Electronic Supplementary Material (ESI) for Chemical Science.

Supporting Information

Butane-1,2,3,4-tetraol-based Amphiphilic Stereoisomers for Membrane Protein Study: Importance of Chirality in the Linker Region

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Supporting Information

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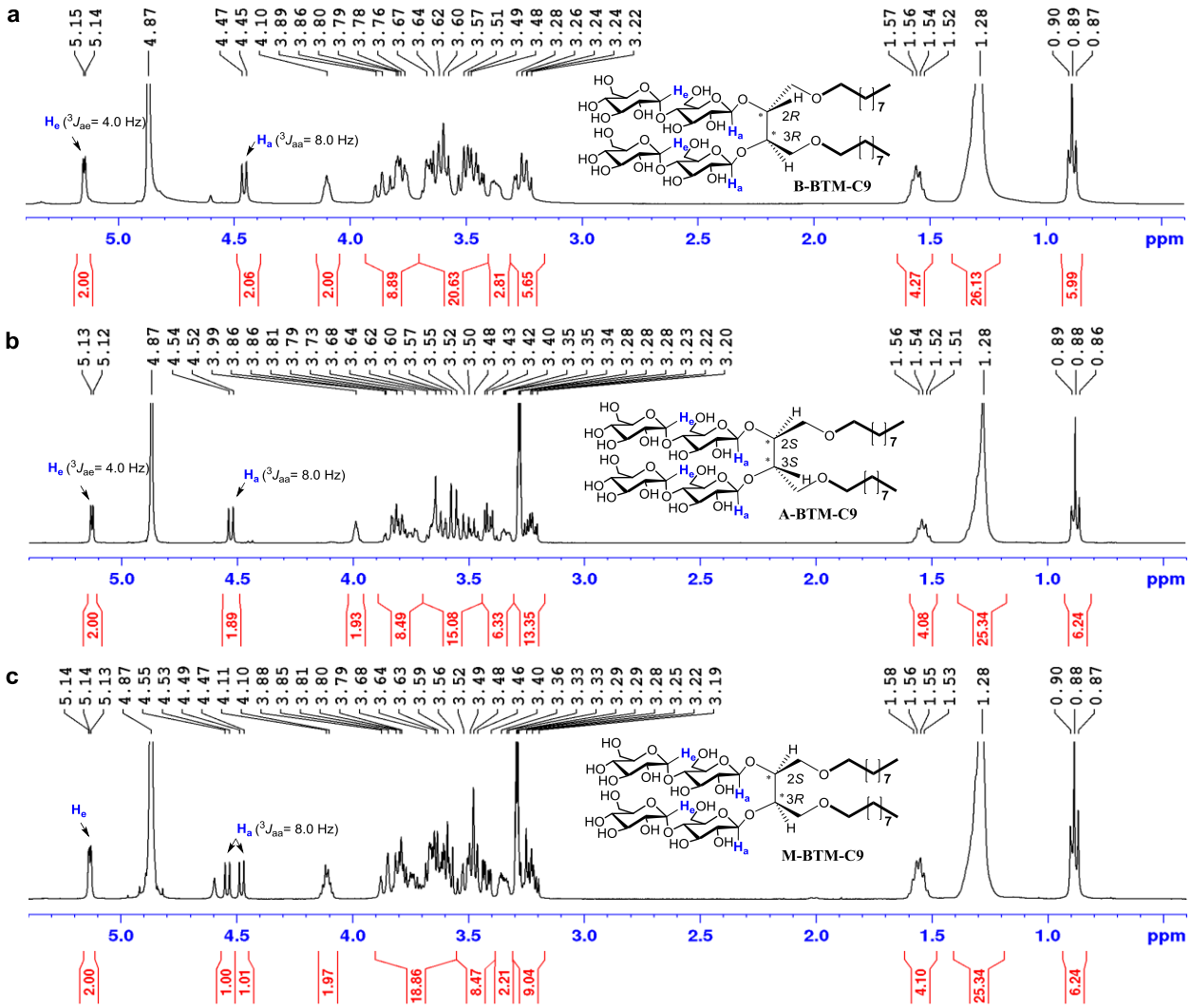
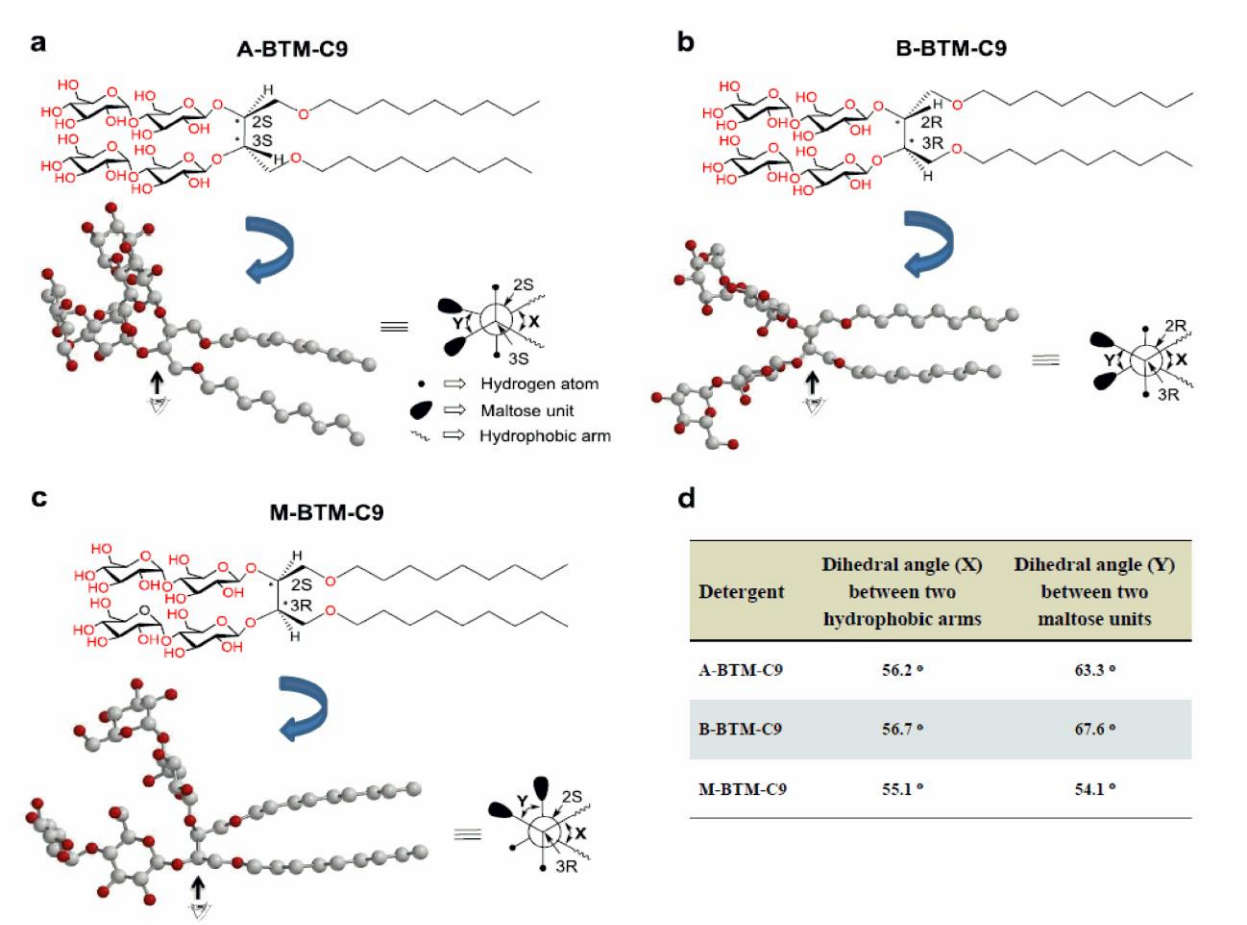


Figure S1. ^{1}H NMR spectra of BTM-C9 isomers ((a) B-BTM-C9, (b) A-BTM-C9 and (c) M-BTM-C9). The high diastereomeric purity of each isomer was confirmed by the respective ^{1}H NMR spectrum. The typical vicinal axial-axial couplings ($^{3}J_{aa}$ ~ 8.0 Hz) were observed in all the isomers for the anomeric proton (H_a), whereas vicinal axial-equatorial couplings ($^{3}J_{ae}$) were observed for another anomeric proton (H_e) with normal values of 4.0 Hz. The two sets of anomeric protons of the maltoside head group labeled H_a and H_e in the chemical structures are different in terms of their location and direction. Assignments of anomeric protons along with the measured coupling constants ($^{3}J_{aa}$ and $^{3}J_{ae}$) are given above the peaks in the spectra. The resonance peak around 5.14 ppm corresponding to H_e of M-BTM-C9 in (c) was a doublet of doublets (dd) with the coupling constants ($^{3}J_{ae}$ = 3.6, 1.2 Hz).



Detergent	Dihedral angle (X) between two hydrophobic arms	Dihedral angle (Y) between two maltose units
A-BTM-C9	56.2 °	63.3 °
B-BTM-C9	56.7 °	67.6 °
M-BTM-C9	55.1 °	54.1 °

Figure S2. Energy-minimized conformations of the BTM-C9 isomers (A-BTM-C9 (a), B-BTM-C9 (b) and M-BTM-C9 (c)). The dihedral angles between the two hydrophobic groups and between the two hydrophilic groups (designated X and Y, respectively) for individual conformations are summarized in the table (d). These energy-minimized structures were obtained by using density functional theory (DFT) calculation at the level of B3LYP/6-31G*. Newman projections were inserted for individual isomers to specify the two dihedral angles of interest.

