

Supporting Information

Maltose Neopentyl Glycol-3 (MNG-3) Analogues for Membrane Protein Study: Implication for Importance of Match/Mismatch between Amphiphile Hydrophobicity and Membrane Protein Propensity

**Kyung Ho Cho,^a Mohd Husri,^b Anowarul Amin,^c Kamil Gotfryd,^d Ho Jin Lee,^a Juyeon Go,^a Claus J.
Loland,^d Lan Guan,^c Bernadette Byrne^b and Pil Seok Chae^{*a}**

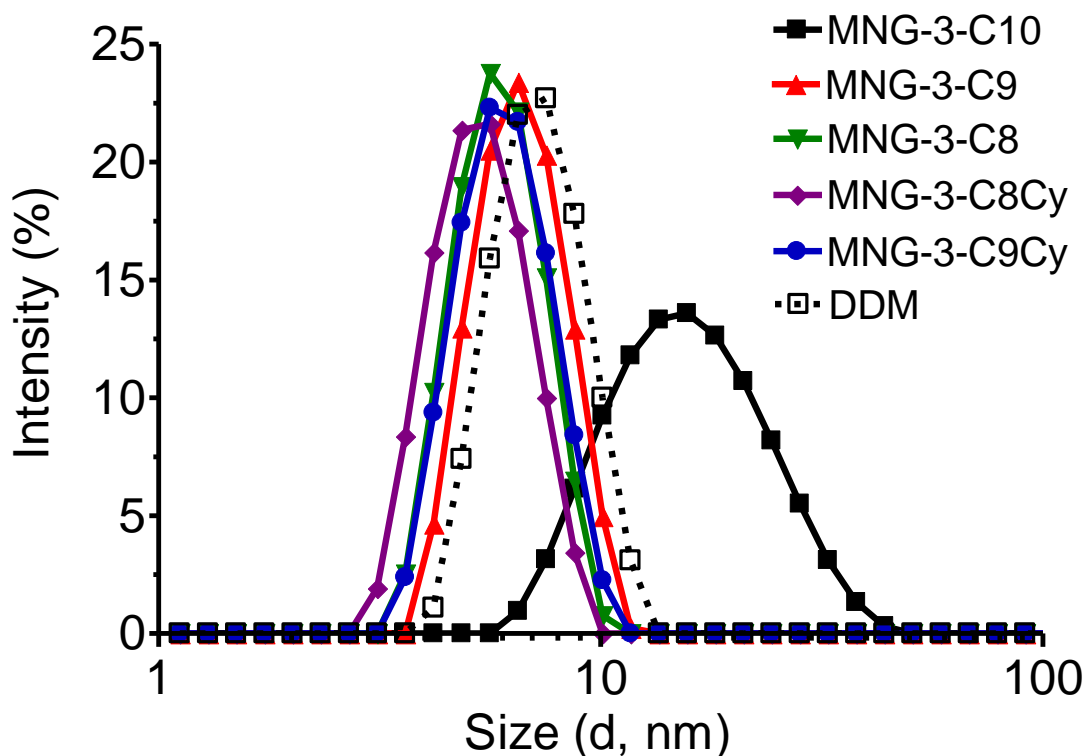


Figure S1. A collective diagram presenting the size distribution (nm) of micelles formed by MNG-3-C10, its alkyl variants (MNG-3-C9, MNG-3-C8, MNG-3-C8Cy and MNG-3-C9Cy) and a conventional detergent (DDM). This diagram was obtained using dynamic light scattering (DLS) where analysis of scattered light intensity fluctuation containing information about the time scale of micelle movements was performed. All detergents were tested at 0.5 wt%, and showed a single micelle distribution, with MNG-3-C10 having the largest micelle formation.

