Supplementary information

Tandem malonate-based glucosides (TMGs) for membrane protein structural studies

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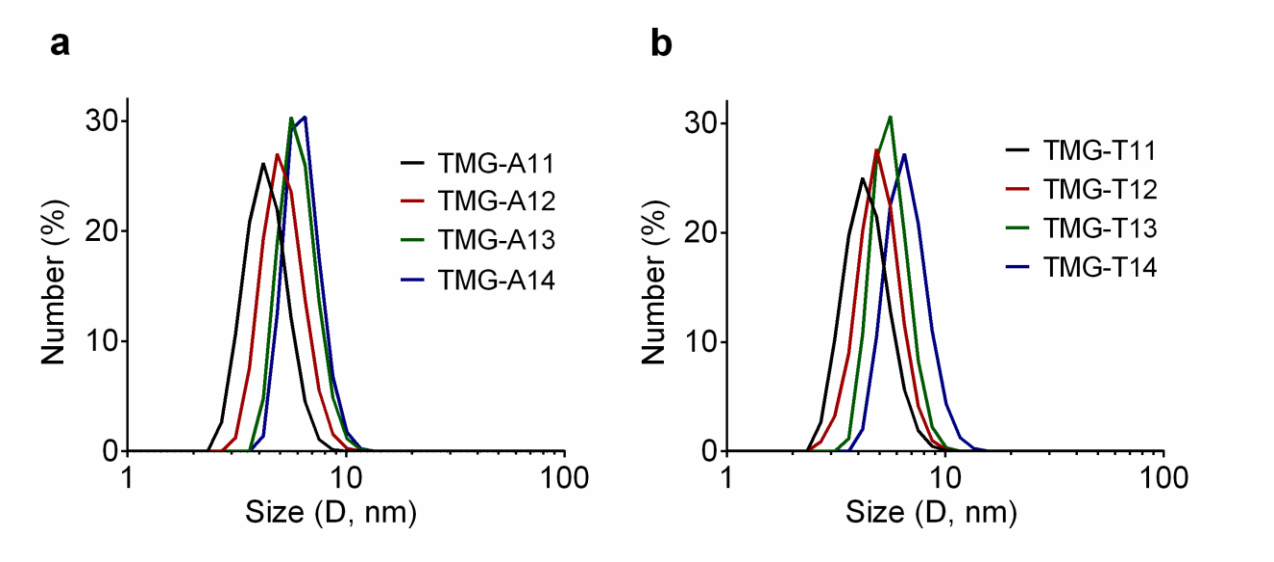
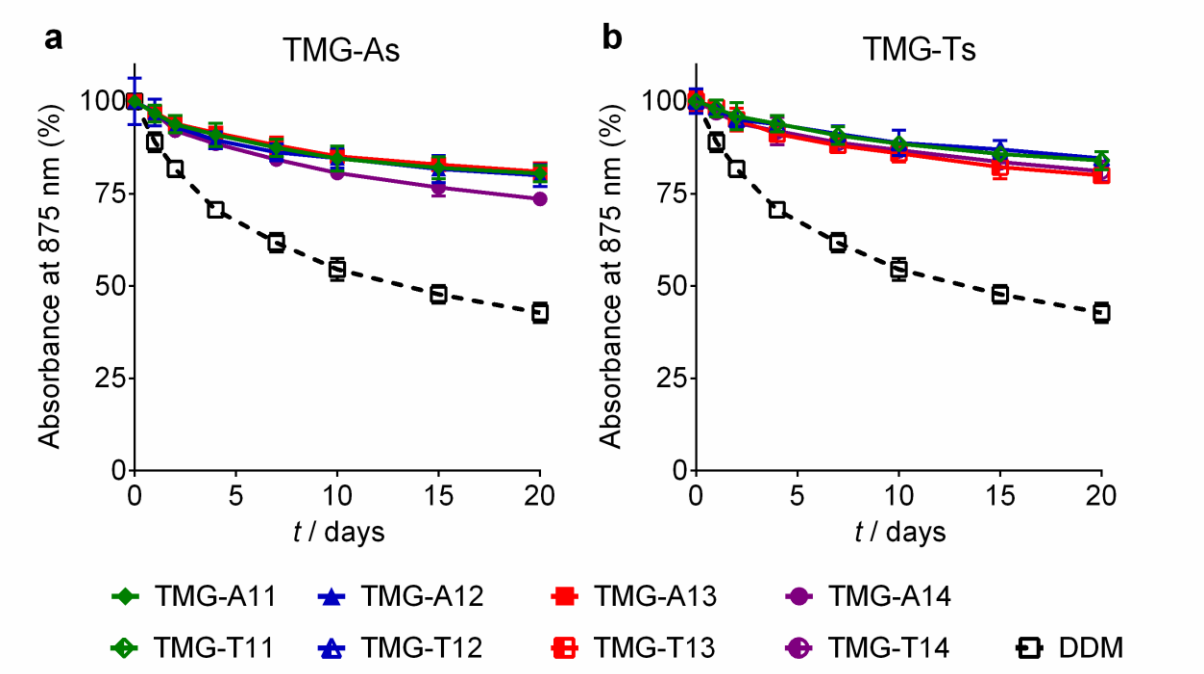
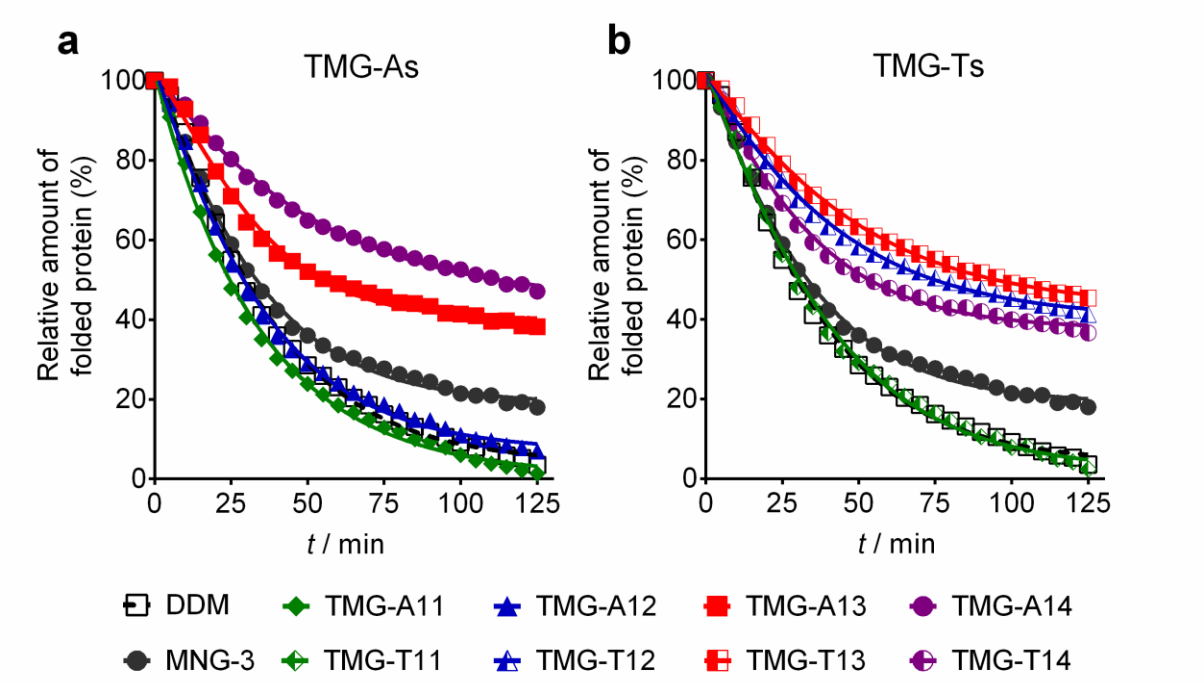


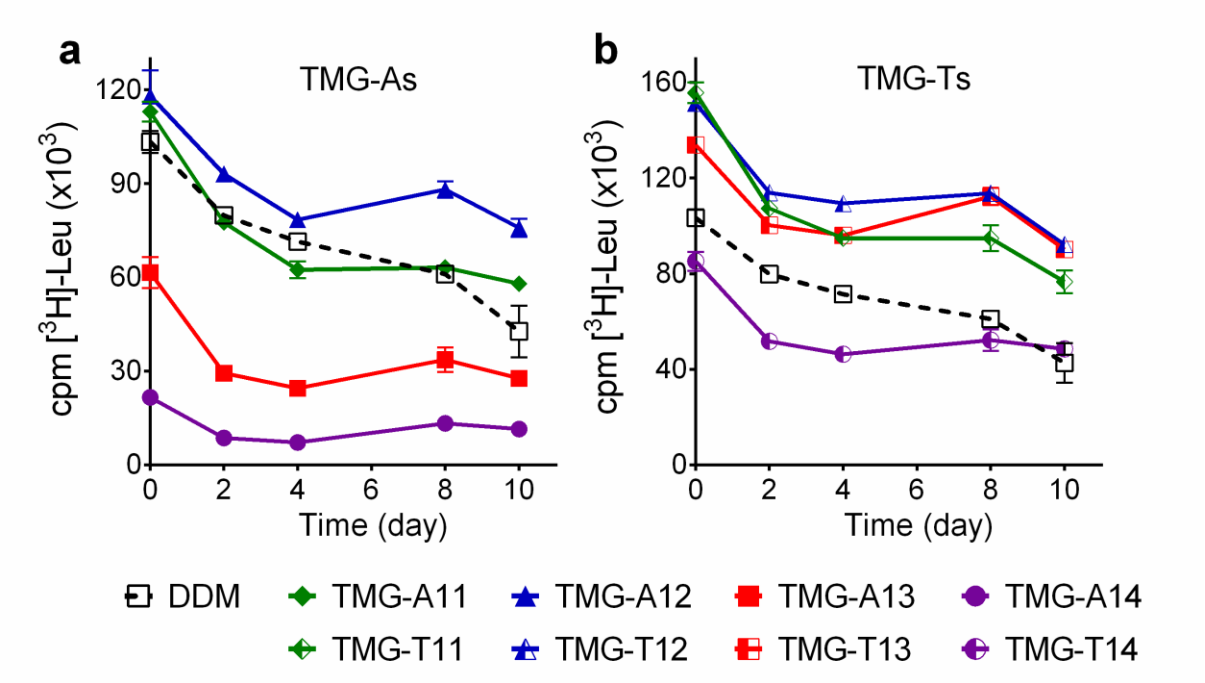
Supplementary Figure S1. Number-averaged dynamic light scattering profiles of micelles formed by TMG-As (a) and TMG-Ts (b). Each TMG-A (TMG-A11, TMG-A12, TMG-A13 or TMG-A14) or TMG-T (TMG-T11, TMG-T12, TMG-T13 or TMG-T14) was used at 1.0 wt%. Time-dependent fluctuation in the scattered light intensity was analyzed by autocorrelation, giving the translational diffusion coefficient (D). The hydrodynamic radii (R_h) of detergent micelles were correlated with the diffusion constant *via* the Stokes-Einstein equation.



