Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

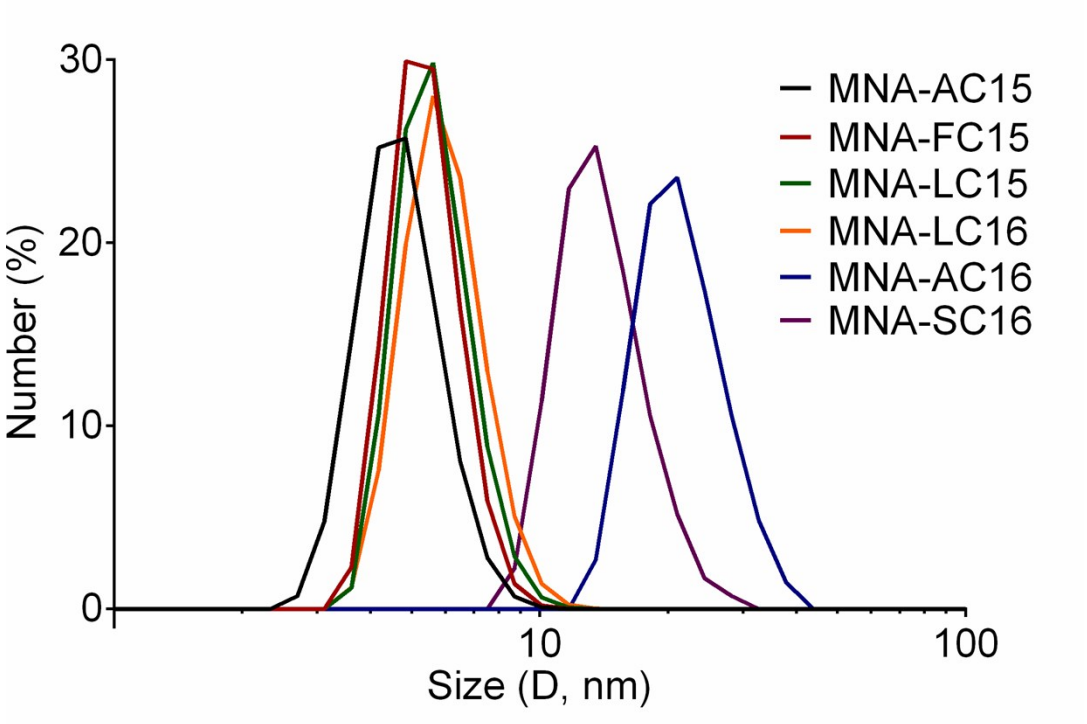


Figure S1. Dynamic light scattering (DLS) profiles for micelles formed by the mannitol-based glucosides (MNA-AC15, MNA-FC15, MNA-LC15, MNA-LC16, MNA-AC16, and MNA-SC16). The agents were used at 1.0 wt% for this measurement. Autocorrelation was used to analyze time-dependent fluctuation in the scattered light intensity.

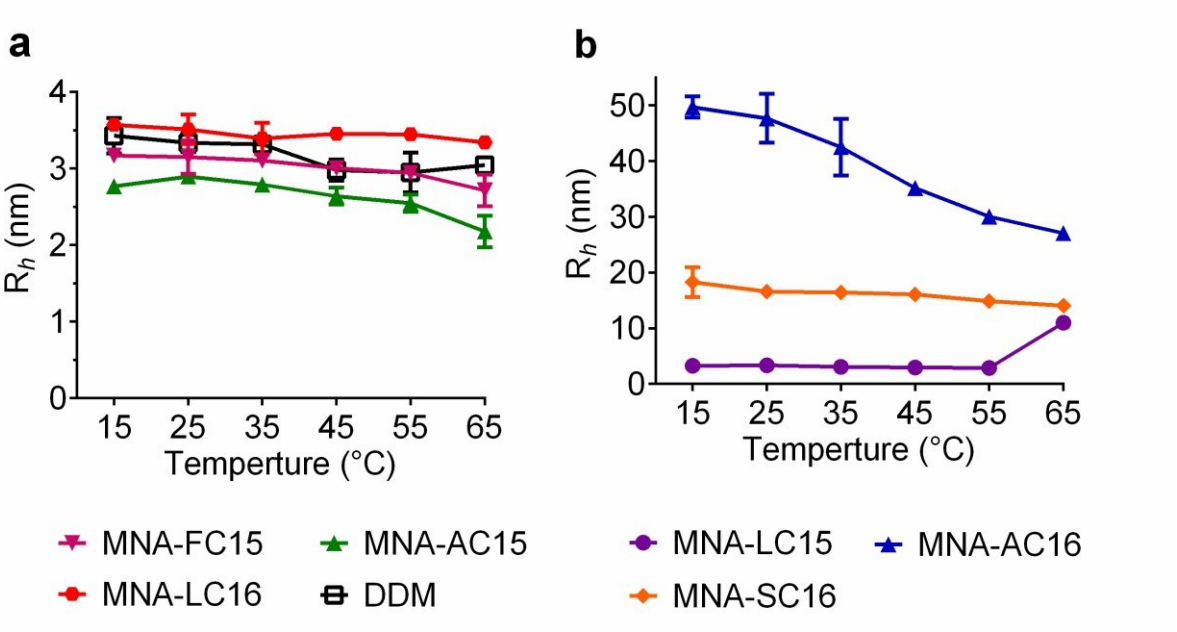


Figure S2. Hydrodynamic radii (R_h) of self-assemblies formed by individual MNAs (MNA-AC15, MNA-FC15, MNA-LC15, MNA-LC16, MNA-AC16, and MNA-SC16) and DDM. The detergents were used at 1.0 wt%. Self-assembly sizes were measured with increasing temperature from 15 ℃ to 65 ℃. Data points are mean ± SEM, n = 4-5.

Table S1. Theoretical values for the hydrophobic thickness of target membrane proteins (LeuT, $MelB_{St}$ and β_2AR).

Membrane proteins	LeuT	$MelB_{St}$	β_2AR
PDB ID	2A65	4M64	2RH1
Hydrophobic thickness (Å)[b]	29.8±0.5	30.4±1.3	31.8±0.9

[a] Protein ID for the crystal structures of membrane proteins. [b] The calculated value for protein hydrophobic thickness, obtained from a web-based server (http://opm.phar.umich.edu/about.php).