Protein stability evaluation

LeuT stability assay

Purification of the wild type of the leucine transporter (LeuT) from *Aquifex aeolicus* was performed according to the protocol described previously.^[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, isolated bacterial membranes were solubilized in 1 % DDM and the protein was bound to nickel-charged affinity resin (Life Technologies, Denmark) followed by elution in 20 mM Tris-HCl (pH 8.0), 1 mM NaCl, 199 mM KCl, 0.05 % DDM and 300 mM imidazole. Subsequently, approx. 1.7 mg/ml protein stock was diluted 10x in identical buffer without imidazole and DDM,

but supplemented with MNG-3-C10, MNG-3-C9, MNG-3-C8, MNG-3-C8Cy and MNG-3-C9Cy at the final concentrations of CMC+0.04 wt% or CMC+0.2 wt%, respectively (DDM was used as control detergent at the same final concentrations). Following storage at RT, protein samples were centrifuged at the indicated time points and the concentration was determined by measuring absorbance at 280 nm. In addition, for the corresponding time points, protein activity was assessed by measuring [³H]-Leu binding using scintillation proximity assay (SPA).^[2] SPA was performed with 5 μ L of the respective protein samples, 20 nM [³H]-Leu and copper chelate (His-Tag) YSi beads (both from PerkinElmer, Denmark) in the buffer containing 200 mM NaCl and the respective test detergents at the concentrations indicated above. [³H]-Leu binding was monitored using MicroBeta liquid scintillation counter (PerkinElmer).

***R. capsulatus* superassembly stability assay**

The stability of the *R. capsulatus* superassembly was assessed according to the published protocol.^[3] Briefly, we used specialized membranes obtained from an engineered strain of *Rhodobacter (R.) capsulatus*, U43[pUHTM86Bgl], with no LHI light-harvesting complex for protein extraction. First, a frozen aliquot of *R. capsulatus* membranes was thawed and homogenized using a glass homogenizer. After a 30 min incubation at 32°C, LHI-RC superassembly was treated with 1.0 wt% DDM and further incubated for an additional 30 min. Following ultracentrifugation at 315,000 *g* at 4°C for 20 min, the DDM-solubilized supernatant was transferred into a new tube containing Ni-NTA resin (pre-equilibrated and stored in buffer containing 10 mM Tris, pH 7.8, and 100 mM NaCl). After 1 h incubation at 4°C, the resin was washed twice with 0.5 mL of binding buffer (a pH 7.8 Tris buffer solution containing 1xCMC DDM). The protein was eluted using 3 x 0.20 mL Buffer B containing 1 M imidazole (pH = 7.8). The DDM-purified protein fractions were combined for the next step. For detergent efficacy evaluation, a small volume (0.05 mL) of the DDM-purified LHI-RC was transferred into 0.95 mL individual MNG solutions at concentrations CMC+0.04 wt% or CMC+0.2 wt% and the resulting solutions were incubated at room temperature for 20 days. UV-Vis spectra of these solutions were taken at regular intervals. Stability was assessed by the absorbance value at 875 nm. Reduction in the absorbance value over time indicates degradation of the complex.

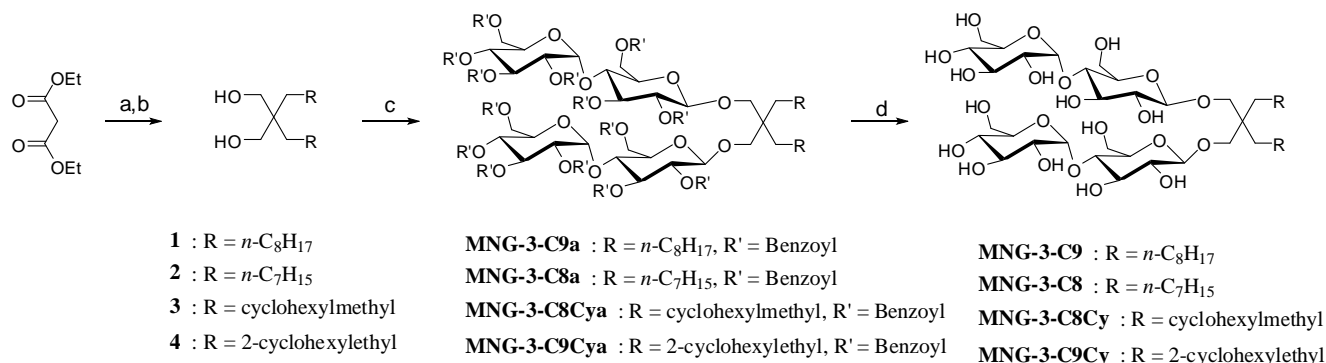
MelB stability assay

Our reported protocol was used to evaluate MelB_{St} stability with DDM and MNGs.^[4] The plasmid pK95 Δ AHB/WT MelB_{St}/CH10 encoding the wild-type MelB with a 10-His tag at the C-terminus and the *E. coli* DW2-R cells ($\Delta melB$ and $\Delta lacZY$) were used for the assay. Cells growth and membrane preparation were carried out as described.^[5] Protein assay was carried out with a BCA kit (Thermo Scientific, Rockford, IL). For the measurement of solubilization efficiency, membrane samples containing MelB_{St} (final protein concentration was 10 mg/mL) were incubated with a solubilization buffer (20 mM Tris, pH 7.5, 200 mM NaCl, 10% glycerol,

