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Supporting Information

Mesitylene-Cored Glucoside Amphiphiles (MGAs) for Membrane Protein Studies: Importance of Alkyl Chain Density in Detergent Efficacy

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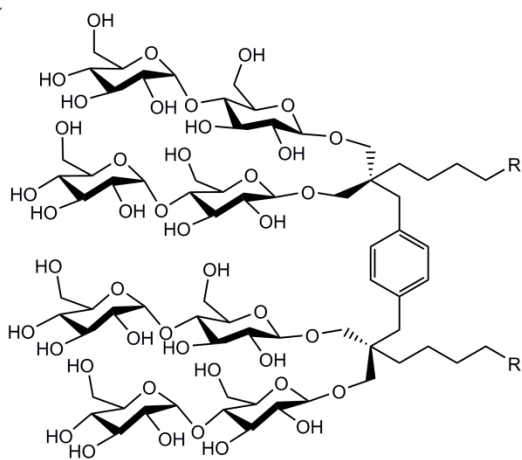
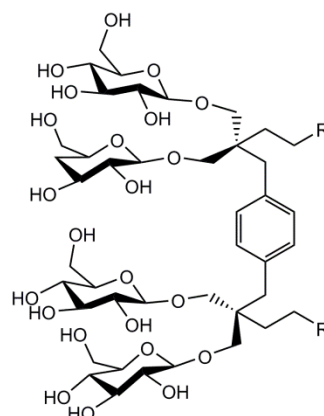
a**XMA-C8** : $R = n\text{-C}_4\text{H}_9$ **XMA-C9** : $R = n\text{-C}_5\text{H}_{11}$ **XMA-C10** : $R = n\text{-C}_6\text{H}_{13}$ **XMA-C11** : $R = n\text{-C}_7\text{H}_{15}$ **XMA-C12** : $R = n\text{-C}_8\text{H}_{17}$ **b****XGA-C4** : $R = n\text{-C}_2\text{H}_5$ **XGA-C5** : $R = n\text{-C}_3\text{H}_7$ **XGA-C6** : $R = n\text{-C}_4\text{H}_9$ **XGA-C7** : $R = n\text{-C}_5\text{H}_{11}$

Figure S1. Chemical structures of (a) xylene-linked maltoside amphiphiles (XMAs; XMA-C8, XMA-C9, XMA-C10, XMA-C11, and XMA-C12) and (b) xylene-linked glucoside amphiphiles (XGAs; XGA-C4, XGA-C5, XGA-C6, XGA-C7).

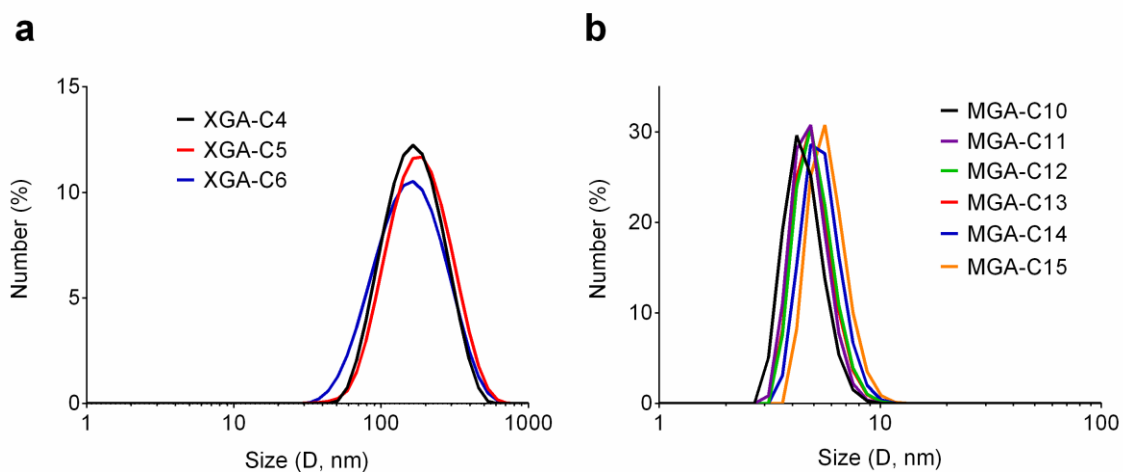


Figure S2. Dynamic light scattering (DLS) profiles of micelles formed by (a) xylene-linked glucoside amphiphiles (XGAs) and (b) mesitylene-cored glucoside amphiphiles (MGAs). These agents showed a single set of populations in their micelle size when used at 1.0 wt %. Time-dependent fluctuation in the scattered light intensity was analyzed by autocorrelation. This analysis gave translational diffusion coefficient (D), from which the hydrodynamic radii (R_h) of detergent micelles were calculated using the Stokes-Einstein equation.

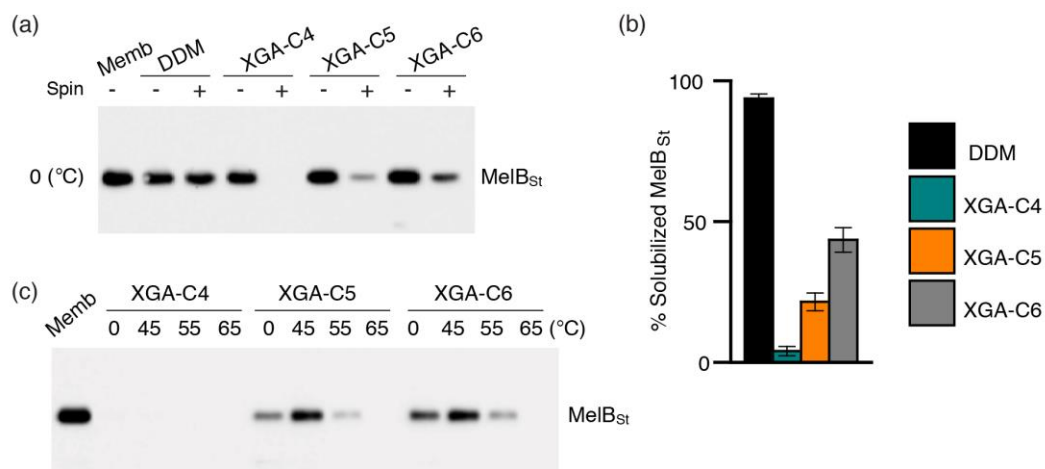


Figure S3. Thermo-stability of MelB_{St} solubilized in xylene-linked glucoside amphiphiles (XGA-C4, XGA-C5, or XGA-C6) or DDM. *E. coli* membranes containing MelB_{St} were treated with 1.5 wt% individual detergents for 90 min at 0°C (a, b) or elevated temperature (45, 55, or 65°C, c). The solubilized protein was analyzed by SDS-PAGE and Western blot. The amount of soluble MelB_{St} in each sample was expressed as a percentage of the total amount of MelB_{St} in the untreated membrane (Memb). Error bars, SEM, $n = 2-4$.

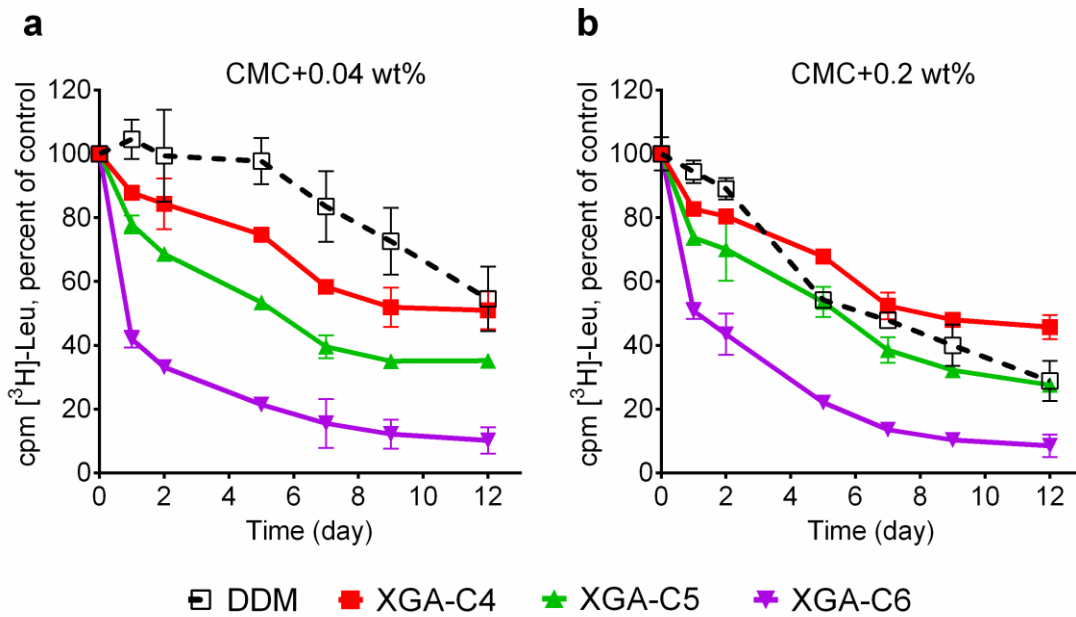


Figure S4. Long-term ligand binding activity for LeuT solubilized in DDM or a XGA (XGA-C4, XGA-C5, or XGA-C6). All detergents were tested at two concentrations: (a) CMC+0.04 wt % and (b) CMC+0.2 wt %. Protein activity was measured via scintillation proximity assay (SPA) using a radio-labelled ligand (³H]-Leucine). The individual detergent-solubilized transporters were incubated for 12 days at room temperature. Error bars, SEM, *n* = 3.

