Supporting Information

Conformationally Preorganized Diastereomeric Norbornane-Based Maltosides for Membrane Protein Study: Implications of Detergent Kink for Micellar Properties

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Supporting Information

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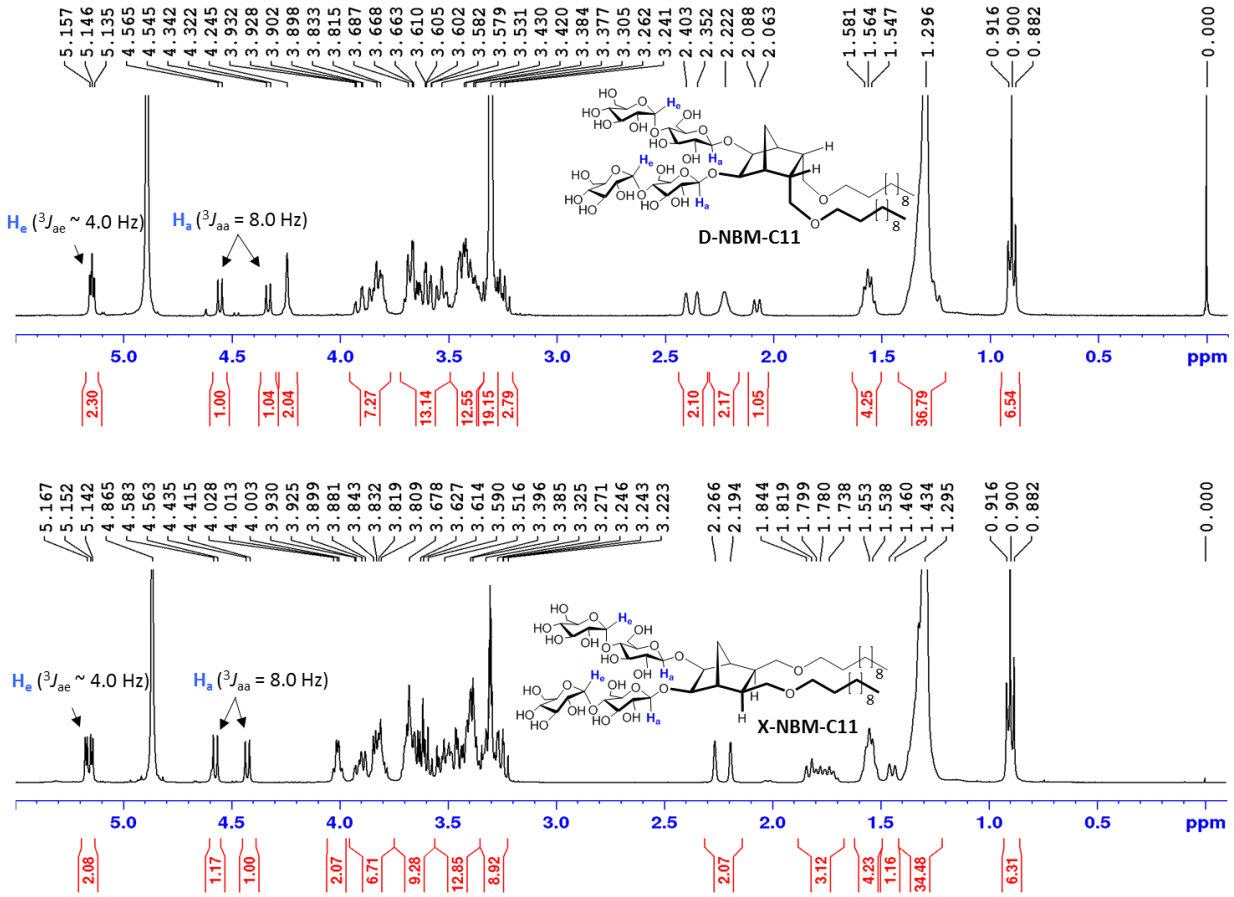


Figure S1. ^{1}H NMR spectra of NBM-C11 isomers (D-NBM-C11 and X-NBM-C11). High isomeric purity of D-NBM-C11 and X-NBM-C11 were confirmed by the individual spectra. The two sets of anomeric protons in the maltoside head group, labeled H_a and H_e in the chemical structures, are different in terms of their chemical shift (δ) and coupling constant (^{3}J). Assignments for anomeric protons along with the coupling constants ($^{3}J_{aa}$ and $^{3}J_{ae}$) are given above the peaks in the individual NMR spectra. The peaks for the anomeric protons (H_e) appeared at ~5.15 ppm with a vicinal axial-equatorial coupling constant ($^{3}J_{ae}$) of ~4.0 Hz, whereas the peaks for the other anomeric protons (H_a) appeared as two seperate doublets individually centered at 4.33 and 4.55 ppm for D-NBM-C11 and 4.42 and 4.57 ppm for X-NBM-C11, with vicinal axial-axial coupling ($^{3}J_{aa}$) of 8.0 Hz in both two isomers. The chemical sthifts and coupling constants observed here are typical for anomeric protons with α- and β-glycosidic bonds (H_e and H_a), respectively.

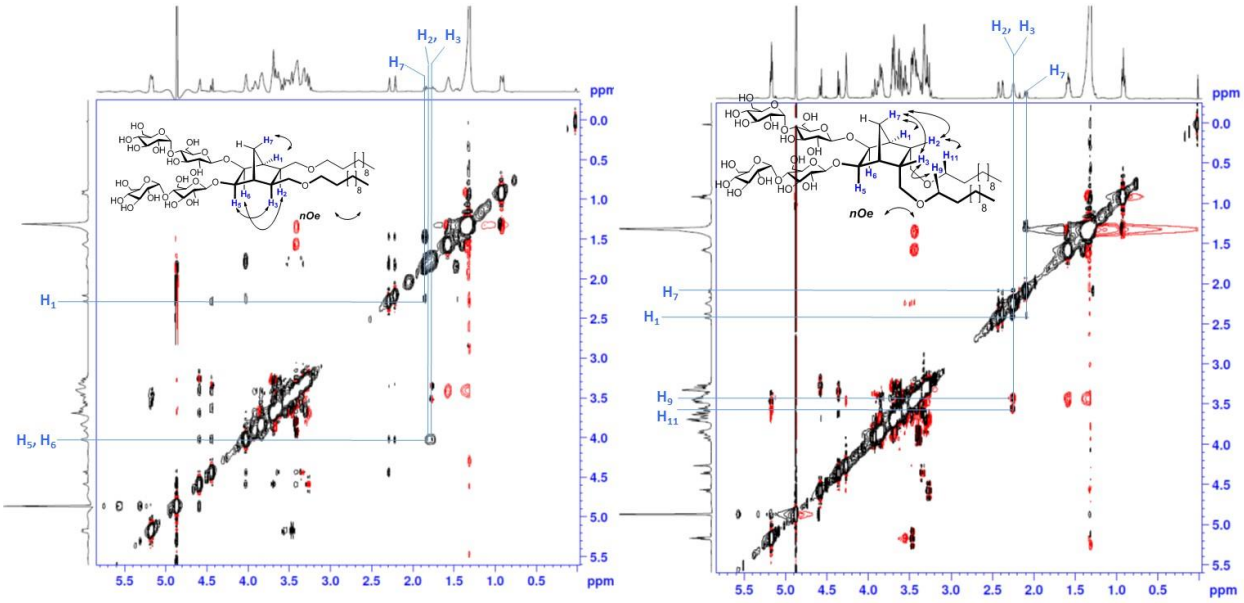


Figure S2. ^{1}H-^{1}H NOESY spectra (400 MHz) of X-/D-NBM-C11 in CD_3OD. X-NBM-C11 gave strong NOE correlation signals between protons (H_2 and H_3) and protons (H_5 and H_6), which are not seen for the D-isomer. In the X-isomer, an NOE correlation signal between H_7 and H_1 was found because of their close proximity in space, but H_7 is too far from protons (H_2 and H_3) to give a detectable signal. In contrast, the NOESY spectra of D-NBM-C11 gave a strong NOE correlation signal between H_7 and protons (H_2 and H_3). Distinctive NOE correlation signals were additionally observed between the protons (H_2 and H_3) and protons (H_9 and H_{11}) in this isomer.

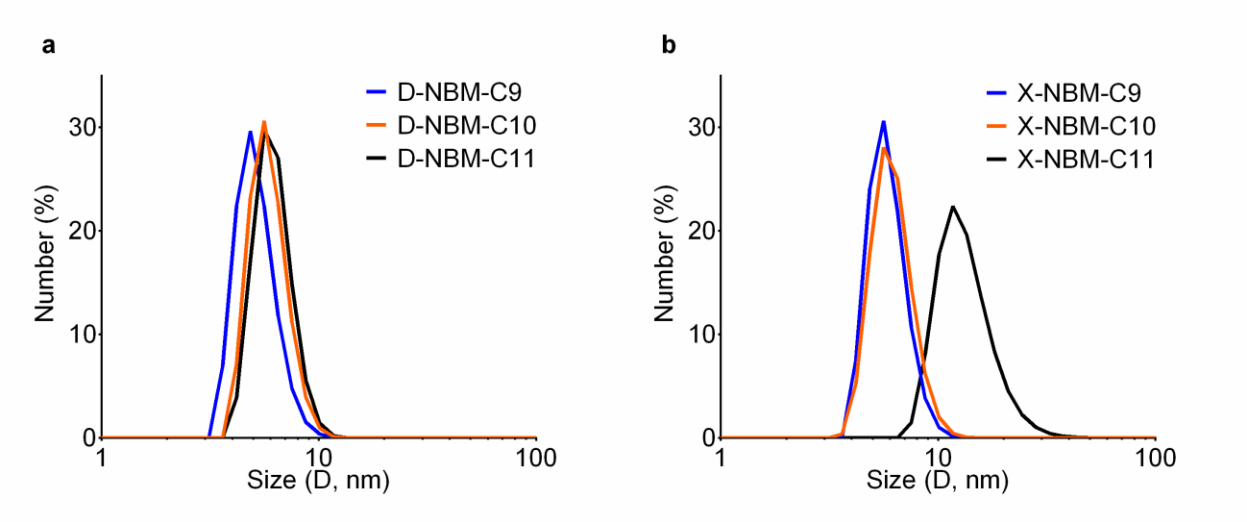


Figure S3. Dynamic light scattering (DLS) profiles at 25°C for micelles formed by NBM isomers with a C9, C10 or C11 alkyl chain. These agents showed a single micelle size population when used at ~1.0 wt%. Autocorrelation analysis for time-dependent fluctuation in the scattered light intensity gave the translational diffusion coefficient (D). The Stokes-Einstein equation was used to calculate the hydrodynamic diameter (D_H) of detergent micelles from the translational diffusion coefficient.