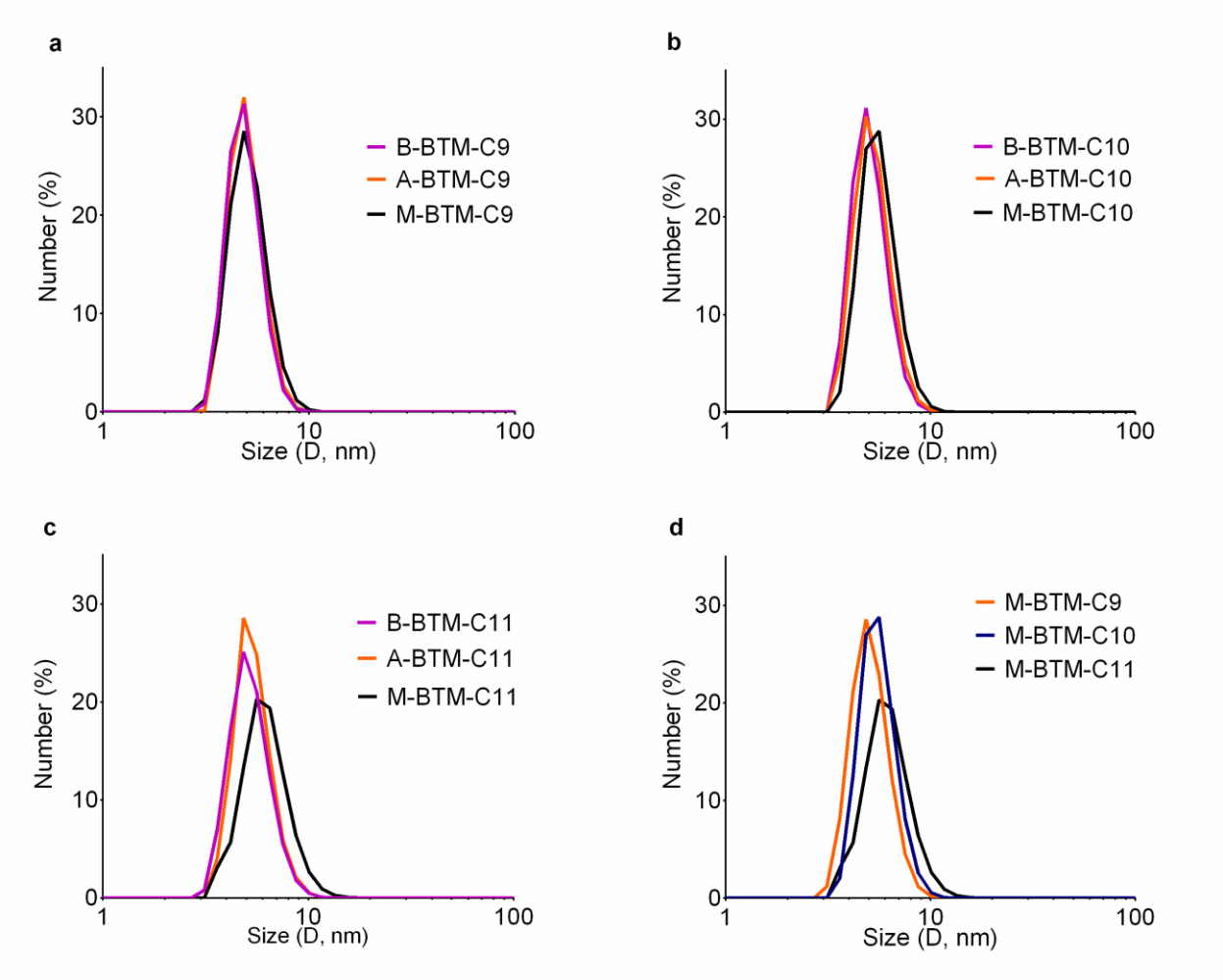


Figure S3. Dynamic light scattering (DLS) profiles for micelles formed by BTM isomers with a C9, C10 or C11 alkyl chain. These agents showed a single micelle size population when used at 1.0 wt%. Autocorrelation analysis for time-dependent fluctuation in the scattered light intensity gave a translational diffusion coefficient (*D*). The Stokes-Einstein equation was used to calculate the hydrodynamic radii (R_h) of detergent micelles from the translational diffusion coefficient.

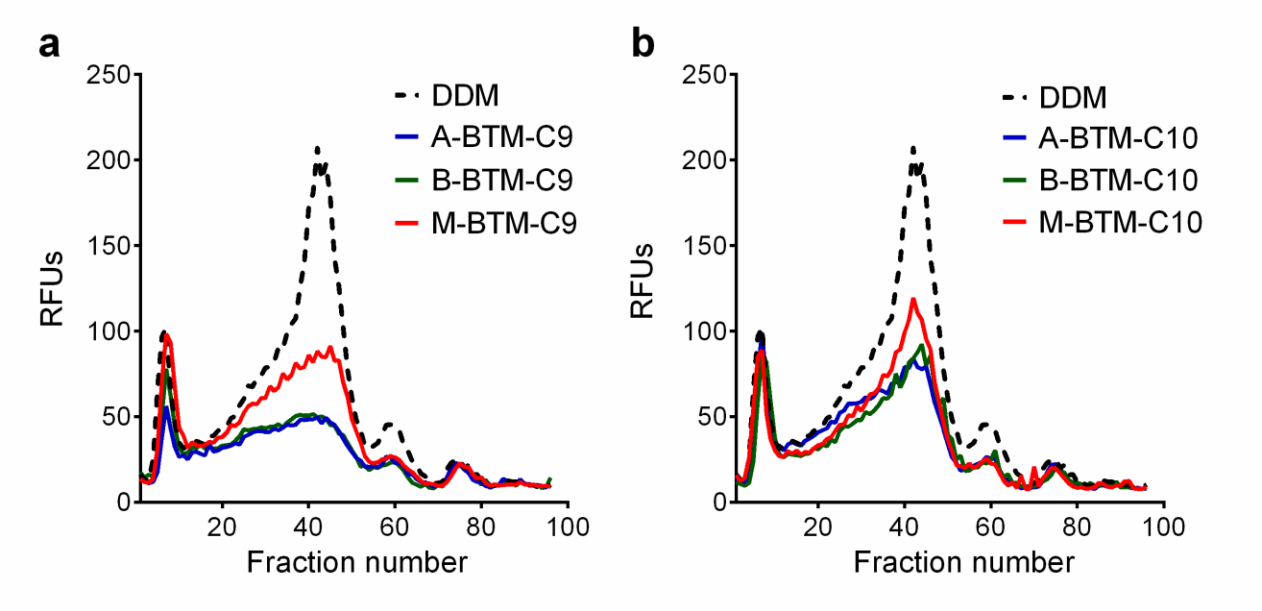


Figure S4. Thermo-stability of UapA solubilized in BTM isomers with (a) a C9 alkyl chain (A-BTM-C9, B-BTM-C9 and M-BTM-C9) or (b) a C10 alkyl chain (A-BTM-C10, B-BTM-C10 and M-BTM-C10). Protein stability was assessed using fluorescence size exclusion chromatography (FSEC) after heat treatment for 10 min at 40℃. The data is representative of two independent experiments.

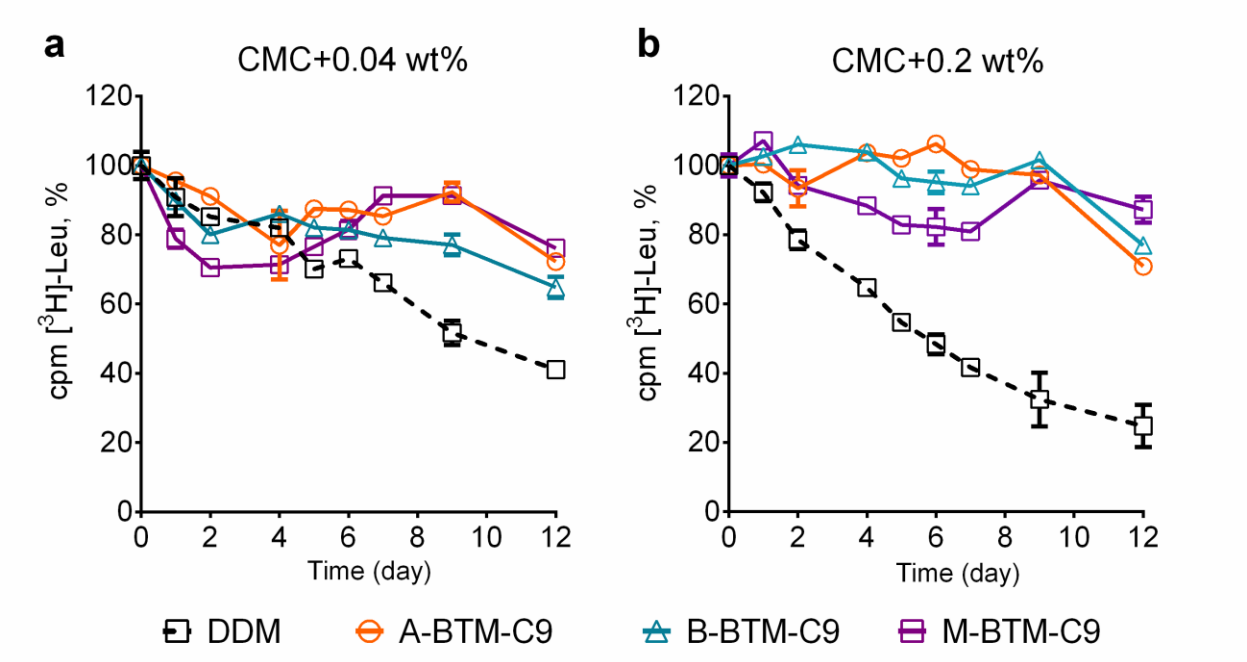


Figure S5. Long-term stability of LeuT solubilized in BTM-C9 isomers or DDM at two different detergent concentrations: (a) CMC+0.04 wt% and (b) CMC+0.2 wt%. The ligand binding activity of the transporter was measured at regular intervals using the radio-labelled ligand, [^{3}H]-Leu, via scintillation proximity assay (SPA) during a 12-day incubation at room temperature. Error bars, SEM, n = 3.

