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Supporting Information

A New Class of Amphiphiles Bearing Rigid Hydrophobic Groups for Solubilization and Stabilization of Membrane Proteins

Pil Seok Chae,*[a] Søren G. F. Rasmussen,[c] Rohini R. Rana,[d] Kamil Gotfryd,[e] Andrew C. Kruse,[c] Aashish Manglik,[c] Kyung Ho Cho,[a] Shailika Nurva,[f] Ulrik Gether,[e] Lan Guan,*[f] Claus J. Loland,*[e] Bernadette Byrne,*[d] Brian K. Kobilka,*[c] and Samuel H. Gellman*[b]

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Supporting Information

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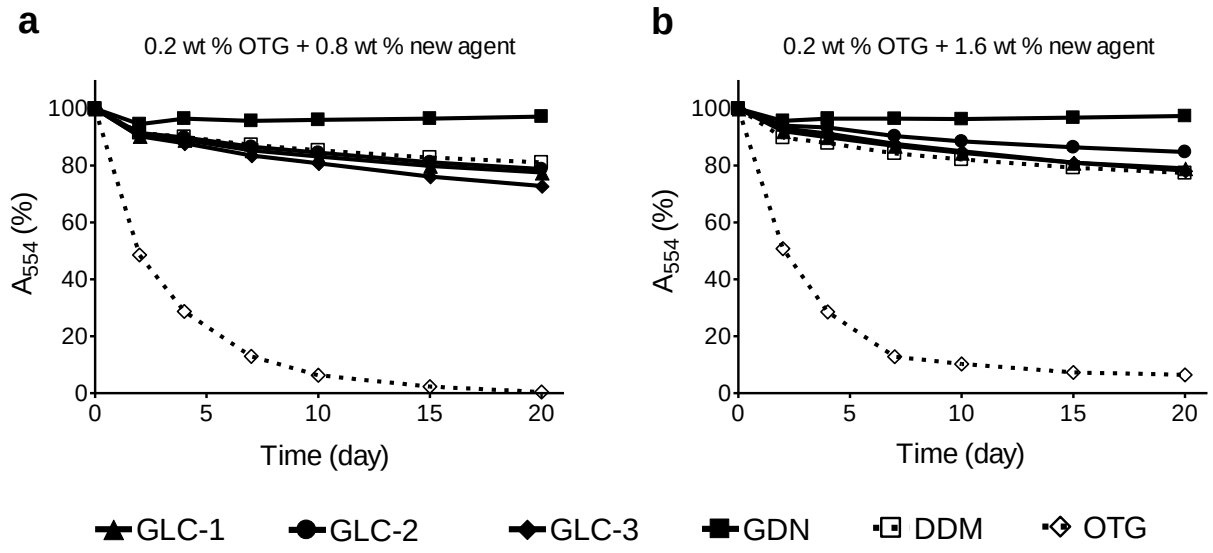


Figure S1. Time course of bR stability at RT. OTG was mixed with each agent in a ratio of (a) 1:4 (0.2 wt % OTG + 0.8 wt % GLCs/GDN) or (b) 1:8 (0.2 wt % OTG + 1.6 wt % GLCs/GDN). Absorbance at 554 nm was used as an indicator of native bR stability. See main text for additional information.

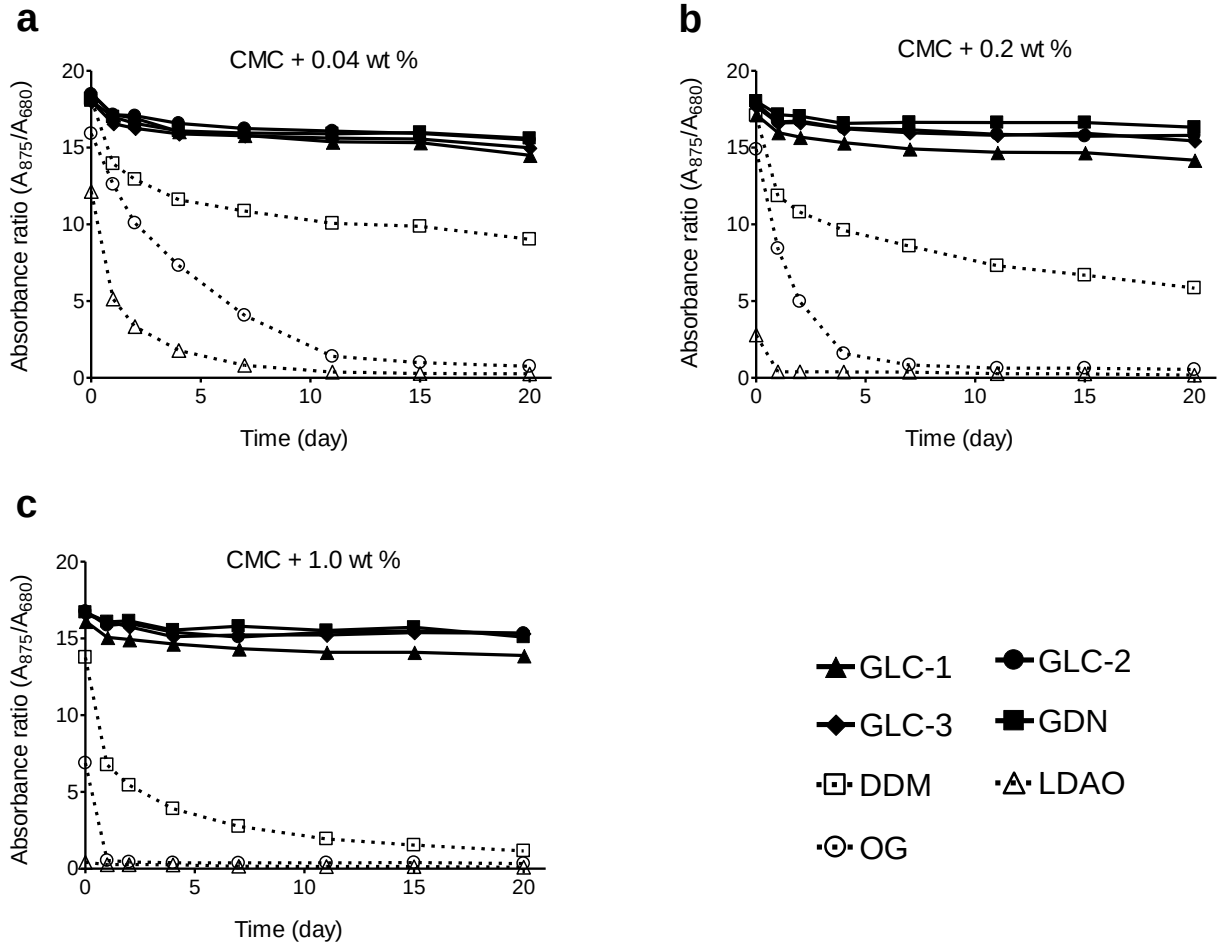


Figure S2. Time course of *R. capuslatus* LHI-RC superassembly stability at RT. The superassembly was purified with each GLC amphiphile (GLC-1, GLC-2 or GLC-3) or with GDN at three different concentrations: (a) CMC + 0.04 wt %, (b) CMC + 0.2 wt % and (c) CMC + 1.0 wt %. The absorbance ratio A_{875}/A_{680} was used as an indicator of superassembly integrity. See main text and ref. 5i for further information.

