Supplementary Information

Highly branched penta-saccharide-bearing amphiphiles for membrane protein studies

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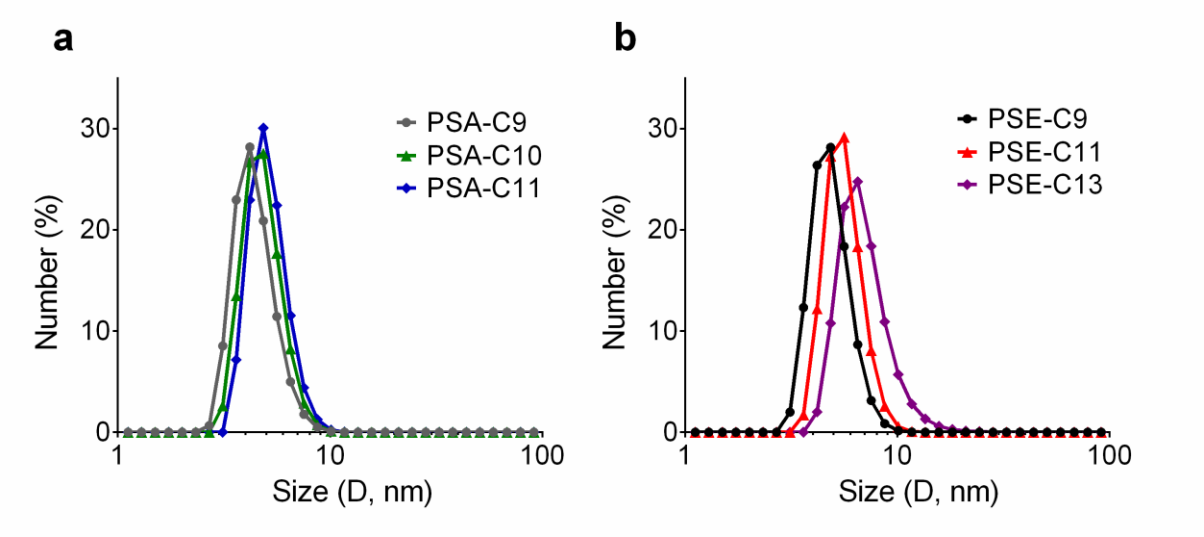


Figure S1. Dynamic light scattering (DLS) profiles for micelles formed by pentasaccharide-bearing amphiphiles (PSAs and PSEs). The novel agents (PSA-C9, PSA-C10, PSA-C11, PSE-C9, PSE-C11 and PSE-C13) were used at 1.0 wt% for this measurement. The time scale of micelle movements analyzed from scattered light intensity was used to calculate the hydrodynamic diameter of the micelles. All these agents formed a relatively monodisperse distribution of micelles.

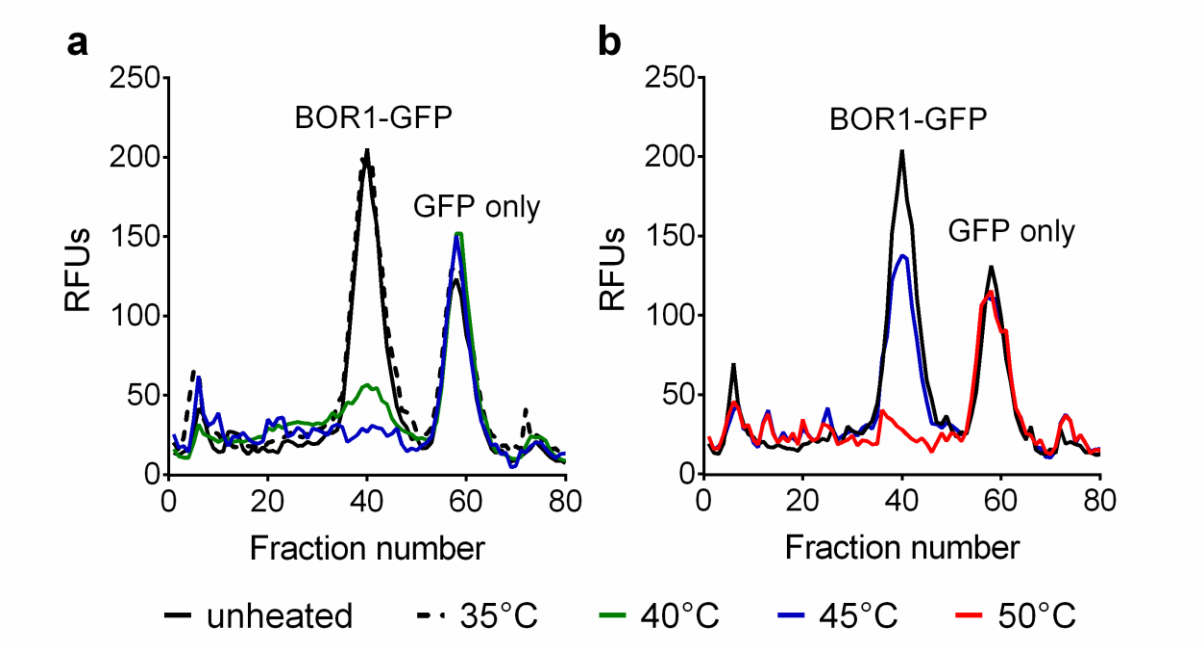


Figure S2. Temperature dependency of BOR1-GFP stability solubilized in DDM (a) and PSE-C11 (b). BOR1-GFP fusion protein was first solubilized by DDM or PSE-C11 at 1.0 wt% and the detergent-solubilized BOR1-GFP fusion protein was heated for 10 min at the designated temperature (35℃, 40℃, 45 ℃ or 50℃). For the DDM-solubilized sample the protein was not heated at 50℃, since there was no recovery of monodispersed sample at 45℃. For the PSE-C11 only sample heated at 45 ℃ or 50℃ is shown. Thermally-treated protein samples were loaded onto the SEC column and each fraction was measured by relative fluorescent units (RFU). The data is representative of two independent experiments.

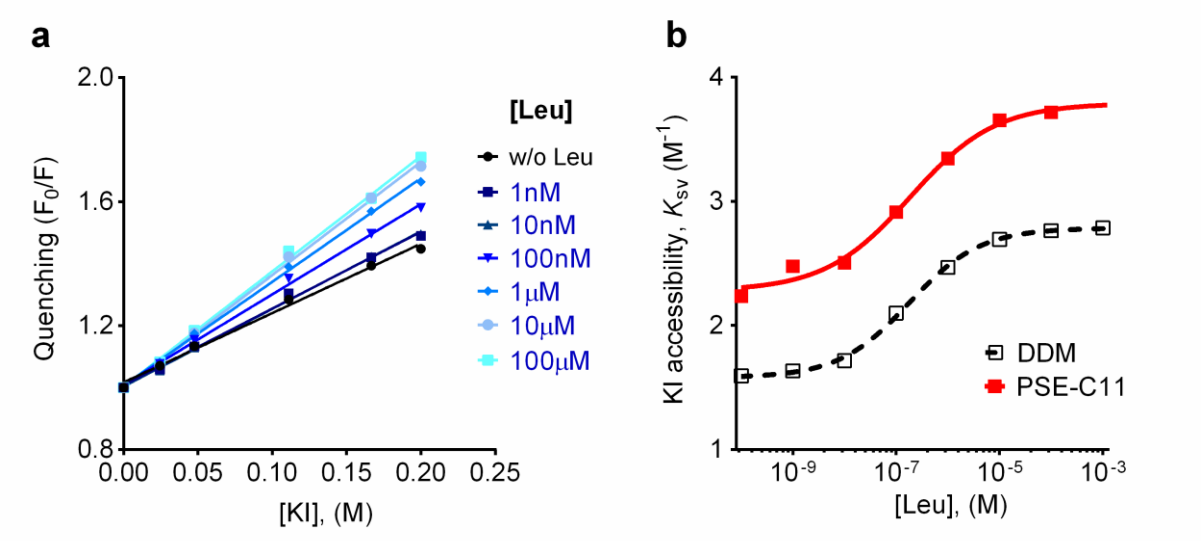


Figure S3. (a) Increase in water accessibility of the LeuT-coupled TMR fluorophore induced by leucine binding and (b) K_{SV} obtained from (a). Iodide quenching of the fluorescence of LeuT E192C^{TMR} solubilized in CMC+0.04 wt% PSE-C11 was measured in the absence of leucine and increasing [Leu] ranging from 1 nM to 100 μM. For detergent comparison, K_{SV} values for DDM and PSE-C11-solubilized transporters were plotted as a function of [Leu]. Data points are means ± s.e.m. with n = 3-4.