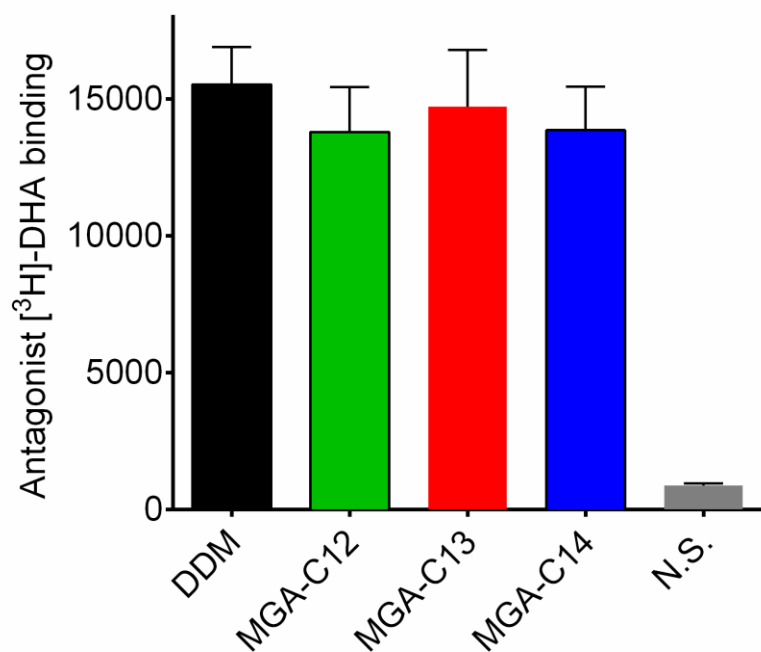


Figure S5. Ligand binding activity of β_2 AR solubilized in DDM and a novel detergent (MGA-C12, MGA-C13, or MGA-C14). 0.1% DDM-purified β_2 AR was diluted into buffer solutions containing individual detergents and receptor activity was measured at regular intervals using the antagonist [³H]-dihydroalprenolol (DHA) over three-day incubation at room temperature. The final detergent concentration was CMC+0.2 wt%. Error bars, SEM, $n = 3$.

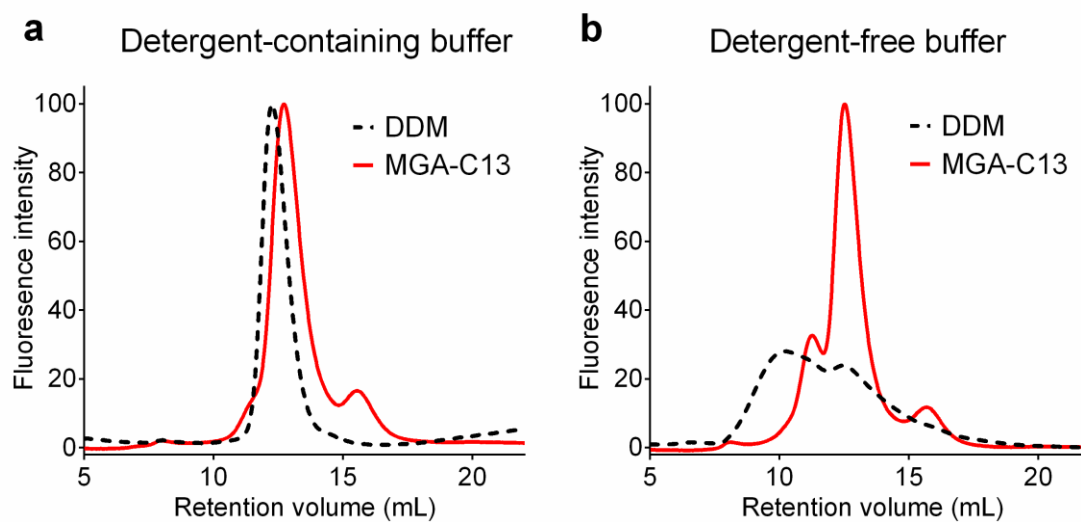


Figure S6. SEC profiles for β_2 AR solubilized in DDM and MGA-C13 in a detergent-containing (a) or detergent-free buffer (b). DDM-purified receptor was subjected to detergent exchange with MGA-C13. DDM- or MGA-C13-solubilized receptor was applied for the SEC column which had been equilibrated with a detergent-containing or detergent-free buffer. The chromatogram of each agent-solubilized receptor was obtained from the intrinsic tryptophan emission at 345 nm.

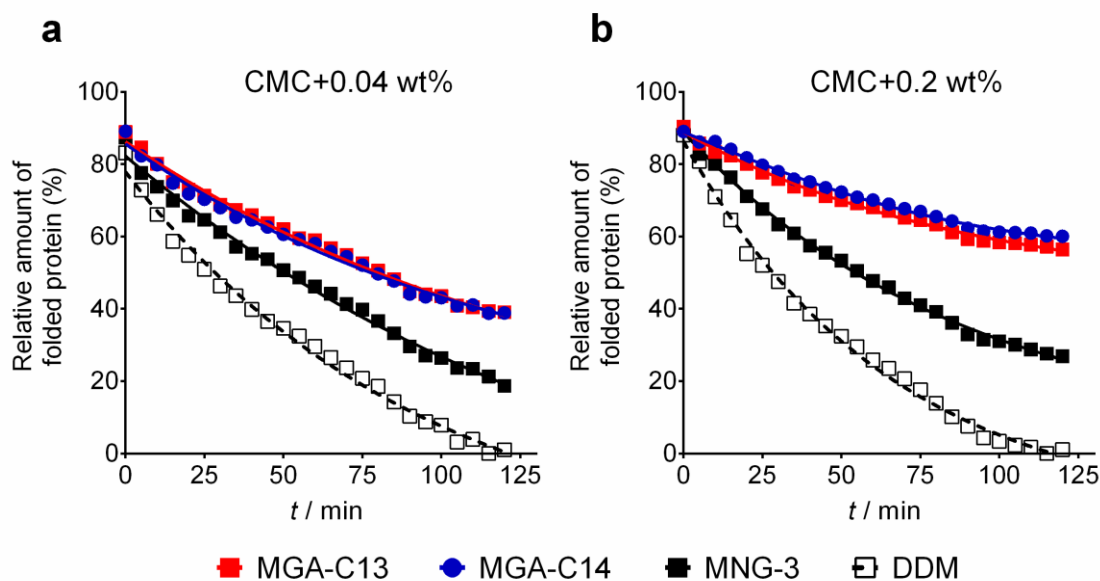


Figure S7. Thermal denaturation profile of UapA solubilized in representative novel MGAs (MGA-C13 and MGA-C14), previously reported MNG-3 and DDM. Detergents were used at two different concentrations: CMC+0.04 wt % (a) and CMC+0.2 wt % (b). The relative amount of folded protein was monitored using the CPM assay in the course of 120 min incubation at 40°C. The data is representative of two independent experiments.

Table S1. Molecular weights (MWs) and critical micelle concentrations (CMCs) of XGAs (XGA-C4, XGA-C5 and XGA-C6) and the hydrodynamic radii (R_h ; $n = 5$) of their micelles.

Detergent	MW ^[a]	CMC (mM)	CMC (wt%)	R_h (nm) ^[b]
XGA-C4	1015.1	~0.8	~0.081	170 ± 11.6
XGA-C5	1043.1	~0.8	~0.083	199 ± 7.1
XGA-C6	1071.2	~0.8	~0.086	177 ± 7.9

[a] Molecular weight of detergents. [b] Hydrodynamic radius of detergents measured at 1.0 wt% by dynamic light scattering.

Protein stability evaluation

UapA thermal denaturation assay

UapAG411V_{Δ1-11} 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, 1 mM xanthine).¹ The protein was concentrated to approximately 10 mg/ml using a 100 kDa molecular weight cut off filter (Millipore). The protein was diluted 1:150 into buffer containing either DDM or a MGA (MGA-C10, MGA-C11, MGA-C12, MGA-C13, MGA-C14, or MGA-C15) at concentrations of CMC + 0.04 wt% or CMC + 0.2 wt% in Greiner 96-well plates. The CPM dye (Invitrogen), stored in DMSO (Sigma), was diluted in dye buffer (20 mM Tris (pH 7.5), 150 mM NaCl, 0.03% DDM, 5 mM EDTA) and 3 μl of the diluted dye was added to individual protein samples. The reaction mixture was incubated for 120 min at 40 °C. The fluorescence emission was monitored using a microplate spectrofluorometer set at excitation and emission wavelengths of 387 nm and 463 nm, respectively. The maximum fluorescence value was used to calculate the percentage of relative folded protein during this incubation period. The relative amounts of folded proteins were plotted against time using GraphPad Prism.

