Electronic Supplementary Information

Synthetic ultra-long chain fatty acyl based amphiphilic lipids as a dual function excipient for production of surfactant-free solid lipid nanoparticles (SF-SLNs): A physico-chemical study

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Materials

Compound **1** to **5** were synthesis in our lab. (+)-Diacetyl-L-tartaric anhydride (≥99%), (S)-(−)-2-Acetoxy succinic anhydride (≥99%), 2-Methyltetrahydrofuran (anhydrous, ≥99%, Inhibitor-free), 2-Methylfuran (99%) and other chemicals were purchased from Sigma-Aldrich (St. Louis, USA). All solvents used were HPLC grade. All water used was de-ionized water obtained from a Milli-Q (Millipore, MA) system.

General of synthesis and analysis methods

General: Compound **1** to **5** were previously synthesis with high purity using Novozym 435 (*Candida antarctica* lipase B) in *t*-BuOH [1]. Various diacetyl tartaric acid esters were synthesized with 0.5 mmol 2-acetoxy succinic anhydride/diacetyl tartaric anhydride and require 1.2 equivalent compounds **1-5** without catalyst. Reactions were performed in a capped glass vial at 50 - 60 °C for 3-12 h with magnetic stirring at 500 rpm. Solvent mixture and 20 w% molecular sieves (4Å, actived at 200 °C over 8 h) were added into the reaction mixture. The reaction mixture was heated slowly up until all the compounds dissolved. Samples were taken from the reaction mixture and analyzed qualitatively by TLC and quantified by HPLC. Variance of conversion values was estimated to be ≤2%. Isolated yields were estimated to be ≤5%.

Thin layer chromatography (TLC) analysis was used to monitor reactions. Samples were taken from the reaction at set time intervals and diluted with 300 μL $CHCl_3$ resulted in the concentration of 2-3 mg/mL. TLC analysis was performed on silica gel 60 TLC plate (5 cm × 10 cm, Merck, Germany) using $CHCl_3$/methanol/ acetic acid /water, 80:10:8:2 (v/v/v) as developing system. Plates were dried and sprayed with 30% of sulfuric acid in methanol and visualized by evaporating the methanol.

High performance liquid chromatography (HPLC) analysis were performed on a Thermo HPLC system (Waltham, MA, USA) equipped with a Supelcosil LC-18 column (250 mm × 4.6 mm × 5 μm, Supelco, USA) to calculate the reaction conversions. The reaction mixtures were detected by a Sedex model 75 evaporative light scattering detector (Sedere, Alfortville, France) at 40 °C. Mobile phase consists of (A) methanol/ACN/ acetic acid (75/25/0.001, v/v/v) and (B) water. Analysis was performed using a mobile phase of 98.5% A and 1.5% B and a flow rate of 1 mL/min. Sample were taken from the reaction and diluted with $CHCl_3$. The calibration standards included diacetyl tartaric anhydride (R^2 = 0.999 0), target compounds (R^2 = 0.997 5) which was high purity compounds purified by column, compound **3** (R^2 = 0.997 3) and behenic acid (R^2 = 0.997 9). They were applied to HPLC both in mixtures and individually.
In a typical HPLC chromatogram of reaction mixture for compound **3b** synthesis, the retention time for substrates, product and side products are: diacetyl tartaric anhydrous: 3.1min; side product **1'** (): 7.2 min; target product (compound **3b**): 8.5 min; Compound **3**: 10.5 min; side product **2'**: 20.2 min;

Structure identifications were carried out using a Bruker Avance III spectrometer at 400 MHz and Bruker mass spectroscopy with ESI-IT quadrupole detection.

Synthetic compounds and characterization data

Synthesis of 2-acetoxy-4-(2-(docosanoyloxy)ethoxy)-4-oxobutanoic acid (**1a**): 230 mg of **1** (0.6 mmol) and 79 mg of 2-acetoxy succinic anhydride (0.5 mmol) were dissolved into 2 mL *n*-hexane and heated slowly up to 50 °C. Then 0.5 mL of 2-Me-THF was added. Stirring was continued for 3 h at 50 °C. At the end of the reaction, the purification of compound **1a** which R_f close to compound **1**, were carried out by recrystallization. Excess solvent was evaporated under reduced pressure and the solid residue was precipitated three times with *n*-hexane. Conversion of the 2-acetoxy succinic anhydride was 87%. Yield of isolated product after purification was 63%.
White solid; R_f 0.74; m.p. 63.34 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ_{TMS} = 0 ppm) δ = 5.48 (t, J = 6.1 Hz, 1H), 4.49 – 4.17 (m, 4H), 2.96 (d, J = 6.1 Hz, 2H), 2.32 (t, J = 7.6 Hz, 2H), 2.15 (s, 3H), 1.66 – 1.56 (m, 2H), 1.32 – 1.23 (m, 36H), 0.88 (t, J = 6.8 Hz, 3H). MS: *m/z* calculated for $C_{30}H_{54}O_8$: 542.38; found: 565.38 ($M + Na^+$).

2-acetoxy-4-(3-(docosanoyloxy)-2-hydroxypropoxy)-4-oxobutanoic acid (**2a**): 248 mg of **2** (0.6 mmol) and 79 mg of 2-acetoxy succinic anhydride (0.5 mmol) were dissolved into 2 mL *n*-hexane and heated slowly up to 60 °C. Then 0.5 mL of 2-Me-THF was added. Stirring was continued for 4 h at 60 °C. After completion of the reaction, the reaction mixture was evaporated and the residue was purified by column chromatography on silica gel (CH_2Cl_2: methanol, 85:15 v/v). The corresponding parts were collected, dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. Conversion of the 2-acetoxy succinic anhydride was 93%. Yield of isolated product after purification was 48%.
White solid; R_f 0.73; m.p. 68.68 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ_{TMS} = 0 ppm) δ = 5.47 (d, J = 46.0 Hz, 1H), 4.48 – 3.94 (m, 5H), 3.13 – 2.74 (m, 2H), 2.34 (t, J = 7.6 Hz, 2H), 2.15 (s, 3H), 1.66 – 1.54 (m, 2H), 1.31 – 1.22 (m, 36H), 0.88 (t, J = 6.8 Hz, 3H). MS: *m/z* calculated for $C_{31}H_{56}O_9$: 572.39; found: 595.39 ($M + Na^+$).

2-acetoxy-4-(4-(docosanoyloxy)-2,3-dihydroxybutoxy)-4-oxobutanoic acid (**3a**): 266 mg of **3** (0.6 mmol) and 79 mg of 2-acetoxy succinic anhydride (0.5 mmol) were dissolved into 6 mL *n*-hexane and heated slowly up to 60 °C. Then 1 mL of 2-Me-THF was added. Stirring was continued for 4 h at 60 °C. The purification was the same as described of **2a**. Conversion of the 2-acetoxy succinic anhydride was 87%. Yield of isolated product after purification was 44%.
White solid; R_f 0.68; m.p. 70.50 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ_{TMS} = 0 ppm) δ = 5.52 – 5.31 (m, 1H), 4.63 – 4.00 (m, 4H), 3.98 – 3.76 (m, 2H), 2.94 (ddd, J = 54.6, 16.5, 5.2 Hz, 2H), 2.34 (dt, J = 17.2, 8.8 Hz, 2H), 2.24 – 2.04 (m, 3H), 1.75 – 1.52 (m, 2H), 1.36 – 1.20 (m, 36H), 0.88 (t, J = 6.8 Hz, 3H). MS: *m/z* calculated for $C_{32}H_{58}O_{10}$: 602.40; found: 625.40 ($M + Na^+$).