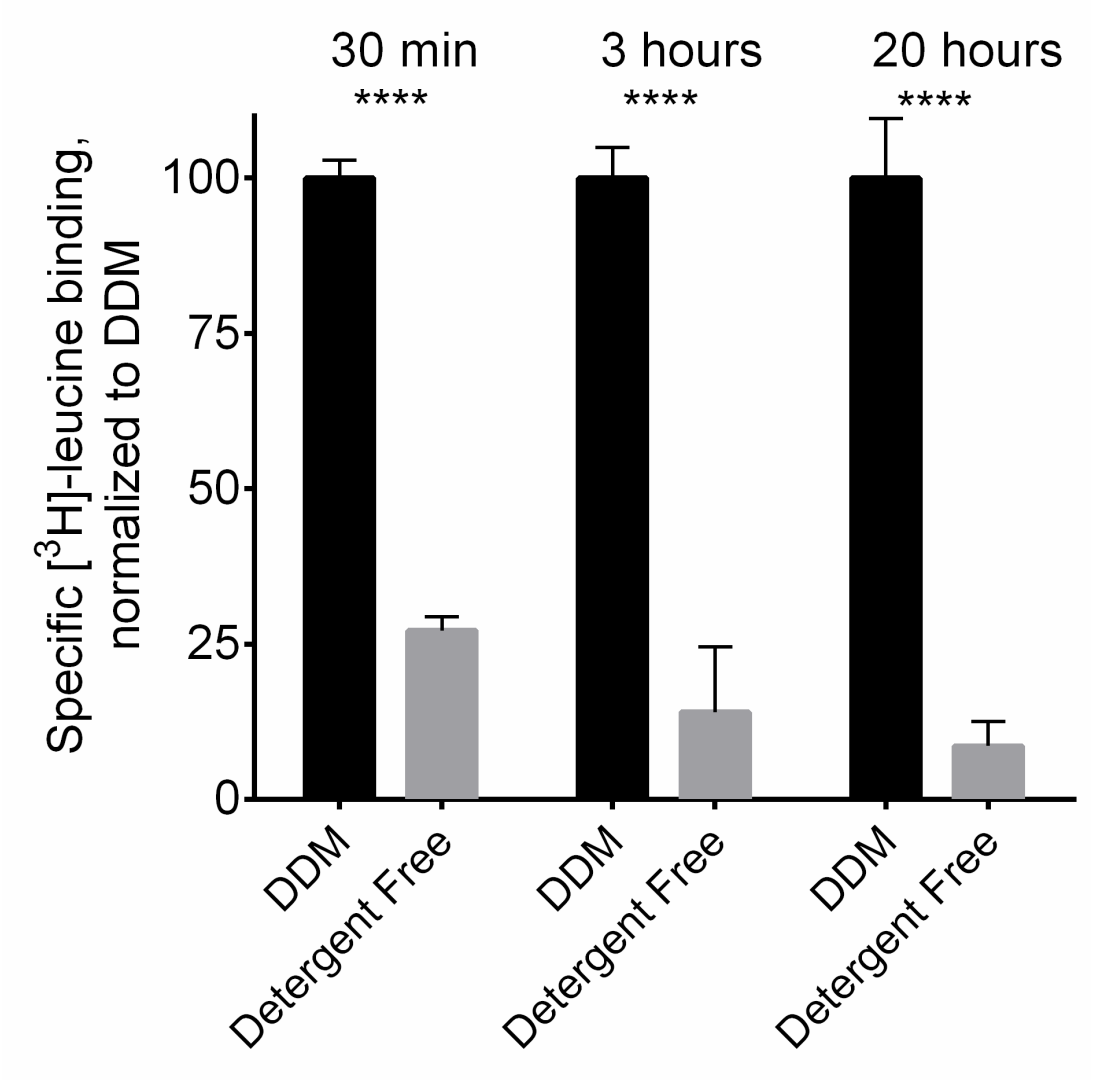
Supplementary Figure S2. Long-term stability of the *R. capsulatus* superassembly solubilized in individual TMG-As (TMG-A11, TMG-A12, TMG-A13 and TMG-A14) (a) and TMG-Ts (TMG-T11, TMG-T12, TMG-T13 and TMG-T14) (b). A conventional detergent (DDM) was used as a control. Protein stability was assessed *via* changes in absorbance value at 875 nm (A_{875}) over a 20-day incubation at 25°C. The results are expressed as % absorbance relative to the value on day 0 (mean ± SEM, n = 2).



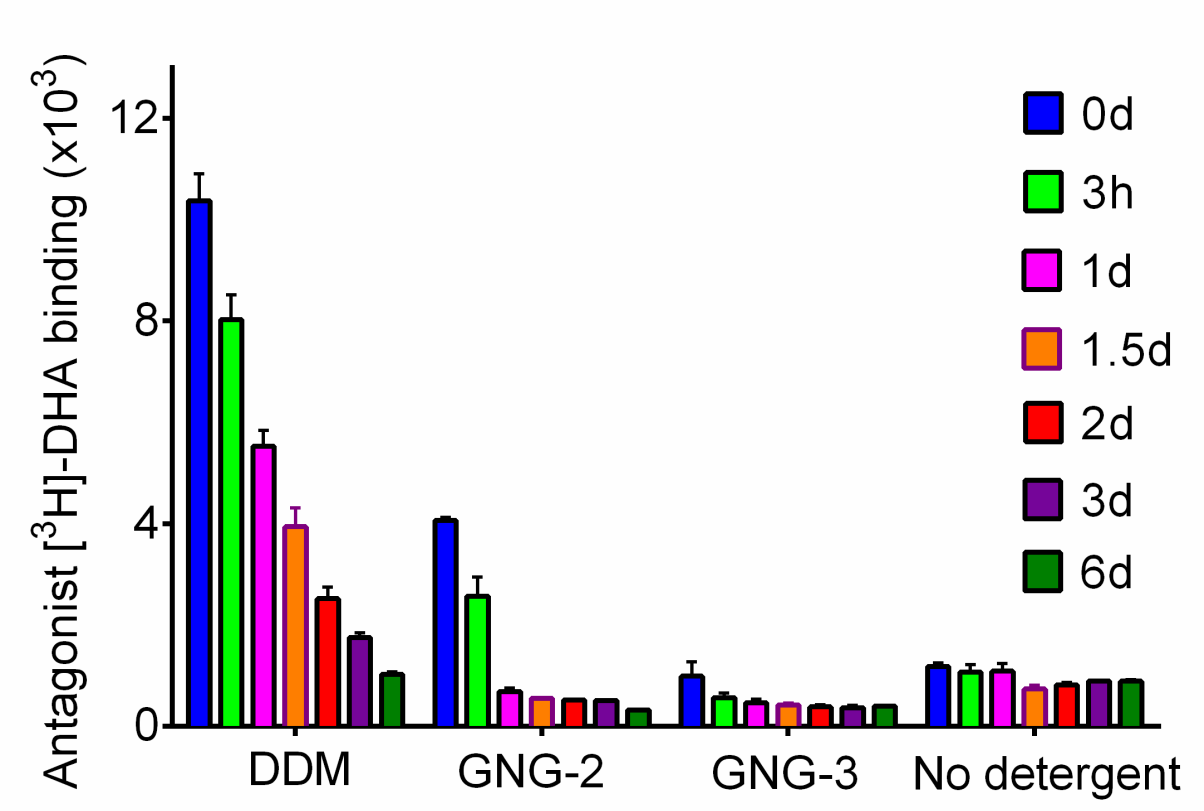
Supplementary Figure S3. Thermal denaturation profiles of UapA solubilized in individual novel agents (TMG-As (a) and TMG-Ts (b)), MNG-3, DDM used at CMC+0.2 wt%. The relative amounts of the folded protein were estimated by measuring solution fluorescence intensity *via* CPM assay during a 125-min incubation at 40°C. Mean standard deviation (n = 3) for DDM, TMG-A11, TMG-A12, TMG-A13, TMG-A14, MNG-3, TMG-T11, TMG-T12, TMG-T13 and TMG-T14 are 2.4, 4.5, 4.3, 3.7, 4.1, 2.7, 3.3, 7.7, 5.3, 5.6, respectively.



