Supporting information for

Expedient Synthesis of Functional Single-Component Glycoliposomes Using Thiol-yne Chemistry

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1 Materials and methods

Reactions were carried out under argon using commercially available ACS grade dioxane which was stored over 4 Å molecular sieve. Commercially available alkanethiols (dodecane- tetradecane- and hexadecanethiol) and azobisisobutyronitrile (or azobiscyanovaleric acid) from Sigma Aldrich Canada LTD were used without further purification. The plant lectin concanavalin A (Con A, that binds to D-mannose) from *Canavalia ensiformis* (Jack bean) was purchased from Sigma-Aldrich. Progress of reactions was monitored by thin-layer chromatography using silica gel 60 F_{254} coated plates (E. Merck). NMR spectra were recorded on Varian Inova AS600 and Bruker Avance III HD 600 MHz spectrometer. Proton and carbon chemical shifts (δ) are reported in ppm relative to the chemical shift of residual $CHCl_3$, which was set at 7.26 ppm (^{1}H) and 77.16 ppm (^{13}C). Coupling constants (*J*) are reported in Hertz (Hz), and the following abbreviations are used for peak multiplicities: singlet (s), doublet (d), doublet of doublets (dd), doublet of doublet with equal coupling constants (t_{ap}), triplet (t), multiplet (m). Chiral centers are denoted with an asterisk (C*). Analysis and assignments were made using COSY and HSQC experiments. High-resolution mass spectra (HRMS) were measured with a LC-MS-TOF spectrometer (Agilent Technologies) in positive and/or negative electrospray mode by the analytical platform of UQAM.

Dynamic light scattering (DLS) was performed with a Malvern Instruments particle sizer (Zetasizer® Nano S, Malvern Instruments, UK) equipped with 4mW He-Ne laser 633 nm and avalanche photodiode positioned at 90° to the beam and temperature controlled cuvette holder. Instrument parameters were determined automatically along with measurement times. Experiments were performed in triplicate at 25°C.

Differential interference contrast and confocal microscopy images were taken using a Nikon A1R confocal microscope with 100X oil CFI NA 1.45 Plan Apochromat λ objective (fluorescence) and 63X oil immersion objective upon differential interference contrast illumination (DIC).. All images were captured with a pinhole size of 59.1 µm, with a calibration of 0.12 µm/pixel (radial resolution of 0.20 µm) and a Z-step of 0.15 µm. Images were captured using NIS-element software (Nikon) and Leica LAS AF imaging software.

Transmission electron microscopy was acquired with Tecani-TEM (FEI, USA). Negative-staining transmission electron miscroscopy was performed as follow:
a total of 10 uL of the suspension of liposomes compound **14** (5 mg/mL, 100 uL THF+900 uL nanopure water) was placed on a carbon-over-Pioloform.

Atomic force microscopy characterization was performed on a Nanoscope V / Multimode 8 Scanning Probe microscope with ScanAsyst. Images were recorded in ScanAsyst mode with a Bruker ScanAsyst-Air probe. Images were visualized, analyzed and edited with Gwyddion 2.36 image software.

2 Synthesis and characterization

General procedure A for the thiol-yne bis-coupling reaction

A microwave vial was loaded with propargyl glycoside **2** (1 mmol, 1 eq.), alkanethiol (4 mmol, 4 eq.) and dioxane (1 ml). The solution was sonicated for 30 minutes under a gentle stream of argon. [1] AIBN[2] (16 mg, 0.1 mmol, 0.1 eq.) was then added to the solution, and the mixture was heated at 100°C for one hour, after which TLC showed complete disappearance of the starting material. After cooling to room temperature, the solution was transferred with the aid of CH_2Cl_2 into a flask, and concentrated under reduced pressure. The pale yellow oil residue was chromatographed (Hexane–EtOAc), to afford the bis-coupling compound as white solid (1:1 diastereomeric mixture).

General procedure B for the thiol-yne mono-coupling reaction

A microwave vial was loaded with propargyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside **2** (100 mg, 0.26 mmol, 1.00 eq.), alkanethiol (1.00 mmol, [3] 1.00 eq.) and dioxane (1 mL). The solution was sonicated under a gentle stream of argon for 30 minutes. AIBN (5 mg, 0.03 mmol, 0.10 eq.) was then added to the solution and the mixture was heated at 100°C for 30 minutes. TLC monitoring showed that the starting sugar was not completely converted, but the reaction could not be brought to completion by extension of the reaction time and thus stopped at this level. After cooling to room temperature, the solution was transferred, with the aid of CH_2Cl_2 into a flask, and concentrated under reduced pressure. The pale yellow oil residue was chromatographed (Hexane–EtOAc), to afford mono-coupled compound as an amorphous solid (99 mg, 0.17 mmol, 65%, intractable 2:1 (*E*)/(*Z*) diastereomeric mixture.

General proceddure C for the thiol-ene coupling reaction on 3-(alkylthio)prop-2-en-yl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside

A microwave vial was loaded with 3-(alkylthio)prop-2-en-yl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (1eq.), alkanethiol (2eq.) and dioxane (0.5 mL/mmol of alkene). The solution was sonicated under a gentle stream of argon for 30 minutes. AIBN (5 mg, 0.03 mmol, 0.10 eq.) was then added to the solution and the mixture was heated at 100°C for 30 minutes. TLC monitoring showed that the starting sugar was not completely converted, but the reaction could not be brought to completion by extension of the reaction time and thus stopped at this level. After cooling to room temperature, the solution was transferred, with the aid of CH_2Cl_2 into a flask, and concentrated under reduced pressure. The pale yellow oil residue was chromatographed (Hexane–EtOAc), to afford bis-coupled compound as an amorphous solid (1:1 diastereomeric mixture)..

General procedure D for the Zeplén deacetylation

Per-acetylated compound was dissolved in 1:1 MeOH–CH_2Cl_2 (4 mL/100 mg), 1M methanolic NaOMe (0.5 mL) was added, and the mixture was stirred at room temperature for 6 hours. The mixture was neutralized with Amberlite H^+-resin, filtered and concentrated, to afford analytically pure, deacetylated compound in >95% yield.

2-Propynyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (2) [1]

2,3-bis(dodecylthio)propyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (3)

Prepared according to general procedure **A**; White foam; 95%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.19 ($2t_{ap}$, 2H, *J* = 9.5 Hz, 2×*H*-3), 5.06 ($2t_{ap}$, 2H, *J* = 9.7 Hz, 2×*H*-4), 4.96, 5.00 (m, 2H, 2×*H*-2), 4.52 (d, 2H, *J* = 8.0 Hz, 2×*H*-1), 4.25 (dd, 2H, *J* = 12.4, 4.2 Hz, 2×*H*-6a), 4.1-4.13 (m, 3H, 2×*H*-6b, OCH_2CH),

[1] Degassing the solvent is essential. Experiments have shown that oxidation of sulphur atoms can occur during the reaction, resulting in decreased yield.

[2] AIBN can be substituted by azobiscyanovaleric acid (ACVA) as initiator.

[3] Alkanethiol was weighed for more accuracy

4.06 (dd, 1H, *J* = 10.0, 5.8 Hz, OC*H*$_2$CH), 3.69 (m, 3H, 2×H-5, OC*H*$_2$CH), 3.60 (dd, 1H, *J* = 9.7, 7.8 Hz, OC*H*$_2$CH), 2.90 (m, 2H, 2×C*H*), 2.76 (m, 4H, 2×SC*H*$_2$CH), 2.53 (m, 8H, SC*H*$_2$-alk), 2.07, 2.04, 2.00, 1.98 (8s, 24H, 8×C*H*$_3$, acetyl), 1.54 (m, 8H, C*H*$_2$-alk), 1.24-1.34 (m, 72H, C*H*$_2$-alk), 0.86 (t, 12H, *J* = 7.1 Hz, 4×C*H*$_3$-alk); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.7, 170.7, 170.4, 170.3, 169.5, 169.5, 169.4, 169.3 (8×*C*=O), 101.4 (*C*-1), 101.0 (*C*-1), 172.9 (*C*-3), 172.8 (*C*-3), 71.9 (2×*C*-5), 71.9 (O*C*H$_2$CH), 71.6 (O*C*H$_2$CH), 71.3 (*C*-2), 71.2 (*C*-2), 68.5 (2×*C*-4), 62.0 (2×*C*-6), 45.8 (*C*H), 45.3 (*C*H), 34.6, 34.6 (2×S*C*H$_2$CH), 33.5-22.8 (44×*C*H$_2$-alk), 20.9, 20.8, 20.7, 20.7 (8×*C*H$_3$, acetyl), 14.2 (4×*C*H$_3$-alk); HRMS [ESI +] $(M+H)^+$ calcd for $C_{41}H_{75}O_{10}S_2$, 791.4796; found, 791.4789

2,3-Bis(dodecylthio)propyl β-D-glucopyranoside (4)

Prepared according to general procedure **D**; white amorphous solid; 96%; ^{1}H NMR (DMSO-d_6 + $CDCl_3$, 600 MHz) δ 4.96-4.90 (m, 6H, 2×O*H*-2, 2×O*H*-3, 2×O*H*-4), 4.48-4.42 (m, 2H, 2×O*H*-6), 4.13 (d, 2H, *J* = 7.8 Hz, 2×*H*-1), 3.90 (dd, 1H, *J* = 10.4, 4.8 Hz, OC*H*$_2$CH), 3.81 (dd, 1H, *J* = 10.5, 7.0 Hz, OC*H*$_2$CH), 3.69 (dd, 1H, *J* = 10.6, 4.6 Hz, OC*H*$_2$CH), 3.64-3.67 (m, 2H, 2×*H*-6a), 3.53 (dd, 1H, *J* = 10.3, 8.3 Hz, OC*H*$_2$CH), 3.41-3.44 (m, 2H, 2×*H*-6b), 3.01-3.14 (m, 6H, 2×*H*-3, 2×*H*-5, 2×*H*-4), 2.89-2.99 (m, 6H, 2×*H*-2, 2×C*H*, 2×SC*H*$_2$CH), 2.66-2.70 (m, 2H, SC*H*$_2$CH), 2.51-2.60 (m, 8H, C*H*$_2$-alk), 1.47-1.53 (m, 8H, C*H*$_2$-alk), 1.24-1.24 (m, 72H, C*H*$_2$-alk), 0.85 (t, 12H, *J* = 7.0 Hz, 4×C*H*$_3$-alk); ^{13}C NMR (DMSO-d_6 + $CDCl_3$) δ 103.6 (*C*-1), 102.8 (*C*-1), 76.9 (2×*C*-5), 76.7 (2×*C*-3), 73.4 (2×*C*-2), 71.1 (O*C*H$_2$CH), 70.3 (O*C*H$_2$CH), 70.0 (2×*C*-4), 61.1 (2×*C*-6), 45.6 (CH), 45.0 (CH), 32.2 (S*C*H$_2$CH), 32.0 (S*C*H$_2$CH), 22.1-31.3 (44×*C*H$_2$-alk), 14.4 (4×*C*H$_3$-alk); HRMS [ESI +] $(M+Na)^+$ calcd for $C_{33}H_{66}O_6S_2Na$ 645.4193 found 645.4182; Anal. Calcd. For $C_{33}H_{66}O_6S_6$: C, 63.62; H, 10.68. Found: C, 62.78; H, 10.69.

2,3-bis(tetradecylthio)propyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (5)

Prepared according to general procedure **A**; white amorphous solid; 93%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.12 (2t$_{ap}$, 2H, *J* = 9.4 Hz, 2×*H*-3), 4.98 (2t$_{ap}$, 2H, *J* = 9.6 Hz, 2×*H*-4), 4.90 (2dd, 2H, *J* = 9.5, 8.0 Hz, 2×*H*-2), 4.46 (d, 2H, *J* = 7.9 Hz, 2×*H*-1), 4.20 (dd, 2H, *J* = 12.3, 4.6 Hz, 2×*H*-6a), 3.96-4.07 (m, 4H, 2×*H*-6b, 2×OC*H*$_2$CH), 3.51-3.67 (m, 4H, 2×*H*-5, 2×OC*H*$_2$CH), 2.77-2.86 (m, 2H, 2×C*H*), 2.62-2.74 (m, 4H, 2×HCC*H*$_2$S), 2.41-2.52 (m, 8H, C*H*$_2$-alk), 1.99, 1.99, 1.96, 1.96, 1.92, 1.92, 1.90, 1.90 (8s, 24H, 8×C*H*$_3$, acetyl), 1.43-1.50 (m, 8H, C*H*$_2$-alk), 1.14-1.27 (m, 88H, C*H*$_2$-alk), 0.78 (t, 12H, *J* = 6.7 Hz, C*H*$_3$-alk); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.4, 170.4, 170.0, 170.0, 169.2, 169.2, 169.1, 169.0 (8×*C*=O), 101.2 (*C*-1), 100.8 (*C*-1), 72.7 (*C*-3), 72.6 (*C*-3), 71.8 (2×*C*-5), 71.7 (O*C*H$_2$CH), 71.4 (O*C*H$_2$CH), 71.1 (2×*C*-2), 68.3 (2×*C*-4),61.8 (2×*C*-6), 45.7 (*C*H), 45.1 (*C*H), 34.4 (2×S*C*H$_2$CH), 22.6-33.3 (52×*C*H$_2$-alk), 20.5-20.6 (8×*C*H$_3$, acetyl), 14.0 (4×*C*H$_3$-alk); HRMS [ESI +] $(M+K)^+$ calcd for $C_{45}H_{82}O_{10}S_2$ 885.4981 found 885.4969

2,3-bis(hexadecylthio)propyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (6)

Prepared according to general procedure **A**; White amorphous solid; 91%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.16 (2t$_{ap}$, 2H, *J* = 9.5 Hz, 2×*H*-3), 5.03 (2t$_{ap}$, 2H, *J* = 9.6 Hz, 2×*H*-4), 4.95 (2dd, 2H, *J* = 9.5, 8.0 Hz, 2×*H*-2), 4.49 (d, 2H, *J* = 7.9 Hz, 2×*H*-1), 4.23 (dd, 2H, *J* = 12.3, 4.7 Hz, 2×*H*-6a), 4.02-4.11 (m, 4H, 2×*H*-6b, 2×OC*H*$_2$CH), 3.54-3.70 (m, 4H, 2×*H*-5, 2×OC*H*$_2$CH), 2.79-2.92 (m, 2H, 2×C*H*), 2.64-2.76 (m, 4H, 2×HCC*H*$_2$S), 2.45-2.54 (m, 8H, C*H*$_2$-alk), 2.03, 2.03, 2.00, 2.00, 1.97, 1.97, 1.95, 1.95 (8s, 24H, 8×C*H*$_3$, acetyl), 1.47-1.54 (m, 8H, C*H*$_2$-alk), 1.20-1.30 (m, 104H, C*H*$_2$-alk), 0.82 (t, 12H, *J* = 6.7 Hz, C*H*$_3$-alk); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.6, 170.6, 170.2, 170.2, 169.3, 169.2 169.2, 169.2 (8×*C*=O), 101.3 (*C*-1), 100.9 (*C*-1), 72.8 (*C*-3), 72.7 (*C*-3), 71.9 (2×*C*-5), 71.8 (O*C*H$_2$CH), 71.5 (O*C*H$_2$CH), 71.2 (2×*C*-2), 68.4 (2×*C*-4), 61.9 (2×*C*-6), 45.7 (*C*H), 45.2 (*C*H), 34.5 (2×S*C*H$_2$CH), 22.7-33.4 (60×*C*H$_2$-alk), 20.7, 20.7, 20.7, 20.7, 20.6, 20.6, 20.6, 20.6 (8×*C*H$_3$, acetyl), 14.1 (4×*C*H$_3$-alk); HRMS [ESI +] $(M+Na)^+$ calcd for $C_{49}H_{90}NaO_{10}S_2$ 925.5868 found 925.5844