2-acetoxy-4-(5-(docosanoyloxy)-2,3,4-trihydroxypentyloxy)-4-oxobutanoic acid (**4a**): 285 mg of **4** (0.6 mmol) and 79 mg of 2-acetoxy succinic anhydride (0.5 mmol) were dissolved into 8 mL *n*-hexane and heated slowly up to 70 °C. Then 1.5 mL of 2-Me-THF was added. Stirring was continued for 4 h at 70 °C. The purification was the same as described of **2a**. Conversion of the 2-acetoxy succinic anhydride was 92%. Yield of isolated product after purification was 39%.
White solid; R_f 0.46; m.p. 67.74 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ_{TMS} = 0 ppm) δ = 5.52 – 5.22 (m, 1H), 4.53 – 4.13 (m, 4H), 4.03 (d, J = 23.6 Hz, 2H), 3.61 (s, 1H), 2.93 (dd, J = 71.2, 12.5 Hz, 2H), 2.34 (t, J = 7.6 Hz, 2H), 2.22 – 2.07 (m, 3H), 1.69 – 1.49 (m, 2H), 1.39 – 1.05 (m, 36H), 0.88 (t, J = 6.8 Hz, 3H). MS: *m/z* calculated for $C_{32}H_{58}O_{10}$: 632.41; found: 655.41 ($M + Na^+$).

2-acetoxy-4-(6-(docosanoyloxy)-2,3,4,5-tetrahydroxyhexyloxy)-4-oxobutanoic acid (**5a**): 302 mg of **5** (0.6 mmol) and 79 mg of 2-acetoxy succinic anhydride (0.5 mmol) were dissolved into 15 mL *n*-hexane and heated slowly up to 90 °C. Then 3 mL of 2-Me-THF was added. Stirring was continued for 12 h at 90 °C. The purification was the same as described of **2a**. Conversion of the 2-acetoxy succinic anhydride was 68%. Yield of isolated product after purification was 26%.
White solid; R_f 0.26; m.p. 66.50 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.45 (dd, J = 10.5, 5.1 Hz, 1H), 4.44 – 4.08 (m, 4H), 4.01 – 3.68 (m, 2H), 3.01 (dd, J = 16.5, 6.4 Hz, 2H), 2.96 – 2.78 (m, 2H), 2.34 (t, J = 7.6 Hz, 2H), 2.15 (d, J = 4.0 Hz, 3H), 1.68 – 1.51 (m, 2H), 1.39 – 1.08 (m, 36H), 0.88 (t, J = 6.7 Hz, 3H). MS: *m/z* calculated for $C_{34}H_{62}O_{12}$: 662.42; found: 685.42 ($M + Na^+$).

2,3-diacetoxy-4-(2-(docosanoyloxy)ethoxy)-4-oxobutanoic acid (**1b**): 230 mg of **1** (0.6 mmol) and 108 mg of diacetyl tartaric anhydride (0.5 mmol) were dissolved into 2 mL *n*-hexane and heated slowly up to 50 °C. Then 0.5 mL of 2-Me-THF was added. Stirring was continued for 3 h at 50 °C. The purification was the same as described of **1a**. Conversion of the diacetyl tartaric anhydride was 92%. Yield of isolated product after purification was 71%.
White solid; R_f 0.63; m.p. 66.78 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ_{TMS} = 0 ppm) δ = 5.75 (d, J = 13.5 Hz, 2H), 4.61 – 4.17 (m, 4H), 2.32 (t, J = 7.6 Hz, 2H), 2.19 (d, J = 8.4 Hz, 6H), 1.74 – 1.48 (m, 2H), 1.47 – 1.10 (m, 36H), 0.89 (t, J = 6.8 Hz, 3H). MS: *m/z* calculated for $C_{32}H_{56}O_{10}$: 600.39; found: 623.39 ($M + Na^+$).

2,3-diacetoxy-4-(3-(docosanoyloxy)-2-hydroxypropoxy)-4-oxobutanoic acid (**2b**): 248 mg of **2** (0.6 mmol) and 108 mg of diacetyl tartaric anhydride (0.5 mmol) were dissolved into 6 mL *n*-hexane and heated slowly up to 60 °C. Then 0.5 mL of 2-Me-THF was added. Stirring was continued for 4 h at 60 °C. The purification was the same as described of **2a**. Conversion of the diacetyl tartaric anhydride was 97%. Yield of isolated product after purification was 57%.
White solid; R_f 0.39; m.p. 69.15 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ_{TMS} = 0 ppm) δ = 5.91 – 4.96 (m, 2H), 4.45 – 3.97 (m, 4H), 3.72 (s, 1H), 2.32 (dd, J = 11.0, 7.5 Hz, 2H), 2.27 – 1.93 (m, 6H), 1.67 – 1.52 (m, 2H), 1.33 – 1.20 (m, 36H), 0.88 (t, J = 6.8 Hz, 3H). MS: *m/z* calculated for $C_{33}H_{58}O_{11}$: 630.4; found: 653.40 ($M + Na^+$).

2,3-diacetoxy-4-(4-(docosanoyloxy)-2,3-dihydroxybutoxy)-4-oxobutanoic acid (**3b**): 266 mg of **3** (0.6 mmol) and 108 mg of diacetyl tartaric anhydride (0.5 mmol) were dissolved into 6 mL *n*-

hexane and heated slowly up to 60 °C. Then 1 mL of 2-Me-THF was added. Stirring was continued for 4 h at 60 °C. The purification was the same as described of **2a**. Conversion of the diacetyl tartaric anhydride was 92%. Yield of isolated product after purification was 38%.
White solid; R_f 0.24; m.p. 69.20 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ_{TMS} = 0 ppm) δ = 5.86 – 5.32 (m, 2H), 4.61 – 4.00 (m, 4H), 3.87 (s, 2H), 2.34 (t, J = 7.5 Hz, 2H), 2.28 – 2.08 (m, 6H), 1.66 – 1.52 (m, 2H), 1.32 – 1.20 (m, 36H), 0.88 (t, J = 6.8 Hz, 3H). MS: *m/z* calculated for $C_{34}H_{60}O_{12}$: 660.41; found: 683.41 ($M + Na^+$).