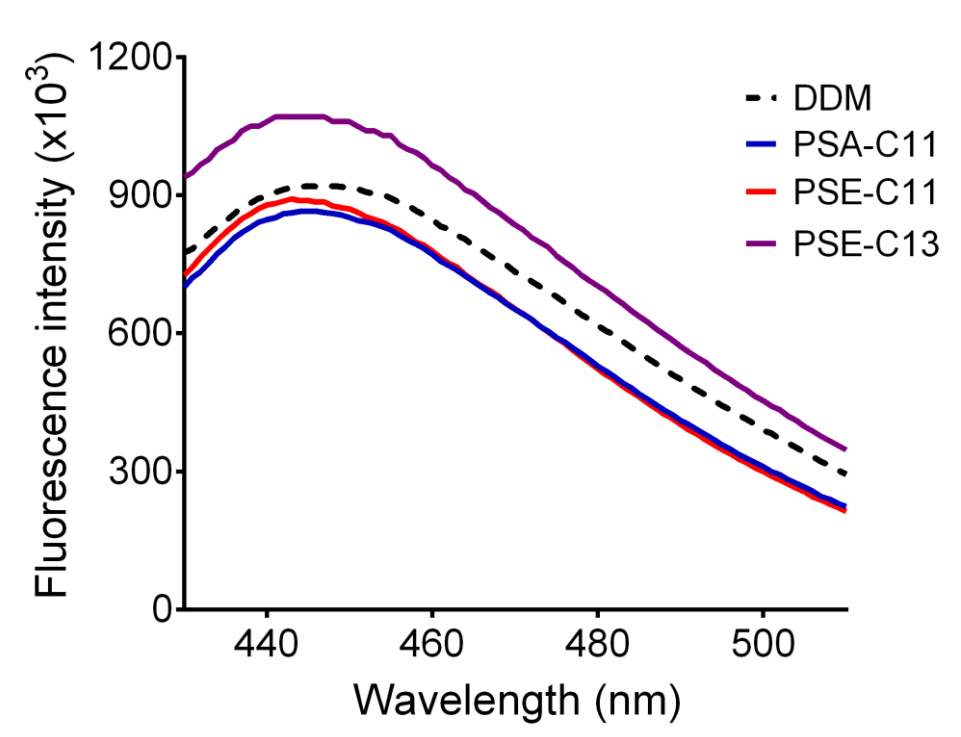


Figure S4. Fluorescence spectra of monobromobimane-labeled β_2AR (mBBr-β_2AR) solubilized in DDM, PSA-C11, PSE-C11, or PSE-C13 in the presence of a high affinity agonist of BI (BI-167107). BI-bound mBBr-β_2AR in DDM was diluted into an aqueous buffer containing DDM, PSA-C11, PSE-C11, or PSE-C13 at 0.1 wt%. After incubation for 30 min, the bimane spectra of the receptor in the individual detergents were measured. The data is representative of three independent experiments.

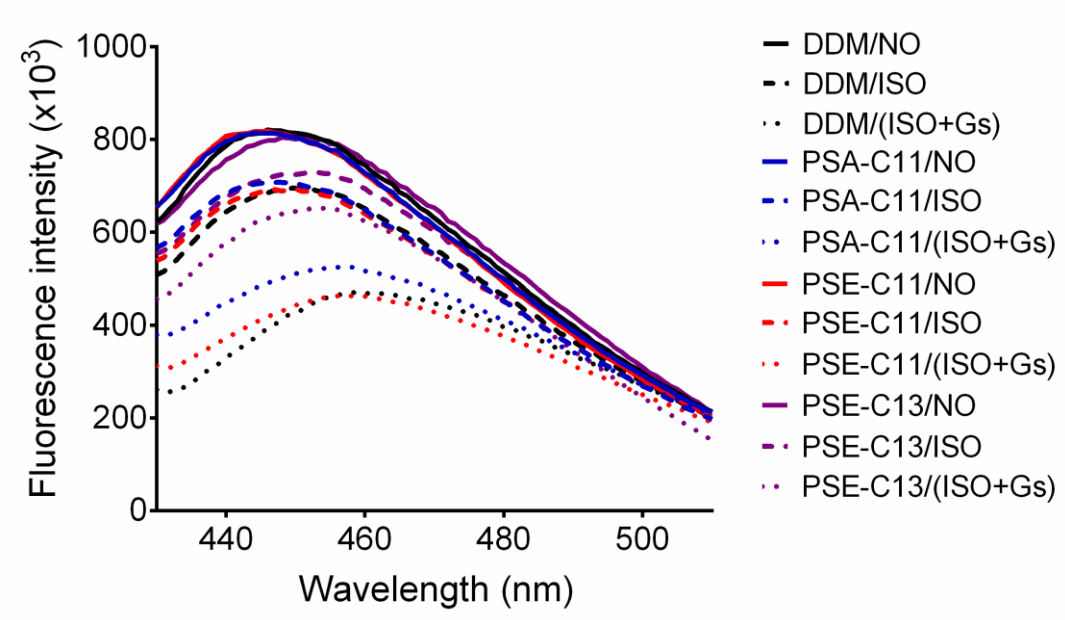


Figure S5. Fluorescence spectra of monobromobimane-labeled β_2AR (mBBr-β_2AR) solubilized in DDM, PSA-C11, PSE-C11, or PSE-C13. Fluorescence spectra of mBBr-β_2AR were measured in the absence of agonist (detergent/NO), the presence of full agonist (isopreoterenol (ISO); detergent/ISO), or a combination of ISO and G_s-protein (detergent/(ISO+G_s)). The data is representative of three independent experiments.

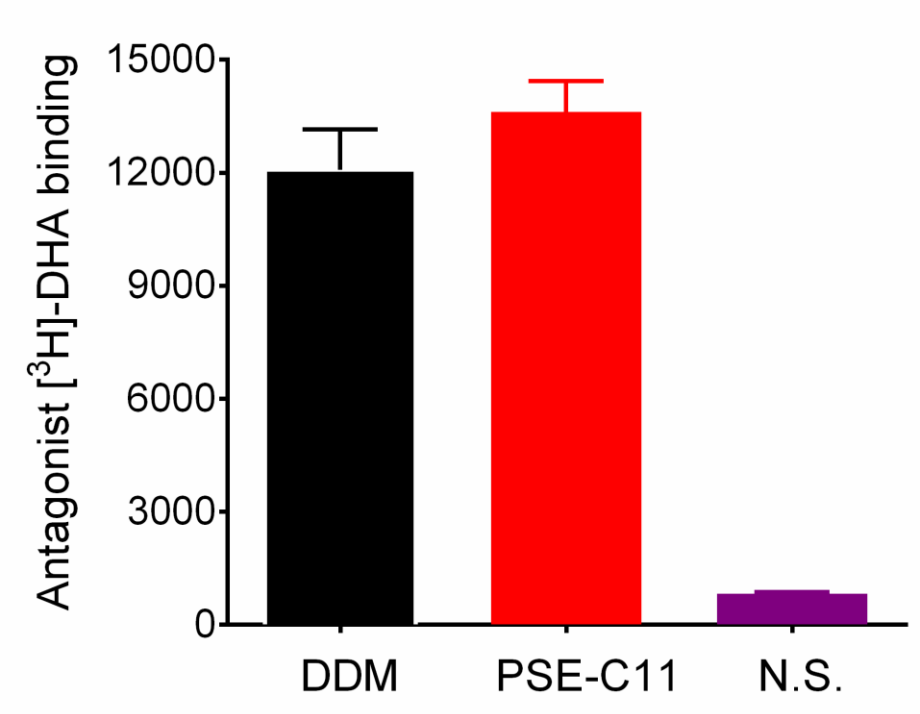


Figure S6. Ligand binding activity for β_2AR solubilized in DDM or PSE-C11. The membrane containing β_2AR was solubilized in 1% DDM or 1% PSE-C11 by detergent treatment. Activity of DDM or PSE-C11-solubilzed receptor was measured by radioligand binding assay using the antagonist [^{3}H]-DHA. N.S. represents nonspecific binding. Each measurement was performed in triplicate.