2,3-Bis(tetradecylthio)propyl β-D-glucopyranoside (7)

Prepared according to general procedure **D**; White powder; >95%; ^{1}H NMR ($CDCl_3$ + CD_3OD, 600 MHz) δ 4.87, 4.84, 4.77, 4.74, 4.43, 4.39 (6 br s, 8H, 8×O*H*), 4.30 (d, 2H, *J* = 7.5 Hz, 2×*H*-1), 4.08 (dd, 1H, *J* = 10.2, 4.2 Hz, OC*H*$_2$CH), 4.03 (dd, 1H, *J* = 10.0, 6.1 Hz, OC*H*$_2$CH), 3.78-3.84 (m, 4H, 2×*H*-6a, 2×*H*-6b), 3.74 (dd, 1H, *J* = 10.2, 5.3 Hz, OC*H*$_2$CH), 3.69 (dd, 1H, *J* = 9.9, 6.6 Hz, OC*H*$_2$CH), 3.48-3.53 (m, 4H, 2×H-3, 2×*H*-4), 3.29-3.34 (m, 4H, 2×*H*-2, 2×*H*-5), 2.95-2.98 (m, 2H, C*H*), 2.75-2.90 (m, 4H, SC*H*$_2$CH), 2.52-2.57 (m, 8H, C*H*$_2$-alk), 1.53-1.58 (m, 8H, C*H*$_2$-alk), 1.23-1.34 (m, 88H, C*H*$_2$-alk), 0.86 (t, 12H, *J* = 6.9 Hz, 2×C*H*$_3$-alk); ^{13}C NMR ($CDCl_3$ + CD_3OD, 150 MHz) δ 103.4 (*C*-1), 103.2 (*C*-1), 76.2 (2×*C*-3), 75.8 (2×*C*-5), 73.5 (2×*C*-2), 71.3 (O*C*H$_2$CH), 71.1 (O*C*H$_2$CH), 69.7 (2×*C*-4), 61.7 (2×*C*-6), 45.8 (*C*H), 45.7 (*C*H), 34.8 (S*C*H$_2$CH), 34.7 (S*C*H$_2$CH), 22.8-33.4 (52×*C*H$_2$-alk), 14.2 (4×*C*H$_3$-alk); HRMS [ESI +] $(M+Na)^+$ calcd for $C_{37}H_{74}NaO_6S_2$ 701.4819 found 701.4811

2,3-Bis(hexadecylthio)propyl β-D-glucopyranoside (8)

Prepared according to general procedure **D**; White powder; >95%; ^{1}H NMR ($CDCl_3$ + CD_3OD, 600 MHz) δ 4.31 (d, 2H, *J* = 7.4 Hz, 2×*H*-1), 4.11 (dd, 1H, *J* = 10.2, 4.2 Hz, OC*H*$_2$CH), 4.06 (dd, 1H, *J* = 10.1, 6.0 Hz, OC*H*$_2$CH), 3.85-3.87 (m, 2H, 2×*H*-6a), 3.77-3.80 (m, 2H, 2×*H*-6b), 3.69-3.75 (m, 2H, 2×OC*H*$_2$CH), 3.49-3.54 (m, 4H, 2×*H*-3, 2×*H*-4), 3.33-3.36 (m, 4H, 2×*H*-2, 2×*H*-5), 2.94-2.99 (m, 2H, C*H*), 2.76-2.89 (m, 4H, SC*H*$_2$CH), 2.52-2.58 (m, 8H, C*H*$_2$-alk), 1.54-1.59 (m, 8H, C*H*$_2$-alk), 1.24-1.35 (m, 104H, C*H*$_2$-alk), 0.86 (t, 12H, *J* = 6.8 Hz, 4×C*H*$_3$-alk); ^{13}C NMR ($CDCl_3$ + CD_3OD, 150 MHz) δ 103.4 (*C*-1), 103.1 (*C*-1), 76.3 (*C*-3), 76.2 (*C*-2), 75.8 (2×*C*-5), 73.7 (*C*-2), 73.6 (*C*-2), 71.2 (O*C*H$_2$CH), 71.1 (O*C*H$_2$CH), 70.1 (*C*-4), 70.0 (*C*-4), 62.1 (*C*-6), 62.0 (*C*-6), 45.8 (*C*H), 45.7 (*C*H), 34.9 (S*C*H$_2$CH), 34.8 (S*C*H$_2$CH), 22.8-34.9 (60×*C*H$_2$-alk), 14.2 (4×*C*H$_3$-alk); HRMS [ESI +] $(M+H)^+$ calcd for $C_{41}H_{83}O_6S_2$ 735.5626 found 735.5655

2-Propynyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (9)[1]

2,3-bis(dodecylthio)propyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (10)

Prepared according to general procedure **A**; White amorphous solid; 91%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.37-5.38 (m, 2H, 2×*H*-4), 5.20 (2dd, 2H, *J* = 10.0, 8.1 Hz, 2×*H*-2), 5.0-5.02 (m, 2H, 2×*H*-3), 4.49 (2d, 2H, *J* = 8.0 Hz, 2×*H*-1), 4.12-4.18 (m, 5H, 2×*H*-6a, 2×*H*-6b, OC*H*$_2$CH), 4.09 (dd, 1H, *J* = 10.2, 5.7 Hz, OC*H*$_2$CH), 3.89-3.91 (m, 2H, 2×*H*-5), 3.70 (dd, 1H, *J* = 10.0, 5.9 Hz, OC*H*$_2$CH), 3.60 (dd, 1H, *J* = 9.9, 7.7 Hz, OC*H*$_2$CH), 2.88-2.96 (m, 2H, 2×C*H*), 2.71-2.85 (m, 4H, 2×SC*H*$_2$CH), 2.51-2.59 (m, 8H, C*H*$_2$-alk), 2.14, 2.14, 2.07, 2.06, 2.04, 2.04, 1.97, 1.97 (8s, 24H, 8×C*H*$_3$, acetyl), 1.53-1.59 (m, 8H, C*H*$_2$-alk), 1.33-1.39 (m, 8H, C*H*$_2$-alk), 1.25-1.30 (m, 64H, C*H*$_2$-alk), 0.87 (t, 12H, *J* = 7.0 Hz, 4×C*H*$_3$-alk); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.5, 170.5, 170.4, 170.4, 170.3, 170.3, 169.5, 169.5 (8×*C*=O), 101.9 (*C*-1), 101.6 (*C*-1), 71.9 (O*C*H$_2$CH), 71.5 (O*C*H$_2$CH), 71.0 (*C*-3), 70.9 (*C*-3), 70.8 (2×*C*-5), 68.9 (2×*C*-2), 67.1 (2×*C*-4), 61.3 (*C*-6), 61.3 (*C*-6), 45.9 (*C*H), 45.4 (*C*-H), 34.7 (2×SC*H*$_2$CH), 22.8-33.6 (44×*C*H$_2$-alk), 21.0, 21.0, 20.8, 20.8, 20.8, 20.8, 20.7, 20.7 (8×*C*H$_3$, acetyl), 14.2 (4×*C*H$_3$-alk); HRMS [ESI +] $(M+Na)^+$ calcd for $C_{41}H_{74}NaO_{10}S_2$ 813.4616 found 813.4586

2,3-Bis(dodecylthio)propyl β-D-galactopyranoside (11)

Prepared according to general procedure **D**; White powder; >95%; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 4.88, 4.64, 4.54 (3 br s, 8H, 8×O*H*), 4.09 (d, 2H, *J* = 7.1 Hz, 2×*H*-1), 3.87 (dd, 1H, *J* = 10.2, 4.5 Hz, OC*H*$_2$CH), 3.79 (dd, 1H, *J* = 10.4, 6.6 Hz, OC*H*$_2$CH), 3.68 (dd, 1H, *J* = 10.4, 4.2 Hz, OC*H*$_2$CH), 3.64 (d, 2H, *J* = 1.9 Hz, 2×*H*-4), 3.51-3.55 (m, 3H, 2×*H*-6a, OC*H*$_2$CH), 3.42-3.45 (m, 2H, 2×*H*-6b), 3.23-3.31 (m, 6H, 2×*H*-2, 2×*H*-3, 2×*H*-5), 2.89-2.95 (m, 4H, 2×C*H*, 2×SC*H*$_2$CH), 2.65-2.69 (m, 2H, 2×SC*H*$_2$CH), 2.51-2.59 (m, 8H, C*H*$_2$-alk), 1.48-1.51 (m, 8H, C*H*$_2$-alk), 1.23-1.33 (m, 72H, C*H*$_2$-alk), 0.85 (t, 12H, *J* = 6.9 Hz, 4×C*H*$_3$-alk); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 104.3 (*C*-1), 103.5 (*C*-1), 75.2 (2×*C*-5), 73.5 (*C*-3), 73.4 (*C*-3), 71.0 (O*C*H$_2$CH), 70.6 (*C*-2), 70.5 (*C*-2), 70.2 (O*C*H$_2$CH), 67.9

(*C*-4), 67.8 (*C*-4), 60.2 (*C*-6), 60.1 (*C*-6), 45.7 (*C*H), 45.1 (*C*H), 34.0 (S*C*H$_2$CH), 33.8 (S*C*H$_2$CH), 22.1-32.2 (44×*C*H$_2$-alk), 13.9 (4×*C*H$_3$-alk); HRMS [ESI +] (M+Na)$^+$ calcd for $C_{33}H_{66}NaO_6S_2$ 645.4193 found 645.4176

2-Propynyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (12)[2]

2,3-bis(dodecylthio)propyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (13)

Prepared according to general procedure **A**; White amorphous solid; 89%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.25-5.33 (m, 6H, 2×*H*-2, 2×*H*-3, 2×*H*-4), 4.84 (br s, 2H, 2×*H*-1), 4.28 (2dd, 2H, *J* = 5.2, 4.0 Hz, 2×*H*-6a), 4.09-4.13 (m, 4H, 2×*H*-6b, 2×*H*-5), 3.88-3.91 (m, 2H, 2×OC*H*$_2$CH), 3.67-3.71 (m, 2H, 2×OC*H*$_2$CH), 2.91-2.97 (m, 2H, 2×C*H*), 2.77-2.86 (m, 4H, 2×SC*H*$_2$CH), 2.53-2.62 (m, 8H, C*H*$_2$-alk), 2.15, 2.15, 2.10, 2.10, 2.03, 2.03, 1.98, 1.98 (8s, 24H, 8×C*H*$_3$, acetyl), 1.54-1.61 (m, 8H, C*H*$_2$-alk), 1.35-1.37 (m, 8H, C*H*$_2$-alk), 1.25-1.30 (m, 64H, C*H*$_2$-alk), 0.87 (t, 12H, *J* = 7.0 Hz, 4×C*H*$_3$-alk); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.8, 170.8, 170.1, 170.1, 169.9, 169.9, 169.9, 169.8 (8×*C*=O), 97.9 (*C*-1), 97.7 (*C*-1), 70.2 (2×OCH$_2$CH), 69.6 (2×OCH$_2$CH), 69.5 (2×*C*-2), 69.2 (2×*C*-3), 66.1 (2×*C*-4), 62.5 (*C*-6), 62.4 (*C*-6), 45.3 (*C*H), 45.1 (*C*H), 35.0 (S*C*H$_2$CH), 34.8 (S*C*H$_2$CH), 22.8-33.4 (22×*C*H$_2$-alk), 20.8-21.0 (8×*C*H$_3$, acetyl), 14.2 (4×*C*H$_3$-alk); HRMS [ESI +] (M+Na)$^+$ calcd for $C_{41}H_{74}NaO_{10}S_2$ 813.4616 found 813.4600

2,3-Bis(dodecylthio)propyl β-D-mannopyranoside (14)

Prepared according to general procedure **D**; White powder; >95%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.10, 5.03, 4.90 (3 br s, 6H, 6×O*H*), 4.84 (br s, 2H, 2×*H*-1), 4.50 (br s, 2H, 2×O*H*), 3.91-3.97 (m, 7H, 2×*H*-2, 2×*H*-4, 2×*H*-6a, OC*H*$_2$CH), 3.80-3.83 (m, 3H, 2×*H*-3, OC*H*$_2$CH), 3.76 (d$_{ap}$, 2H, *J* = 11.4 Hz, 2×*H*-6b), 3.67 (dd, 1H, *J* = 10.0, 4.6 Hz, OC*H*$_2$CH), 3.55-3.59 (m, 3H, 2×*H*-5, OC*H*$_2$CH), 2.86-2.92 (m, 2H, C*H*), 2.74-2.83 (m, 4H, 2×SC*H*$_2$CH), 2.51-2.58 (m, 8H, C*H*$_2$-alk), 1.54-1.59 (m, 8H, C*H*$_2$-alk), 1.25-1.37 (m, 72H, C*H*$_2$-alk), 0.87 (t, 12H, *J* = 7.0 Hz, 4×C*H*$_3$-alk); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 100.5 (*C*-1), 100.2 (*C*-1), 72.7 (*C*-5), 72.6 (*C*-5), 71.7 (2×*C*-3), 71.0 (*C*-2), 70.9 (*C*-2), 69.3 (OCH$_2$CH), 69.0 (OCH$_2$CH), 66.0 (2×*C*-4), 60.9 (2×*C*-6), 45.5 (*C*H), 45.4 (*C*H), 34.9 (S*C*H$_2$CH), 34.8 (S*C*H$_2$CH), 22.8-33.4 (44×*C*H$_2$-alk), 14.3 (4×*C*H$_3$-alk); HRMS [ESI +] (M+Na)$^+$ calcd for $C_{33}H_{66}NaO_6S_2$ 645.4193 found 645.4167

2-Propynyl 4-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl]-2,3,6-tri-*O*-acetyl-β-D-galactopyranoside (15)[1]

2,3-bis(dodecylthio)propyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside)-β-D-glucopyranoside (16)