2,3-diacetoxy-4-(5-(docosanoyloxy)-2,3,4-trihydroxypentyloxy)-4-oxobutanoic acid (**4b**): 285 mg of **4** (0.6 mmol) and 108 mg of diacetyl tartaric anhydride (0.5 mmol) were dissolved into 8 mL *n*-hexane and heated slowly up to 70 °C. Then 1.5 mL of 2-Me-THF was added. Stirring was continued for 4 h at 70 °C. The purification was the same as described of **2a**. Conversion of the diacetyl tartaric anhydride was 96%. Yield of isolated product after purification was 38%.
White solid; R_f 0.19; m.p. 67.09 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ_{TMS} = 0 ppm) δ = 5.64 – 5.22 (m, 2H), 4.44 – 4.07 (m, 4H), 4.09 – 3.86 (m, 2H), 3.61 (s, 1H), 2.43 – 2.24 (m, 2H), 2.16 (d, J = 8.5 Hz, 6H), 1.73 – 1.44 (m, 2H), 1.27 – 1.24 (m, 36H), 0.88 (t, J = 6.8 Hz, 3H). MS: *m/z* calcd for $C_{35}H_{62}O_{13}$: 690.42; found: 713.42 ($M + Na^+$).

2,3-diacetoxy-4-(6-(docosanoyloxy)-2,3,4,5-tetrahydroxyhexyloxy)-4-oxobutanoic acid (**5b**): 302 mg of **5** (0.6 mmol) and 108 mg of diacetyl tartaric anhydride (0.5 mmol) were dissolved into 15 mL *n*-hexane and heated slowly up to 90 °C. Then 3.5 mL of 2-Me-THF was added. Stirring was continued for 12 h at 90 °C. The purification was the same as described of **2a**. Conversion of the 2-acetoxy succinic anhydride was 81%. Yield of isolated product after purification was 21%.
White solid; R_f 0.13; m.p. 68.10 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ_{TMS} = 0 ppm) δ = 5.59 – 5.16 (m, 2H), 4.61 – 3.99 (m, 4H), 3.99 – 3.77 (m, 2H), 3.77 – 3.42 (m, 2H), 2.33 (d, J = 5.8 Hz, 2H), 2.28 – 1.96 (m, 6H), 1.72 – 1.44 (m, 2H), 1.32 – 1.19 (m, 36H), 0.88 (t, J = 6.8 Hz, 3H). MS: *m/z* calculated for $C_{36}H_{64}O_{14}$: 720.43; found: 743.43 ($M + NH4^+$).

Differential scanning calorimetry measurement

The thermal properties of the synthetic compounds were analyzed using Differential scanning calorimetry (DSC) instrument on Pyris 6 system (Perkin-Elmer Cetus, Norwalk, USA). The synthetic compounds were dried under low pressure over night, and then encapsulated in aluminum pans. The measurement was under an atmosphere of nitrogen with flow of 20 mL/min. The heating and cooling profile was: 1) initial temperature 20 °C; 2) ramp 20 °C /min to 120 °C; 3) isothermal for 5 min; 4) ramp 5 °C /min to -60 °C; 5) isothermal for 10 min; and 6) ramp 5 °C /min to 120 °C. The DSC scans were evaluated by using MicroCal Origin 9.0 software.

Temp-Ramp- Fourier transform infrared spectroscopy measurement

Fourier transform infrared spectroscopy (FT-IR) is applied for determining the molecular organization of the synthetic compounds. Spectra were recorded using an ATR-FTIR (PIKE, Madison, WI; Bruker, Ettlingen, Germany). The synthetic compounds were dried under low pressure over night, and then pressed onto a ZnSe ATR crystal mounted in a trough plate. The ATR crystal was coupled with an Auto Pro Temperature Controller (Pike Technologies, Madison, WI) for gradual heating of the crystal from 30 °C to 56 °C. Spectra were collected with a spectral resolution of 4 cm^{-1} with 8 scans over the range of 4000-600 cm^{-1}. The FTIR spectra were analyzed by using MicroCal Origin 9.0 software.

Determination of critical micelle concentration

Critical micelle concentration (CMC) values of the synthetic compounds were determined using pyrene fluorescence method [2] by a Varian Cary Eclipse Fluorescence spectrometer (Agilent Technology, California, USA). All the synthetic compound solutions were prepared using water previously saturated with pyrene (10^{-6} mmol/mL) in different concentrations (2, 1, 0.5, 0.1, 0.05 and 0.01 mg/mL). Emission spectra of pyrene were obtained by exciting the samples at 334 nm. The fluorescence intensity ratio of I_1/I_3 (I_1=372.5 nm, I_3=383 nm) was plotted against sample solution concentration. The concentration at which the first break occurs was the CMC value [3] of the compound in water.

Preparation of surfactant-free solid lipid nanoparticles

The pre o/w emulsion was prepared by melting 20 mg lipid materials at temperature 5 °C above the melting point (68-75 °C) in a 25 mL round bottle. Then 2 mL warm Milli Q water was added into the round bottle result a 1 mg/mL concentration. The SF-SLN was prepared either by mixing for 5 min using IKA RCT basic magnetic stirring (IKA, Staufen, Germany) at 1000 rpm or by first magnetic stirring, then mixing for 10 s using a QSonica Q500 Sonicator (QSonica, Newtown, CT) at 20 kHz/500 Watts. After that, the warm nanoemulsion was cooled to room temperature, resulting in the formation of solid nanoparticles.

Dynamic light scattering

The mean particle sizes and polydispersity index (PDI) of the SF-SLN were measured using Photon Correlation Spectroscopy with a Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, UK). The freshly prepared nanoparticles were analyzed in triplicate with a reference refractive index of 1.553 at 25 °C. The zeta potential of the SF-SLN was measured using Laser Doppler velocimetry with a Zetasizer Nano ZS. The sample was dispersed in PBS buffer (0.01 M, pH=7.4) and analyzed in triplicate.

Atomic force microscopy imaging

Atomic force microscopy (AFM) images were obtained using MultiMode SPM (Veeco Instruments, Santa Barbara, CA) operated in tapping mode in air. AFM imaging was performed at scan frequencies of 1 Hz and optimized feedback parameters. Silicon cantilevers (OMCL-AC160TS-R3, Olympus, Tokyo, Japan) were used with a typical resonance frequency of 300 kHz and a spring constant of 26.1 N/m. The resolution of the AFM scans was 512 × 512 pixels. 10 µL sample was taken from freshly prepared SF-SLN and deposited onto a freshly cleaved mica surface, and left to be air dried for overnight. For each mica, images of 300 × 300 nm were obtained from separate places.

Transmission electron microscopy imaging

The interior structure of the SF-SLN was characterized by Transmission electron microscopy (TEM, Tecnai G2 Spirit, FEI Co.). A 5 µl of freshly prepared samples were dropped on a 300 mesh carbon-coated copper grid (Ted Pella, Inc., Redding, CA) then incubated at 60 °C for 30 min. TEM images were obtained at 120 kV.