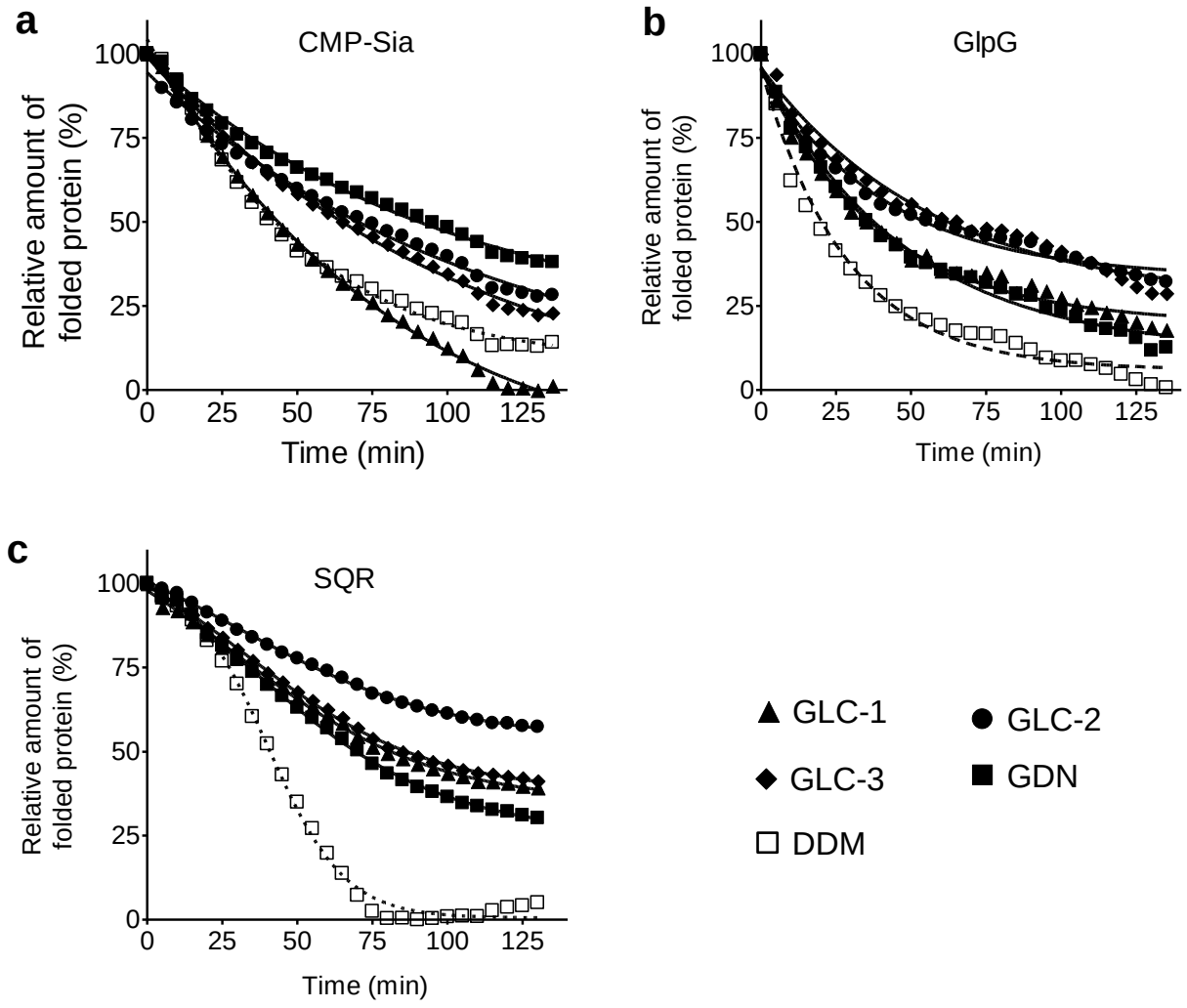


Figure S3. CPM assays for (a) CMP-Sia, (b) GlpG and (c) SQR solubilized with new amphiphiles or DDM. The coumarin moiety of CPM is internally quenched by the maleimide unit, but the coumarin becomes fluorescent following reaction with Cys side chain thiol groups exposed upon protein unfolding. The CPM assay can therefore be used to monitor the extent of protein unfolding. CMP-Sia and GlpG were initially extracted from the native membrane with 1% DDM in PBS, 10 mM imidazole (pH 8.0), 150 mM NaCl, 10% glycerol, and isolated in 20 mM Tris (pH 7.5), 150 mM NaCl containing 0.03% DDM. SQR was extracted from the membrane using 2% $C_{12}E_9$ in 20 mM potassium phosphate (pH 7.4), 0.2 M EDTA, and isolated in 20 mM Tris (pH 7.6), 0.2% decyl-β-D-maltoside (DM). The purified proteins (CMP-Sia (6 mg/ml), GlpG (5 mg/ml) and SQR (12 mg/ml)) were diluted 1:150 in 20 mM Tris (pH 7.5), 150 mM NaCl containing CMC + 0.04 wt % amphiphile or DDM. The CPM analysis was performed over 130 min at 30°C using a microplate spectrofluorometer set at an excitation wavelength of 387 nm and an emission wavelength of 463 nm. Measurements were taken every 5 min after automatic agitation of the plate. The vertical axes in these graphs have no absolute meaning. The "Relative amount of folded protein" in each case is defined as follows: 100% corresponds to the fluorescence emission intensity at time = 0 min; 0% corresponds to the lowest value measured among

the amphiphile-treated samples for each protein during the 130 min assay period. Thus, for CMP-Sia, 0% is defined by the end-point measurement for protein solubilized with GLC-1. For GlpG and SQR, 0% is defined by the lowest value measured for protein solubilized with DDM. In no case can the "0%" value be interpreted as indicating that the protein is fully unfolded. This point is demonstrated by the gel filtration results shown for CMP-Sia in the main text (Figure 3), which indicate that ~50% of the protein solubilized with DDM remains intact at the end of the incubation period; however, in Figure S3a, DDM-solubilized CMP-Sia is indicated to contain ~20% "relative amount of folded protein" under the conditions used for the gel filtration analysis.

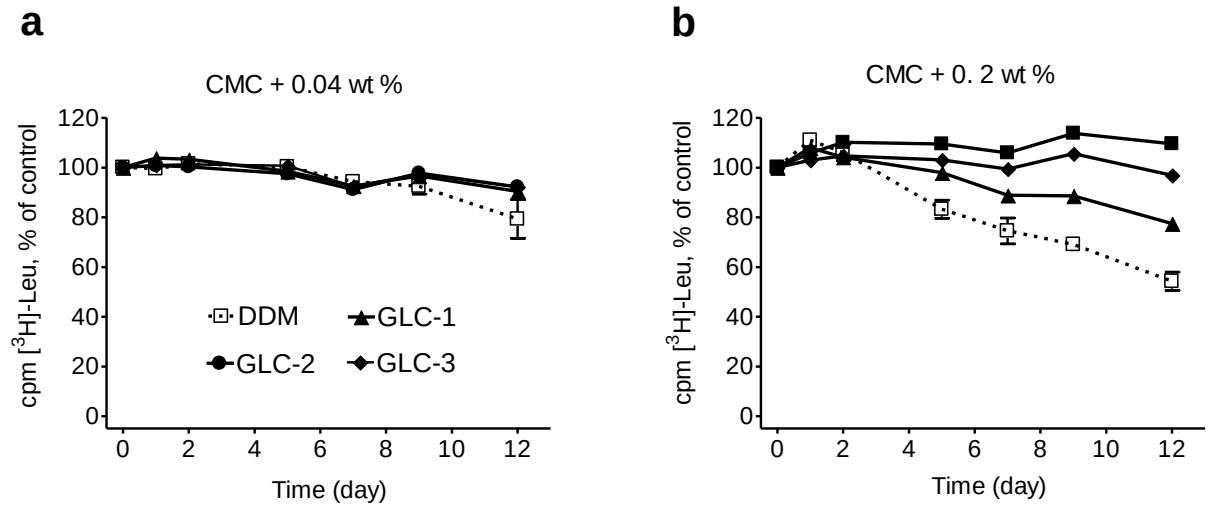


Figure S4. Time course activity (scintillation proximity assay (SPA), based on [^{3}H]-Leu binding) of LeuT at RT. The assay was conducted for protein solubilized with GLC amphiphiles (GLC-1, GLC-2 or GLC-3) or DDM at (a) CMC + 0.04 wt % and (b) CMC + 0.2 wt %. Results are expressed as % activity relative to the day 0 measurements (mean ± s.e.m., $n = 2$).

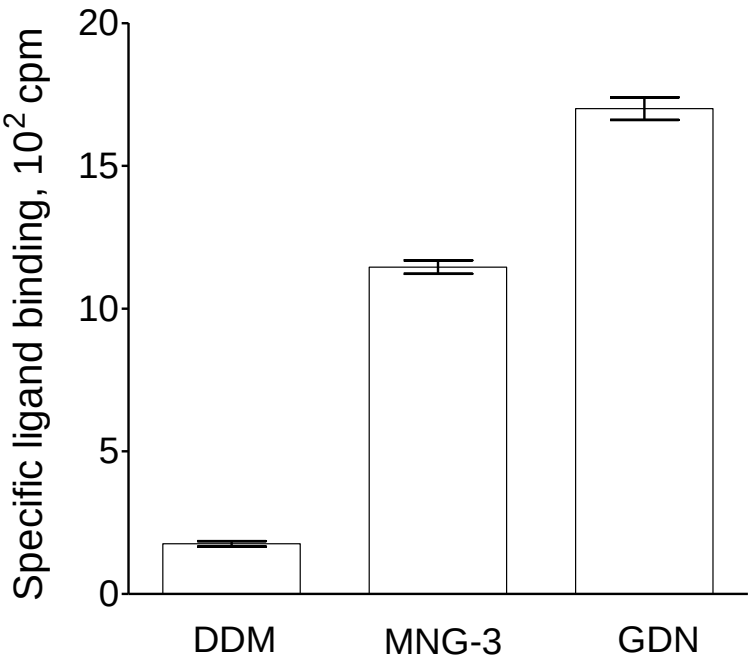


Figure S5. Activity of δ-opioid receptor-T4L (δOR-T4L) solubilized with DDM, MNG-3 or GDN. The receptor was extracted with 1.0 wt % of amphiphile, and activity was measured by radioligand-binding assay using the antagonist [^{3}H]-diprenorphine.

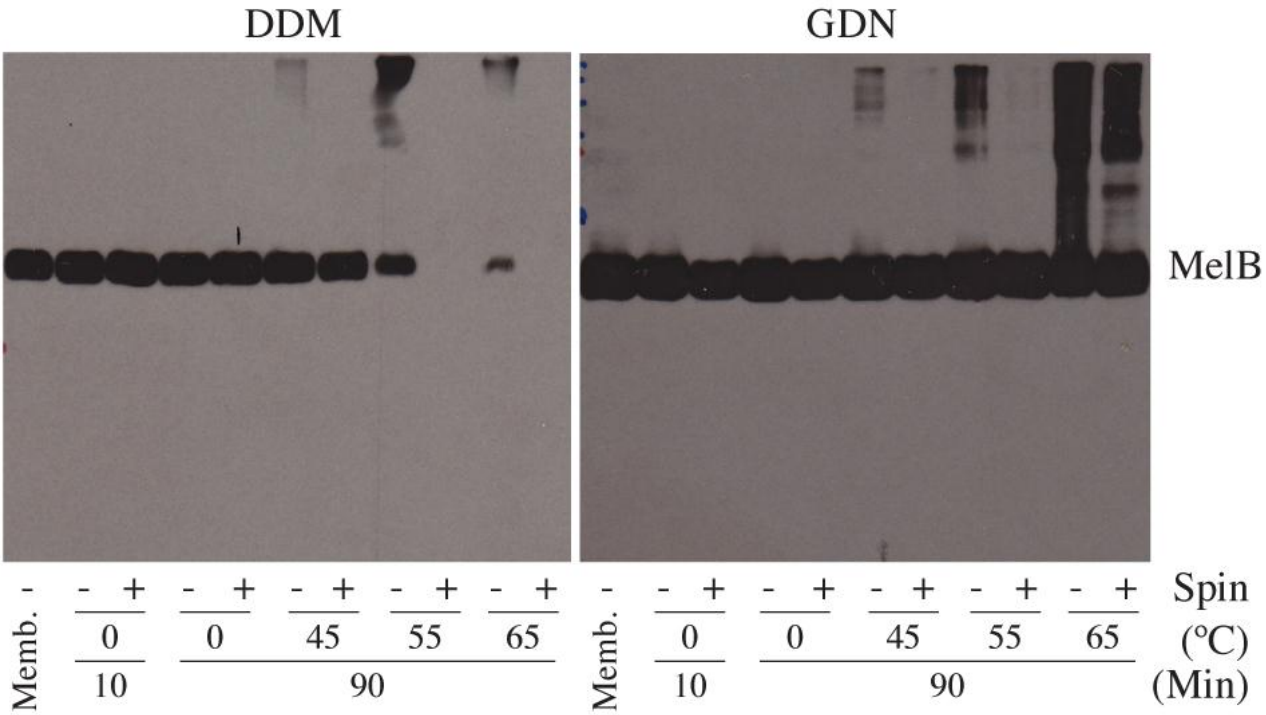


Figure S6. SDS-12% PAGE and Western blot analysis of MelB of *E. coli*. MelB protein was expressed in *E. coli* and extracted with DDM or GDN. The samples were then separated by SDS-PAGE, and MelB was detected by Western blotting using anti-histidine tag antibody. Each sample included 10 μg protein. For solubilization extracts obtained under each set of conditions, one sample was subjected to ultracentrifugation (+) and a comparison sample was not (−). As a control, an untreated membrane sample (no ultracentrifugation) was included in each gel.