Detergent CMC determination by diphenylhexatriene (DPH) encapsulation

The MNAs variants were dissolved in distilled, deionized water to prepare 5.0 mM stock solution. From the stock solutions a series of detergent solutions with different concentrations were prepared. From each detergent sample 200 μL was taken and poured into 96-well plate in duplicates. Similarly, 3.0 mg of DPH was dissolved in 5.0 mL THF to prepare DPH stock solution. From the DPH stock solution, working solution was prepared by adding 50 μL of the stock solution into 950 μL of distilled water. From the DPH work solution an aliquot of 2.0 μL was added into each well containing a detergent solution for dye encapsulation. The DPH containing detergent solution was incubated for 15~20 min at room temperature. Fluorescence intensities of the detergent solution were measured at 430 nm upon excitation at 358 nm by using Synergy Mx Monochromator-Based Multi-Mode Microplate reader. From the fluorescence intensities, CMC values were determined by plotting florescence intensities as a function of detergent concentrations.

Detergent micelle size measurement by dynamic light scattering (DLS) experiment

The MNA variants were dissolved in distilled, deionized water to give detergent concentration of 1.0 wt%. These MNA solutions were filtered through a syringe filter with a pore size of 0.22 μm. Hydrodynamic radii of the micelles produced by the MNAs were measured by using Malvern Zeta Sizer Nano ZS90 particle analyzer. A He-Ne laser with 633 nm wavelength and a 5 mW power was used as a light source and the scattered light was collected at 90°angle. All the readings were recorded at room temperature. The translational diffusion coefficient and hydrodynamic radius (R_h) of detergent micelles was calculated by autocorrelation analysis of scattered light intensity as a function of time. R_h values were expressed as mean ± SD (n =5).

Protein stability evaluation

LeuT stability assay

LeuT stability assay Wild type of the leucine transporter (LeuT) from *Aquifex aeolicus* was purified according to the previously described protocol.[1] LeuT was expressed in *E. coli* C41(DE3) transformed with pET16b encoding C-terminally 8xHis-tagged transporter (expression plasmid was kindly provided by Dr E. Gouaux, Vollum Institute, Portland, Oregon, USA). Briefly, protein of the isolated bacterial membranes was solubilized in 1% DDM, bound to Ni^{2+}-NTA resin (Life Technologies, Denmark) and eluted in 20 mM Tris-HCl (pH 8.0), 1 mM NaCl, 199 mM KCl, 0.05 % DDM and 300 mM imidazole. Subsequently, approximately 1.5 mg/ml protein stock was diluted 10 times into an identical buffer without DDM and imidazole, but supplemented with MNA variants and DDM (a positive control) at the final concentrations of CMC + 0.04 wt% or CMC + 0.2 wt%, respectively. Protein samples were stored at room temperature and were centrifuged at the indicated time prior to protein activity measurement. Protein activity was determined by measuring [^{3}H]-Leu binding using

scintillation proximity assay (SPA).[2] Briefly, assay was performed with 5 µL of the respective protein samples in the buffer containing 200 mM NaCl, 20 nM [^{3}H]-Leu and copper chelate (His-Tag) YSi beads (both from PerkinElmer, Denmark) and the test compounds at the indicated concentration. Total [^{3}H]-Leu binding was measured using MicroBeta liquid scintillation counter (PerkinElmer).

$MelB_{St}$ solubilization, thermal stability and ligand binding assays

MelB solubilization and thermal stability assay E. coli DW2 strain (Δ*melB* and Δ*lacZY*) harboring pK95ΔAHB/WT $MelB_{St}$/CH10 plasmid which encodes the wild-type melibiose permease of *Salmonella typhimurium* ($MelB_{St}$) with a 10-His tag at the C-terminus was used for protein production.[3] Cell growth and membrane preparation were carried out as described.[4] Protein assay was carried out with a Micro BCA kit (Thermo Scientific, Rockford, IL). The membrane samples containing $MelB_{St}$ (final total membrane protein concentration was 10 mg/mL) in a solubilization buffer (20 mM sodium phosphate, pH 7.5, 200 mM NaCl, 10% glycerol and 20 mM melibiose) were mixed with individual detergents (DDM or MNAs) at 1.5 % (w/v). The membranes were extracted with a given detergent for 90 min at 0 ºC, and subjected to ultracentrifugation. The supernatants were then further incubated with increasing temperatures (45, 55, or 65 ºC) for 90 min, and then ultracentrifuged again. Insoluble fractions were removed by ultracentrifugation, and all ultracentrifugation steps were carried out at 355,590 *g* in a Beckman OptimaTM MAX Ultracentrifuge using a TLA-100 rotor for 30 min at 4 ºC. Equal amount (20 µg) of membrane proteins were analyzed for each condition and were loaded on SDS-15% PAGE. $MelB_{St}$ was visualized by immunoblotting with a Penta-His- HRP antibody (Qiagen, Germantown, MD) as described.[4]

Preparation of right-side-out (RSO) vesicles RSO membrane vesicles were prepared from *E. coli* DW2 cells containing $MelB_{St}$ or $MelB_{Ec}$ by osmotic lysis,[4-6] resuspended with 100 mM KPi (pH 7.5), and stored at -80 °C.

Trp→D^2G FRET assay RSO membrane vesicles in 100 mM KPi, pH 7.5, and 50 mM NaCl at a protein concentration of 1 mg/ml were solubilized with an indicated detergent (1.0%) at 23 °C for 30 min and ultracentrifuged using TLA 120.2 rotor at >300,000 g for 45 min at 4 ºC. The soluble fractions (supernatant) were used for Trp→D^2G FRET measurements with an Amico-Bowman Series 2 (AB2) Spectrofluorometer. Trp residues were excited at 290 nm, and the emission was recorded at 465 nm for $MelB_{Ec}$ or 490 nm for $MelB_{St}$. On a time trace, 10 µM D^2G (kindly provided by Drs. Gérard Leblanc and H. Ronald Kaback) and excess of MelB or equal volume of water were added at 1-min- and 2-min time points, respectively.