MelB solubilization and thermal stability assay

Our reported protocol was used to evaluate DDM and MGAs for MelB_{St} stability.² The plasmid pK95ΔAHB/WT MelB_{St}/CH10 encoding the wild-type MelB with a 10-His tag at the C-terminus and *Salmonella typhimurium* (MelB_{St}) DW2 cells (Δ*melB* and Δ*lacZY*) were used to express the protein. Cell growth and membrane preparation were carried out as described.³ Protein assay was carried out with a Micro BCA kit (Thermo Scientific, Rockford, IL). For the measurement of protein solubilization, membrane samples containing MelB_{St} (the final protein concentration was 10 mg/mL) were incubated with a solubilization buffer (20 mM sodium phosphate, pH 7.5, 200 mM NaCl, 10% glycerol, 20 mM melibiose) containing 1.5 % (w/v) DDM or a MGA (MGA-C10, MGA-C11, MGA-C12, MGA-C13, MGA-C14, or MGA-C15) at four different temperatures (0, 45, 55, and 65 °C) for 90 min. Ultracentrifugation was carried out at 355,590 g in a Beckman Optima™ MAX Ultracentrifuge with a TLA-100 rotor for 45 min at 4 °C. 20 μg proteins were 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 Health Care Life Science).

LeuT stability assay

Purification of the wild type of the leucine transporter (LeuT) from *Aquifex aeolicus* was performed according to the protocol described previously.⁴ 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). After isolation of bacterial membranes, the protein was solubilized by treatment of 1.0 % DDM. The DDM-solubilized protein was bound to Ni²⁺-NTA resin (Life Technologies, Denmark) and eluted in 20 mM Tris-HCl (pH 8.0), 1 mM NaCl, 199 mM KCl, 0.05 % DDM and 300 mM imidazole. Subsequently, approx. 1.5 mg/ml protein stock was diluted ten-fold in identical buffer without DDM and imidazole, but supplemented with a MGA (MGA-C10, MGA-C11, MGA-C12, MGA-C13, MGA-C14, or MGA-C15) and DDM (control). The final detergent concentrations became 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 ligand binding activity of the transporter was determined via scintillation proximity assay (SPA) using [³H]-Leucine.⁵ Assay was performed with buffer containing 450 mM NaCl and the respective MGAs at the concentrations indicated above. SPA reaction was carried out in the presence of 20 nM [³H]-Leu and 1.25 mg/ml copper chelate (His-Tag) YSi beads (both from PerkinElmer, Denmark). Total [³H]-Leu binding for the respective samples was measured using a MicroBeta liquid scintillation counter (PerkinElmer).

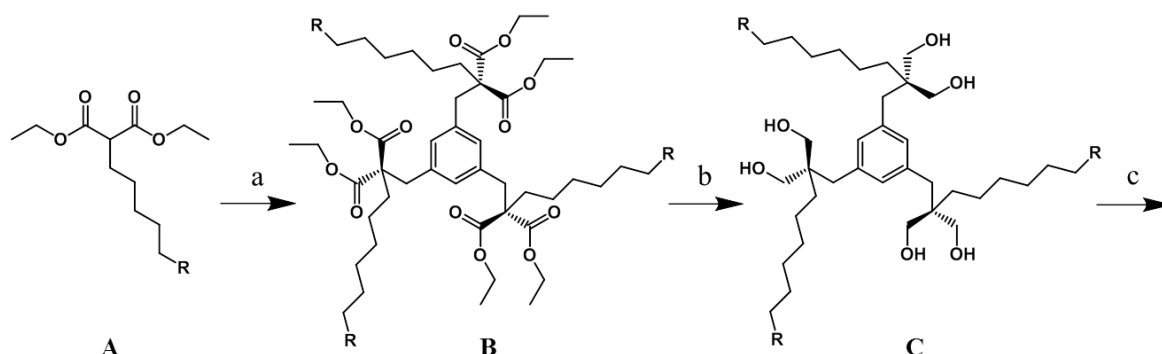
β₂AR stability assay

Soluble radioligand binding The β₂AR was purified using 0.1% DDM. DDM-purified β₂AR was diluted into buffer solutions containing individual detergents (DDM and MGAs) to reach detergent concentration of CMC + 0.2 wt%. β₂AR in the respective detergents was stored for 3 days at room temperature and its ligand binding capacity was measured at regular intervals by incubating with 10 nM of radioactive [³H]-dihydroalprenolol (DHA) for 30 min at room temperature. The mixture was loaded onto a G-50 column and collected the flow-through with certain amount of binding buffer (20 mM HEPES pH 7.5, 100 mM NaCl, supplemented with 0.5 mg/ml BSA), and further filled with 15 ml scintillation fluid. Receptor-bound [³H]-DHA was measured with a scintillation counter (Beckman). Non-specific binding of [³H]-DHA was calculated by adding 1 μM of alprenolol (Sigma) in the same binding condition. The binding capacity of [³H]-DHA for individual MGA-solubilized β₂AR was expressed as a column graph.

Size exclusion chromatography (SEC) experiment The purified β₂AR in 0.1% DDM was mixed with DDM/MGA-C13 detergent buffer (20 mM HEPES pH 7.5, 100 mM NaCl, 0.2% respective detergent) for incubation of 30 min. The samples were further applied to a Superdex-200 10/300 GL column (GE healthcare) at 0.5 ml/min and fluorescence intensity at 345 nm was recorded with excitation wavelength of 295 nm. The running buffer contains 20 mM HEPES pH 7.5, 100 mM NaCl in the presence or absence of individual detergents (DDM and MGA-C13).

Preparation of Mesitylene-cored Glucoside Amphiphiles (MGAs)

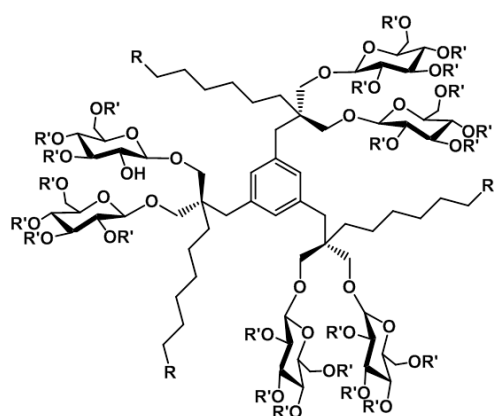
Supplementary scheme



1 : R = (CH₂)₅CH₃
 2 : R = (CH₂)₆CH₃
 3 : R = (CH₂)₇CH₃
 4 : R = (CH₂)₈CH₃
 5 : R = (CH₂)₉CH₃
 6 : R = (CH₂)₁₀CH₃

7 : R = (CH₂)₅CH₃
 8 : R = (CH₂)₆CH₃
 9 : R = (CH₂)₇CH₃
 10 : R = (CH₂)₈CH₃
 11 : R = (CH₂)₉CH₃
 12 : R = (CH₂)₁₀CH₃

13 : R = (CH₂)₅CH₃
 14 : R = (CH₂)₆CH₃
 15 : R = (CH₂)₇CH₃
 16 : R = (CH₂)₈CH₃
 17 : R = (CH₂)₉CH₃
 18 : R = (CH₂)₁₀CH₃



D {
 MGA-C10a : R = (CH₂)₅CH₃, R' = benzoyl
 MGA-C11a : R = (CH₂)₆CH₃, R' = benzoyl
 MGA-C12a : R = (CH₂)₇CH₃, R' = benzoyl
 MGA-C13a : R = (CH₂)₈CH₃, R' = benzoyl
 MGA-C14a : R = (CH₂)₉CH₃, R' = benzoyl
 MGA-C15a : R = (CH₂)₁₀CH₃, R' = benzoyl

E {
 MGA-C10 : R = (CH₂)₅CH₃, R' = H
 MGA-C11 : R = (CH₂)₆CH₃, R' = H
 MGA-C12 : R = (CH₂)₇CH₃, R' = H
 MGA-C13 : R = (CH₂)₈CH₃, R' = H
 MGA-C14 : R = (CH₂)₉CH₃, R' = H
 MGA-C15 : R = (CH₂)₁₀CH₃, R' = H

(a) NaH, 1,3,5-tris(bromomethyl)benzene, THF, DMF, room temperature; (b) LiAlH₄, THF, 50°C; (c) Perbenzoylated glucosylbromide, AgOTf, CH₂Cl₂, -45°C → room temperature; (d) NaOMe, MeOH, room temperature.

General procedure for preparation of monoalkylated diethylmalonate (A)

K₂CO₃ (5.0 equiv.) was added to diethyl malonate (5.0 equiv.) in a 1:1 mixture of THF (20 mL) and DMF (20 mL) at 0°C. The mixture was stirred until evolution of gas ceases. To this solution was added 1-bromoalkane (1.0 equiv.). The mixture was heated 60°C for 18 hrs. The resulting solution was extracted with diethyl ether and washed with 1.0 M aqueous HCl (100 mL) and brine (100 mL). The combine organic layer was dried over anhydrous Na₂SO₄, and the solvent was removed by rotary evaporation. The residue was purified by silica gel column chromatography (EtOAc/hexane) providing the desired product as an oily liquid.