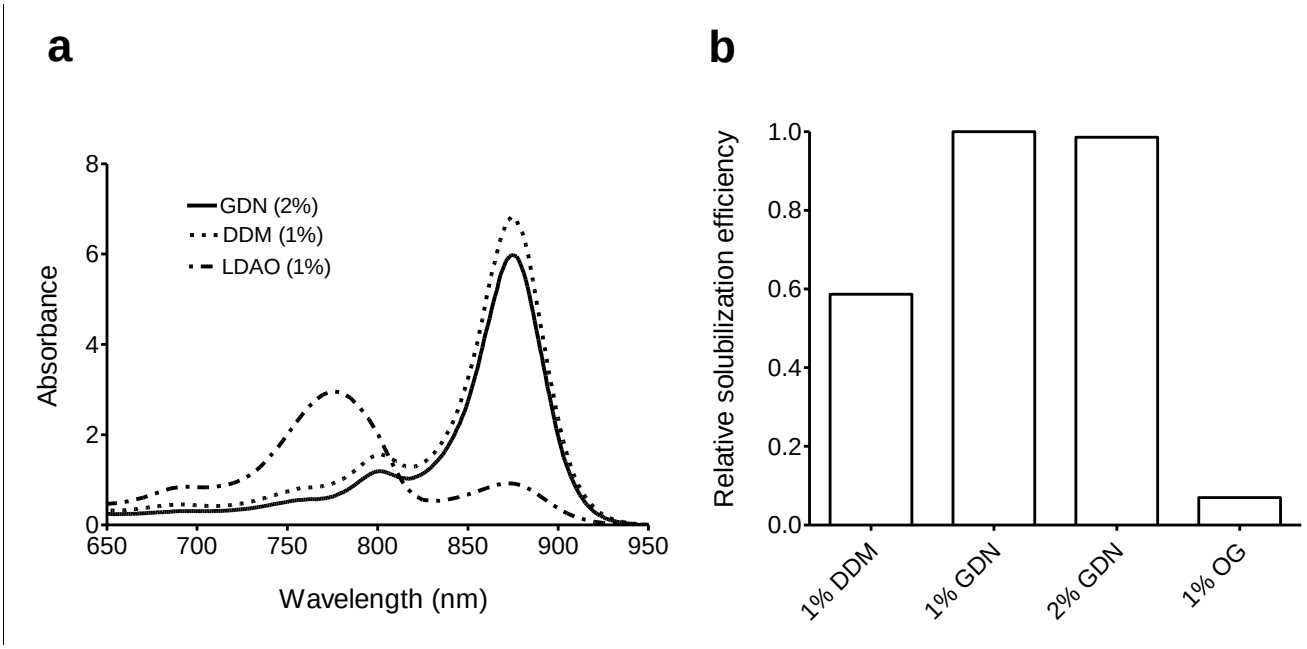


Figure S7. Characterization of (a) LHI-RC superassembly and (b) β_2AR WT extracted with GDN or conventional detergents (DDM, laurydimethylamine-*N*-oxide (LDAO), or *n*-octyl-β-D-glucopyranoside (OG)). The superassembly integrity was assessed via spectrophotometry (see main text), and β_2AR WT was detected by Western blotting using M1 antibody.

Protein stability evaluation

Bacteriorhodopsin stability

The procedure for the bR stability assay followed the reported protocol.[1] Frozen aliquots of purple membranes containing bR at 184 μM were thawed at room temperature and solubilzed by using a octylthioglucoside (OTG) solution for 24 hr at 4℃ in a dark room. For this purpose, OTG (CMC = 0.28 wt %) was used at 2.0 wt % in 10 mM sodium phosphate (pH 6.9). Membrane debris were then removed from the solubilized material via ultracentrifugation at 200,000 *g* at 4℃ for 20 min. The supernatant, including bR protein, was transferred into individual DDM, GLC, or GDN solutions, giving final concentration of OTG : new amphiphiles = 0.2 wt % : 0.8 wt % (1:4) or 0.2 wt % : 1.6 wt % (1:8). The stability of bR in each solution was monitored by measuring absorbance at 554 nm over 20 days.

Solubilization and stability assay for *R. capsulatus* superassembly

The solubilization and stability of the *R. capsulatus* superassembly were assessed according to the published protocol.[2] Briefly, we used specialized photosynthetic membranes obtained from an engineered strain of *Rhodobacter (R.) capsulatus*, U43[pUHTM86Bgl], lacking the LHII light-harvesting complex. We began solubilization of the LHI-RC superassembly by thawing, homogenizing, and incubating frozen aliquots of *R. capsulatus* membranes at 32˚C for 30 min. Subsequent 30-min incubation was performed after adding DDM or LDAO at 1.0 wt % or GDN at 2.0 wt % in the solid form. The solution was then subjected to ultracentrifugation at 315,000 *g* at 4℃ for 30 min to remove membrane debris. To assess solubilization efficiency, UV-Vis spectra of the solubilized protein solutions were measured in a range of 650 ~ 950 nm.

For the stability assay, DDM-solubilized material was transferred into a new microcentrifuge tube containing Ni-NTA resin (Qiagen, Inc.; Valencia, CA; pre-equilibriated and stored in an equal volume of buffer containing 10 mM Tris, pH 7.8, and 100 mM NaCl). After a 1 h incubation at 4℃ for binding, the resins were washed twice with 0.5 mL of binding buffer (a pH 7.8 Tris solution containing DDM at 1xCMC) and eluted three times with 0.20 mL elution buffer aliquots containing 1 M imidazole (otherwise, this buffer was identical to binding buffer; the pH of each solution was readjusted to pH = 7.8). The DDM-purified solutions were collected and diluted with 0.4 mL of the binding buffer. Then small aliquots (0.05 mL) of the DDM-purified protein solutions were mixed with 0.95 mL GLC or GDN solutions at concentrations CMC+0.04 wt %, CMC+ 0.2 wt % or CMC + 1.0 wt %. UV-Vis spectra of these solutions were monitored at room temperature over 20 days. Protein degradation was assessed by measuring the 875 nm/680 nm absorbance ratio.

Membrane Solubilization and Protein Purification (CMP-Sia, GlpG and SQR)

CMP-Sia and GlpG were expressed as fusion proteins with a C-terminal GFP-His tag in in *Saccharomyces cerevisiae* and *Escherichia coli* respectively. All steps were carried out at 4 °C. Membranes containing CMP-Sia and GlpG were resuspended in PBS, 10 mM imidazole pH 8.0, 150 mM NaCl, 10% glycerol and solubilised in 1% DDM for 1 hr with mild agitation. Supernatant containing DDM-solubilised protein was harvested after

ultracentrifugation at 100,000 *g* for 45 min. The GFP-His fusions, CMP-Sia and GlpG were individually bound to Ni^{2+}-NTA resin (1 ml per 1 mg of GFP fusion) pre-equilibrated with Buffer A (PBS, 10 mM Imidazole pH 8.0, 150 mM NaCl, 10% glycerol, 0.03% DDM) using stirred mixing for 2-3 hr. The resin was washed with 10 CV of Buffer A, then 35 CV of Buffer A supplemented with 30 mM imidazole, followed by elution using 2-3 CV of Buffer A supplemented with 250 mM Imidazole. Equal amounts of His-tagged TEV protease was added to the GFP-His fusions in the eluate, and the samples dialysed overnight against Buffer B (20 mM Tris (pH 7.5), 150 mM NaCl, 0.03% DDM). Cleaved CMP-Sia and GlpG were isolated in the flowthrough fractions using reverse Ni^{2+}-NTA binding. Samples were concentrated to a 0.5 ml volume using centrifugal concentrators, and submitted to a final polishing gel filtration step using a Superdex 200 10/300 column pre-equilibrated with Buffer B. CMP-Sia and GlpG were concentrated to 6 mg/ml and 5mg/ml respectively, using molecular weight cut-off filters.