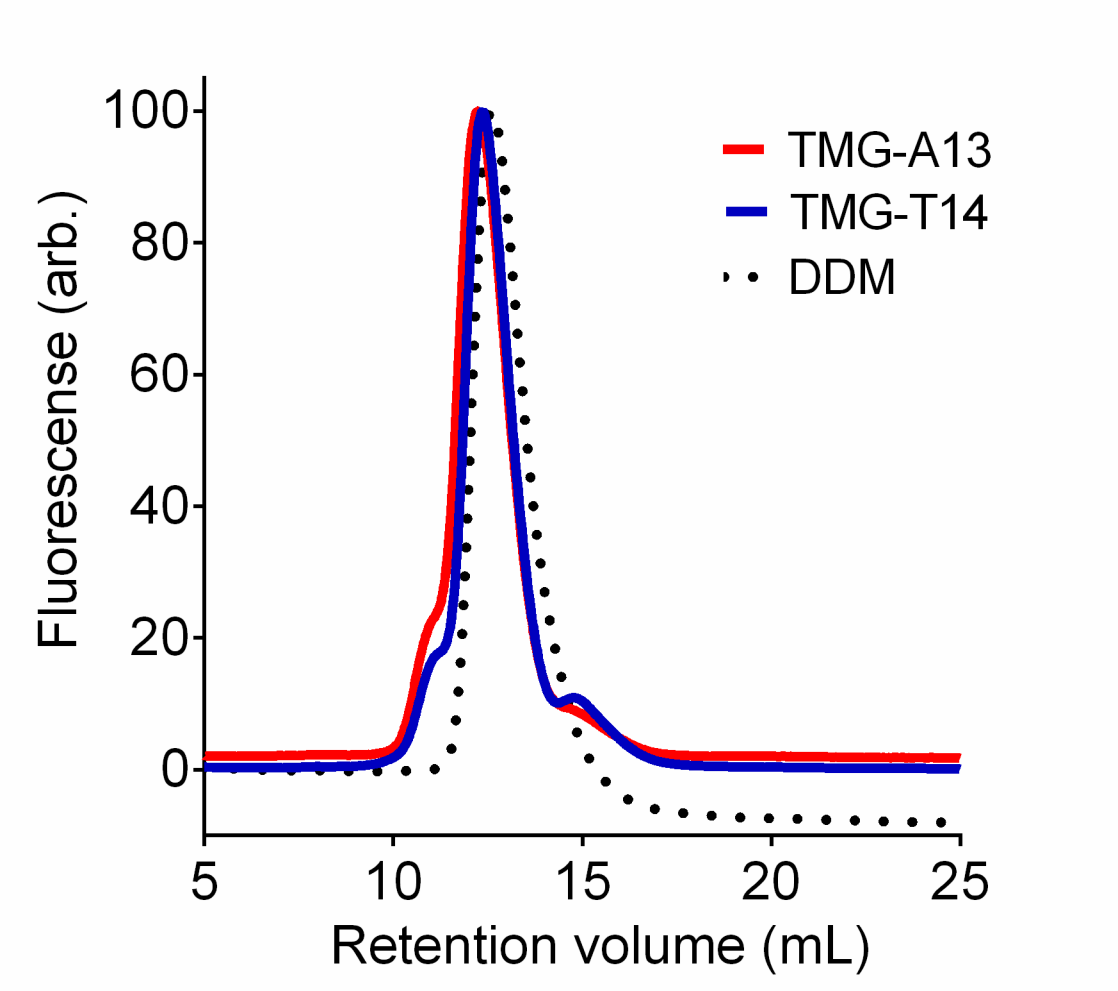
Supplementary Figure S4. Long-term stability of LeuT solubilized in DDM, individual TMG-As (TMG-A11, TMG-A12, TMG-A13 and TMG-A14), (a) or individual TMG-Ts (TMG-T11, TMG-T12, TMG-T13 and TMG-T14) (b). LeuT stability was measured based on the ability of the transporter to bind a radiolabelled substrate ([^{3}H]-Leu) *via* scintillation proximity assay (SPA). The transporter activity was measured in regular intervals over a 10-day incubation at room temperature. The results are expressed as specific binding of [^{3}H]-Leu (mean ± SEM, n = 2). All detergents were used at CMC+0.2 wt% for comparison.



Supplementary Figure S5. Time course LeuT stability solubilized in DDM or detergent-free condition. LeuT stability was measured based on the ability of the transporter to bind a radiolabeled substrate ([^{3}H]-Leu) *via* scintillation proximity assay (SPA). Transporter activity was measured in regular intervals over a 20-hour incubation at room temperature. The data of detergent-free condition expressed as specific binding of [^{3}H]-Leu were normalized to those of DDM (mean ± SEM, n = 3) at the individual time points.



Supplementary Figure S6. Time course β_2AR stability solubilized in DDM, GNG-2, GNG-3 or detergent-free condition. β_2AR stability was monitored by measuring the ability of the receptor to bind the antagonist ([^{3}H]-DHA). Receptor activity was measured in regular intervals in the course of a 6-day incubation at room temperature. Error bars, SEM, n = 3.



Supplementary Figure S7. Size exclusion chromatography (SEC) profiles of β_2AR in selected novel detergents (TMG-A13 and TMG-T14) and DDM. Detergent was exchanged from DDM to a TMG (TMG-A13 or TMG-T14) before applying each detergent-solubilized receptor for SEC column.

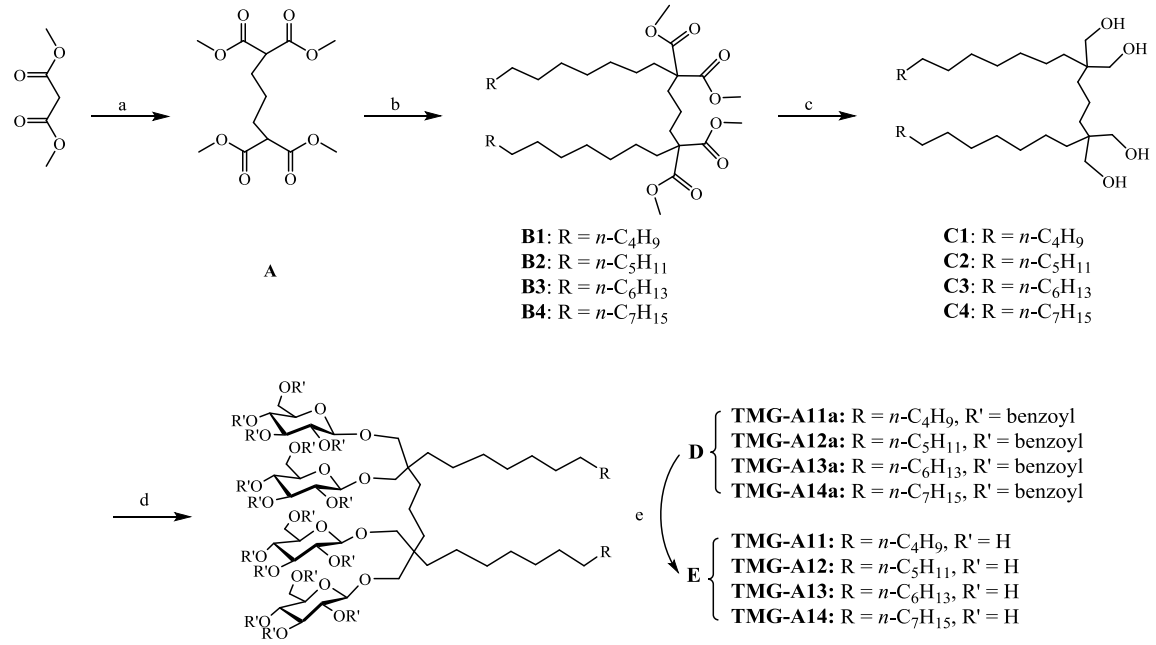
Table S1. Concentrations of target membrane proteins (μM) and residual DDM (wt%) after sample dilution into buffer solutions containing the individual novel agents (TMG-As and TMG-Ts).

Protein	Protein concentration (μM)	Residual DDM concentration (wt%)[a]
LHI-RC	0.2	0.005[a]
UapA	0.55	0.011[a]
LeuT	2.2	0.030[b]
β_2AR	0.002	0.0007[a]

[a] Value was calculated assuming that 400 DDMs are associated with a single protein. [b] Value was obtained using the 226 DDMs/protein, known for LeuT.[1]

Supplementary synthetic protocol

Supplementary Scheme 1



a) DMF, K_2CO_3, 1,3-diiodopropane, 100 ^oC; b) DMF, NaH, RI, 50 ^oC; c) THF, $LiAlH_4$, RT; d) DCM, 2,4,6-collidine, AgOTf, perbenzoylated glucosylbromide, 0 ^oC; e) NaOMe, MeOH/DCM, RT.

General protocol for the synthesis of tetramethyl pentane-1,1,5,5-tetracarboxylate (***A**; step a*)[2]

This reaction was carried out according to the reported protocol with slight modifications. To a stirring solution of K_2CO_3 (2.34 g, 16.9 mmol, 2.5 equiv.) and 1,3-diiodopopane (6.76 mmol, 1 equiv.) in anhydrous DMF (20 mL) was added dimethyl malonate (16.9 mmol, 2.5 equiv.). After 24 h of stirring at room temperature, the reaction vessel was transferred to preheated oil bath at 100 ^oC and further stirred for an additional 4 h. After completion (monitored by TLC) of the reaction, the reaction mixture was diluted with ether (100 mL), washed with water (2 x 100 mL) and brine (100 mL), and dried with anhydrous Na_2SO_4. Removal of solvent followed by column chromatography (EtOAc/hexane) on silica gel gave colorless oil (**A**) which solidified was on standing.

*General protocol for the alkylation of tetramethyl pentane-1,1,5,5-tetracarboxylate (**B**; step b)*

Alkylation of the tetramethyl pentane-1,1,5,5-tetracarboxylate (**A**) was carried out according to the literature.[3] To a stirring suspension of NaH (15.8 mmol, 2.4 equiv.) in dry DMF (25 mL)

was added tetramethyl pentane-1,1,5,5-tetracarboxylate (6.57 mmol, 1 equiv.). After 15 min of stirring, 1-iodoalkane (15.8 mmol, 2.4 equiv.) was added and stirred at room temperature overnight, followed by 5 h stirring at 50 ^{o}C. After reaction completion (monitored by TLC), the reaction was quenched by adding ice-cold saturated NH_4Cl and extracted with diethyl ether (150 mL). The organic layer was washed with water (2 x 100 mL), brine (100 mL) and dried with anhydrous Na_2SO_4. After complete evaporation of the solvent, the residue was purified by silica gel column chromatography (EtOAc/hexane) providing dialkylated tetramethyl pentane-1,1,5,5-tetracarboxylate (**B**) as an oily liquid.

*General protocol for the reduction of alkylated tetramethyl pentane-1,1,5,5-tetracarboxylate (**C**; step c)*

To a stirring suspension of $LiAlH_4$ (18.72 mmol, 6 equiv) in anhydrous THF (20 mL) at 0 ^{o}C was added a solution of dialkylated tetramethyl pentane-1,1,5,5-tetracarboxylate (C; 3.12 mmol, 1 equiv.) in THF (15 mL) dropwise over 15 min. The mixture was stirred at room temperature for 6 h. After completion (monitored by TLC), the reaction was successively quenched with MeOH, water, a 1 N aqueous HCl solution at 0 °C. The organic layer was extracted with DCM (200 mL) and washed with water (2 x 150 mL), brine (100 mL) and dried with anhydrous Na_2SO_4. After evaporation of organic solvent, the reaction mixture was purified by silica gel column chromatography (EtOAc/hexane), providing dialkyl-containing tetraol (**C**), as a white crystalline solid.