NMR spectra of compounds

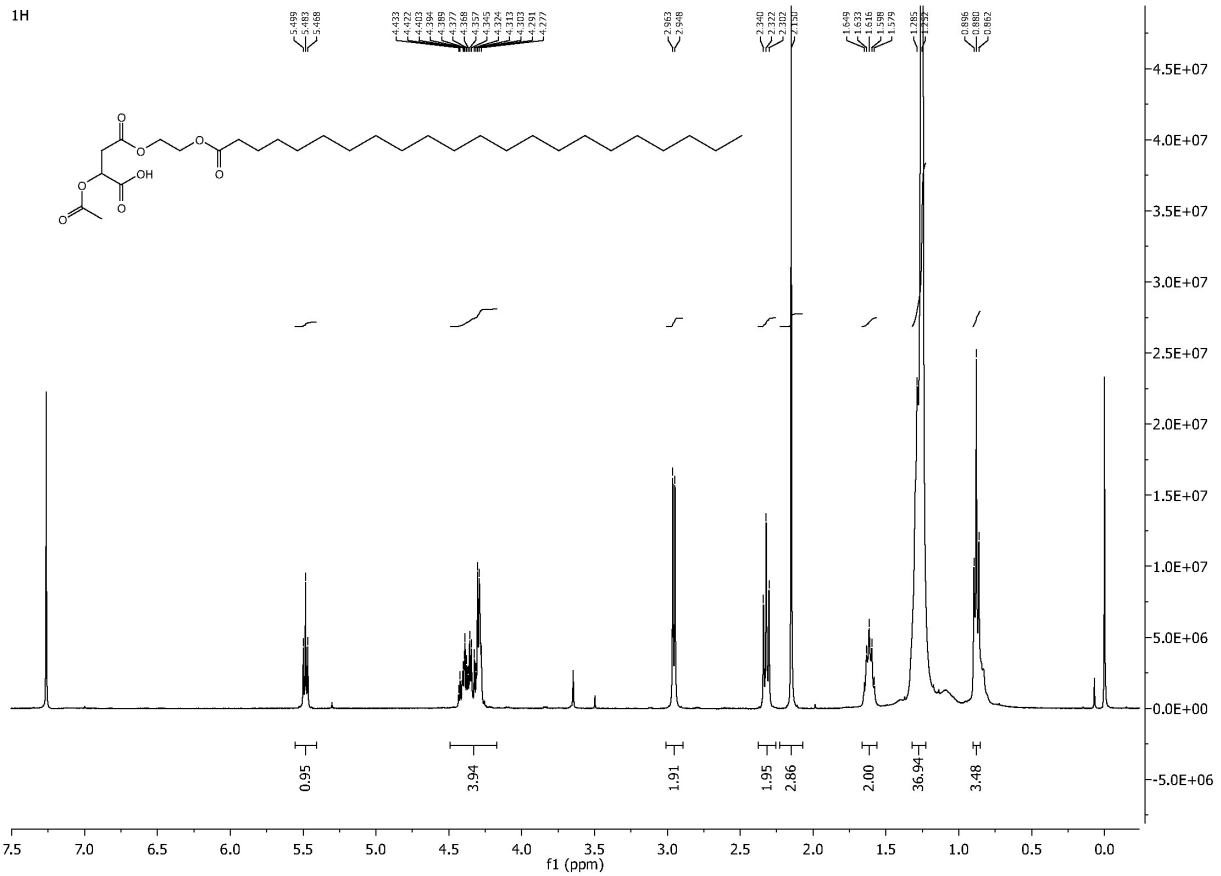

Figure S1: ^{1}H NMR spectrum of **1a** in $CDCl_3$.

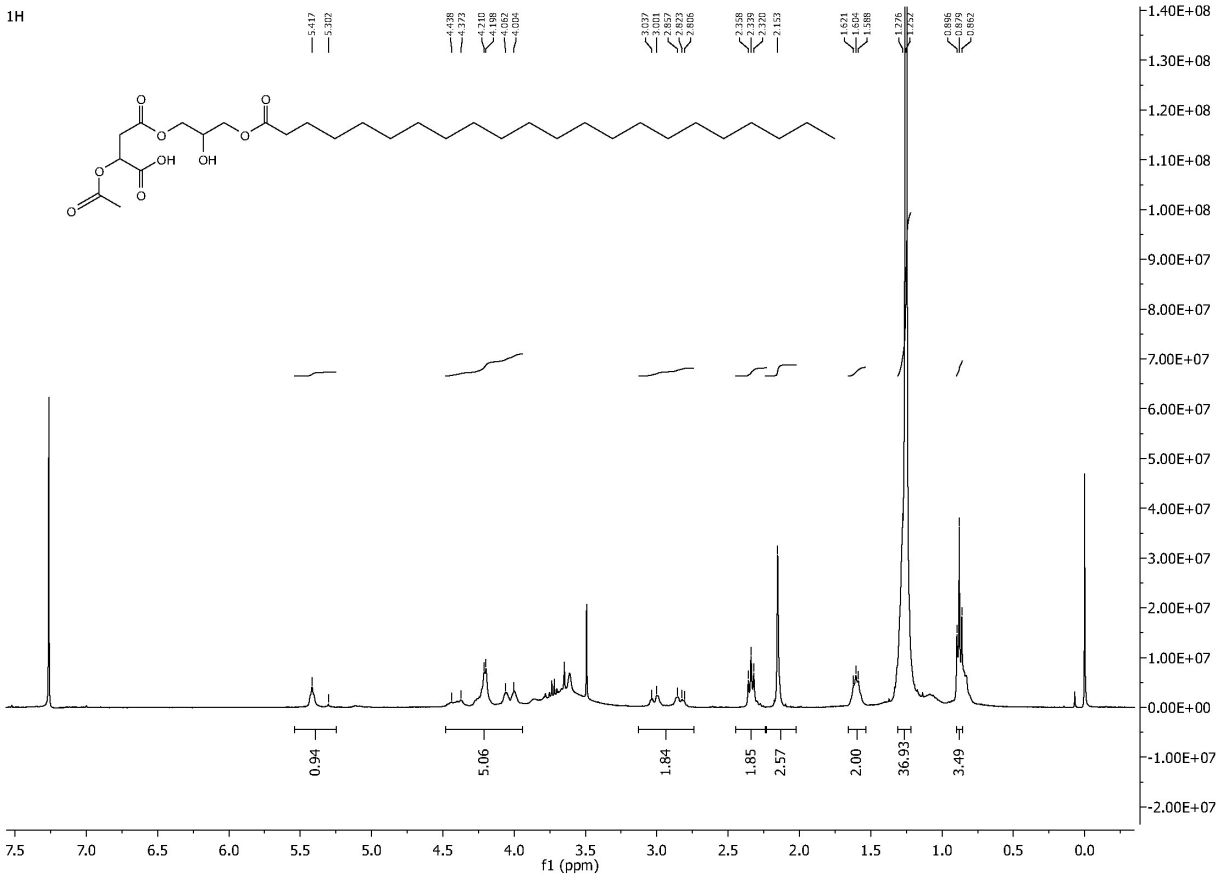

Figure S2: ^{1}H NMR spectrum of **2a** in $CDCl_3$.