SQR was expressed in *E. coli* as an untagged construct. Membranes (~400 mg) containing SQR were resuspended in 20 mM potassium phosphate (pH 7.4), 0.2 M EDTA and solubilised in 2% $C_{12}E_9$ for 15 min. Supernatant containing detergent-solubilised protein was harvested following ultracentrifugation at 100,000*g* for 45 min, and filtered through a 0.2 μm filter. SQR was bound to pre-equilibrated Q-sepharose Fast Flow resin in an XK26/20 column (~ 24 ml). The column was washed with 2 CV of Buffer C (20 mM potassium phosphate (pH 7.4), 0.2 M EDTA, 0.05% $C_{12}E_9$), 2 CV of Buffer C supplemented with 100 mM NaCl, followed by elution using a (100-350) mM NaCl gradient. Fractions containing SQR were concentrated using an Amicon stirred cell concentrator, and filtered. The SQR was then applied onto a Phoros 50 HQ resin using an XK16/20 column (~20 ml) pre-equilibrated with Buffer C, followed by a Sephacryl 300 26/60 pre-equilibrated with buffer D (20 mM potassium phosphate (pH 7.4), 0.05% $C_{12}E_9$). The final buffer exchange was performed on a Superdex 200 10/300 gel filtration column pre-equilibrated with 20 mM Tris (pH 7.6), 0.2% decyl-β-D-maltoside (DM). SQR was concentrated to 12 mg/ml using molecular weight cut-off filters.

Samples for CPM Assay and Gel Filtration Analysis

CPM dye (Invitrogen), stored in DMSO (Sigma), was diluted (1:100) in Buffer B supplemented with 5 mM EDTA. Test amphiphiles or DDM were used at CMC+0.04 wt% concentrations in 20 mM Tris (pH 7.5), 150 mM NaCl. 1 μl of the purified protein; CMP-Sia (6 mg/ml), GlpG (5 mg/ml) and SQR (12 mg/ml) was individually added to test buffers (150 μl) in Greiner 96-well plates, and left for equilibration at RT for 5 min, before adding 3 μl diluted CPM dye. For gel filtration analysis, 10 μl of purified CMP-Sia (6 mg/ml) was diluted in 1000 μl test buffer. Test buffer (20 mM Tris (pH 7.5), 150 mM NaCl) included DDM or GDN at 0.042 wt % (corresponds to CMC + 0.033 wt % for DDM and CMC + 0.040 wt % for GDN). 500 μl aliquots of the diluted protein were applied onto a Superdex 200 30/100 gel filtration column, before and after incubation at 30 °C for 2 hr. The column was pre-equilibrated with the respective test buffer prior to sample loading.

LeuT functional assay

LeuT activity was measured according to the reported procedure.[2] The wild type of the leucine transporter (LeuT) from *Aquifex aeolicus* was expressed in *E. coli* C41(DE3) harboring pET16b encoding LeuT WT-His_8, essentially as described (2). Plasmid was kindly provided by E. Gouaux (Vollum Institute, Portland, Oregon, USA). Briefly, after isolation of bacterial membranes followed by solubilization in 1 % DDM, the LeuT was purified by nickel affinity chromatography in 20 mM Tris-HCl (pH 8.0), 1 mM NaCl, 199 mM KCl, 0.05 % DDM. Subsequently, approx. 1.5 mg/ml protein stock was diluted ten-fold in same buffer without DDM, but containing GDN, GLC-1, GLC-2 or GLC-3 in final concentrations of CMC + 0.04 wt % or CMC + 0.2 wt %, respectively. In control samples, DDM was used at the above-mentioned final concentrations. Following protein storage at RT, at the indicated time points, samples were centrifuged and the protein concentration was assessed by determining absorbance at 280 nm. Concomitantly, for the corresponding time points, [^{3}H]-Leu binding was measured using scintillation proximity assay (SPA) (2). Briefly, SPA reaction mixture consisted of 5 µL from the respective protein samples, 20 nM [^{3}H]-Leu and copper chelate (His-Tag) YSi beads (both from PerkinElmer, USA). Binding was assessed in 200 mM NaCl in the presence of tested compounds at the above-mentioned concentrations, and monitored using MicroBeta liquid scintillation counter (PerkinElmer).

β_2AR-T4L thermostability

A receptor fusion protein of T4 lysozyme inserted in the 3rd intracellular loop of the β_2AR[4] was cloned into BestBac baculovirus (Expression Systems, CA) and expressed in Sf9 insect cell cultures. The receptor was solubilized and purified in DDM as previously described[5]. Briefly, the receptor was purified in a three step procedure, M1 FLAG antibody affinity chromatography followed by alprenolol-Sepharose chromatography ending in a second M1 chromatography step. The fluorescent inverse agonist carazolol was bound to the receptor on the second M1 resin following extensive washing in buffer (0.1% DDM, 100 mM NaCl, 20 mM HEPES, pH 7.5) containing 30 µM carazolol. The eluted and carazolol-bound receptor was dialyzed against buffer containing 1 µM carazolol to reduce free carazolol concentration. The receptor was spin concentrated to 7 mg/ml (≈ 140 µM). For stability measurements the carazolol-bound receptor was diluted below the CMC for DDM by adding 3 µL of the concentrated receptor in a quartz cuvette containing 600 µL buffer (100 mM NaCl, 20 mM HEPES, pH 7.5) with amphiphiles at various concentrations above their CMC. The cuvette was placed in a Spex FluoroMax-3 spectrofluorometer (Jobin Yvon Inc.) under *Peltier* temperature control. Fluorescence emission from carazolol was obtained following 5 min incubations from 25 to 85°C in twelve continuous 5°C increments. Excitation was set at 325 nm, and emission was measured from 335 to 400 nm with an integration time of 0.3 s nm^{-1} using a bandpass of 1 nm for both excitation and emission. The 341:356 nm peak ratio was calculated and graphed using Microsoft Excel and GraphPad Prism software.

Solubilization and stability assay of β_2AR WT

A gene encoding amino-terminally FLAG epitope tagged β_2AR was expressed in Sf9 cells by baculovirus, with no ligand present during culture. Cells were infected at a density of 4 X 10^6 cells/mL and then cultured for 48 hours prior to harvesting by centrifugation. Cells were resuspended and lysed by osmotic shock with a low ionic strength buffer (20 mM Tris pH 7.5, 1 mM EDTA). The lysed cells were aliquoted 35 mg per aliquot, then

frozen. For extraction tests, 300 μL of solubilization buffer (20 mM HEPES pH 7.5, 100 mM NaCl) containing each amphiphile was added to each aliquot, which was then homogenized by pipet followed by grinding with a glass dounce tissue homogenizer. After a two-hour incubation at 4 ºC, samples were centrifuged at maximum speed in a tabletop microcentrifuge to pellet insoluble material. Supernatant was removed and assayed for protein concentration by D_C protein assay (Bio-Rad).

The amount of functional receptor was quantified by incubation for 1 hour with 10 nM ^{3}H dihydroalprenolol. Samples were then separated by gel filtration over G-50 resin and radioactivity was quantified by liquid scintillation. Nonspecific binding was measured in the presence of 10 μM alprenolol. Assays were performed in triplicate at time points indicated. G-50 filtration was performed in buffer containing 20 mM HEPES pH 7.5, 100 mM NaCl, 10-fold CMC of the detergent tested. All binding assays were performed with ice cold buffers.

Solubilization of δ-opioid receptor-T4L fusion (δOR-T4L)

FLAG epitope tagged δOR-T4L was expressed in Sf9 insect cells using baculovirus particles generated by the pFastBac vector system (Invitrogen). Insect cells were infected and cultured as for the β_2AR and cells were lysed by osmotic shock as done for cells expressing β_2AR. Lysed cells were used for extraction tests by adding 40 mg of cells to 200 μL of solubilization buffer (20 mM HEPES pH 7.5, 100 mM NaCl) containing each amphiphile. Cell membranes were homogenized in solubilization buffer by 20 passes through a narrow bore needle coupled to a 1 ml syringe. Solubilization reactions were then incubated at 4 ºC for two hours and then centrifuged at maximum speed in a tabletop microcentrifuge. The amount of functional receptor after solubilization was quantified by incubation for 1 hour with 10 nM ^{3}H diprenorphine. Binding assays using gel-filtration were carried out as for β_2AR, with the exception that 10 μM naloxone was used to determine nonspecific binding.

Solubilization and thermostability assay of MelB

The reported protocol[3] was used to evaluate MelB stability with DDM and GDN. Vector pK35ΔAHB/WT MelB/CH6 encoding the wild-type MelB with a 6-His tag at the C-terminus and *E. coli* DW2-R cells (Δ*melB* and Δ*lacZY*) are used for the assay. Cells were harvested, resuspended in a buffer containing 20 mM Tris, pH 7.5, 200 mM NaCl and 10% glycerol. The harvested cells were subjected to French press and centrifugation at 20,000 g for 15 min. Subsequently, membranes were obtained via ultracentrifugation at 43,000 rpm for 3 hr in the Beckman rotor, Type *45 Ti rotor*. A protein assay was carried out with a BCA kit (Thermo Scientific, Rockford, IL). For the measurement of solubilization efficiency, membrane samples containing MelB were incubated with a solubilization buffer (20 mM Tris, 200 mM NaCl, 10% glycerol, 20 mM melibiose, pH, 7.5) and 1.5 wt % DDM or GDN at 0ºC for 10 min. The final protein concentration was 10 mg/mL. For the MelB thermostability, the samples were incubated for 90 min at the four different temperatures (0, 45, 55, and 65ºC). After ultracentrifugation at 355,590 g in a Beckman Optima™ MAX Ultracentrifuge using a TLA-100 rotor for 45 min at 4ºC, 10 μg protein before and after spin for each condition was analyzed by SDS-12% PAGE and immunoblotted with Penta-His-HRP antibody (Qiagen, Germantown, MD).

CMP-Sia solubilization

CMP-Sia was expressed as a fusion protein with a C-terminal GFP in FGY217 *Saccharomyces cerevisiae* cells. Cell lysis was conducted by using a cell disruptor (Constant Systems) and the protein samples were subjected to centrifugation at 15,000 *g* for 10 mins to remove unbroken cells and debris. Subsequently, the membranes were harvested by ultracentrifugation at 150,000 *g* for 45 min. The membranes were resuspended in 50 mM Tris–HCl (pH 7.6), 1 mM EDTA, 0.6 M sorbitol and the protein concentration was estimated using a BCA kit (Pierce). The membranes were incubated with OG or DDM at 1.0%, or GDN at 2.0% for 1 hr at 4°C. A fluorescence value was measured for each sample before and after ultracentrifugation at 150,000 *g* for 1 h. The solubilization efficiency was calculated via the fluorescence measurements of the soluble supernatant/the total sample.