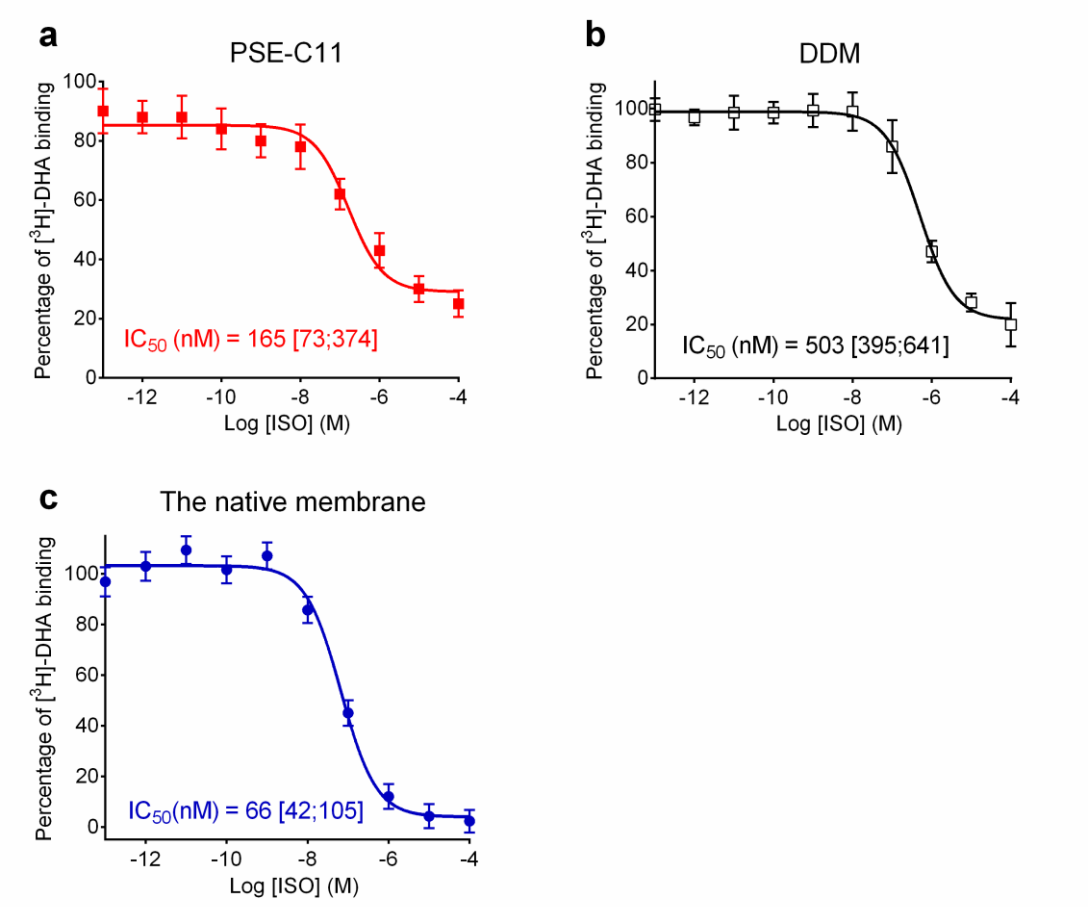


Figure S7. ISO competition binding to β_2AR in PSE-C11 micelles (a), DDM micelles (b) and in the native membrane (c). PSE-C11 or DDM sample was prepared by solubilizing β_2AR with 1.0 wt% PSE-C11 or DDM and by purifying the receptor in the same detergent at 20 × CMC via an M1 anti-Flag tag affinity column. IC_{50} values of ISO were calculated from data fitting to a one-site binding model and are shown as means [s.e.m. interval]. All data are from three independent experiments

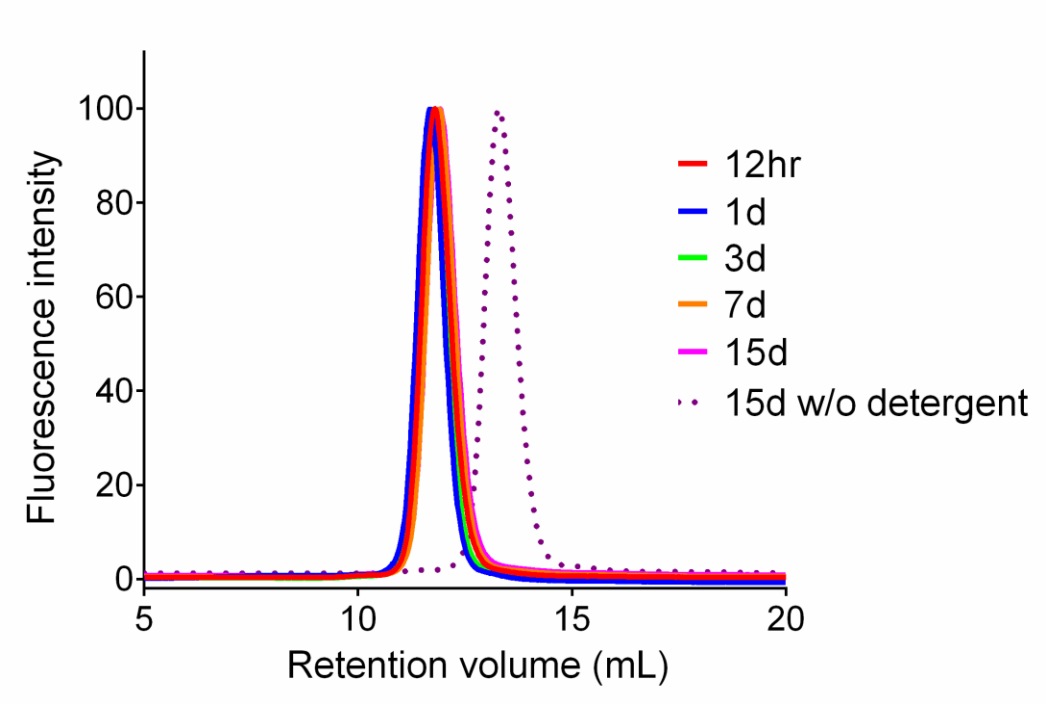


Figure S8. Time course SEC profiles of the T4L-β_2AR-G_s complex in PSE-C11. The T4L-β_2AR-G_s complex was prepared with DDM-purified T4L-β_2AR and the G_s heterotrimer. After detergent exchange with PSE-C11, the stability of the purified complex was assessed using analytical gel filtration over 15 days. Running buffer with or without PSE-C11 was used to measure stability of the complex in the detergent solution or at detergent free condition.

Table S1 Values of iodide quenching-response constants (EC_{50}) and ΔK_{SV} of LeuT E192C^{TMR} calculated from site-directed fluorescence quenching spectroscopy experiments. The EC_{50} and ΔK_{SV} are shown as means [s.e.m. interval]. All data are from three to four independent experiments.

	EC_{50} (nM)	ΔK_{SV} (M^{-1})
DDM	178 [126;251]	1.2 [1.12;1.29]
PSE-C11	186 [110;314]	1.5 [1.34;1.70]

Detergent evaluation with membrane proteins

BOR1 thermal stability assay

The BOR1 from *Arabidopsis thaliana* was expressed as a fusion protein with a C-terminal GFP tag in *Saccharomyces cerevisiae* FGY217 cells. The cells were grown in URA- media supplemented with 0.1% glucose. Protein expression was induced with addition of 2% galactose followed by incubation for 18 hours at 20℃ as previously described.[1] The cells were harvested and used to prepare membranes as previously described.[2] The membranes containing BOR1-GFP fusion proteins were diluted to a final total protein concentration of 2.8 mg ml^{-1} in PBS (pH 7.4) supplemented with either 1% DDM or 1% individual PSAs and PSEs. The samples were incubated with gentle rocking for 1 hour at 4 ℃ and then the insoluble material was removed by centrifugation at 14 000 g for 1 hour at 4 ℃. The supernatants containing the solubilized protein samples were incubated at the designated temperature (35 ℃, 40 ℃, 45 ℃, or 50 ℃) for 10 minutes and heavily aggregated protein was removed by centrifugation at 14 000 g for 10 minutes at 4 °C. A 200 µl aliquot of the supernatant was injected onto a Superose 6 10/300 column equilibrated with 20 mM Tris-HCl (pH 7.5), 150 mM NaCl and 0.03% DDM. The individual elution fractions were collected from a retention volume of 6.4 ml (i.e after 6.4 ml of flow-through) in 200 µl fractions in a clear bottom 96-well plate. The GFP fluorescence of each fraction was measured using an excitation wavelength of 470 nm and an emission wavelength of 512 nm.

LeuT

Stability assay According to the protocol described previousely,[3] purification of the wild type of the leucine transporter (LeuT) from *Aquifex aeolicus* was performed. 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). Shortly, protein was bound to Ni^{2+}-NTA resin (Life Technologies, Denmark) after bacterial membranes containing LeuT was treated with 1 % DDM. The resin-bound transporter was eluted in 20 mM Tris-HCl (pH 8.0), 1 mM NaCl, 199 mM KCl, 0.05 % DDM and 300 mM imidazole. Subsequently, approximately 1.5 mg/ml protein stock was diluted 10 times in an identical buffer without DDM and imidazole, but supplemented with PSAs/PSEs (PSA-C10, PSA-C11, PSE-C9, PSE-C11 and PSE-C13) and DDM (control) at the final concentrations of CMC + 0.04 wt % or CMC + 0.2 wt %, respectively. The protein samples were stored at room temperature and were centrifuged at the indicated time points. Protein activity was determined by measuring [^{3}H]-Leu binding using scintillation proximity assay (SPA).[4] Briefly, SPA was performed with 5 µL of the respective protein samples, 20 nM [^{3}H]-Leu and 1.25 mg/ml copper chelate (His-Tag) YSi beads (both from Perkin Elmer, Denmark) in buffer containing 450 mM NaCl and the respective test detergents at above-mentioned concentrations. [^{3}H]-Leu binding was determined using a MicroBeta liquid scintillation counter (Perkin Elmer).

Construction, expression, purification and fluorescent labeling The leucine transporter mutant (LeuT E192C^{TMR}) was generated, purified and labelled as previously described.[5] Briefly the residue E192 was mutated to a cysteine using QuikChange (Agilent Technologies) to allow fluorescent labelling using maleimide chemistry. The LeuT variant was expressed in *Escherichia coli* C41 (DE3). The

membranes were isolated by disruption, and LeuT was solubilized in 1 wt% DDM, followed by immobilization on Chelating Sepharose Fast Flow resin (GE Healthcare, Little Chalfont, UK). The bound protein was washed and incubated with excess tetramethylrhodamin-5-maleimide (TMR, Life Technologies, Carlsbad, California, USA) for 16 hours at 4℃. Subsequently LeuT was eluted in buffer A containing 300 mM imidazole, 20 mM Tris-HCl (pH 8.0), 1 mM NaCl, 199 mM KCl and 0.05 % DDM.