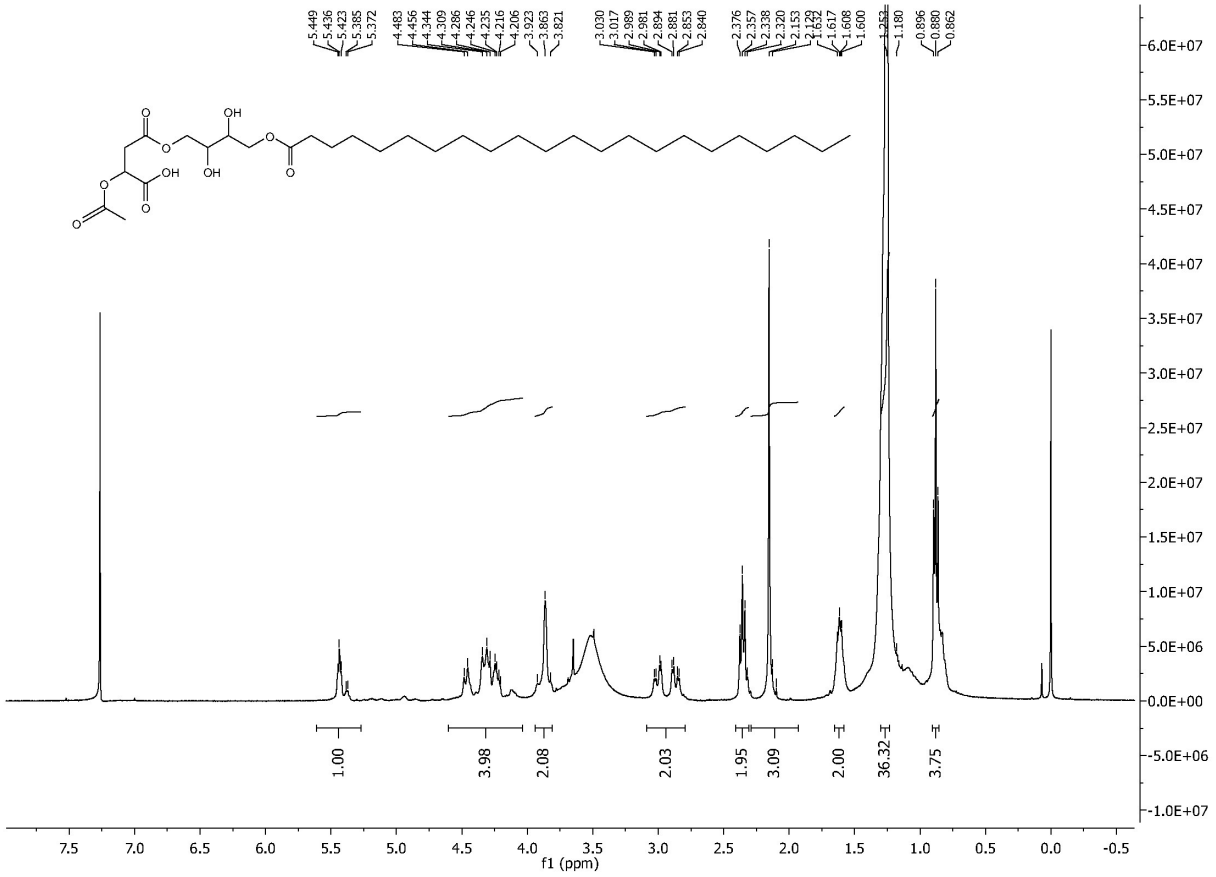

Figure S3: ^{1}H NMR spectrum of **3a** in $CDCl_3$.

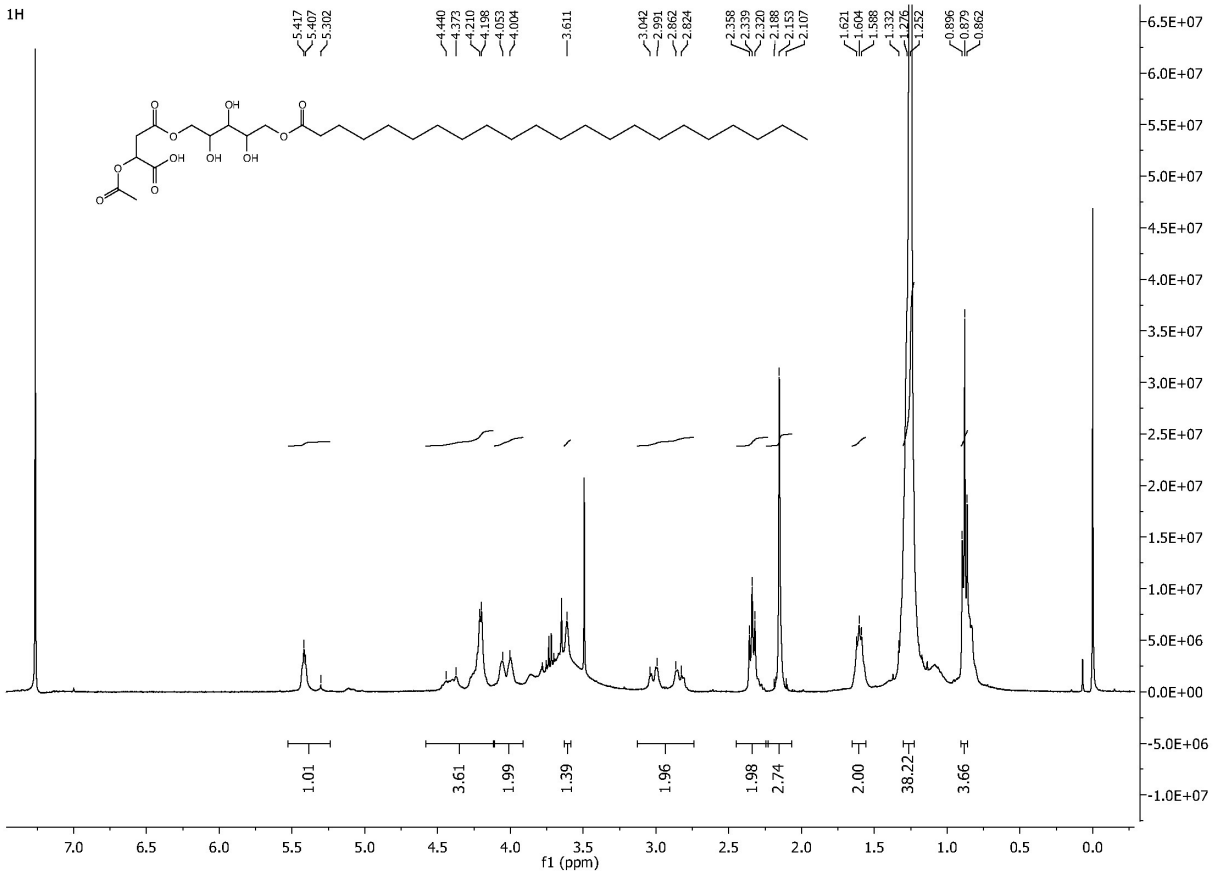

Figure S4: ^{1}H NMR spectrum of **4a** in $CDCl_3$.