Amphiphile Synthesis

Synthesis of perbenzoylated maltosylbromide

This compound was prepared by following the reported protocolfor perbenzoylated lactosylbromide[6] with a slight modification as follows. To a solution of maltose monohydrate (30g, 0.083 mol) in pyridine (300 mL) was added slowly benzoyl chloride (106 mL, 0.92 mol) and a catalytic amount (~0.2 g) of dimethylaminopyridine (DMAP) at 0 °C. The resulting solution was allowed to warm up to RT and stirred for 20 hr at the same temperature. The solution was taken up with EtOAC (300 mL) and was washed with an iced aqueous 2N HCl solution until the aqueous phase became acidic. The neutralized organic layer was washed with brine (2 x 200 mL). The collected organic layer was dried over anhydrous Na_2SO_4 and removed by rotary evaporation to give crude syrup. This crude syrup was used for the next reaction without further purification. The crude was dissolved in dried CH_2Cl_2 (100 mL) and to the solution was added 33 wt % HBr–acetic acid (100 mL) at 0 °C under N_2 condition. The mixture was stirred at 0 °C for 4 hr. The solution was washed with iced water and saturated $NaHCO_3$ solution until the aqueous layer became slightly basic. The neutralized organic solution was washed with brine, dried over anhydrous Na_2SO_4 and removed by rotary evaporation to make crude syrup. The syrup was dissolved in ether (~ 500 mL) and stored at RT until white precipitates was formed. The white precipitates were collected on the glass filter and washed with ether three times. The filtered solid was dried *in vacuo* to afford perbenzoylated maltosylbromide as a white solid (80 g, 80% in two steps). This product was used for the next reaction without further purification. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.13-8.06 (m, 2H), 8.02-7.96 (m, 2H), 7.91-7.84 (m, 4H), 7.77-7.64 (m, 4H), 7.69-7.63 (m, 2H), 7.63-7.15 (m, 21H), 6.76 (d, *J* = 3.7 Hz, 2H), 6.16 (t, *J* = 9.4 Hz, 2H), 6.10 (t, *J* = 9.2 Hz, 1H), 5.79 (d, *J* = 4.0 Hz, 1H), 5.68 (t, *J* = 9.6 Hz, 1H), 5.28 (dd, *J* = 10.8, 4.0 Hz, 1H), 5.09 (dd, *J* = 10.0, 3.8 Hz, 1H), 4.96-4.87 (m, 1H), 4.84-4.75 (m, 1H), 4.72-4.62 (m, 3H), 4.59-4.39 (m, 1H).

General procedure for glycosylation reactions[7]

This reaction was performed according to a reported method with slight modifications. A mixture of the alcohol to be glycosylated, AgOTf (2.4 equiv.), and 2,4,6-collidine (1.8 equiv.) in anhydrous CH_2Cl_2 (40 mL) was stirred at -45°C. A solution of perbenzoylated maltosylbromide (2.4 equiv.) in CH_2Cl_2 (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 stirred for 1.5 h. After completion of the reaction (as detected by TLC), pyridine was added to the reaction mixture, which was then diluted with CH_2Cl_2 (40 mL) and filtered through celite. The filtrate was washed successively with 1 M aqueous $Na_2S_2O_3$ solution (40 mL), 0.1 M aqueous HCl solution (40 mL), and brine (2 x 40 mL). Then the organic layer was dried with anhydrous Na_2SO_4, and the solvent was removed by rotary

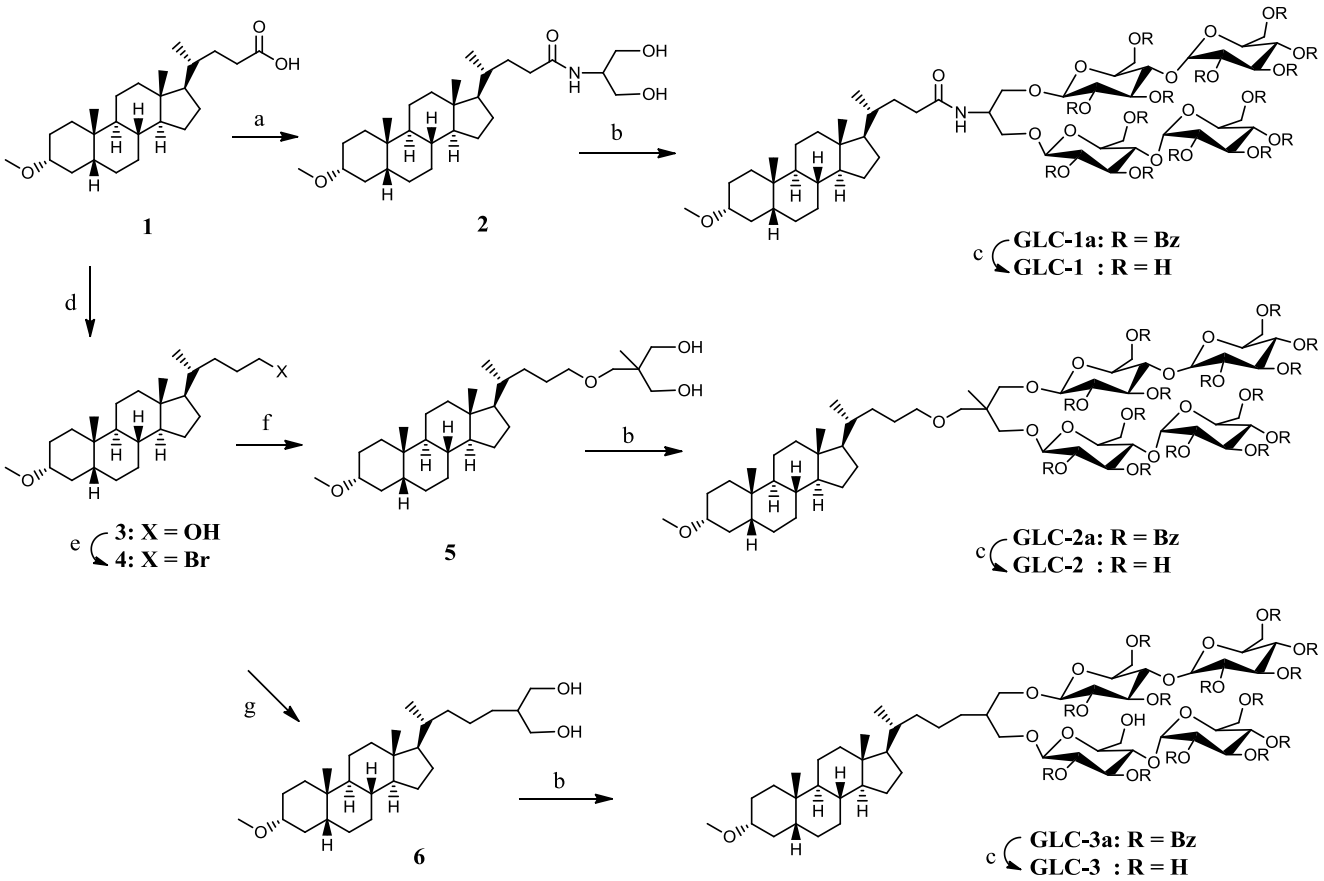
evaporation. The residue was purified by silica gel column chromatography (eluting with EtOAc/hexane) to provide the desired product as a glassy solid.

General Procedure for de-*O*-benzoylations[7]

O-Benzoylated compounds were 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 6 h at RT, 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 was achieved by recrystallization from CH_2Cl_2/MeOH/diethyl ether, to provide the fully de-*O*-benzoylated product as a white solid.

Synthesis and characterization of GLC amphiphiles

Synthetic Scheme 1



(a) EDC • HCl, HOBt, DMF, RT, 2 days; (b) perbenzoylated maltosylbromide, AgOTf, CH_2Cl_2, -45°C → RT, 3 hr; (c) NaOMe, MeOH, RT, 4 hr; (d) $LiAlH_4$, THF, RT, 1 day; (e) CBr_4, Ph_3P, MeCN:THF, RT, 15 hr; (f) 1,1,1- Tris(hydroxymethyl)ethane, NaH, 60 °C, 2 hr; (g) diethylmalonate, NaH, THF, RT, 15 hr; $LiAlH_4$, THF, RT, 1 day.

Compound 1 was synthesized by a modified version of a reported protocol.[8] **^{1}H NMR** (300 MHz, $CDCl_3$): δ 3.26 (s, 3H), 3.24-3.08 (m, 1H), 2.46-2.32 (m, 1H), 2.32-2.16 (m, 1H), 1.96-1.50 (m, 10H), 1.50-0.94 (m, 18H), 0.94-0.82 (m, 6H), 0.64 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 80.7, 56.7, 56.2, 55.7, 43.0, 42.3, 40.6, 40.4, 36.1, 35.6, 35.5, 35.1, 32.9, 31.2, 31.0, 28.4, 27.6, 27.0, 26.6, 24.4, 23.6, 21.0, 18.5, 12.3 ; **MS (MALDI-TOF)**: calcd. for $C_{25}H_{42}O_3$ $[M+Na]^+$ 413.3027, found 413.3017.