Prepared according to general procedure **A**; White amorphous solid; 90%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.32 (d$_{ap}$, 2H, *J* = 3.0 Hz, 2×*H*-4'), 5.17 (2t$_{ap}$, 2H, *J* = 9.3 Hz, 2×*H*-3), 5.1 (dd, 2H, 10.4, 7.9 Hz, 2×*H*-2'), 4.93 (dd, 2H, *J* = 10.4, 3.5 Hz, 2×*H*-3'), 4.88 (t$_{ap}$, 1H, *J* = 7.7 Hz, *H*-2), 4.87 (t$_{ap}$, 1H, *J* = 7.8 Hz, *H*-2), 4.48 (d, 2H, *J* = 7.9 Hz, 2×*H*-1), 4.44-4.46 (m, 4H, 2×H-1', 2×*H*-6a), 4.04-4.12 (m, 7H, 2×*H*-6b, 2×*H*-6'a, 2×*H*-6'b, OC*H*$_2$CH), 4.00 (dd, 1H, *J* = 10.1, 4.8 Hz, OC*H*$_2$CH), 3.85 (tap, 2H, *J* = 6.9 Hz, 2×*H*-5'), 3.77 (m, 2H, 2×*H*-4), 3.69 (dd, 1H, *J* = 10.2, 5.6, OC*H*$_2$CH), 3.56-3.59 (m, 3H, 2×*H*-5), 2.84-2.91 (m, 2H, 2×C*H*), 2.67-2.80 (m, 4H, 2×SC*H*$_2$CH), 2.48-2.57 (m, 8H, C*H*$_2$-alk), 2.13, 2.10, 2.04, 2.03, 2.03, 2.02, 1.94 (7s, 42H, CH$_3$, acetyl), 1.50-1.56 (m, 8H, C*H*$_2$-alk), 1.31-1.34 (m, 8H, C*H*$_2$-alk), 1.23-1.29 (m, 64H, C*H*$_2$-alk), 0.85 (t, 12H, *J* = 7.0 Hz, 4×C*H*$_3$-alk); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.4, 170.4, 70.4, 170.4, 170.2, 170.2, 170.1, 170.1, 169.9, 169.8, 169.7, 169.6, 169.2, 169.2 (14×*C*=O), 101.2 (2×*C*-1'), 101.1 (*C*-1), 100.7 (*C*-1), 76.3 (2×*C*-4), 72.9 (2×*C*-3), 72.8 (2×*C*-5), 71.7 (2×*C*-2, OCH$_2$CH), 71.5 (OCH$_2$CH), 71.1 (2×*C*-3'), 70.8 (2×*C*-5'), 69.2 (2×*C*-2'), 66.7 (2×*C*-4'), 62.1 (2×*C*-6), 60.9 (2×*C*-6'), 45.8 (*C*H), 45.3 (*C*H), 34.6 (S*C*H$_2$CH), 34.6 (S*C*H$_2$CH), 22.8-33.5 (44×*C*H$_2$-

alk), 21.0, 21.0, 20.9, 20.9, 20.9, 20.8, 20.7, 20.7, 20.7, 20.7, 20.7, 20.7, 20.6, 20.6 (14×*C*H_3, acetyl), 14.2 (4×*C*H_3-alk); HRMS [ESI +] $(M+Na)^+$ calcd for $C_{53}H_{90}NaO_{18}S_2$ 1101.5461 found 1101.5491

2,3-bis(dodecylthio)propyl (β-D-galactopyranoside)-β-D-glucopyranoside (17)

Prepared according to general procedure **D**; White powder; >95%; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 5.12, 4.83, 4.69, 4.66, 4.53, 4.51 (6 br s, 14H, 14×O*H*), 4.22 (d, 2H, *J* = 7.8 Hz, 2×*H*-1), 4.20 (d, 2H, *J* = 6.9 Hz, 2×*H*-1'), 3.91 (dd, 1H, *J* = 10.3, 4.7 Hz, OCH_2CH), 3.82 (dd, 1H, *J* = 10.5, 6.9 Hz, OCH_2CH), 3.74 (dd, 2H, 2×*H*-6a), 3.70 (dd ,1H, *J* = 10.6, 4.6 Hz, OCH_2CH), 3.58-3.61 (m, 4H, 2×*H*-4', 2×*H*-6b), 3.55 (dd, 1H, *J* = 10.4, 2.2 Hz, OCH_2CH), 3.46-3.53 (m, 4H, 2×*H*-6a', 2×*H*-6b'), 3.44-3.46 (m, 2H, 2×*H*-5'), 3.27-3.32 (m, 10H, 2×*H*-3, 2×*H*-4, 2×*H*-5, 2×*H*-2', 2×*H*-3'), 2.99-3.03 (m, 2H, 2×*H*-2), 2.94-2.98 (m, 2H, 2×C*H*), 2.88-2.93 (m, 2H, S*C*H_2CH), 2.68 (2dd, 2H, *J* = 13.3, 6.5 Hz, 2×S*C*H_2CH), 2.51-2.60 (m, 8H, CH_2-alk), 1.47-1.53 (m, 8H, CH_2-alk), 1.24-1.33 (m, 72H, CH_2-alk), 0.85 (t, 12H, *J* = 7.0 Hz, 4×CH_3-alk); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 103.9 (2×*C*-1'), 103.3 (*C*-1), 102.5 (*C*-1), 80.8 (2×*C*-4), 75.5 (*C*-5'), 75.0 (2×*C*-3'), 74.8 (2×*C*-5), 73.2 (2×*C*-3), 73.1 (2×*C*-2), 71.2 (O*C*H_2CH), 70.6 (O*C*H_2CH), 70.5 (2×*C*-2'), 68.1 (2×*C*-4'), 60.5 (2×*C*-6), 60.4 (2×*C*-6'), 45.6 (*C*H), 45.0 (*C*H), 33.9 (S*C*H_2CH), 33.8 (S*C*H_2CH), 22.1-32.2 (44×*C*H_2-alk), 14.0 (4×*C*H_3-alk); HRMS [ESI +] $(M+Na)^+$ calcd for $C_{39}H_{76}NaO_{11}S_2$ 807.4721 found 807.4705

3-(dodecylthio)prop-2-enyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (18)

Prepared according to general procedure **B**; White amorphous solid; 65%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.20 (d, 1H, *J* = 15.2 Hz, HC=C*H*$S_{(E)}$) 6.16 (d, 1H, *J* = 9.7 Hz, HC=C*H*$S_{(Z)}$) 5.54 (ddd, 1H, *J* = 9.5, 7.1, 6.0 Hz, *H*C=C$HS_{(Z)}$), 5.45 (ddd, 1H, *J* = 15.1, 6.4, 6.4 Hz, *H*C=C$HS_{(E)}$), 5.14 (t_{ap}, 2H, *J* = 9.5 Hz, 2×*H*-3), 5.03 (t_{ap}, 1H, *J* = 9.6 Hz, *H*-$4_{(Z)}$), 5.02 (t_{ap}, 1H, *J* = 9.7 Hz, *H*-$4_{(E)}$), 4.92 (m, 2H, 2×*H*-2), 4.50 (d, 1H, *J* = 8.1 Hz, *H*-$1_{(E)}$) 4.48 (d, 1H, *J* = 8.1 Hz, *H*-$1_{(Z)}$), 4.18-4.26 (m, 4H, 2×*H*-6a, 2×OCH_2), 4.03-4.09 (m, 4H, 2×*H*-6b, 2×OCH_2), 3.61-3.65 (m, 2H, 2×*H*-5), 2.59-2.63 (m, 4H, 2×SCH_2), 2.02, 2.02, 1.98, 1.97, 1.97, 1.95, 1.95, 1.93 (8s, 24H, 8×CH_3, acetyl), 1.53-1.59 (m, 4H, 2×CH_2-alk), 1.30-1.33 (m, 4H, 2×CH_2-alk), 1.19-1.22 (m, 32H, CH_2-alk), 0.81 (t, 6H, *J* = 6.9 Hz, 2×CH_3-alk);^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.6, 170.6, 170.2, 170.2, 169.3, 169.3, 169.3, 169.2(8×*C*=O), 131.2 (*C*sp^2-$H_{(Z)}$), 130.0 (*C*sp^2-$H_{(E)}$), 123.0 (*C*sp^2-$H_{(Z)}$), 120.9 (*C*sp^2-$H_{(E)}$), 99.3 (*C*-$1_{(Z)}$), 99.1 (*C*-$1_{(E)}$), 72.9 (*C*-$3_{(Z)}$), 72.8 (*C*-$3_{(E)}$), 71.9 (*C*-$5_{(Z)}$), 71.8 (*C*-$5_{(E)}$), 71.2 (2×*C*-2), 69.6 (O*C*$H_{2(E)}$), 68.4 (*C*-$4_{(Z)}$), 68.4 (*C*-$4_{(E)}$), 65.7 (O*C*$H_{2(Z)}$), 61.9 (*C*-$6_{(E)}$), 61.9 (*C*-$6_{(Z)}$), 34.2 (S*C*H_2-alk), 32.0 (S*C*H_2-alk), 22.6-31.9 (20×*C*H_2-alk), 20.7, 20.7, 20.7, 20.6, 20.6, 20.6, 20.5, 20.5 (8×*C*H_3 acetyl), 14.1 (2×*C*H_3-alk).;HRMS [ESI $^-$] $(M+HCOO)^-$ calcd for $C_{30}H_{49}O_{12}S$ 633.2950 found 633.2938.

3-(hexadecylthio)prop-2-en-yl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (19)

Prepared according to general procedure **B**; White amorphus solid; 64%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.15 (m, 2H, 2×HC=C*H*S), 5.45 (m, 2H, 2×*H*C=CHS), 5.11 (t_{ap}, 2H, *J* = 13.4 Hz, 2×*H*-3), 4.99 (2t_{ap}, 2H, *J* = 9.6 Hz, 2×*H*-4), 4.90 (dd, 1H, *J* = 9.4, 8.1 Hz, *H*-$2_{(E)}$), 4.88 (dd, 1H, *J* = 9.4, 8.1 Hz, *H*-$2_{(Z)}$), 4.48 (d, 1H, *J* = 7.9 Hz, *H*-$1_{(E)}$), 4.46 (d, 1H, *J* = 7.9 Hz, *H*-$1_{(Z)}$), 4.15-4.27 (m, 4H, 2×*H*-6a, 2×OCH_2), 3.98-4.10 (m, 4H, 2×*H*-6b, 2×OCH_2), 3.58-3.64 (m, 2H, 2×*H*-5), 2.55-2.61 (m, 4H, 2×CH_2-alk), 1.99, 1.94, 1.92, 1.90 (4s, 24H, 8×CH_3, acetyl), 1.47-1.58 (m, 4H, CH_2-alk), 1.16-1.29 (m, 52H, CH_2-alk), 0.78 (t, 6H, *J* = 6.6 Hz, CH_3-alk); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.4, 170.4, 170.1, 170.0, 169.2, 169.2, 169.1, 169.1 (8×*C*=O), 131.1 (HC=*C*$HS_{(Z)}$), 129.9 (HC=*C*$HS_{(E)}$), 122.9 (H*C*=C$HS_{(Z)}$), 120.1 (H*C*=C$HS_{(E)}$), 99.2 (*C*-$1_{(Z)}$), 99.0 (*C*-$1_{(E)}$), 72.9 (*C*-$3_{(Z)}$), 72.8 (*C*-$3_{(E)}$), 71.8 (*C*-$5_{(Z)}$), 71.7 (*C*-$5_{(E)}$), 71.2 (2×*C*-2), 69.5 (O*C*$H_{2(E)}$), 68.3 (*C*-$4_{(Z)}$), 68.3 (*C*-$4_{(E)}$), 65.6 (O*C*$H_{2(Z)}$), 61.8 (2×*C*-6), 22.6-34.1 (30×*C*H_2-alk), 20.4-20.6 (8×*C*H_3, acetyl), 14.0 (2×*C*H_3-alk); HRMS [ESI +] $(M+H)^+$ calcd for $C_{33}H_{57}O_{10}S$ 645.3667 found 645.3662

(2-hexadecylthio-3-dodecanoylthio)propyl 2,3,4,6- tetra-*O*-acetyl-β-D-glucopyranoside (20)

Prepared according to general procedure **C**; White amorphous solid; 90%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.12 ($2t_{ap}$, 2H, *J* = 9.5 Hz, 2×*H*-3), 4.99 ($2t_{ap}$, 2H, *J* = 9.7 Hz, 2×*H*-4), 4.90 (2dd, 2H, *J* = 9.5, 8.0 Hz, 2×*H*-2), 4.46 (d, 2H, *J* = 7.9 Hz, 2×*H*-1), 4.19 (dd, 2H, *J* = 12.3, 4.6 Hz, 2×*H*-6a), 3.96-4.06 (m, 4H, 2×*H*-6b, 2×CH_2CH), 3.50-3.66 (m, 4H, 2×*H*-5, 2×CH_2CH), 2.76-2.85 (m, 2H, 2×C*H*), 2.59-2.74 (m, 4H, 2×SCH_2CH), 2.40-2.50 (m, 8H, CH_2-alk), 1.98, 1.98, 1.96, 1.95, 1.92, 1.92, 1.90, 1.90 (8s, 24H, 8×CH_3, acetyl), 1.42-1.49 (m, 8H, CH_2-alk), 1.16-1.26 (m, 88H, CH_2-alk), 0.78 (t, 12H, *J* = 6.6 Hz, 4×CH_3-alk); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.3, 170.3, 170.0, 170.0, 169.2, 169.2, 169.0, 169.0 (8×*C*=O), 101.2 (*C*-1), 100.8 (*C*-1), 72.7 (*C*-3), 72.6 (*C*-3), 71.7 (2×*C*-5), 71.6 (O*C*H_2CH), 71.3 (O*C*H_2CH), 71.1 (2×*C*-2), 68.3 (2×*C*-4), 61.8 (2×*C*-6), 45.6 (*C*H), 45.1 (*C*H), 34.4 (2×S*C*H_2CH), 22.6-33.3 (26×*C*H_2-alk), 20.4-20.6 (8×*C*H_3, acetyl), 14.0 (4×*C*H_3-alk); HRMS [ESI +] $(M+Na)^+$ calcd for $C_{45}H_{82}NaO_{10}S_2$ 869.5242 found 869.5251

(2-dodecylthio-3-hexadecylthio)propyl 2,3,4,6- tetra-*O*-acetyl-β-D-glucopyranoside (21)

