Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information

Sialylation of lactosyl lipids in membrane microdomains by T. cruzi trans-sialidase

Gavin T. Noble, Faye L. Craven, Maria Dolores Segarra-Maset, Juana Elizabeth Reyes Martínez, Robert Šardzík, Sabine L. Flitsch* and Simon J. Webb*

Manchester Institute of Biotechnology and School of Chemistry, The University of Manchester, 131 Princess Street, Manchester, M1 7DN, United Kingdom.

S.1. Experimental procedures

General Procedures: NMR spectra were recorded on Bruker Avance 300, DPX400 and Avance II+ 500 spectrometers at 25 °C and calibrated to residual solvent. Spectra were assigned with appropriate ^{1}H, ^{13}C, DEPT, COSY, HSQC, HMQC and HMBC NMR experiments. Chemical shifts are in ppm, coupling constants in Hertz (Hz) and multiplicities indicated with: singlet (s), doublet (d), triplet (t), double doublet (dd) and multiplet (m). ES+ mass spectra were obtained with Micromass Prospec and Micromass Platform spectrometers. IR spectra were recorded using PerkinElmer Spectrum RX I and Bruker ALPHA-P FT-IR spectrometers. Unless otherwise specified, emission spectra were recorded at 37 °C from 360 to 600 nm (excitation 346 nm) using a Perkin Elmer LS55 luminescence spectrometer with Julabo F25 waterbath. UV-visible absorption spectra were recorded using a Jasco V-660 spectrophotometer. Fluorescence micrographs were produced using a Zeiss Axio Imager A1 fluorescence microscope fitted with a Canon Powershot G6 digital camera. MALDI-ToF/ToF measurements were made using a Bruker Daltonics Ultraflex II spectrometer (Bremen, Germany). HPLC data were obtained on an Agilent 1200 series LC system with a G1315B diode-array detector (DAD). LC/MS measurements were made using an Agilent 110 series LC system with an attached G1315B DAD and G1956B LC/MSD SL unit. A Phenomenex Luna C18(2) 250×2 mm 5 micron column was used for all HPLC and LC/MS experiments. GUVs were electroformed using an Agilent 33210A 10 MHz function arbitrary waveform generator. PD-10 gel permeation columns were purchased from GE Healthcare and Vivaspin 500 ultracentifugation filters were obtained from Sartorius Stedin Biotech. 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Hexadecafluoro-10-(pyren-1-ylmethoxy)decyloxy)acetic acid was prepared using literature methods.[S1] 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl(1-4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide, 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl(1-4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-1-benzyl *N*-(2-hydroxyethyl)carbamate and 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl(1-4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl-1-(2-hydroxyethyl)amine were synthesised according to literature methods.[S2] Fetuin from fetal calf serum was obtained from Sigma-

Aldrich. Fluorescein-labeled *Maackia amurensis* leukoagglutinin was provided by Vector Laboratories. *Trypanosoma cruzi* trans-sialidase (TcTS) was subcloned by S. Kaloo (University of Manchester) in *E. coli*. DMPC and DPPC were supplied by Avanti Polar Lipids. Other reagents were purchased from Sigma-Aldrich and used as received unless otherwise stated.

2-((2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Hexadecafluoro-10-(pyren-1-ylmethoxy)decyl)oxy-*N*-(2-(2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyl-(1'-4')-2'',3'',6''-tri-*O*-acetyl-β-D-glucopyranosyl)oxyethyl))acetamide (**8**). 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Hexadecafluoro-10-(pyren-1-ylmethoxy)-decyloxy)-acetic acid (**6**, 1 eq.), dicyclohexylcarbodiimide (DCC, 1.3 eq.) and *N*-hydroxysuccinimide (NHS, 1.3 eq.) were dissolved in dry CH_2Cl_2 under N_2. 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 precipitate and 2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl(1-4)-2,3,6-tri-*O*-acetyl-*β*-D-glucopyranosyl-1-(2-hydroxyethyl)amine **7** (1.5 eq.) was added in dry CH_2Cl_2 (1 mL). The mixture was stirred under N_2 overnight. The mixture was filtered again and the solvent was evaporated under reduced pressure. The crude mixture was then purified using two rounds of flash column chromatography (first 19:1 $CHCl_3$/MeOH on silica, then 3:2:1 ethyl acetate/$CHCl_3$/cyclohexane on silica followed by MeOH flush to recover the product). This yielded the desired acetyl protected glycolipid **8** as a light yellow oil (55 mg, 58%); **TLC** R_f 0.55 (19:1 $CHCl_3$/methanol); **^{1}H NMR** (400 MHz, $CDCl_3$, 25 °C) : δ_H 1.96 (3H, s, Ac CH_3), 2.02 (3H, s, Ac CH_3), 2.03-2.04 (9H, s, 3 × Ac CH_3), 2.10 (3H, s, Ac CH_3), 2.15 (3H, s, Ac CH_3), 3.49-3.55 (2H, m, C$H_2$$NH_2$), 3.59 (1H, ddd, 3*J* (H,H) = 1.9, 3.6, 9.7 Hz, H-5), 3.63-3.69 (1H, m, C$H_a$$H_b$$CH_2$), 3.75 (1H, dd, 3*J* (H,H) = 9.2, 9.7 Hz, H-4), 3.82-3.87 (2H, m, $CH_a$$H_b$$CH_2$, H‴-5), 4.02 (2H, t, 3*J* (H,F) = 13.7 Hz, CH_2CF_2), 4.04 (2H, t, 3*J* (H,F) = 14.3 Hz, CH_2CF_2), 4.05-4.12 (3H, m, CH_2-6", H-6a), 4.13 (2H, s, CH_2CONH), 4.46 (1H, d, 3*J* (H,H) = 7.7 Hz, H-1), 4.47 (1H, d, 3*J* (H,H) = 7.7 Hz, H-1"), 4.50 (1H, dd, ^{3}*J* (H,H) = 1.7, 11.7 Hz, H-6b), 4.87 (1H, dd, 3*J* (H,H) = 8.0, 9.3 Hz, H-2), 4.95 (1H, dd, 3*J* (H,H) =