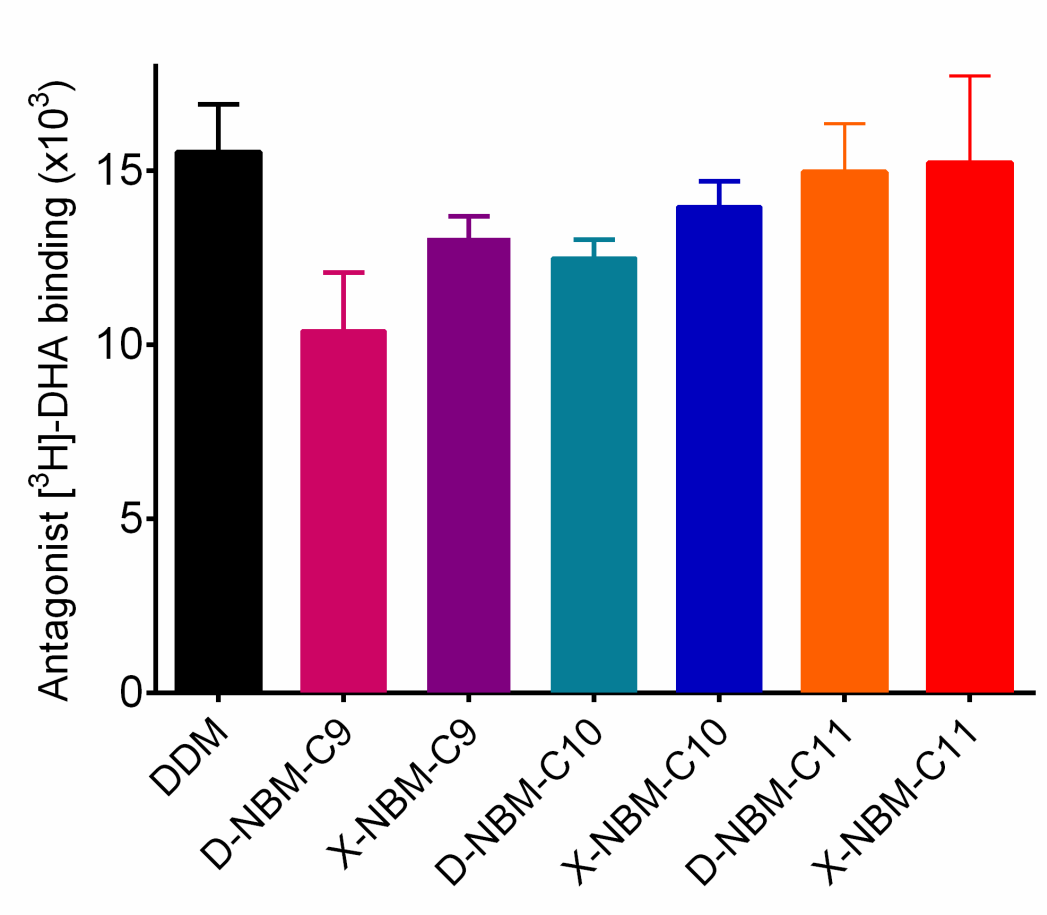


Figure S4. Ligand binding activity of β_2AR solubilized in DDM or an NBM (X-NBM-C9, D-NBM-C9, X-NBM-C10, D-NBM-C10, X-NBM-C11, or D-NBM-C11). DDM was used as a positive control. DDM-purified receptor was diluted into buffer solution containing individual NBMs to reach a final concentration of CMC+0.2 wt%. The ligand binding activity of the receptor was measured using the antagonist, [^{3}H]-dihydroalprenolol (DHA), 30-min after dilution. Error bars, SEM, n = 3.

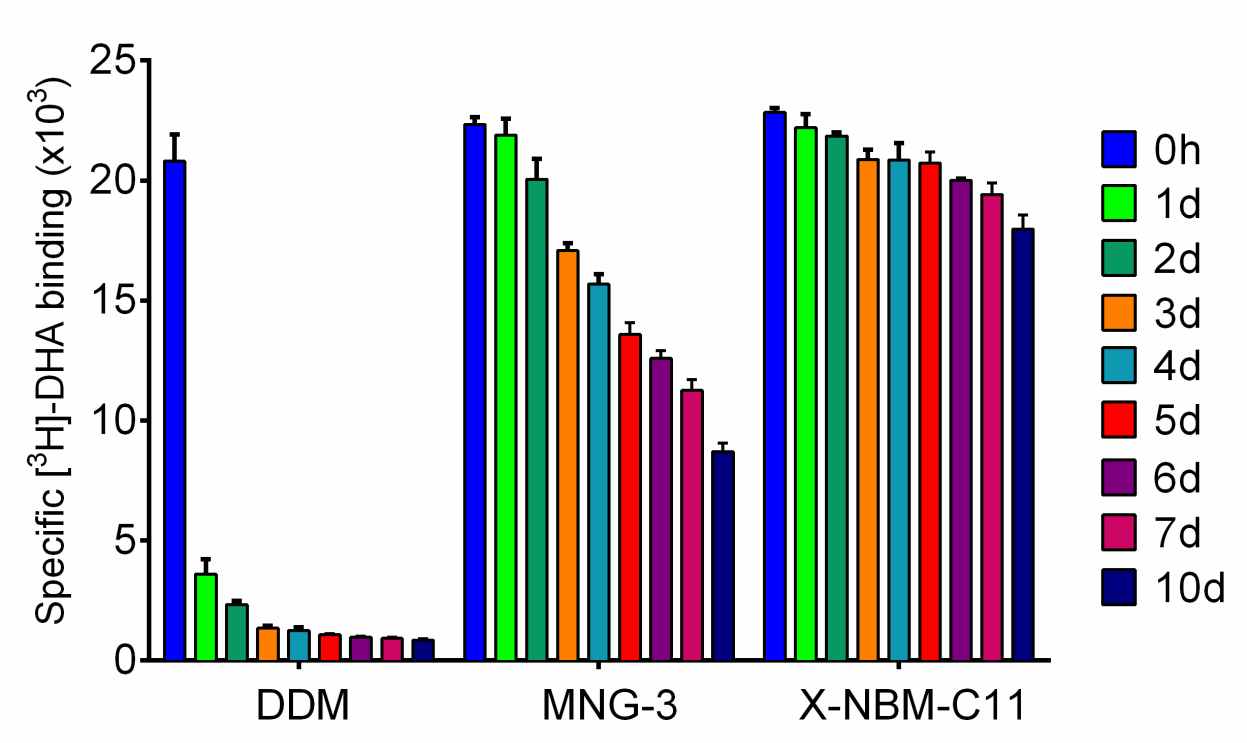


Figure S5. Long-term stability of β_2AR solubilized in DDM, MNG-3, or a representative NBM (X-NBM-C11). DDM-purified receptor was diluted into buffer solutions containing individual NBMs to reach a final concentration of CMC+0.2 wt%. The specific ligand binding activity of the receptor was measured using the antagonist [^{3}H]-dihydroalprenolol (DHA) following a 30-min dilution. The activity of the receptor was further measured at regular intervals during a 10-day incubation at room temperature. Error bars, SEM, n = 3.

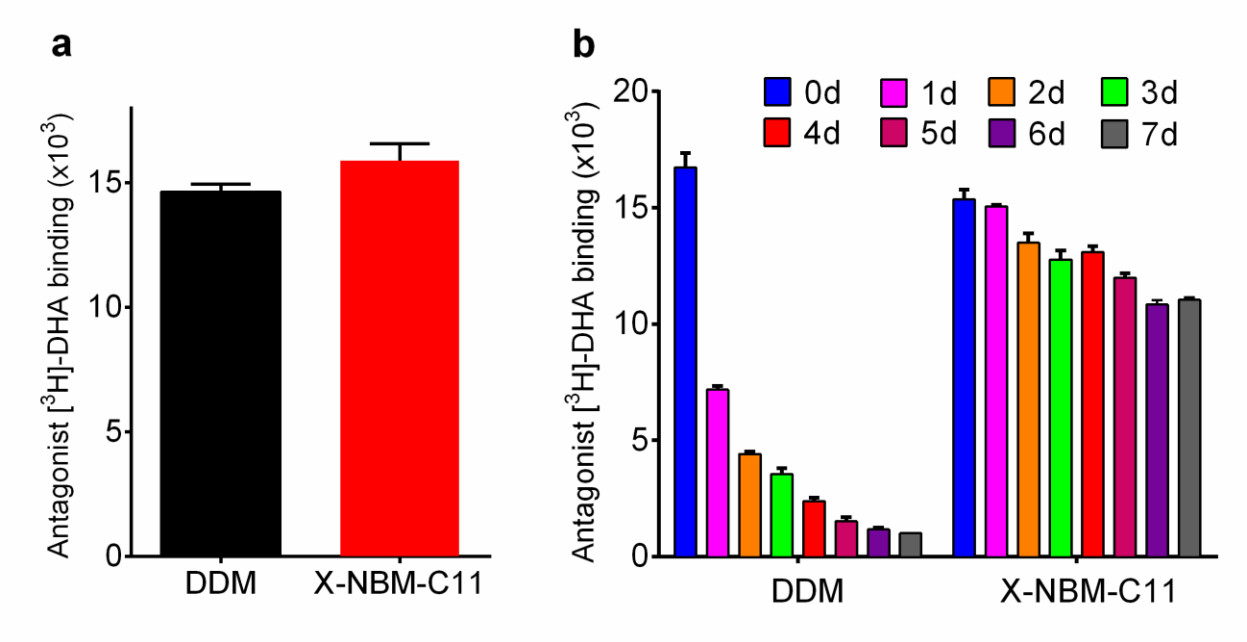


Figure S6. The ligand binding activities of β_2AR solubilised into DDM or X-NBM-C11. Receptor activity was measured immediately after extraction from the membrane (a) or monitored at regular intervals during a 7-day incubation at room temperature (b). The antagonist, [^{3}H]-dihydroalprenolol (DHA), was used as a ligand to measure receptor activity via radioligand binding assay. For the long-term experiments, receptor activity was measured at regular intervals during the incubation. Error bars, SEM, n = 3.