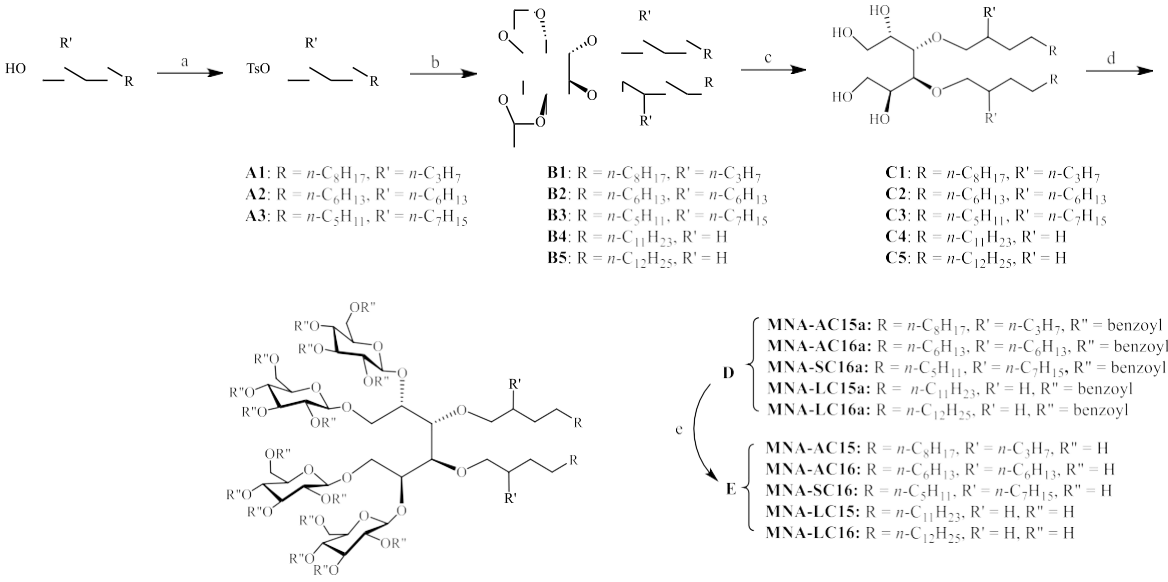
Long-term stability measurement of β_2AR

Long-term stability measurement The β_2AR was purified in 0.1% DDM and concentrated to around 10 mg/ml (approximately200 µM).[5] The DDM purified β_2AR was used to prepare a

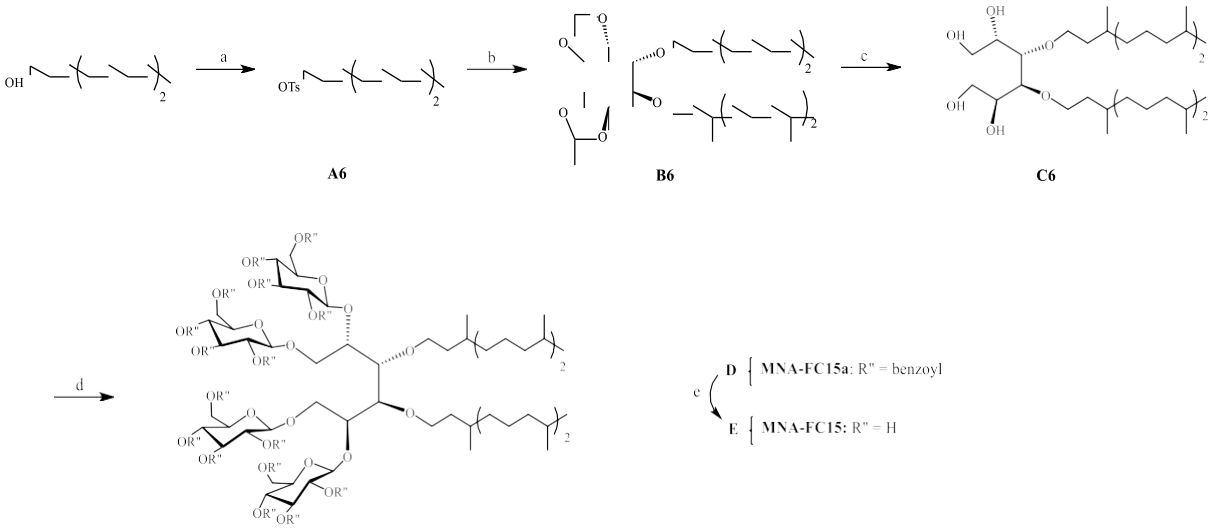
master binding mixture containing 10 nM [^{3}H]-dihydroalprenolol (DHA) supplemented with 0.5 mg/ml BSA, in 0.2% DDM or MNAs, respectively. The activity of the detergent-purified receptor at 0.2 pmol was monitored at the regular intervals during eight-days of incubation. The receptor activity was measured by the soluble radioligand binding assay described below. The receptor purified in DDM or the test detergent (MNA-LC13, MNA-AC16, MNA-LC16 or MNA-SC16) was incubated with 10 nM of [^{3}H]-DHA for 30 min at room temperature. The mixture was loaded on a G-50 column and collected the follow-through with 1 ml binding buffer (20 mM HEPES pH 7.5, 100 mM NaCl, supplemented with 0.5 mg/ml BSA and 20×CMC individual detergents), and further filled with 15 ml scintillation fluid. Receptor-bound [^{3}H]-DHA was measured with a scintillation counter (Beckman). Non-specific binding of [^{3}H]-DHA was calculated by adding 2 μM alprenolol (Sigma) in the same binding reaction. The binding capacity of [^{3}H]-DHA was measured as column graph. Each experiment was performed in triplicate.

Amphiphiles synthesis

Supplementary scheme 1



Supplementary scheme 2



a) Pyridine, *p*-toluenesulfonyl chloride, R.T; b) DMF, 1,2:5,6-di-isopropylidene-D-mannitol, NaH, RBr/ROTs, R.T.; (c) DCM, MeOH, *p*-TSA, R.T; (d) DCM, 2,4,6-collidine, AgOTf, perbenzoylated glucosylbromide, 0 °C; (e) DCM, MeOH, NaOMe, R.T.

General protocol for the synthesis of toslated mono-ol (**A**; step a).[6] The reaction was carried out according to the literature protocol.[6] Briefly, to a stirring solution of mono-ol (10 mmol) dissolved in pyridine (10 mL) was added *p*-toluenesulfonyl chloride (15 mmol) and left the reaction mixture to stir for 4 h at room temperature. After completion of the reaction, was

quenched with ethylene glycol. The organic layer was extracted with ether, washed with 1 N HCl (3 x 100 mL), $NaHCO_3$, water and brine successively and dried with anhydrous Na_2SO_4. The solvent was evaporated and the crude product was passed through column chromatography to give the desired compound (**A**) as oily liquid.