Prepared according to general procedure **C**; White amorphous solid; 92%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.11 ($2t_{ap}$, 2H, *J* = 9.5 Hz, 2×*H*-3), 4.97 ($2t_{ap}$, 2H, *J* = 9.6 Hz, 2×*H*-4), 4.88 (2dd, 2H, *J* = 9.5, 8.0 Hz, 2×*H*-2), 4.45 (d, 2H, *J* = 7.9 Hz, 2×*H*-1), 4.18 (dd, 2H, *J* = 12.3, 4.6 Hz, 2×*H*-6a), 3.98-4.05 (m, 4H, 2×*H*-6b, 2×OCH_2CH), 3.49-3.65 (m, 4H, 2×*H*-5, 2×OCH_2CH), 2.75-2.84 (m, 2H, 2×C*H*), 2.58-2.73 (m, 4H, 2×SCH_2CH), 2.39-2.49 (m, 8H, CH_2-alk), 1.97, 1.97, 1.95, 1.94, 1.91, 1.91, 1.89, 1.89 (8s, 24H, 8×CH_3, acetyl), 1.41-1.48 (m, 8H, CH_2-alk), 1.12-1.25 (m, 88H, CH_2-alk), 0.77 (t, 12H, *J* = 6.6 Hz, 4×CH_3-alk); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.3, 170.3, 170.0, 169.9, 169.2, 169.1, 169.0, 168.9 (8×*C*=O), 101.1 (*C*-1), 100.8 (*C*-1), 72.7 (*C*-3), 72.6 (*C*-3), 71.7 (2×*C*-5), 71.6 (O*C*H_2CH), 71.3 (O*C*H_2CH), 71.0 (2×*C*-2), 68.3 (2×*C*-4), 61.7 (2×*C*-6), 45.6 (*C*H), 45.0 (*C*H), 34.3 (2×S*C*H_2CH), 22.6-31.8 (52×*C*H_2-alk), 20.4-20.6 (8×*C*H_3, acetyl), 14.0 (4×*C*H_3-alk); HRMS [ESI +] $(M+Na)^+$ calcd for $C_{45}H_{82}NaO_{10}S_2$ 869.5242 found 869.5224

(2-hexadecylthio-3-dodecanoylthio)propyl β-D-glucopyranoside (22)

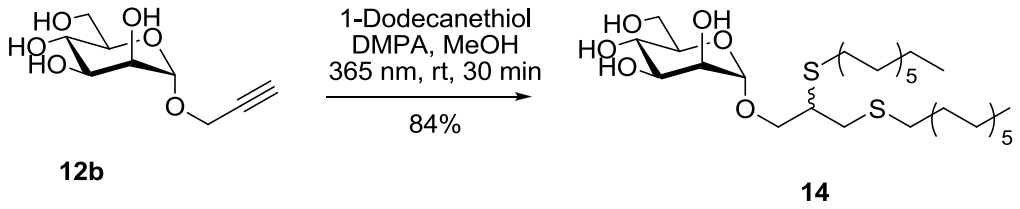
Prepared according to general procedure **D**; White powder; >95%; ^{1}H NMR ($CDCl_3$ + CD_3OD, 600 MHz) δ 4.77, 4.74, 4.67, 4.63, 4.35 (5 br s, 8H, 8×O*H*), 4.31 (d, 2H, *J* = 6.5 Hz, 2×*H*-1), 4.04-4.10 (m, 2H, OCH_2CH), 3.80-3.83 (m, 4H, 2×*H*-6a, 2×*H*-6b), 3.69-3.76 (m, 2H, OCH_2CH), 3.48-3.54 (m, 4H, 2×*H*-3, 2×*H*-4), 3.30-3.42 (m, 4H, 2×*H*-2, 2×*H*-5), 2.95-2.98 (m, 2H, 2×C*H*), 2.76-2.90 (m, 4H, 2×SCH_2CH), 2.52-2.55 (m, 8H, CH_2-alk), 1.55-1.57 (m, 8H, CH_2-alk), 1.24-1.35 (m, 88H, CH_2-alk), 0.86 (t, 12H, *J* = 6.1 Hz, 4×CH_3-alk); ^{13}C NMR ($CDCl_3$ + CD_3OD, 150 MHz) δ 103.4 (*C*-1), 103.2 (*C*-1), 76.3 (2×*C*-3), 75.8 (2×*C*-5), 73.5 (2×*C*-2), 71.3 (O*C*H_2CH), 71.1 (O*C*H_2CH), 69.7 (2×*C*-4), 61.8 (2×*C*-6), 45.8 (*C*H), 45.7 (*C*H), 34.8 (S*C*H_2CH), 34.7 (S*C*H_2CH), 22.8-33.4 (52×*C*H_2-alk), 14.2 (4×*C*H_3-alk); HRMS [ESI +] $(M+H)^+$ calcd for $C_{37}H_{75}O_6S_2$ 679.5000 found 679.5012

(2- dodecanoylthio-3-hexadecylthio)propyl β-D-glucopyranoside (23)

Prepared according to general procedure **D**; White powder; >95%; ^{1}H NMR ($CDCl_3$ + CD_3OD, 600 MHz) δ 5.03, 5.00, 4.89, 4.86, 4.52, 4.48 (6 br s, 8H, 8×O*H*), 4.31 (d, 2H, *J* = 7.7 Hz, 2×*H*-1), 4.08 (dd, 1H, *J* = 10.3, 4.5 Hz, OCH_2CH), 4.02 (dd, 1H, *J* = 10.3, 6.2 Hz, OCH_2CH), 3.79-3.82 (m, 4H, 2×*H*-6a, 2×*H*-6b), 3.75 (dd, 1H, *J* = 10.4, 5.3 Hz, OCH_2CH), 3.70 (dd, 1H, *J* = 10.3, 6.6 Hz, OCH_2CH), 3.48-3.54 (m, 4H, 2×*H*-3, 2×*H*-4), 3.33-3.36 (m, 2H, 2×*H*-2), 3.28-3.30 (m, 2H, 2×*H*-5), 2.94-2.98 (m, 2H, 2×C*H*), 2.75-2.91 (m, 4H, 2×SCH_2CH), 2.52-2.57 (m, 8H, CH_2-alk), 1.53-1.58 (m, 8H, CH_2-alk), 1.24-1.35 (m, 88H, CH_2-alk), 0.86 (t, 12H, *J* = 7.0 Hz, 4×CH_3-alk); ^{13}C NMR ($CDCl_3$ + CD_3OD, 150 MHz) δ 103.4 (*C*-1), 103.2 (*C*-1), 76.3 (2×*C*-3), 75.8 (2×*C*-5), 73.5 (2×*C*-2), 71.3 (O*C*H_2CH), 71.1 (O*C*H_2CH), 69.6 (2×*C*-4), 61.7 (2×*C*-6), 34.8 (*C*H), 34.7 (*C*H), 33.4 (S*C*H_2CH), 33.3 (S*C*H_2CH), 22.8-32.0 (52×*C*H_2-alk), 22.8 (4×*C*H_3-alk); HRMS [ESI +] $(M+H)^+$ calcd for $C_{37}H_{75}O_6S_2$ 679.5000 found 679.4973

Typical Thiol-Yne reaction on unprotected sugars: case of compound 14

Unprotected propargyl α–D-mannopyranoside **12** (44 mg, 0.2 mmol, 1.0 equiv.), 1-decanethiol (240 uL, 1.0 mmol, 5.0 equiv.) and DMPA (2,2-dimethoxy-2-phenylacetophenone, 0.08 mmol, 0.4 equiv.) were dissolved in MeOH (2.0 mL) in UV quartz cell. The mixture was stirred under UV light (365 nm) at room temperature for 30 min. The solvent was evaporated in vacuum. The crude residue was purified by silica gel flash chromatography using CH_2Cl_2-MeOH (94: 6). The desired compound **14** was isolated as a white solid (105 mg, 0.17 mmol, 84%). R*f* = 0.2 (CH_2Cl_2-MeOH 94: 6). Its physical and spectroscopic data were identical to the one described from the fully acetylated compound.



12b **14**

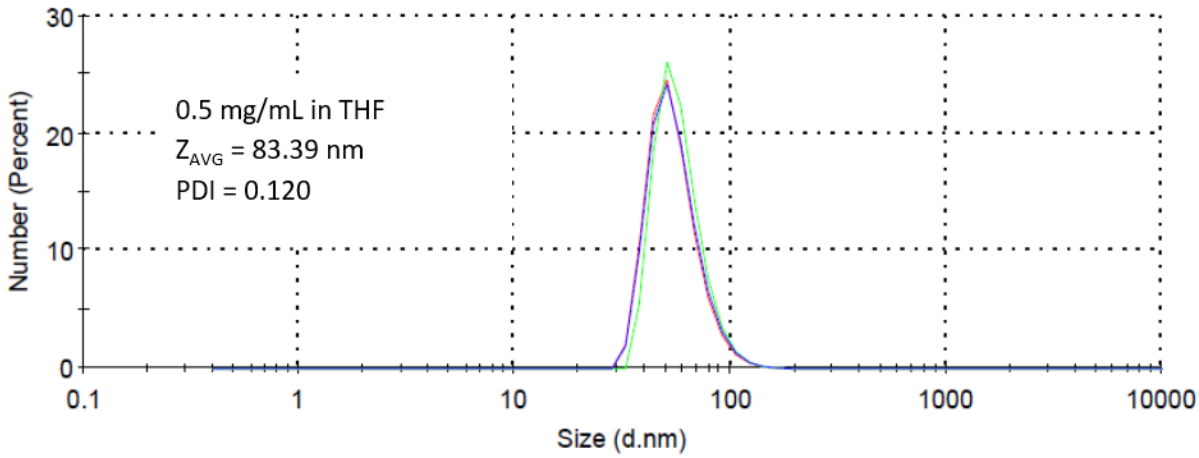
3 Preparation of liposomes and giant vesicles

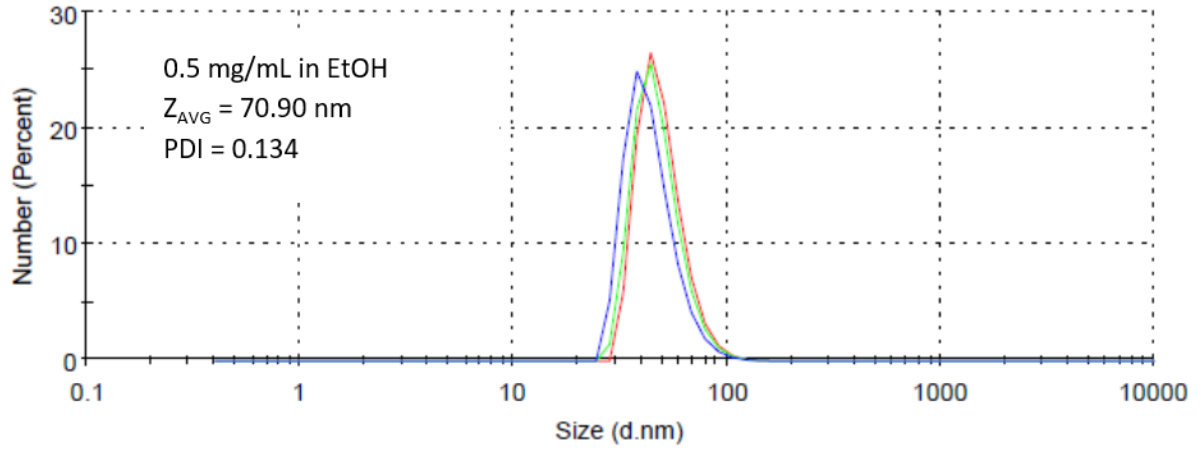
- Liposomes (small vesicles) were prepared by the injection method : 100µL of a solution of neoglycolipid in a water miscible solvent (typically THF or EtOH) were injected *via* micropipet in 2mL of nanopure water, followed by 5 seconds of vortexing
- Giant vesicles were prepared by film hydration : 25 µL of a 5mg/mL solution of neoglycolipid in $CHCl_3$ were uniformly deposited on a confocal microscopy slide and left to evaporate at room temperature for 16 hours. The slide was then immersed in nanopure water, heated at 60°C and left to hydrate overnight. For confocal microscopy visualization, 5µL of a 0.1 mg/mL solution of hydrophobic nile red were added to the neoglycolipid solution prior to evaporation on the microscope slide.

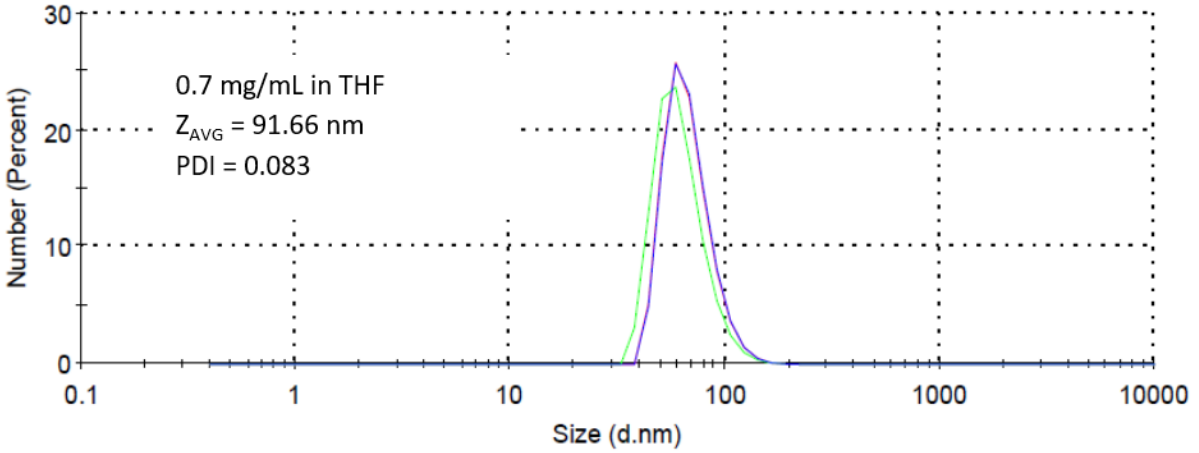
4 Spectra of dynamic light scattering of the assemblies

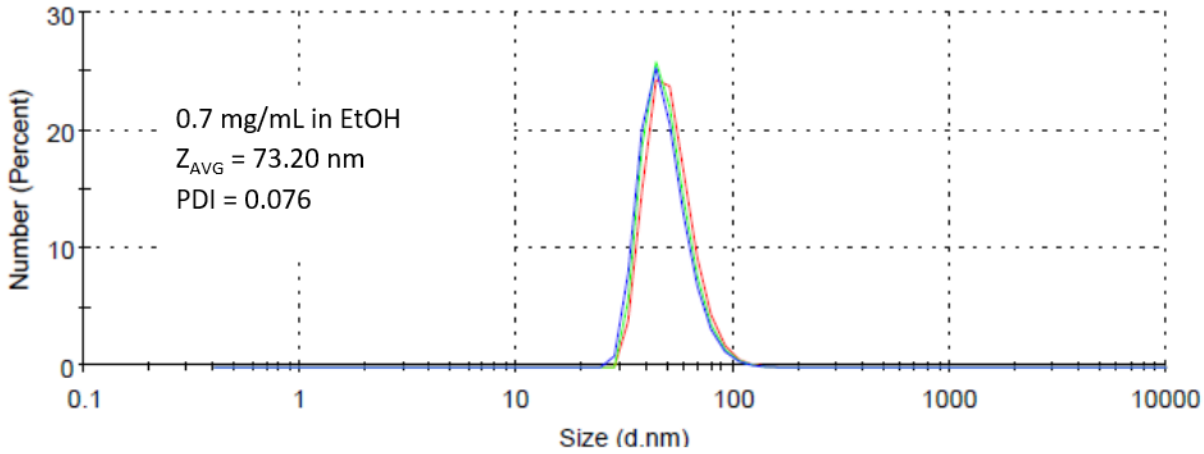
- DLS spectra of Glucose12/12 at various concentrations in THF and EtOH