General procedure for glycosycosylation reactions[4] *(**D**; step d)*

Glycosylation was carried out according to a literature,[4] with some modifications. Briefly to a stirring solution of an alcohol derivative (C), and 2,4,6-collidine (3.0 equiv.) in CH_2Cl_2 (15 mL) was added AgOTf (5.0 equiv.) at 0 ^{o}C and stirred for 10 min. To this mixture a solution of perbenzoylated glucosylbromide (5.0 equiv) in CH_2Cl_2 (5 mL) was added dropwise. The reaction was allowed to stir for 30 min at 0 ^{o}C. After completion of the reaction (as detected by TLC), pyridine was added to quench the reaction. The reaction mixture was diluted with CH_2Cl_2 (20 mL) before being filtered over celite. The filtrate was washed successively with a 1 M aqueous $Na_2S_2O_3$ solution (40 mL), a 0.1 M aqueous HCl solution (40 mL), and brine (3 x 40 mL). Then the organic layer was dried with anhydrous Na_2SO_4 and the solvent was removed by rotary evaporation. The residue was purified by silica gel column chromatography (EtOAc/hexane), providing the desired product (**D**) as a glassy solid.

De-O-benzoylation under Zemplén's conditions[4] *(**E**; step e)*

De-*O*-benzoylation reaction was carried out by following a literature protocol.[4] *O*-benzoylated compound (**D**) was dissolved in anhydrous CH_2Cl_2 and MeOH was added

dropwise untill appearance of persistent precipitation. To the reaction mixture was added the required amount of a methanolic solution of 0.5 M NaOMe such that the final concentration of NaOMe was 0.05 M. Methanolic solution was added in such a way that precipitation was avoided. The reaction mixture was allowed to stir for 6 h at room temperature. After completion of reaction (monitored by TLC) the reaction mixture was neutralized with amberlite IR-120 (H^+ form) resin. The resin was removed by filtration and washed with MeOH, and solvent was removed from the combined filtrate *in vacuo*. The residue was purified by recrystallization using CH_2Cl_2/MeOH/diethyl ether, affording fully de-*O*-benzoylated product (**E**) as a white solid.

Tetramethyl pentane-1,1,5,5-tetracarboxylate (A) was synthesized according to the general synthetic procedure for the synthesis of tetramethyl pentane-1,1,5,5-tetracarboxylate in 93% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.73 (s, 12H), 3.36 (t, *J* = 8.0 Hz, 2H), 1.96-1.90 (m, 4H), 1.39-1.33 (m, 2H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 170.0, 52.6, 51.4, 28.4, 25.1.

Tetramethyl heptacosane-12,12,16,16-tetracarboxylate (B1) was synthesized according to the general synthetic protocol for the alkylation of tetramethyl pentane-1,1,5,5-tetracarboxylate in 82% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.69 (s, 12H), 1.88-1.78 (m, 8H), 1.38-1.10 (m, 46H), 0.84 (t, *J* = 7.1 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 172.4, 57.7, 52.5, 34.3, 33.0, 32.9, 32.7, 32.1, 30.0, 29.8, 29.6, 29.0, 28.4, 24.3, 22.9, 19.1, 14.3.

Tetramethyl nonacosane-13,13,17,17-tetracarboxylate (B2) was synthesized according to the general synthetic protocol for the alkylation of tetramethyl pentane-1,1,5,5-tetracarboxylate in 83% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.70 (s, 12H), 1.89-1.80 (m, 8H), 1.28-1.10 (m, 50H), 0.88 (t, *J* = 7.1 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 172.3, 57.7, 52.5, 32.9, 32.7, 32.1, 30.7, 30.0, 29.9, 29.8, 29.6, 28.8, 24.3, 22.9, 19.1, 14.3.

Tetramethyl hentriacontane-14,14,18,18-tetracarboxylate (B3) was synthesized according to the general synthetic protocol for the alkylation of tetramethyl pentane-1,1,5,5-tetracarboxylate in 85% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.68 (s, 12H), 1.87-1.80 (m, 8H), 1.38-1.32 (m, 2H), 1.28 (s, 54H), 1.25-1.10 (m, 6H), 0.87 (t, *J* = 7.1 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 172.4, 57.6, 52.5, 34.3, 33.1, 32.9, 32.7, 32.1, 30.0, 29.9, 29.7, 29.6, 29.0, 28.4, 24.3, 22.9, 19.1, 14.3.

Tetramethyl tritriacontane-15,15,19,19-tetracarboxylate (B4) was synthesized according to the general synthetic protocol for the alkylation of tetramethyl pentane-1,1,5,5-tetracarboxylate in 87%. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.69 (s, 12H), 1.89-1.80 (m, 8H), 1.26-1.05 (m, 58H), 0.87 (t, *J* = 7.1 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 172.4, 57.7, 52.5, 32.9, 32.8, 32.1, 30.0, 29.9, 29.8, 29.6, 24.3, 22.9, 19.1, 14.3.

2,6-bis(hydroxymethyl)-2,6-diundecylheptane-1,7-diol (C1) was synthesized according to the general synthetic procedure for the reduction of alkylated tetramethyl pentane-1,1,5,5-tetracarboxylate in 83% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.54 (s, 8H), 1.68-1.25 (m, 46H), 0.88 (t, *J* = 6.8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 32.1, 31.8, 30.8, 30.0, 29.8, 29.6, 27.0, 22.9, 19.1, 14.3.

2,6-didodecyl-2,6-bis(hydroxymethyl)heptane-1,7-diol (C2) was synthesized according to the general synthetic procedure for the reduction of alkylated tetramethyl pentane-1,1,5,5-tetracarboxylate in 83% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.49 (s, 8H), 1.66-1.24 (m, 50H), 0.88 (t, *J* = 6.8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 32.2, 31.9, 30.8, 30.0, 29.9, 29.6, 27.2, 23.0, 22.9, 14.4.

2,6-bis(hydroxymethyl)-2,6-ditridecylheptane-1,7-diol (C3) was synthesized according to the general synthetic procedure for the reduction of alkylated tetramethyl pentane-1,1,5,5-tetracarboxylate in 85% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.49 (s, 8H), 1.67-1.10 (m, 54H), 0.88 (t, *J* = 6.8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 32.2, 31.8, 30.9, 30.1, 29.9, 29.6, 27.0, 26.6, 22.9, 14.3.

2,6-bis(hydroxymethyl)-2,6-ditetradecylheptane-1,7-diol (C4) was synthesized according to the general synthetic procedure for the reduction of alkylated tetramethyl pentane-1,1,5,5-tetracarboxylate in 86% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.55 (s, 8H), 1.67-1.16 (m, 58H), 0.88 (t, *J* = 6.8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 32.2, 31.9, 31.1, 30.1, 29.9, 29.6, 27.1, 26.7, 22.9, 14.3.

TMG-A11a was synthesized according to the general synthetic procedure for the glycosylation reaction in 54% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.23-8.19 (m, 2H), 8.10-8.02 (m, 4H), 8.01-7.85 (m, 16H), 7.84-7.76 (m, 8H), 7.53-7.39 (m, 10H), 7.38-7.28 (m, 10H), 7.27-7.18 (m, 16H), 7.17-7.10 (m, 8H), 7.09-7.05 (m, 6H), 5.72-5.57 (m, 6H), 5.56-5.52 (m, 2H), 4.62-4.42 (m, 6H), 3.81-3.76 (m, 2H), 3.49-3.43 (m, 2H), 3.19-3.01 (m, 2H), 2.96-2.93 (m, 2H), 1.45-0.91 (m, 46H), 0.88 (t, *J* = 6.7 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.1, 166.0, 165.9, 165.8, 165.2, 164.8, 164.7, 133.7, 133.5, 133.3, 133.0, 130.2, 130.1, 129.9, 129.8, 129.7, 129.4, 129.1, 129.0, 128.8, 128.7, 128.6, 128.5, 128.4, 128.1, 128.0, 101.9, 101.6, 101.5, 72.9, 72.7, 72.5, 72.0, 71.7, 71.3, 70.0, 69.7, 69.5, 69.3, 62.9, 60.5, 40.6, 32.1, 31.0, 30.9, 30.7, 30.4, 30.0, 29.6, 22.8, 22.5, 14.3.

TMG-A12a was synthesized according to the general synthetic procedure for the glycosylation reaction in 53% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.18-8.16 (m, 2H), 8.02-7.94 (m, 4H), 7.92-7.72 (m, 16H), 7.71-7.56 (m, 8H), 7.54-7.48 (m, 10H), 7.43-7.37 (m, 10H), 7.29-7.24 (m, 16H), 7.21-7.10 (m, 8H), 5.66-5.41 (m, 8H), 4.48-4.34 (m, 6H), 3.79-

3.74 (m, 2H), 3.51-3.45 (m, 2H), 2.94-2.90 (m, 2H), 1.27-1.15 (m, 50H), 0.86 (t, *J* = 7.1 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.0, 165.9, 165.8, 165.7, 165.4, 165.2, 164.8, 164.7, 164.2, 133.7, 133.5, 133.2, 133.0, 130.4, 130.2, 130.0, 129.8, 129.7, 129.6, 129.4, 129.1, 129.0, 128.8, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 101.6, 101.5, 72.8, 72.6, 72.5, 72.0, 71.7, 71.4, 70.0, 69.7, 69.5, 69.3, 62.3, 40.6, 32.0, 30.9, 30.7, 30.4, 30.0, 29.9, 29.5, 22.8, 22.5, 14.3.