General procedure for the synthesis of 3,4-O-di-alkyl-1,2:5,6-di-O-isopropylidene-D-mannitol (**B**; step b)[7,8]

To a stirring suspension of sodium hydride (3 equiv.) in dry DMF was added 1,2:5,6-di-*O*-isopropylidene-D-mannitol and left for stirring at 0 ^oC for 30 min. To this stirring solution was added tosylated mono-ol (**A**) or commercially available alkyl halide (2.3 equiv.) and the reaction mixture was allowed to stir at room temperature for an additional 6 h. After completion (monitored by TLC), the reaction was quenched with a few drops of water. The organic compound was extracted with ether, washed with water (2 x 100 mL), brine (50 mL) and dried with anhydrous Na_2SO_4. The solvent was evaporated by rotary evaporator and the product (**B**) was purified through chromatographic separation.

General procedure for the synthesis of 3, 4-O-di-alkyl-D-mannitols (**C**; step c)[7,8]

3,4-*O*-di-alkyl-1,2:5,6-di-*O*-isopropylidene-D-mannitol (**B**) was dissolved in 1:1 mixture of CH_2Cl_2 and MeOH (50 mL) and was added *p*-toluenesulfonic acid (*p*-TSA) monohydrate (200 mg). The reaction was left to stir at room temperature for 6 h. After the completion of reaction, solid $NaHCO_3$ was added with vigorous stirring to neutralize the reaction mixture. The solution was filtered, evaporated by rotary evaporator and the product (**C**) was purified with silica gel column chromatography (EtOAc/Hexane).

General procedure for glycosylation reactions (**D**; step d)[8,9]

Glycosylation was carried out according to the literature with slight modification.[8] An alcohol derivative (**C**) and 2,4,6-collidine (3.0 equiv.) was dissolved in CH_2Cl_2 (15 mL) at room temperature and molecular sieves were added. To this mixture AgOTf (4.8 equiv.) was added at 0 °C and then a solution of perbenzoylated glucosylbromide (4.8 equiv.) in CH_2Cl_2 (3 mL) was added dropwise. The reaction was continued to stir for 30 min at 0 °C. After completion (as detected by TLC), pyridine was added to quench the reaction and 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). 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), which provided the desired product (**D**) as a glassy solid.

General procedure for de-O-benzoylations under Zemplén's conditions (**E**; step e)[8,9]

The *O*-benzoylated compound was dissolved in anhydrous CH_2Cl_2 and then MeOH was added dropwise before precipitation appeared persistently. The required amount of a methanolic

solution of 0.5 M NaOMe was added slowly such that the final concentration of NaOMe was 0.05 M. During this period methanol and NaOMe were added alternatively in such a way that precipitation is avoided. The reaction mixture was stirred for 6 h at room temperature, and then neutralized with Amberlite IR-120 (H^+ form) resin. The resin was removed by filtration, washed with MeOH, and solvent was removed from the 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.

2-propyldodecyl 4-methylbenzenesulfonate **(A1)** was synthesized according to the general synthetic procedure for the synthesis of tosylated mono-ol in 92% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.78 (d, *J* = 8.3 Hz, 2H), 7.34 (d, *J* = 7.9 Hz, 2H), 3.90 (d, *J* = 5.4 Hz, 2H), 2.45 (s, 3H), 1.42-1.06 (m, 22H), 0.91-0.87 (m, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 130.0, 128.1, 73.1, 37.6, 33.0, 32.8, 32.1, 30.7, 30.0, 29.8, 29.7, 29.6, 29.5, 29.0, 27.0, 25.5, 22.8, 21.8, 19.8, 14.3.

2-hexyldecyl 4-methylbenzenesulfonate **(A2)** was synthesized according to the general synthetic procedure for the synthesis of tosylated mono-ol in 92% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.78 (d, *J* = 8.3 Hz, 2H), 7.34 (d, *J* = 7.9 Hz, 2H), 3.90 (d, *J* = 5.4 Hz, 2H), 2.45 (s, 3H), 1.31-1.13 (m, 24H), 0.88-0.85 (m, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 130.0, 128.1, 73.0, 37.8, 32.1, 32.0, 30.8, 30.0, 29.7, 29.6, 29.5, 29.0, 26.6, 22.9, 22.8, 21.8, 14.3.

2-heptylnonyl 4-methylbenzenesulfonate **(A3)** was synthesized according to the general synthetic procedure for the preparation of tosylated mono-ol in 92% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.79 (d, *J* = 8.3 Hz, 2H), 7.34 (d, *J* = 7.9 Hz, 2H), 3.90 (d, *J* = 5.4 Hz, 2H), 2.45 (s, 3H), 1.35-1.06 (m, 24H), 0.88 (t, *J* = 7.0 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 130.0, 128.1, 73.0, 37.7, 32.0, 30.7, 29.9, 29.4, 26.6, 22.9, 21.8, 14.3.

3,7,11-trimethyldodecyl 4-methylbenzenesulfonate (**A6***)* was synthesized according to the general synthetic procedure for the synthesis of tosylated mono-ol in 89 % yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.78 (d, *J* = 8.2 Hz, 2H), 7.33 (d, *J* = 8.0 Hz, 2H), 4.12-4.01 (m, 2H), 2.44 (s, 3H), 2.14-1.78 (m, 4H), 1.75-1.52 (m, 6H), 1.51-1.12 (m, 18H), 1.11-0.71 (m, 18H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 129.9, 127.9, 69.1, 43.3, 40.0, 39.9, 39.4, 38.9, 38.7, 37.4, 37.3, 37.2, 37.0, 36.8, 36.6, 36.2, 36.1, 35.8, 32.8, 32.4, 32.2, 31.8, 30.4, 29.2, 28.9, 28.2, 28.0, 27.7, 25.8, 25.2, 25.1, 24.9, 24.4, 23.5, 22.7, 21.6, 19.8, 19.7, 19.2, 19.1, 17.7, 15.8.