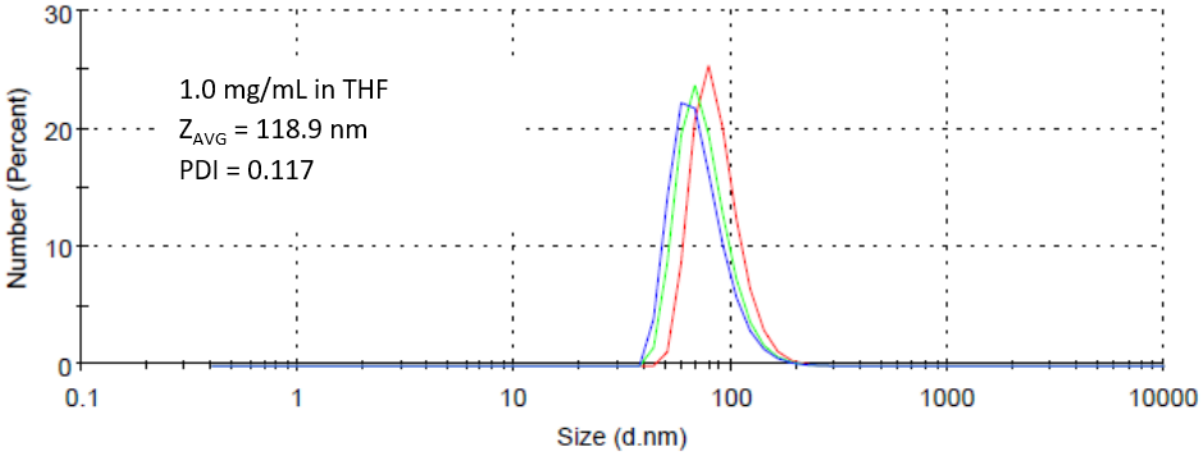
  Different solutions of **4** in THF and EtOH were prepared from 0.5 mg/mL to 10 mg/mL. 100µL of these preparations were injected in a constant 2mL of nanopure water followed by 5 seconds of vortexing.

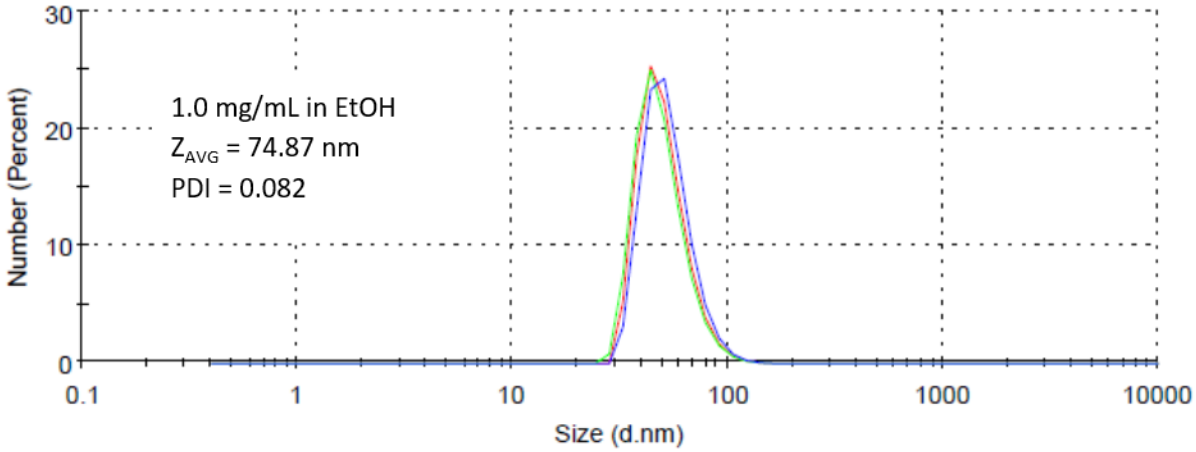

0.5 mg/mL in THF
Z_{AVG} = 83.39 nm
PDI = 0.120
Number (Percent)
Size (d.nm)
0
10
20
30
0.1
1
10
100
1000
10000

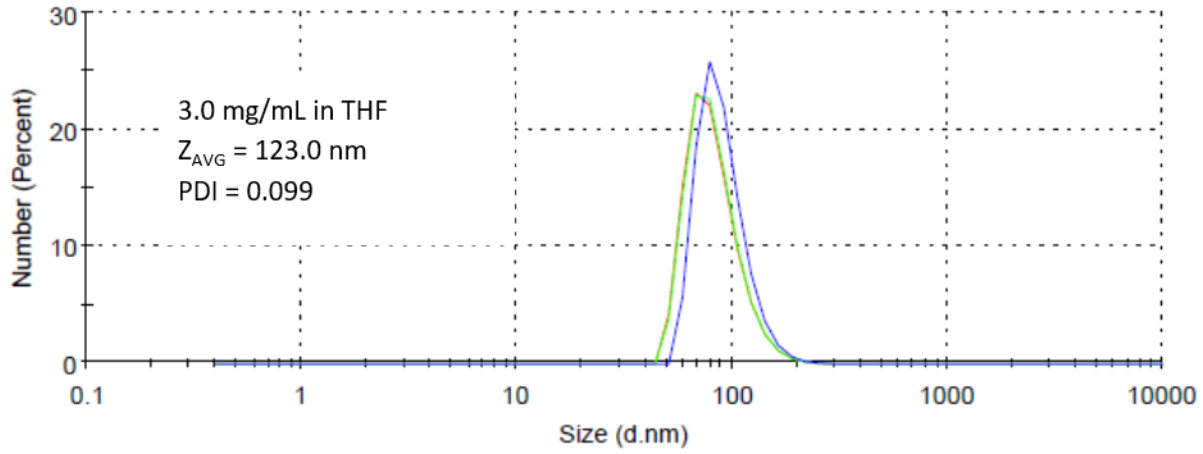

0.5 mg/mL in EtOH
Z_{AVG} = 70.90 nm
PDI = 0.134
Number (Percent)
Size (d.nm)
0
10
20
30
0.1
1
10
100
1000
10000

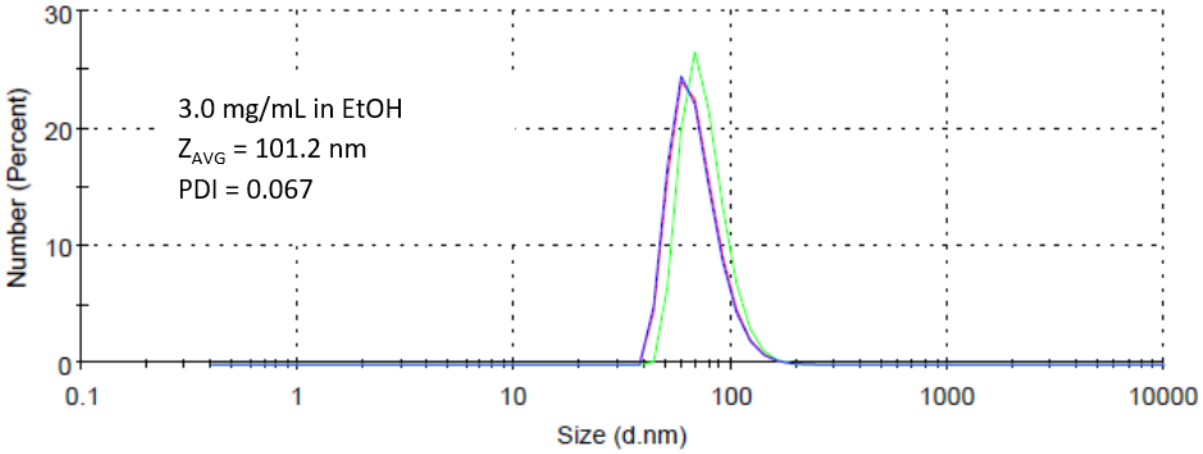

0.7 mg/mL in THF
Z_{AVG} = 91.66 nm
PDI = 0.083
Number (Percent)
Size (d.nm)
0
10
20
30
0.1
1
10
100
1000
10000

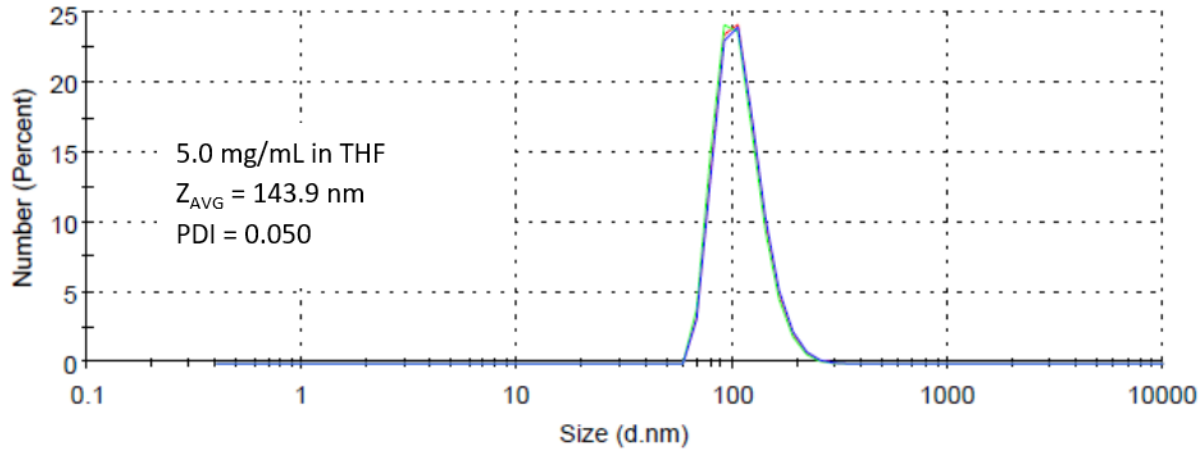

0.7 mg/mL in EtOH
Z_{AVG} = 73.20 nm
PDI = 0.076
Number (Percent)
Size (d.nm)
0
10
20
30
0.1
1
10
100
1000
10000

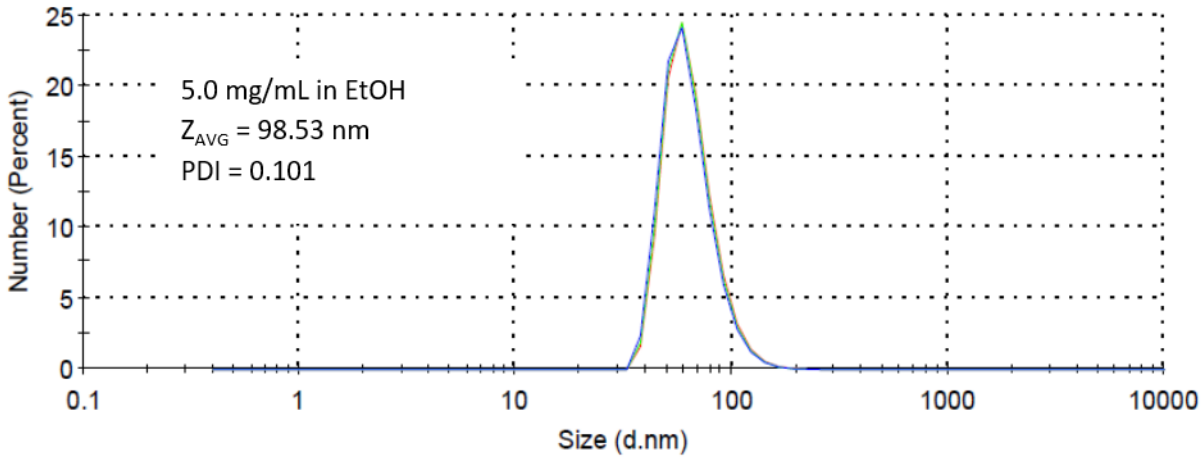

1.0 mg/mL in THF
Z_{AVG} = 118.9 nm
PDI = 0.117
Number (Percent)
Size (d.nm)
0
10
20
30
0.1
1
10
100
1000
10000

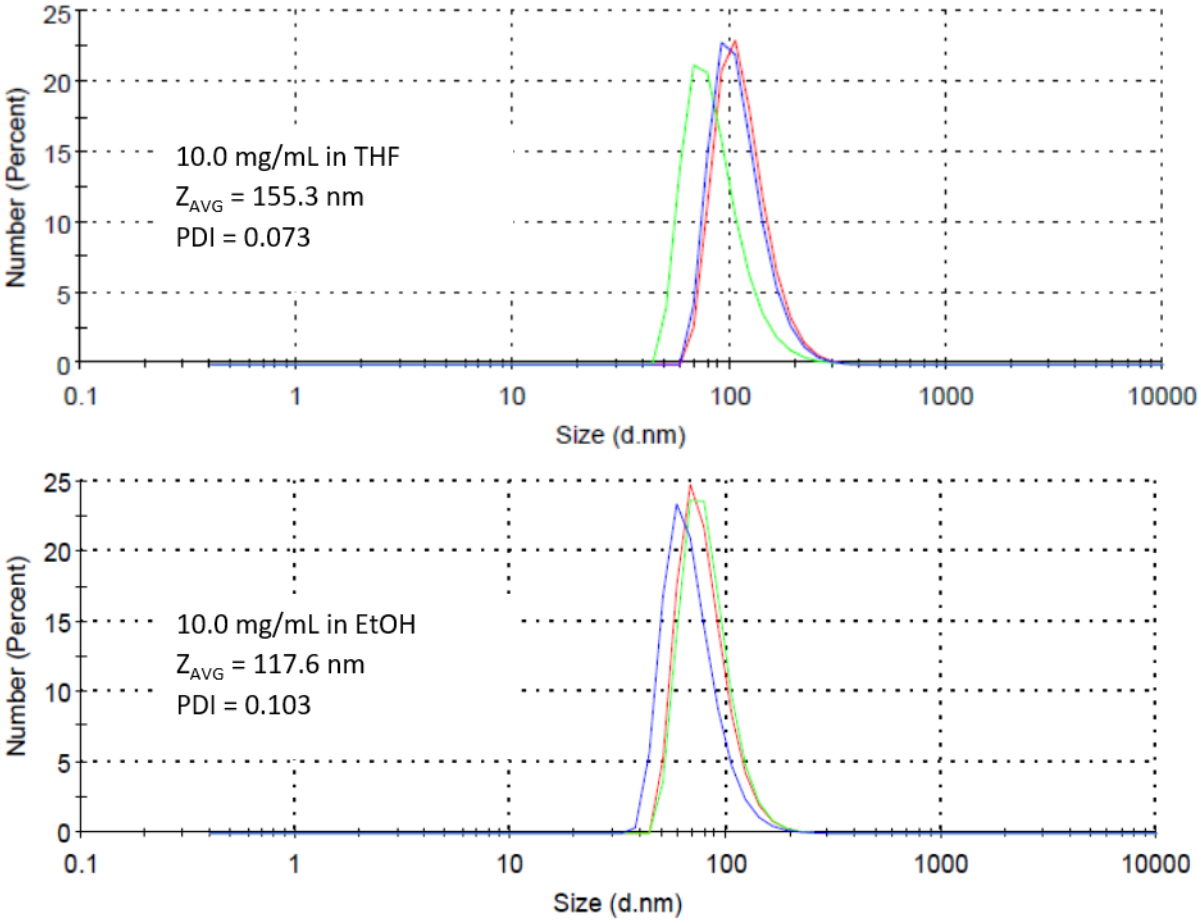

1.0 mg/mL in EtOH
Z_{AVG} = 74.87 nm
PDI = 0.082
Number (Percent)
Size (d.nm)
0
10
20
30
0.1
1
10
100
1000
10000