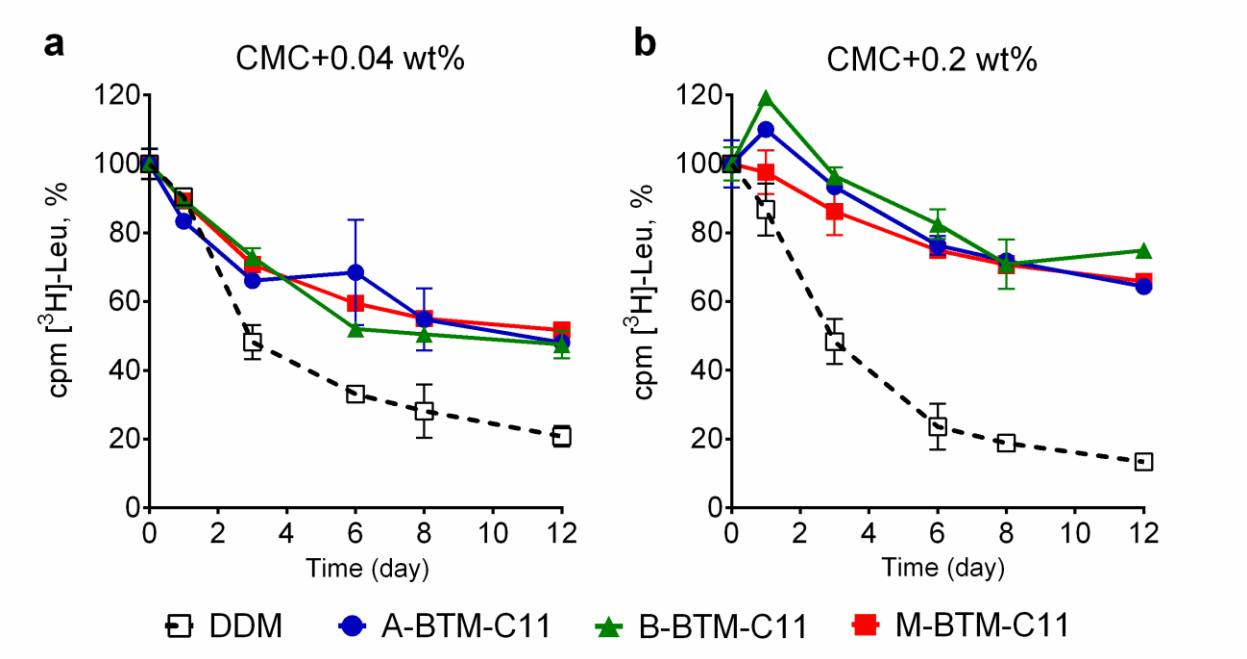


Figure S6. Long-term stability of LeuT solubilized in BTM-C11 isomers or DDM at two different detergent concentrations: (a) CMC+0.04 wt% and (b) CMC+0.2 wt%. The ligand binding activity of the transporter was measured at regular intervals using the radio-labelled ligand, [^{3}H]-Leu, via scintillation proximity assay (SPA) during a 12-day incubation at room temperature. Error bars, SEM, n = 3.

Table S1. Theoretical values for the hydrophobic thickness of membrane proteins (UapA, LeuT, $MelB_{St}$, and β_2AR) and the best alkyl chain length found for the BTM agents.

Membrane proteins	UapA	LeuT	$MelB_{St}$	β_2AR
PDB ID[a]	5I6C	2A65	4M64	2RH1
Hydrophobic thickness (Å)[b]	~29.2±1.0	29.8±0.5	30.4±1.3	~31.8±0.9
Optimal detergent alkyl chain length for the BTMs	C11	C10	C11	C11

[a] protein ID for the crystal structures of target membrane proteins. [b]The value obtained from web based server (http://opm.phar.umich.edu/about.php) that gives information on protein orientation and hydrophobic thickness in membranes.

Detergent CMC determination by diphenylhexatriene (DPH) encapsulation

5.0 mM BTM stock solutions were prepared in deionized and distilled water. A series of detergent solutions were prepared with a range of concentrations from the stock solutions. 200 μL of each detergent sample was transferred to a 96-well plate in duplicates. A DPH stock solution was prepared by dissolving 3.0 mg DPH in 5.0 mL THF. 50 μL of the stock solution was added to 950 μL of distilled water to prepare a DPH working solution and then 2.0 μL DPH work solution was added into each well containing a detergent solution for dye encapsulation. After 15 ~ 20 min incubation at room temperature, fluorescence intensities were measured at 430 nm upon excitation at 358 nm using a Synergy Mx Monochromator Based Multi-Mode Microplate reader. Detergent 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 BTMs were dissolved in distilled and deionized water to give a detergent concentration of 1.0 wt%. These BTM solutions were filtered by a syringe filter with a pore size of 0.22 μm. Hydrodynamic radii of the micelles produced by the BTMs were measured using a Malvern Zeta Sizer Nano ZS90 particle analyzer. With a maximum power of 5Mw, a He-Ne laser set at 633 nm was used as light source. The scattered light was collected at the angle of 90°. Temperature was kept constant at 25 ℃ throughout all experiments. The translational diffusion coefficient and hydrodynamic radius (R_h) of detergent micelles was calculated by autocorrelation analysis on time-dependent scattered light intensity. Hydrodynamic radius (R_h) values for micelles formed by individual detergents (BTMs and DDM) were expressed as mean ± SD (n = 4).

Protein stability evaluation

Fluorescence size exclusion chromatography (FSEC)

UapAG411V$_{\Delta 1\text{-}11}$ (referred to as UapA) was expressed as a GFP fusion in *Saccharomyces cerevisiae* strain FGY217 and isolated as described previously in sample buffer (20 mM Tris (pH 7.5), 150 mM NaCl, 0.03% DDM, 0.6 mM xanthine).[1] Membranes containing UapA were resuspended in PBS, 10 mM Imidazole pH 8.0, 150 mM NaCl, 10% glycerol and the protein concentration measured. The membranes were adjusted to a concentration of 1 mg/ml and 1 ml aliquots were incubated individually with DDM and BTMs at a final detergent concentration of 1.0 wt % for 10 min at 40 °C with mild agitation. 100 µl aliquots were removed from each tube, and a fluorescence reading was taken for each sample before and after ultracentrifugation at 150,000 g for 10 min to remove insoluble material. The remaining soluble fraction for each condition was submitted to fluorescent SEC (FSEC) using a Superose 6 column (GE Healthcare) equilibrated with buffer containing the appropriate agent (DDM or a BTM).

LeuT stability assay

Purification of the wild type of the leucine transporter (LeuT) from *Aquifex aeolicus* was performed according to the protocol described previousely.[2] 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, after isolation of bacterial membranes and solubilisation in 1 % DDM, protein was 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, approx. 1.5 mg/ml protein stock was diluted 10 times in identical buffer without DDM and imidazole, but supplemented with individual BTMs and DDM (control) at the final concentrations of CMC + 0.04 wt% or CMC + 0.2 wt%, respectively. Protein samples were stored at room temperature and, at the indicated time points, were centrifuged and protein activity was determined by measuring [^{3}H]-Leu binding using scintillation proximity assay (SPA).[3] Assay was performed with 5 µL of the respective protein samples in the buffer containing 200 mM NaCl and the respective test compounds at the concentrations indicated above. SPA reaction was carried out in the presence of 20 nM [^{3}H]-Leu and copper chelate (His-Tag) YSi beads (both from PerkinElmer, Denmark). Total [^{3}H]-Leu binding for the respective samples was measured using MicroBeta liquid scintillation counter (PerkinElmer).

β_2AR stability assay

The β_2AR in 0.1% DDM was purified based on the protocol as reported previously[4] and finally concentrated to around 10 mg/ml (approximately 200 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/BTMs, respectively. The activity of the detergent-purified receptor at 0.2 pmol was monitored at regular intervals during three-day of incubation at room temperature. The receptor activity was measured by the soluble radioligand binding assay described below. The receptor purified in

DDM or individual BTMs 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.