20 mM melibiose) and 1.5 wt % DDM or MNGs at 0°C for 10 min. For the assessment of the protein thermostability, the samples were incubated for 90 min at the four different temperatures (0, 45, 55, and 65 °C). After ultracentrifugation at 355,590 g in a Beckman Optima™ MAX Ultracentrifuge using a TLA-100 rotor for 45 min at 4°C, 20 µg proteins before and after spin for each condition were separated by SDS-16% PAGE, followed by immunoblotted with a Penta-His-HRP antibody (Qiagen, Germantown, MD). MelB_{St} was detected using SupperSignal West Pico chemiluminescent substrate by the ImageQuant LAS 4000 Biomolecular Imager (GE Health Care Lifer Science)

Amphiphile Synthesis

Supplementary scheme



(a) NaH, alkyl iodide (RI), THF, room temperature; (b) LiAlH₄, THF, room temperature; (c) perbenzoylated maltosylbromide, AgOTf, 2,4,6-Collidine, CH₂Cl₂, -45°C → room temperature; (d) NaOMe, MeOH, room temperature

General procedure for dialkylation and reductions (step a & b)

This reaction was synthesized according to a literature method^[6] with slight modification. To a solution of diethyl malonate (1.04 mL, 6.9 mmol) in THF (40 mL) was added dropwise a solution of NaH (0.82g, 21 mmol) in THF at 0 °C and left stirring for 20 min. After addition of iodoalkane (2.6 equiv.), the reaction mixture was stirred at room temperature for 48 h, quenched by adding ice-cold saturated NH₄Cl (100 mL) and then extracted with diethyl ether (2 x 50 mL). The organic layer was washed with brine and dried with anhydrous Na₂SO₄. After complete evaporation of solvent, LiAlH₄ (0.52 g, 14.0 mmol) was added slowly to the residue dissolved in THF (50mL) at 0°C. The mixture was stirred at room temperature for 4 h, quenched with MeOH, water, a 1 N aqueous HCl solution successively at 0°C and then extracted with diethyl ether (2 x 50 mL). The combined organic layer was washed with brine and dried with anhydrous Na₂SO₄. The residue was purified by silica gel column chromatography (EtOAc/hexane) providing alkyl-containing diol as a white solid.

General procedure for glycosylation reactions (step c)^[7]

This reaction was performed according to a literature method¹ with slight modification. A mixture of alcohol derivative, AgOTf (2.4 equiv.), 2,4,6-collidine (1.8 equiv.) in anhydrous CH₂Cl₂ (40 mL) was stirred at -45°C. A solution of perbenzoylated maltosylbromide (2.4 equiv.) in CH₂Cl₂ (40 mL) was added dropwise over 0.5 h to this suspension. Stirring was continued for 0.5 h at -45°C, and then the reaction mixture was allowed to warm to 0 °C and left stirring for 1.5 h. After completion of reaction (as detected by TLC), pyridine was added to the reaction mixture, and it was diluted with CH₂Cl₂ (40 mL) before being filtered over celite. The filtrate was washed successively with a 1 M aqueous Na₂S₂O₃ solution (40 mL), a 0.1 M aqueous HCl solution (40 mL),

and brine (2 x 40 mL). Then the organic layer was dried with anhydrous Na₂SO₄ and the solvents were removed by rotary evaporation. The residue was purified by silica gel column chromatography (EtOAc/hexane) providing desired product as a glassy solid.

General Procedure for the de-*O*-benzoylations under Zemplén's condition (step d)^[7]

The *O*-benzoylated compounds were dissolved in MeOH and then 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 left stirring for 6 h at room temperature, and then 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 silica gel column chromatography (MeOH/CH₂Cl₂). Further purification carried out by recrystallization using CH₂Cl₂/MeOH/diethyl ether afforded fully de-*O*-benzoylated product as a white solid.