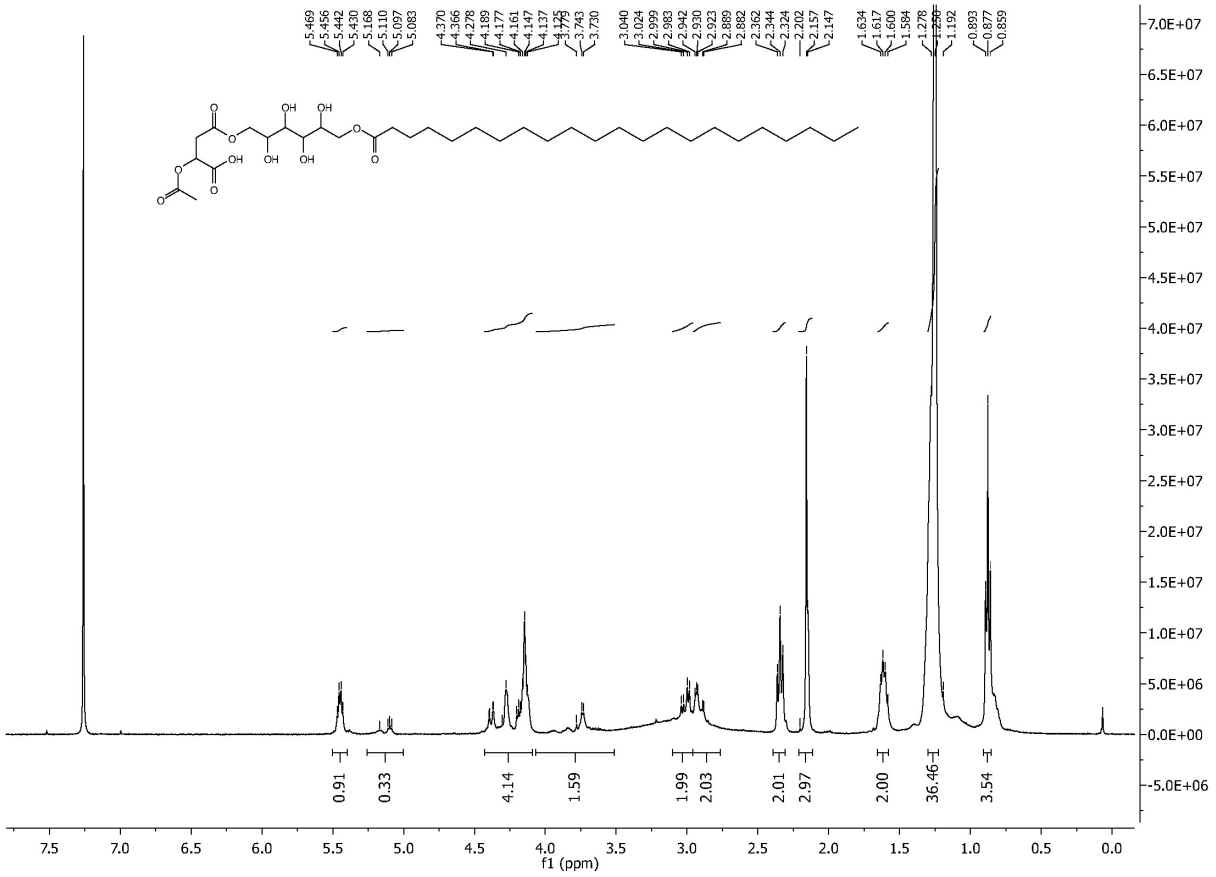

Figure S5: ^{1}H NMR spectrum of **5a** in $CDCl_3$.

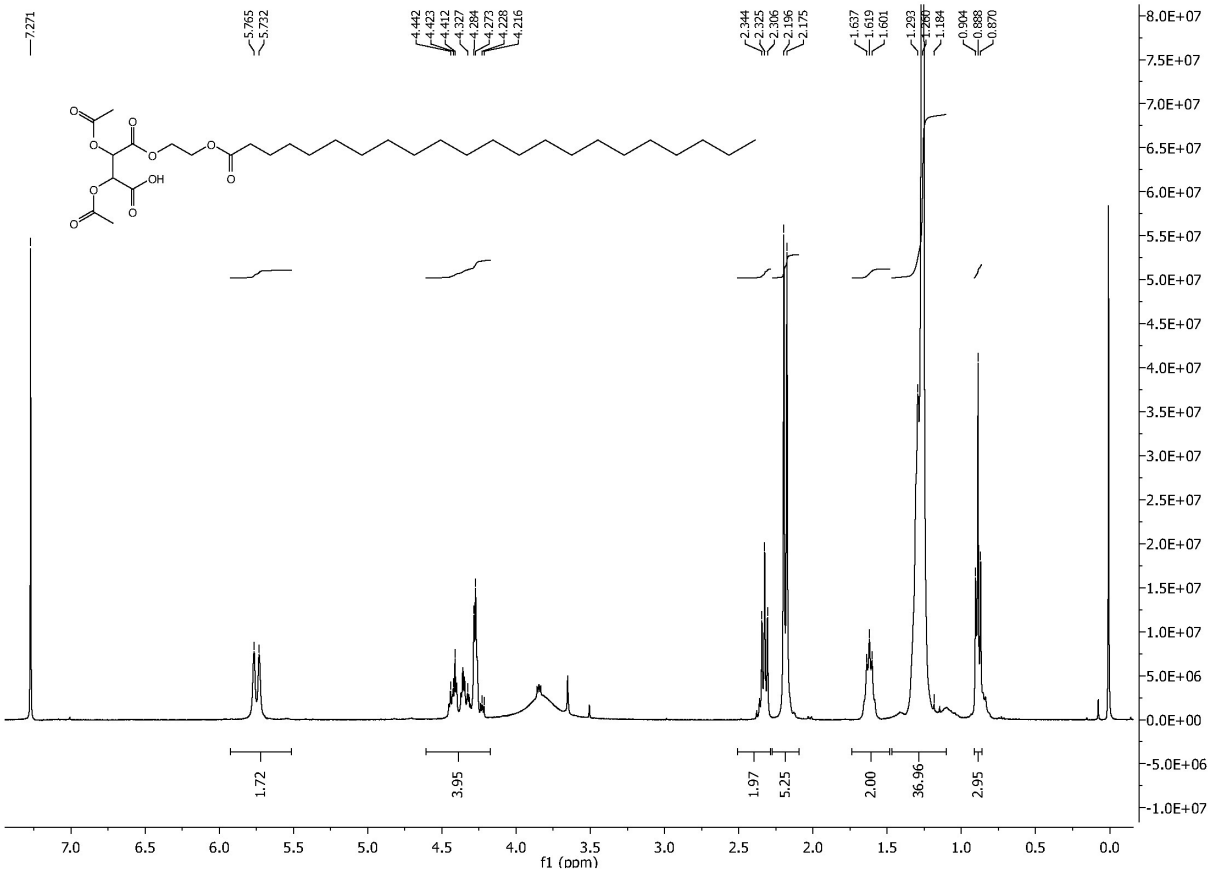

Figure S6: ^{1}H NMR spectrum of **1b** in $CDCl_3$.