Compound 2

Methylated lithocholic acid (**1**) (1.5 g, 3.8 mmol), serinol (0.41 g, 4.6 mmol) and 1-hydroxybenzotriazole monohydrate (HOBt) (0.61 g, 4.6 mmol) were dissolved in anhydrous DMF (30 mL). 1-(3-(Dimethylamino)propyl)-3-ethylcarbodiimide hydrochoride (EDC • HC1) (0.87 g, 4.55 mmol) was added in small portions at 0°C, and the resulting solution was stirred at room temperature for 20 h. The solution was diluted with EtOAc (100 mL) and then washed successively with 1 M aqueous $NaHCO_3$ (100 mL), 0.1 M aqueous HCl (100 mL) and brine (2 x 100 mL). The organic layer

was dried with anhydrous Na_2SO_4, and solvent was removed by rotary evaporation. The crude product was precipitated with ether (100 mL), and the resulting solid was collected and dried *in vacuo* to afford amide-containing diol (**2**) as a white solid (1.60 g, 91%). This product was used for the next reaction without purification. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 6.86 (d, *J* = 7.9 Hz, 1H), 3.88-3.80 (m, 4H), 3.76-3.64 (m, 2H), 3.64-3.52 (m, 2H), 3.36 (s, 3H), 3.26-3.12 (m, 1H), 2.34-2.21 (m, 1H), 2.17-2.05 (m, 1H), 1.98-1.50 (m, 9H), 1.48-0.94 (m, 16H), 0.94-0.84 (m, 6H), 0.65 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 175.4, 80.7, 61.4, 56.5, 56.0, 55.4, 52.5, 49.3, 49.0, 48.7, 48.4, 42.8, 42.1, 40.4, 40.2, 35.9, 35.6, 35.2, 34.9, 32.7, 31.8, 28.2, 27.3, 26.7, 26.4, 24.2, 23.4, 20.8, 18.3, 12.0; **MS (MALDI-TOF)**: calcd. for $C_{28}H_{49}NO_4$ $[M+Na]^+$ 486.3554, found 486.3570.

Compound 3

$LiAlH_4$ (0.44 g, 1.5 mmol) was added slowly to compound **1** (1.5 g, 3.8 mmol) dissolved in THF (50 mL) at 0°C. The mixture was stirred at RT for 1 day, 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 Na_2SO_4. The residue was purified by silica gel column chromatography (EtOAc/hexane) providing a desired product (**3**) as a white solid (1.3 g, 89 %). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 3.60 (t, *J* = 6.0 Hz, 2H), 3.34 (s, 3H), 3.21-3.11 (m, 1H), 2.01-1.51 (m, 10H), 1.50-0.96 (m, 18H), 0.96-0.82 (m, 6H), 0.64 (m, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 80.6, 63.7, 56.7, 56.4, 55.7, 42.9, 42.2, 40.5, 40.4, 36.0 35.8, 35.5, 35.1, 32.9, 32.0, 29.6, 28.5, 27.5, 27.0, 26.6, 24.4, 23.6, 21.0, 18.8, 12.2 ; **MS (MALDI-TOF)**: calcd. for $C_{25}H_{44}O_2$ $[M+NH_4]^+$ 394.3680, found 394.3683.

Compound 4

To a solution of alcohol (**3**) (0.88 g, 2.3 mmol) and carbon tetrabromide (0.79 g, 3.0 mmol) in CH_2Cl_2 (100 mL) was added triphenylphosphine (Ph_3P) at 0 °C. The solution was stirred at 0°C for 1 hr and then continued the stirring at RT for 15 hr. The solvent was evaporated, and then a 1:15 mixture of CH_2Cl_2 and hexane (100 mL) was added to the residue to dissolve the crude product by not triphenylphosphine oxide. After filtration and evaporation, the residue was purified by silica gel column chromatography (eluting with EtOAc/hexane) to provide the desired product (**4**) as a white solid (0.92 g, 90 %). **^{1}H NMR** (300 MHz, CD_3OD): δ 3.43-3.29 (m, 5H), 3.21-3.09 (m, 1H), 2.01-1.47(m, 11H), 1.47-0.99 (m, 17H), 0.99-0.84 (m, 7H), 0.64 (s, 3H); **^{13}C NMR** (75 MHz, CD_3OD): δ 80.6, 56.8, 56.7, 56.3, 55.8, 42.9, 42.3, 40.4, 36.1, 35.5, 35.4, 35.1, 34.8, 34.7, 33.0, 29.9, 28.5, 27.6, 27.0, 26.6, 24.4, 23.6, 21.0, 18.9, 12.3; **MS (MALDI-TOF)**: calcd. for $C_{25}H_{43}O_2Br$ $[M+NH_4]^+$ 456.2836, found 456.2118.

Compound 5

1,1,1- Tris(hydroxymethyl)ethane (1.3 g, 11.2 mmol) is dissolved in 40 mL of DMF, and NaH (0.45 g, 11.2 mmol) was added. Bromide **4** (1.6 g, 3.7 mmol) was added to this solution, and the mixture was stirred for 2 hr at 60 °C. After adding water (100 mL), the resulting solution was extracted with ether (2 x 100 mL). The combined organic layer was washed with brine and dried with anhydrous Na_2SO_4. Solvent was removed, and the residue was purified by silica gel column chromatography (eluting with EtOAc/hexane) to provide diol (**5**) as a white solid (1.1 g, 60 %). **^{1}H NMR** (300 MHz, CD_3OD): δ 3.69 (d, *J* = 11.0 Hz, 2H), 3.56(d, *J* = 11.0 Hz, 2H), 3.46-3.34 (m, 7H), 3.21-3.11 (m, 1H), 3.01-2.79 (br s, 2H), 2.01-1.57 (m, 9H), 1.57-0.92 (m, 20H), 0.92-0.71(m, 11H), 0.63 (s, 3H); **^{13}C NMR** (75 MHz, CD_3OD): δ 80.6, 77.0, 72.5, 67.9, 56.6, 56.2, 56.2, 55.6, 42.9, 42.8, 42.2, 40.9, 40.8, 40.5, 40.3, 36.0, 35.7, 35.4, 35.1, 35.0, 32.3, 28.4, 27.4, 26.9, 26.5, 26.2, 24.3, 23.5, 20.9, 18.8, 18.6, 17.3, 12.2; **MS (MALDI-TOF)**: calcd. for $C_{30}H_{54}O_4$ $[M+H]^+$ 479.4095, found 479.4096.

Compound 6

To a solution of bromide **4** (0.92 g, 2.1 mmol) and diethyl malonate (1.6 g, 10.4 mmol) in a 1:1 mixture of THF and DMF (80 mL) was added K_2CO_3 (1.5 g, 10.5 mmol). The mixture was heated at 90 °C for 15 hr, and then water (100 mL) was added at 0°C. The resulting solution was extracted with diethyl ether (2 x 100 mL). The combined organic layer was washed with brine and dried with anhydrous Na_2SO_4. After removal of solvent, the crude product was used for the next reaction without purification. The crude product was dissolved in THF (50 mL), and $LiAlH_4$ (0.52 g, 14.0 mmol) was added slowly to the solution at 0°C. The mixture was stirred at RT for 1 day, and then excess hydride was quenched by addition of MeOH, water and 1 N aqueous HCl successively at 0°C. The resulting solution was extracted with diethyl ether (2 x 50 mL). The combined organic layer was washed with brine and dried with anhydrous Na_2SO_4. After removal of solvent, the residue was purified by silica gel column chromatography (eluting with EtOAc/hexane) to provide diol (**6**) as a white solid (0.85 g, 93% (two steps)). **^{1}H NMR** (300 MHz, CD_3OD): 3.89-3.81 (m, 2H), 3.67-3.59 (m, 2H), 3.35 (s, 3H), 3.24-3.10 (m, 1H), 3.54 (br s, 2H), 2.02-1.48 (m, 10H), 1.48-0.92 (m, 25H), 0.92-0.71 (m, 10H), 0.63 (s, 3H); **^{13}C NMR** (75 MHz, CD_3OD): δ 80.7, 67.1, 66.8, 56.7, 56.5, 55.7, 42.9, 42.3, 40.6, 40.4, 36.4, 36.1, 35.9, 35.5, 35.1, 33.0, 28.6, 28.4, 27.5, 27.0, 26.6, 24.4, 24.0, 23.6, 21.0, 18.8, 12.2; **MS (MALDI-TOF)**: calcd. for $C_{28}H_{50}O_3$ $[M+ NH_4]^+$ 452.4099, found 452.4102.

GLC-1a was synthesized from diol **2** according to the general procedure for glycosylation. **^{1}H NMR** (300 MHz, CD_3OD): 5.77 (d, *J* = 8.1 Hz, 1H), 5.44-5.19 (m, 5H), 5.06 (dt, *J* = 10.2, 2.0 Hz, 2H), 4.90-

4.75 (m, 4H), 4.55-4.43 (m, 4H), 4.32-4.15 (m, 5H), 4.09-3.93 (m, 6H), 3.80-3.67 (m, 4H), 3.56-3.46 (m, 1H), 3.35 (m, 3H), 3.23-3.09 (m, 1h), 2.16 (s, 6H), 2.11 (s, 6H), 2.08-1.93 (m, 32H), 1.93-0.95 (m, 29H), 0.95-0.80 (m, 8H), 0.63 (s, 3H); **^{13}C NMR** (75 MHz, CD_3OD): δ 173.5, 170.7, 170.6, 170.5, 170.3, 170.2, 170.1, 169.9, 169.8, 169.6, 101.0, 100.8, 95.8, 80.6, 75.4, 75.2, 73.0, 72.9, 72.6, 72.5, 72.4, 72.2, 70.2, 69.5, 68.7, 68.6, 68.2, 62.9, 61.6, 56.2, 55.7, 42.9, 42.2, 40.5, 40.4, 36.0, 35.7, 35.0, 33.5, 33.0, 31.7, 28.4, 27.5, 27.0, 24.4, 23.6, 21.1, 21.0, 20.9, 20.8, 18.6, 12.2; **MS (MALDI-TOF)**: calcd. for $C_{150}H_{145}NO_{38}$ $[M+Na]^+$ 2590.9, found 2591.4.