MelB$_{St}$ solubilization and thermo-stability assay

The plasmid pK95ΔAHB/WT MelB$_{St}$/CH10) was used to express *Salmonella typhimurium* melibiose permease (MelB$_{st}$) with a 10-His tag at the C-terminus in *E. coli* DW2 cells (Δ*melB* and Δ*lacZY*). Cell growth and membrane preparation were carried out as described in a previous report.[6] Protein assay was performed with a Micro BCA kit (Thermo Scientific, Rockford, IL). The reported protocol was used to evaluate three isomers of BTM-C11 (A-BTM-C11, B-BTM-C11 and M-BTM-C11) and DDM for MelB$_{St}$ stability.[7] Membrane samples containing MelB$_{St}$ (the final protein concentration was 10 mg/mL) were incubated with a solubilization buffer (20 mM sodium phosphate, pH 7.5, 200 mM NaCl, 10% glycerol, 20 mM melibiose) containing1.5 % (w/v) DDM or an isomer of BTM-C11 (A-BTM-C11, B-BTM-C11, or M-BTM-C11) at four temperatures (0, 45, 55, and 65℃) for 90 min. In order to remove insoluble material, ultracentrifugation was carried out at 355,590 g in a Beckman Optima™ MAX Ultracentrifuge with a TLA-100 rotor for 45 min at 4 ℃. The soluble portions were separated by SDS-16% PAGE, followed by immunoblotting with a Penta-His-HRP antibody (Qiagen, Germantown, MD). A membrane fraction containing 20 μg of proteins without treatment was used to present the total MelB; the treated sample was loaded onto each well at equal volume. MelB$_{St}$ was detected using SuperSignal West Pico chemiluminescent substrate by the ImageQuant LAS 4000 Biomolecular Imager (GE Health Care Life Science).

Amphiphiles Synthesis

General procedure for dialkylation

NaH (3.0 equiv.) and **(*E*)-but-2-ene-1,4-diol** or **(*Z*)-but-2-ene-1,4-diol** (1 equiv., 500mg) were dissolved in DMF (15mL) at 0 ℃. Alkyl iodide (2.9 equiv.) was added dropwise, and the resulting solution was stirred at 80 ℃ for 3days. After completion of the reaction (as detected by TLC), the solution was diluted with diethyl ether (150 mL) and the washed successively with 1M aqueous HCl (2 x 20 mL) and brine (100 mL). The organic layer was dried with anhydrous Na_2SO4, and the solvent was removed by rotary evaporation. The residue was purified by silica gel column chromatography (EtOAc/hexane) providing a desired product (**A** or **H**) as a liquid.

General procedure for Sharpless asymmetric dihydroxylation[8]

A 25-mL round-bottomed flask, equipped with a magnetic stirrer, was charged with 5 mL of *tert*-butyl alcohol, 5 mL of water, and 1.4 g of AD-mix-β or AD-mix-α. Stirring at room temperature produced two clear phases; the lower aqueous phase appears bright yellow. Methanesulfonamide (95 mg, 1 equiv based on 1 mmol of olefin) was added at this point. The mixture was cooled to 0 ℃ whereupon some of the dissolved salts precipitated. One mmol of trans-olefin **A** was added at once, and the heterogeneous slurry was stirred vigorously at 0 ℃ for 48 h (progress was monitored by TLC). While the mixture was stirred at 0 ℃, solid sodium sulfite (1.5 g) was added and the mixture was allowed to warm to room temperature and stirred for 30-60 min. Ethyl acetate (10 mL) was added to the reaction mixture, and after separation of the layers, the aqueous phase was further extracted with the organic solvent (3 X 15 mL). The combined organic layers were washed with 2 N KOH. The combined organic extracts were dried over anhydrous sodium sulfate and concentrated to give the diol and the ligand. This crude product was purified by flash chromatography (silica gel, EtOAc/hexanes; the ligand does not move in this solvent system) to afford the optically active 1,2-diol (**B** or **C**) in 90-95% yield.

General procedure for Upjohn Dihydroxylation

A solution of NMO (1.5 equiv.) in water (50 wt %) was added to a mixture of THF and water (15 mL of a 9:1 mixture) at 0 ℃. Compound **H** (500 mg, 1 equiv.) was then added in one portion, the mixture allowed to stir for 15 minutes and then OsO_4 (1.4 mL of a 2.5 wt % solution in ^{t}BuOH) was added dropwise by syringe over 20 minutes. The mixture was stirred at room temperature for 12 h. The reaction was quenched by the addition of sodium sulfite (8 g) and diluted with water (30 mL). The solution was then extracted with EtOAc (2 × 70 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated

in vacuo and the residue was purified by silica gel column chromatography (EtOAc/hexane) providing a desired meso 1,2-diol (**I**) as an orange gum.

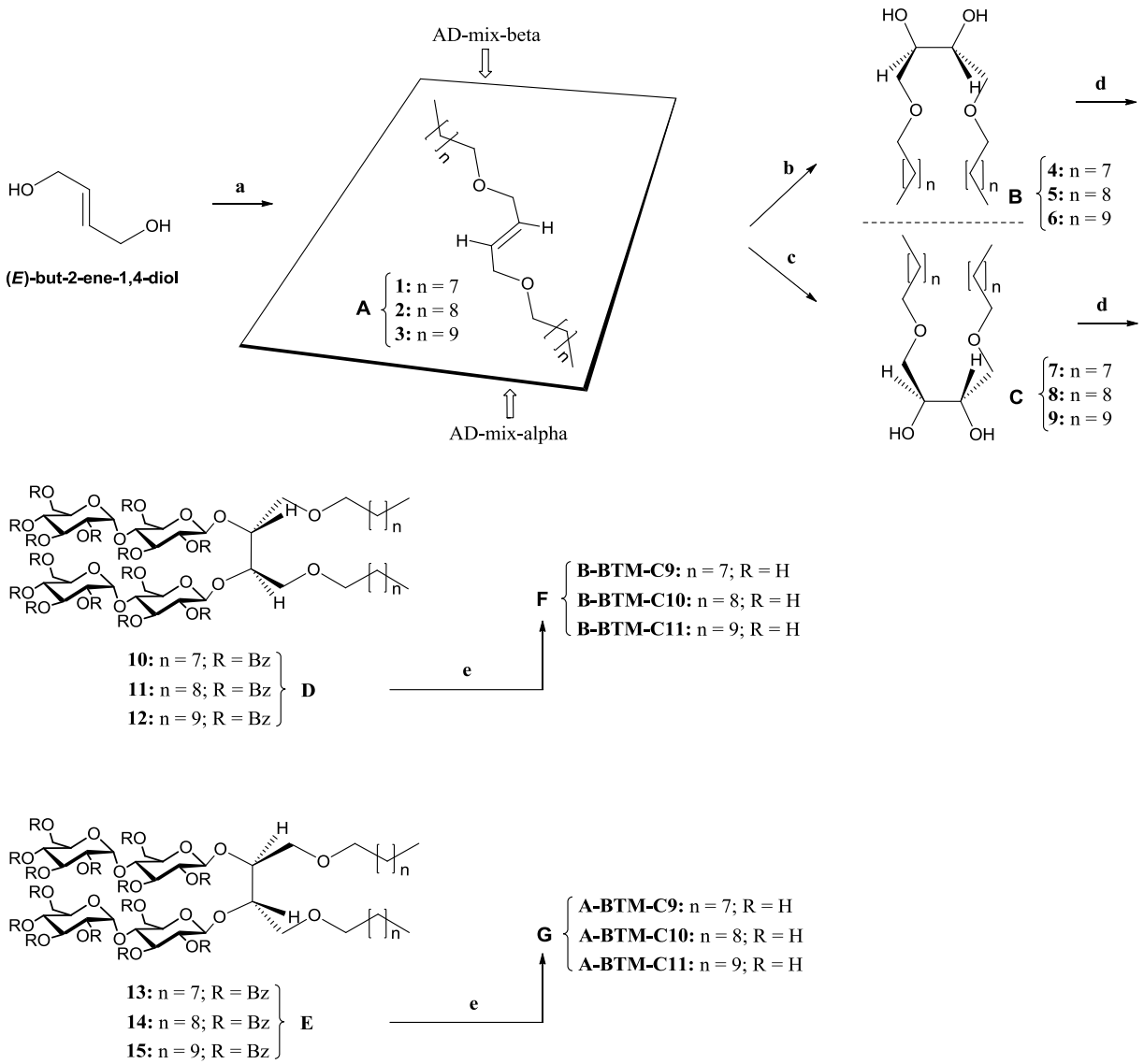
General procedures for glycosylation reaction

This procedure followed a literature method[9] with slight modification. A mixture of diol (1 equiv., 250 mg), AgOTf (2.4 equiv.) and 2,4,5-collidine (1.0 equiv.) in anhydrous CH_2Cl_2 (40 mL) was stirred at -45 ℃. A solution of perbenzoylated maltosylbromide (2.4 equiv.) in CH_2Cl_2 (10 mL) was added dropwise over 0.5h to this suspension. Stirring was continued for 0.5 h at -45℃, and then the reaction mixture was allowed to warm to 0℃ and left stirring for 1 hr. After completion of the reaction, pyridine was added to the reaction mixture, and it was diluted with CH_2Cl_2 (40 mL) before being filtered through celite. The filtrate was washed successively with a 1 M aqueous $Na_2S_2O_3$ (40 mL), a 0.1 M aqueous HCl solution (40 mL), and brine (2 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 glycosylated product as a glossy white solid.

General procedures for deprotection reaction

This procedure followed the de-*O*-benzoylation or de-*O*-acetylation under Zemplén's conditions.[9] The O-protected glycosylated compound was dissolved in MeOH and treated with the required amount of a methanolic solution of 0.5 M NaOMe such that the final concentration of NaOMe was 0.05 M. The reaction mixture was stirred for 14 h at room temperature, and then neutralized with Amberlite IR-120 resin (H^+ form). The resin was removed by filtration and washed with MeOH, and the solvent was removed from the combined filtrate *in vacuo*. The residue was purified by silica gel column chromatography (eluting with MeOH/CH_2Cl_2). Further purification, by recrystallization using CH_2Cl_2/MeOH/diethyl ether, afforded fully deprotected product as a white solid.