Site-directed fluorescence quenching spectroscopy experiments Fluorescence-based experiments were performed with 0.5 μg ml^{-1} fluorescently labelled LeuT diluted buffer B (500 mM NaCl, 20 mM Tris-HCl (pH 8)) supplemented with CMC + 0.04 wt% DDM or PSE-C11. Aliquots of LeuT with increasing concentration of leucine were incubated on shaker at room temperature for 1 hour prior to measurements. Steady-state fluorescence intensities were recorded on a FluoroMax4 (Horiba Scientific, Edison, New Jersey, USA) at λ_{em} = 572 nm and excitation source at λ_{ex} = 552 nm at 20℃. Quencher-titration was carried out by successive addition of small aliquots containing 1 mM KI in buffer B supplemented with 10 mM $Na_2S_2O_3$. Fluorescence intensities (F) were corrected for sample dilution, normalized to the initial intensity of the sample (F_0), and analyzed by linear regression in GraphPad Prism 6.0 (GraphPad Software). The degree of accessibility was obtained from the Stern-Volmer equation: $F_0/F = 1 + K_{SV} \times [Q]$, where F_0/F is the normalized fluorescence quenching, K_{SV} is the Stern-Volmer constant and [Q] is the quencher concentration.

MelB

Solubilization and thermal stability assay E. coli DW2 strain (Δ*melB* and Δ*lacZY*) harboring pK95ΔAHB/WT $MelB_{St}$/CH10, which encodes the wild-type melibiose permease of *Salmonella typhimurium* ($MelB_{st}$) with a 10-His tag at the C-terminus, was used for protein production.[6] Cell growth and membrane preparation were carried out as described.[7] Protein assay was carried out with a Micro BCA kit (Thermo Scientific, Rockford, IL). The membrane samples containing $MelB_{St}$ (final total membrane protein concentration was 10 mg/mL) in a solubilization buffer (20 mM sodium phosphate, pH 7.5, 200 mM NaCl, 10% glycerol and 20 mM melibiose) were mixed with individual detergents (DDM, PSAs or PSEs) at 1.5 % (w/v). The extractions were incubated at four different temperatures (0, 45, 55, and 65 ℃) for 90 min. Insoluble fractions were removed by ultracentrifugation at 355,590 *g* in a Beckman OptimaTM MAX Ultracentrifuge using a TLA-100 rotor for 45 min at 4 ℃. Equal amount of total membrane proteins (20 μg) were analyzed for each condition, and equal volume of solutions were loaded on SDS-15% PAGE. $MelB_{St}$ was detected by immunoblotting with a Penta-His-HRP antibody (Qiagen, Germantown, MD) as described.

Preparation of RSO vesicles and Trp→D^2G FRET assay RSO membrane vesicles were prepared from *E. coli* DW2 cells containing $MelB_{St}$ or $MelB_{Ec}$ by osmotic lysis.[8-10] RSO membrane vesicles in a buffer (pH 7.5) containing 100 mM KP_i and 100 mM NaCl at a protein concentration of 1 mg/ml were treated with 1.0 % DDM or PSE-C11 at 23 ℃ for 30 min and subjected to ultracentrifugation using TLA 120.2 rotor at >300,000 g for 45 min at 4 ℃. The supernatants were directly used for Trp→D^2G FRET experiments using an Amico-Bowman Series 2 (AB2) Spectrofluorometer. Trp residues were excited at 290 nm and emission was recorded at 465 nm and 490 nm for $MelB_{Ec}$ and $MelB_{St}$,

respectively. On a time trace, 10 μM D^2G and excess melibiose or equal volume of water were added at 60-sec and 120-sec point, respectively..

β_2AR and β_2AR-G_s complex

Detergent incubation assay Monobromobimane (mBBr)-labeled β_2AR (mainly at Cys265) was used to measure changes of fluorescence spectra induced by local conformational changes near to TM6.[11] 0.5 μl BI (agonist)-bound monobromobimane (mBBr)-labeled β_2AR in 0.1% DDM at 50 μM, was diluted with 500 μl the new detergents buffer (PSA-C11, PSE-C11 and PSE-C13) at 0.1%. After incubating the protein samples for 30 min, the mBBr spectra were measured and compared with the spectra of mBBr-labeled receptor in 0.1% DDM. The bimane fluorescence was measured by excitation at 370 nm, and emission spectra was recorded from 430 to 510 nm at 1-nm increments with 0.5 nm s^{-1} integration on a Spex FluoroMax-3 spectrofluorometer (Jobin Yvon Inc.) in photon counting mode set at a 4-nm emission bandwidth pass. The mBBr response in DDM was used as a positive control. The data shows a representative in three independent experiments.

G_s-protein coupling assay 0.5 μl unliganded mBBr-labeled receptor at 50 μM was diluted with 500 μl 0.1% new detergent buffer for 15 min at room temperature. This dilution produces the final receptor concentration of 50 nM. 2 μM isoproterenol (ISO) was added to these solutions and the resulting solutions were incubated for another 15 min. After further addition of 250 nM G_s, the protein samples were incubated for another 20 min at room temperature. The bimane fluorescence was measured by excitation at 370 nm, and emission spectra was recorded from 430 to 510 nm at 1-nm increments with 0.5 nm s^{-1} integration on a Spex FluoroMax-3 spectrofluorometer (Jobin Yvon Inc.) in photon counting mode set at a 4-nm emission bandwidth pass. The mBBr response in 0.1% DDM was used as positive control. The data shows a representative in three independent experiments.

Long-term stability measurement The β_2AR purified in 0.1% DDM was concentrated to around 10 mg/ml (approximately 200 μM).[12] 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/PSA/PSE, respectively. The activity of the detergent-purified receptor at 0.2 pmol was monitored at the regular intervals during four-day of incubation. The protein samples were initially incubated on ice during the first two days and then transferred to room temperature in the next two days. The receptor activity was measured by the soluble radioligand binding assay described below. The receptor purified in DDM or a new detergent (PSA-C11, PSE-C11, or PSE-C13) 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.

Size exclusion chromatography (SEC) experiment The β_2AR in 0.1% DDM was loaded on M1 Flag column in the presence of 2 mM $CaCl_2$ and washed the column with DDM/PSA/PSE detergent buffer

(20 mM HEPES pH 7.5, 100 mM NaCl, 0.2% respective detergent). The receptor was eluted in 20×CMC DDM/PSA/PSE with 5 mM EDTA and 0.2 mg/ml free Flag peptide. The eluate was further applied to superdex-200 10/300 GL column (GE healthcare) at 0.5 ml/min and UV absorbance at 280 nm was recorded. The running buffer contains 20 mM HEPES pH 7.5, 100 mM NaCl, 20×CMC individual detergents (DDM, PSA-C11, PSE-C11, and PSE-C13).

Solubilization assay & ISO competition binding[13] 10 ml PSE-C11 detergent buffer (20 mM HEPES pH 7.5, 100 mM NaCl, 1.0% PSE-C11) was added into 1 gram insect cell (*Sf*9) cell pellet expressing β_2AR. The mixture was stirred for solubilization for 1 hr. After centrifugation at 12,000g for 20 min, the supernatant was collected and loaded on M1 Flag column in the presence of 2 mM $CaCl_2$. The column was washed with PSE-C11 buffer (20 mM HEPES pH 7.5, 100 mM NaCl, 0.2% detergent). The receptor was eluted in 20×CMC PSE-C11, 5 mM EDTA and 0.2 mg/ml free Flag peptide. The receptor activity solubilized and purified in DDM and PSE-C11 were measured by incubating 0.2 pmol β_2AR in individual detergents with 10 nM of [^{3}H]-DHA for 30 min at room temperature. The next procedures were followed as in long-term stability assay. Each measurement was performed in triplicate. The receptor in 20×CMC PSE-C11 or 20×CMC DDM was also applied to SEC in detergent-free buffer (20 mM HEPES pH 7.5, 100 mM NaCl). For competition binding, pure DDM or PSE-C11-solubilised β_2AR and membranes expressing β_2AR were incubated with [^{3}H]-DHA (2 nM of the final concentration) and increasing concentrations (10^{-13}-10^{-4} M of the final concentration) of (−)-isoproterenol for 1 hr at room temperature before harvesting onto GF/B filters. Competition data were fitted to a one-site binding model and IC_{50} of isoproterenol was calculated using GraphPad prism.