GLC-2a was synthesized from diol **5** according to the general procedure for glycosylation. **^{1}H NMR** (300 MHz, CD_3OD): 8.12-8.02 (m, 4H), 8.02-7.95 (m, 7H), 7.95 -7.88 (m, 4H), 7.88-7.83 (m, 4H), 7.83-7.77 (m, 4H), 7.77-7.67 (m, 4H), 7.67-7.16 (m, 45H), 6.13 (t, *J* = 10.0 Hz, 2H), 5.72-5.60 (m, 4H), 5.39 (t, *J* = 9.5 Hz, 2H), 5.20-5.08 (m, 4H), 4.70-4.43 (m, 4H), 4.40-4.16 (m, 8H), 3.56-3.37 (m, 5H), 3.34 (s, 3H), 3.26-3.12 (m, 2H), 3.06 (q, *J* = 10.1 Hz, 2H), 2.98-2.89 (m, 3H), 2.84 (d, *J* = 9.2 Hz, 1H), 1.95-0.95 (m, 34H), 0.95-0.82 (m, 6H), 0.82-0.73 (m, 6H), 0.59 (s, 3H); **^{13}C NMR** (75 MHz, CD_3OD): δ 166.3, 166.2, 166.0, 165.7, 165.2, 165.0, 133.9, 133.7, 133.6, 133.4, 133.3, 133.2, 130.1, 129.9, 129.8, 129.6, 129.3, 129.2, 129.1, 129.0, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3, 101.1, 101.0, 95.9, 80.6, 74.8, 73.6, 72.5, 72.4, 72.3, 71.4, 70.0, 69.2, 69.1, 66.0, 63.5, 62.7, 56.6, 56.3, 55.7, 42.8, 42.2, 40.5, 40.4, 40.3, 36.0, 35.6, 35.5, 35.1, 33.0, 32.2, 28.4, 27.5, 27.0, 26.6, 26.2, 24.4, 23.6, 21.0, 18.8, 17.2, 15.4, 14.4, 12.2; **MS (MALDI-TOF)**: calcd. for $C_{152}H_{150}O_{38}$ $[M+Na]^+$ 2606.0, found 2606.5.

GLC-3a was synthesized from diol **6** according to the general procedure for glycosylation. **^{1}H NMR** (300 MHz, CD_3OD): 8.10-7.90 (m, 15H), 7.89-7.83 (m, 4H), 7.83-7.76 (m, 4H), 7.76-7.68 (m, 4H), 7.68-7.13 (m, 42H), 6.13 (t, *J* = 10.0 Hz, 2H), 5.74-5.59 (m, 4H), 5.41-5.30 (m, 2H), 5.21-5.06 (m, 4H), 4.72-4.48 (m, 4H), 4.41-4.16 (m, 8H), 3.69-3.56 (m, 2H), 3.34 (s, 3H), 3.34-3.25 (m, 2H), 3.21-3.10 (m, 1H), 3.10-2.92 (m, 2H), 2.79 (t, *J* = 9.8 Hz, 1H), 1.92-1.44 (m, 11H), 1.44-1.09 (m, 15H), 1.09-0.79 (m, 14H), 0.79-0.69 (m, 3H), 0.56 (s, 3H) **^{13}C NMR** (75 MHz, CD_3OD): δ 166.3, 166.0, 165.7, 165.2, 165.1, 165.0, 133.7, 133.6, 133.4, 133.3, 130.2, 130.1, 130.0, 129.8, 129.7, 129.6, 129.5, 129.4, 129.2, 129.1, 129.0, 128.6, 128.4, 101.2, 95.8, 80.6, 77.4, 74.8, 74.7, 72.2, 71.4, 70.0, 69.1, 62.7, 60.6, 56.6, 55.7, 42.8, 42.3, 40.5, 40.3, 36.0, 35.9, 35.5, 35.1, 33.0, 28.5, 28.2, 27.5, 27.0, 24.4, 23.9, 23.6, 21.2, 21.0, 18.7, 14.4, 12.2; **MS (MALDI-TOF)**: calcd. for $C_{150}H_{146}O_{37}$ $[M+Na]^+$ 2561.9, found 2562.5.

GLC-1 was synthesized from **GLC-1a** according to the general procedure for de-*O*-benzoylation. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 5.15 (d, *J* = 3.6 Hz, 2H), 4.33 (t, *J* = 7.0 Hz, 2H), 4.01-3.74 (m, 9H), 3.73-3.15 (m, 25H), 2.36-2.20 (m, 1H), 2.20-2.06 (m, 1H), 2.06-1.52 (m, 10H), 1.52-1.02 (m, 18H), 1.02-0.88 (m, 7H), 0.69 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 176.9, 103.0, 82.1, 81.4, 77.8, 76.7, 75.2,

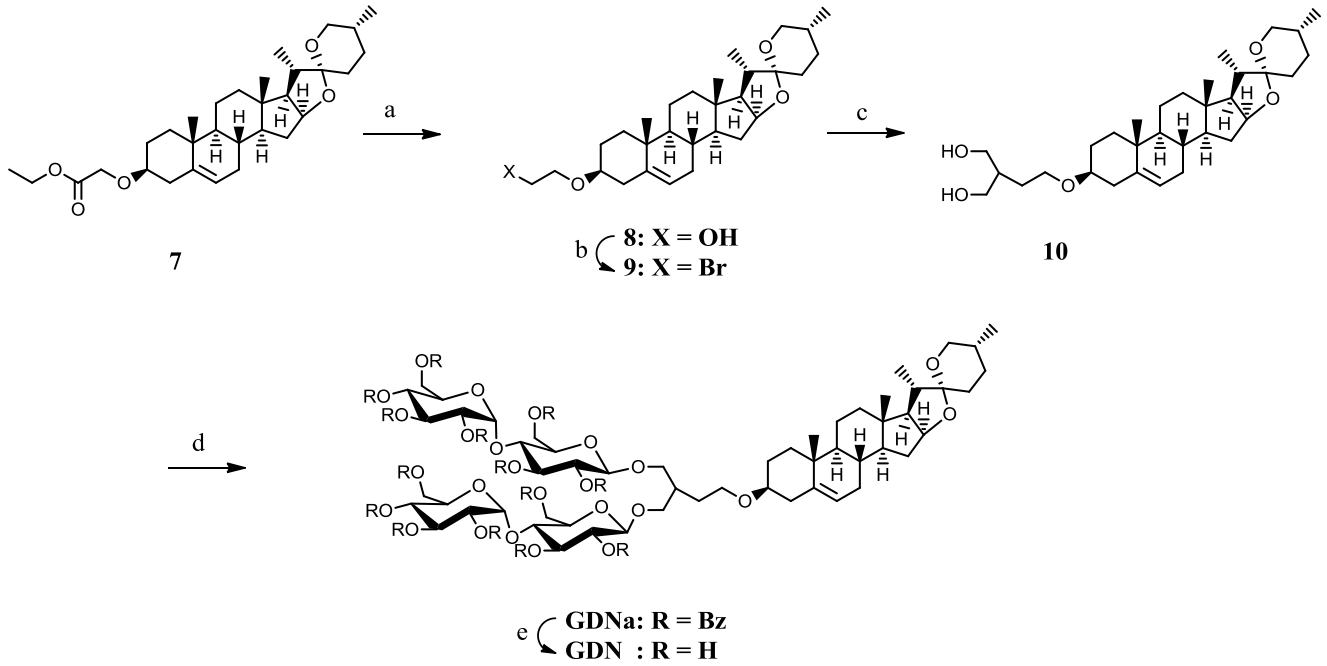
74.9, 74.8, 74.7, 74.2, 71.6, 62.8, 58.0, 57.5, 56.0, 44.0, 43.5, 42.0, 37.3, 37.0, 36.1, 28.5, 27.8, 24.1, 22.1, 19.1, 12.8; **MS (MALDI-TOF)**: calcd. for $C_{52}H_{89}O_{24}$ $[M+Na]^+$ 1134.5667, found 1134.5703.

GLC-2 was synthesized from **GLC-2a** according to the general procedure for de-*O*-benzoylation. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 5.19 (d, *J* = 3.6 Hz, 2H), 4.35 (t, *J* = 7.7 Hz, 2H), 4.00-3.77 (m, 8H), 3.77-3.51 (m, 12H), 3.51-3.14 (m, 15H), 2.11-1.05 (m, 26H), 1.05-0.91 (m, 9H), 0.72 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 105.1, 103.0, 82.2, 81.5, 78.0, 76.7, 75.2, 74.9, 74.3, 73.6, 73.4, 73.2, 71.6, 62.9, 62.3, 58.0, 57.8, 56.0, 44.0, 43.5, 42.1, 42.0, 41.7, 37.4, 37.0, 36.4, 36.1, 34.0, 33.7, 29.6, 28.5, 27.9, 27.8, 27.5, 25.4, 24.1, 22.1, 19.4, 18.0, 12.7 ; **MS (MALDI-TOF)**: calcd. for $C_{54}H_{94}O_{24}$ $[M+Na]^+$ 1149.6027, found 1149.6029.

GLC-3 was synthesized from **GLC-3a** according to the general procedure for de-*O*-benzoylation. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 5.16 (d, *J* = 3.6 Hz, 2H), 4.32 (t, *J* = 7.7 Hz, 2H), 3.99-3.74 (m, 8H), 3.74-3.55 (m, 10H), 3.55-3.15 (m, 12H), 2.10-1.02 (m, 31H), 1.02-0.83 (m, 7H), 0.68 (s, 3H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 104.9, 104.8, 103.0, 82.2, 81.5, 78.0, 76.7, 75.2, 74.9, 74.3, 71.6, 71.4, 62.9, 62.4, 58.0, 57.9, 56.0, 44.0, 43.6, 42.0, 41.7, 40.9, 37.6, 37.4, 37.2, 36.4, 36.1, 34.0, 30.0, 29.6, 28.5, 28.0, 27.8, 25.4, 24.8, 24.1, 22.1, 19.4, 12.7 ; **MS (MALDI-TOF)**: calcd. for $C_{52}H_{90}O_{23}$ $[M+Na]^+$ 1105.5766, found 1105.5719.