(1S,2S)-1,2-bis((S)-2,2-dimethyl-1,3-dioxolan-4-yl)-1,2-bis((2-propyldodecyl)oxy)ethane (**B1**) was prepared according to the general procedure for the synthesis of 3,4-*O*-di-alkyl-1,2:5,6-di-*O*-isopropylidene-D-mannitols in 83% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.24-4.14 (m, 2H), 4.11-4.02 (m, 2H), 3.97-3.87 (m, 2H), 3.62-3.48 (m, 4H), 3.47-3.42 (m, 2H), 1.61-1.45 (m, 2H), 1.40 (s, 6H), 1.35 (s, 6H), 1.32-1.16 (m, 44H), 0.88 (t, *J* = 6.5 Hz, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 108.7, 75.9, 73.3, 73.1, 67.1, 67.0, 38.9, 37.8, 33.9, 33.8, 32.1, 31.7, 31.5, 30.8, 30.5, 30.3, 30.0, 29.9, 29.8, 29.7, 29.6, 27.1, 26.9, 26.6, 25.5, 22.9, 20.1, 14.7, 14.3.

(1S,2S)-1,2-bis((S)-2,2-dimethyl-1,3-dioxolan-4-yl)-1,2-bis((2-hexyldecyl)oxy)ethane (**B2**) was prepared according to the general procedure for the synthesis of 3,4-*O*-di-alkyl-1,2:5,6-di-*O*-isopropylidene-D-mannitols with 83% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.21-4.16 (m, 2H), 4.08-4.04 (m, 2H), 3.93-3.89 (m, 2H), 3.52-3.49 (m, 4H), 3.46-3.44 (m, 2H), 1.57-1.47 (m, 2H), 1.40 (s, 6H), 1.34 (s, 6H), 1.32-1.15 (m, 48H), 0.88 (t, *J* = 6.4 Hz, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 108.6, 80.4, 76.1, 76.0, 67.1, 39.1, 32.1, 31.5, 30.4, 30.0, 29.9, 29.6, 27.1, 27.0, 26.9, 25.5, 22.9, 14.3.

(1S,2S)-1,2-bis((S)-2,2-dimethyl-1,3-dioxolan-4-yl)-1,2-bis((2-heptylnonyl)oxy)ethane (**B3**) was prepared according to the general procedure for the synthesis of 3,4-*O*-di-alkyl-1,2:5,6-di-*O*-isopropylidene-D-mannitols with 83% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.20-4.17 (m, 2H), 4.08-4.04 (m, 2H), 3.93-3.89 (m, 2H), 3.52-3.49 (m, 4H), 3.47-3.45 (m, 2H), 1.58-1.51 (m, 2H), 1.41 (s, 6H), 1.34 (s, 6H), 1.32-1.14 (m, 48H), 0.88 (t, *J* = 6.4 Hz, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 108.6, 80.4, 76.2, 76.0, 67.1, 39.2, 32.1, 31.4, 30.4, 30.0, 29.8, 29.7, 27.1, 27.0, 25.5, 22.9, 14.3.

(1S,2S)-1,2-bis((S)-2,2-dimethyl-1,3-dioxolan-4-yl)-1,2-bis(pentadecyloxy)ethane (**B4**) was prepared according to the general procedure for the synthesis of 3,4-*O*-di-alkyl-1,2:5,6-di-*O*-isopropylidene-D-mannitols in 91% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.28-4.15 (m, 2H), 4.11-4.06 (m, 2H), 3.97-3.93 (m, 2H), 3.65-3.55 (m, 4H), 3.54-3.48 (m, 2H), 1.62-1.49 (m, 4H), 1.41 (s, 6H), 1.35 (s, 6H), 1.33-1.16 (m, 48 H), 0.88 (t, *J* = 6.6 Hz, 6H); **^{13}C NMR** (100 MHz, CD_3OD): δ 108.6, 80.5, 76.1, 73.6, 67.0, 58.7, 33.0, 32.1, 30.6, 30.5, 29.9, 29.8, 29.6, 26.8, 26.3, 26.0, 25.7, 22.8, 14.3.

(1S,2S)-1,2-bis((S)-2,2-dimethyl-1,3-dioxolan-4-yl)-1,2-bis(hexadecyloxy)ethane (**B5**) was prepared according to the general procedure for the synthesis of 3,4-*O*-di-alkyl-1,2:5,6-di-*O*-isopropylidene-D-mannitols in 91% yield. Compared the NMR spectra with the reported data all the peaks were in agreement.[2]

(1S,2S)-1,2-bis((S)-2,2-dimethyl-1,3-dioxolan-4-yl)-1,2-bis((3,7,11-trimethyldodecyl)oxy)-ethane (**B6**) was prepared according to the general procedure for the synthesis of 3,4-*O*-di-alkyl-1,2:5,6-di-*O*-isopropylidene-D-mannitols in 87% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.19-4.16 (m, 2H), 4.11-4.06 (m, 2H), 4.02-3.92 (m, 2H), 3.78-3.68 (m, 4H), 3.67-3.56 (m, 2H), 2.01-1.91 (m, 2H), 1.68-1.47 (m, 8H), 1.41-1.34 (m, 18H), 1.31-1.12 (m, 20H), 0.97-0.83 (m, 20H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 109.0, 78.1, 76.2, 72.3, 67.7, 66.5, 39.5, 37.5, 32.9, 28.1, 27.0, 26.8, 25.7, 22.8, 19.9, 19.7.

(2S,3S,4S,5S)-3,4-bis((2-propyldodecyl)oxy)hexane-1,2,5,6-tetraol (**C1**) was synthesized according to the general procedure for the synthesis of 3, 4-*O*-di-alkyl-D-mannitol in 92% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.03-3.96 (m, 2H), 3.81-3.78 (m, 2H), 3.72-3.67 (m, 4H), 3.62-3.58 (m, 2H), 3.51-3.47 (m, 2H), 1.61-1.47 (m, 2H), 1.36-1.18 (m, 44H), 0.88 (t, *J* = 7.0 Hz,

12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 76.3, 71.8, 63.3, 38.5, 33.6, 32.1, 32.0, 31.3, 30.1, 29.8, 29.6, 27.0, 22.9, 20.1, 14.6, 14.3.