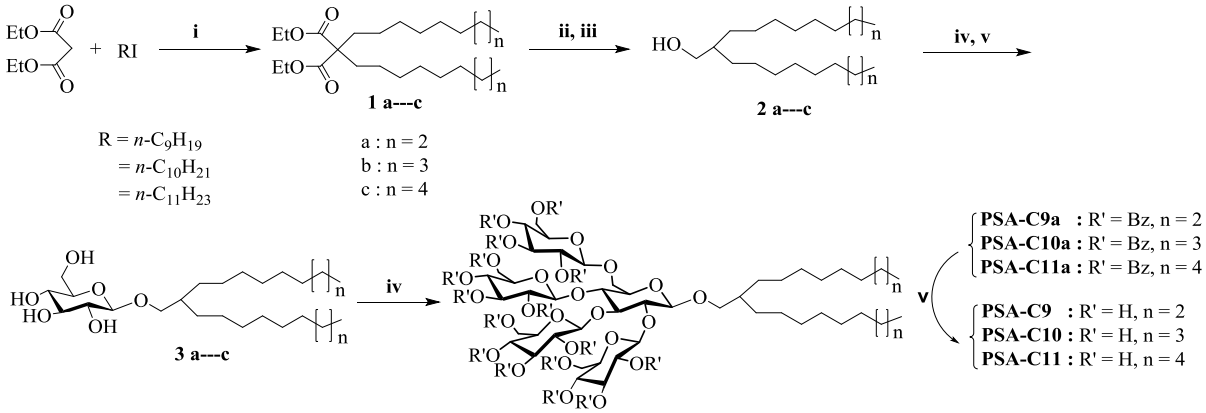
Purification and stability measurement on T4L-β_2AR-G_s complex in PSE-C11 100 μM T4L-β_2AR in 0.1% DDM was mixed with 120 μM G_s heterotrimer for 30 min at room temperature. 0.5 unit apyrase (NEB) and 2 mM $MgCl_2$ was added into to facilitate complex formation for 1 hr. 1% PSE-C11 was further added in the mixture to achieve 0.8% final concentration for incubation of 30 min to initiate detergent exchange from DDM to PSE-C11. The protein solution was loaded on M1 Flag column, washed with a series of buffers in different molar ratio of 0.1% DDM buffer to 0.5% PSE-C11 buffer to allow complete detergent exchange from DDM to PSE-C11, finally eluated with 0.05% (100xCMC) PSE-C11 buffer. A preparative gel filtration was carried out to purify the T4L-β_2AR-G_s complex with running buffer (20mM HEPES pH 7.5, 100 mM NaCl, 0.005% PSE-C11, 1 μM BI, 100 μM TCEP). To measure the stability of the β_2AR-G_s complex in PSE-C11, analytical gel filtrations were performed in a time-dependent manner on 12 hr, 1 d, 3 d, 7 d, 15 d with the same formulation of running buffer as above-mentioned. After 15 day of incubation, analytical gel filtration was done using detergent-free buffer that has the same formulation but without PSE-C11 detergent.

Negative stain EM analysis of T4L-β_2AR-G_s solubilized in PSE-C11 T4L-β_2AR-G_s was prepared for electron microscopy using the conventional negative staining protocol,[14] and imaged at room temperature with a Tecnai T12 electron microscope operated at 120 kV suing low-dose procedures. Images were recorded at a magnification of 71,138x and a defocus value of ~ 1.5 μm on a Gatan US4000 CCD camera. All images were binned (2x2 pixels) to obtain a pixel size of 4.16 Å on the specimen level. Particles were manually excised using e2boxer (part of the EMAN2 software suite).[15]

2D reference-free alignment and classification of particle projections was performed using ISAC. 3,904 projections of T4L-β_2AR-G_s were subjected to ISAC,[16] producing 69 classes consistent over two-way matching and accounting for 3,606 particle projections.

Amphiphile preparation

Supplementary scheme I



i) NaH, 1-iodoalkane (RI), THF, RT; **ii)** LiCl, DMSO, H_2O, 175 °C; **iii)** $LiAlH_4$, THF, RT; **iv)** AgOTf, 2,4,6-collidine, DCM, perbenzoylated glucosylbromide, -45 °C to RT; **v)** NaOMe, MeOH, RT.

General protocol for synthesis of dialkylated diethylmalonate (**1a–c**)

This reaction was carried out according to a method available in the literature[6] with slight modification. NaH (30 mmol) in THF was added to a solution of diethyl malonate (10 mmol) in THF (40 mL) at 0 °C and left stirring for 20 min. After addition of 1-iodoalkane (25 mmol), the reaction mixture was stirred at room temperature for 48 h, quenched by adding ice-cold saturated NH_4Cl and then extracted with diethyl ether. The organic layer was washed with brine and dried with anhydrous Na_2SO_4. After complete evaporation of the solvent, the residue was purified by silica gel column chromatography (EtOAc/hexane) providing dialkylated diethylmalonate as an oily liquid.

Diethyl 2,2-dinonylmalonate (**1a**) was prepared in 90 % yield according to the general procedure for preparation of dialkylated diethylmalonate. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.16 (q, *J* = 8.0 Hz, 4H), 1.85 (q, *J* = 8.8 Hz, 4H), 1.30-1.21 (m, 28H), 1.16 (t, *J* = 8.0 Hz, 6H), 0.87 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.3, 61.1, 57.7, 32.2, 32.1, 31.8, 30.0, 29.8, 29.7, 29.5, 24.0, 22.9, 14.3.

Diethyl 2,2-didecylmalonate (**1b**) was prepared in 92 % yield according to the general procedure for preparation of dialkylated diethylmalonate. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.16 (q, *J* = 8.0 Hz, 4H), 1.85 (q, *J* = 8.8 Hz, 4H), 1.30-1.21 (m, 32H), 1.16 (t, *J* = 8.0 Hz, 6H), 0.87 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.3, 61.1, 57.7, 32.2, 32.1, 30.0, 29.8, 29.7, 29.5, 24.0, 22.9, 14.3.

Diethyl 2,2-diundecylmalonate (**1c**) was prepared in 90 % yield according to the general procedure for preparation of dialkylated diethylmalonate. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.16 (q, *J* = 8.0 Hz, 4H), 1.85 (q, *J* = 8.8 Hz, 4H), 1.30-1.21 (m, 36H), 1.16 (t, *J* = 8.0 Hz, 6H), 0.87 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, CDCl3): δ 172.2, 61.1, 57.7, 32.2, 32.1, 30.0, 29.8, 29.8, 29.5, 29.5, 24.1, 22.9, 14.3.

General procedure for synthesis of dialkylated mono-ol (**2a–c**)

To a solution of dialkylated malonate (**1a–c**; 6.9 mmol) in DMSO were added LiCl (15.2 mmol) and

H_2O (7.7 mmol). The mixture was heated at 175 ℃ for 12 h, cooled, diluted with H_2O. The organic layer obtained by extraction with diethyl ether was washed with water and brine, dried over anhydrous Na_2SO_4. After complete evaporation of the solvent, $LiAlH_4$ (21.3 mmol) was added slowly to the residue dissolved in THF (30 mL) at 0 ℃. The mixture was stirred at room temperature for 4 h, quenched with MeOH, water, a 1 N aqueous HCl solution successively at 0 ℃ and then extracted with diethyl ether two times. The combined organic layer was washed with brine and dried with anhydrous Na_2SO_4. The reaction mixture was purified by silica gel column chromatography (EtOAc/hexane) providing dialkyl-containing mono-ol (**2a–c**) as an oily liquid (80 to 86 % (two steps)).

2-nonylundecan-1-ol (**2a**) was prepared in 82 % yield according to the general procedure for preparation of dialkylated mono-ol. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.54 (d, *J* = 4.0 Hz, 2H), 1.50-1.40 (m, 1H), 1.37-1.20 (m, 32H), 0.88 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 65.7, 40.5, 31.9, 30.9, 30.1, 29.6, 29.3, 26.9, 22.7, 14.1.

2-decyldodecan-1-ol (**2b**) was prepared in 86 % yield according to the general procedure for preparation of dialkylated mono-ol. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.55 (d, *J* = 4.0 Hz, 2H), 1.50-1.40 (m, 1H), 1.37-1.20 (m, 36H), 0.88 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 65.6, 40.5, 31.9, 30.9, 30.1, 29.7, 29.3, 26.9, 22.7, 14.0.

2-undecyltridecan-1-ol (**2c**) was prepared in 85 % yield according to the general procedure for preparation of dialkylated mono-ol. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.55 (d, *J* = 4.0 Hz, 2H), 1.50-1.40 (m, 1H), 1.37-1.20 (m, 40H), 0.88 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 65.7, 40.6, 32.1, 31.1, 30.3, 29.9, 29.8, 29.5, 27.0, 22.8, 14.2.

General procedure for glycosylation and de-O-benzoylation under Zemplén's condition (**3a–c**)

This reaction was carried out according to a literature method[17] with some modifications. Briefly, a mixture of a mono-ol derivative (**2a–c)**, AgOTf (1.2 or 4.5 equiv.), 2,4,6-collidine (0.7 or 2.0 equiv.) in anhydrous CH_2Cl_2 (30 mL) was stirred at -45 ℃. Then perbenzoylated glucosylbromide (1.2 or 4.5 equiv.) in CH_2Cl_2 (30 mL) was added dropwise over 0.5 h into this suspension. The reaction was left to warm to 0 ℃ for 1.5 h. The reaction was monitored by TLC. After completion of reaction (as detected by TLC), pyridine was added to the reaction mixture. Reaction mixture was diluted with CH_2Cl_2 (30 mL) before being filtered over celite. The filtrate was washed successively with a 1 M aqueous $Na_2S_2O_3$ solution (30 mL), a 0.1 M aqueous HCl solution (30 mL), and brine (30 mL). Then the organic layer was dried with anhydrous Na_2SO_4 and the solvents were removed by rotary evaporation. The glycosylated residues 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/CH_2Cl_2) as a white solid (84 to 88 % (two steps)).