2,2-dinonyl-propane-1,3-diol (1) was synthesized according to the general procedure for dialkylation and reductions. Yield: 90% (two steps). ¹H NMR (300 MHz, CDCl₃): δ 3.54 (s, 4H), 2.93 (s, 2H), 1.38-1.08 (m, 32H), 0.88 (t, *J* = 6.8 Hz, 6H); ¹³C NMR (75 MHz, CDCl₃): δ 69.7, 41.2, 32.1, 31.0, 30.8, 29.8, 29.5, 23.1, 22.9, 14.3; **HRMS (ESI)**: calcd. for C₂₁H₄₄O₂Na [M+Na]⁺ 351.3224, found 351.3210.

2,2-dioctyl-propane-1,3-diol (2) was synthesized according to the protocol for the preparation of compound **1** by using 1-iodooctane instead of 1-iodononane. Yield: 91% (two steps); ¹H NMR (300 MHz, CDCl₃): δ 3.57 (s, 4H), 2.28 (s, 2H), 1.38-1.08 (m, 28H), 0.88 (t, *J* = 6.8 Hz, 6H); ¹³C NMR (75 MHz, CDCl₃): δ 69.5, 41.2, 32.1, 30.9, 30.8, 29.8, 29.5, 23.1, 22.9, 14.3; **HRMS (ESI)**: calcd. for C₁₉H₄₀O₂Na [M+Na]⁺ 323.2921, found 323.2913.

2,2-bis(2-cyclohexylethyl)-propane-1,3-diol (3) was synthesized according to the protocol for the preparation of compound **1** by using 2-iodoethylcyclohexane instead of iodononane. Yield: 85% (two steps); ¹H NMR (300 MHz, CDCl₃): δ 3.55 (d, *J* = 5.2 Hz, 4H), 2.12 (s, 2H), 1.78-1.52 (m, 10H), 1.30-0.98 (m, 16H), 0.98-0.77 (m, 4H); ¹³C NMR (75 MHz, CDCl₃): δ 69.6, 40.9, 38.7, 33.7, 30.5, 27.9, 26.9, 26.6; **HRMS (ESI)**: calcd. for C₁₉H₃₆O₂Na [M+Na]⁺ 319.2608, found 319.2600.

2,2-bis(3-cyclohexylpropyl)-propane-1,3-diol (4) was synthesized according to the protocol for the preparation of compound **1** by using 3-iodopropylcyclohexane instead of iodononane. Yield: 87% (two steps); ¹H NMR (300 MHz, CDCl₃): δ 3.56 (d, *J* = 5.2 Hz, 4H), 2.43 (s, 2H), 1.78-1.53 (m, 10H), 1.30-1.03 (m, 20H),

0.95-0.78 (m, 4H); ^{13}C NMR (75 MHz, CDCl_3): δ 69.7, 41.3, 38.6, 37.7, 33.7, 31.3, 26.9, 26.6, 20.2; **HRMS (ESI)**: calcd. for $\text{C}_{21}\text{H}_{40}\text{O}_2\text{Na}$ $[\text{M}+\text{Na}]^+$ 347.2921, found 347.2917.

MNG-3-C9a was synthesized according to the general procedure for glycosylation. Yield: 93%; ^1H NMR (300 MHz, CDCl_3): δ 8.09-8.02 (m, 4H), 8.02-7.95 (m, 8H), 7.95-7.89 (m, 4H), 7.89-7.83 (m, 4H), 7.83-7.76 (m, 4H), 7.74-7.67 (m, 4H), 7.67-7.46 (m, 14H), 7.46-7.34 (m, 18H), 7.33-7.17 (m, 12H), 6.13 (t, $J = 10.0$ Hz, 2H), 5.70-5.61 (m, 4H), 5.35 (t, $J = 9.5$ Hz, 2H), 5.21-5.07 (m, 4H), 4.70-4.51 (m, 4H), 4.40-4.29 (m, 4H), 4.29-4.17 (m, 4H), 3.40-3.27 (m, 4H), 2.97-2.86 (m, 2H), 2.71 (d, $J = 8.8$ Hz, 2H), 1.37-0.91 (m, 32H), 0.86 (t, $J = 6.9$ Hz, 6H); ^{13}C NMR (75 MHz, CDCl_3): δ 166.3, 166.0, 165.7, 165.2, 165.0, 133.9, 133.7, 133.6, 133.4, 133.3, 130.2, 130.1, 130.0, 129.9, 129.8, 129.7, 129.6, 129.3, 129.2, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 100.9, 95.9, 74.8, 72.5, 72.3, 72.1, 71.5, 71.3, 70.0, 69.2, 63.4, 62.7, 40.4, 32.1, 30.6, 30.2, 29.8, 29.7, 29.5, 22.9, 22.3, 14.3; **MS (MALDI-TOF)**: calcd. for $\text{C}_{143}\text{H}_{140}\text{O}_{36}\text{Na}$ $[\text{M}+\text{Na}]^+$ 2456.6, found 2456.4.