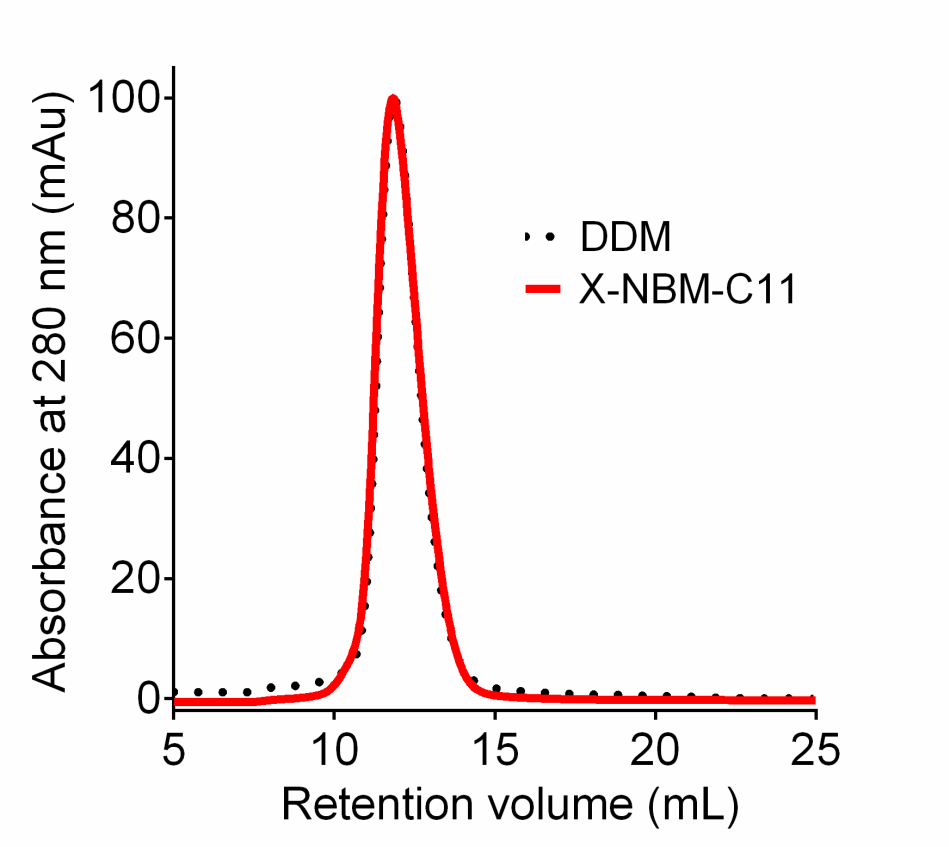


Figure S7. SEC profiles for DDM or X-NBM-C11-solubilized β_2AR after detergent exchange. DDM- or X-NBM-C11-solubilized receptor was applied to a superdex-200 GL column. The two different detergents produced detergent-receptor complexes of virtually identical size.

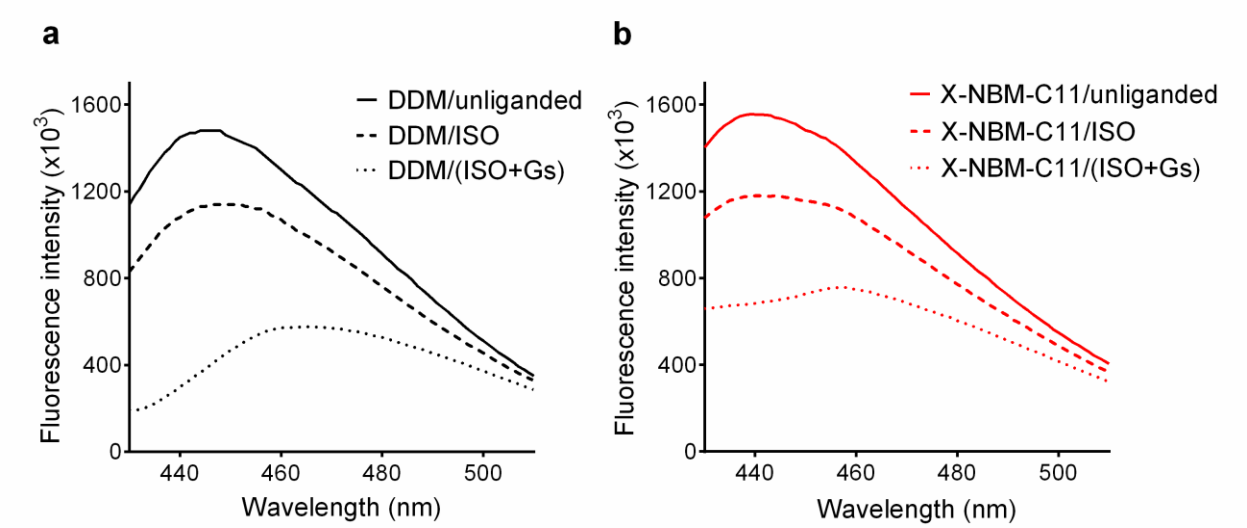


Figure S8. Fluorescence spectra of monobromobimane-labeled β_2AR (mBBr-β_2AR) solubilized in DDM or X-NBM-C11. Fluorescence spectra of mBBr-β_2AR were measured in the absence of agonist (detergent/unliganded), the presence of full agonist (isoproterenol (ISO); detergent/ISO), or a combination of ISO and G_s-protein (detergent/(ISO+G_s)). The data is representative of three independent experiments. Minor differences in the spectra produced by the protein samples in the two detergents (DDM and X-NBM-C11) are likely to be due to inherent detergent characteristics rather than a fundamental difference in receptor response to the presense of the agonist alone (detergent/ISO) or combined with G_s-protein (detergent/(ISO+G_s).

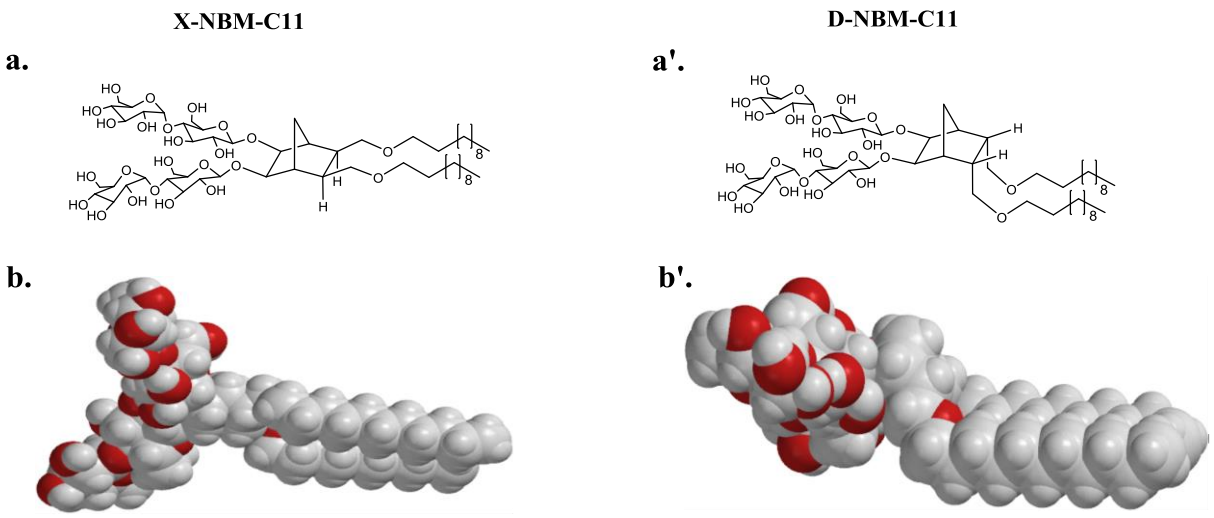


Figure S9. Chemical structures (a and a') of X-NBM-C11 and D-NBM-C11 and space-filling models (b and b') for their molecular structures. Atoms are indicated by different colors and size (gray-large for carbon atoms, gray-small for hydrogen atoms and red for oxygen atoms). As a result of the *exo*-connection, the overall molecular shape of X-NBM-C11 is comparatively straight. In contrast, the D-NBM-C11 is kinked between the norbornane linker and the alkyl chains due to the *endo*-connection.