3.3, 10.4 Hz, H-3"), 5.10 (1H, dd, ^{3}J (H,H) = 7.9, 10.4 Hz, H-2"), 5.19 (1H, dd, 3J (H,H) = 9.2, 9.3 Hz, H-3), 5.33 (1H, dd, 3J (H,H) = 0.7, 3.0 Hz, H-4"), 5.40 (2H, s, Ar-CH_2), 6.72 (1H, t, 3J (H,H) = 5.6 Hz, CONH), 7.99-8.36 (9H, m, 9 × Ar CH); **^{13}C NMR** (100 MHz, $CDCl_3$, 25 °C) : δ_C 20.6-20.9 (7 × CH_3), 38.9, 60.9, 62.0, 66.6 (t, ^{2}J (C,F) = 24.7 Hz, CH$_2$CF$_2$), 66.7, 68.2 (t, ^{2}J (C,F) = 24.4 Hz, CH$_2$CF$_2$), 68.5, 69.3, 70.9, 71.1, 71.7, 72.0, 72.9, 73.0, 73.2, 76.4, 100.6, 101.2, 123.2, 124.6, 124.7, 125.1, 125.6 (2 × CH), 126.3, 127.4 (2 × CH), 128.0, 128.3, 129.3, 129.7, 130.9, 131.3, 132.0, 168.2, 169.2-170.3 (7 × CO) (CF_2 signals are weak complex multiplets); **IR** υ (cm^{-1}) 1020, 1263, 1577, 1610, 2933.

2-((2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Hexadecafluoro-10-(pyren-1-ylmethoxy)decyl)oxy-*N*-(2-(β-D-galactopyranosyl -(1'-4')-β-D-glucopyranosyl)oxyethyl))acetamide (**1**). 2-((2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Hexadecafluoro-10-(pyren-1-ylmethoxy)decyl)oxy-*N*- (2- (2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyl-(1'-4')-2'',3'',6''-tri-*O*-acetyl-β-D-glucopyranosyl)oxyethyl))-acetamide (**8**, 20 mg) was dissolved in methanol (2 mL). NaOMe/MeOH solution (15% w/v, 0.2 mL) was then added slowly to the stirring lipid solution, and the mixture stirred for 0.5 h. Ion-exchange resin (Amberlite IR-120) was then added until the solution reached pH 7. The resin was then removed by filtration through cotton wool and the ethanol removed under reduced pressure to yield glycolipid **1** as a light yellow solid (8.3 mg, 53%); **TLC** R_f 0.0 (19:1 $CHCl_3$/methanol); **^{1}H NMR** (400 MHz, DMF-d_7, 25 °C) : δ_H 3.18 (1H, ddd, ^{3}J (H,H) = 4.0, 8.2, 8.2 Hz, H-2), 3.41-3.58 (7H, m, CH_2NH, H-2", 4 × sugar CH), 3.61- 3.92 (10H, m, CH_2CH$_2$NH, CH_2-6, CH_2-6", 2 × sugar CH, OH-6, OH-6"), 4.24 (2H, s, CH_2CONH), 4.35 (1H, d, ^{3}J (H,H) = 7.8 Hz, H-1), 4.37 (1H, d, ^{3}J (H,H) = 7.6 Hz, H-1), 4.43 (2H, t, ^{3}J (H,F) = 13.9 Hz, CH_2CF_2), 4.50 (2H, t, ^{3}J (H,F) = 14.6 Hz, CH_2CF_2), 4.70- 4.77 (3H, m, OH-3, OH-3", OH-4"), 5.29 (1H, d, ^{3}J (H,H) = 4.1 Hz, OH-2), 5.36 (1H, d, ^{3}J (H,H) = 4.4 Hz, OH-2"), 5.54 (2H, s, Ar-CH_2), 7.97 (1H, t, ^{3}J (H,H) = 5.8 Hz, CONH), 8.11-8.50 (9H, m, 9 × Ar CH); **^{13}C NMR** (100 MHz, DMF-d_7, 25 °C) : δ_C 39.1, 61.5, 61.7, 66.7 (t, ^{2}J = 25.2 Hz, CH$_2$CF$_2$), 68.0 (t, ^{2}J = 25.8 Hz, CH$_2$CF$_2$), 68.5, 69.2, 71.6, 71.7, 72.6, 74.2, 74.4, 75.8 (2 × CH), 76.5, 81.5, 103.8, 104.9, 123.8, 124.6, 124.9, 125.1, 125.9 (2 × CH), 126.7, 127.8, 127.9, 128.1, 128.3, 129.7, 130.9, 131.1, 131.6, 131.9, 168.9 (CF_2

signals are weak complex multiplets); **MS** (MALDI) *m/z*: 1124.463 (100 %) $[M+Na]^+$; **IR** υ (cm^{-1}) 1020, 1266, 1624, 2921, 3340.