TMG-A13a was synthesized according to the general synthetic procedure for the glycosylation reaction in 53% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.23-8.19 (m, 2H), 8.01-7.95 (m, 4H), 7.93-7.85 (m, 16H), 7.73-7.70 (m, 8H), 7.54-7.48 (m, 6H), 7.47-7.42 (m, 10H), 7.41-7.35 (m, 16H), 7.34-7.23 (m, 8H), 7.21-7.17 (m, 6H), 5.65-5.59 (m, 8H), 5.52-5.24 (m, 2H), 4.46-4.33 (m, 6H), 3.81-3.73 (m, 2H), 3.51-3.46 (m, 2H), 3.10-3.07 (m, 2H), 2.93-2.88 (m, 2H), 1.48-0.93 (m, 54H), 0.86 (t, *J* = 6.9 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.1, 166.0, 165.9, 165.2, 165.1, 164.8, 164.7, 133.7, 133.5, 133.3, 133.2, 130.2, 130.0, 129.8, 129.7, 129.5, 129.3, 129.1, 129.0, 128.7, 128.6, 128.5, 128.4, 128.3, 128.1, 101.6, 101.5, 72.6, 72.0, 71.6, 71.4, 70.0, 69.7, 63.3, 62.9, 60.4, 53.6, 40.6, 32.0, 30.9, 30.7, 30.4, 30.0, 29.9, 29.8, 29.6, 22.8, 22.5, 14.3.

TMG-A14a was synthesized according to the general synthetic procedure for the glycosylation reaction in 52% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.24-8.20 (m, 2H), 8.02-7.96 (m, 4H), 7.95-7.89 (m, 16H), 7.75-7.71 (m, 8H), 7.54-7.48 (m, 6H), 7.47-7.41 (m, 10H), 7.39-7.35 (m, 16H), 7.34-7.23 (m, 8H), 7.21-7.17 (m, 6H), 5.65-5.60 (m, 8H), 5.54-5.43 (m, 2H), 4.46-4.33 (m, 6H), 3.82-3.73 (m, 2H), 3.53-3.47 (m, 2H), 3.14-3.09 (m, 2H), 2.95-2.86 (m, 2H), 1.49-0.94 (m, 58H), 0.86 (t, *J* = 7.1 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.1, 166.0, 165.9, 165.2, 165.1, 164.8, 164.7, 133.7, 133.5, 133.3, 133.2, 130.2, 130.0, 129.8, 129.7, 129.5, 129.3, 129.1, 129.0, 128.7, 128.6, 128.5, 128.4, 128.3, 128.1, 101.6, 101.5, 72.6, 72.0, 71.6, 71.4, 70.0, 69.7, 69.2, 63.3, 63.0, 62.6, 60.4, 53.6, 40.6, 32.0, 30.9, 30.7, 30.4, 30.0, 29.9, 29.8, 29.5, 22.8, 22.5, 21.1, 14.9, 14.3.

TMG-A11 was synthesized according to the general synthetic procedure for De-*O*-benzoylation under Zemplén's condition in 95% yield. **^{1}H NMR** (400 MHz, CD_3OD): δ 4.34 (d, *J* = 4.0 Hz, 4H), 3.92-3.85 (m, 4H), 3.75-3.63 (m, 8H), 3.48-3.35 (m, 4H), 3.25-3.19 (m, 4H), 1.33-1.22 (m, 46H), 0.88 (t, *J* = 7.2 Hz, 6H); **^{13}C NMR** (100 MHz, CD_3OD): δ 105.0, 78.2, 77.8 , 75.3, 75.2, 73.3, 71.7, 62.9, 42.3, 33.2, 32.6, 32.0, 31.9, 31.2, 31.1, 31.0, 30.7, 23.9, 14.6; **HRMS (EI):** calcd. for $C_{55}H_{104}O_{24}[M+Na]^+$ 1149.4130, found 1149.9616.

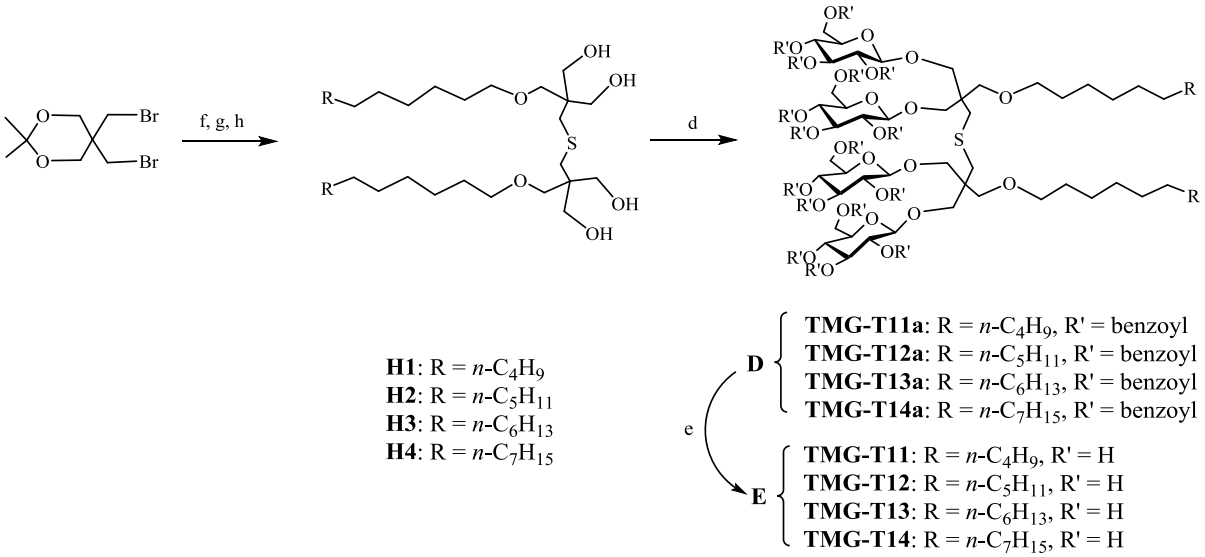
TMG-A12 was synthesized according to the general synthetic procedure for De-*O*-benzoylation under Zemplén's condition in 95% yield. **^{1}H NMR** (400 MHz, CD_3OD): δ 4.32

(d, *J* = 4.0 Hz, 4H), 3.88-3.83 (m, 4H), 3.75-3.65 (m, 8H), 3.48-3.35 (m, 4H), 3.24-3.17 (m, 4H), 1.39-1.15 (m, 50H), 0.88 (t, *J* = 6.6 Hz, 6H); **^{13}C NMR** (100 MHz, CD_3OD): δ 105.1, 78.3, 78.1, 77.9, 75.4, 75.2, 73.3, 71.9, 62.9, 42.3, 33.3, 32.7, 32.1, 31.2, 31.1, 31.0, 30.7, 24.0, 16.9, 14.7; **HRMS (EI):** calcd. for $C_{57}H_{108}O_{24}[M+Na]^+$ 1177.4670, found 1177.7233.

TMG-A13 was synthesized according to the general synthetic procedure for De-*O*-benzoylation under Zemplén's condition in 96% yield. **^{1}H NMR** (400 MHz, CD_3OD): δ 4.32 (d, *J* = 4.0 Hz, 4H), 3.88-3.82 (m, 4H), 3.75-3.63 (m, 8H), 3.48-3.35 (m, 4H), 3.22-3.18 (m, 4H), 1.34-1.12 (m, 54H), 0.90 (t, *J* = 8.0 Hz, 6H); **^{13}C NMR** (100 MHz, CD_3OD): δ 105.1, 78.3, 77.9, 75.4, 75.2, 73.4, 71.8, 62.9, 42.4, 33.3, 32.7, 32.1, 32.0, 31.2, 31.1, 31.0, 24.0, 14.7; **HRMS (EI):** calcd. for $C_{59}H_{112}O_{24}[M+Na]^+$ 1205.5210, found 1205.754.

TMG-A14 was synthesized according to the general synthetic procedure for De-*O*-benzoylation under Zemplén's condition in 96% yield. **^{1}H NMR** (400 MHz, CD_3OD): δ 4.34 (d, *J* = 4.0 Hz, 4H), 3.88-3.84 (m, 4H), 3.75-3.65 (m, 8H), 3.48-3.35 (m, 4H), 3.22-3.18 (m, 4H), 1.31-1.12 (m, 58H), 0.90 (t, *J* = 8.0 Hz, 6H); **^{13}C NMR** (100 MHz, CD_3OD): δ 105.1, 78.2, 77.8, 75.3, 75.2, 73.3, 71.8, 62.9, 42.4, 33.2, 32.6, 32.1, 31.9, 31.2, 31.1, 31.0, 30.7, 24.0, 14.7; **HRMS (EI):** calcd. for $C_{61}H_{116}O_{24}[M+Na]^+$ 1233.5750, found 1233.7858.

Supplementary Scheme 2



f) DMF, NaH, 1-iodoalkane, RT to 100 ^oC; g) DMF, KI, $Na_2S.9H_2O$, 90 ^oC; h) THF, $LiAlH_4$, RT; d) DCM, 2,4,6-collidine, AgOTf, perbenzoylated glucosylbromide, 0 ^oC; e) NaOMe, MeOH/DCM, RT.

General procedure for the synthesis of 2,2'-(thiobis(methylene))bis(2-(alkyloxy)methyl)propane-1,3-diol) (***H; step f-h***)[5]

This compound was synthesized according to the literatures[5] with slight modification. To a solution of 1-alkanol (11.6 mmol, 1 equiv.) in DMF (25 mL) was added NaH (11.6 mmol, 1.2 equiv., 60%) at 0 ℃. After stirring the mixture at room temperature for 30 min was 5,5-bis-bromomethyl-2,2-dimethyl-[1,3]dioxane (11.6 mmol, 1 equiv.) added. The reaction vessel was transferred to preheated oil bath at 100 ℃ and stirred further for 15 hr. After completion of the reaction (monitored by TLC), the reaction mixture was cooled to room temperature, quenched with ice-cold H_2O (50 mL) and extracted with ether (3 × 100 mL). The combined organic layer washed with brine (2 ×150 mL), dried with anhydrous Na_2SO_4 and then concentrated by rotary evaporation. The crude product (5.08 mmol, 1 equiv.) was dissolved in DMF (20 mL) and KI (5.08 mmol, 1 equiv.) was added to the solution. To this mixture was added $Na_2S.9H_2O$ (0.6 equiv.) in water (5 mL) followed by additional DMF (20 mL) and the mixture was stirred under N_2 at 90 ℃ for 20 hours. After cooling, the mixture was poured into water (300 mL) and extracted with ether (150 mL). The extract was washed sequentially with water (300 mL), 2.5% NaOH solution (300 mL), and brine (100 mL), dried with anhydrous Na_2SO_4. The reaction mixture was stirred with 3g silica gel, filtered and concentrated by rotary evaporation. To the residue dissolved in 1:1 mixture of CH_2Cl_2 and MeOH (50 mL) was added *p*-toluenesulfonic acid (*p*-TSA) monohydrate (200 mg) and left stirring at room temperature for 6 hr. After completion the reaction mixture was neutralized with $NaHCO_3$ solution, filtered and dried by rotary evaporation. Flash column chromatography (EtOAc/hexane) affords thioether-containing tetraol (**H**) as a white solid.