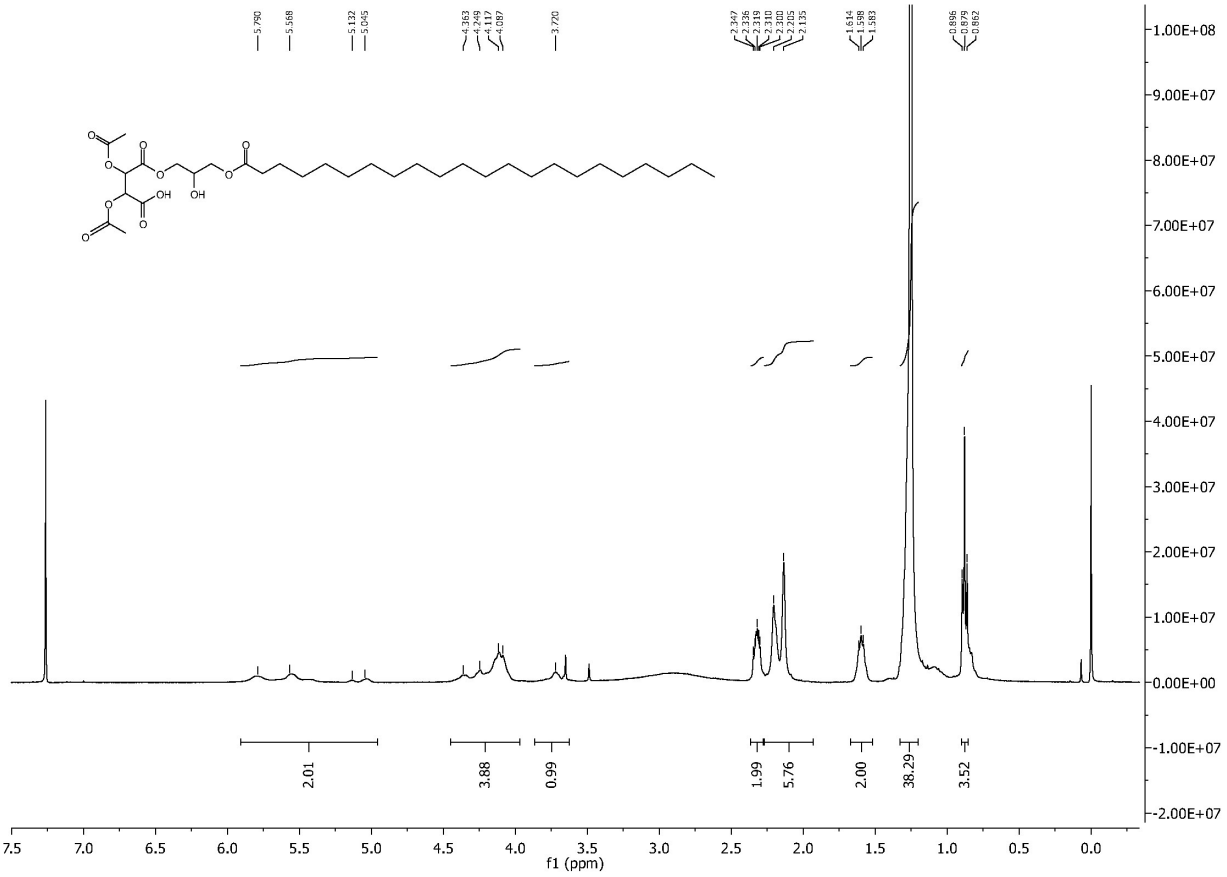

Figure S7: ^{1}H NMR spectrum of **2b** in $CDCl_3$.

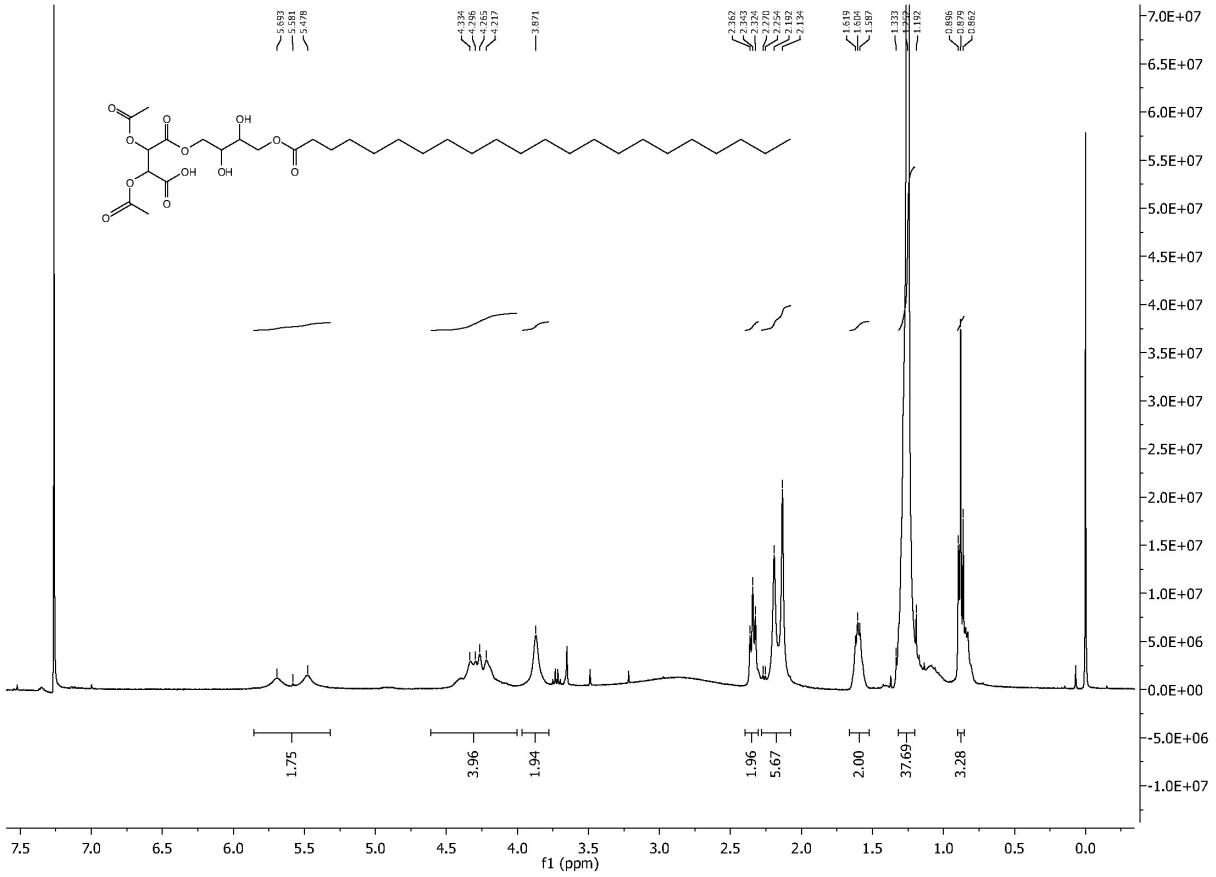

Figure S8: ^{1}H NMR spectrum of **3b** in $CDCl_3$.