General procedure for introduction of mesitylene linker (step a; A→B)

NaH (4.5 equiv.) and monoalkylated diethylmalonate (**A**, 3.3 equiv.) were dissolved in a 1:2 mixture of THF (15 mL) and DMF (30 mL) at 0 °C. 1,3,5-tris(bromomethyl)benzene (1.0 equiv.) was added in small portions, and the resulting solution was stirred at room temperature for 6 hrs. After completion of the reaction (as detected by TLC), the solution was diluted with diethyl ether (50 mL) and the washed successively with 1.0 M aqueous HCl (20 mL) and brine (100 mL). The organic layer was dried with anhydrous Na₂SO₄, and the solvent was removed by rotary evaporation. The residue was purified by silica gel column chromatography (EtOAc/hexane) providing the desired product as an oily liquid.

General procedure for reduction of esters by using LAH (step b; B→C)

LiAlH₄ (7.2 equiv.) was added slowly to **B** (1.0 equiv.) dissolved in THF (20 mL) at 0 °C. The mixture was stirred at 50 °C for 24 hrs, quenched with MeOH, water, a 1.0 M aqueous HCl solution successively at 0 °C and then extracted with diethyl ether (2 x 30mL). The combined organic layer was washed with brine and dried with anhydrous Na₂SO₄. The residue was purified by silica gel column chromatography (EtOAc/hexane) providing a desired product as a white solid.

General procedure for glycosylation reactions (step c; C→D)⁶

A mixture of alcohol derivative **C** to be glycosylated, AgOTf (8.0 equiv.) and 2,4,6-collidine (2.0 equiv.) in anhydrous CH₂Cl₂ (40mL) was stirred at -45°C. A solution of perbenzoylated glucosylbromide (8.0 equiv.) in CH₂Cl₂ (40 mL) was added dropwise over 0.5h to this suspension. Stirring was continued for 0.5 h at -45 °C, and then the reaction mixture was allowed to warm to 0 °C and left stirring for 1.5 hrs. After completion of the reaction (as detected by TLC), pyridine was added to the reaction mixture, which was then diluted with CH₂Cl₂ (40 mL) and filtered through celite. The filtrate was washed successively with a 1 M aqueous Na₂SO₄ (40 mL), a 1.0 M aqueous HCl solution (40 mL), and brine (2 x 40 mL). The organic layer was dried with anhydrous Na₂SO₄, 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** as a white solid.

General Procedure for the de-O-benzoylations under Zemlén's condition (step d; D→E)⁶

The *O*-protected **D** 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 reached 0.05 M. The reaction mixture was stirred for 6 h at room temperature, and then neutralized with Amberlite IR-120 (H⁺ form) resin. The resin was removed by filtration and washed with MeOH, and solvent was removed from the combined filtrate in vacuo. The residue was purified by silica gel column chromatography (MeOH/CH₂Cl₂). Further purification was achieved by recrystallization using CH₂Cl₂/MeOH/diethyl ether, afforded the fully deprotected product **E** as a white solid.

Diethyl 2-decylmalonate (**1**) was prepared in 93 % yield according to the general procedure for

preparation of monoalkylated diethylmalonate. **¹H NMR** (400 MHz, CDCl₃): δ 4.22-4.17 (m, 4H), 3.31 (t, *J* = 7.6Hz, 1H), 1.89-1.87 (m, 2H), 1.30-1.21 (m, 22H), 0.88 (t, *J* = 7.2Hz, 3H); **¹³C NMR** (100 MHz, CDCl₃): δ 169.8, 61.5, 52.3, 31.1, 29.8, 29.7, 29.5, 29.4, 28.9, 27.5, 22.9, 14.3.

Diethyl 2-undecylmalonate (**2**) was prepared in 90 % yield according to the general procedure for preparation of monoalkylated diethylmalonate. **¹H NMR** (400 MHz, CDCl₃): δ 4.23-4.16 (m, 4H), 3.31 (t, *J* = 7.6Hz, 1H), 1.89-1.87 (m, 2H), 1.31-1.25 (m, 24H), 0.88 (t, *J* = 7.2Hz, 3H); **¹³C NMR** (100 MHz, CDCl₃): δ 169.8, 61.5, 52.3, 32.1, 29.8, 29.7, 29.6, 29.4, 28.9, 27.5, 22.9, 14.3.

Diethyl 2-dodecylmalonate (**3**) was prepared in 91 % yield according to the general procedure for preparation of monoalkylated diethylmalonate. **¹H NMR** (400 MHz, CDCl₃): δ 4.22-4.16 (m, 4H), 3.31 (t, *J* = 7.6 Hz, 1H), 1.90-1.87 (m, 2H), 1.30-1.25 (m, 26H), 0.87 (t, *J* = 7.2 Hz, 3H); **¹³C NMR** (100 MHz, CDCl₃): δ 169.9, 61.5, 52.3, 32.1, 29.9, 29.7, 29.6, 29.5, 29.4, 28.9, 27.5, 22.9, 14.4.

Diethyl 2-tridecylmalonate (**4**) was prepared in 88 % yield according to the general procedure for preparation of monoalkylated diethylmalonate. **¹H NMR** (400 MHz, CDCl₃): δ 4.22-4.16 (m, 4H), 3.31 (t, *J* = 7.6 Hz, 1H), 1.90-1.87 (m, 2H), 1.30-1.25 (m, 28H), 0.87 (t, *J* = 7.2 Hz, 3H); **¹³C NMR** (100 MHz, CDCl₃): δ 169.9, 61.5, 52.3, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 28.9, 27.5, 22.9, 14.4, 14.3.

Diethyl 2-tetradecylmalonate (**5**) was prepared in 89 % yield according to the general procedure for preparation of monoalkylated diethyl malonate. **¹H NMR** (400 MHz, CDCl₃): δ 4.22-4.16 (m, 4H), 3.31 (t, *J* = 7.6 Hz, 1H), 1.90-1.87 (m, 2H), 1.30-1.25 (m, 30H), 0.87 (t, *J* = 7.2 Hz, 3H); **¹³C NMR** (100 MHz, CDCl₃): 169.9, 61.5, 52.3, 32.1, 29.9, 29.7, 29.6, 29.5, 29.4, 28.9, 27.5, 22.9, 14.4.

Diethyl 2-pentadecylmalonate (**6**) was prepared in 86 % yield according to the general procedure for preparation of monoalkylated diethylmalonate. **¹H NMR** (400 MHz, CDCl₃): δ 4.22-4.16 (m, 4H), 3.31 (t, *J* = 7.6 Hz, 1H), 1.90-1.87 (m, 2H), 1.30-1.25 (m, 28H), 0.87 (t, *J* = 7.2 Hz, 3H); **¹³C NMR** (100 MHz, CDCl₃): δ 169.9, 61.5, 52.3, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 28.9, 27.5, 22.9, 14.4, 14.3.

Hexaethyl 2,2',2''-(benzene-1,3,5-triyltris(methylene))tris(2-decylmalonate) (**7**) was prepared in 75 % yield according to the general procedure for introduction of mesitylene linker. **¹H NMR** (400 MHz, CDCl₃): δ 6.68 (s, 3H), 4.22-4.09 (m, 12H), 3.09 (s, 6H), 1.74-1.71 (m, 6H), 1.31-1.20 (m, 66H), 0.88 (t, *J* = 7.2Hz, 9H); **¹³C NMR** (100 MHz, CDCl₃): δ 171.5, 136.3, 130.5, 61.3, 58.9, 38.4, 32.2, 32.1, 30.0, 29.8, 29.7, 29.5, 24.5, 22.9, 14.3.

Hexaethyl 2,2',2''-(benzene-1,3,5-triyltris(methylene))tris(2-undecylmalonate) (**8**) was prepared in 76 % yield according to the general procedure for introduction of mesitylene linker. **¹H NMR** (400 MHz, CDCl₃): δ 6.68 (s, 3H), 4.22-4.09 (m, 12H), 3.09 (s, 6H), 1.74-1.71 (m, 6H), 1.31-1.20 (m, 72H), 0.88 (t, *J* = 7.2Hz, 9H); **¹³C NMR** (100 MHz, CDCl₃): δ 171.5, 136.3, 130.5, 61.3, 58.9, 38.4, 32.1, 30.0, 29.9, 29.8, 29.7, 29.6, 24.5, 22.9, 14.3.

Hexaethyl 2,2',2''-(benzene-1,3,5-triyltris(methylene))tris(2-dodecylmalonate) (**9**) was prepared in 73 % yield according to the general procedure for introduction of mesitylene linker. ¹H NMR (400 MHz, CDCl₃): δ 6.68 (s, 3H), 4.22-4.09 (m, 12H), 3.09 (s, 6H), 1.74-1.71 (m, 6H), 1.31-1.20 (m, 78H), 0.88 (t, *J* = 7.2Hz, 9H); ¹³C NMR (100 MHz, CDCl₃): δ 171.5, 136.3, 130.5, 61.3, 58.9, 38.4, 32.1, 30.0, 29.9, 29.8, 29.7, 29.6, 24.5, 22.9, 14.3.