2,2'-(thiobis(methylene))bis(2-((undecyloxy)methyl)propane-1,3-diol) (H1) was synthesized according to the general synthetic procedure for the synthesis of 2,2'-(thiobis(methylene))bis(2-(alkyloxy)methyl)propane-1,3-diol) in 60% (three steps) yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.65 (s, 4H), 3.51-3.31 (m, 8H), 3.30 (s, 8H), 1.58-1.49 (m, 4H), 1.29 (s, 32H), 0.89 (t, *J* = 6.8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 73.8, 73.3, 72.7, 66.1, 56.2, 49.8, 47.0, 45.4, 45.2, 35.6, 33.2, 30.9, 30.8, 30.6, 28.8, 27.5, 23.9, 21.5, 14.6.

2,2'-(thiobis(methylene))bis(2-((dodecyloxy)methyl)propane-1,3-diol) (H2) was synthesized according to the general synthetic procedure for the synthesis of 2,2'-(thiobis(methylene))bis(2-(alkyloxy)methyl)propane-1,3-diol) in 62% yield (three steps)**. ^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.92 (s, 4H), 3.73-3.62 (m, 8H), 3.39 (s, 8H), 1.61-1.50 (m, 4H), 1.29 (s, 36H), 0.88 (t, *J* = 6.8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 73.9, 73.4, 72.2, 67.3, 64.9, 64.5, 48.2, 45.4, 45.1, 40.4, 35.6, 32.1, 29.8, 29.6, 29.5, 28.9, 26.3, 22.8, 14.3.

2,2'-(thiobis(methylene))bis(2-((tridecyloxy)methyl)propane-1,3-diol) (H3) was synthesized according to the general synthetic procedure for the synthesis of 2,2'-(thiobis(methylene))bis(2-(alkyloxy)methyl)propane-1,3-diol) in 63% yield (three steps)**. ^{1}H**

NMR (400 MHz, $CDCl_3$): δ 3.92 (s, 4H), 3.75-3.63 (m, 8H), 3.41 (s, 8H), 1.61-1.51 (m, 4H), 1.29 (s, 40H), 0.88 (t, *J* = 6.8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 73.9, 73.3, 72.2, 67.5, 64.9, 64.6, 64.4, 48.2, 45.4, 45.1, 44.6, 40.7, 40.4, 35.6, 32.1, 29.8, 29.6, 29.5, 28.9, 26.2, 22.8, 17.3, 14.3.

2,2'-(thiobis(methylene))bis(2-((tetradecyloxy)methyl)propane-1,3-diol) (H4) was synthesized according to the general synthetic procedure for the synthesis of 2,2'-(thiobis(methylene))bis(2-(alkyloxy)methyl)propane-1,3-diol in 65% yield (three steps). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.94 (s, 4H), 3.78-3.51 (m, 8H), 3.35 (s, 8H), 1.52-1.36 (m, 4H), 1.23 (s, 44H), 0.88 (t, *J* = 6.8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 73.7, 73.2, 72.2, 71.8, 67.6, 67.1, 64.9, 64.7, 64.4, 48.3, 45.4, 45.1, 44.7, 40.8, 40.4, 38.8, 35.5, 32.0, 29.8, 29.6, 29.5, 28.8, 26.2, 22.8, 17.2, 14.3.

TMG-T11a was synthesized according to the general synthetic procedure for the glycosylation reaction in 52% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.35-8.31 (m, 2H), 8.16-8.14 (m, 4H), 7.99-7.91 (m, 16H), 7.90-7.78 (m, 8H), 7.72-7.63 (m, 10H), 7.52-7.44 (m, 10H), 7.42-7.32 (m, 16H), 7.31-7.23 (m, 12H), 6.91-6.78 (m, 2H), 5.82-5.78 (m, 6H), 5.77-5.52 (m, 4H), 4.61-4.39 (m, 8H), 3.92-3.72 (m, 4H), 3.53-3.42 (m, 4H), 3.41-3.12 (m, 8H), 1.49-1.32 (m, 4H), 1.31-0.97 (m, 32H), 0.86 (t, *J* = 8.0 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.1, 166.0, 165.8, 165.7, 165.4, 165.2, 165.0, 164.9, 164.7, 133.8, 133.7, 133.4, 133.2, 133.1, 130.5, 130.3, 130.0, 129.8, 129.7, 129.6, 129.4, 129.3, 129.2, 129.1, 129.0, 128.7, 128.6, 128.5, 128.4, 128.0, 72.7, 72.6, 72.5, 72.1, 71.8, 71.6, 71.5, 70.0, 69.9, 60.5, 45.2, 32.1, 29.9, 29.8, 29.7, 29.6, 26.2, 22.8, 14.3.

TMG-T12a was synthesized according to the general synthetic procedure for the glycosylation reaction in 52% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.37-8.31 (m, 2H), 8.19-8.11 (m, 4H), 8.01-7.91 (m, 16H), 7.81-7.76 (m, 8H), 7.74-7.62 (m, 12H), 7.51-7.42 (m, 10H), 7.41-7.34 (m, 16H), 7.33-7.19 (m, 12H), 6.91-6.78 (m, 2H), 5.79-5.65 (m, 6H), 5.64-5.46 (m, 4H), 4.59-4.42 (m, 8H), 3.89-3.71 (m, 4H), 3.43-3.22 (m, 4H), 3.21-3.12 (m, 8H), 1.49-1.32 (m, 4H), 1.31-0.97 (m, 36H), 0.86 (t, *J* = 6.4 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 165.4, 165.2, 165.0, 164.9, 133.8, 133.5, 133.3, 133.2, 133.1, 130.5, 130.3, 130.1, 129.9, 129.8, 129.7, 129.5, 129.3, 129.2, 128.8, 128.6, 128.5, 128.4, 128.1, 101.7, 101.1, 72.8, 72.6, 72.2, 71.9, 71.6, 71.5, 70.1, 69.9, 63.3, 45.2, 32.2, 30.0, 29.9, 29.8, 29.7, 29.6, 26.3, 22.9, 14.4.

TMG-T13a was synthesized according to the general synthetic procedure for the glycosylation reaction in 51% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.37-8.31 (m, 2H), 8.21-8.12 (m, 4H), 8.01-7.91 (m, 16H), 7.83-7.76 (m, 8H), 7.72-7.62 (m, 12H), 7.50-7.42 (m, 10H), 7.41-7.35 (m, 16H), 7.34-7.20 (m, 12H), 6.83-6.76 (m, 2H), 5.81-5.64 (m, 6H), 5.63-

5.48 (m, 4H), 4.59-4.38 (m, 8H), 3.87-3.71 (m, 4H), 3.43-3.22 (m, 4H), 3.21-3.12 (m, 8H), 1.49-1.32 (m, 4H), 1.31-1.08 (m, 40H), 0.86 (t, *J* = 6.4 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.1, 165.9, 165.7, 165.4, 165.2, 165.0, 164.9, 133.7, 133.4, 133.2, 133.1, 133.0, 130.5, 130.3, 130.0, 129.9, 129.8, 129.7, 129.6, 129.4, 129.3, 129.2, 129.1, 129.0, 128.8, 128.7, 128.6, 128.5, 128.4, 128.0, 72.8, 71.6, 71.5, 71.4, 60.5, 45.2, 32.1, 29.9, 29.8, 29.7, 29.5, 26.2, 22.8, 21.2, 14.3.

TMG-T14a was synthesized according to the general synthetic procedure for the glycosylation reaction in 51% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.37-8.32 (m, 2H), 8.21-8.14 (m, 4H), 8.01-7.89 (m, 16H), 7.83-7.78 (m, 8H), 7.73-7.64 (m, 12H), 7.52-7.40 (m, 10H), 7.39-7.32 (m, 16H), 7.31-7.18 (m, 12H), 6.83-6.76 (m, 2H), 5.81-5.63 (m, 6H), 5.62-5.49 (m, 4H), 4.57-4.34 (m, 8H), 3.88-3.72 (m, 4H), 3.45-3.23 (m, 4H), 3.22-3.12 (m, 8H), 1.50-1.32 (m, 4H), 1.31-1.04 (m, 44H), 0.86 (t, *J* = 6.8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 165.9, 165.8, 165.4, 165.2, 165.0, 164.9, 133.7, 133.5, 133.2, 133.1, 130.5, 130.3, 130.0, 129.9, 129.6, 129.4, 129.3, 129.2, 129.1, 129.0, 128.8, 128.6, 128.5, 128.4, 128.2, 128.0, 72.5, 72.3, 72.2, 71.8, 71.6, 71.5, 70.6, 69.9, 69.8, 69.2, 68.0, 63.9, 63.2, 45.2, 34.4, 32.1, 29.9, 29.8, 29.7, 29.6, 26.3, 22.9, 14.3.

TMG-T11 was synthesized according to the general synthetic procedure for De-*O*-benzoylation under Zemplén's condition in 94% yield. **^{1}H NMR** (400 MHz, CD_3OD): δ 4.37-4.31 (m, 4H), 3.95-3.83 (m, 8H), 3.78-3.62 (m, 4H), 3.61-3.51 (m, 4H), 3.49-3.35 (m, 12H), 3.31-3.19 (m, 4H), 1.61-1.49 (m, 4H), 1.41-1.20 (m, 32H), 0.90 (t, *J* = 7.2 Hz, 6H); **^{13}C NMR** (100 MHz, CD_3OD): δ 105.0, 104.8, 78.0, 77.8 , 75.2, 72.6, 71.6, 71.5, 71.4, 71.2, 70.7, 70.6, 63.0, 62.8, 46.4, 46.0, 33.2, 32.2, 31.0, 30.9, 30.8, 30.6, 27.5, 23.8, 14.6; **HRMS (EI):** calcd. for $C_{56}H_{106}O_{26}S[M+Na]^+$ 1227.4980 found 1227.6697.