A compound (**3a**) was prepared in 86 % yield according to the general procedure for glycosylation

and de-*O*-benzoylations. ^{1}H NMR (400 MHz, CD_3OD): δ 4.22 (d, *J* = 8.0 Hz, 1H), 3.85-3.82 (m, 2H), 3.72-3.66 (m, 1H), 3.40-3.30 (m, 3H), 3.26-3.15 (m, 2H), 1.61 (br s, 1H), 1.38 (s, 2H), 1.30-1.26 (m, 30H), 0.90 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ 104.8, 78.1, 77.8, 75.1, 74.1, 71.6, 62.8, 39.6, 33.2, 32.3, 31.3, 30.9, 30.6, 27.9, 23.9, 14.7

A compound (**3b**) was prepared in 88 % yield according to the general procedure for glycosylation and de-*O*-benzoylations. ^{1}H NMR (400 MHz, CD_3OD): δ 4.22 (d, *J* = 8.0 Hz, 1H), 3.85-3.82 (m, 2H), 3.72-3.66 (m, 1H), 3.39-3.30 (m, 3H), 3.26-3.15 (m, 2H), 1.61 (br s, 1H), 1.38 (s, 2H), 1.30-1.26 (m, 34H), 0.90 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ 104.8, 78.1, 77.8, 75.1, 74.1, 71.6, 62.82, 39.6, 33.2, 32.3, 32.2, 31.3, 30.9, 30.9, 30.6, 27.9, 23.9, 14.7.

A compound (**3c**) was prepared in 88 % yield according to the general procedure for glycosylation and de-*O*-benzoylations. ^{1}H NMR (400 MHz, CD_3OD): δ 4.22 (d, *J* = 8.0 Hz, 1H), 3.85-3.82 (m, 2H), 3.72-3.66 (m, 1H), 3.39-3.30 (m, 3H), 3.26-3.15 (m, 2H), 1.61 (br s, 1H), 1.38 (s, 2H), 1.30-1.26 (m, 38H), 0.89 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ 104.8, 78.2, 77.8, 75.1, 74.1, 71.6, 62.8, 39.6, 33.2, 32.3, 32.2, 31.3, 31.0, 30.9, 30.9, 30.6, 27.9, 23.9, 14.7.

PSA-C9a was synthesized according to the general procedure for glycosylation. Yield: 75 %; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26 (d, *J* = 8.0 Hz, 2H), 8.20-7.61 (m, 30H), 7.60-7.55 (m, 2H), 7.43-7.18 (m, 46H), 5.95 (t, *J* = 8.0 Hz, 1H), 5.90-5.81 (m, 3H), 5.80-5.70 (m, 2H), 5.60-5.45 (m, 6H), 4.99-4.80 (m, 5H), 4.72-4.62 (d, *J* = 8.0 Hz, 2H), 4.60-4.50 (m, 4H), 4.40-4.32 (m, 1H), 4.20-4.00 (m, 5H), 3.92 (t, *J* = 8.0 Hz, 1H), 3.82-3.75 (m, 3H), 3.68-3.64 (m, 1H), 3.39-3.32 (m, 1H), 3.12-3.01 (m, 1H), 2.91-2.88 (m, 1H), 2.71-2.65 (m, 1H), 1.32-1.10 (m, 32H), 0.84 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.2, 166.1, 166.0, 165.9, 165.8, 165.7, 165.2, 165.1, 164.9, 164.5, 164.4, 133.6, 133.4, 133.3, 133.2, 133.0, 130.2, 129.9, 129.8, 129.7, 129.6, 129.5, 129.3, 129.1, 129.0, 128.9, 128.6, 128.5, 128.4, 128.3, 128.2, 101.4, 100.5, 99.9, 99.8, 78.0, 75.9, 74.9, 73.2, 73.0, 72.8, 72.5, 72.4, 72.0, 71.8, 71.2, 70.6, 70.3, 70.0, 69.2, 38.3 32.0, 31.2, 30.9, 30.5, 30.4, 30.0, 29.9, 29.8, 29.5, 27.0, 26.8, 22.7, 14.2.

PSA-C10a was synthesized according to the general procedure for glycosylation. Yield: 75 %; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26 (d, *J* = 8.0 Hz, 2H), 8.20-7.61 (m, 30H), 7.60-7.55 (m, 2H), 7.43-7.18 (m, 46H), 5.95 (t, *J* = 8.0 Hz, 1H), 5.90-5.81 (m, 3H), 5.80-5.70 (m, 2H), 5.60-5.45 (m, 6H), 4.99-4.80 (m, 5H), 4.72-4.62 (d, *J* = 8.0 Hz, 2H), 4.60-4.50 (m, 4H), 4.40-4.32 (m, 1H), 4.20-4.00 (m, 5H), 3.92 (t, *J* = 8.0 Hz, 1H), 3.82-3.75 (m, 3H), 3.68-3.64 (m, 1H), 3.39-3.32 (m, 1H), 3.12-3.01 (m, 1H), 2.91-2.88 (m, 1H), 2.71-2.65 (m, 1H), 1.32-1.09 (m, 36H), 0.84 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.2, 166.1, 166.0, 165.9, 165.8, 165.7, 165.2, 165.2, 165.1, 164.9, 164.5, 164.4, 133.6, 133.4, 133.3, 133.2, 133.0, 130.2, 129.9, 129.8, 129.7, 129.6, 129.5, 129.3, 129.1, 129.0, 128.9, 128.6, 128.5, 128.4, 128.3, 128.2, 101.4, 100.5, 99.9, 99.8, 78.0, 75.9, 74.9, 73.2, 73.0, 72.8, 72.5, 72.4, 72.0, 71.8, 71.2, 70.6, 70.3, 70.0, 69.2, 38.3, 32.0, 31.2, 30.9, 30.5, 30.3, 30.0, 29.9, 29.8, 29.5, 27.0, 26.8, 22.8, 14.2.

PSA-C11a was synthesized according to the general procedure for glycosylation. Yield: 72 %; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26 (d, *J* = 8.0 Hz, 2H), 8.20-7.61 (m, 30H), 7.60-7.55 (m, 2H), 7.45-7.20 (m, 46H), 5.95 (t, *J* = 8.0 Hz, 1H), 5.90-5.81 (m, 3H), 5.80-5.70 (m, 2H), 5.60-5.45 (m, 6H),

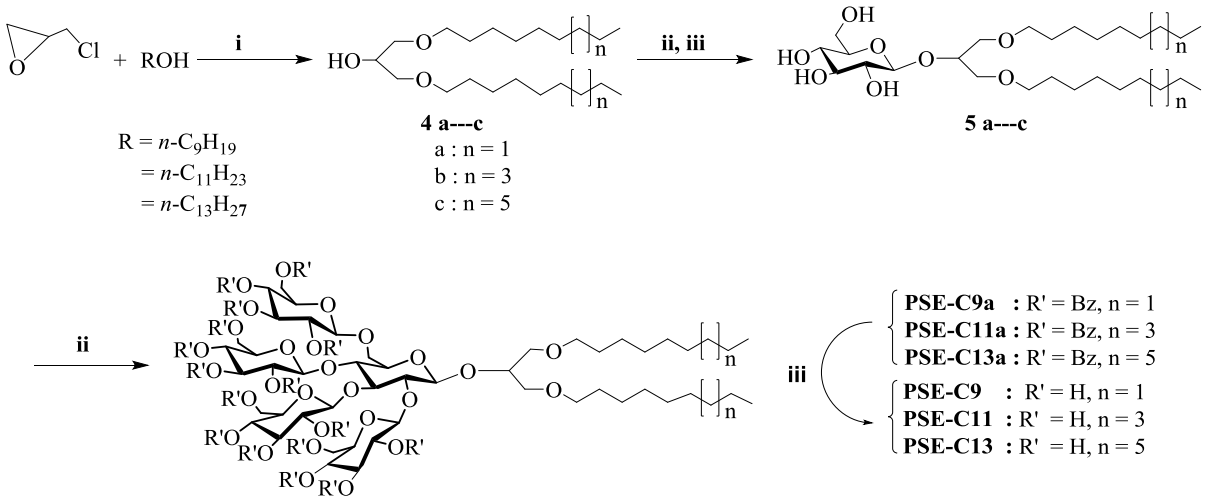
4.99-4.80 (m, 5H), 4.72-4.62 (d, *J* = 8.0 Hz, 2H), 4.60-4.50 (m, 4H), 4.40-4.32 (m, 1H), 4.20-4.00 (m, 5H), 3.92 (t, *J* = 8.0 Hz, 1H), 3.82-3.75 (m, 3H), 3.68-3.64 (m, 1H), 3.39-3.32 (m, 1H), 3.12-3.01 (m, 1H), 2.91-2.88 (m, 1H), 2.71-2.65 (m, 1H), 1.35-1.09 (m, 40H), 0.84 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.2, 166.1, 166.0, 165.9, 165.8, 165.7, 165.2, 165.2, 165.1, 164.9, 164.5, 164.4, 133.6, 133.4, 133.3, 133.2, 133.0, 130.2, 129.9, 129.8, 129.7, 129.6, 129.5, 129.3, 129.1, 129.0, 128.9, 128.6, 128.5, 128.4, 128.3, 128.2, 101.4, 100.5, 99.9, 99.8, 78.0, 75.9, 74.9, 73.2, 73.0, 72.8, 72.5, 72.4, 72.0, 71.8, 71.2, 70.6, 70.3, 70.0, 69.2, 38.3, 32.0, 31.2, 30.9, 30.5, 30.4, 30.0, 29.9, 29.8, 29.4, 27.0, 26.7, 22.8, 14.2.