(2S,3S,4S,5S)-3,4-bis((2-hexyldecyl)oxy)hexane-1,2,5,6-tetraol (**C2**) was synthesized according to the general procedure for the synthesis of 3, 4-*O*-di-alkyl-D-mannitol in 92% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.01-3.98 (m, 2H), 3.81-3.71 (m, 2H), 3.72-3.66 (m, 4H), 3.61-3.58 (m, 2H), 3.51-3.46 (m, 2H), 1.62-1.49 (m, 2H), 1.36-1.18 (m, 48H), 0.88 (t, *J* = 6.6 Hz, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 76.4, 71.8, 63.3, 38.7, 33.6, 32.1, 32.0, 31.3, 30.1, 29.8, 29.5, 27.0, 22.9, 20.1, 14.3.

(2S,3S,4S,5S)-3,4-bis((2-heptylnonyl)oxy)hexane-1,2,5,6-tetraol (**C3**) was synthesized according to the general procedure for the synthesis of 3, 4-*O*-di-alkyl-D-mannitol in 92% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.01-3.88 (m, 2H), 3.82-3.78 (m, 2H), 3.71-3.64 (m, 4H), 3.62-3.57 (m, 2H), 3.53-3.46 (m, 2H), 1.58-1.47 (m, 2H), 1.35-1.17 (m, 48H), 0.88 (t, *J* = 6.6 Hz, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 75.0, 71.8, 64.0, 63.3, 38.7, 37.7, 32.0, 31.4, 30.7, 30.1, 29.9, 29.5, 27.0, 22.9, 20.1, 14.3.

(2S,3S,4S,5S)-3,4-bis(pentadecyloxy)hexane-1,2,5,6-tetraol (**C4)** was prepared according to the general procedure for the synthesis of 3, 4-*O*-di-alkyl-D-mannitol in 93% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.02-3.96 (m, 2H), 3.82-3.78 (m, 2H), 3.75-3.71 (m, 4H), 3.69-3.65 (m, 2H), 3.61-3.56 (m, 2H), 1.60-1.51 (m, 4H), 1.34-1.17 (m, 48H), 0.88 (t, *J* = 7.2 Hz, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 72.5, 71.7, 63.2, 32.0, 30.4, 30.2, 29.9, 29.8, 29.7, 29.5, 26.3, 22.8, 14.3.

(2S,3S,4S,5S)-3,4-bis(hexadecyloxy)hexane-1,2,5,6-tetraol (**C5**) was prepared according to the general procedure for the synthesis of 3, 4-*O*-di-alkyl-D-mannitol in 93% yield. The NMR spectra of the product are in well agreement with the reported spectra.[2]

(2S,3S,4S,5S)-3,4-bis((3,7,11-trimethyldodecyl)oxy)hexane-1,2,5,6-tetraol (**C6**) was prepared according to the general procedure for the synthesis of 3, 4-*O*-di-alkyl-D-mannitol in 81% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.98-3.92 (m, 4H), 3.82-3.78 (m, 2H), 3.74-3.69 (m, 4H), 3.65-3.61 (m, 2H), 2.01-1.93 (m, 4H), 1.68-1.63 (m, 2H), 1.61-1.43 (m, 8H), 1.38-1.21 (m, 12H), 1.20-1.11 (m, 6H), 0.92-0.80 (m, 18H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 71.7, 70.6, 63.4, 40.1, 39.5, 39.3, 39.1, 38.8, 37.6, 37.4, 37.2, 33.0, 32.6, 29.9, 28.1, 27.8, 26.0, 25.8, 25.0, 24.5, 22.8, 21.2.

MNA-AC15a was synthesized according to the general procedure for glycosylation reactions in 59% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ; 8.31-8.27 (m, 2H), 8.25-8.12 (m, 4H), 8.11-8.04 (m, 8H), 8.01-7.92 (m, 12H), 7.91-7.82 (m, 8H), 7.72-7.62 (m, 10H), 7.56-7.46 (m, 8H), 7.44-7.39 (m, 8H), 7.38-7.31 (m, 12H), 7.30-7.21 (m, 10H), 7.19-7.14 (m, 2H), 5.78-5.68 (m, 2H), 5.56- 5.46 (m, 8H), 4.67-4.56 (m, 2H), 4.51-4.32 (m, 10H), 4.22-4.17 (m, 4H), 3.55-3.46 (m, 4H), 3.34-3.31 (m, 2H), 3.08-3.00 (m, 2H), 1.52-1.48 (m, 2H), 1.47-0.98 (m, 44 H), 0.86-0.73

(m, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.1, 165.9, 165.8, 165.7, 165.3, 165.1, 164.9, 164.7, 133.7, 133.5, 133.3, 133.1, 130.1, 129.9, 128.8, 128.6, 128.4, 128.3, 72.8, 72.6, 71.6, 69.8, 63.0, 62.7, 38.6, 33.3, 31.2, 30.4, 30.0, 29.9, 29.6, 27.3, 26.3, 22.8, 20.1, 14.7, 14.3.

MNA-AC16a was synthesized according to the general procedure for glycosylation reactions in 59% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.28-8.25 (m, 2H), 8.19-8.16 (m, 4H), 8.11-7.98 (m, 8H), 7.91-7.82 (m, 18H), 7.72-7.62 (m, 8H), 7.57-7.45 (m, 8H), 7.43-7.35 (m, 14H), 7.32-7.21 (m, 20H), 5.78-5.67 (m, 2H), 5.65-5.56 (m, 8H), 4.62-4.59 (m, 2H), 4.46-4.32 (m, 10H) 4.16-4.11 (m, 2H), 3.87-3.84 (m, 2H), 3.53-3.44 (m, 4H), 3.31-3.29 (m, 2H), 3.07-2.99 (m, 2H), 1.48-1.39 (m, 2H), 1.31-0.97 (m, 48H), 0.88-0.81 (m, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.1, 165.9, 165.8, 165.2, 164.8, 133.5, 133.3, 133.1, 130.2, 129.9, 129.7, 129.6, 129.4, 128.8, 128.5, 128.4, 72.9, 71.6, 69.9, 32.2, 32.0 30.5, 30.1, 30.0, 29.8, 26.9, 23.0, 22.9, 14.3.