TMG-T12 was synthesized according to the general synthetic procedure for De-*O*-benzoylation under Zemplén's condition in 95% yield. **^{1}H NMR** (400 MHz, CD_3OD): δ 4.38-31 (m, 4H), 3.97-3.84 (m, 8H), 3.74-3.68 (m, 4H), 3.65-3.53 (m, 4H), 3.47-3.37 (m, 12H), 3.29-3.17 (m, 4H), 1.61-1.49 (m, 4H), 1.41-1.20 (m, 36H), 0.90 (t, *J* = 7.2 Hz, 6H); **^{13}C NMR** (100 MHz, CD_3OD): δ 105.1, 105.0, 78.0, 77.8, 75.2, 72.7, 71.7, 71.6, 71.2, 70.8, 70.6, 70.4, 62.8, 46.4, 33.2, 31.0, 30.9, 30.8, 30.7, 27.6, 23.9, 14.7; **HRMS (EI):** calcd. for $C_{58}H_{110}O_{26}S[M+Na]^+$ 1255.5520 found 1255.7006.

TMG-T13 was synthesized according to the general synthetic procedure for De-*O*-benzoylation under Zemplén's condition in 96% yield. **^{1}H NMR** (400 MHz, CD_3OD): δ 4.37-4.28 (m, 4H), 3.98-3.84 (m, 8H), 3.73-3.62 (m, 4H), 3.62-3.51 (m, 4H), 3.47-3.38 (m, 12H), 3.27-3.17 (m, 4H), 1.62-1.49 (m, 4H), 1.41-1.20 (m, 40H), 0.90 (t, *J* = 7.2 Hz, 6H); **^{13}C**

NMR (100 MHz, CD_3OD): δ 106.0, 105.0, 104.9, 78.1, 78.0, 77.8 , 75.2, 74.7, 72.8, 72.7, 71.6, 71.5, 71.1, 62.8, 62.7, 46.4, 45.7, 33.2, 31.0, 30.9, 30.6, 27.5, 23.9, 14.6; **HRMS (EI):** calcd. for $C_{60}H_{114}O_{26}S[M+Na]^+$ 1283.7319 found 1283.7316.

TMG-T14 was synthesized according to the general synthetic procedure for De-*O*-benzoylation under Zemplén's condition in 96% yield. **^{1}H NMR** (400 MHz, CD_3OD): δ 4.38-4.28 (m, 4H), 3.97-3.82 (m, 8H), 3.74-3.65 (m, 4H), 3.64-3.51 (m, 4H), 3.48-3.38 (m, 12H), 3.29-3.16 (m, 4H), 1.62-1.50 (m, 4H), 1.42-1.18 (m, 44H), 0.90 (t, *J* = 7.2 Hz, 6H); **^{13}C NMR** (100 MHz, CD_3OD): δ 105.1, 105.0, 78.1, 77.8, 75.2, 72.7, 71.7, 71.6, 71.2, 70.8, 70.6, 70.4, 62.8, 46.4, 33.2, 31.0, 30.9, 30.8, 30.6, 27.6, 23.9, 14.7. **HRMS (EI):** calcd. for $C_{62}H_{118}O_{26}S[M+Na]^+$ 1311.6600 found 1311.7534.

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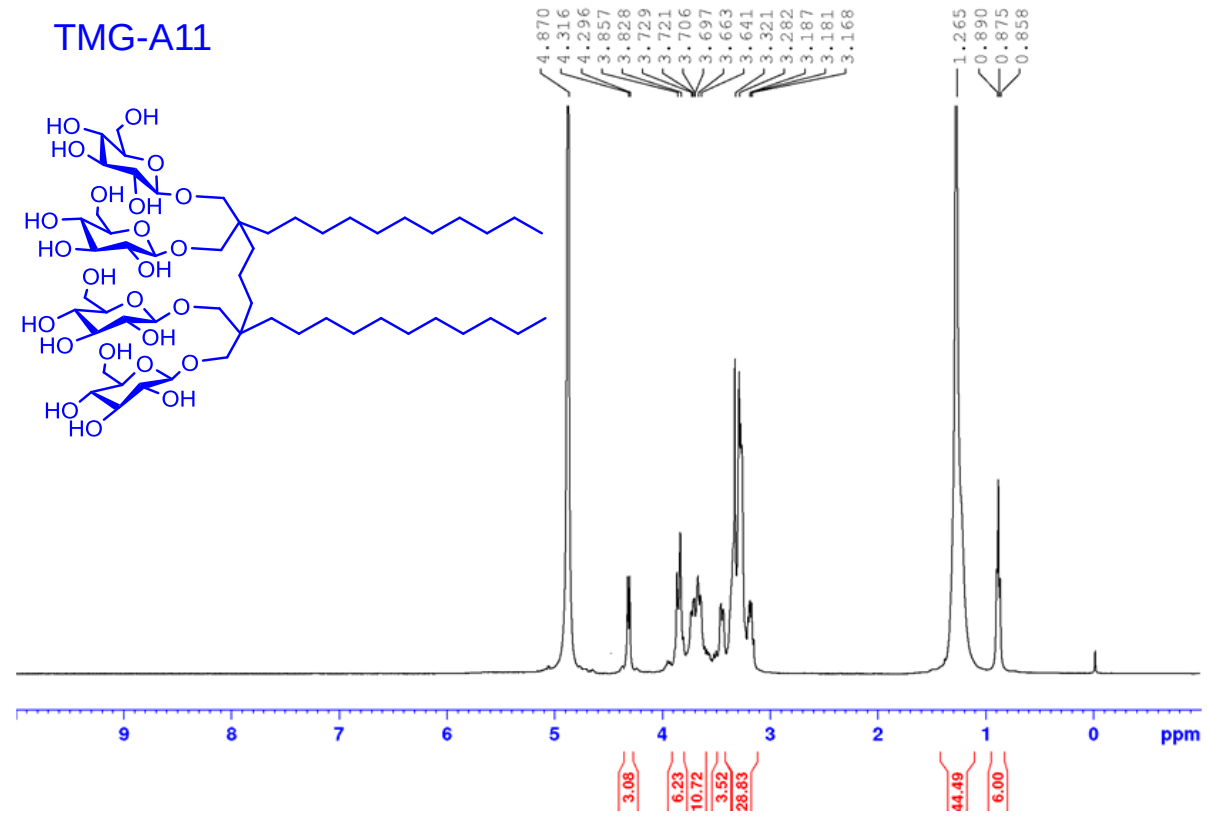
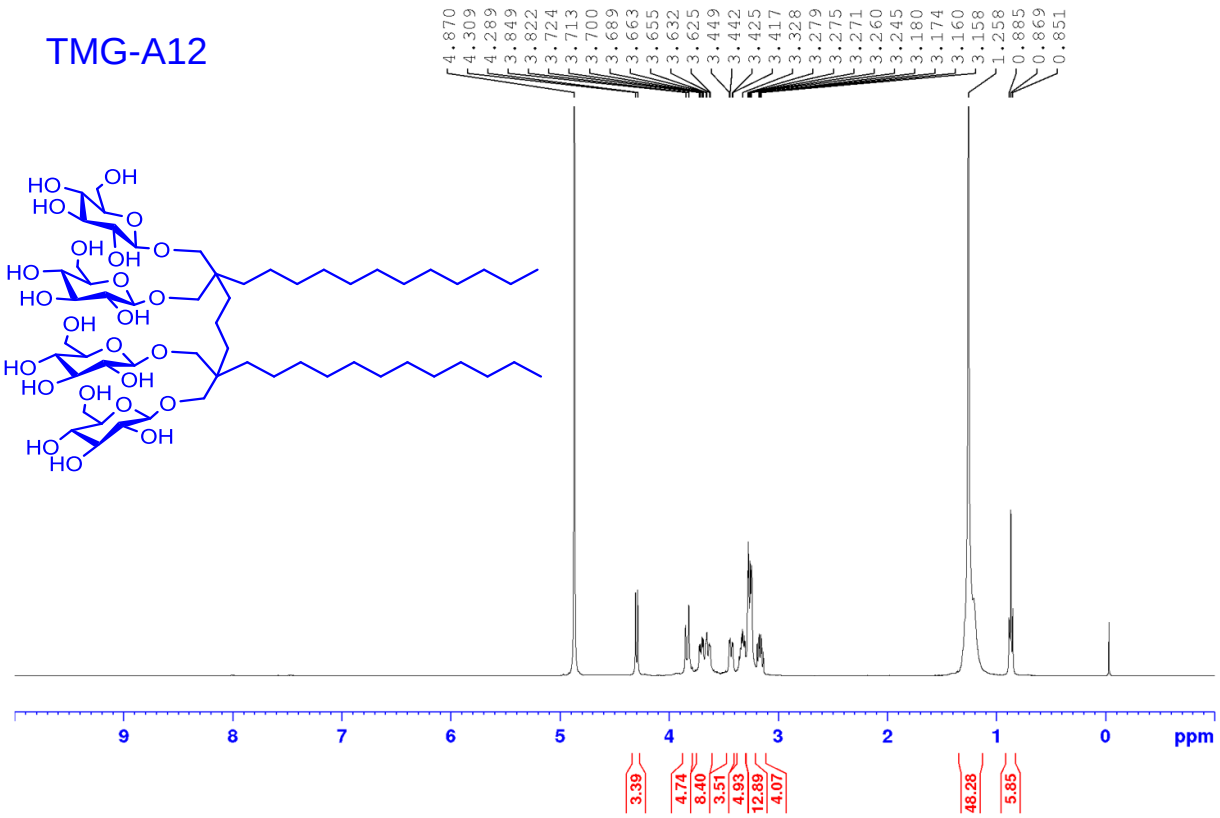
^{1}H NMR spectra of TMG amphipihles