***Trypanosoma cruzi trans-sialidase* (*TcTS*) expression**: Transformed *E. coli* BL21(DE3) cells containing the TcTS clone encoding the catalytic N-terminal domain of wild-type TcTS (TcTS6-11/2)[S3] cloned in pTrcHisA expression vector[S4] were grown and the over-expressed recombinant TcTS purified according to published techniques.[S5]

LUV preparation: The appropriate lipid mixture (1.80 μmol total lipid; DMPC 1.22 mg, DPPC 1.32 mg, DMPC/cholesterol 0.61 mg/0.35 mg) and lipid **1** (0.22 mg, 0.20 μmol) were dissolved in spectroscopic grade $CHCl_3$ (1 mL) and the solvent removed *in vacuo* to generate a thin film. The lipid film was further dried under vacuum and phosphate buffer added (1 mL, 100 mM, pH 7.4 at 37 °C). The lipids were dispersed into the buffer solution by vortex mixing for 15-20 minutes. Unilamellar vesicles were formed by extrusion (19 times) through an 800 nm pore polycarbonate membrane, above the T_m, using an Avestin Liposofast extrusion apparatus.

General reaction procedure: Vesicle suspension containing **1** at 1 or ~9% mol/mol loading (100 μL, ~180 μM **1** in 100 mM phosphate buffer, pH 7.4 at 37 °C), TcTS solution (1 or 5 μL, 3.91 μM, 100 mM phosphate buffer, pH 7.4 at 37 °C) and fetuin (1 mg or 5 mg, equivalent to 1.25 mM and 6 mM sialic acid respectively[S6]) were mixed in a 200 μL Eppendorf tube or small volume mass spectrometry vial and incubated at 37 °C. The enzyme concentration was estimated using its UV absorption at 280 nm;[S7] the molar extinction coefficient was estimated at ε = 147,250 $Lmol^{-1}cm^{-1}$ from the tryptophan and tyrosine residues in the protein amino acid sequence.[S8]

MALDI-ToF/ToF analysis: Samples were prepared as for β4GalT1 analysis,[S9] but MALDI-ToF spectra were taken in both reflector-positive and linear-positive modes.

Reaction rate determination –HPLC: Enzymatic reactions were performed as outlined in the general TcTS reaction procedure and were analyzed using a similar HPLC procedure used for β4GalT1 analysis.[S9] Doped vesicles (~9% mol/mol **1** in the appropriate matrix, 100 μL, 2.0 mM total lipid in phosphate buffer pH 7.4 at 37 °C), sialic acid donor (fetuin, 5 mg, or MuNANA, 5.8 μL of 0.1 M) and TcTS (5 μL, ~3.9 μM in phosphate buffer pH 7.4 at 37 °C) were added to a mass spectrometry vial with a reduced volume insert. The analytical HPLC was programmed to inject 5 μL aliquots from the vial onto the column every 30 minutes for 7 h. The experiments were run using reverse phase solvent system conditions (50:50 acetonitrile/ water (+ 0.1% formic acid)). Percent conversions were determined using the ratio of product peak area to the combined product and starting material area.

Product determination – LC/MS: Enzymatic reactions were performed as above, combined with a modification of the LC/MS procedure employed previously.[S9] The HPLC DAD module was set to detect the pyrene UV-visible absorption at 346 nm and the MS detector set to detect m/z 1126 ([**1** + H + Na]$^+$), then 1391 ([**2** + H]$^+$). Correlation of the HPLC peak for Lac-lipid **1** and 1126 m/z was observed, the peak for product **2** correlated with 1391 m/z in the mass spectrometric trace.

GUV preparation: Two heated ITO coated slides were evenly coated with DMPC (18 μL, 10 mM) and glycolipid (22 μL, 0.9 mM) from stock solutions of known concentration in $CHCl_3$. The slides were dried under vacuum for 1 h to give a thin lipid film. A rubber spacer was carefully placed on top of each slide, and a second ITO slide to form a chamber. The chambers were secured with binder clips and fitted with short strips of copper tape at the top corners of the chamber. The chambers were filled with glucose solution (550-600 μL, 300 mOsm) and sealed using modelling clay. The copper strips were connected to electrodes, and vesicles were electroformed at 30 °C. After electroformation, GUVs were mixed with sucrose solution (200 μL, 300 mOsm) and visualized by epi-fluorescence microscopy in custom-made chambers.

S.2. Spectra of novel compounds

2-((2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Hexadecafluoro-10-(pyren-1-ylmethoxy)decyl)oxy-*N*- (2-(2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyl-(1'-4')-2'',3'',6''-tri-*O*-acetyl-β-D-glucopyranosyl)oxyethyl))acetamide (8).