MNA-SC16a was synthesized according to the general procedure for glycosylation reactions in 51% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ, 8.02-7.98 (m, 2H), 7.97-7.93 (m, 4H), 7.82-7.78 (m, 8H), 7.77-7.41 (m, 18H), 7.36-7.30 (m, 8H), 7.29-7.24 (m, 8H), 7.23-7.20 (m, 14H), 7.19-7.08 (m, 20H), 6.08 (t, J =10 Hz, 2H), 5.85- 5.56 (m, 8H), 5.41-5.21 (m, 4H), 4.56-4.32 (m, 12H) 4.17-4.07 (m, 4H), 3.41-3.29 (m, 4H), 1.48-1.39 (m, 2H), 1.38-0.98 (m, 48H), 0.96-0.82 (m, 12H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 165.9, 165.7, 165.2, 165.0, 164.8 133.8, 133.4, 133.1, 132.9, 130.0, 129.9, 129.8, 129.6, 129.4, 129.0, 128.8, 128.6, 128.4, 71.4, 70.0, 69.8, 68.6, 62.3, 38.7, 32.2, 31.0, 30.2, 29.5, 27.0, 22.9, 14.3.

MNA-LC15a was synthesized according to the general procedure for glycosylation reactions in 52% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.23-8.20 (m, 2H), 8.10-7.98 (m, 4H), 7.97-7.93 (m, 4H), 7.91-7.89 (m, 8H), 7.88-7.83 (m, 14H), 7.61-7.56 (m, 6H), 7.55-7.43 (m, 12H), 7.41-7.34 (m, 28H), 7.33-7.24 (m, 4H), 5.76-5.73 (m, 2H), 5.57-5.52 (m, 4H), 5.48-5.41 (m, 4H), 4.80-4.76 (m, 2H), 4.49-4.45 (m, 6H), 4.31-4.22 (m, 6H), 4.03-3.93 (m, 2H), 3.56-3.49 (m, 5H), 3.38-3.32 (m, 2H), 3.31-3.17 (m, 4H), 1.51-1.32 (m, 2H), 1.27-1.12 (m, 48H), 0.86 (t, J = 7.6 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 165.9, 165.8, 165.6, 165.1, 165.0, 164.8, 133.6, 133.4, 133.3, 133.1, 130.2, 130.0, 129.9, 129.7, 129.6, 129.4, 129.2, 129.0, 128.9, 128.7, 128.5, 128.4, 128.3, 128.1, 82.4, 80.1, 73.3, 72.9, 72.8, 72.6, 71.8, 71.6, 71.1, 69.9, 63.2, 32.0, 30.2, 29.9, 29.8, 29.4, 26.2, 22.8, 14.2.

MNA-LC16a was synthesized according to the general procedure for glycosylation reactions in 52% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.27-8.22 (m, 2H), 8.11-8.00 (m, 4H), 7.98-7.93 (m, 4H), 7.92-7.89 (m, 8H), 7.88-7.82 (m, 14H), 7.62-7.56 (m, 6H), 7.55-7.42 (m, 12H), 7.41-7.33 (m, 28H), 7.32-7.25 (m, 4H), 5.79-5.75 (m, 2H), 5.58-5.52 (m, 4H), 5.48-5.42 (m, 4H), 4.80-4.76 (m, 2H), 4.49-4.45 (m, 6H), 4.32-4.24 (m, 6H), 4.03-3.97 (m, 2H), 3.58-3.51 (m, 5H), 3.42-3.36 (m, 2H), 3.32-3.18 (m, 4H), 1.49-1.38 (m, 2H), 1.32-1.06 (m, 52H), 0.87 (t, J = 7.0 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.0, 165.9, 165.7, 165.2, 165.1, 164.9, 133.7, 133.5, 133.4, 133.3, 133.2, 130.1, 130.0, 129.9, 129.8, 129.6, 129.5, 129.3, 129.0, 128.7, 128.5,

128.1, 82.5, 80.2, 73.4, 72.9, 72.7, 72.4, 71.9, 71.6, 71.1, 69.9, 32.0, 30.3, 30.0, 29.9, 29.8, 29.5, 26.2, 22.8, 14.3.

MNA-FC15a was synthesized according to the general procedure for the glycosylation reactions in 52% yield. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.79-5.74 (m, 2H), 5.59-5.52 (m, 4H), 5.51-5.39 (m, 4H), 4.75-4.63 (m, 2H), 4.51-4.41 (m, 6H), 4.39-4.22 (m, 6H), 3.98-3.84 (m, 2H), 3.62-3.48 (m, 8H), 3.21-3.02 (m, 3H), 2.02-1.80 (m, 6H), 1.78-1.41 (m, 10H), 1.40-0.91 (m, 24H), 0.90-0.56 (m, 24H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.0, 165.9, 165.8, 165.2, 165.1, 164.9, 133.8, 133.5, 133.3, 133.2, 130.2, 130.1, 129.9, 129.8, 129.7, 129.5, 129.3, 129.2, 129.1, 128.6, 128.5, 125.4, 124.7, 101.6, 100.8, 82.9, 72.9, 72.7, 72.4, 71.8, 71.7, 70.9, 69.9, 63.2, 63.0, 39.6, 39.3, 39.0, 38.0, 37.7, 33.1, 29.9, 28.2, 28.1, 27.8, 26.0, 25.9, 25.8, 25.7, 25.0, 22.9, 22.8, 19.8, 19.5, 19.3, 17.8, 16.1.

MNA-AC15 was synthesized according to the general procedure for de-*O*-benzoylations under Zemplén's conditions in 97% yield. **^{1}H NMR** (400 MHz, $(CD_3)_2SO$): δ 4.95-4.88 (m, 2H), 4.54-4.43 (m, 10H), 4.27-4.21 (m, 2H), 4.04-3.97 (m, 4H), 3.88-3.84 (m, 2H), 3.76-3.72 (m, 2H), 3.68-3.48 (m, 16H), 3.21-3.09 (m, 12H), 3.01-2.91 (m, 4H), 1.51-1.38 (m, 2H), 1.36-1.10 (m, 44H), 0.91-0.76 (m, 12H); **^{13}C NMR** (100 MHz, $(CD_3)_2SO$): δ 103.8, 80.4, 79.2, 76.9, 76.5, 74.0, 73.7, 70.0, 60.9, 33.2, 31.3, 30.8, 29.6, 29.2, 29.1, 28.8, 26.5, 22.1, 14.4, 14.0; **HRMS (EI)**: calcd. for $C_{60}H_{114}O_{26}[M+Na]^+$ 1251.5460, found 1251.7499.