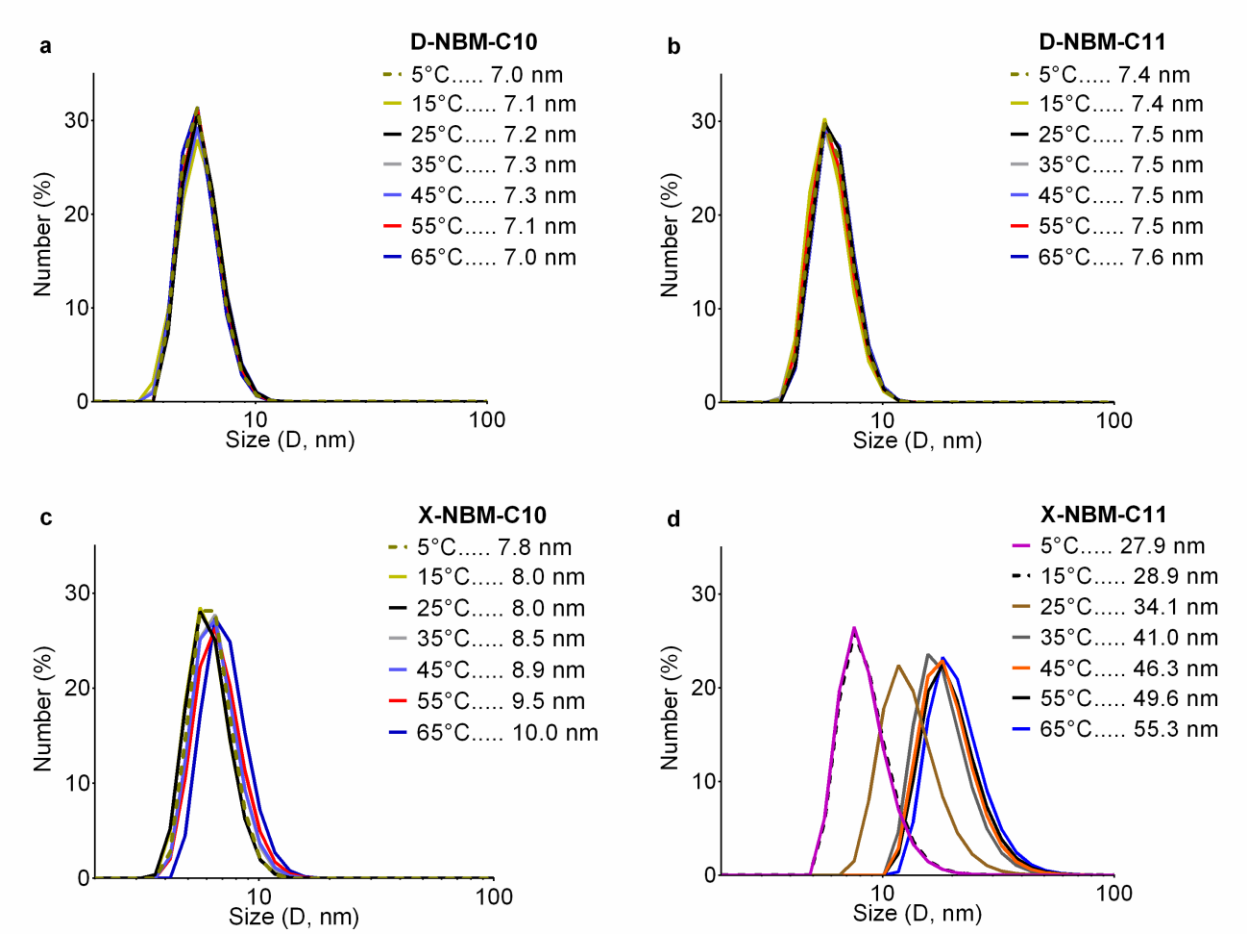


Figure S10. Changes in the DLS profiles of the NBM isomers (D-NBM-C10 (a), D-NBM-C11 (b), X-NBM-C10 (c) and X-NBM-C11(d)) with changing temperature (5°C to 65°C). These results indicate that X-isomers only (C10/C11) are subjected to temperature-dependent changes in micelle size.

Detergent CMC determination by diphenylhexatriene (DPH) encapsulation

5.0 mM NBM stock solutions were prepared in deionized and distilled water. A series of detergent solutions were prepared with a range of concentrations from the stock solutions. 200 μL of each detergent sample was transferred to a 96-well plate in duplicate. A DPH stock solution was prepared by dissolving 3.0 mg DPH in 5.0 mL THF. 50 μL of the stock solution was added into 950 μL of distilled water to prepare a DPH working solution and then 2.0 μL DPH work solution was added into each well containing a detergent solution for dye encapsulation. After 15 ~ 20 min incubation at room temperature, fluorescence intensities were measured at 430 nm following excitation at 358 nm using a Synergy Mx Monochromator Based Multi-Mode Microplate reader. Detergent CMC values were determined by plotting florescence intensities as a function of detergent concentrations.

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

The NBMs were dissolved in distilled and deionized water to give a detergent concentration of 1.0 wt%. These NBM solutions were filtered by a syringe filter with a pore size of 0.22 μm. Hydrodynamic diameter of the micelles produced by the NBMs were measured at variable temperature using a Malvern Zeta Sizer Nano ZS90 particle analyzer. With a maximum power of 5 MW, a He-Ne laser set at 633 nm was used as the light source. The scattered light was collected at an angle of 90°. The translational diffusion coefficient and hydrodynamic diameter (D_H) of the detergent micelles was calculated by autocorrelation analysis on time-dependent scattered light intensity. Hydrodynamic diameter (D_H) values for micelles formed by individual detergents (NBMs and DDM) were expressed as mean ± SD (n = 4).

Protein stability evaluation

Thermal denaturation assay

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

LeuT stability assay

Purification of the wild type leucine transporter (LeuT) from *Aquifex aeolicus* was performed according to the protocol described before.[2] LeuT was expressed in *E. coli* C41(DE3) transformed with pET16b encoding C-terminally 8xHis-tagged transporter. After isolation of bacterial membranes, the protein was

solubilized by treatment of 1.0 wt% of DDM. The DDM-solubilized protein was bound to Ni^{2+}-NTA resin (Life Technologies, Denmark) and eluted in 20 mM Tris-HCl (pH 8.0), 1 mM NaCl, 199 mM KCl, 0.05 % DDM and 300 mM imidazole. Approx. 1.5 mg/ml of the purified protein stock was diluted ten-fold in identical buffer without DDM and imidazole, but supplemented with individual NBMs or DDM (control). The final detergent concentration was either CMC + 0.04 wt% or CMC + 0.2 wt%. Protein samples were stored for 12 days at room temperature and, at the indicated time points, were centrifuged and the substrate binding activity of the transporter was determined via scintillation proximity assay (SPA) using [^{3}H]-Leucine.[3] The assay was performed with buffer containing 450 mM NaCl and the respective NBM isomers at the concentrations specified above. The SPA reaction was carried out in the presence of 20 nM [^{3}H]-Leu and 1.25 mg/ml copper chelate (His-Tag) YSi beads (from PerkinElmer, Denmark). Total [^{3}H]-Leu binding for the respective samples was measured using a MicroBeta liquid scintillation counter (PerkinElmer).

β_2AR stability assay

Long-term stability measurement: Long-term stability was measured using a previously published method.[4] The β_2AR purified in 0.1 wt% of DDM was concentrated to around 10 mg.ml^{-1} (~200 µM) and was used to make a main binding mixture containing 10 nM [^{3}H]- dihydroalprenolol (DHA) and 0.5 mg.ml^{-1} BSA. The β_2AR was added to each of representative NBMs (D-NBM-C11 and X-NBM-C11), DDM, or MNG-3 to make the final concentration at CMC+0.2 wt%. β_2AR receptor in individual detergents was incubated for 30 min at room temperature followed by loading onto a G-50 column. The individual fractions were collected in 1 ml binding buffer (20 mM HEPES pH 7.5, 100 mM NaCl, containing 0.5 mg.ml^{-1} BSA and 20 × CMC individual detergents). Each fraction was supplemented with 15 ml scintillation fluid and receptor-bound [^{3}H]-DHA measured using a scintillation counter (Beckman). The binding capacity of the receptor for [^{3}H]-DHA is shown as a bar chart. Alternatively, β_2AR was extracted from the membrane using 1.0 wt% of DDM or X-NBM-C11 and purified in 0.2 wt% for the same individual detergents. Receptor stability as assessed by ligand binding ability was measured at regular intervals during a 7-day incubation at room temperature. All experiments were carried out in triplicate.

Size exclusion chromatography (SEC) experiment: The β_2AR in 0.1 wt% of DDM was loaded onto a M1 Flag column in the presence of 2 mM $CaCl_2$ and the column was washed with DDM or X-NBM-C11 detergent buffer (20 mM HEPES pH 7.5, 100 mM NaCl, 0.2% respective detergent). The receptor was eluted in 20×CMC DDM/ X-NBM-C11 with 5 mM EDTA and 0.2 mg/ml free Flag peptide. The eluate was further applied to a Superdex-200 10/300 GL column (GE healthcare) at 0.5 ml.min^{-1} and UV absorbance at 280 nm was recorded. The running buffer contained 20 mM HEPES pH 7.5, 100 mM NaCl, 20×CMC individual detergents (DDM and X-NBM-C11).

Solubilization assay 10 ml detergent buffer (20 mM HEPES pH 7.5, 100 mM NaCl, 1.0% X-NBM-C11 or DDM) was added into 1 gram insect cell (*Sf*9) cell pellet expressing β_2AR. The mixture was stirred for 1 hr. After centrifugation at 12,000g for 20 min, the supernatant containing the solubilized material was collected and loaded onto an M1 Flag column in the presence of 2 mM $CaCl_2$. The column was washed with individual detergent buffers (20 mM HEPES pH 7.5, 100 mM NaCl, 0.2% detergent). The receptor

was eluted in 20×CMC X-NBM-C11/DDM, 5 mM EDTA and 0.2 mg/ml free Flag peptide. The level of active receptor present in both the DDM and X-NBM-C11 were measured by incubating 0.2 pmol β_2AR in the 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.

G_s-protein coupling assay 0.5 µl unliganded mBBr-labeled receptor at 50 µM in DDM was diluted with 500 µl 0.1% NBM or DDM containing buffer and incubated for 15 min at room temperature. This dilution gives a final receptor concentration of 50 nM. 2 µM isoproterenol (ISO) was added and the resulting solutions incubated for a further 15 min at room temperature. After further addition of 250 nM G_s, the protein samples were incubated for an additional 20 min at room temperature. The bimane fluorescence was measured by excitation at 370 nm, and emission spectra were 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.

Purification and stability measurement on β_2AR-G_s complex in X-NBM-C11 100 µM 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 to facilitate complex formation and the solution incubated for a further 1 hr. 1% X-NBM-C11 was then added to give a final concentration of 0.8% and the sample incubated for a further 30 min to initiate detergent exchange from DDM to X-NBM-C11. The protein solution was loaded onto an M1 Flag column, washed with a series of buffers with different molar ratios of 0.1% DDM buffer to 0.5% X-NBM-C11 buffer to allow complete detergent exchange from DDM to X-NBM-C11, and the protein finally eluted with 0.05% (70xCMC) X-NBM-C11 buffer. A preparative gel filtration was carried out to purify the β_2AR-G_s complex with running buffer (20mM HEPES pH 7.5, 100 mM NaCl, 0.005% X-NBM-C11, 1 µM BI, 100 µM TCEP). To measure the stability of the β_2AR-G_s complex in X-NBM-C11, analytical gel filtrations were performed at regular intervals (0, 7, 14, 21 D) with the same formulation of running buffer as above-mentioned. After a 21-day of incubation, analytical gel filtration was done using detergent-free buffer that has the same formulation but without X-NBM-C11.