Supplementary scheme 1



(a) alkyl iodide, NaH, 79-85%; (b) AD-mix-β, $CH_3SO_2NH_2$, ^tBuOH, H_2O, 0°C, 5 days, 90-95%; (c) AD-mix-α, $CH_3SO_2NH_2$, ^tBuOH, H_2O, 0°C, 5 days, 90-95%; (d) Perbenzoylated malotsylbromide, AgOTf, DCM, -45°C → 0°C, 82-88%; (e) NaOMe, MeOH, room temperature, 14 hr, 91-95%.

Compound **1** was prepared in 85% yield according to the general procedure for dialkylation. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.81-5.80 (m, 2H), 3.97 (dd, *J* = 3.2 Hz, 1.6 Hz, 4H), 3.41 (t, *J* = 6.8 Hz, 4H), 1.59-1.56 (m, 4H), 1.40-1.27 (m, 24H), 0.88 (t, *J* = 6.8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 129.7, 71.0. 70.8, 32.1, 30.0, 29.8, 29.7, 29.5, 26.4, 22.9, 14.3.

Compound **2** was prepared in 80% yield according to the general procedure for dialkylation. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.81-5.79 (m, 2H), 3.97 (dd, *J* = 4 Hz, 1.6 Hz, 4H), 3.41 (t, *J* = 8 Hz, 4H), 1.60-1.55 (m, 4H), 1.38-1.26 (m, 28H), 0.88 (t, *J* = 8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 129.7, 71.0. 70.7, 32.1, 30.0, 29.8 (2C), 29.7, 29.5, 26.4, 22.9, 14.3.

Compound **3** was prepared in 79% yield according to the general procedure for dialkylation. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.82-5.80 (m, 2H), 3.97 (dd, *J* = 4 Hz, 1.6 Hz, 4H), 3.41 (t, *J* = 8 Hz, 4H), 1.61-1.54 (m, 4H), 1.40-1.26 (m, 34H), 0.88 (t, *J* = 8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 129.7, 71.0. 70.8, 32.1, 30.0, 29.8 (2C), 29.7, 29.6, 26.4, 22.9, 14.3.

Compound **4** was prepared in 94% yield according to the general procedure for Sharpless asymmetric dihydroxylation using AD-mix-β. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.85-3.80 (m, 2H), 3.58-3.54 (m, 4H), 3.49-3.45 (m, 4H), 2.92 (d, *J* = 4.8 Hz, 2H), 1.58 (app. t, *J* = 7.2 Hz, 4H), 1.39-1.20 (m, 24H), 0.88 (t, *J* = 6.4 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 73.0, 72.1, 70.9, 32.1, 29.8 (2C), 29.7, 29.5, 26.3, 22.9, 14.3; $[\alpha]_D^{20}$=-2.871 degcm^{3}g^{-1}dm^{-1} (*c*=1.22 gcm^{-3} in acetone)

Compound **5** was prepared in 95% yield according to the general procedure for Sharpless asymmetric dihydroxylation using AD-mix-β. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.85-3.80 (m, 2H), 3.59-3.54 (m, 4H), 3.49-3.45 (m, 4H), 3.08 (d, *J* = 4 Hz, 2H), 1.58 (app. t, *J* = 8 Hz, 4H), 1.38-1.22 (m, 28H), 0.88 (t, *J* = 8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 72.8, 72.0, 70.7, 32.0, 29.7 (2C), 29.6, 29.5, 26.2, 22.8, 14.2; $[\alpha]_D^{20}$=-3.301 degcm^{3}g^{-1}dm^{-1} (*c*=1.11 gcm^{-3} in acetone)

Compound **6** was prepared in 90% yield according to the general procedure for Sharpless asymmetric dihydroxylation using AD-mix-β. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.85-3.81 (m, 2H), 3.55-3.51 (m, 4H), 3.46-3.43 (m, 4H), 2.98 (d, *J* = 4 Hz, 2H), 1.56 (app. t, *J* = 8 Hz, 4H), 1.36-1.24 (m, 32H), 0.86 (t, *J* = 8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 72.9, 72.0, 70.8, 32.1, 29.8 (2C), 29.7, 29.5, 26.3, 22.9, 14.3; $[\alpha]_D^{20}$=-3.654 degcm^{3}g^{-1}dm^{-1} (*c*=0.60 gcm^{-3} in acetone)

Compound **7** was prepared in 90% yield according to the general procedure for Sharpless asymmetric dihydroxylation using AD-mix-α. **^{1}H** and **^{13}C NMR** are identical for compound **7** and compound **4 s**ince they are enantiomers; $[\alpha]_D^{20}$=+2.874 degcm^{3}g^{-1}dm^{-1} (*c*=1.24 gcm^{-3} in acetone)

Compound **8** was prepared in 94% yield according to the general procedure for Sharpless asymmetric dihydroxylation using AD-mix-α. **^{1}H** and **^{13}C NMR** are identical for compound **8** and compound **5 s**ince they are enantiomers; $[\alpha]_D^{20}$=+3.311 degcm^{3}g^{-1}dm^{-1} (*c*=1.19 gcm^{-3} in acetone)

Compound **9** was prepared in 95% yield according to the general procedure for Sharpless asymmetric dihydroxylation using AD-mix-α. **^{1}H** and **^{13}C NMR** are identical for compound **9** and compound **6 s**ince they are enantiomers; $[\alpha]_D^{20}$=+3.652 degcm^{3}g^{-1}dm^{-1} (*c*=0.75 gcm^{-3} in acetone)

Compound **10** was prepared in 85% yield according to the general procedure for glycosylation reaction. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.21-7.77 (m, 26H), 7.67-7.65 (m, 6H), 7.53-7.20 (m, 42H), 6.17 (t, *J* = 9.6 Hz, 2H), 5.79-5.70 (m, 6H), 5.31 (d, *J* = 8 Hz, 2H), 5.24 (t, *J* = 6.8 Hz, 2H), 5.04 (d, *J* = 12 Hz, 2H), 4.93 (d, *J* = 8 Hz, 2H), 4.67 (d, *J* = 8 Hz, 2H), 4.57-4.50 (m, 4H), 4.43-4.33 (m, 4H), 3.99 (d, *J* = 4 Hz, 2H), 3.87 (d, *J* = 8 Hz, 2H), 3.45 (d, *J* = 10 Hz, 2H), 3.20 (t, *J* = 8 Hz, 2H), 2.94-2.85 (m, 4H), 1.29-1.07 (m, 24H), 1.00-0.92 (m, 4H), 0.88 (t, *J* = 4 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.2, 166.0, 165.8, 165.6, 165.2, 165.1, 133.9, 133.5, 133.3, 133.2, 132.9, 130.1 (2C), 129.9 (2C), 129.8, 129.7 (2C), 129.5, 129.1, 129.0 (2C), 128.9, 128.7, 128.5, 128.3, 128.2 (2C), 100.8, 96.6, 79.7, 75.1, 73.6, 72.7, 72.5, 71.5, 71.1, 70.3, 70.1, 69.3, 63.4, 63.6, 62.7, 32.0, 29.7, 29.6 (2C), 29.4, 26.0, 22.8, 14.3.

Compound **11** was prepared in 88% yield according to the general procedure for glycosylation reaction. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.20-7.77 (m, 25H), 7.67-7.65 (m, 6H), 7.55-7.17 (m, 44H), 6.17 (t, *J* = 10 Hz, 2H), 5.77-5.71 (m, 6H), 5.33 (d, *J* = 8 Hz, 2H), 5.24 (t, *J* = 6.8 Hz, 2H), 5.06 (d, *J* = 10 Hz, 2H), 4.68 (d, *J* = 4 Hz, 2H), 4.67 (d, *J* = 8 Hz, 2H), 4.53-4.50 (m, 4H), 4.43-4.36 (m, 4H), 3.99 (d, *J* = 6 Hz, 2H), 3.89 (d, *J* = 8 Hz, 2H), 3.45 (d, *J* = 10 Hz, 2H), 3.20 (t, *J* = 4 Hz, 2H), 2.92-2.85 (m, 4H), 1.25-1.07 (m, 32H), 0.98-0.90 (m, 4H), 0.88 (t, *J* = 4 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.1, 166.0, 165.7, 165.6, 165.1 (2C), 133.9, 133.6, 133.3, 133.1, 132.9, 130.1 (2C), 129.9 (2C), 129.8 (2C), 129.7 (2C), 129.5, 129.1, 129.0 (2C), 128.8, 128.7, 128.6, 125.5(2C), 128.3, 128.2 (2C), 100.8, 96.6, 79.7, 75.1, 73.6 (2C), 72.7, 72.5, 71.5, 71.0, 70.3, 70.1, 69.3, 63.4, 63.6, 62.6, 32.1, 29.7 (2C), 29.6 (2C), 29.5, 26.0, 22.8, 14.3.

