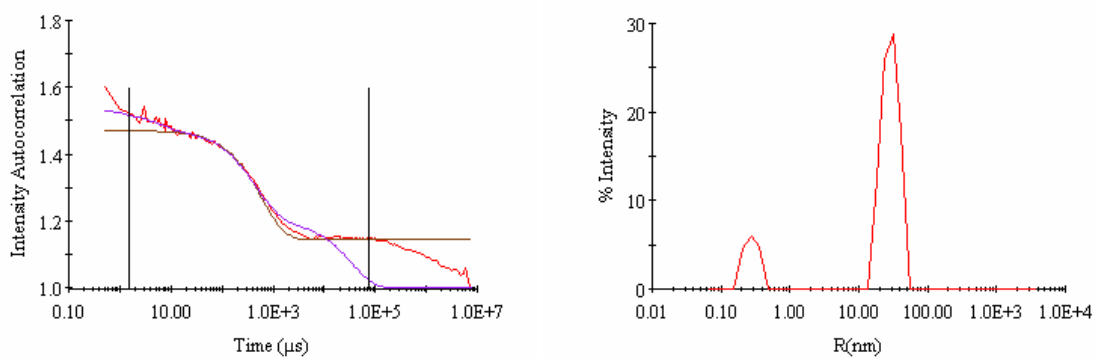
**2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Hexadecafluoro-10-(pyren-1-methoxy)-decycloxy-N-(2-(2-(2- $\alpha$ -D-(2,3,4,6-tetra-O-acetyl)-mannopyranosyl-ethoxy)ethoxy)ethyl)-amide****2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Hexadecafluoro-10-(pyren-1-methoxy)-decycloxy-N-(2-(2-(2- $\alpha$ -D-mannopyranosyl-ethoxy)ethoxy)ethyl)-amide (3)**

**2,3,4,6-tetra-O-acetyl-1-(2-benzoyloxycarbonylamino-ethoxy)-galactose (S9)****2,3,4,6-tetra-O-acetyl-1-(2-amino-ethoxy)-galactose (S10)**

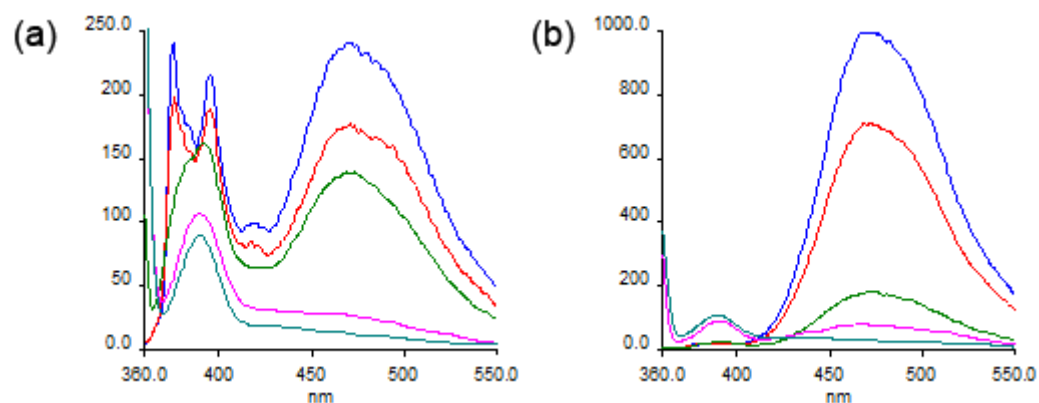
**2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Hexadecafluoro-10-(pyren-1-ethoxy)-decycloxy-*N*-(2- $\alpha$ -D-(2,3,4,6-tetra-*O*-acetyl)-galactopyranosyl-ethyl)-acetamide****2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Hexadecafluoro-10-(pyren-1-ethoxy)-decycloxy-*N*-(2- $\alpha$ -D-galactopyranosyl-ethyl)-acetamide (S11)**

**S3. Dynamic Light Scattering of 2 and 3 in aqueous buffer**

**Fig S1.** Autocorrelation (experimental trace (-) cumulant fit (-) and regularization fit (-)) and calculated hydrodynamic radius of lipid **2** in aqueous buffer.