Negative stain EM analysis of β_2AR-G_s solubilized in X-NBM-C11 β_2AR-G_s was prepared for electron microscopy using the conventional negative staining protocol,[5] and imaged at room temperature with a Tecnai T12 electron microscope operated at 120 kV using low-dose procedures. Images were recorded at a magnification of 71,138x and a defocus value of ~ 1.4 µm on a Gatan US4000 CCD camera. All images were binned (2x2 pixels) to obtain a pixel size of 4.16 Å at the specimen level. Particles were manually excised using e2boxer (part of the EMAN2 software suite).[6] 2D reference-free alignment and classification of particle projections was performed using ISAC.[7] 14,556 projections of β_2AR-G_s were subjected to ISAC, producing 199 classes consistent over two-way matching and accounting for 10,100 particle projections.

$MelB_{st}$ solubilization and thermal stability assay

A published protocol was used to evaluate DDM and selected NBMs with MelB from *Salmonella typhimurium* ($MelB_{St}$).[8] The plasmid pK95ΔAHB/WT $MelB_{St}$/CH10 encoding the wild-type $MelB_{St}$ with a C-terminal 10-His tag was expressed in DW2 cells (*ΔmelB* and *ΔlacZY*). Cell growth and membrane

preparation were carried out as reported.[9] Protein assays were carried out with a Micro BCA kit (Thermo Scientific, Rockford, IL). For the measurement of solubilization/stability, membrane samples containing $MelB_{St}$ (a final protein concentration was 10 $mg.mL^{-1}$) in a solubilization buffer (20 mM sodium phosphate, pH 7.5, 200 mM NaCl, 10% glycerol, 20 mM melibiose) were mixed with 1.5 wt% of DDM or individual NBMs (X-NBM-C10, D-NBM-C10, X-NBM-C11, D-NBM-C11) and the resulting solutios were incubated for 90 min at four different temperatures (0, 45, 55, and 65 ℃). Following ultracentrifugation at 355,590 g in a Beckman Optima™ MAX Ultracentrifuge using a TLA-100 rotor for 45 min at 4 ℃, 20 µg of each protein sample was separated by SDS-16% PAGE, followed by immunoblotting with a Penta-His-HRP antibody (Qiagen, Germantown, MD). $MelB_{St}$ was detected using SuperSignal West Pico chemiluminescent substrate by the ImageQuant LAS 4000 Biomolecular Imager (GE Healthcare Life Science).

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.[10-12] The 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 D-XBM-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 applied for Trp→D^2G FRET experiments using an Amico-Bowman Series 2 (AB2) Spectrofluorometer. Tryptophan residues were excited at 290 nm, and Trp→D^2G FRET was recorded at 465 nm and 490 nm for $MelB_{Ec}$ and $MelB_{St}$,[12] respectively. On a time trace, 10 µM D^2G and excess melibiose or equal volume of water were added at 1-min and 2-min points, respectively.

Amphiphile Synthesis

General procedure for dialkylation (step a)

NaH (3.0 equiv.) and **compound A** or **G** (1 equiv., 500 mg) were dissolved in DMF (15mL) at 0℃. Alkyl iodide (2.9 equiv.) was added dropwise, and the resulting solution was stirred at 70℃ for 3 days. After completion of the reaction (as detected by TLC), the solution was diluted with diethyl ether (150 mL) and the washed successively with 1 M aqueous HCl (2 x 20 mL) and brine (100 mL). The organic layer was dried with anhydrous Na_2SO_4, and the solvent was removed by rotary evaporation. The residue was purified by silica gel column chromatography (EtOAc/hexane) providing a desired product (**B** or **H**) as a liquid.

General procedure for Upjohn Dihydroxylation (step b)

A solution of NMO (1.5 equiv.) in water (50 wt. %) was added to a mixture of THF and water (15 mL of a 9:1 mixture) at 0℃. C**ompound B** or **H** (500 mg, 1.5 equiv.) was then added in one portion, the mixture allowed to stir for 15 minutes and then OsO_4 (1.4 mL of a 2.5 wt. % solution in t-BuOH) was added dropwise by syringe over 20 minutes. The mixture was stirred at room temperature for 5days. The reaction was quenched by the addition of sodium sulfite (8.0 g) and diluted with water (30 mL). The solution was then extracted with EtOAc (2 × 70 mL). The combined organic extracts were dried with anhydrious Na_2SO_4 and concentrated in vacuo and the residue was purified by silica gel column chromatography (EtOAc/hexane) providing a desired diol (**C** or **I**) as an orange gum.

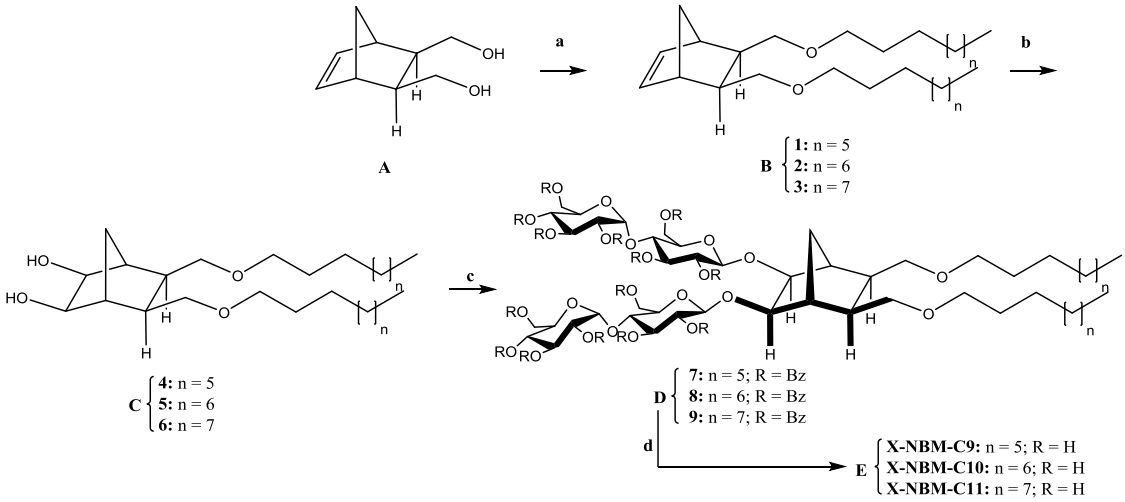
General procedures for glycosylation reactions (step c)

This procedure followed a literature method[13] with slight modification. A mixture of **compound C** or **I** (1 equiv., 250 mg), AgOTf (2.4 equiv.) and collidine (1.0 equiv.) in anhydrous CH_2Cl_2 (40 mL) was stirred at -45℃. A solution of 2.4 equiv. of perbenzoylatedmaltosylbromide (synthesized[9]from D-(+)-Maltose monohydrate) in CH_2Cl_2 (10 mL) was added dropwise over 0.5 h to this suspension. Stirring was continued for 0.5 h at -45℃, and then the reaction mixture was allowed to warm to 0℃ and left stirring for 1 h. After completion of the reaction, pyridine was added to the reaction mixture, and it was diluted with CH_2Cl_2 (40 mL) before being filtered through celite. The filtrate was washed successively with a 1 M aqueous $Na_2S_2O_3$ (40 mL), a 0.1 M aqueous HCl solution (40 mL), and brine (2 x 40 mL). The organic layer was dried with anhydrous Na_2SO_4, and the solvent was removed by rotary evaporation. The residue was purified by silica gel column chromatography (EtOAc/hexane), which provided the desired product (**D** or **J**) as a white solid.

General procedures for deprotection reactions (step d)

This procedure followed the de-O-benzoylation or de-O-acetylation under Zemplén's conditions.[14] The O-protected **compounds D** or **J** 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 14 h at room temperature, and then neutralized with Amberlite IR-120 resin (H^+ form). The resin was removed by filtration and washed with MeOH, and the solvent was removed from the combined filtrate *in vacuo*. The residue was purified by silica gel column chromatography (eluting with MeOH/CH_2Cl_2). Further purification, by recrystallization using CH_2Cl_2/MeOH/diethyl ether, afforded fully deprotected product (**E** or **K**) as a white solid.

Supplementary scheme 1



(a) alkyl iodide, NaH, 0°C → 70°C, 78-83%; (b) OsO_4, NMO, THF, H_2O, 0°C → room temperature, 5 days, 90-95%; (c) perbenzoylated malotsylbromide, AgOTf, DCM, -45°C → 0°C, 80-87%; (d) NaOMe, MeOH, room temperature, 14 hr, 89-97%.

Compound **1** was prepared in 82% yield according to the general procedure for dialkylation. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 6.15 (t, *J* = 4.2 Hz, 2H), 3.57 (dd, *J* = 8.0 Hz, 4.0 Hz, 2H), 3.44-3.34 (m, 4H), 3.27 (app. t, *J* = 8.2 Hz, 2H), 2.75 (t, *J* = 4.1 Hz, 2H), 1.77-1.75 (m, 2H), 1.59-1.55 (m, 4H), 1.48 (d, *J* = 8.1 Hz, 1H), 1.40-1.30 (m, 27H), 0.88 (t, *J* = 8.6 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 137.5, 72.3, 71.4, 45.0, 42.9, 40.7, 30.0, 29.8, 29.7, 29.5, 26.5, 22.9, 14.3.

Compound **2** was prepared in 78% yield according to the general procedure for dialkylation. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 6.15 (t, *J* = 4.5 Hz, 2H), 3.55 (dd, *J* = 8.0 Hz, 4.0 Hz, 2H), 3.40-3.30 (m, 4H), 3.27 (app. t, *J* = 8.6 Hz, 2H), 2.74 (t, *J* = 4.1 Hz, 2H), 1.77-1.75 (m, 2H), 1.59-1.55 (m, 4H), 1.47 (d, *J* = 8.2 Hz, 1H), 1.40-1.28 (m, 30H), 0.89 (t, *J* = 7.8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 137.5, 72.3, 71.4, 45.1, 42.9, 40.7, 32.1, 30.0, 29.9, 29.8, 29.7, 29.6, 26.5, 22.9, 14.3.