Hexaethyl 2,2',2''-(benzene-1,3,5-triyltris(methylene))tris(2-tridecylmalonate) (**10**) was prepared in 70 % yield according to the general procedure for introduction of mesitylene linker. ¹H NMR (400 MHz, CDCl₃): δ 6.68 (s, 3H), 4.22-4.09 (m, 12H), 3.09 (s, 6H), 1.74-1.71 (m, 6H), 1.31-1.20 (m, 86H), 0.88 (t, *J* = 7.2Hz, 9H); ¹³C NMR (100 MHz, CDCl₃): δ 171.5, 136.3, 130.5, 61.3, 58.9, 38.4, 32.1, 30.0, 29.9, 29.8, 29.7, 29.6, 24.5, 22.9, 14.3.

Hexaethyl 2,2',2''-(benzene-1,3,5-triyltris(methylene))tris(2-tetradecylmalonate) (**11**) was prepared in 73 % yield according to the general procedure for introduction of mesitylene linker. ¹H NMR (400 MHz, CDCl₃): δ 6.68 (s, 3H), 4.22-4.09 (m, 12H), 3.09 (s, 6H), 1.74-1.71 (m, 6H), 1.31-1.20 (m, 92H), 0.88 (t, *J* = 7.2Hz, 9H); ¹³C NMR (100 MHz, CDCl₃): δ 171.5, 136.3, 130.5, 61.3, 58.9, 38.4, 32.1, 30.0, 29.9, 29.8, 29.7, 29.6, 24.5, 22.9, 14.3.

Hexaethyl 2,2',2''-(benzene-1,3,5-triyltris(methylene))tris(2-pentadecylmalonate) (**12**) was prepared in 71 % yield according to the general procedure for introduction of mesitylene linker. ¹H NMR (400 MHz, CDCl₃): δ 6.68 (s, 3H), 4.22-4.09 (m, 12H), 3.09 (s, 6H), 1.74-1.71 (m, 6H), 1.31-1.20 (m, 98H), 0.88 (t, *J* = 7.2Hz, 9H); ¹³C NMR (100 MHz, CDCl₃): δ 171.5, 136.3, 130.5, 61.3, 58.9, 38.4, 32.1, 30.0, 29.9, 29.8, 29.7, 29.6, 24.5, 22.9, 14.3.

2,2',2''-(benzene-1,3,5-triyltris(methylene))tris(2-decylpropane-1,3-diol) (**13**) was prepared in 74 % yield according to the general procedure for reduction of esters by using LAH. . ¹H NMR (400 MHz, CD₃OD): δ 6.92 (s, 3H), 3.41 (s, 12H), 2.55 (s, 6H), 1.34-1.12 (m, 72H), 0.89 (t, *J* = 6.8Hz, 9H); ¹³C NMR (100 MHz, CD₃OD): δ 138.6, 131.8, 66.2, 44.3, 38.1, 33.3, 32.4, 32.1, 31.1, 31.0, 30.7, 24.3, 23.9, 14.7.

2,2',2''-(benzene-1,3,5-triyltris(methylene))tris(2-undecylpropane-1,3-diol) (**14**) was prepared in 75 % yield according to the general procedure for reduction of esters by using LAH. . ¹H NMR (400 MHz, CD₃OD): δ 6.92 (s, 3H), 3.41 (s, 12H), 2.55 (s, 6H), 1.34-1.12 (m, 78H), 0.89 (t, *J* = 6.8Hz, 9H); ¹³C NMR (100 MHz, CD₃OD): δ 138.6, 131.8, 66.2, 44.3, 38.1, 33.3, 32.4, 32.1, 31.1, 31.0, 30.7, 24.3, 23.9, 14.7.

2,2',2''-(benzene-1,3,5-triyltris(methylene))tris(2-dodecylpropane-1,3-diol) (**15**) was prepared in 72 % yield according to the general procedure for reduction of esters by using LAH. . ¹H NMR (400 MHz, CD₃OD): δ 6.92 (s, 3H), 3.41 (s, 12H), 2.55 (s, 6H), 1.34-1.12 (m, 84H), 0.89 (t, *J* = 6.8Hz, 9H); ¹³C NMR (100 MHz, CD₃OD): δ 138.6, 131.8, 66.2, 44.3, 38.1, 33.3, 32.4, 32.1, 31.1, 31.0, 30.7, 24.3, 23.9, 14.7.

2,2',2''-(benzene-1,3,5-triyltris(methylene))tris(2-tridecylpropane-1,3-diol) (**16**) was prepared in 74 % yield according to the general procedure for reduction of esters by using LAH. . ¹H NMR (400 MHz, CD₃OD): δ 6.92 (s, 3H), 3.41 (s, 12H), 2.55 (s, 6H), 1.34-1.12 (m, 90H), 0.89 (t, *J* = 6.8Hz, 9H); ¹³C NMR (100 MHz, CD₃OD): δ 138.6, 131.8, 66.2, 44.3, 38.1, 33.3, 32.4, 32.1, 31.1, 31.0, 30.7, 24.3, 23.9, 14.7.

2,2',2''-(benzene-1,3,5-triyltris(methylene))tris(2-tetradecylpropane-1,3-diol) (**17**) was prepared in 70 % yield according to the general procedure for reduction of esters by using LAH. . ¹H NMR (400 MHz, CD₃OD): δ 6.92 (s, 3H), 3.41 (s, 12H), 2.55 (s, 6H), 1.34-1.12 (m, 96H), 0.89 (t, *J* = 6.8Hz, 9H); ¹³C NMR (100 MHz, CD₃OD): δ 138.6, 131.8, 66.2, 44.3, 38.1, 33.3, 32.4, 32.1, 31.1, 31.0, 30.7, 24.3, 23.9, 14.7.

2,2',2''-(benzene-1,3,5-triyltris(methylene))tris(2-pentadecylpropane-1,3-diol) (**18**) was prepared in 72 % yield according to the general procedure for reduction of esters by using LAH. . ¹H NMR (400 MHz, CD₃OD): δ 6.92 (s, 3H), 3.41 (s, 12H), 2.55 (s, 6H), 1.34-1.12 (m, 102H), 0.89 (t, *J* = 6.8Hz, 9H); ¹³C NMR (100 MHz, CD₃OD): δ 138.6, 131.8, 66.2, 44.3, 38.1, 33.3, 32.4, 32.1, 31.1, 31.0, 30.7, 24.3, 23.9, 14.7.

MGA-C10a was prepared in 50 % yield according to the general procedure for glycosylation reaction. The ¹H NMR spectrum of this compound dissolved in CDCl₃ or DMSO-d₆ showed broad peaks with low resolutions because of the substantial aggregation.

MGA-C11a was prepared in 51 % yield according to the general procedure for glycosylation reaction. The ¹H NMR spectrum of this compound dissolved in CDCl₃ or DMSO-d₆ showed broad peaks with low resolutions because of the substantial aggregation.

MGA-C12a was prepared in 51 % yield according to the general procedure for glycosylation reaction. The ¹H NMR spectrum of this compound dissolved in CDCl₃ or DMSO-d₆ showed broad peaks with low resolutions because of the substantial aggregation.

MGA-C13a was prepared in 47 % yield according to the general procedure for glycosylation reaction. The ¹H NMR spectrum of this compound dissolved in CDCl₃ or DMSO-d₆ showed broad peaks with low resolutions because of the substantial aggregation.

MGA-C14a was prepared in 48 % yield according to the general procedure for glycosylation reaction. The ¹H NMR spectrum of this compound dissolved in CDCl₃ or DMSO-d₆ showed broad peaks with low resolutions because of the substantial aggregation.

MGA-C15a was prepared in 45 % yield according to the general procedure for glycosylation reaction. The ¹H NMR spectrum of this compound dissolved in CDCl₃ or DMSO-d₆ showed broad peaks with low resolutions because of the substantial aggregation.

MGA-C10 was prepared in 94 % yield according to the general procedure for deprotection reactions.

¹H NMR (400 MHz, CD₃OD): δ 7.02 (s, 3H), 4.38-4.35 (m, 6H), 3.90-3.87 (m, 6H), 3.81-3.67 (m, 12H), 3.51-3.46 (m, 6H), 3.42-3.22 (m, 12H), 2.69 (s, 6H), 1.41-1.16 (m, 54H), 0.90 (t, *J* = 7.2Hz, 9H); **¹³C NMR** (100MHz, CD₃OD): δ 138.4, 105.2, 105.1, 78.3, 77.9, 75.4, 73.4, 71.8, 71.7, 62.9, 43.6, 38.4, 33.3, 32.9, 32.2, 31.3, 31.1, 30.8, 24.4, 23.9, 14.7; **HRMS (EI)**: calcd. for C₈₄H₁₅₀O₃₆[M+Na]⁺ 1757.9805, found 1757.9810.

MGA-C11 was prepared in 92 % yield according to the general procedure for deprotection reactions. **¹H NMR** (400 MHz, CD₃OD): δ 7.02 (s, 3H), 4.38-4.35 (m, 6H), 3.90-3.87 (m, 6H), 3.81-3.67 (m, 12H), 3.51-3.46 (m, 6H), 3.42-3.22 (m, 12H), 2.69 (s, 6H), 1.41-1.16 (m, 60H), 0.90 (t, *J* = 7.2Hz, 9H); **¹³C NMR** (100 MHz, CD₃OD): δ 138.3, 132.3, 105.2, 105.1, 78.3, 77.9, 75.4, 73.4, 71.9, 71.8, 62.9, 43.6, 38.4, 33.3, 32.9, 32.2, 31.2, 31.1, 30.8, 24.3, 23.9, 14.7; **HRMS (EI)**: calcd. for C₈₇H₁₅₆O₃₆[M+Na]⁺ 1801.0308, found 1801.0337.