**Fig S2.** Autocorrelation (experimental trace (-) cumulant fit (-) and regularization fit (-)) and calculated hydrodynamic radius of lipid **3** in aqueous buffer.

**S4. Excimer formation for lipids 2 and 3 in aqueous buffer and CAC determination**

**Fig S3.** Fluorescence spectra of (a) lipid 2 and (b) lipid 3 in aqueous buffer (20 mM MOPS, 100 mM NaCl, pH 7.4) at 25 °C at concentrations of 20 μM (-), 2 μM (-), 200 nM (-), 20 nM (-) and 2 nM(-).

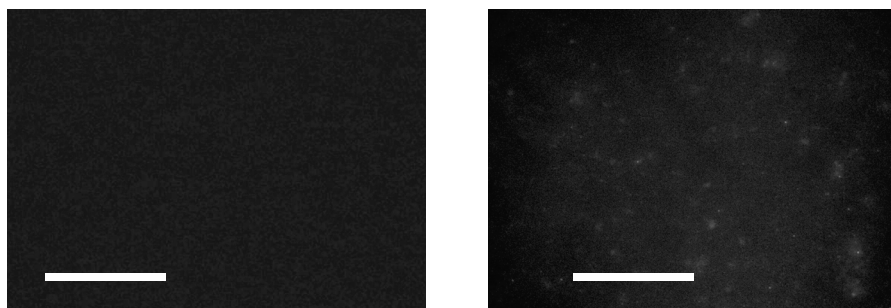


**Fig S4.** E/M of (a) lipid 2 and (b) lipid 3 vs. log[lipid] for critical aggregation concentration (CAC) determination. The intercept of the two linear fits corresponds to the CAC value.

### **S5. Observation of lipid rafts of 2 by fluorescence microscopy**

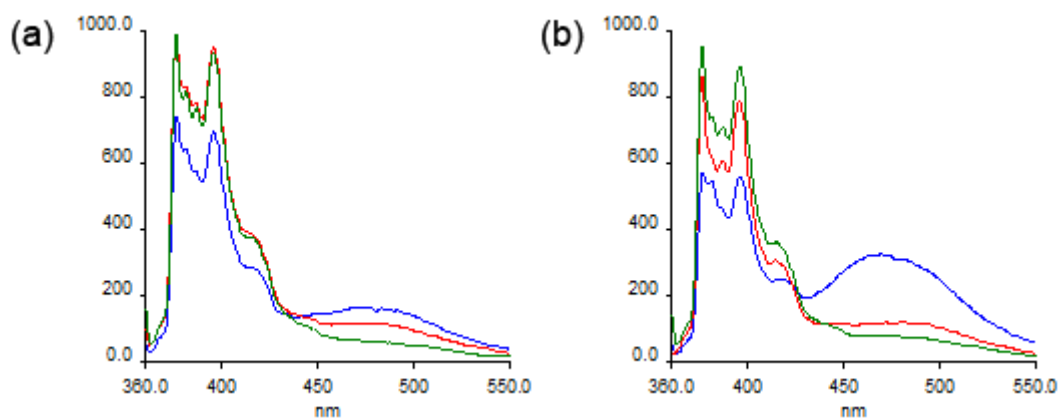
#### **Preparation of solid supported lipid films**

To prepare the solid support, glass slides were cleaned with piranha solution and oven-dried. Any remaining residue was removed from the slides by washing in absolute ethanol followed by further drying overnight at 110 °C. A section of a polycarbonate fluorescence cell was clamped firmly onto a glass slide to provide a temporary, square-based well with an internal footprint of 1 cm<sup>2</sup>. The bilayers were prepared by incubating a suspension of unilamellar vesicles prepared by sonication (200 µL, 2 mM lipid in 20 mM MOPS buffer, 100 mM NaCl, pH 7.4) within this well for an hour. After incubation, the sample was rinsed with MOPS buffer and the plastic well was removed to leave a hydrated film on the glass surface. A cover-slip was affixed with varnish to prepare the sample for *epi*-fluorescence and confocal microscopy.