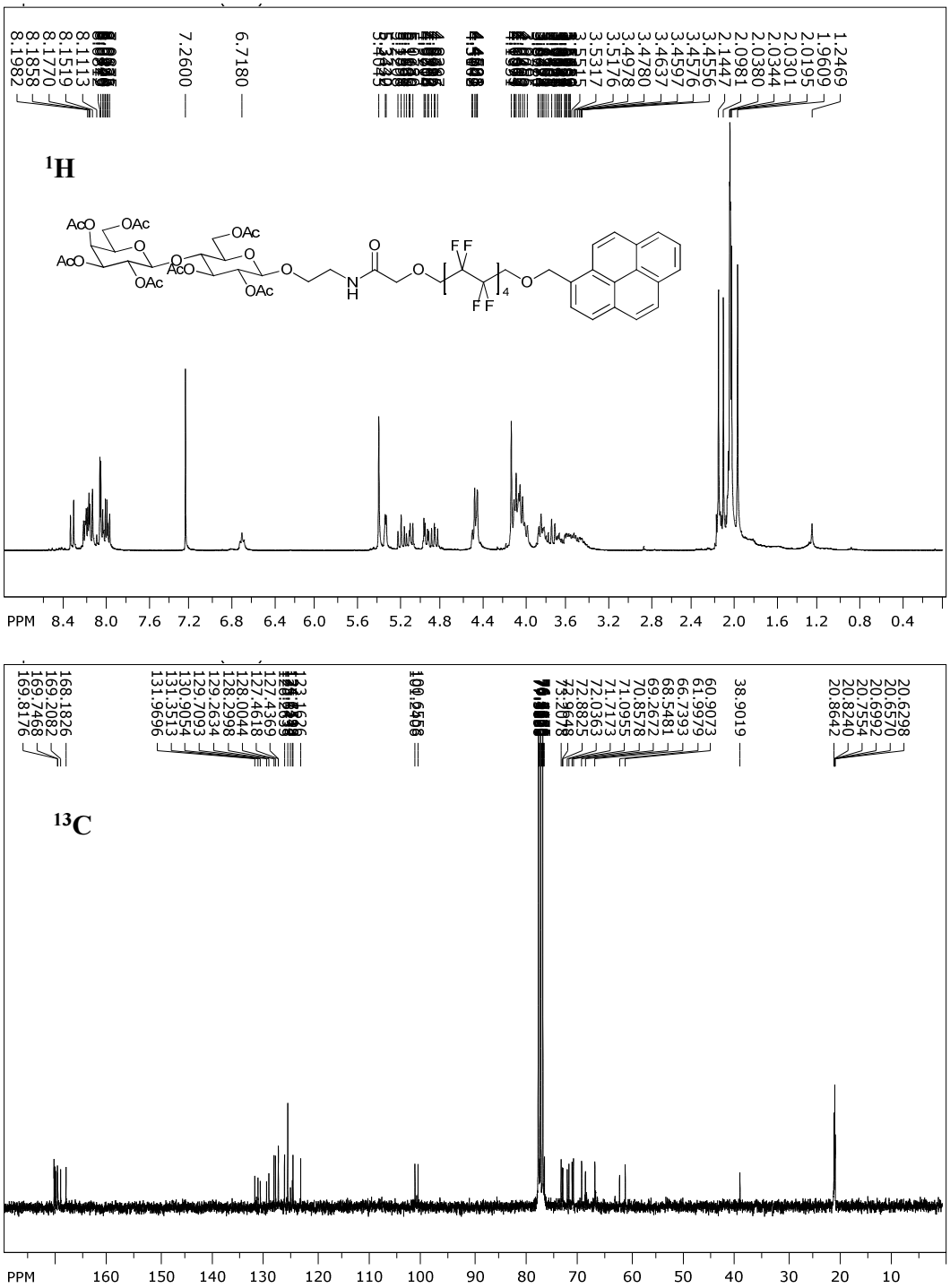

Figure S.1. ^{1}H and ^{13}C NMR in $CDCl_3$ of 2-((2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Hexadecafluoro-10-(pyren-1-ylmethoxy)decyl)oxy-*N*- (2- (2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyl-(1'-4')-2'',3'',6''-tri-*O*-acetyl-β-D-glucopyranosyl)oxyethyl))acetamide **8**.

2-((2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Hexadecafluoro-10-(pyren-1-ylmethoxy)decyl)oxy-*N*-(2-(β-D-galactopyranosyl-(1'-4')-β-D-glucopyranosyl)oxyethyl))acetamide (1).