MGA-C12 was prepared in 93 % yield according to the general procedure for deprotection reactions. **¹H NMR** (400 MHz, CD₃OD): δ 7.02 (s, 3H), 4.38-4.35 (m, 6H), 3.90-3.87 (m, 6H), 3.81-3.67 (m, 12H), 3.51-3.46 (m, 6H), 3.42-3.22 (m, 12H), 2.69 (s, 6H), 1.41-1.16 (m, 66H), 0.90 (t, *J* = 7.2Hz, 9H); **¹³C NMR** (100 MHz, CD₃OD): δ 138.4, 105.2, 105.1, 78.3, 77.9, 75.3, 73.3, 71.7, 62.9, 43.6, 33.3, 33.0, 31.3, 31.2, 31.1, 30.1, 24.4, 14.7; **HRMS (EI)**: calcd. for C₉₀H₁₆₁O₃₆[M+Na]⁺ 1842.0700, found 1841.0668.

MGA-C13 was prepared in 91 % yield according to the general procedure for deprotection reactions. **¹H NMR** (400 MHz, CD₃OD): δ 7.02 (s, 3H), 4.38-4.35 (m, 6H), 3.90-3.87 (m, 6H), 3.81-3.67 (m, 12H), 3.51-3.46 (m, 6H), 3.42-3.22 (m, 12H), 2.69 (s, 6H), 1.41-1.16 (m, 72H), 0.90 (t, *J* = 7.2Hz, 9H); **¹³C NMR** (100 MHz, CD₃OD): δ 138.4, 105.2, 105.1, 78.3, 77.9, 75.3, 71.8, 71.7, 62.9, 43.6, 33.3, 31.3, 31.2, 31.1, 30.8, 24.4, 23.9, 14.7; **HRMS (EI)**: calcd. for C₉₃H₁₆₈O₃₆[M+Na]⁺ 1885.1247, found 1885.0898.

MGA-C14 was prepared in 93 % yield according to the general procedure for deprotection reactions. **¹H NMR** (400 MHz, CD₃OD): δ 7.02 (s, 3H), 4.38-4.35 (m, 6H), 3.90-3.87 (m, 6H), 3.81-3.67 (m, 12H), 3.51-3.46 (m, 6H), 3.42-3.22 (m, 12H), 2.69 (s, 6H), 1.41-1.16 (m, 78H), 0.90 (t, *J* = 7.2Hz, 9H); **¹³C NMR** (100 MHz, CD₃OD): δ 168.4, 132.3, 105.2, 105.1, 78.3, 77.9, 75.3, 73.5, 73.3, 71.8, 71.7, 62.8, 43.6, 38.4, 33.4, 32.3, 31.3, 31.2, 31.1, 30.8, 24.4, 23.9, 14.7; **HRMS (EI)**: calcd. for C₉₆H₁₇₄O₃₆[M+Na]⁺ 1927.1717, found 1927.1675.

MGA-C15 was prepared in 92 % yield according to the general procedure for deprotection reactions. **¹H NMR** (400 MHz, CD₃OD): δ 7.02 (s, 3H), 4.38-4.35 (m, 6H), 3.90-3.87 (m, 6H), 3.81-3.67 (m, 12H), 3.51-3.46 (m, 6H), 3.42-3.22 (m, 12H), 2.69 (s, 6H), 1.41-1.16 (m, 84H), 0.90 (t, *J* = 7.2Hz, 9H); **¹³C NMR** (100 MHz, CD₃OD): δ 168.4, 132.3, 105.2, 105.1, 78.3, 77.9, 75.3, 73.5, 73.3, 71.8, 71.7, 62.8, 43.6, 38.4, 33.3, 32.2, 31.3, 31.2, 31.1, 30.8, 24.4, 23.9, 14.7; **HRMS (EI)**: calcd. for C₉₉H₁₈₀O₃₆[M+Na]⁺ 1969.2186, found 1968.2430.

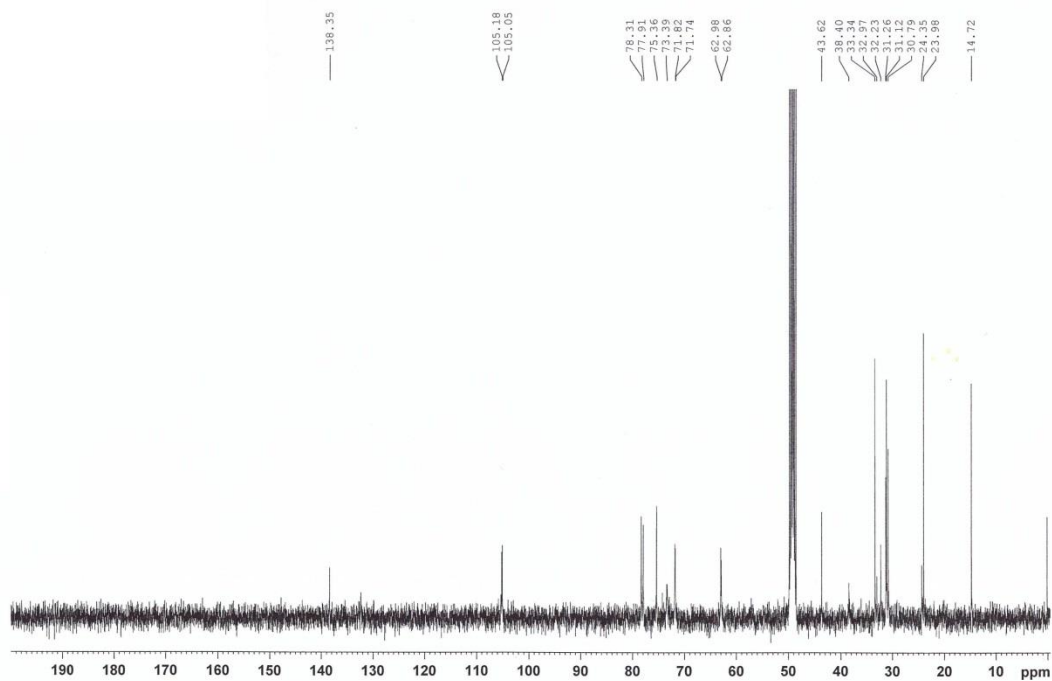
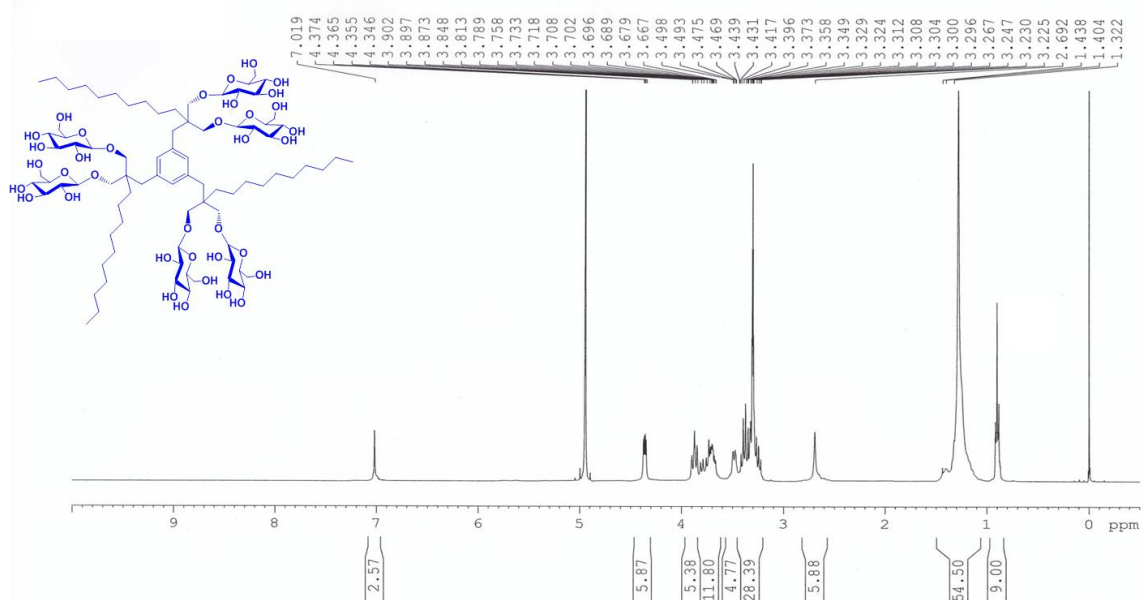
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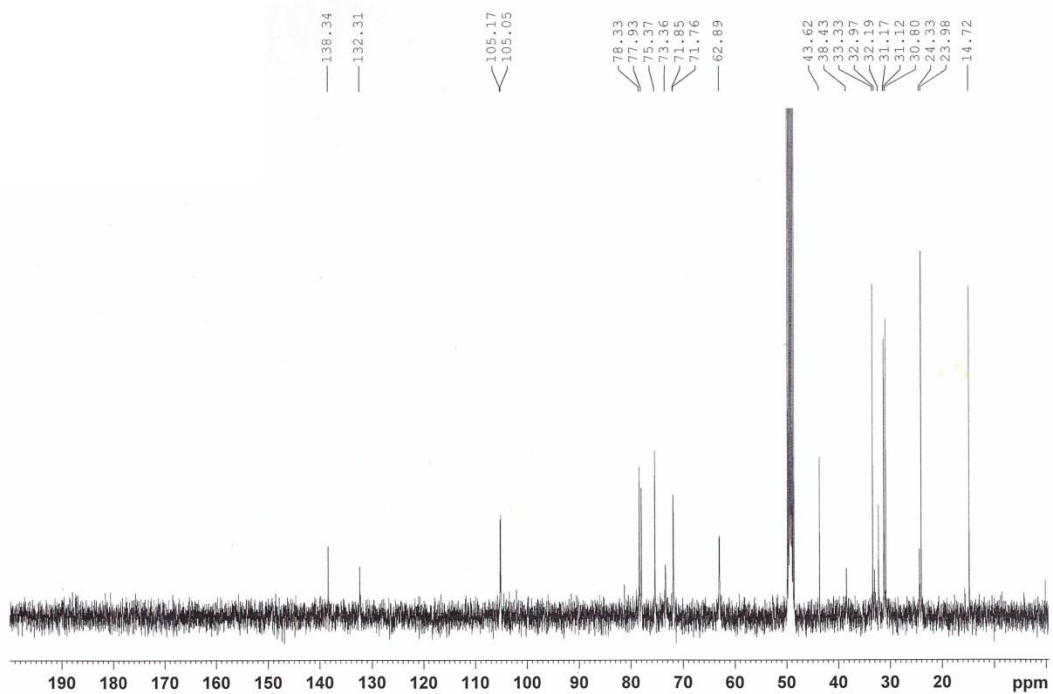
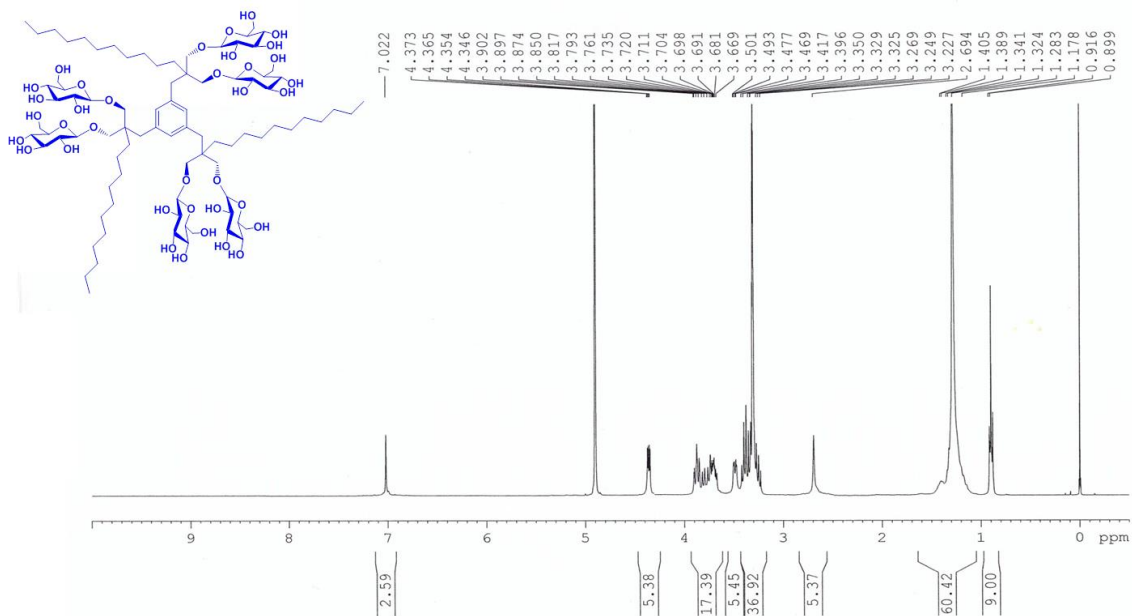
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^1H and ^{13}C NMR spectra of MGAs (MGA-C10, MGA-C11, MGA-C12, MGA-C13, MGA-C14, or MGA-C15)