Synthesis and characterization of GDN amphiphile

Synthetic Scheme 2



(a) $LiAlH_4$, THF, RT, 1 day; (b) CBr_4, Ph_3P, CH_2Cl_2, RT, 15 hr; (c) diethylmalonate, NaH, THF, RT, 1day; $LiAlH_4$, THF, RT, 1 day; (d) perbenzoylated maltosylbromide, AgOTf, CH_2Cl_2, -45°C → RT, 3 hr; (e) NaOMe, MeOH, RT, 4 hr.

Compound 7

Ethyl diazoacetate (1.8 g, 15.7 mmol) was added to a solution of diosgenin (5.0 g, 12.1 mmol) dissolved in anhydrous CH_2Cl_2 (100 mL) under N_2 atmosphere. BF_3• etherate (0.083g, 0.67 mmol) was then added to the solution and then the resulting reaction mixture at RT for 1.5 day. The reaction mixture was quenched with a saturated aqueous $NaHCO_3$ solution and extracted with EtOAc (200 mL). The organic layer was washed with water (200 mL) and dried with anhydrous Na_2SO_4. After removal of solvent, the residue was purified by silica gel column chromatography (eluting with EtOAc/hexane) to provide the desired product (**7**) as a white solid (3.6 g, 60%). **^{1}H NMR** (300 MHz, $CDCl_3$): δ 5.35 (d, *J* = 5.2 Hz, 1H), 4.41 (q, *J* = 7.3 Hz, 1H), 4.20 (q, *J* = 7.0 Hz, 2H), 4.12 (s, 2H), 3.52-3.42 (m, 1H), 3.37 (t, *J* = 10.6 Hz, 1H), 3.28-3.18 (m, 1H), 2.46-2.34 (m, 1H), 2.34-2.18 (m, 1H), 2.07-1.80 (m, 5H), 1.80-1.34 (m, 13H), 1.28 (t, *J* = 7.2 Hz, 3H), 1.30-1.04 (m, 3H), 1.04-0.89 (m, 8H), 0.89-0.72 (m, 6H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 171.1, 140.8, 124.0, 121.9, 109.5, 81.0, 80.2, 67.0, 66.0, 62.3, 61.0, 56.7, 50.3, 41.8, 40.5, 40.0, 38.9, 37.3, 37.2, 32.3, 32.1, 31.6, 30.5, 29.0, 28.3, 21.0, 19.6, 17.3, 16.5, 14.7, 14.4 ; **MS (MALDI-TOF)**: calcd. for $C_{31}H_{48}O_5$ $[M+NH_4]^+$ 518.3840, found 518.3837.

Compound 8 was synthesized from **7** via a protocol similar to that used to prepare **3** from **1**. Yield: 89 %; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 5.35 (d, *J* = 5.2 Hz, 1H), 4.41 (q, *J* = 7.3 Hz, 1H), 3.72 (t, *J* = 4.6 Hz, 2H), 3.59 (t, *J* = 4.6 Hz, 2H), 3.52-3.42 (m, 1H), 3.37 (t, *J* = 10.6 Hz, 1H), 3.26-3.11 (m, 1H), 2.45-2.31 (m, 1H), 2.26-2.14 (m, 1H), 2.08-1.82 (m, 6H), 1.82-1.37 (m, 12H), 1.37-1.05 (m, 4H), 1.05-0.88 (m, 8H), 0.85-0.69 (m, 6H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 141.0, 121.7, 109.5, 81.0, 79.6, 69.2, 68.3, 67.0, 62.3, 56.7, 50.3, 41.8, 40.5, 40.0, 37.4, 37.2, 32.3, 32.1, 31.6, 30.5, 29.0, 28.6, 21.1, 19.6, 17.3, 16.5, 14.7 ; **MS (MALDI-TOF)**: calcd. for $C_{29}H_{46}O_4$ $[M+NH_4]^+$ 476.3735, found 476.3739.

Compound 9 was synthesized from **8** via a protocol similar to that used to prepare **4** from **3**. Yield: 86 %; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 5.35 (s, 1H), 4.41 (q, *J* = 7.3 Hz, 1H), 3.78 (t, *J* = 6.8 Hz, 2H), 3.52-3.32 (m, 4H), 3.27-3.15 (m, 1H), 2.42-2.31 (m, 1H), 2.29-2.16 (m, 1H), 2.08-1.81 (m, 5H), 1.81-1.37 (m, 12H), 1.36-1.05 (m, 4H), 1.05-0.92 (7H), 0.92-0.73 (m, 7H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 140.9, 121.8, 109.5, 81.0, 79.8, 68.3, 67.1, 62.3, 56.7, 50.3, 41.8, 40.5, 40.0, 39.3, 37.4, 37.2, 32.3, 32.1, 31.6, 31.1, 30.5, 29.0, 28.6, 21.1, 19.6, 17.4, 16.5, 14.7; **MS (MALDI-TOF)**: calcd. for $C_{29}H_{45}O_3Br[M+H]^+$ 521.2625, found 521.2621.

Compound 10 was synthesized from **9** via a protocol similar to that used to prepare **6** from **4**. Yield (two steps): 90 %; **^{1}H NMR** (300 MHz, $CDCl_3$): δ 5.34 (d, = 5.2 Hz, 1H), 4.41 (q, = 7.4 Hz, 1H), 3.78-3.63 (m, 4H), 3.57 (t, = 5.6 Hz, 2H), 3.52-3.42 (m, 1H), 3.37 (t, = 10.6 Hz, 1H), 3.27-3.08 (m, 1H), 2.87 (s, 2h), 2.45-2.30 (m, 1H), 2.27-2.12 (m, 1H), 2.08-1.34 (m, 21H), 1.34-1.05 (m, 4H), 1.05-0.88 (m, 8H), 0.88-0.72 (m, 7H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 140.8, 121.8, 109.5, 81.0, 79.6, 67.1, 66.4, 65.4, 62.3, 56.7, 41.8, 50.3, 40.5, 40.1, 39.1, 37.3, 37.2, 32.3, 32.0, 31.6, 30.5, 29.5, 29.0, 28.5, 21.1, 19.6, 17.3, 16.5, 14.7; **MS (MALDI-TOF)**: calcd. for $C_{32}H_{52}O_5[M+Na]^+$ 539.3707, found 539.3714.

GDNa was synthesized from **10** according to the general procedure for glycosylation. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 8.14-7.90 (m, 15H), 7.90-7.83 (m, 4H), 7.83-7.77 (m, 4H), 7.77-7.68 (m, 4H), 7.68-7.15 (m, 42H), 6.13 (t, *J* =10.0 Hz, 2H), 5.73-5.59 (m, 4H), 5.35 (q, *J* =9.7 Hz, 2H), 5.29-5.03 (m, 8H), 4.71-4.48 (m, 4H), 4.48-4.14 (m, 9H), 3.69-3.57 (m, 2H), 3.53-3.23 (m, 7H), 3.13-2.92 (m, 4H), 2.85-2.74 (m, 1H), 2.32-2.20 (m, 1H), 2.20-1.81 (m, 5H), 1.80-1.70 (m, 5H), 1.70-1.55 (m, 4H), 1.55-1.40 (m, 4H), 1.40-1.02 (m, 8H), 1.02-0.94 (m, 3H), 0.94-0.83 (m, 6H), 0.83-0.70 (m, 5H); **^{13}C NMR** (75 MHz, $CDCl_3$): δ 166.3, 166.2, 166.0, 165.6, 165.2, 165.1, 165.0, 141.1, 133.7, 133.6, 133.4, 133.3, 130.1, 129.9, 129.8, 129.6, 129.5, 129.3, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.4, 128.3, 121.3, 109.3, 101.1, 95.8, 81.0, 78.9, 74.7, 72.3, 72.2, 70.0, 69.2, 69.1, 67.0, 62.7, 60.6, 56.7, 50.2, 41.8, 40.4, 40.0, 37.3, 37.1, 32.2, 32.0, 31.6, 30.5, 29.0, 28.5, 21.0, 19.5, 17.3, 16.5, 15.5, 14.7; **MS (MALDI-TOF)**: calcd. for $C_{154}H_{148}O_{39}$ $[M+Na]^+$ 2643.9, found 2644.6.

GDN was synthesized from **GDNa** according to the general procedure for de-*O*-benzoylation. **^{1}H NMR** (300 MHz, $CDCl_3$): δ 5.37 (d, *J* = 5.2 Hz, 1H), 5.15 (d, *J* = 3.4 Hz, 2H), 4.39 (q, *J* = 7.7 Hz, 1H), 4.10 (d, *J* = 7.6 Hz, 2H) 3.98-3.74 (m, 8H), 3.72-3.54 (m, 12H), 3.54-3.47 (m, 3H), 3.47-3.40 (m, 3H), 3.40-3.32 (m, 2H), 3.32-3.08 (m, 5H), 2.43-2.30 (m, 1H), 2.20-1.82 (m, 3H), 1.82-1.06 (m, 18 H), 1.06-1.00 (m, 4H), 0.96 (d, *J* = 6.9 Hz, 4H), 0.85-0.74 (m, 6H) **^{13}C NMR** (75 MHz, $CDCl_3$): δ 142.2, 122.6, 110.7, 104.9, 104.7, 103.1, 82.4, 81.5, 80.6, 78.0, 76.7, 75.2, 75.0, 74.3, 71.7, 70.8, 68.0, 67.2, 63.9, 62.9, 62.4, 58.0, 51.8, 43.1, 41.6, 41.8, 40.4, 38.6, 38.3, 38.0, 33.3, 33.0, 32.9, 32.6, 31.6, 30.0, 29.9, 29.7, 22.2, 20.0, 17.7, 16.9, 15.1; **MS (MALDI-TOF)**: calcd. for $C_{56}H_{92}O_{25}$ $[M+Na]^+$ 1187.5820, found 1187.5769.

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