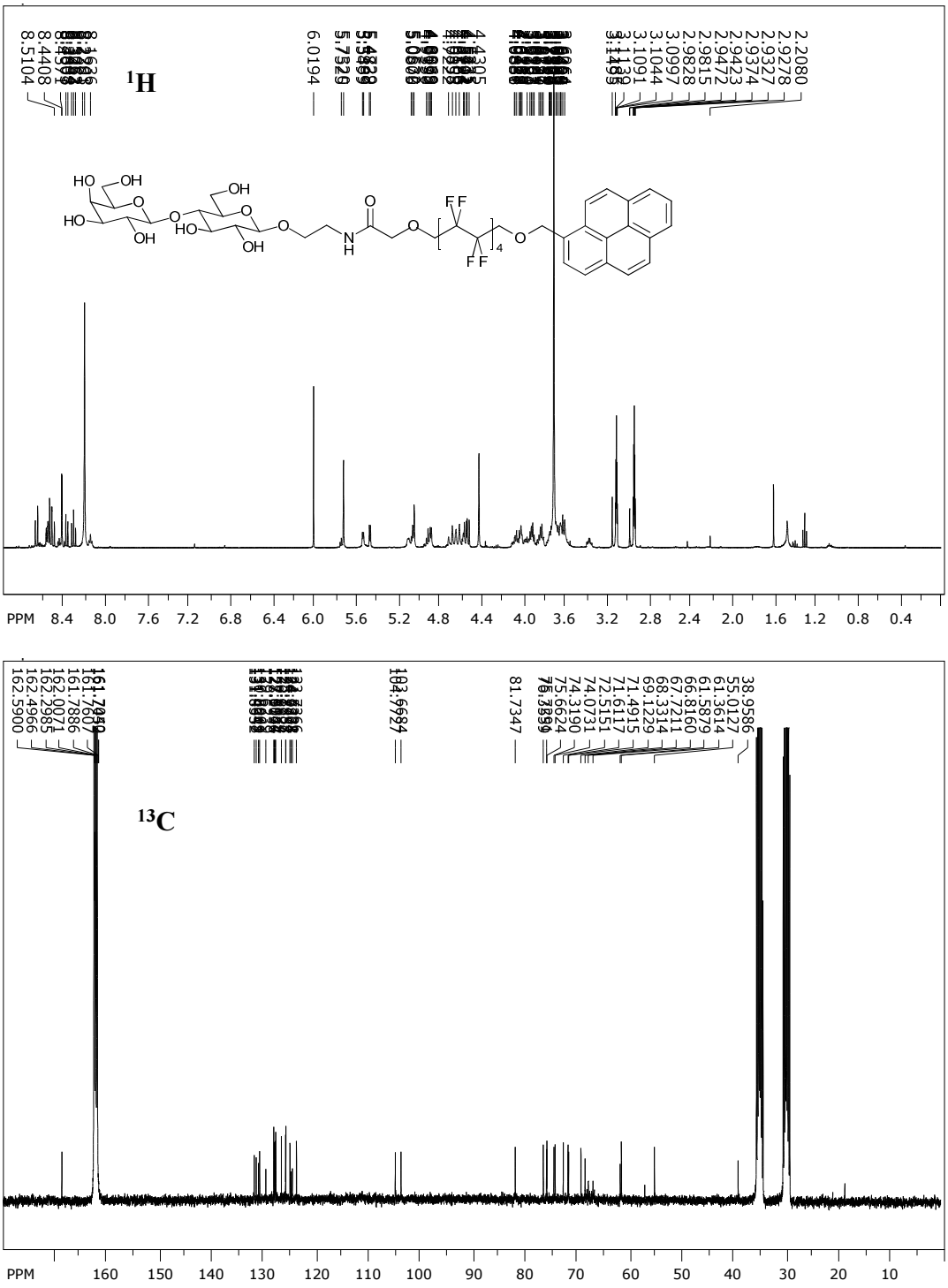

Figure S.2. ^{1}H and ^{13}C NMR in DMF-d_7 of 2-((2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-Hexadecafluoro-10-(pyren-1-ylmethoxy)decyl)oxy-*N*-(2-(β-D-galactopyranosyl-(1'-4')-β-D-glucopyranosyl)oxyethyl))acetamide **1**.

S.3. Determination of the critical aggregation concentration (CAC) of lipid **1**

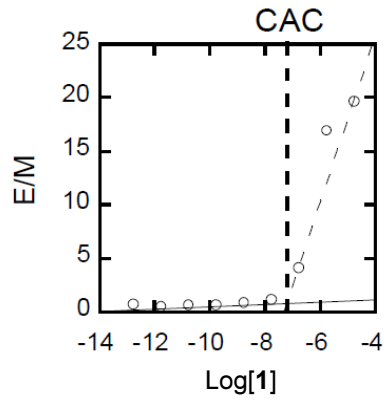

Figure S.3 CAC determination: plot of E/M vs. log[lipid **1**].

Lac-lipid **1** was suspended in phosphate buffer (20 µM **1**, 100 mM phosphate buffer, pH 7.4 at 37 °C) by sonication of a thin lipid film. Lac-lipid **1** self-assembled in aqueous buffer to give a high E/M ratio of 19.5. Plotting the E/M ratios after dilution vs. log[**1**] (Figure S.3) gave a CAC of 49.5 nM.

S.4. Determining the rate of "flip-flop" of lipid **1** in DMPC, DMPC/chol and DPPC bilayers at 37 °C

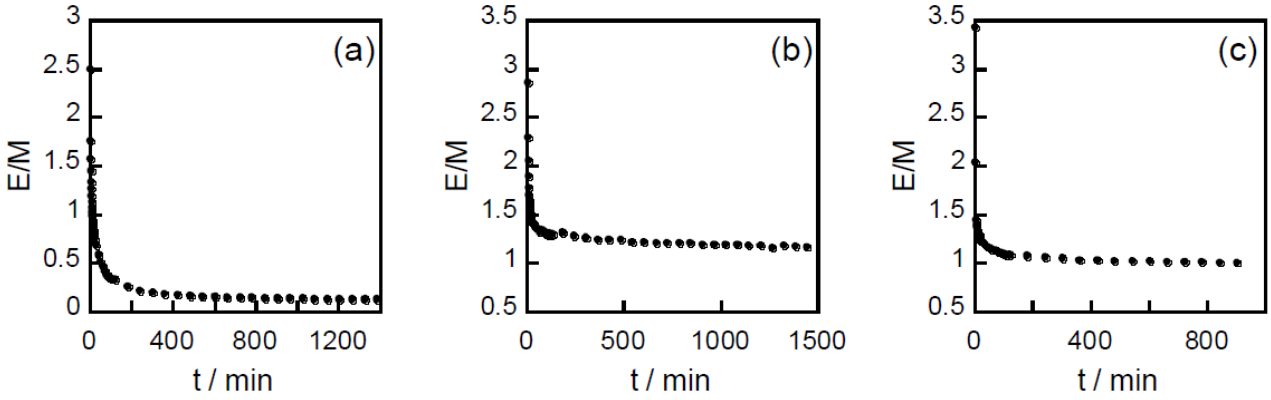

Figure S.4 Transverse diffusion of Lac-lipid **1** in (a) DMPC, (b) DMPC/chol and (c) DPPC vesicles showing the decline in E/M over several hours.

Lac-lipid **1** in ethanol (40 µL, 1 mM) was added to blank vesicles composed of DMPC, DMPC/chol or DPPC (2 mL, 2 mM total lipid) in buffer (50 mM MES, pH 7 at 37 °C) to give a loading of 1% mol/mol. Fluorescence spectra were taken at regular intervals over 20 h. Immediately after addition the E/M ratios dropped from 20.9 to 0.5 in DMPC, 1.5 in DMPC/chol and 1.3 in DPPC as the lipids inserted and dispersed in the fluid phase membrane (Figure S.4). The rate of the slower second decline corresponded to flip-flop half-lives of approximately $t_{½}$ = 1.5 hours in DMPC, $t_{½}$ = 7 hours in DMPC/chol and $t_{½}$ = 5 hours in DPPC as the more rigid phases slowed the rate of flip-flop.