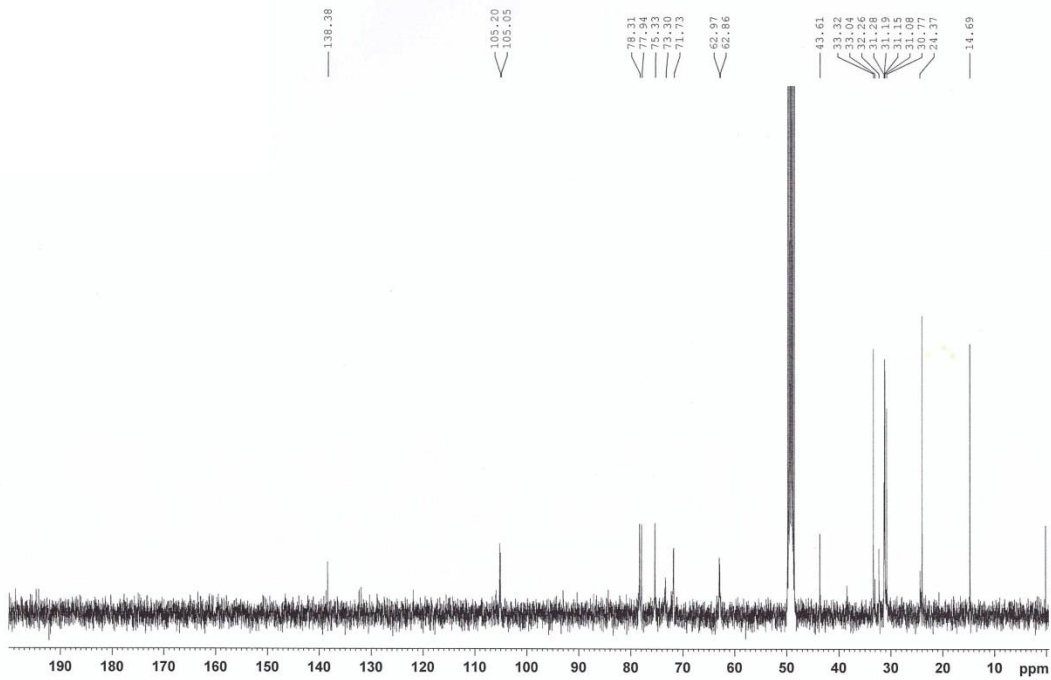
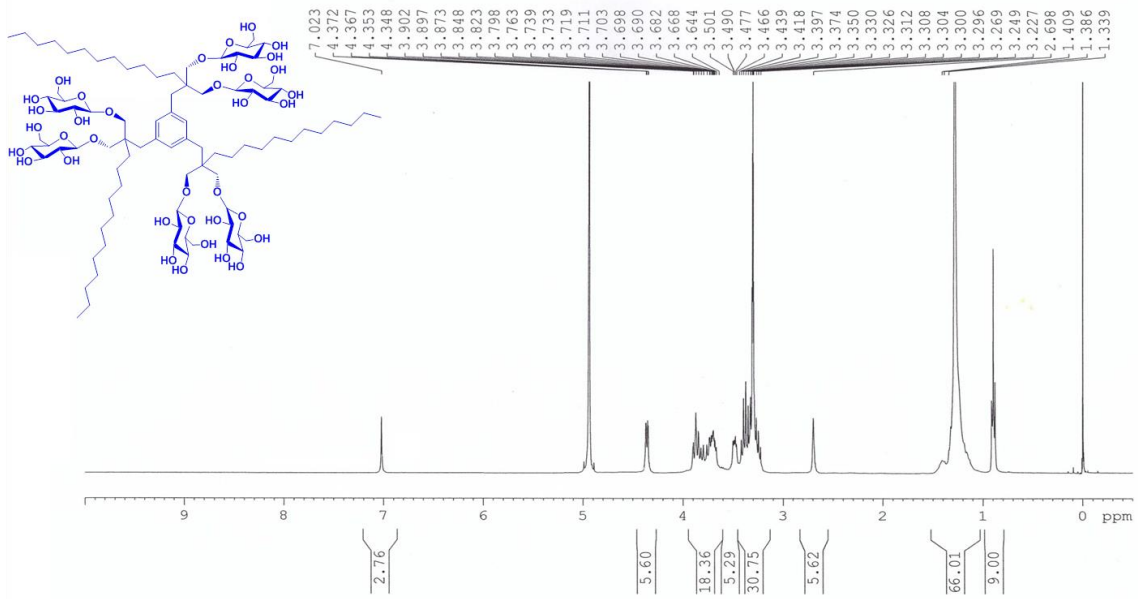
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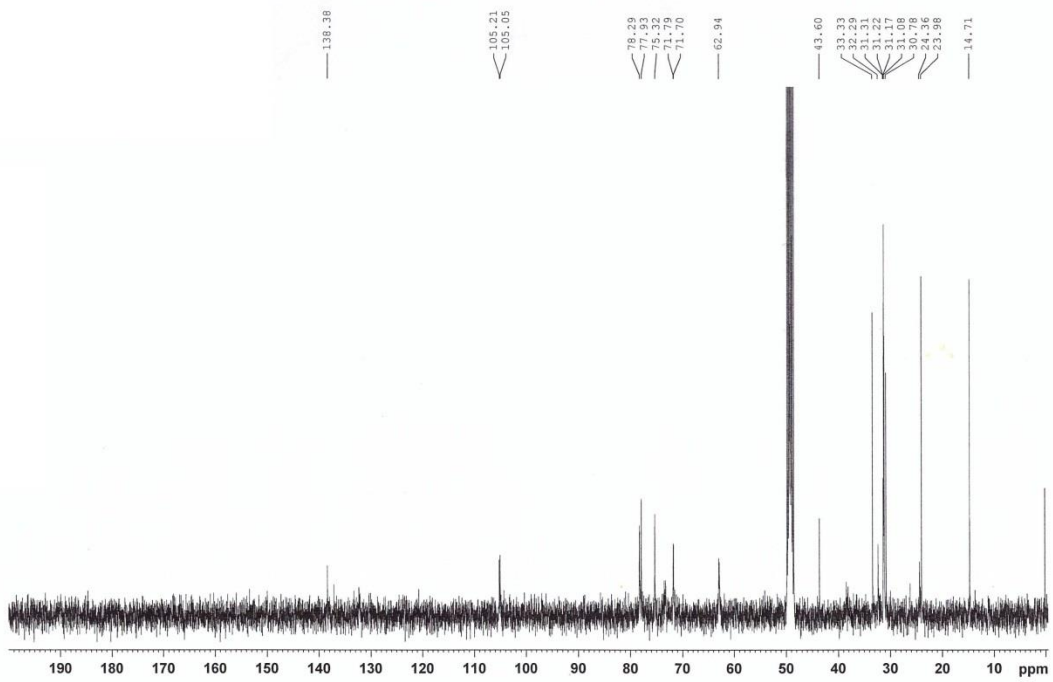
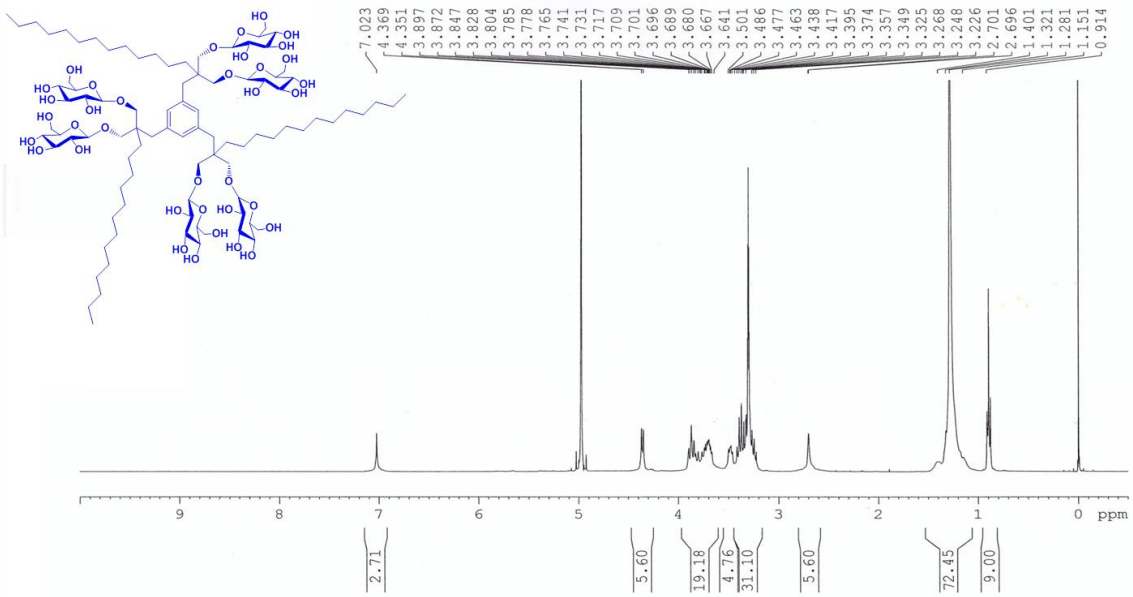