3.0 mg/mL in THF
Z_{AVG} = 123.0 nm
PDI = 0.099
Number (Percent)
Size (d.nm)
0
10
20
30
0.1
1
10
100
1000
10000


3.0 mg/mL in EtOH
Z_{AVG} = 101.2 nm
PDI = 0.067
Number (Percent)
Size (d.nm)
0
10
20
30
0.1
1
10
100
1000
10000


5.0 mg/mL in THF
Z_{AVG} = 143.9 nm
PDI = 0.050
Number (Percent)
Size (d.nm)
0
5
10
15
20
25
0.1
1
10
100
1000
10000


5.0 mg/mL in EtOH
Z_{AVG} = 98.53 nm
PDI = 0.101
Number (Percent)
Size (d.nm)
0
5
10
15
20
25
0.1
1
10
100
1000
10000



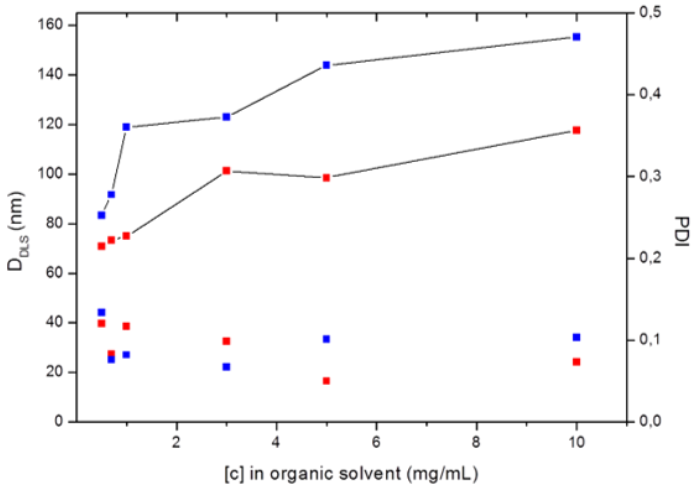
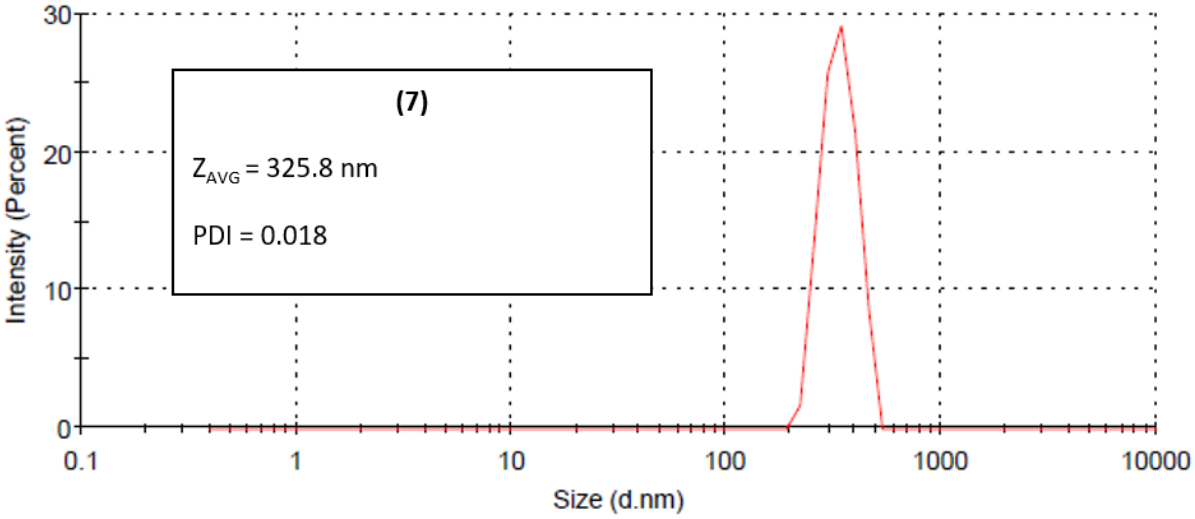
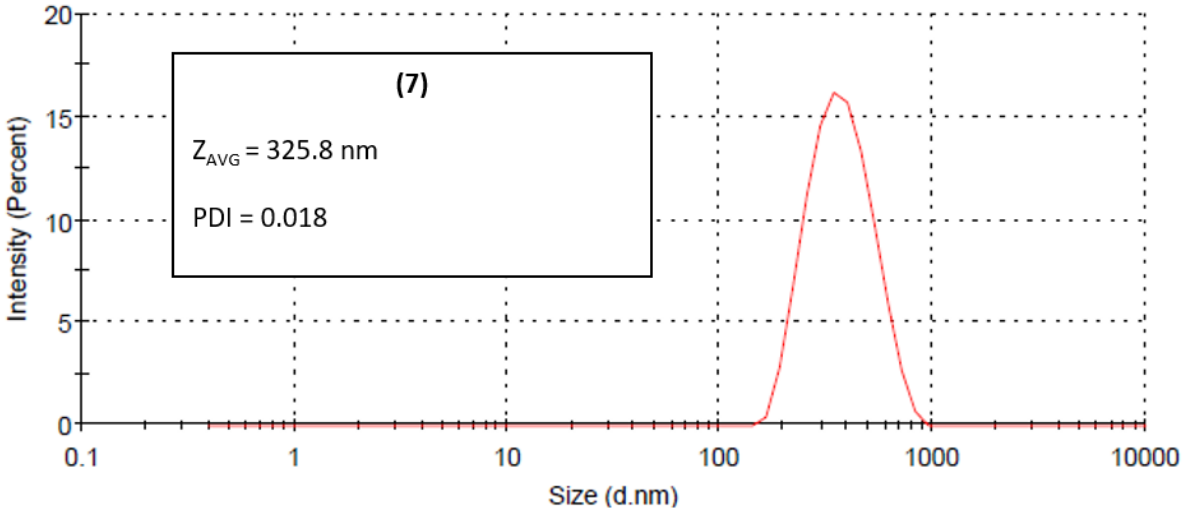
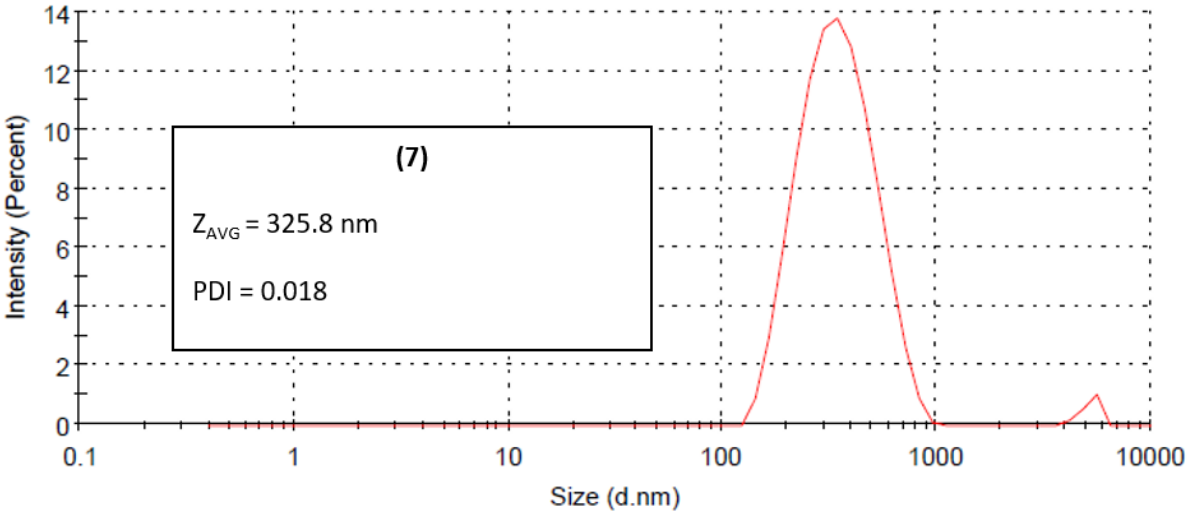


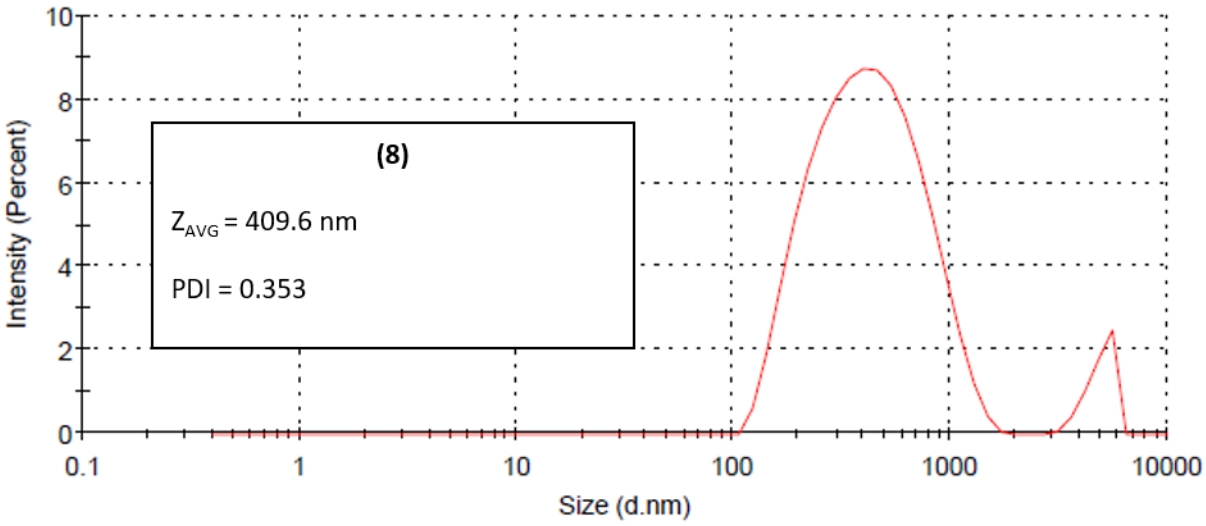
Figure S1A. Diameter (D_{DLS})—Starting concentration relationship of the glycoliposomes formed by injection of a THF (blue line) and EtOH (red line) solution of compound **4** in nanopure water. Polydispersity indexes(PDI) are reported for each set of measures in THF (blue dots) and EtOH (red dots).

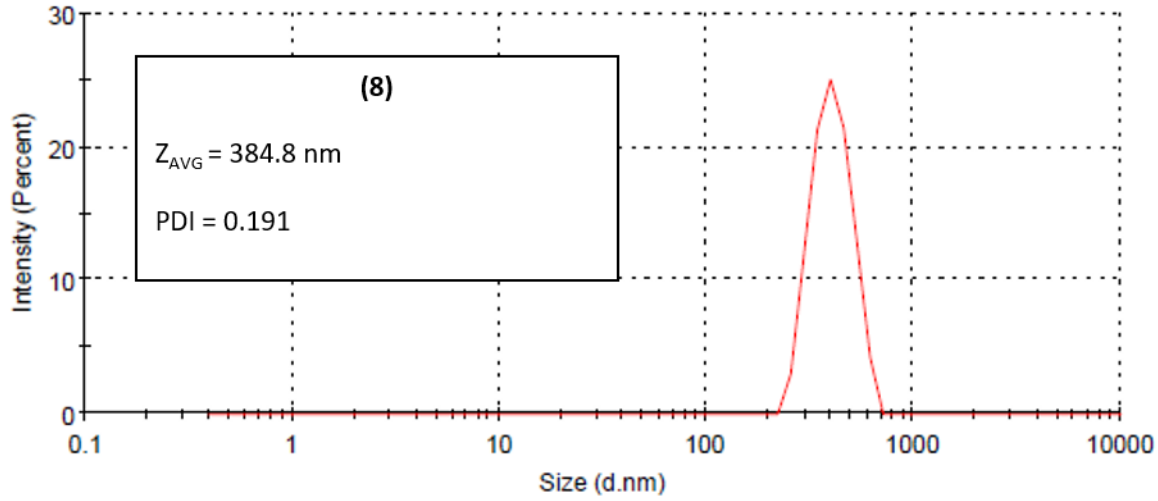

(7)
Z_{AVG} = 325.8 nm
PDI = 0.018
Intensity (Percent)
Size (d.nm)
0
10
20
30
0.1
1
10
100
1000
10000

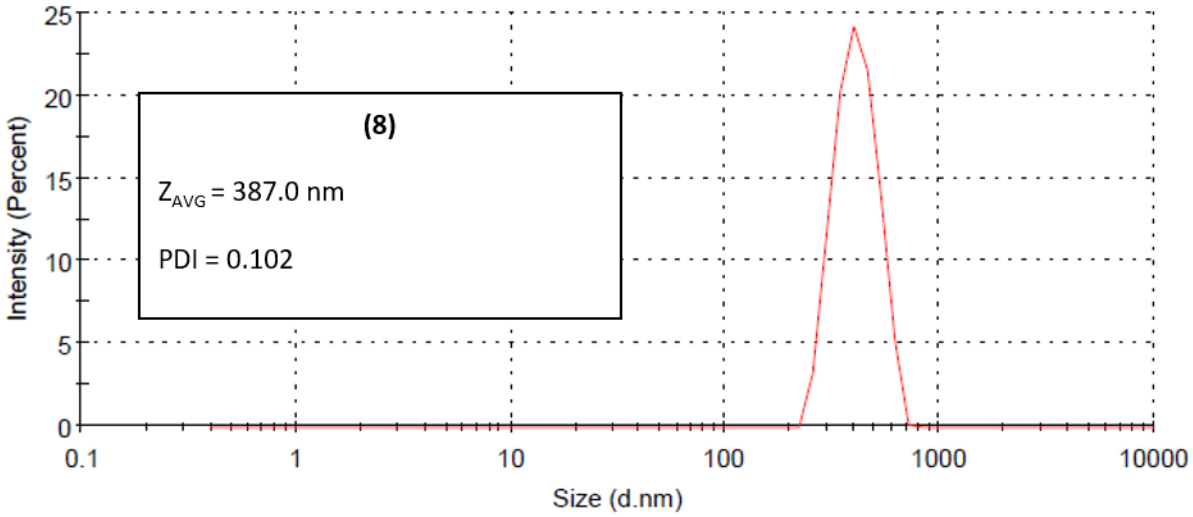

(7)
Z_{AVG} = 325.8 nm
PDI = 0.018
Intensity (Percent)
Size (d.nm)
0
5
10
15
20
0.1
1
10
100
1000
10000