S.5. Fluorescence spectra of Lac-lipid **1** in different bilayer compositions

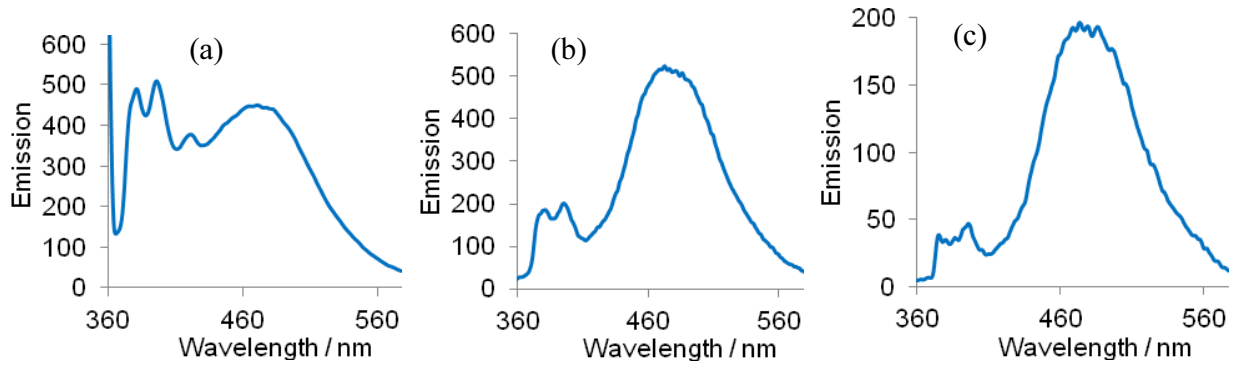

Figure S.5 Fluorescence spectra of Lac-lipid **1** in vesicles composed of (a) DMPC at 9.5 mol %, (b) DMPC/chol at 8.5 mol % (c), DPPC at 8.7 mol %.

S.6. Dependence of sialylation on TcTS and fetuin concentration

Bilayer composition	E/M	Fetuin (mg)	TcTS (μL, 3.91 μM)	% sialylation after 18 h
DMPC (9.5 mol %)	0.80 ± 0.15	1	1	8.5
		5	5	34.8
DMPC/chol (8.5 mol %)	3.9 ± 0.2	1	1	7.4
		5	5	21.4
DPPC (8.7 mol %)	3.0 ± 0.4	1	1	7.6
		5	5	30.4

Table S.1 Percent sialylation of Lac-lipid **1** after reaction with TcTS and fetuin at 37 °C. The amount of TcTS and fetuin used is indicated.

S.7. Representative HPLC traces of lipid 1 after reaction with TcTS and fetuin

Monitoring vesicle reactions by HPLC: Injects (5 µL, with a 10 µL CH_3OH needle wash) were performed on a 30 minute gradient of 50:50 CH_3CN (+ 0.1% TFA)/H_2O (+ 0.1% TFA) to 80% THF (+ 0.1% TFA) on a Phenomenex Luna C18 column (150 × 4.6 mm, 5 µm) fitted with a guard column, with a flow rate of 0.8 mL/min. The HPLC DAD module was set to detect the pyrenyl UV-visible absorption at 346 nm. This was repeated every half hour for 8 hours and at 24 hours. Percent conversions were determined using the ratio of product peak area to the combined product and starting material area.

The best wavelength to monitor the conversion of **1** to **2** by HPLC was the strong pyrene absorption at 346 nm. The extinction co-efficient for lipid **1** at 346 nm in 1:1 $CHCl_3$:MeOH is $(2.9 \pm 0.2) \times 10^4$ $M^{-1}cm^{-1}$, and the product lipid **2** will have a very similar extinction co-efficient (e.g. GlcNAc capped analogue lipid **3** has $\varepsilon = (3.6 \pm 0.3) \times 10^4$ $M^{-1}cm^{-1}$ and a galactose capped analogue lipid **3** has $\varepsilon = (3.9 \pm 0.3) \times 10^4$ $M^{-1}cm^{-1}$).