Compound **3** was prepared in 83% yield according to the general procedure for dialkylation. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 6.14 (t, *J* = 4.2 Hz, 2H), 3.56 (dd, *J* = 8.0 Hz, 4.0 Hz, 2H), 3.41-3.36 (m, 4H), 3.27 (app. t, *J* = 7.8 Hz, 2H), 2.74 (t, *J* = 4.4 Hz, 2H), 1.77-1.75 (m, 2H), 1.60-1.53 (m, 4H), 1.48 (d, *J* = 8.2 Hz, 1H), 1.40-1.20 (m, 41H), 0.88 (t, *J* = 7.9 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 137.5, 72.3, 71.4, 45.0, 42.9, 40.7, 32.1, 30.0, 29.8 (2C), 29.7, 29.6, 29.5 (2C), 26.5, 22.9, 14.3.

Compound **4** was prepared in 90% yield according to the general procedure for upjohn dihydroxylation. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.71 (br s, 2H), 3.51 (br s, 2H), 3.42 (dd, *J* = 8.0 Hz, 4.0 Hz, 2H), 3.37-3.32 (m, 4H), 3.22 (app. t, *J* = 7.7 Hz, 2H), 2.09 (br s, 2H), 1.75-1.73 (m, 2H), 1.61 (d, *J* = 8 Hz, 1H), 1.54-1.49 (m, 4H), 1.40-1.20 (m, 27H), 0.86 (t, *J* = 8.1 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 74.3, 71.4, 70.3, 46.9, 40.6, 32.1, 29.8 (2C), 29.7, 29.5, 27.5, 26.4, 22.9, 14.3

Compound **5** was prepared in 95% yield according to the general procedure for upjohn dihydroxylation. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.89 (br s, 2H), 3.67 (br s, 2H), 3.39 (dd, *J* = 8.0 Hz, 4.1 Hz, 2H), 3.37-

3.31 (m, 4H), 3.20 (app. t, *J* = 7.7 Hz, 2H), 2.06 (br s, 2H), 1.72-1.68 (m, 2H), 1.55 (d, *J* = 8.1 Hz, 1H), 1.51-1.48 (m, 4H), 1.35-1.25 (m, 31H), 0.84 (t, *J* = 8.2 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 74.1, 71.4, 70.3, 46.8, 40.5, 32.0, 29.8, 29.7, 29.6, 29.5, 27.5, 26.4, 22.8, 14.2

Compound **6** was prepared in 91% yield according to the general procedure for upjohn dihydroxylation. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.84 (br s, 2H), 3.71 (br s, 2H), 3.44 (dd, *J* = 8.1 Hz, 4.1 Hz, 2H), 3.40-3.31 (m, 4H), 3.24 (app. t, *J* = 7.8 Hz, 2H), 2.10 (br s, 2H), 1.80-1.70 (m, 2H), 1.62 (d, *J* = 8.0 Hz, 1H), 1.56-1.51 (m, 4H), 1.40-1.20 (m, 34H), 0.88 (t, *J* = 7.9 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 74.2, 71.4, 70.3, 46.8, 40.5, 32.1, 29.8, 29.6, 29.5, 27.5, 26.4, 22.8, 14.3

Compound **7** was prepared in 80% yield according to the general procedure for glycosylation reaction. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.12-7.78 (m, 9H), 7.71-7.50 (m, 14H), 7.42-7.12 (m, 43H), 6.12 (t, *J* = 7.7 Hz, 1H), 6.09 (t, *J* = 7.6 Hz, 1H), 5.79-5.55 (m, 5H), 5.54-5.48 (m, 2H), 5.39-5.33 (m, 3H), 5.03 (br s, 1H), 4.84-4.75 (m, 2H), 4.61-4.25 (m, 10H), 4.11-3.79 (m, 3H), 3.41-3.39 (m, 1H), 3.25-3.16 (m, 4H), 3.06-3.00 (m, 2H), 2.00-1.98 (m, 2H), 1.58-1.39 (m, 5H), 1.38-1.20 (m, 21H), 1.00-1.11 (m, 1H), 0.87 (t, *J* = 8.0 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.2, 165.9, 165.8, 165.7, 165.6, 165.4, 165.1, 164.9, 164.7, 164.3, 133.4, 133.1, 130.0, 129.9, 129.8, 129.7, 129.6, 129.5 (2C), 129.0, 128.9 (2C), 128.8 (2C), 128.7, 128.6, 128.5 (2C), 128.4, 128.2 (2C), 128.1, 128.0, 99.0, 98.1, 96.9, 96.4, 81.0, 79.2, 72.4, 71.3, 71.0 (2C), 69.2, 69.1, 69.0, 64.1, 62.6, 60.4, 31.9, 29.8, 29.7, 29.6 (2C), 29.5, 29.4 (2C), 26.3, 26.2, 22.8, 22.7, 21.1, 14.2 (2C).

Compound **8** was prepared in 85% yield according to the general procedure for glycosylation reaction.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10-7.77 (m, 9H), 7.75-7.43 (m, 14H), 7.42-7.17 (m, 40H), 6.18 (t, *J* = 8.0 Hz, 1H), 6.08 (t, *J* = 7.8 Hz, 1H), 5.75-5.64 (m, 5H), 5.51-5.44 (m, 2H), 5.36-5.32 (m, 3H), 5.99 (br s, 1H), 4.80-4.70 (m, 2H), 4.60-4.18 (m, 10H), 3.84-3.53 (m, 3H), 3.40-3.34 (m, 1H), 3.23-3.13 (m, 4H), 3.04-2.97 (m, 2H), 1.96-1.95 (m, 2H), 1.55-1.47 (m, 5H), 1.40-1.20 (m, 22H), 1.05-0.95 (m, 1H), 0.89 (t, *J* = 7.7 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.3 (2C), 166.0 165.9, 165.8, 165.7, 165.5, 165.4, 165.2, 165.0, 164.8, 164.4, 133.5 (2C), 133.4, 133.2, 130.2, 130.1, 130.0 (2C), 129.9 (2C), 129.8, 129.7 (2C), 129.6, 129.1, 128.9 (2C), 128.7, 128.6, 128.5 (2C), 128.4, 128.3, 128.2, 128.1, 99.9, 98.2, 96.9, 96.8, 81.2, 78.9, 71.4, 71.2, 70.7, 70.3, 70.1, 69.3, 69.2, 69.1, 64.2, 62.7, 40.3, 32.1 (2C), 29.9, 29.8 (2C), 29.7 (2C), 29.5, 26.5, 26.3, 22.9, 22.8, 14.3 (2C).

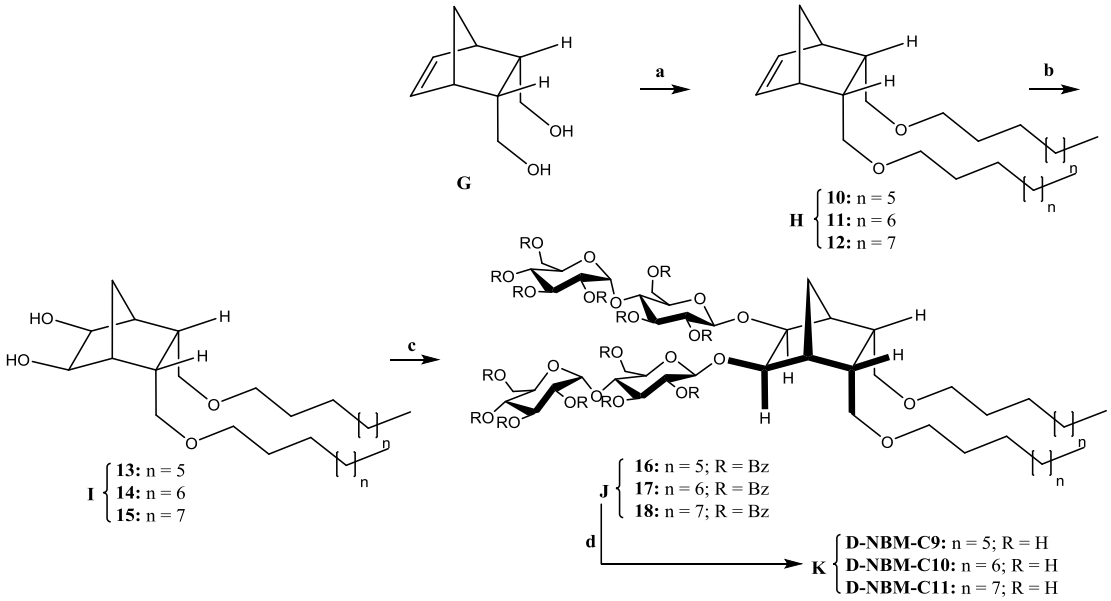
Compound **9** was prepared in 87% yield according to the general procedure for glycosylation reaction. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.09-7.87 (m, 9H), 7.77-7.51 (m, 15H), 7.42-7.14 (m, 48H), 6.16 (t, *J* = 8.1 Hz, 1H), 6.08 (t, *J* = 7.9 Hz, 1H), 5.76-5.61 (m, 5H), 5.51-5.44 (m, 2H), 5.32-5.29 (m, 3H), 5.00 (br s, 1H), 4.83-4.71 (m, 2H), 4.59-4.21 (m, 11H), 3.93-3.71 (m, 3H), 3.40-3.34 (m, 1H), 3.29-3.10 (m, 4H), 3.09-2.95 (m, 2H), 1.97-1.95 (m, 2H), 1.55-1.47 (m, 6H), 1.40-1.20 (m, 33H), 1.02-0.92 (m, 1H), 0.86 (t, *J* = 7.8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.3 (2C), 166.1, 165.9, 165.8, 165.7, 165.5, 165.3, 165.2, 165.1, 164.8, 164.4, 133.4, 133.3, 133.2 (2C), 133.0, 130.2, 130.1, 129.9 (2C), 129.8, 129.7, 129.6, 129.2, 129.0, 128.9 (2C), 128.7, 128.6 (2C), 128.5 (2C), 128.4, 128.3, 128.2, 128.1, 99.7, 98.5, 97.3, 97.0, 80.7, 79.6, 71.8, 71.4, 71.2, 70.7, 70.6, 70.3, 70.1, 69.4, 69.3, 69.2, 69.1, 64.2, 62.7, 40.5, 40.3, 32.1 (2C), 29.9, 29.8, 29.7 (2C), 29.6, 29.5, 28.6, 26.4 (2C), 22.9, 14.3.