PSA-C9 was synthesized according to the general procedure for de-*O*-benzoylation. Yield: 91 %; ^{1}H NMR (400 MHz, CD_3OD): δ 4.97 (d, *J* = 8.0 Hz, 1H), 4.79 (d, *J* = 8.0 Hz, 1H), 4.68 (d, *J* = 8.0 Hz, 1H), 4.46 (d, *J* = 8.0 Hz, 1H), 4.40 (d, *J* = 8.0 Hz, 1H), 4.28 (d, *J* = 8.0 Hz, 1H), 4.09 (t, *J* = 8.0 Hz, 1H), 3.90-3.78 (m, 8H), 3.70-3.62 (m, 5H), 3.45-3.27 (m, 18H), 1.60 (br s, 1H), 1.39-1.20 (m, 32H), 0.90 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ 104.7, 103.6, 103.3, 102.3, 81.7, 79.8, 78.3, 78.1, 77.8, 76.1, 75.9, 75.2, 75.1, 75.0, 74.5, 71.6, 63.1, 62.9, 62.5, 39.5, 33.2, 31.3, 30.9, 30.6, 28.0, 27.8, 23.8, 14.6; HRMS (EI): calcd. for $C_{50}H_{92}O_{26}$ $[M+Na]^+$ 1131.5775, found 1131.5778.

PSA-C10 was synthesized according to the general procedure de-*O*-benzoylation. Yield: 92 %; ^{1}H NMR (400 MHz, CD_3OD): δ 4.97 (d, *J* = 8.0 Hz, 1H), 4.79 (d, *J* = 8.0 Hz, 1H), 4.68 (d, *J* = 8.0 Hz, 1H), 4.46 (d, *J* = 8.0 Hz, 1H), 4.40 (d, *J* = 8.0 Hz, 1H), 4.28 (d, *J* = 8.0 Hz, 1H), 4.09 (t, *J* = 8.0 Hz, 1H), 3.90-3.78 (m, 8H), 3.70-3.61 (m, 5H), 3.45-3.26 (m, 18H), 1.60 (br s, 1H), 1.42-1.20 (m, 36H), 0.90 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ 104.7, 103.6, 103.3, 102.3, 81.7, 79.8, 78.3, 78.1, 77.8, 76.1, 75.9, 75.2, 75.1, 75.0, 74.5, 71.6, 63.1, 62.9, 62.5, 39.5, 33.2, 31.3, 30.9, 30.6, 28.0, 27.8, 23.8, 14.6; HRMS (EI): calcd. for $C_{52}H_{96}O_{26}$ $[M+Na]^+$ 1159.6088, found 1159.6086.

PSA-C11 was synthesized according to the general procedure for de-*O*-benzoylation. Yield: 90 %; ^{1}H NMR (400 MHz, CD_3OD): δ 4.97 (d, *J* = 8.0 Hz, 1H), 4.79 (d, *J* = 8.0 Hz, 1H), 4.68 (d, *J* = 8.0 Hz, 1H), 4.46 (d, *J* = 8.0 Hz, 1H), 4.40 (d, *J* = 8.0 Hz, 1H), 4.28 (d, *J* = 8.0 Hz, 1H), 4.09 (t, *J* = 8.0 Hz, 1H), 3.90-3.78 (m, 8H), 3.70-3.62 (m, 5H), 3.45-3.26 (m, 18H), 1.60 (br s, 1H), 1.42-1.20 (m, 40H), 0.90 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ 104.8, 103.6, 103.4, 102.4, 81.7, 79.8, 78.3, 78.1, 77.8, 76.1, 75.9, 75.2, 75.1, 75.0, 74.5, 71.7,71.3, 69.6, 63.1, 62.9, 62.6, 39.5, 33.2, 31.3, 30.9, 30.6, 28.0, 27.8, 23.9, 14.6; HRMS (EI): calcd. for $C_{54}H_{100}O_{26}$ $[M+Na]^+$ 1187.6401, found 1187.6396.

Supplementary scheme II



i) NaOH, ROH, 120 ℃; **ii)** AgOTf, 2,4,6-collidine, DCM, perbenzoylated glucosylbromide, -45 ℃ to RT; **iii)** NaOMe, MeOH, RT.

General procedure for synthesis of alcohol derivatives (**4a–c)**

This reaction was carried out according to a literature method[18] with slight modification. Epichlorohydrine (0.124 mmol) was added to an alcohol solution (0.43 mmol) with NaOH (0.25 mmol) under argon. The mixture was heated at 120 ℃ and stirred overnight at this temperature. On cooling to room temperature, the reaction mixture was diluted with 40 mL distilled water and the aqueous phase was extracted with CH_2Cl_2. The organic phase was dried with anhydrous Na_2SO_4 and evaporated to dryness by roto-evaporator. Target compounds (**4a–c)** were isolated as oily residues by vacuum distillation.

1,3-bis(nonyloxy)propan-2-ol (**4a**) was prepared in 77 % yield according to the general procedure for preparation of alcohol derivatives. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.95–3.93 (m, 1H), 3.47–3.43 (m, 8H), 2.47 (d, J = 4.0 Hz, 1H), 1.57–1.54 (m, 4H), 1.30–1.26 (m, 24H), 0.88 (t, J = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 71.9, 71.7, 70.0, 32.1, 29.7, 29.6, 29.5, 29.4, 26.2, 22.7, 14.3.

1,3-bis(undecyloxy)propan-2-ol (**4b**) was prepared in 77 % yield according to the general procedure for preparation of alcohol derivatives. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.95–3.93 (m, 1H), 3.43–3.40 (m, 8H), 2.47 (d, J = 4.0 Hz, 1H), 1.57–1.54 (m, 4H), 1.32–1.25 (m, 32H), 0.88 (t, J = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 71.9, 71.7, 69.5, 31.9, 29.7, 29.5, 29.4, 26.1, 22.7, 14.3.

1,3-bis(tridecyloxy)propan-2-ol (**4c**) was prepared in 75 % yield according to the general procedure for preparation of alcohol derivatives. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.95–3.92 (m, 1H), 3.45–3.40 (m, 8H), 2.47 (d, J = 4.0 Hz, 1H), 1.57–1.54 (m, 4H), 1.32–1.25 (m, 40H), 0.88 (t, J = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 71.9, 71.7, 70.0, 32.1, 29.7, 29.6, 29.5, 29.4, 26.2, 22.9, 14.3.

Compounds (**5a–c)** and (**PSE-C9, PSE-C11 and PSE-C13)** were synthesized according to the same procedure as described for compounds (**3a–c)** and **(PSA-C9, PSA-C10 and PSA-C11)** in **supplementary scheme I**.

A compound **(5a)** was synthesized according to the general procedure glycosylation and de-*O*-benzoylation. Yield: 86 %; ^{1}H NMR (400 MHz, CD_3OD): δ 4.45 (d, *J* = 8.0 Hz, 1H), 4.01 (quint, *J* = 8.0 Hz 1H), 3.87-3.82 (m, 1H), 3.60-3.56 (m, 4H), 3.52-3.46 (m, 4H), 3.36-3.20 (m, 4H), 3.18 (t, *J* = 8.0 Hz, 1H), 1.60-1.55 (m, 4H), 1.42-1.24 (m, 24H), 0.90 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ 104.1, 103.9, 80.1, 78.4, 78.2, 77.9, 75.2, 72.7, 71.9, 71.8, 71.5, 71.3, 71.1, 62.9, 33.2, 30.9, 30.4, 27.4, 27.3, 23.8, 14.6.

A compound (**5b)** was synthesized according to the general procedure for glycosylation and de-*O*-benzoylation. Yield: 86 %; ^{1}H NMR (400 MHz, CD_3OD): δ 4.46 (d, *J* = 8.0 Hz, 1H), 3.99 (quint, *J* = 8.0 Hz 1H), 3.88-3.81 (m, 1H), 3.65-3.56 (m, 4H), 3.52-3.46 (m, 4H), 3.36-3.20 (m, 4H), 3.18 (t, *J* = 8.0 Hz, 1H), 1.60-1.55 (m, 4H), 1.42-1.24 (m, 32H), 0.90 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ 104.0, 103.9, 80.1, 79.9, 78.3, 78.2, 77.8, 75.1, 72.7, 71.9, 71.8, 71.5, 71.3, 71.1, 62.8, 33.2, 31.0, 30.9, 30.8, 30.4, 27.4, 27.3, 23.8, 14.7.

A compound (**5c)** was synthesized according to the general procedure for glycosylation and de-*O*-benzoylation. Yield: 85 %; ^{1}H NMR (400 MHz, CD_3OD): δ 4.46 (d, *J* = 8.0 Hz, 1H), 3.99 (quint, *J* = 8.0 Hz 1H), 3.87-3.81 (m, 1H), 3.65-3.56 (m, 4H), 3.52-3.46 (m, 4H), 3.36-3.20 (m, 4H), 3.18 (t, *J* = 8.0 Hz, 1H), 1.60-1.55 (m, 4H), 1.42-1.24 (m, 40H), 0.89 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ 104.0, 103.9, 80.1, 79.9, 78.3, 78.2, 77.8, 75.1, 72.7, 71.9, 71.8, 71.5, 71.3, 71.1, 62.8, 33.2, 31.0, 30.9, 30.8, 30.7, 27.5, 27.4, 27.3, 23.8, 14.7.