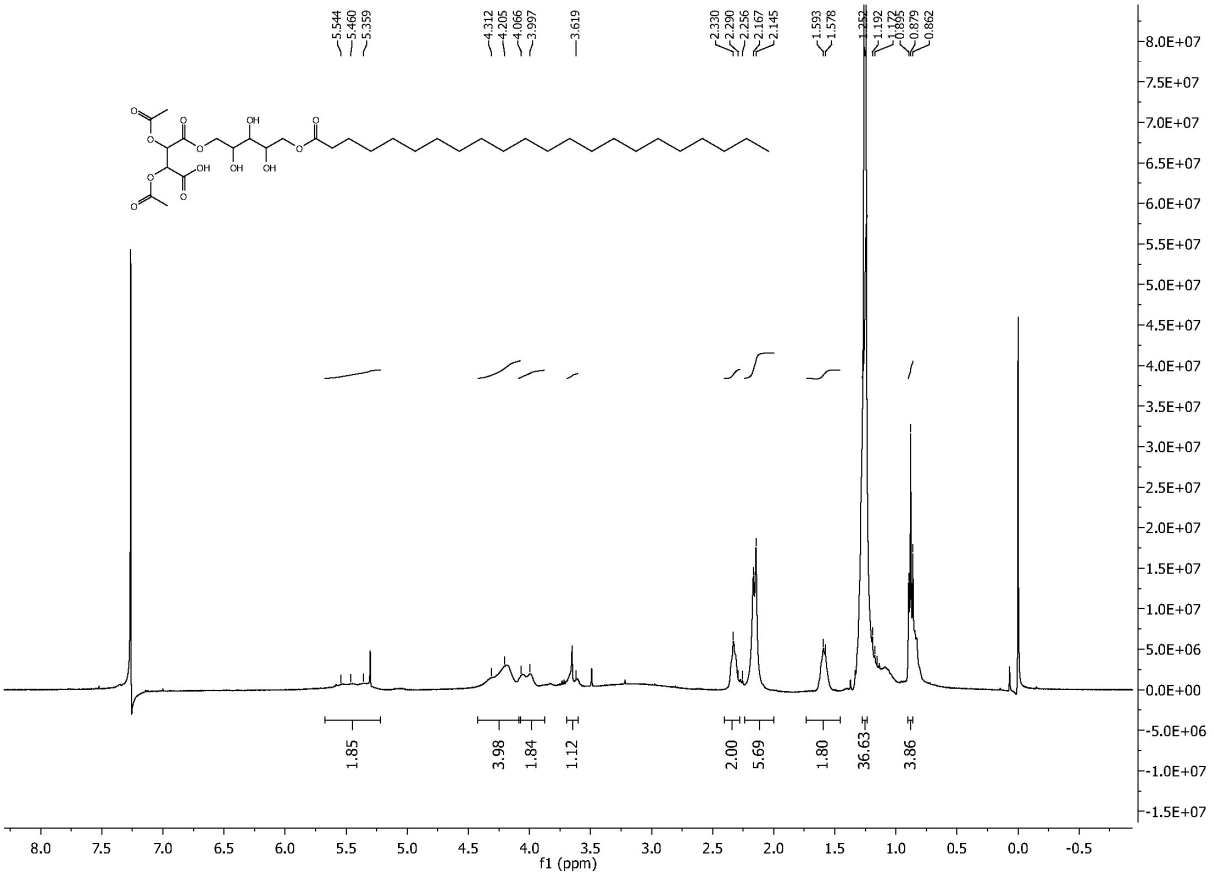

Figure S9: ^{1}H NMR spectrum of **4b** in $CDCl_3$.

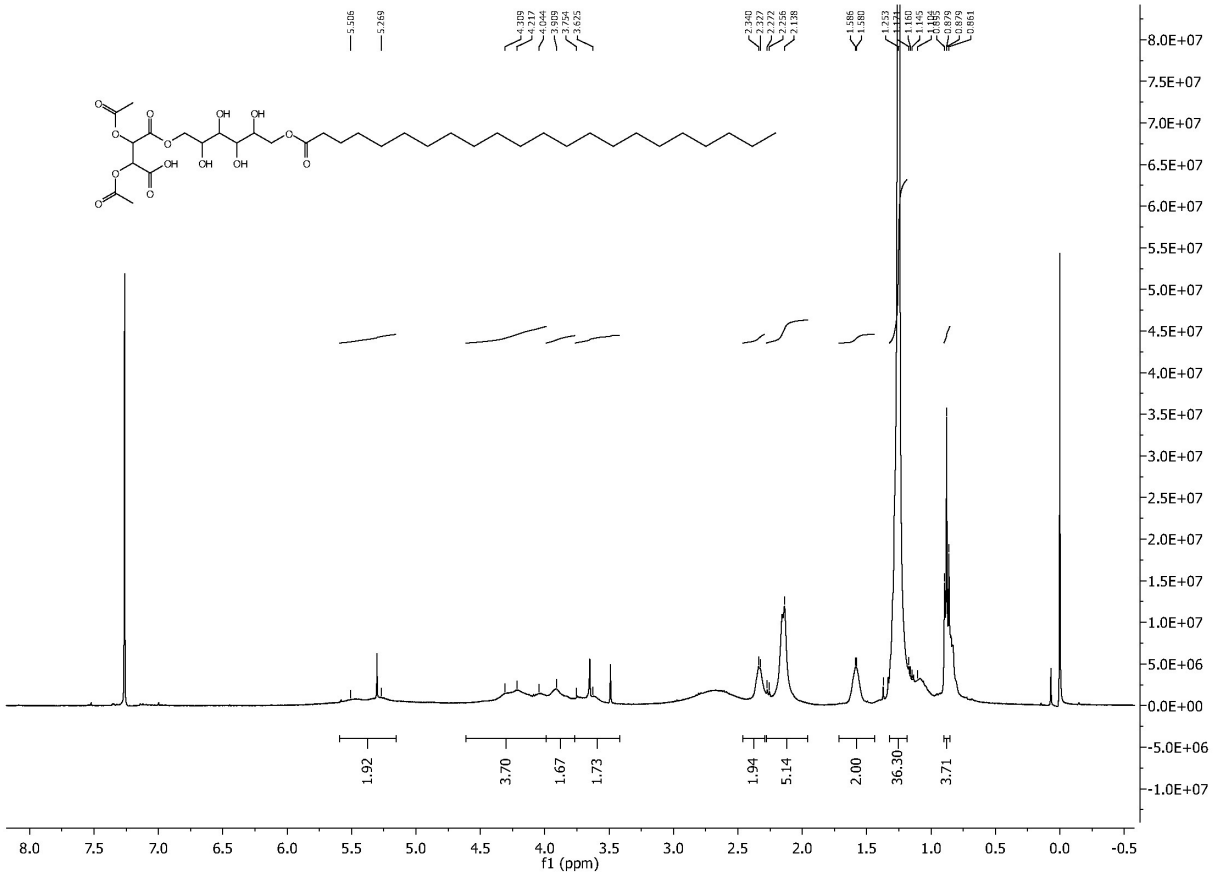

Figure S10: ^{1}H NMR spectrum of **5b** in $CDCl_3$.

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