X-NBM-C9 was prepared in 95% yield according to the general procedure for deprotection reaction. 1H NMR (400 MHz, CD_3OD): δ 5.16 (dd, *J* = 12.0 Hz, 4.0 Hz, 2H), 4.57 (d, *J* = 8.0 Hz, 1H), 4.42 (d, *J* = 8.0 Hz, 1H), 4.03-3.99 (m, 2H), 3.93-3.78 (m, 7H), 3.67-3.59 (m, 10H), 3.54-3.22 (m, 24H), 2.27 (br s, 1H), 2.19 (br s, 1H), 1.85-1.74 (m, 3H), 1.55 (app. t, *J* = 8.0 Hz, 4H), 1.45 (d, *J* = 12.0 Hz, 1H), 1.40-1.22 (m, 26H), 0.90 (t, *J* = 8.0 Hz, 6H); **^{13}C NMR** (100 MHz, CD_3OD): δ 103.4, 103.3, 103.0, 82.8, 82.1, 81.5, 81.3, 77.9, 77.7, 76.9, 76.7, 75.4, 75.2, 74.9, 74.2 (2C), 72.3, 72.2, 71.5, 62.8, 62.4, 47.5, 45.4, 42.1, 41.8, 33.2, 30.9 (2C), 30.7, 30.6, 27.6, 27.5, 23.9, 14.6; HRMS (EI): calcd. for $C_{51}H_{92}O_{24}Na^+$ $[M+Na]^+$ 1111.5876, found 1111.5873.

X-NBM-C10 was prepared in 89% yield according to the general procedure for deprotection reaction. 1H NMR (400 MHz, CD_3OD): δ 5.15 (dd, *J* = 12.0 Hz, 4.0 Hz, 2H), 4.57 (d, *J* = 8.0 Hz, 1H), 4.43 (d, *J* = 8.0 Hz, 1H), 4.03-3.98 (m, 2H), 3.92-3.81 (m, 7H), 3.65-3.59 (m, 10H), 3.53-3.22 (m, 29H), 2.27 (br s, 1H), 2.20 (br s, 1H), 1.84-1.74 (m, 3H), 1.55 (app. t, *J* = 8.0 Hz, 4H), 1.46 (d, *J* = 12.0 Hz, 1H), 1.40-1.22 (m, 31H), 0.90 (t, *J* = 8.0 Hz, 6H); **^{13}C NMR** (100 MHz, CD_3OD): δ 103.5, 103.2, 103.0, 82.9, 82.5, 81.8, 81.6, 81.4, 81.3, 77.8, 77.7, 76.9, 76.7, 76.6, 75.2, 75.0, 74.9, 74.1, 72.2, 71.6, 71.5, 62.7, 62.1, 47.5, 45.4, 42.0, 41.8, 33.2, 30.9 (3C), 30.7, 30.6, 29.7, 27.5 (2C), 23.9, 14.6; HRMS (EI): calcd. for $C_{53}H_{96}O_{24}Na^+$ $[M+Na]^+$ 1139.6189, found 1139.6187.

X-NBM-C11 was prepared in 97% yield according to the general procedure for deprotection reaction. 1H NMR (400 MHz, CD_3OD): δ 5.16 (dd, *J* = 12.0 Hz, 4.0 Hz, 2H), 4.57 (d, *J* = 8.0 Hz, 1H), 4.43 (d, *J* = 8.0 Hz, 1H), 4.03-3.98 (m, 2H), 3.93-3.81 (m, 7H), 3.68-3.58 (m, 9H), 3.53-3.22 (m, 22H), 2.27 (br s, 1H), 2.19 (br s, 1H), 1.84-1.74 (m, 3H), 1.54 (app. t, *J* = 4.0 Hz, 4H), 1.45 (d, *J* = 10.4 Hz, 1H), 1.40-1.22 (m, 35H), 0.90 (t, *J* = 6.4 Hz, 6H); **^{13}C NMR** (100 MHz, CD_3OD): δ 103.5, 103.3, 103.1, 82.9, 82.2, 81.6, 81.4, 77.9, 77.7, 76.9, 76.8, 75.5, 75.2, 74.9, 74.3, 74.2, 72.3 (2C), 71.6, 62.9, 62.4, 47.5, 45.5, 42.1, 41.9, 33.2, 30.9 (2C), 30.8, 30.7, 29.6, 27.6 (2C), 23.9, 14.6; HRMS (EI): calcd. for $C_{55}H_{100}O_{24}Na^+$ $[M+Na]^+$ 1167.6502, found 1167.6499.

Supplementary scheme 2



(a) alkyl iodide, NaH, 0°C → 70°C, 78-83%; (b) OsO_4, NMO, THF, H_2O, 0°C → room temperature, 5 days, 91-94%; (c) perbenzoylated malotsylbromide, AgOTf, DCM, -45°C → 0°C, 78-85%; (d) NaOMe, MeOH, room temperature, 14 hr, 90-96%.

Compound **10** was prepared in 81% yield according to the general procedure for dialkylation. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 6.12 (s, 2H), 3.38-3.28 (m, 4H), 3.22 (dd, *J* = 12.0 Hz, 8.0 Hz, 2H), 3.00 (t, *J* = 7.8 Hz, 2H), 2.91 (br s, 2H), 2.45 (br s, 2H), 1.59-1.50 (m, 4H), 1.44 (d, *J* = 7.9 Hz, 1H), 1.39-1.22 (m, 28H), 0.88 (t, *J* = 8.0 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 135.5, 71.3, 71.0, 49.2, 45.8, 41.7, 32.1, 30.0, 29.8, 29.7, 29.5, 26.5, 22.9, 14.3.

Compound **11** was prepared in 78% yield according to the general procedure for dialkylation. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 6.08 (s, 2H), 3.38-3.27 (m, 4H), 3.20 (dd, *J* = 12.0 Hz, 8.0 Hz, 2H), 2.97 (t, *J* = 8.1 Hz, 2H), 2.88 (br s, 2H), 2.42 (br s, 2H), 1.53-1.45 (m, 4H), 1.43 (d, *J* = 8.0 Hz, 1H), 1.38-1.24 (m, 30H), 0.85 (t, *J* = 8.0 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 135.4, 71.2, 71.0, 49.2, 45.8, 41.6, 32.1, 29.9, 29.8 (2C), 29.7, 29.5, 26.4, 22.9, 14.3.

Compound **12** was prepared in 83% yield according to the general procedure for dialkylation. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 6.11 (s, 2H), 3.37-3.30 (m, 4H), 3.22 (dd, *J* = 11.8 Hz, 8.0 Hz, 2H), 3.00 (t, *J* = 8.0 Hz, 2H), 2.91 (br s, 2H), 2.45 (br s, 2H), 1.56-1.45 (m, 4H), 1.46 (d, *J* = 12.2 Hz, 1H), 1.38-1.21 (m, 34H), 0.88 (t, *J* = 7.9 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 135.4, 71.2, 71.0, 49.2, 45.8, 41.6, 32.1, 29.9, 29.8, 29.7, 29.6, 26.5, 22.9, 14.3.

Compound **13** was prepared in 91% yield according to the general procedure for upjohn dihydroxylation. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.99 (br s, 2H), 3.44 (dd, *J* = 8.0 Hz, 4.0 Hz, 2H), 3.71 (t, *J* = 7.8 Hz, 6H), 3.29 (br s, 2H), 2.25 (br s, 4H), 1.88 (d, *J* = 10.4 Hz, 1H), 1.60-1.51 (m, 4H), 1.33-1.22 (m, 24H), 1.20 (d,

J = 10.4 Hz, 1H), 0.88 (t, *J* = 8.4 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 71.5, 69.5, 68.1, 47.2, 38.7, 33.2, 32.1, 29.9, 29.8, 29.7, 29.5, 26.4, 22.8, 14.3.

Compound **14** was prepared in 94% yield according to the general procedure for upjohn dihydroxylation. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 3.92 (br s, 2H), 3.65 (br s, 2H), 3.39 (dd, *J* = 8.0 Hz, 4.2 Hz, 2H), 3.32 (t, *J* = 8.0 Hz, 6H), 2.19 (br s, 4H), 1.83 (d, *J* = 12 Hz, 1H), 1.52-1.48 (m, 4H), 1.33-1.17 (m, 30H), 1.14 (d, *J* = 11.7 Hz, 1H), 0.83 (t, *J* = 7.8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 71.4, 69.3, 68.4, 47.1, 38.6, 33.2, 32.1, 29.8 (2C), 29.7, 29.6, 29.5, 26.3, 22.8, 14.2.

Compound **15** was prepared in 93% yield according to the general procedure for upjohn dihydroxylation. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 4.00 (br s, 2H), 3.44 (dd, *J* = 8.2 Hz, 4.1 Hz, 2H), 3.37 (t, *J* = 7.9 Hz, 6H), 3.13 (br s, 2H), 2.26 (br s, 4H), 1.88 (d, *J* = 7.8 Hz, 1H), 1.60-1.53 (m, 4H), 1.39-1.17 (m, 34H), 0.88 (t, *J* = 7.8 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 71.5, 69.6, 68.1, 47.3, 38.7, 33.2, 32.1, 29.9, 29.8, 29.7, 29.5, 26.4, 22.9, 14.3.