Compound **12** was prepared in 84% yield according to the general procedure for glycosylation reaction. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.19-7.70 (m, 24H), 7.67-7.65 (m, 6H), 7.55-7.19 (m, 43H), 6.17 (t, *J* = 8 Hz, 2H), 5.79-5.70 (m, 6H), 5.32 (d, *J* = 8 Hz, 2H), 5.23 (t, *J* = 6.8 Hz, 2H), 5.03 (d, *J* = 12 Hz, 2H), 4.94 (d, *J* = 4 Hz, 2H), 4.56 (d, *J* = 8 Hz, 2H), 4.57-4.50 (m, 4H), 4.43-4.35 (m, 4H), 3.99 (d, *J* = 8 Hz, 2H), 3.87 (d, *J* = 8 Hz, 2H), 3.45 (d, *J* = 10 Hz, 2H), 3.21 (t, *J* = 4 Hz, 2H), 2.93-2.85 (m, 4H), 1.26-1.13 (m, 34H), 1.00-0.92 (m, 4H), 0.87 (t, *J* = 4 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.2, 166.1, 165.7 (2C), 165.6, 165.2, 165.1, 133.8, 133.6(2C), 133.3, 133.2, 132.8, 130.1 (2C), 129.9 (2C), 129.8 (2C), 129.7, 129.5, 129.1, 129.0 (2C), 128.9, 128.7 (2C), 128.5, 128.3, 128.2 (2C), 100.8, 96.6, 79.7, 75.1, 73.6, 72.7, 72.5, 71.5, 71.0, 70.3, 70.1, 69.3, 63.4, 63.6, 62.7, 32.1, 29.8 (2C), 29.7, 29.6, 29.5, 29.4, 26.0, 22.9, 14.3.

Compound **13** was prepared in 83% yield according to the general procedure for glycosylation reaction. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.19-7.73 (m, 26H), 7.70-7.59 (m, 6H), 7.58-7.18 (m, 4H), 6.10 (t, *J* = 8 1Hz, 2H), 5.72-5.67 (m, 6H), 5.31 (t, *J* = 8 Hz, 2H), 5.20 (d, *J* = 6.8 Hz, 2H), 4.71 (d, *J* = 8 Hz, 2H), 4.57-4.54 (m, 4H), 4.44-4.33 (m, 6H), 4.29-4.24 (m, 2H), 3.76 (br s, 2H), 3.41-3.32 (m, 4H), 3.17-3.10 (m, 5H), 1.33-1.14 (m, 27H), 0.88 (t, *J* = 8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.2, 166.1, 165.9, 165.8, 165.7, 165.2 (2C), 165.1, 133.7, 133.6 (2C), 133.5 (2C), 133.3, 133.1 (2C), 130.0 (2C), 129.9 (2C), 129.7, 129.6, 129.5, 129.4, 129.3, 129.0, 128.8 (2C), 128.7, 128.5, 128.2, 100.7, 96.5, 79.0, 75.0, 73.1, 73.0, 72.2, 71.8, 71.4, 70.5, 69.9, 69.2 (2C), 63.3, 62.6, 32.1, 29.8, 29.7 (2C), 29.6, 29.5, 26.2, 22.9, 14.3.

Compound **14** was prepared in 82% yield according to the general procedure for glycosylation reaction. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.07-7.72 (m, 24H), 7.70-7.59 (m, 6H), 7.58-7.18 (m, 42H), 6.12 (t, *J* = 10 Hz, 2H), 5.70-5.66 (m, 6H), 5.30 (t, *J* = 8 Hz, 2H), 5.22 (d, *J* = 8 Hz, 2H), 4.72 (d, *J* = 10 Hz, 2H), 4.57-4.56 (m, 4H), 4.40-4.30 (m, 6H), 4.29-4.24 (m, 2H), 3.79 (br s, 2H), 3.44-3.32 (m, 4H), 3.20-3.09 (m, 5H), 1.34-1.16 (m, 35H), 0.88 (t, *J* = 6.8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.1, 166.0, 165.9, 165.7, 165.2 (2C), 165.0, 133.7, 133.6, 133.5 (2C), 133.3, 133.2, 130.0 (2C), 129.9 (2C), 129.7, 129.6, 129.5, 129.4, 129.1, 129.0, 128.8 (2C), 128.7, 128.5, 128.3, 100.6, 96.4, 79.0, 75.0, 73.1, 73.0, 72.2, 71.8, 71.4, 70.5, 69.9, 69.2 (2C), 63.3, 62.6, 32.1, 29.8 (3C), 29.7, 29.6, 29.5, 26.2, 22.9, 14.3.

Compound **15** was prepared in 87% yield according to the general procedure for glycosylation reaction. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.17-7.70 (m, 26H), 7.68-7.55 (m, 6H), 7.58-7.16 (m, 44H), 6.13 (t, *J* = 8 Hz, 2H), 5.72-5.67 (m, 6H), 5.32 (t, *J* = 8 Hz, 2H), 5.27 (d, *J* = 6.8 Hz, 2H), 4.79 (d, *J* = 8 Hz, 2H), 4.57-4.54 (m, 4H), 4.40-4.30 (m, 6H), 4.28-4.24 (m, 2H), 3.88 (br s, 2H), 3.44-3.32 (m, 4H), 3.21-3.09 (m, 5H), 1.33-1.14 (m, 38H), 0.94 (app. t, *J* = 4 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.1, 166.0, 165.9, 165.7, 165.2 (2C), 165.1, 133.7, 133.6, 133.5 (2C), 133.3, 133.1 (2C), 130.0 (2C), 129.9 (2C), 129.8 (2C), 129.6, 129.5, 129.4, 129.1 (2C), 128.8 (2C), 128.7, 128.5, 128.3, 100.6, 96.4, 79.0, 75.0, 73.1, 73.0, 72.1, 71.7, 71.3, 70.5, 69.9, 69.1, 62.6, 60.4, 32.0, 29.8, 29.7, 29.6 (3C), 29.5, 26.1, 22.8, 21.1, 14.2.

B-BTM-C9 was prepared in 94% yield according to the general procedure for deprotection reactions. **^{1}H NMR** (400MHz, CD_3OD): δ 5.15 (d, *J* =4 Hz, 2H), 4.46 (d, *J* = 8 Hz, 2H), 4.10 (br s, 2H), 3.80-3.67 (m, 9H), 3.55-3.15 (m, 28H), 1.48-1.45 (m, 4H), 1.28-1.14 (m, 28H), 0.89 (t, *J* = 6.4 Hz, 6H); **^{13}C NMR** (100MHz, CD_3OD): δ 104.6, 103.0, 81.4, 79.2, 77.7, 76.8, 75.1, 74.9 (2C), 74.2, 72.7, 71.5, 71.2, 62.8, 62.5, 62.3, 33.2, 30.9 (2C), 30.8, 30.6, 27.4, 23.9, 14.6; HRMS (EI): calcd. for $C_{46}H_{86}O_{24}Na^+$ $[M+Na]^+$ 1045.5407, found 1045.5411.

B-BTM-C10 was prepared in 92% yield according to the general procedure for deprotection reactions. **^{1}H NMR** (400MHz, CD_3OD): δ 5.15 (d, *J* =4 Hz, 2H), 4.46 (d, *J* = 8 Hz, 2H), 4.11 (br s, 2H), 3.91-3.81 (m, 9H), 3.68-3.24 (m, 27H), 1.61-1.54 (m, 4H), 1.38-1.23 (m, 30H), 0.90 (t, *J* = 6.8 Hz, 6H); **^{13}C NMR** (100MHz, CD_3OD): δ 104.6, 102.9, 81.3, 79.1, 77.7, 76.7, 75.1, 74.8 (2C), 74.1, 72.6, 71.4, 71.1, 62.7, 62.5, 33.2, 30.9, 30.8, 30.7, 30.6, 27.4, 23.8, 14.7; HRMS (EI): calcd. for $C_{48}H_{90}O_{24}Na^+$ $[M+Na]^+$ 1073.5720, found 1073.5718.

B-BTM-C11 was prepared in 95% yield according to the general procedure for deprotection reactions. **^{1}H NMR** (400MHz, CD_3OD): δ 5.16 (d, *J* =4 Hz, 2H), 4.46 (d, *J* = 8 Hz, 2H), 4.10 (br s, 2H), 3.90-3.79 (m, 8H), 3.68-3.22 (m, 26H), 1.59-1.54 (m, 4H), 1.38-1.23 (m, 32H), 0.90 (t, *J* = 8 Hz, 6H); **^{13}C NMR** (100MHz, CD_3OD): δ 104.6, 103.0, 81.5, 79.2, 77.8, 76.8, 75.2, 74.9 (2C), 74.2, 72.7, 71.5, 71.2, 62.8, 62.6, 33.2, 30.8, 30.7, 27.5, 23.9, 14.6; HRMS (EI): calcd. for $C_{50}H_{94}O_{24}Na^+$ $[M+Na]^+$ 1101.6033, found 1101.6035.