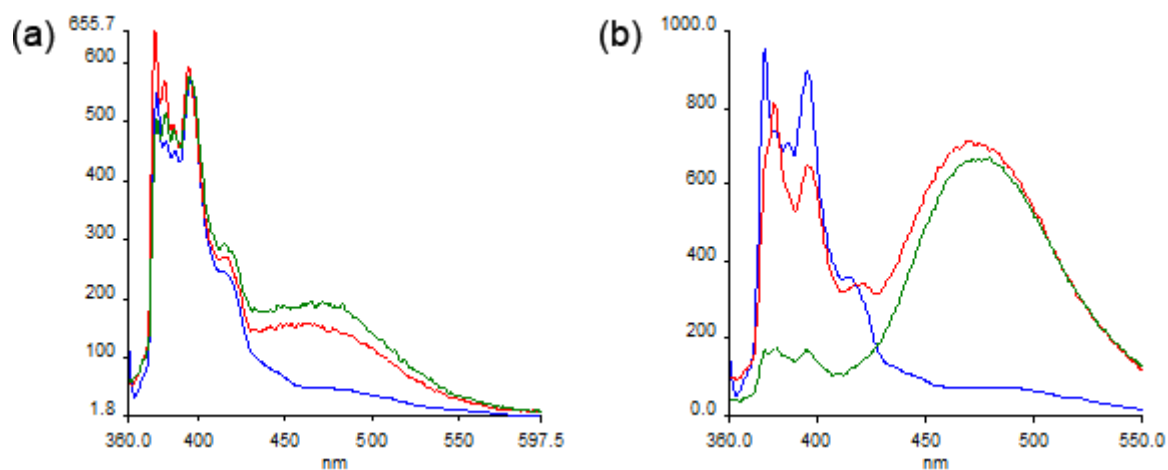


**Fig S5.** Fluorescence micrographs of solid supported bilayers. Bilayers composed of DMPC with 1 mol% lipid **2** (left) exhibited a dark blue fluorescence under the DAPI filter. Whereas bilayers composed of 50 % mol/mol DMPC/cholesterol doped with 1 mol% **2** (right) contained patches of pyrene excimer, indicating phase separation of **2**. Scale bar represents 10 µm.

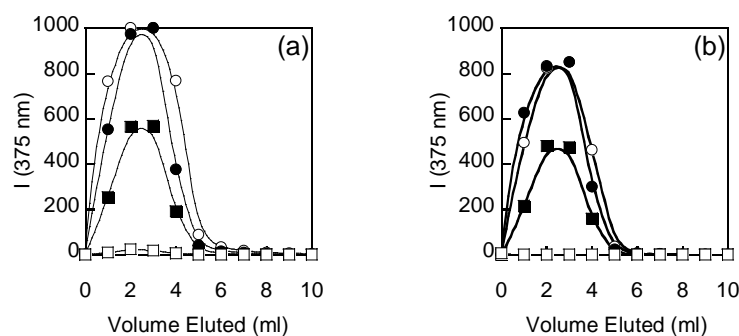
### **S6. Fluorescence spectra of 1, 2 and 5 % mol/mol mannose lipid loadings**



**Fig S6.** Fluorescence spectra of (a) lipid **2** and (b) lipid **3** in DMPC at membrane loadings of 1 % mol/mol (-), 2 % mol/mol (-) and 5 % mol/mol (-).

**S7. Excimer formation due to phase separation of 2 and 3 in DMPC/cholesterol vesicles.**

**Fig S7.** Fluorescence spectra of 1 % mol/mol (a) lipid 2 and (b) lipid 3 in vesicles composed of DMPC (-), 70/30 DMPC/cholesterol (-) and 50/50 DMPC/cholesterol (-) at 2 mM total lipid at 25 °C.