MNG-3-C8a was synthesized according to the general procedure for glycosylation. Yield: 92%; ^1H NMR (300 MHz, CDCl_3): δ 8.09-8.03 (m, 4H), 8.03-7.95 (m, 8H), 7.95-7.89 (m, 4H), 7.89-7.83 (m, 4H), 7.83-7.76 (m, 4H), 7.75-7.67 (m, 4H), 7.67-7.46 (m, 14H), 7.46-7.33 (m, 18H), 7.33-7.16 (m, 12H), 6.13 (t, $J = 10.0$ Hz, 2H), 5.71-5.60 (m, 4H), 5.35 (t, $J = 9.6$ Hz, 2H), 5.21-5.07 (m, 4H), 4.70-4.51 (m, 4H), 4.42-4.30 (m, 4H), 4.30-4.17 (m, 4H), 3.40-3.28 (m, 4H), 3.00-2.87 (m, 2H), 2.72 (d, $J = 8.7$ Hz, 2H), 1.36-0.91 (m, 28H), 0.86 (t, $J = 6.9$ Hz, 6H); ^{13}C NMR (75 MHz, CDCl_3): δ 166.3, 166.0, 165.7, 165.2, 165.0, 133.9, 133.7, 133.6, 133.3, 130.2, 130.1, 130.0, 129.8, 129.7, 129.6, 129.3, 129.2, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 100.9, 95.9, 74.8, 72.5, 72.3, 72.1, 71.5, 71.3, 70.0, 69.2, 63.4, 62.8, 40.4, 32.1, 30.5, 30.2, 29.6, 29.5, 22.9, 22.3, 14.3; **MS (MALDI-TOF)**: calcd. for $\text{C}_{141}\text{H}_{136}\text{O}_{36}\text{Na}$ $[\text{M}+\text{Na}]^+$ 2428.6, found 2428.3.

MNG-3-C8Cya was synthesized according to the general procedure for glycosylation. Yield: 92%; ^1H NMR (300 MHz, CDCl_3): δ 8.16-8.08 (m, 4H), 8.02-7.94 (m, 4H), 7.92-7.82 (m, 8H), 7.78-7.71 (m, 4H), 7.71-7.64 (m, 4H), 7.62-7.38 (m, 24H), 7.38-7.15 (m, 28H), 6.20-6.02 (m, 4H), 5.89-5.59 (m, 6H), 5.34-5.19 (m, 2H), 5.18-5.05 (m, 4H), 5.05-4.83 (m, 3H), 4.82-4.68 (m, 2H), 4.68-4.57 (m, 2H), 4.55-4.35 (m, 8H), 4.32-4.19 (m, 2H), 4.17-4.06 (m, 1H), 3.30-3.15 (m, 2H), 1.76-1.47 (m, 6H), 1.39-1.02 (m, 12H), 0.98-0.67 (m, 12H), ^{13}C NMR (75 MHz, CDCl_3): δ 166.3, 166.0, 165.7, 165.3, 165.2, 165.0, 133.9, 133.7, 133.6, 133.4, 133.3, 130.3, 130.1, 130.0, 129.9, 129.8, 129.7, 129.6, 129.4, 129.2, 129.0, 128.9, 128.8, 128.6, 128.5, 95.9, 74.5, 72.3, 72.2, 71.5, 69.2, 62.8, 40.4, 32.1, 30.6, 30.3, 29.9, 29.8, 29.7, 29.6, 22.9, 22.3, 14.3; **MS (MALDI-TOF)**: calcd. for $\text{C}_{141}\text{H}_{132}\text{O}_{36}\text{Na}$ $[\text{M}+\text{Na}]^+$ 2423.8, found 2424.5.