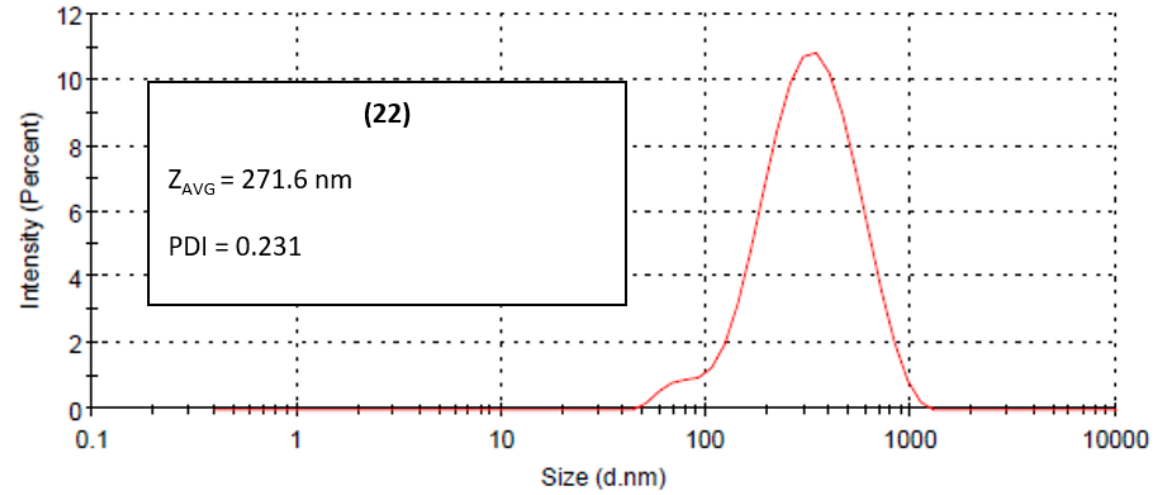

(7)
Z_{AVG} = 325.8 nm
PDI = 0.018
Intensity (Percent)
Size (d.nm)
0
2
4
6
8
10
12
14
0.1
1
10
100
1000
10000

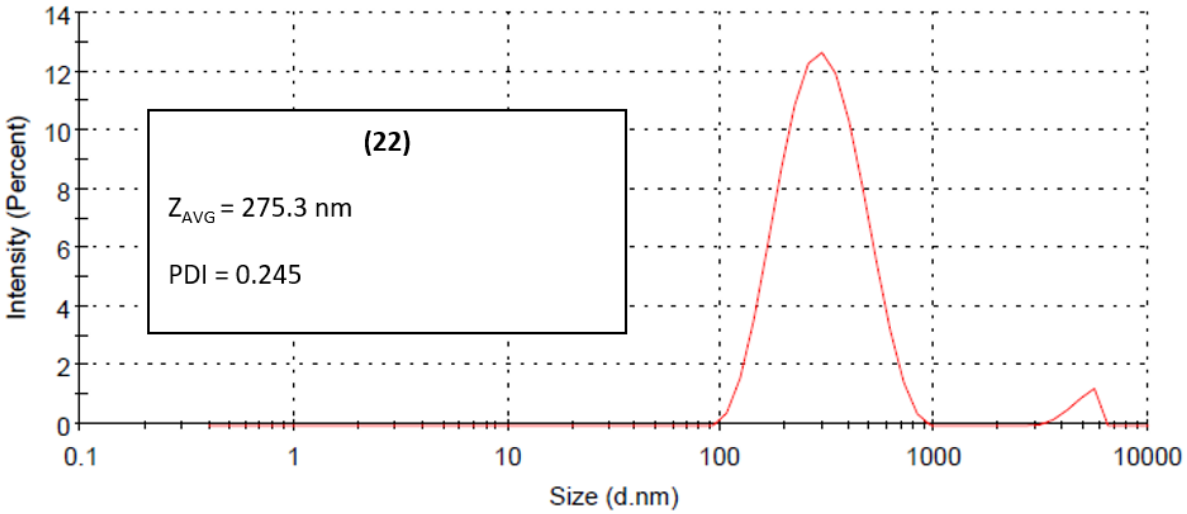
- DLS spectra of the different carbohydrates and different lipid chains. 100µL of a 5mg/mL solution of the neoglycolipid were injected in 2 mL of nanopure water followed by 5 seconds of vortexing.








(22)
Z_{AVG} = 271.6 nm
PDI = 0.231
Intensity (Percent)
Size (d.nm)
0
2
4
6
8
10
12
0.1
1
10
100
1000
10000


(22)
Z_{AVG} = 275.3 nm
PDI = 0.245
Intensity (Percent)
Size (d.nm)
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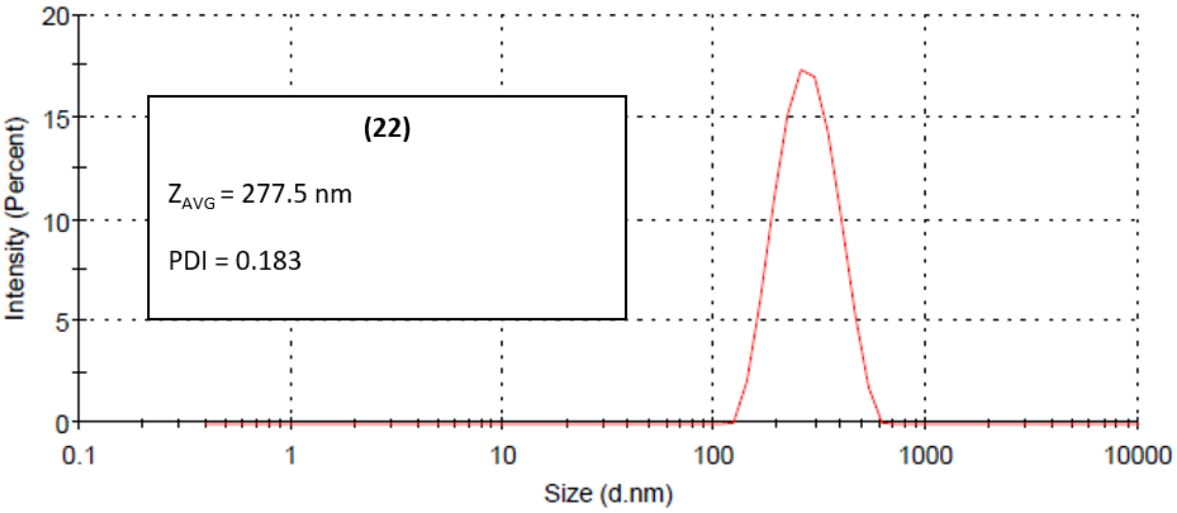
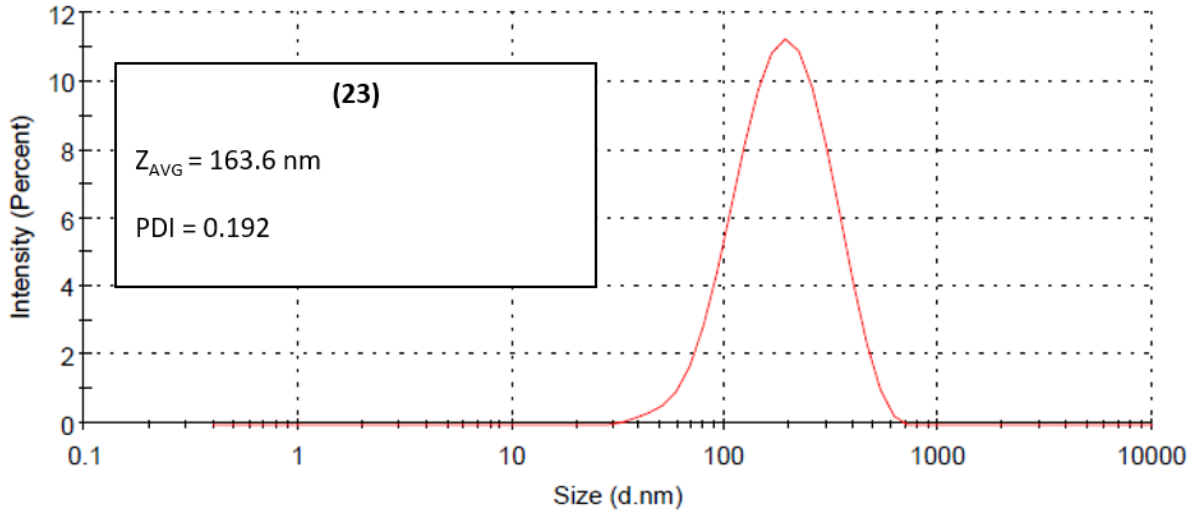
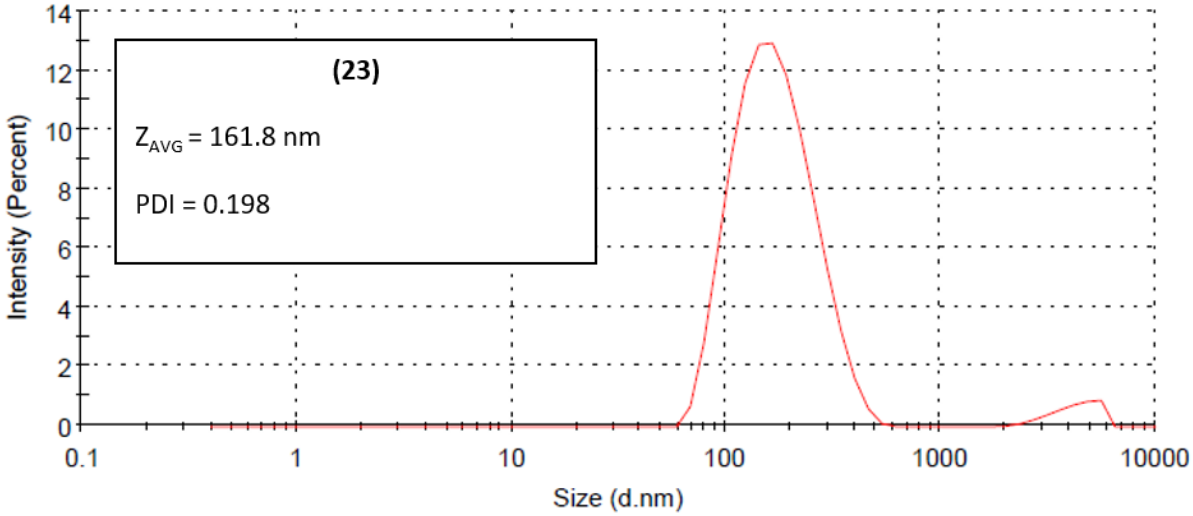
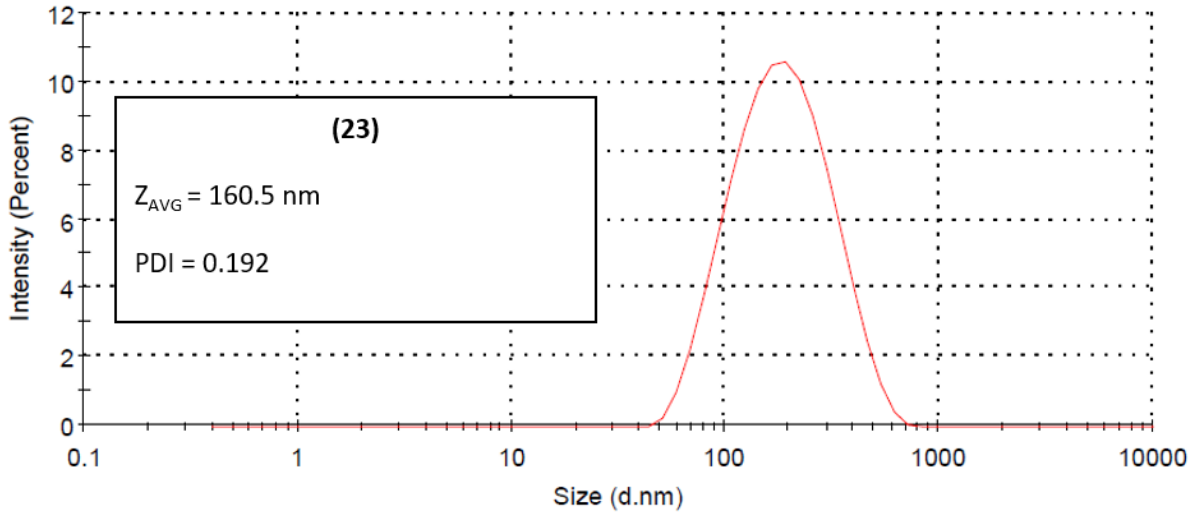
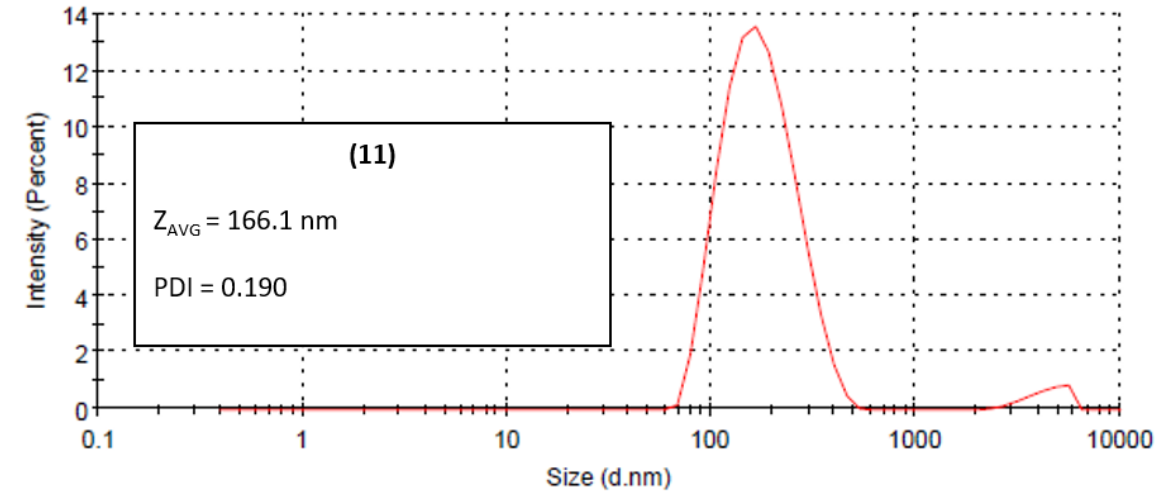
2
4
6
8
10
12
14
0.1
1
10
100
1000
10000