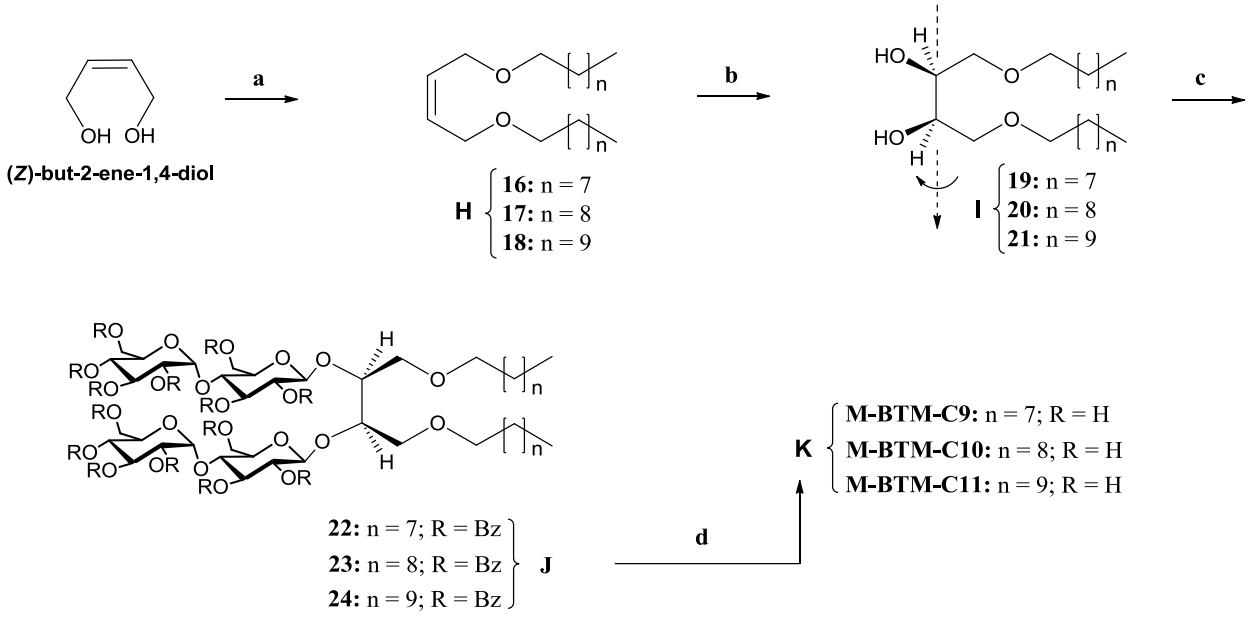
A-BTM-C9 was prepared in 92% yield according to the general procedure for deprotection reactions. **^{1}H NMR** (400MHz, CD_3OD): δ 5.13 (d, *J* =4 Hz, 2H), 4.53 (d, *J* = 8 Hz, 2H), 4.02 (br s, 2H), 3.86-3.59 (m,

21H), 3.50-3.25 (m, 14H), 1.58-1.55 (m, 4H), 1.30 (br s, 24H), 0.88 (t, *J* = 6.4 Hz, 6H); **^{13}C NMR** (100MHz, CD_3OD): δ 105.3, 103.1, 81.3, 79.9, 78.0, 76.8, 75.2, 75.1, 74.9, 74.3, 72.7, 71.6, 62.9, 62.3, 33.2, 30.9 (2C), 30.8, 30.6, 27.6, 23.9, 14.6; HRMS (EI): calcd. for $C_{46}H_{86}O_{24}Na^+$ $[M+Na]^+$ 1045.5407, found 1045.5410.

A-BTM-C10 was prepared in 91% yield according to the general procedure for deprotection reactions. **^{1}H NMR** (400MHz, CD_3OD): δ 5.08 (d, *J* =3.6 Hz, 2H), 4.47 (d, *J* = 8 Hz, 2H), 3.91 (br s, 2H), 3.76-3.34 (m, 29H), 3.20-3.15 (m, 4H), 1.49-1.45 (m, 4H), 1.20 (br s, 28H), 0.81 (t, *J* = 6.8 Hz, 6H); **^{13}C NMR** (100MHz, CD_3OD): δ 105.2, 103.0, 81.2, 80.0, 78.0, 77.9, 76.7, 75.2, 75.1, 74.8, 74.2, 72.6, 71.6, 71.5, 62.8, 62.3, 33.2, 30.9 (2C), 30.8 (2C), 30.6, 27.5, 27.4, 23.9, 14.6; HRMS (EI): calcd. for $C_{48}H_{90}O_{24}Na^+$ $[M+Na]^+$ 1073.5720, found 1073.5718.

A-BTM-C11 was prepared in 95% yield according to the general procedure for deprotection reactions. **^{1}H NMR** (400MHz, CD_3OD): δ 5.17 (app. s, 2H), 4.57 (d, *J* = 8 Hz, 2H), 4.01 (br s, 2H), 3.86-3.43 (m, 34H), 3.31-3.27 (m, 6H), 1.58-1.55 (m, 4H), 1.29 (br s, 34H), 0.90 (t, *J* = 8 Hz, 6H); **^{13}C NMR** (100MHz, CD_3OD): δ 105.2, 103.0, 81.2, 80.0, 77.9, 76.7, 75.1 (2C), 74.8, 74.2, 72.6, 71.6, 71.5, 62.8, 62.3, 33.2, 31.0, 30.9 (2C), 30.8 (2C), 30.6, 27.5, 23.9, 14.6; HRMS (EI): calcd. for $C_{50}H_{94}O_{24}Na^+$ $[M+Na]^+$ 1101.6033, found 1101.6036.

Supplementary scheme 2



(a) alkyl iodide, NaH, 78-84%; (b) OsO_4, NMO, THF, H_2O, 0°C → room temperature, 12 h, 89-91%; (c) Perbenzoylated malotsylbromide, AgOTf, DCM, -45°C → 0°C, 82-86%; (d) NaOMe, MeOH, room temperature, 14 hr, 90-95%.

Compound **16** was prepared in 82% yield according to the general procedure for dialkylation. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.71-5.70 (m, 2H), 4.04 (d, *J* = 4.8 Hz, 4H), 3.41 (t, *J* = 6.8 Hz, 4H), 1.61-1.54 (m,

4H), 1.39-1.27 (m, 24H), 0.88 (t, *J* = 6.8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 129.7, 70.8, 66.6, 32.1, 30.0, 29.8, 29.7, 29.5, 26.4, 22.9, 14.3.

Compound **17** was prepared in 78% yield according to the general procedure for dialkylation. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.72-5.69 (m, 2H), 4.03 (d, *J* = 4 Hz, 4H), 3.40 (t, *J* = 8 Hz, 4H), 1.60-1.53 (m, 4H), 1.39-1.25 (m, 28H), 0.87 (t, *J* = 8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 129.7, 70.8, 66.7, 32.1, 30.0, 29.8 (2C), 29.7, 29.5, 26.4, 22.9, 14.3.

Compound **18** was prepared in 84% yield according to the general procedure for dialkylation. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 5.72-5.70 (m, 2H), 4.04 (d, *J* = 4 Hz, 4H), 3.41 (t, *J* = 8 Hz, 4H), 1.57-1.53 (m, 4H), 1.39-1.26 (m, 34H), 0.88 (t, *J* = 8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 129.7, 70.8, 66.7, 32.1, 30.0, 29.7, 29.6, 26.4, 22.9, 14.3.

Compound **19** was prepared in 91% yield according to the general procedure for upjohn dihydroxylation. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.77 (br s, 2H), 3.62-3.55 (m, 4H), 3.47 (t, *J* = 8 Hz, 4H), 2.90 (d, *J* = 4 Hz, 2H), 1.61-1.52 (m, 4H), 1.48-1.22 (m, 24H), 0.88 (t, *J* = 8 Hz, 6H) ; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 72.2, 71.8, 71.2, 32.0, 29.7 (2C), 29.6, 29.4, 26.2, 22.8, 14.2.

Compound **20** was prepared in 90% yield according to the general procedure for upjohn dihydroxylation. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.78 (br s, 2H), 3.62-3.56 (m, 4H), 3.47 (t, *J* = 8 Hz, 4H), 2.86 (d, *J* = 4 Hz, 2H), 1.62-1.53 (m, 4H), 1.40-1.26 (m, 28H), 0.88 (t, *J* = 8 Hz, 6H) ; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 72.2, 72.0, 71.3, 32.1, 29.8(2C), 29.7, 29.5, 26.3, 22.9, 14.3.

Compound **21** was prepared in 89% yield according to the general procedure for upjohn dihydroxylation. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.78 (br s, 2H), 3.61-3.57 (m, 4H), 3.47 (t, *J* = 8 Hz, 4H), 2.89 (d, *J* = 1.6 Hz, 2H), 1.62-1.54 (m, 4H), 1.40-1.26 (m, 32H), 0.88 (t, *J* = 8 Hz, 6H) ; **^{13}C NMR** (100 MHz, $CDCl_3$): δ 72.2, 71.9, 71.3, 32.1, 29.8(2C), 29.7, 29.5, 26.3, 22.9, 14.3.

Compound **22** was prepared in 82% yield according to the general procedure for glycosylation reaction. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.11-7.67 (m, 27H), 7.62-7.19 (m, 42H), 6.97 (t, *J* = 8 Hz, 1H), 6.91 (t, *J* = 8 Hz, 1H), 6.19-6.09 (m, 2H), 5.91-5.67 (m, 4H), 5.32-5.21 (m, 4H), 5.00-4.70 (m, 5H), 4.55-4.32 (m, 5H), 4.30-4.19 (m, 4H), 4.03 (br s, 2H), 3.44-3.19 (m, 4H), 2.93 (app. t, *J* = 4 Hz, 1H), 2.46 (t, *J* = 6.8 Hz, 2H), 1.40-1.00 (m, 22H), 0.92-0.85 (m, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.0, 165.8, 165.7 (2C), 165.6, 165.3, 165.2, 165.1 (2C), 164.9, 133.7, 133.6, 133.5, 133.4, 133.3 (2C), 133.2 (2C), 133.0, 132.8, 130.4, 130.2, 130.0 (4C), 129.9 (2C), 129.8, 129.7, 129.6 (2C), 129.2 (2C), 129.1 (2C), 129.0, 128.9 (2C), 128.8, 128.7 (2C), 128.6, 128.5 (2C), 128.4, 128.3, 128.2 (2C), 128.1, 100.7, 99.4, 96.6, 95.8, 78.8, 75.6, 74.8, 73.6, 72.8 (2C), 72.7, 72.4, 71.6, 71.4, 71.1, 70.6, 70.3, 70.2, 70.1, 69.2, 69.1, 63.7, 63.6, 62.7, 32.1, 29.8, 29.7 (2C), 29.6, 29.5 (2C), 29.4, 26.1, 25.9, 22.9 (2C), 14.3 (2C).