MNA-AC16 was synthesized according to the general procedure for de-*O*-benzoylations under Zemplén's conditions in 96% yield. **^{1}H NMR** (400 MHz, $(CD_3)_2SO$): δ 5.08-5.04 (m, 2H), 4.98-4.85 (m, 10H), 4.51-4.46 (m, 2H), 4.45-4.39 (m, 2H), 4.30-4.19 (m, 4H), 4.09-3.98 (m, 2H), 3.90-3.81 (m, 2H), 3.71-3.58 (m, 6H), 3.52-3.41 (m, 8H), 3.19-3.11 (m, 12H), 3.02-2.91 (m, 4H), 1.50-1.39 (m, 2H), 1.34-1.10 (m, 48H), 0.85 (t, J = 6.9 Hz, 12H); **^{13}C NMR** (100 MHz, $(CD_3)_2SO$): δ 103.8, 103.3, 80.4, 79.1, 76.9, 76.7, 76.5, 74.0, 73.7, 69.9, 60.9, 31.4, 30.8, 29.6, 29.3, 29.1 28.8, 26.5, 22.2, 14.0; **HRMS (EI)**: calcd. for $C_{62}H_{118}O_{26}[M+Na]^+$ 1279.6000, found 1279.7812.

MNA-SC16 was synthesized according to the general procedure for de-*O*-benzoylations under Zemplén's conditions in 95% yield. **^{1}H NMR** (400 MHz, CD_3OD): δ 4.61 (d, J = 7.8 Hz, 2H), 4.50 (d, J = 7.8 Hz, 2H), 4.21-4.15 (m, 2H), 4.08-3.98 (m, 2H), 3.95-3.88 (m, 2H), 3.87-3.76 (m, 4H), 3.75-3.65 (m, 8H), 3.62-3.53 (m, 4H), 3.42-3.34 (m, 16H), 3.26-3.18 (m, 8H), 1.59-1.49 (m, 2H), 1.48-1.18 (m, 48H), 0.90 (t, J = 7.0 Hz, 12H); **^{13}C NMR** (100 MHz, CD_3OD): δ 105.5, 105.4, 83.4, 81.7, 78.5, 78.2, 78.1, 77.9, 75.4, 71.7, 71.5, 62.9, 62.6, 40.4, 33.4, 32.7, 32.6, 31.5, 30.8, 28.4, 28.2, 24.0, 14.7; **HRMS (EI)**: calcd. for $C_{62}H_{118}O_{26}[M+Na]^+$ 1279.6000, found 1279.7805.

MNA-LC15 was synthesized according to the general procedure for de-*O*-benzoylations under Zemplén's conditions in 96% yield. **^{1}H NMR** (400 MHz, CD_3OD): δ 4.62 (d, J = 7.8 Hz, 2H), 4.40 (d, J = 7.8 Hz, 2H), 4.24-4.18 (m, 2H), 4.09-4.01 (m, 2H), 3.95-3.88 (m, 2H), 3.87-3.77

(m, 4H), 3.76-3.68 (m, 8H), 3.67-3.62 (m, 4H), 3.42-3.32 (m, 16H), 3.24-3.16 (m, 8H), 1.60-1.48 (m, 2H), 1.42-1.22 (m, 48H), 0.90 (t, *J* = 7.0 Hz, 6H); **^{13}C NMR** (100 MHz, CD_3OD): δ 105.2, 104.8, 83.3, 81.4, 78.3, 78.1, 77.9, 75.7, 75.4, 71.7, 71.5, 62.8, 62.5, 33.2, 31.6, 31.0, 30.9, 30.6, 27.5, 23.9, 14.6; **HRMS (EI)**: calcd. for $C_{60}H_{114}O_{26}[M+Na]^+$ 1251.5460, found 1251.7499.

MNA-LC16 was synthesized according to the general procedure for de-*O*-benzoylations under Zemplén's conditions in 95% yield. **^{1}H NMR** (400 MHz, CD_3OD): δ 4.62 (d, *J* = 7.8 Hz, 2H), 4.39 (d, *J* = 7.8 Hz, 2H), 4.21-4.18 (m, 2H), 4.10-4.01 (m, 2H), 3.95-3.90 (m, 2H), 3.89-3.85 (m, 4H), 3.83-3.73 (m, 8H), 3.72-3.63 (m, 4H), 3.42-3.34 (m, 16H), 3.25-3.15 (m, 8H), 1.61-1.49 (m, 2H), 1.48-1.19 (m, 52H), 0.90 (t, *J* = 7.0 Hz, 6H); **^{13}C NMR** (100 MHz, CD_3OD): δ 105.3, 104.8, 82.8, 81.0, 78.4, 78.2, 78.1, 78.0, 75.4, 74.6, 71.8, 71.6, 62.9, 62.7, 33.3, 31.7, 31.0, 30.7, 27.5, 14.6; **HRMS (EI)**: calcd. for $C_{62}H_{118}O_{26}[M+Na]^+$ 1279.6000, found 1279.7806.

MNA-FC15 was synthesized according to the general procedure for de-*O*-benzoylations under Zemplén's conditions in 96% yield. **^{1}H NMR** (400 MHz, $(CD_3)_2SO$): δ 5.07-5.02 (m, 2H), 4.99-4.85 (m, 10H), 4.50-4.45 (m, 2H), 4.45-4.39 (m, 4H), 4.27-4.22 (m, 4H), 4.06-4.01 (m, 2H), 3.88-3.83 (m, 2H), 3.69-3.59 (m, 6H), 3.58-3.44 (m, 6H), 3.21-3.11 (m, 12H), 3.02-2.92 (m, 4H), 2.02-1.86 (m, 4H), 1.65-1.59 (m, 2H), 1.55-1.42 (m, 8H), 1.41-1.17 (m, 14H), 1.16-1.01 (m, 8H), 0.98-0.76 (m, 24H); **^{13}C NMR** (100 MHz, $(CD_3)_2SO$): δ 103.6, 103.1, 80.6, 79.0, 76.6, 76.4, 73.9, 73.7, 70.0, 69.9, 69.6, 60.9, 37.9, 32.0, 29.1, 27.2, 27.1, 26.9, 25.4, 25.1, 25.0, 24.0, 23.7, 22.4, 22.3, 19.4, 15.5; **HRMS (EI)**: calcd. for $C_{61}H_{116}O_{25}[M+Na]^+$ 1248.7806, found 1247.7186.

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