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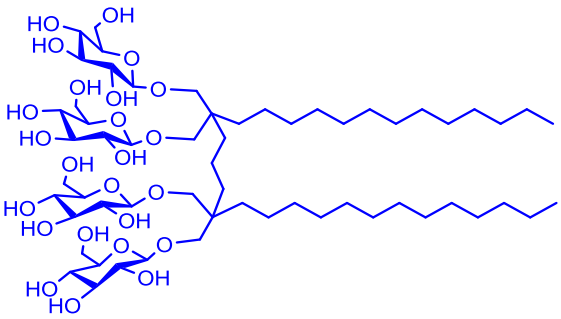

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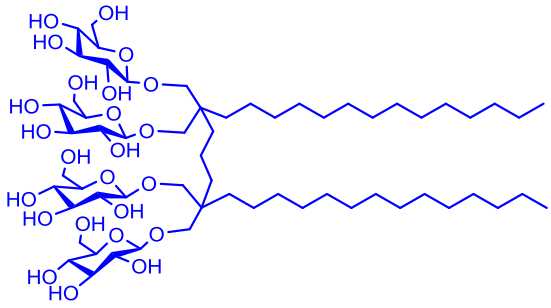

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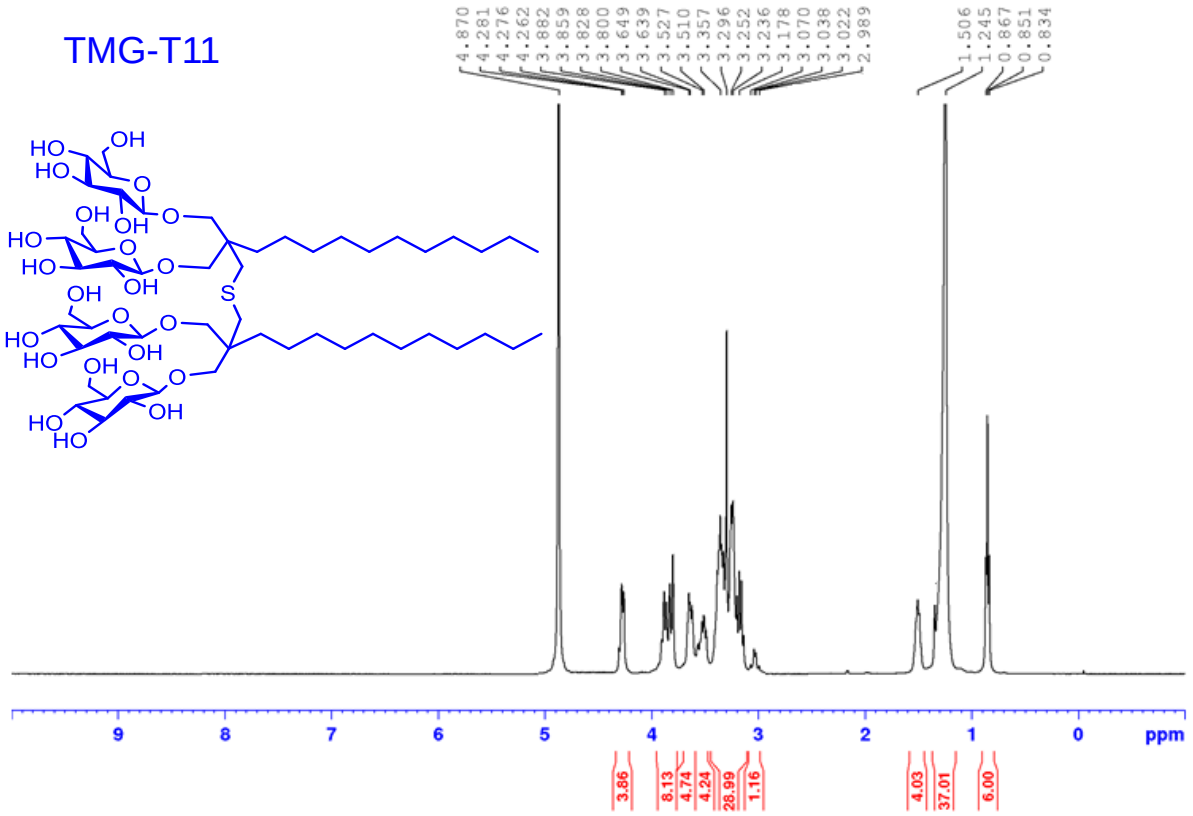

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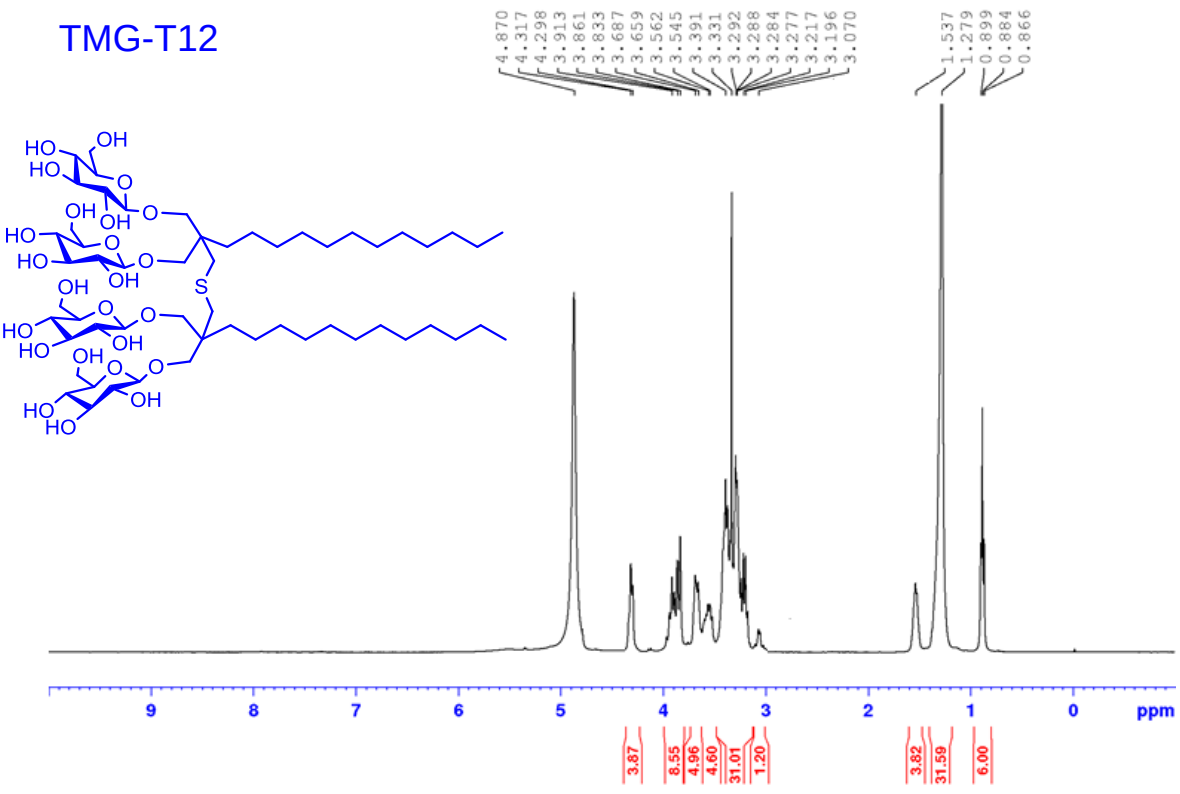

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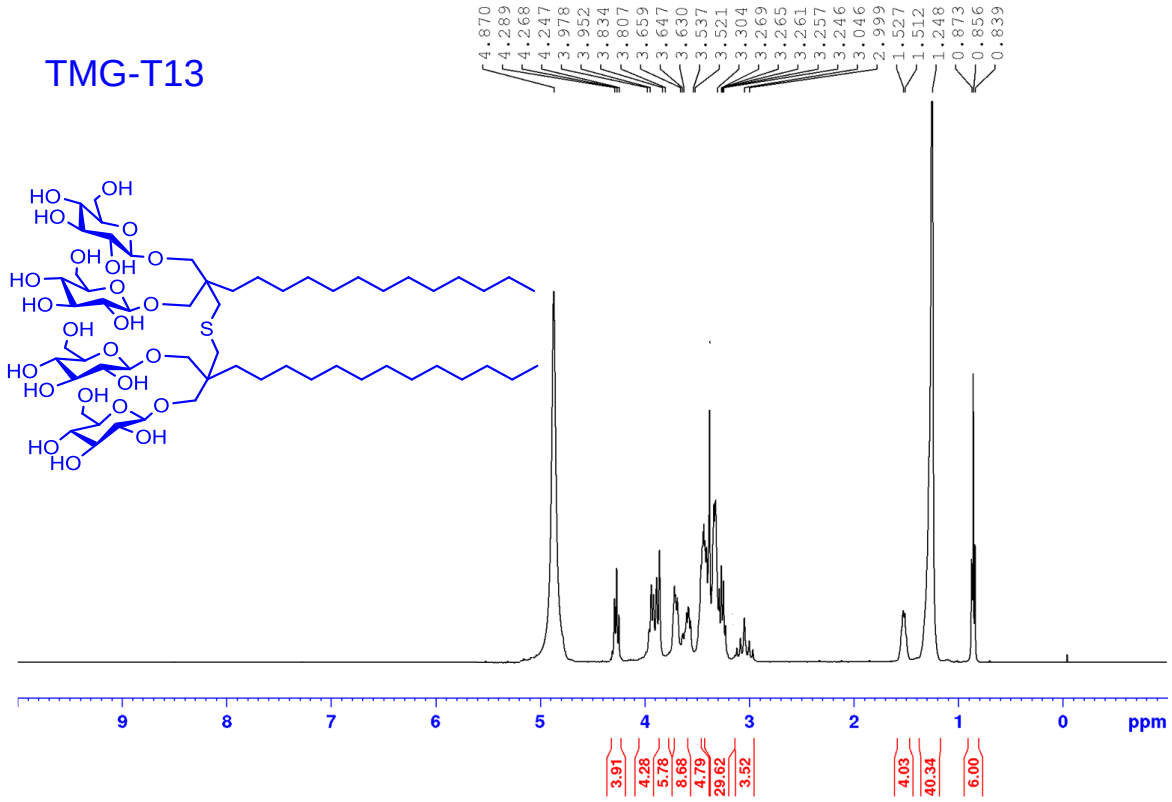

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