MNG-3-C9Cya was synthesized according to the general procedure for glycosylation. Yield: 90%; $^1\text{H NMR}$ (300 MHz, CDCl_3): δ 8.11-8.03 (m, 4H), 8.03-7.96 (m, 8H), 7.96-7.90 (m, 4H), 7.90-7.83 (m, 4H), 7.83-7.77 (m, 4H), 7.77-7.69 (m, 4H), 7.67-7.47 (m, 14H), 7.47-7.33 (m, 18H), 7.33-7.14 (m, 12H), 6.14 (t, $J = 10.0$ Hz, 2H), 5.76-5.57 (m, 4H), 5.35 (t, $J = 9.6$ Hz, 2H), 5.24-5.06 (m, 4H), 4.71-4.52 (m, 4H), 4.41-4.17 (m, 8H), 3.42-3.24 (m, 4H), 3.02-2.86 (m, 2H), 2.72 (d, $J = 8.7$ Hz, 2H), 1.69-1.45 (m, 10H), 1.21-0.58 (m, 24H); $^{13}\text{C NMR}$ (75 MHz, CDCl_3): δ 166.3, 166.1, 166.0, 165.7, 165.3, 165.0, 133.9, 133.8, 133.7, 133.6, 133.4, 133.3, 130.2, 130.1, 129.9, 129.8, 129.7, 129.4, 129.2, 129.1, 128.9, 128.8, 128.5, 100.9, 95.9, 74.9, 72.5, 72.3, 72.2, 71.5, 71.2, 70.0, 69.2, 63.5, 62.8, 40.6, 38.2, 37.5, 33.5, 30.2, 27.0, 26.6, 19.3; **MS (MALDI-TOF)**: calcd. for $\text{C}_{143}\text{H}_{136}\text{O}_{36}\text{Na}$ $[\text{M}+\text{Na}]^+$ 2451.9, found 2452.1.

MNG-3-C9 was synthesized according to the general procedure for de-*O*-benzoylation. Yield: 95%; $^1\text{H NMR}$ (300 MHz, CD_3OD): δ 5.18 (d, $J = 3.8$ Hz, 2H), 4.39 (d, $J = 7.9$ Hz, 2H), 3.99-3.79 (m, 6H), 3.79-3.60 (m, 10H), 3.60-3.37 (m, 8H), 3.37-3.21 (m, 6H), 1.48-1.13 (br s, 32H), 0.93 (t, $J = 6.8$ Hz, 6H); $^{13}\text{C NMR}$ (75 MHz, CD_3OD): δ 105.1, 103.1, 81.6, 78.1, 76.7, 75.3, 75.0, 74.4, 73.3, 71.7, 62.9, 62.4, 42.3, 33.3, 31.8, 31.7, 30.9, 30.8, 30.6, 23.9, 23.6, 14.6; **HRMS (ESI)**: calcd. for $\text{C}_{45}\text{H}_{84}\text{O}_{22}\text{Na}$ $[\text{M}+\text{Na}]^+$ 999.5347, found 999.5352.

MNG-3-C8 was synthesized according to the general procedure for de-*O*-benzoylation. Yield: 93%; $^1\text{H NMR}$ (300 MHz, CD_3OD): δ 5.19 (d, $J = 3.8$ Hz, 2H), 4.39 (d, $J = 7.9$ Hz, 2H), 3.99-3.79 (m, 6H), 3.79-3.60 (m, 10H), 3.60-3.37 (m, 8H), 3.37-3.21 (m, 4H), 1.48-1.13 (br s, 28H), 0.94 (t, $J = 6.8$ Hz, 6H); $^{13}\text{C NMR}$ (75 MHz, CD_3OD): δ 105.1, 103.1, 81.6, 78.1, 76.6, 75.2, 75.0, 74.9, 74.3, 73.3, 71.7, 62.9, 62.4, 42.3, 33.2, 31.8, 30.8, 30.6, 23.9, 23.7, 14.6; **HRMS (ESI)**: calcd. for $\text{C}_{43}\text{H}_{80}\text{O}_{22}\text{Na}$ $[\text{M}+\text{Na}]^+$ 971.5034, found 971.5010.