Compound **16** was prepared in 78% yield according to the general procedure for glycosylation reaction. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.11-8.09 (m, 10H), 7.99-7.68 (m, 14H), 7.52-7.16 (m, 45H), 6.08 (t, *J* = 8.4 Hz, 1H), 6.06 (t, *J* = 7.8 Hz, 1H), 5.77-5.64 (m, 5H), 5.54-5.45 (m, 3H), 5.38-5.31 (m, 2H), 4.96 (br s, 1H), 4.87-4.84 (m, 1H), 4.69-4.44 (m, 9H), 4.33-4.28 (m, 3H), 4.04-3.97(m, 2H), 3.87-3.78 (m, 2H), 3.49-3.31 (m, 3H), 3.20-3.03 (m, 4H), 2.22 (br s, 1H), 2.18 (br s, 1H), 2.05 (br s, 2H), 1.66-1.58 (m, 3H), 1.43-1.23 (m, 23H), 0.87 (t, *J* = 6.4 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.2 (2C), 166.0, 165.9, 165.8, 165.7, 165.5, 165.2 (2C), 165.0, 164.7, 164.4, 133.4 (2C), 133.2, 133.1, 133.0, 130.2, 130.1, 130.0, 129.9 (2C), 129.7 (2C), 129.5 (2C), 129.1, 129.0, 128.9 (2C), 128.8, 128.7 (2C), 128.6, 128.5 (2C), 128.3 (2C), 128.2, 128.1, 99.6, 98.7, 97.3, 97.0, 75.3, 75.0, 74.9, 74.3, 73.9, 72.8, 72.5, 71.9, 71.6, 71.3, 71.1, 70.6, 70.2, 70.1, 69.3, 69.2, 69.1, 68.2, 67.5, 64.7, 64.1, 62.7, 62.6, 46.5, 44.7, 38.6, 37.9, 33.9, 32.0, 30.1, 29.8 (3C), 29.6 (2C), 29.4, 26.5, 26.4, 22.8, 14.3 (2C).

Compound **17** was prepared in 84% yield according to the general procedure for glycosylation reaction.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11-8.06 (m, 10H), 7.99-7.70 (m, 15H), 7.51-7.18 (m, 44H), 6.18 (t, *J* = 8.2 Hz, 1H), 6.08 (t, *J* = 7.8 Hz, 1H), 5.78-5.67 (m, 5H), 5.52-5.46 (m, 3H), 5.34-5.31 (m, 2H), 4.97 (br s, 1H), 4.87-4.84 (m, 1H), 4.67-4.44 (m, 9H), 4.33-4.29 (m, 3H), 4.04-3.98 (m, 2H), 3.82-3.78 (m, 2H), 3.49-3.33 (m, 3H), 3.21-3.03 (m, 4H), 2.22 (br s, 1H), 2.18 (br s, 1H), 2.06 (br s, 2H), 1.66-1.60 (m, 2H), 1.44-1.23 (m, 26H), 0.87 (app. t, *J* = 6.4 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.2 (2C), 166.0, 165.9, 165.8, 165.7, 165.5, 165.2 (2C), 165.0, 164.7, 164.4, 133.4, 133.2, 133.1, 133.0, 130.1, 130.0, 129.9, 129.8, 129.7 (3C), 129.5, 129.4, 129.1, 129.0, 128.9 (2C), 128.8, 128.7 (2C), 128.6, 128.5, 128.3 (2C), 128.2, 128.1, 99.6, 98.6, 97.3, 97.0, 75.4, 75.3, 75.0, 74.9, 74.2, 73.8, 72.8, 72.5, 71.8, 71.6, 71.3, 71.2, 71.1, 70.6, 70.2, 69.3, 69.2 (2C), 69.0, 68.2, 67.5, 64.6, 64.0, 62.7, 62.6, 46.5, 44.7, 38.5, 37.8, 33.9, 32.1, 30.1, 29.9, 29.8, 29.7, 29.6 (2C), 29.5, 26.5, 26.4, 22.8 (2C), 14.3.

Compound **18** was prepared in 85% yield according to the general procedure for glycosylation reaction.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11-7.99 (m, 10H), 7.89-7.68 (m, 14H), 7.50-7.16 (m, 46H), 6.15 (t, *J* = 8.1 Hz, 1H), 6.08 (t, *J* = 7.9 Hz, 1H), 5.78-5.67 (m, 5H), 5.51-5.45 (m, 3H), 5.34-5.31 (m, 2H), 4.96 (br s,

1H), 4.87-4.84 (m, 1H), 4.67-4.44 (m, 9H), 4.33-4.29 (m, 3H), 4.04-4.02 (m, 2H), 3.81-3.79 (m, 2H), 3.49-3.30 (m, 3H), 3.15-3.00 (m, 4H), 2.22 (br s, 1H), 2.18 (br s, 1H), 2.05 (br s, 2H), 1.64-1.61 (m, 3H), 1.49-1.23 (m, 33H), 0.87 (app. t, *J* = 4.0 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.2 (2C), 166.0, 165.9, 165.8, 165.7, 165.5, 165.2 (2C), 165.0, 164.8, 164.4, 133.4 (2C), 133.2, 133.1, 130.2, 130.1, 130.0, 129.9 (2C), 129.7 (3C), 129.5 (2C), 129.1, 129.0, 128.9 (2C), 128.8, 128.7 (2C), 128.6, 128.5 (3C), 128.3 (2C), 128.2, 128.1, 99.6, 98.7, 97.3, 97.0, 75.3, 75.0, 74.9, 74.3, 73.9, 72.8, 72.5, 71.8, 71.6, 71.3, 71.2, 71.1, 70.6, 70.2, 69.2 (2C), 69.1, 68.2, 67.5, 64.7, 64.1, 62.7, 62.6, 60.5, 46.5, 44.7, 38.6, 37.8, 33.9, 32.1 (2C), 30.1, 29.9, 29.8, 29.7, 29.6, 29.5 (2C), 26.5, 26.4, 22.8, 14.3.

D-NBM-C9 was prepared in 92% yield according to the general procedure for deprotection reaction. **^{1}H NMR** (400 MHz, CD_3OD): δ 5.08 (t, *J* = 4.8 Hz, 2H), 4.48 (d, *J* = 8.0 Hz, 1H), 4.26 (d, *J* = 8.0 Hz, 1H), 4.16 (br s, 2H), 3.81-3.73 (m, 7H), 3.66-3.43 (m, 12H), 3.37-3.15 (m, 17H), 2.33 (br s, 1H), 2.27 (br s, 1H), 2.15 (br s, 2H), 1.99 (d, *J* = 9.6 Hz, 1H), 1.48 (app. t, *J* = 6.8 Hz, 4H), 1.32-1.12 (m, 26H), 0.82 (app. t, *J* = 5.6 Hz, 6H); **^{13}C NMR** (100 MHz, CD_3OD): δ 103.8, 103.5, 103.1, 103.0, 81.4, 78.2, 78.0, 77.9, 77.7, 77.0, 76.7, 75.4, 75.2, 74.9, 74.2, 74.1, 72.3 (2C), 71.5, 69.5, 69.1, 62.8, 62.3, 48.0, 45.7, 39.9 (2C), 35.4, 33.2 (2C), 31.0, 30.9, 30.8, 30.7, 30.6, 27.6, 27.5, 23.9, 14.6; HRMS (EI): calcd. for $C_{51}H_{92}O_{24}Na^+$ $[M+Na]^+$ 1111.5876, found 1111.5872

D-NBM-C10 was prepared in 96% yield according to the general procedure for deprotection reaction. **^{1}H NMR** (400 MHz, CD_3OD): δ 5.15 (t, *J* = 4.0 Hz, 2H), 4.56 (d, *J* = 8.0 Hz, 1H), 4.33 (d, *J* = 8.0 Hz, 1H), 4.24 (br s, 2H), 3.93-3.79 (m, 7H), 3.69-3.53 (m, 13H), 3.45-3.22 (m, 19H), 2.40 (br s, 1H), 2.35 (br s, 1H), 2.22 (br s, 2H), 2.08 (d, *J* = 12.0 Hz, 1H), 1.56 (app. t, *J* = 4.0 Hz, 4H), 1.40-1.20 (m, 30H), 0.90 (t, *J* = 8.0 Hz, 6H); **^{13}C NMR** (100 MHz, CD_3OD): δ 103.9, 103.6, 103.2, 103.1, 81.5, 78.2, 78.0, 77.9, 77.8, 77.1, 76.8, 75.4, 75.2, 74.9, 74.3, 74.2, 72.4, 72.3, 71.6, 69.5, 69.1, 62.9, 62.4, 48.0, 45.7, 40.0, 39.9, 35.4, 33.2, 31.0 (2C), 30.9, 30.8 (2C), 30.7, 30.6, 27.6 (2C), 23.9, 14.6; HRMS (EI): calcd. for $C_{53}H_{96}O_{24}Na^+$ $[M+Na]^+$ 1139.6189, found 1139.6187.

D-NBM-C11 was prepared in 90% yield according to the general procedure for deprotection reaction. **^{1}H NMR** (400 MHz, CD_3OD): δ 5.15 (app. t, *J* = 4.4 Hz, 2H), 4.56 (d, *J* = 8.0 Hz, 1H), 4.34 (d, *J* = 8.0 Hz, 1H), 4.25 (br s, 2H), 3.93-3.82 (m, 7H), 3.69-3.53 (m, 13H), 3.43-3.24 (m, 27H), 2.41 (br s, 1H), 2.36 (br s, 1H), 2.22 (br s, 2H), 2.08 (d, *J* = 10.0 Hz, 1H), 1.56 (app. t, *J* = 4.0 Hz, 4H), 1.42-1.22 (m, 37H), 0.90 (t, *J* = 6.4 Hz, 6H); **^{13}C NMR** (100 MHz, CD_3OD): δ 103.9, 103.6, 103.2, 81.5, 81.4, 78.3, 78.2, 77.8, 77.7, 77.0, 76.8, 75.4, 75.1, 74.9, 74.2, 74.1, 72.4, 72.3, 71.6, 69.5, 69.1, 62.8, 62.3, 48.0, 45.7, 39.9, 35.4, 33.2, 31.0 (2C), 30.9 (2C), 30.8, 30.7, 30.6, 27.6 (2C), 23.9, 14.6; HRMS (EI): calcd. for $C_{55}H_{100}O_{24}Na^+$ $[M+Na]^+$ 1167.6502, found 1167.6500.

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