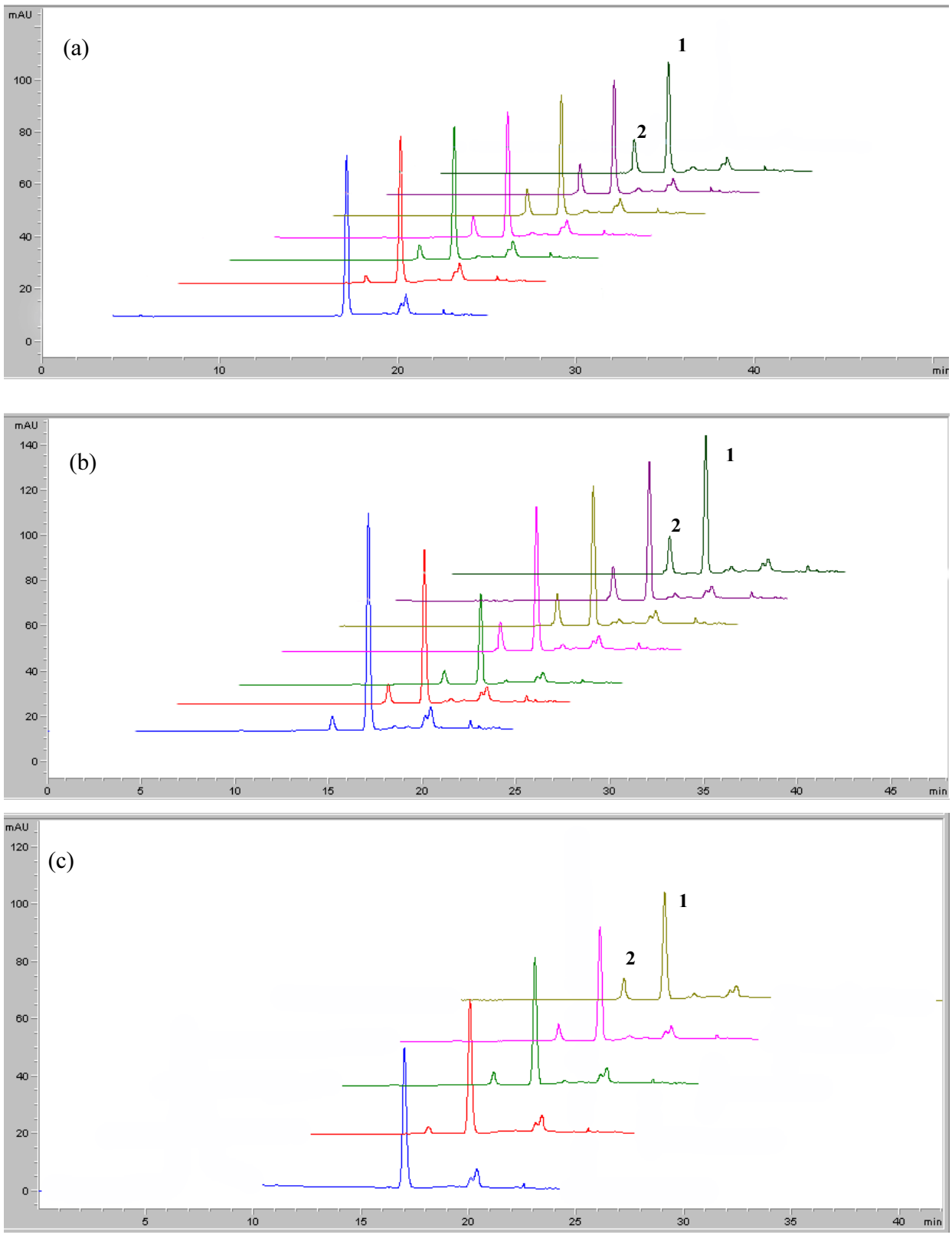

Figure S.6. Representative HPLC traces at 346 nm for the conversion of lipid **1** to **2** in (a) DMPC at 9.5 mol % loading, (b) DMPC/chol at 8.5 mol % loading, and DPPC at 8.7 mol % loading over 4 h. Traces shown were recorded at 0.5 h intervals for DMPC and DMPC/chol, every 1 h for DPPC.

S.8. Experiments using 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (MUNANA) as sialic acid donor

Analogous reactions to those completed with fetuin were performed using lipid **1** embedded in phospholipid vesicles (DMPC, DPPC and 50:50 DMPC/cholesterol, 100 µL, ~ 9 % mol/mol **1**, 2.0 mM total lipid – in phosphate buffer pH 7.4 at 37 °C), TcTS (5 µL, ~3.91 µM in phosphate buffer pH 7.4 at 37 °C) and sialic acid donor 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (MuNANA, 5.8 µL of 100 mM stock, to give 5.2 mM).

The reaction progress was monitored by HPLC, injects (5 µL, with a 10 µL CH_3OH needle wash) were performed on a 30 minute gradient 50:50 CH_3CN (+0.1% TFA)/H_2O (+0.1% TFA) to 80% CH_3CN (+0.1%) with a 0.8 mL/min flow rate on a Phenomenex Luna C18 column fitted with a guard column. The HPLC DAD module was again set to detect the pyrenyl UV-visible absorption at 346 nm, percent conversions calculated from the peak area ratio of product to starting material. Fluorescence spectroscopy was used to monitor the release of methylumbelliferone product emission at 450 nm.

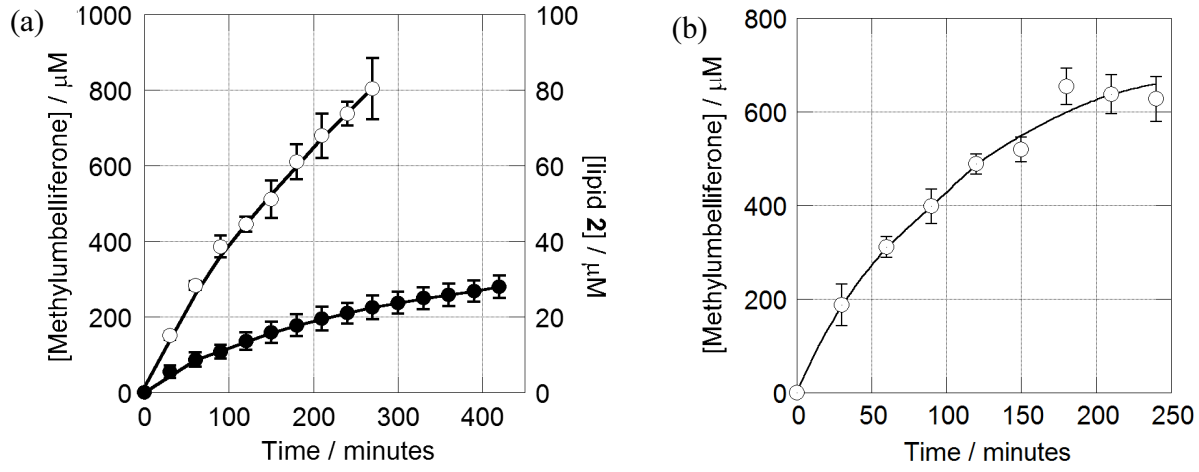

Figure S.7. (a) (●) Rate of conversion of vesicle-bound **1** to **2** by TcTS/MUNANA at 9.5 % mol/mol **1** in DPPC; (○) Rate of production of methylumbelliferone from MUNANA by TcTS with 9.5 % mol/mol **1** in DPPC present. (b) Rate of production of methylumbelliferone from MUNANA by TcTS in buffer without substrate. Standard error bars are shown.