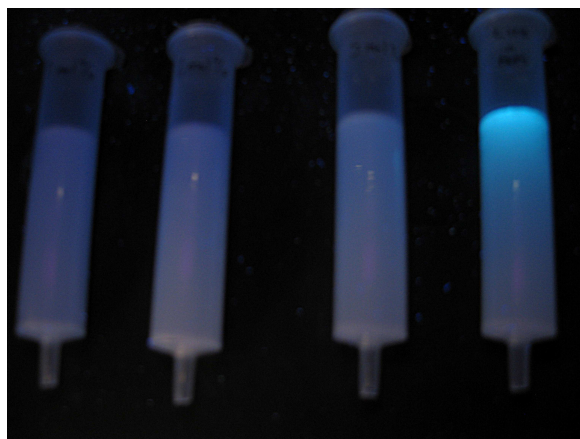
**S8. Demonstration of complete incorporation of 2 and 3 in DMPC vesicles at 1, 2 and 5 % mol/mol; gel permeation chromatography.**

**Fig S8.** GPC elution profile of lipid 2 and lipid 3 in DMPC vesicles at membrane loadings of 1 mol% ( $\square$ ), 2 mol% ( $\bullet$ ) and 5 mol% ( $\blacksquare$ ) and in buffer ( $\square$ ). No fluorescent material was eluted if 2 or 3 were not incorporated in vesicles (Fig S6, S7).



**Fig S9.** GPC columns after elution of lipid 3 with 10 ml buffer. From left to right; 1 % mol/mol, 2 % mol/mol and 5 % mol/mol in DMPC vesicles and as a micellar suspension in buffer.





**Fig S10.** GPC columns after elution of lipid 3 with 10 ml buffer under long wave UV light. From left to right; 1 % mol/mol, 2 % mol/mol and 5 % mol/mol in DMPC vesicles and as a micellar suspension in buffer.

### **S9. Determination of $K$ for 4-methylumbelliferyl- $\alpha$ -D-mannopyranoside (MUM) binding to Con A**

Concanavalin A/MUM solution (1.57 mM Con A, 2  $\mu$ M MUM, 15  $\times$  10  $\mu$ L titres) was added to a buffered solution of MUM (2 mL, 2  $\mu$ M MUM, 20 mM MOPS, 100 mM NaCl, pH 7.4). Fluorescence spectra were recorded (ex. at 317 nm) 60 s after each addition. The emission at 375 nm was fitted to a Stern-Volmer plot, which gave  $K = (3.37 \pm 0.09) \times 10^4 \text{ M}^{-1}$  ( $K_{\text{stat}} = K$ ).

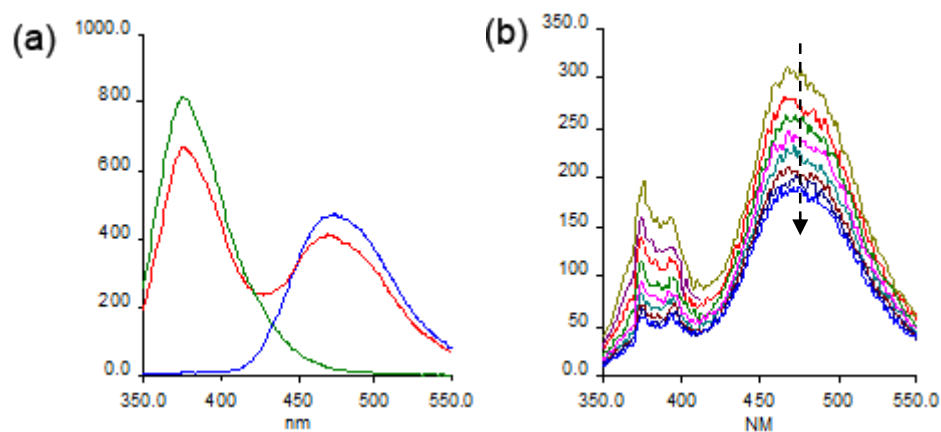
### **S10. Determination of $K$ for 2 and 3 binding to Con A in solution; competition experiments with MUM.**