MNG-3-C8Cy was synthesized according to the general procedure for de-*O*-benzoylation. Yield: 90%; $^1\text{H NMR}$ (300 MHz, CD_3OD): δ 5.15 (d, $J = 3.8$ Hz, 2H), 4.35 (d, $J = 7.9$ Hz, 2H), 3.99-3.79 (m, 6H), 3.79-3.60 (m, 10H), 3.60-3.37 (m, 8H), 3.32-3.22 (m, 4H), 1.85-1.58 (m, 10H), 1.43-1.03 (m, 16H), 1.03-0.80 (m, 4H); $^{13}\text{C NMR}$ (75 MHz, CD_3OD): δ 105.0, 103.0, 81.5, 78.1, 76.6, 75.2, 74.9, 74.3, 71.6, 62.9, 62.4, 42.0, 39.8, 34.9, 34.7, 31.2, 28.7, 28.0, 27.7; **HRMS (ESI)**: calcd. for $\text{C}_{43}\text{H}_{76}\text{O}_{22}\text{Na}$ $[\text{M}+\text{Na}]^+$ 967.4721, found 967.4695.

MNG-3-C9Cy was synthesized according to the general procedure for de-*O*-benzoylation. Yield: 93%; $^1\text{H NMR}$ (300 MHz, CD_3OD): δ 5.15 (d, $J = 3.8$ Hz, 2H), 4.35 (d, $J = 7.9$ Hz, 2H), 3.99-3.79 (m, 6H), 3.79-3.60 (m, 10H), 3.60-3.37 (m, 8H), 3.32-3.22 (m, 4H), 1.80-1.58 (m, 10H), 1.42-1.04 (m, 20H), 0.95-0.80 (m, 4H); $^{13}\text{C NMR}$ (75 MHz, CD_3OD): δ 105.1, 103.1, 81.6, 78.1, 76.7, 75.3, 75.0, 74.9, 74.3, 73.3, 71.7, 62.9, 62.4, 42.3, 39.6, 39.0, 34.8, 32.1, 28.0, 27.7; **HRMS (ESI)**: calcd. for $\text{C}_{45}\text{H}_{80}\text{O}_{22}\text{Na}$ $[\text{M}+\text{Na}]^+$ 995.5034, found 995.5043.

References

- [1] P. S. Chae, A. C. Kruse, K. Gotfryd, R. R. Rana, K. H. Cho, S. G. F. Rasmussen, H. E. Bae, R. Chandra, U. Gether, L. Guan, B. K. Kobilka, C. J. Loland, B. Byrne, S. H. Gellman, *Chem. Eur. J.* **2013**, *19*, 15645-15651.
- [2] M. Quick, J. A. Javitch, *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 3603-3608.
- [3] P. D. Laible, C. Kirmaier, C. S. Udawatte, S. J. Hofman, D. Holten, D. K. Hanson, *Biochemistry* **2003**, *42*, 1718-1730.
- [4] P. S. Chae, S. G. F. Rasmussen, R. R. Rana, K. Gotfryd, R. Chandra, M. A. Goren, A. C. Kruse, S. Nurva, C. J. Loland, Y. Pierre, D. Drew, J.-L. Popot, D. Picot, B. G. Fox, L. Guan, U. Gether, B. Byrne, B. Kobilka, S. H. Gellman, *Nat. Methods* **2010**, *7*, 1003-1008.
- [5] A. S. Ethayathulla, M. S. Yousef, A. Amin, G. Leblanc, H. R. Kaback, L. Guan *Nat. Commun.* **2014**;5:3009. doi: 10.1038/ncomms4009.
- [6] Y. S. Kim, R. R. Kane, C. L. Beno, S. Ropmano, G. Mendez, M. F. Hawthorne, M.F. *Tetrahedron Lett.* **1995**, *36*, 5147-5150.
- [7] P. R. Ashton, S. E. Boyd, C. L. Brown, N. Jayaraman, S. A. Nepogodiev, J. F. Stoddart, *Chem.-Eur. J.* **1996**, *2*, 1115-1128.