Compound **23** was prepared in 84% yield according to the general procedure for glycosylation reaction. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.15-7.62 (m, 27H), 7.60-7.12 (m, 41H), 6.08 (t, *J* = 8 Hz, 1H), 6.90 (t, *J* = 8 Hz, 1H), 6.19-6.12 (m, 2H), 5.45-5.62 (m, 4H), 5.36-5.20 (m, 4H), 5.02-4.71 (m, 5H), 4.52-4.35 (m,

5H), 4.32-4.19 (m, 4H), 4.05 (br s, 2H), 3.49-3.25 (m, 4H), 2.95 (app. t, *J* = 4 Hz, 1H), 2.47 (t, *J* = 4 Hz, 2H), 1.43-1.08 (m, 26H), 0.96-0.86 (m, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.2, 165.8, 165.7 (2C), 165.6, 165.5, 165.3, 165.1 (2C), 164.9, 133.8, 133.6, 133.5, 133.4, 133.3 (2C), 133.2 (2C), 133.1, 132.7, 130.4, 130.2, 130.0 (3C), 129.9 (3C), 129.8, 129.7, 129.6 (2C), 129.2 (3C), 129.1, 129.0, 128.9 (2C), 128.8, 128.7 (2C), 128.6, 128.5 (2C), 128.4, 128.3, 128.2 (2C), 128.1, 100.7, 99.4, 96.6, 95.8, 78.8, 75.6, 74.8, 73.6, 72.8 (2C), 72.7, 72.4, 71.5, 71.4, 71.1, 70.7, 70.3, 70.2, 70.0, 69.2, 69.0, 63.7, 63.6, 62.6, 32.1 (2C), 29.7 (2C), 29.6, 29.5, 29.4, 26.1, 25.9, 22.9, 22.8, 14.3 (2C).

Compound **24** was prepared in 86% yield according to the general procedure for glycosylation reaction. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.12-7.74 (m, 27H), 7.65-7.12 (m, 41H), 6.92 (t, *J* = 8 Hz, 1H), 6.91 (t, *J* = 8 Hz, 1H), 6.15-6.11 (m, 2H), 5.92-5.60 (m, 6H), 5.39-5.19 (m, 4H), 5.12-4.79 (m, 5H), 4.58-4.35 (m, 5H), 4.33-4.15 (m, 4H), 4.03 (br s, 2H), 3.48-3.19 (m, 4H), 2.94 (app. t, *J* = 4 Hz, 1H), 2.46 (t, *J* = 6.4 Hz, 2H), 1.45-1.10 (m, 32H), 0.97-0.85 (m, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.3, 165.8 (2C), 165.7 (2C), 165.6, 165.5, 165.2, 165.1 (2C), 164.9, 133.8, 133.6, 133.5, 133.4 (2C), 133.3 (2C), 133.2, 133.0, 132.7, 130.4, 130.1, 130.0 (4C), 129.9 (2C), 129.8, 129.7 (2C), 129.6, 129.2 (2C), 129.1, 129.0, 128.9 (2C), 128.8 (2C), 128.7 (2C), 128.6, 128.5, 128.4 (2C), 128.3, 128.2 (2C), 128.1, 100.7, 99.5, 96.6, 95.8, 78.8, 75.6, 74.7, 73.6, 72.8 (2C), 72.7, 72.5, 71.5, 71.4, 71.2, 70.6, 70.3 (2C), 70.2, 70.1, 69.1 (2C), 63.7, 63.6, 62.6, 32.1 (2C), 29.8 (4C), 29.7, 29.6, 29.5, 29.4, 26.2, 25.9, 22.9 (2C), 14.3.

M-BTM-C9 was prepared in 94% yield according to the general procedure for deprotection reactions. **^{1}H NMR** (400MHz, CD_3OD): δ 5.14-5.13 (dd, *J* = 3.6, 1.2 Hz, 2H), 4.54 (d, *J* = 8.0 Hz, 1H), 4.48 (d, *J* = 8.0 Hz, 1H), 4.16-4.10 (m, 2H), 3.89-3.43 (m, 27H), 3.40-3.33 (m, 2H), 3.31-3.21 (m, 9H), 1.61-1.52 (m, 4H), 1.40-1.26 (m, 25H), 0.88 (t, *J* = 6.4 Hz, 6H); **^{13}C NMR** (100MHz, CD_3OD): δ 104.4, 104.2, 103.0, 81.3, 79.6, 79.5, 77.8, 77.7, 76.7, 75.1 (2C), 75.0, 74.9, 74.2, 72.7, 72.6, 71.7, 71.5, 71.1, 62.8, 62.4, 62.3, 33.2, 30.9 (3C), 30.8 (2C), 30.6, 27.5 (2C), 23.9, 14.7; HRMS (EI): calcd. for $C_{46}H_{86}O_{24}Na^+$ $[M+Na]^+$ 1045.5407, found 1045.5411.

M-BTM-C10 was prepared in 95% yield according to the general procedure for deprotection reactions. **^{1}H NMR** (400MHz, CD_3OD): δ 5.16 (br s, 2H), 4.56 (d, *J* = 8 Hz, 1H), 4.50 (d, *J* = 8 Hz, 1H), 4.14-4.11 (m, 2H), 3.89-3.46 (m, 30H), 3.38-3.37 (m, 2H), 3.30-3.25 (m, 6H), 1.62-1.53 (m, 4H), 1.40-1.24 (m, 30H), 0.90 (t, *J* = 8 Hz, 6H); **^{13}C NMR** (100MHz, CD_3OD): δ 104.4, 104.2, 103.1, 103.0, 81.4, 81.3, 79.6, 79.5, 77.8, 77.7, 76.7, 75.1 (2C), 75.0, 74.9, 74.2, 72.7, 72.6, 71.7, 71.5, 71.1, 62.8, 62.4, 62.3, 33.2, 31.0 (2C), 30.9 (2C), 30.8 (2C), 30.7, 30.6, 27.5 (2C), 23.9, 14.7; HRMS (EI): calcd. for $C_{48}H_{90}O_{24}Na^+$ $[M+Na]^+$ 1073.5720, found 1073.5723.

M-BTM-C11 was prepared in 94% yield according to the general procedure for deprotection reactions. **^{1}H NMR** (400MHz, CD_3OD): δ 5.16 (br s, 2H), 4.57 (d, *J* = 4 Hz, 1H), 4.50 (d, *J* = 8 Hz, 1H), 4.15-4.12 (m, 2H), 3.86-3.49 (m, 28H), 3.38-3.32 (m, 2H), 3.31-3.22 (m, 6H), 1.60-1.53 (m, 4H), 1.40-1.29 (m, 34H), 0.90 (t, *J* = 8 Hz, 6H); **^{13}C NMR** (100MHz, CD_3OD): δ 104.4, 104.2, 103.0 (2C), 81.4, 81.3, 79.6, 79.5, 77.8, 77.7 (2C), 76.7, 75.1, 75.0 (2C), 74.9, 74.2, 72.7, 72.6, 71.7, 71.5, 71.1, 62.8, 62.4, 62.3, 33.2,

31.0 (2C), 30.9 (3C), 30.8 (2C), 30.7, 27.5 (2C), 23.9, 14.7; HRMS (EI): calcd. for $C_{50}H_{94}O_{24}Na^+$ $[M+Na]^+$ 1101.6033, found 1101.6034.

References

[1] J. Leung, A. D. Cameron, G. Diallinas and B. Byrne, *Mol. Membr. Biol.*, 2013, **30**, 32–42.

[2] G. Deckert, P. V. Warren, T. Gaasterland, W. G. Young, A. L. Lenox, D. E. Graham, R. Overbeek, M. A. Snead, M. Keller, M. Aujay, R. Huber, R. A. Feldman, J. M. Short, G. J. Olsen and R. V. Swanson, *Nature*, 1998, **392**, 353–358.

[3] M. Quick and J. A. Javitch, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 3603-3608.

[4] D. M. Rosenbaum, V. Cherezov, M. A. Hanson, S. G. Rasmussen, F. S. Thian, T. S. Kobilka, H. J. Choi, X. J. Yao, W. I. Weis, R. C. Stevens and B. K. Bobilka, *Science*, 2007, **318**, 1266–1273.

[5] G. Swaminath, J. Steenhuis, B. Kobilka and T. W. Lee, *Mol. Pharmacol*., 2002, **61**, 65-72.

[6] A. S. Ethayathulla, M. S. Yousef, A. Amin, G. Leblanc, H. R. Kaback, L. Guan, *Nat. Commun.* 2014, **5**, 3009.

[7] P. S. Chae, et al., *Nat. Methods* 2010, **7**, 1003-1008.

[8] B. K. Sharpless, W. Amberg, Y. L. Bennani, G. A. Crispino, J. Hartung, K.-S. Jeong, H.-L. Kwong, K. Morikawa, Z.-M. Wang, D. Xu and X.-L. Zhang, *J. Org. Chem.*, 1992, **57**, 2768-2771.

[9] P. R. Ashton, S. E. Boyd, C. L. Brown, N. Jayaraman, S. A. Nepogodiev and J. F. Stoddart, *Chem.-Eur. J*., 1996, **2**, 1115-1128.