Titres of Con A/MUM solution with 2 or 3 (15  $\times$  10  $\mu$ L titres, 1.57 mM Con A, 20  $\mu$ M 2 or 3, 2  $\mu$ M MUM) were added to a solution of MUM with 2 or 3 (2  $\mu$ M MUM, 20  $\mu$ M 2 or 3, 2 mL, 20 mM MOPS, 100 mM NaCl, pH 7.4). Fluorescence spectra were recorded (ex. at 317 nm) 60 s after each addition. The emission of MUM (375 nm) and pyrene monomer (379 and 395 nm) overlaps when excited at 317 nm, while that of the pyrene excimer was clear. Therefore analogous titrations in the absence of MUM were carried out to give E/M ratios that allowed the determination of the contribution of pyrene monomer and thus MUM to the fluorescence at 375 nm. In a modification of the procedure of Landschoot *et al*,<sup>6</sup> a Scatchard plot was employed to determine  $K$  from the resulting data. Equations S1 and S2 were used to resolve values for the response (amount of bound ligand,  $r$ ) and amount of unbound pyrene lipid  $[P]_f$  ( $[2]_f$  or  $[3]_f$ ) at each titre point.

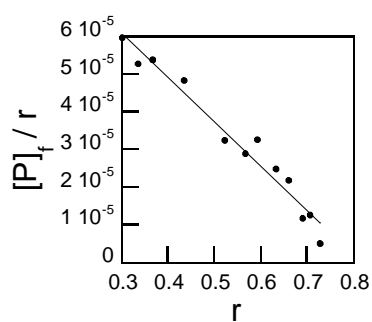
$$r = 1 - \frac{B}{[C]} \left( [U] + \frac{1}{K_{cu} - BK_{cu}} \right) \quad (\text{S1})$$

$$[P]_f = [P] - [C] - B \left( [U] - \frac{1}{K_{cu} - BK_{cu}} \right) \quad (\text{S2})$$

Where  $[C]$ ,  $[U]$  and  $[P]$  are the total concentrations of Con A, MUM and pyrene lipid respectively.  $K_{cu}$  is the association constant of MUM with Con A and  $B = 1 - F_u(0)/F_u$  ( $F_u(0)$  is the initial MUM fluorescence at 375 nm). Plotting  $r/[P]_f$  vs.  $r$  gave a linear plot with a gradient of  $-K$  (Fig. S9) which gave  $K = (5.4 \pm 0.5) \times 10^3 \text{ M}^{-1}$  for 2 and  $K$  was  $(6.9 \pm 1.1) \times 10^3 \text{ M}^{-1}$  for 3.



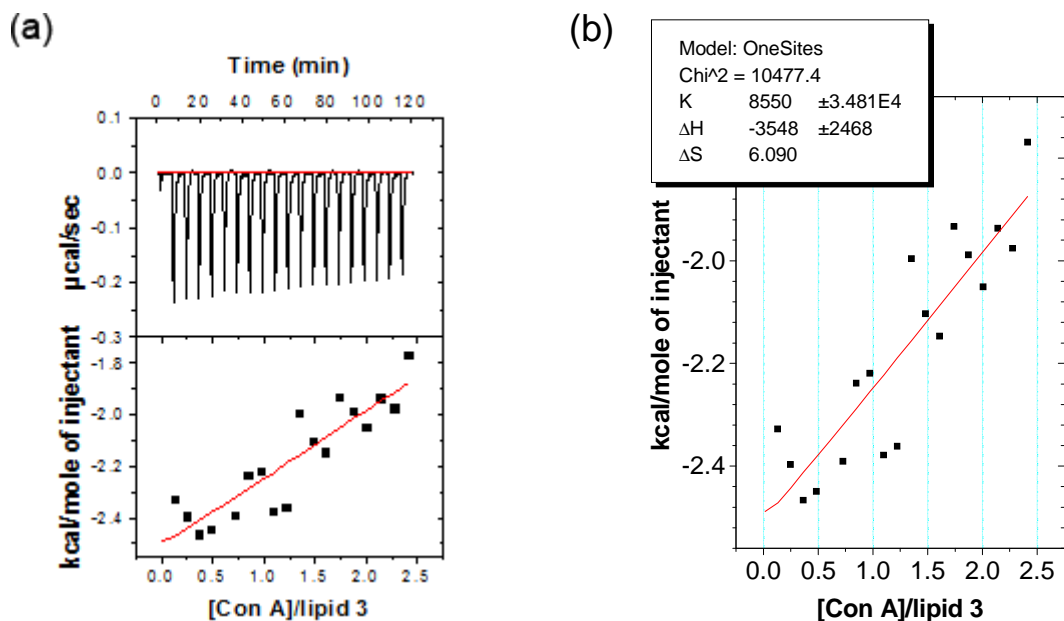
**Fig S11.** (a) Fluorescence spectra of MUM (20  $\mu$ M) (-), lipid **3** (20  $\mu$ M) (-) and both (20  $\mu$ M of each) (-) in aqueous buffer (20 mM MOPS, 100 mM NaCl, pH 7.4) at 25  $^{\circ}$ C. (b) Fluorescence competition experiment with increasing [Con A].