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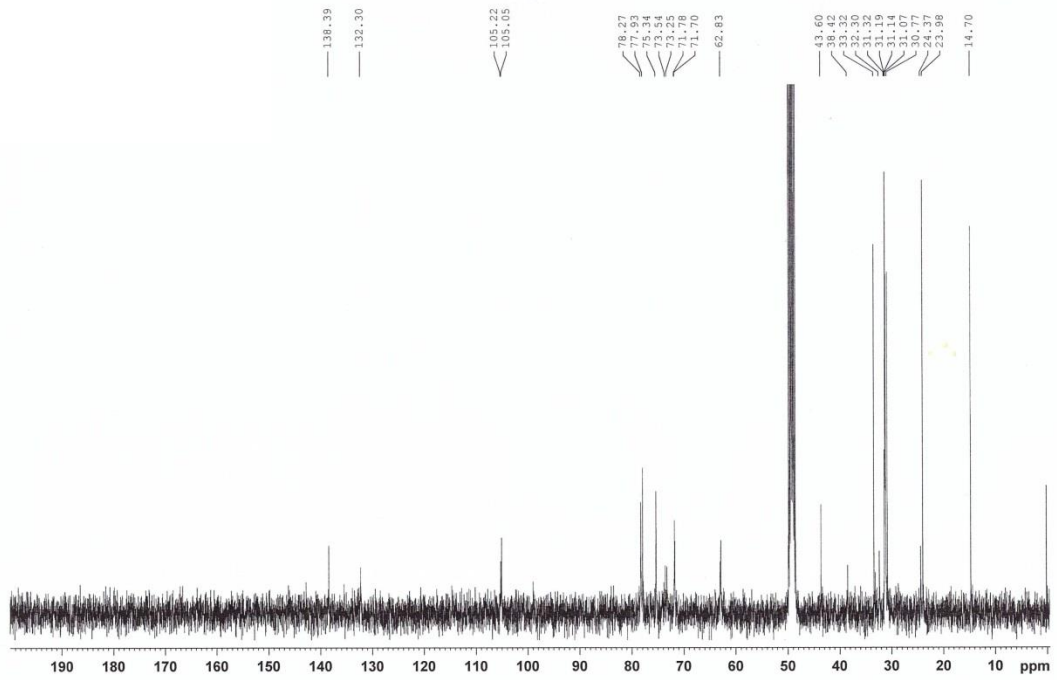
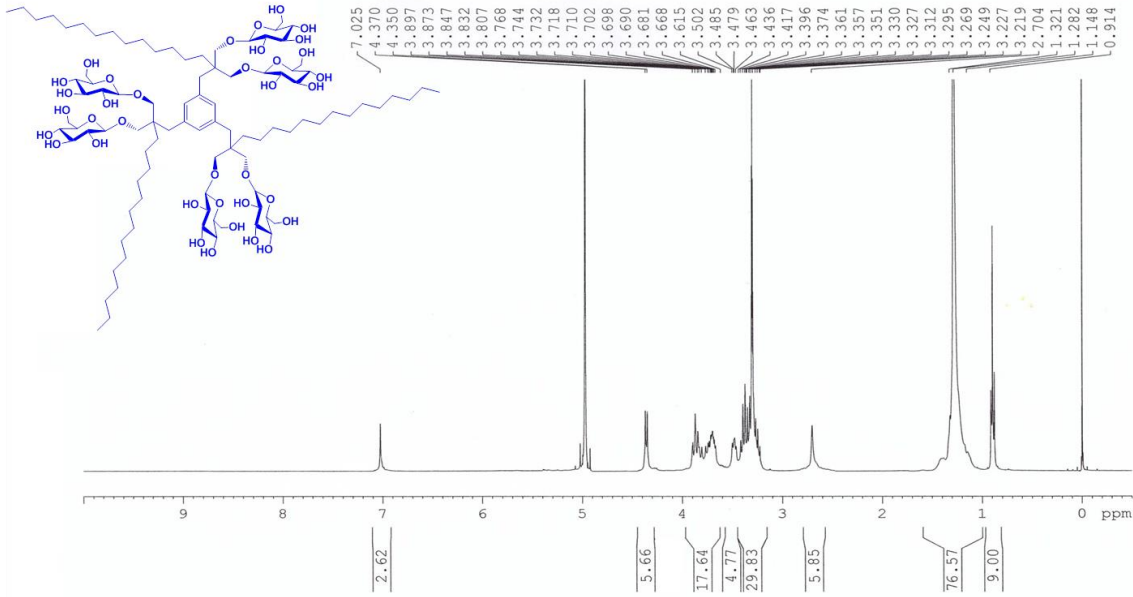
MGA-C12



MGA-C13



MGA-C14



MGA-C15