Measurement of the rate of production of fluorescent methylumbelliferone by TcTS both in the presence and absence of lipid **1** in vesicles showed that hydrolysis dominated the reactivity of MUNANA with TcTS (Figure S.7(a)(○) compared to Figure S.7(b)(○)), giving ~ 34× more hydrolysis than trans-sialylation in the first 3 h.

S.9. Comparative solution phase experiments with benzyl lactose

Analogous solution phase reactions were performed using benzyl lactose (100 µL, 200 mM), fetuin (5 mg) and TcTS (5 µL). Reaction progress was monitored using HPLC, injects (5 µL) were conducted with a 15 minute gradient 50:50 CH_3CN/H_2O to 80% CH_3CN, flow rate 0.8 mL/min on a Phenomenex Luna C18 column. HPLC DAD monitor detected the benzyl lactose UV-visible absorption maxima 257 nm.

(a) (b)

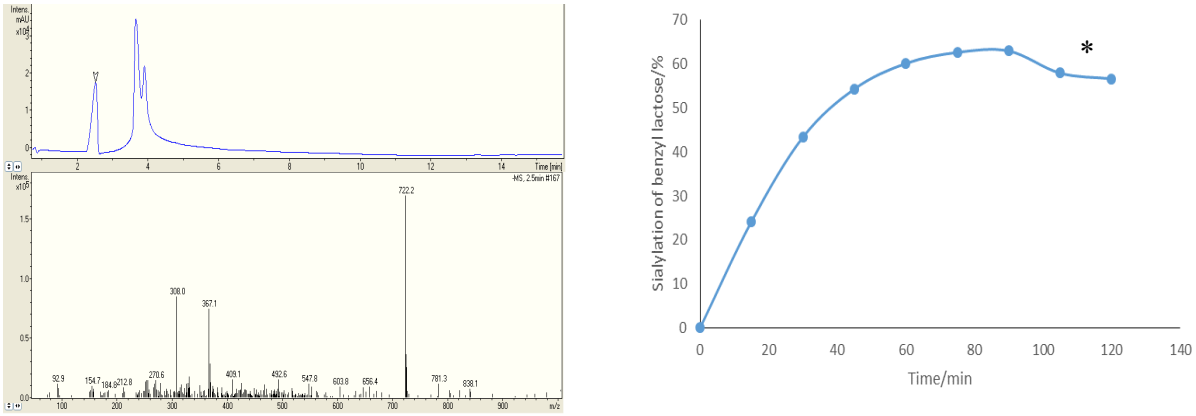

Figure S.8. (a) LC/MS trace showing sialylated benzyl lactose product at 2.3 min and *m/z* 722.2 $[M]^+$ (ES). (b) Rate of sialylation of benzyl lactose by TcTS/fetuin in PBS. (*) Drop after 90 mins suggested to be due to competing hydrolysis of the product **2**.

S.10. MALDI-ToF/ToF of 1 in DMPC vesicles after fetuin/TcTS treatment (10 mg/mL and 39 nM)

MALDI-ToF/ToF analysis using linear-positive mode with 2,4,6-trihydroxyacetophenone as the matrix.

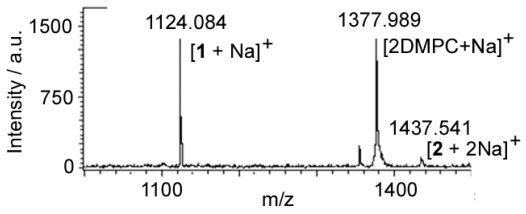

Figure S.9. MALDI-ToF/ToF MS spectrum of conversion of **1** in DMPC vesicles (9.5 % mol/mol) to **2** after fetuin/TcTS treatment (10 mg/mL and 39 nM). Peaks displayed are $[\mathbf{1} + Na]^+$ (m/z 1124), $[2DMPC + Na]^+$ (m/z 1378) and $[\mathbf{2} + 2Na]^+$ (m/z 1437).

S.11. References

S1 S. J. Webb, K. Greenaway, M. Bayati and L. Trembleau, *Org. Biomol. Chem.* 2006, **4**, 2399-2407.

S2 R. Šardzík, G. T. Noble, M. J. Weissenborn, A. Martin, S. J. Webb and S. L. Flitsch, *Beilstein J. Org. Chem*. 2010, **6**, 699-703.

S3 M. L. Cremona, D. O. Sánchez, A. C. Frasch and O. Campetella, *Gene*, 1995, **160**, 123-128.

S4 A. Buschiazzo, O. Campetella and A. C. C. Frasch, *Glycobiology*, 1997, **7**, 1167–1173.

S5 R. Šardzík, A. P. Green, N. Laurent, P. Both, C. Fontana, J. Voglmeir, M. J. Weissenborn, R. Haddoub, P. Grassi, S. M. Haslam, G. Widmalm and S. L. Flitsch, *J. Am. Chem. Soc*., 2012, **134**, 4521–4524.

S6 Lee *et al* estimated that 126 nmol sialic acid is available per mg of fetuin, corresponding to around 75% of total sialic acid on the protein. See: S.-G. Lee, D.-H. Shin and B.-G. Kim, *Enzyme Microb. Technol.*, 2002, **31**, 742-746.

S7 S. C. Gill and P. H. von Hippel, *Anal. Biochem.*, 1989, **182**, 319-326.

S8 T. A. Pitcovsky, J. Mucci, P. Alvarez, M. S. Leguizamón, O. Burrone, P. M. Alzari and O. Campetella, *Infect. Immun.*, 2001, **69**, 1869-1875.

S9 G. T. Noble, F. L. Craven, J. Voglmeir, R. Šardzík, S. L. Flitsch and S. J. Webb, *J. Am. Chem. Soc*. 2012, **134**, 13010-13017.