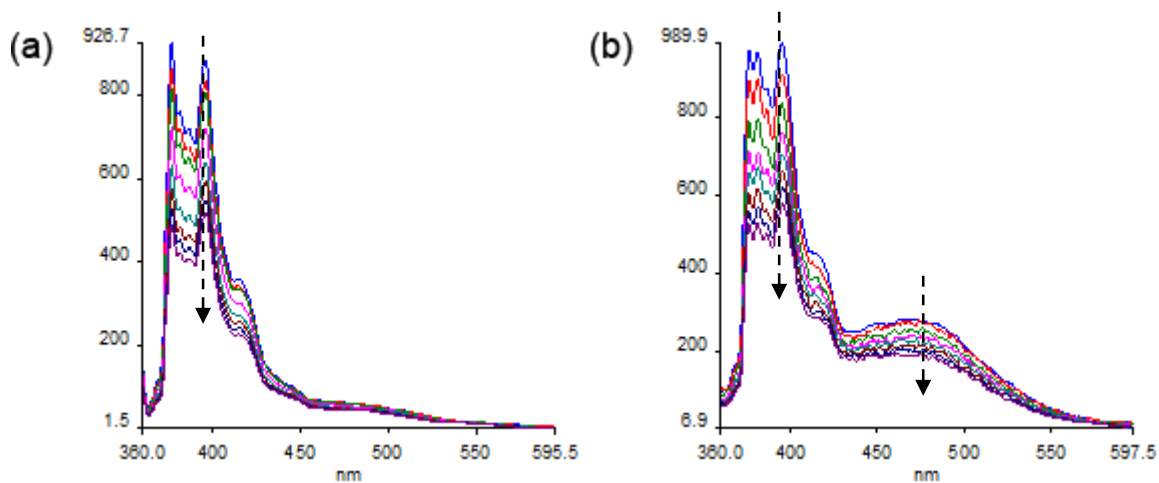
**Fig S12.** Representative Scatchard plot from the MUM vs. lipid **3** competition titration.

**S11. Isothermal titration calorimetry (ITC) data for the titration of Con A with lipid 3**

Isothermal titration calorimetry was carried out using a Microcal VP-ITC calorimeter, with a cell volume of 1.4384 mL.  $24 \times 12 \mu\text{L}$  titres of a 2 mM solution of **3** (20 mM MOPS, 1 mM  $\text{MnCl}_2$ , 1 mM  $\text{CaCl}_2$ , 100 mM NaCl, pH 7.4, 5 vol % DMSO) were injected into the cell containing 200  $\mu\text{M}$  Con A (20 mM MOPS, 1 mM  $\text{MnCl}_2$ , 1 mM  $\text{CaCl}_2$ , 100 mM NaCl, pH 7.4, 5 vol % DMSO), at 25 °C, using a reference power of 12 and stirring at 310 rpm. Lipid **3** was also titrated into neat buffer to obtain heats of dilution. The heats of dilution were subtracted from the titration with Con A and curve-fitted using a single-site binding model with Origin-ITC (version 5).



**Fig S13.** ITC traces for the titration of lipid **3** into Con A (a). Heats of dilution were subtracted and the data fit to a single site binding model (b).

**S12. Representative changes in emission spectra during titrations of lipids 2 and 3 in phospholipid vesicles with Con A.**

**Fig S14.** Fluorescence quenching titrations with 1 % mol/mol lipid 2 in vesicles composed of (a) DMPC and (b) 50/50 DMPC/cholesterol at 2 mM total lipid at 25 °C with increasing Con A concentration.

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