PSE-C9a was synthesized according to the general procedure glycosylation. Yield: 82 %; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (d, *J* = 8.0 Hz, 2H), 8.16-7.98 (m, 26H), 7.80-7.70 (m, 4H), 7.60-7.52 (m, 2H), 7.50-7.18 (m, 46H), 5.91-5.81 (m, 3H), 5.73-5.61 (m, 5H), 5.58-5.52 (m, 2H), 5.48-5.44 (m, 2H), 5.07 (d, *J* = 7.6 Hz, 2H), 4.96-4.71 (m, 3H), 4.69 (d, *J* = 3.2 Hz, 1H), 4.65-4.56 (m, 3H), 4.52-4.39 (m, 4H), 4.23-3.96 (m, 6H), 3.83-3.76 (m, 3H), 3.68 (t, *J* = 8.0 Hz, 1H), 3.52-3.23 (m, 8H), 3.15 (br s, 1H), 3.01 (br s, 1H), 1.71-1.62 (m, 2H), 1.49-1.42 (m, 2H), 1.40-1.17 (m, 24H), 0.84 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.2, 166.0, 165.8, 165.7, 165.2, 165.1, 164.6, 164.5, 133.6, 133.4, 133.3, 133.1, 132.9, 130.2, 129.9, 129.4, 129.3, 129.2, 129.1, 129.0, 128.6, 128.5, 128.4, 128.3, 128.2, 101.6, 100.5, 99.5, 99.3, 74.9, 73.4, 72.9, 72.8, 72.5, 72.2, 71.9, 71.7, 71.6, 71.2, 70.7, 70.3, 69.4, 63.8, 63.6, 62.9, 60.4, 53.6, 32.0, 29.9, 29.8, 29.7, 29.5, 26.2, 22.8, 14.3, 14.1.

PSE-C11a was synthesized according to the general procedure for glycosylation. Yield: 82 %; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (d, *J* = 8.0 Hz, 2H), 8.16-7.98 (m, 26H), 7.80-7.70 (m, 4H), 7.60-7.52 (m, 2H), 7.50-7.18 (m, 46H), 5.92-5.81 (m, 3H), 5.73-5.61 (m, 5H), 5.58-5.52 (m, 2H), 5.48-5.44 (m, 2H), 5.07 (d, *J* = 7.6 Hz, 2H), 4.96-4.71 (m, 3H), 4.69 (d, *J* = 3.2 Hz, 1H), 4.65-4.58 (m, 3H), 4.52-4.39 (m, 4H), 4.23-3.95 (m, 6H), 3.83-3.76 (m, 3H), 3.68 (t, *J* = 8.0 Hz, 1H), 3.52-3.23 (m, 8H), 3.09 (br s, 1H), 3.01 (br s, 1H), 1.71-1.62 (m, 2H), 1.49-1.42 (m, 2H), 1.41-1.18 (m, 32H), 0.84 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.2, 166.0, 165.8, 165.7, 165.2, 165.1, 164.6, 164.5, 133.6, 133.4, 133.3, 133.1, 132.9, 130.2, 129.9, 129.4, 129.3, 129.2, 129.1, 129.0, 128.6, 128.5, 128.4, 128.3, 128.2, 101.6, 100.5, 99.5, 99.3, 74.9, 73.4, 72.9, 72.8, 72.5, 71.9, 71.2, 71.7, 71.6, 71.2, 70.7, 70.3, 69.4, 63.8, 63.6, 62.9, 60.4, 53.6, 32.0, 29.9, 29.8, 29.7, 29.5, 26.3, 26.2, 22.8, 14.3, 14.1.

PSE-C13a was synthesized according to the general procedure for glycosylation. Yield: 80 %; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (d, *J* = 8.0 Hz, 2H), 8.16-7.98 (m, 26H), 7.80-7.70 (m, 4H), 7.60-7.52 (m, 2H), 7.50-7.18 (m, 46H), 5.92-5.81 (m, 3H), 5.73-5.61 (m, 5H), 5.58-5.52 (m, 2H), 5.48-5.44 (m, 2H), 5.07 (d, *J* = 7.6 Hz, 2H), 4.96-4.70 (m, 3H), 4.69 (d, *J* = 3.2 Hz, 1H), 4.65-4.58 (m, 3H), 4.52-4.39 (m, 4H), 4.23-3.96 (m, 6H), 3.83-3.76 (m, 3H), 3.68 (t, *J* = 8.0 Hz, 1H), 3.52-3.23 (m, 8H), 3.09 (br s, 1H), 3.01 (br s, 1H), 1.71-1.62 (m, 2H), 1.49-1.42 (m, 2H), 1.41-1.18 (m, 40H), 0.84 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.2, 166.0, 165.8, 165.7, 165.2, 165.2, 165.1, 165.1, 164.6, 164.5, 133.6, 133.4, 133.3, 133.1, 132.9, 130.2, 129.9, 129.4, 129.3, 129.2, 129.1, 129.0, 128.6, 128.5, 128.4, 128.3, 128.2, 101.6, 100.5, 99.5, 99.3, 74.9, 73.4, 72.9, 72.8, 72.5, 71.9, 71.2, 71.7, 71.6, 71.2, 70.7, 70.3, 69.4, 63.8, 63.6, 62.9, 60.4, 53.6, 32.0, 29.9, 29.8, 29.7, 29.5, 26.3, 26.2, 22.8, 14.3.

PSE-C9 was synthesized according to the general procedure for de-*O*-benzoylation. Yield: 92 %; ^{1}H NMR (400 MHz, CD_3OD): δ 4.95 (d, *J* = 8.0 Hz, 1H), 4.76 (d, *J* = 8.0 Hz, 1H), 4.71 (d, *J* = 7.2 Hz, 1H), 4.66 (d, *J* = 8.0 Hz, 1H), 4.38 (d, *J* = 8.0 Hz, 1H), 4.26 (d, *J* = 9.6 Hz, 1H), 4.01 (t, *J* = 8.0 Hz, 1H), 4.99-3.80 (m, 8H), 3.72-3.55 (m, 8H), 3.51-3.19 (m, 20H), 1.58 (m, 4H), 1.39-1.20 (m, 24H), 0.90 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ 104.8, 103.7, 103.1, 102.6, 102.4, 81.8, 79.7, 78.2, 78.1, 78.0, 77.9, 77.8, 76.0, 75.9, 75.2, 75.0, 72.8, 72.7, 71.8, 71.7, 71.6, 71.5, 71.1, 69.8, 63.0, 62.9, 62.6, 33.2, 30.9, 30.8, 30.7, 30.6, 27.4, 23.8, 14.6; HRMS (EI): calcd. for $C_{51}H_{94}O_{28}$ $[M+Na]^+$ 1177.5829, found 1177.5833.

PSE-C11 was synthesized according to the general procedure for de-*O*-benzoylation. Yield: 92 %; ^{1}H NMR (400 MHz, CD_3OD): δ 4.97 (d, *J* = 7.6 Hz, 1H), 4.77 (d, *J* = 8.0 Hz, 1H), 4.70 (d, *J* = 7.2 Hz, 1H), 4.67 (d, *J* = 7.6 Hz, 1H), 4.38 (d, *J* = 8.0 Hz, 1H), 4.26 (d, *J* = 10.4 Hz, 1H), 4.09 (t, *J* = 8.4 Hz, 1H), 4.00-3.68 (m, 8H), 3.70-3.55 (m, 8H), 3.50-3.20 (m, 20H), 1.57 (m, 4H), 1.39-1.20 (m, 32H), 0.89 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ 104.8, 103.6, 103.1, 102.6, 102.4, 81.8, 79.7, 78.2, 78.1, 78.0, 77.9, 77.8, 76.0, 75.9, 75.2, 75.0, 72.8, 72.7, 71.8, 71.7, 71.6, 71.5, 71.1, 69.8, 63.0, 62.9, 62.6, 33.2, 30.9, 30.9, 30.8, 30.7, 30.6, 27.4, 23.8, 14.6; HRMS (EI): calcd. for $C_{55}H_{102}O_{28}$ $[M+Na]^+$ 1233.6455, found 1233.6451.

PSE-C13 was synthesized according to the general procedure for de-*O*-benzoylation. Yield: 90 %; ^{1}H NMR (400 MHz, CD_3OD): δ 4.95 (d, *J* = 7.6 Hz, 1H), 4.76 (d, *J* = 7.6 Hz, 1H), 4.71 (d, *J* = 7.2 Hz, 1H), 4.66 (d, *J* = 7.6 Hz, 1H), 4.38 (d, *J* = 8.0 Hz, 1H), 4.26 (d, *J* = 9.6 Hz, 1H), 4.08 (t, *J* = 8.0 Hz, 1H), 4.03-3.80 (m, 8H), 3.72-3.55 (m, 8H), 3.50-3.19 (m, 20H), 1.58 (m, 4H), 1.39-1.20 (m, 40H), 0.89 (t, *J* = 8.0 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD): δ 104.9, 103.6, 103.1, 102.6, 102.4, 81.8, 79.7, 78.2, 78.1, 78.0, 77.9, 77.8, 76.0, 75.9, 75.2, 75.0, 72.8, 72.7, 71.8, 71.7, 71.6, 71.5, 71.1, 69.8, 63.0, 62.9, 62.6, 33.2, 31.1, 30.9, 30.8, 30.7, 30.6, 27.5, 23.9, 14.6. HRMS (EI): calcd. for $C_{59}H_{110}O_{28}$ $[M+Na]^+$ 1289.7081, found 1289.7078.

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