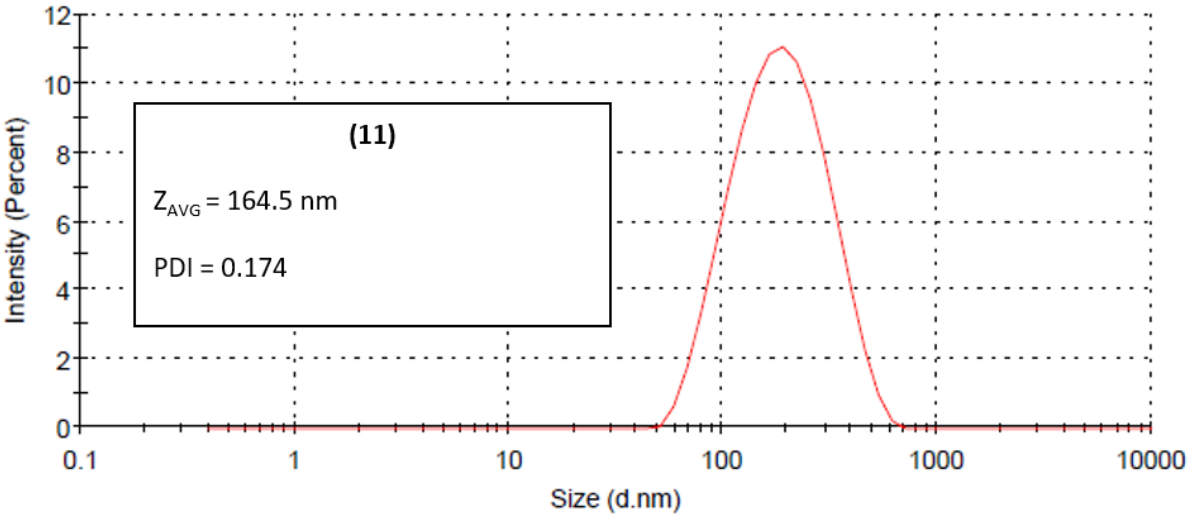

(22)
Z_{AVG} = 277.5 nm
PDI = 0.183
Intensity (Percent)
Size (d.nm)
0
5
10
15
20
0.1
1
10
100
1000
10000

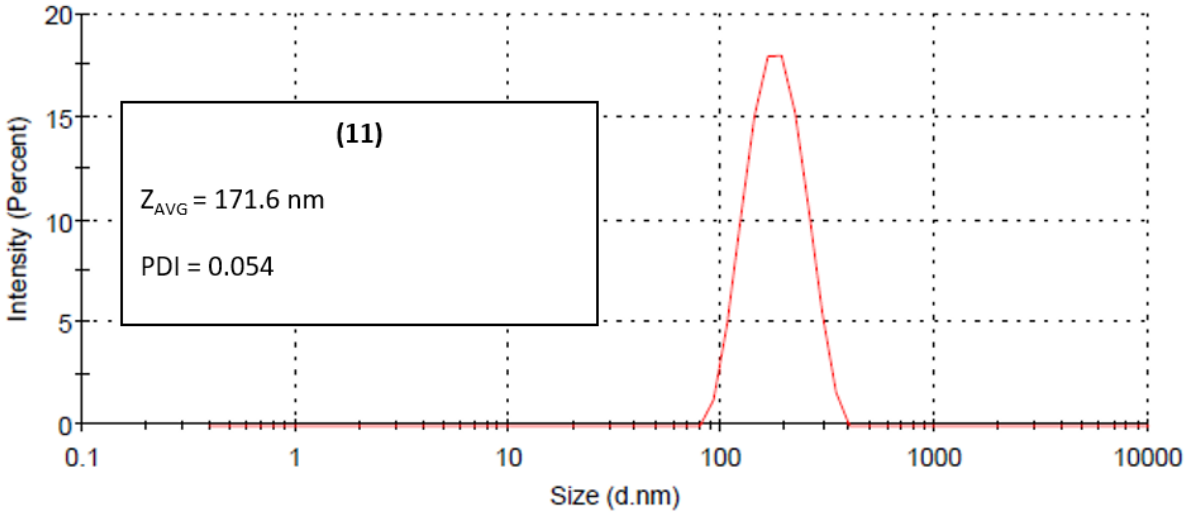

(23)
Z_{AVG} = 163.6 nm
PDI = 0.192
Intensity (Percent)
Size (d.nm)
0
2
4
6
8
10
12
0.1
1
10
100
1000
10000

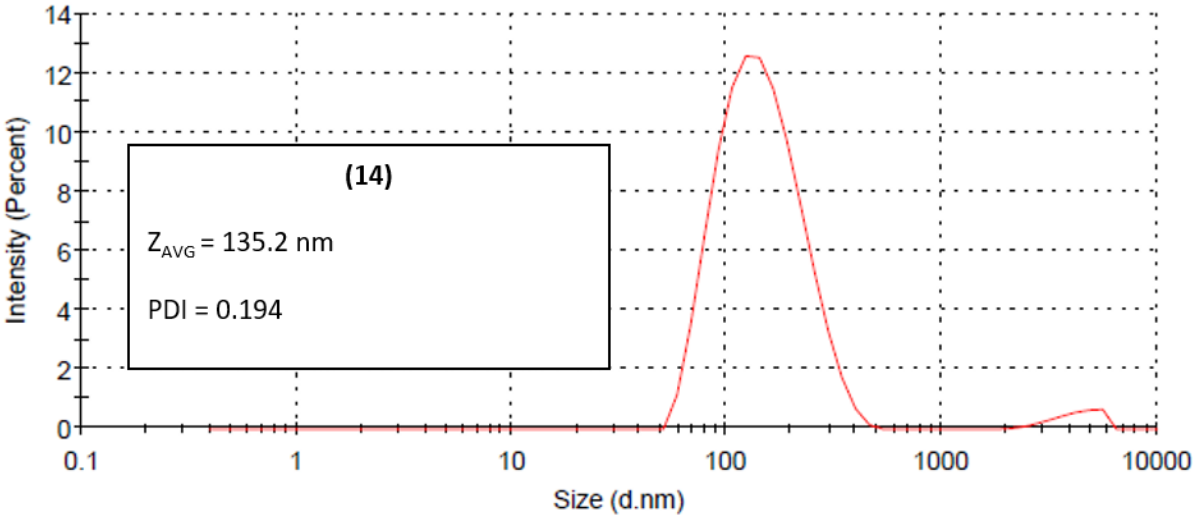

(23)
Z_{AVG} = 161.8 nm
PDI = 0.198
Intensity (Percent)
Size (d.nm)
0
2
4
6
8
10
12
14
0.1
1
10
100
1000
10000

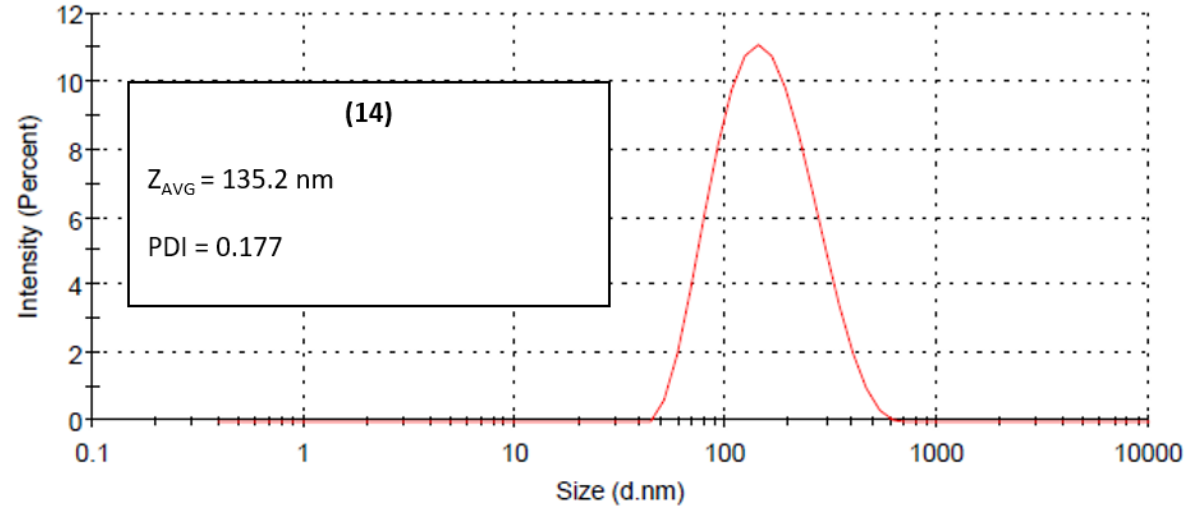

(23)
Z_{AVG} = 160.5 nm
PDI = 0.192
Intensity (Percent)
Size (d.nm)
0
2
4
6
8
10
12
0.1
1
10
100
1000
10000

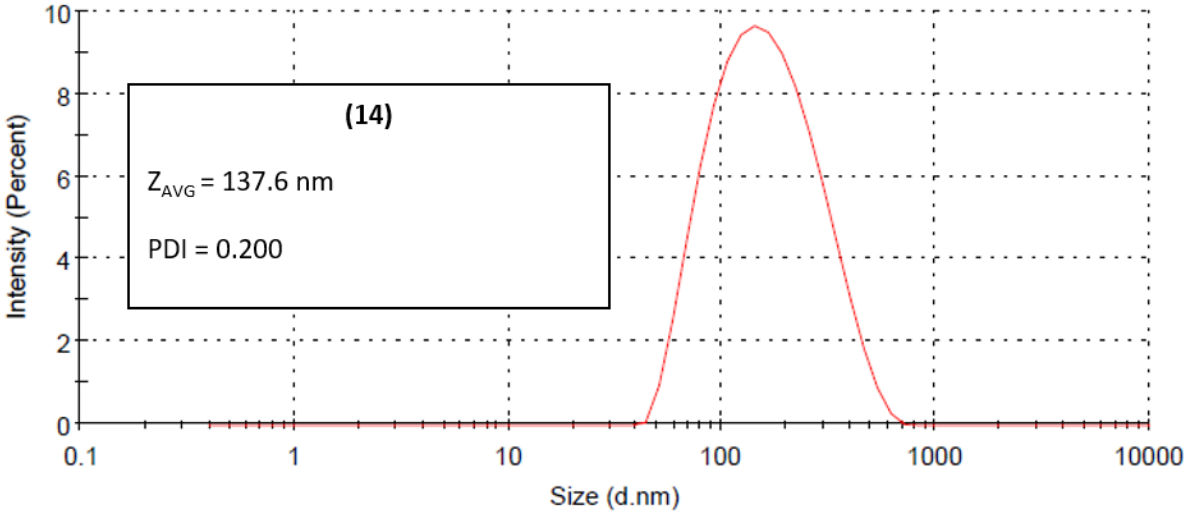

(11)
Z_{AVG} = 166.1 nm
PDI = 0.190
Intensity (Percent)
Size (d.nm)

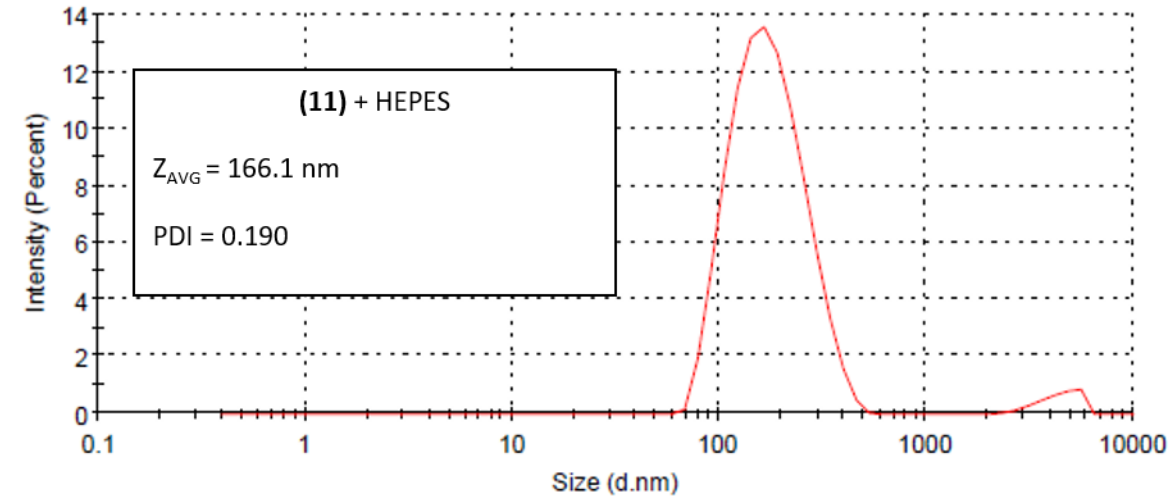

(11)
Z_{AVG} = 164.5 nm
PDI = 0.174
Intensity (Percent)
Size (d.nm)

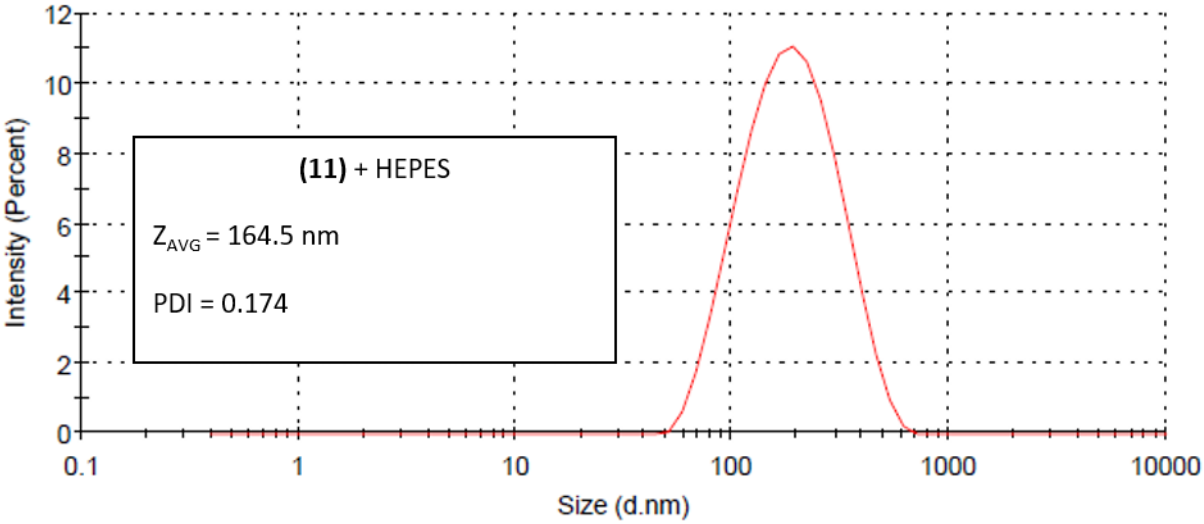

(11)
Z_{AVG} = 171.6 nm
PDI = 0.054
Intensity (Percent)
Size (d.nm)

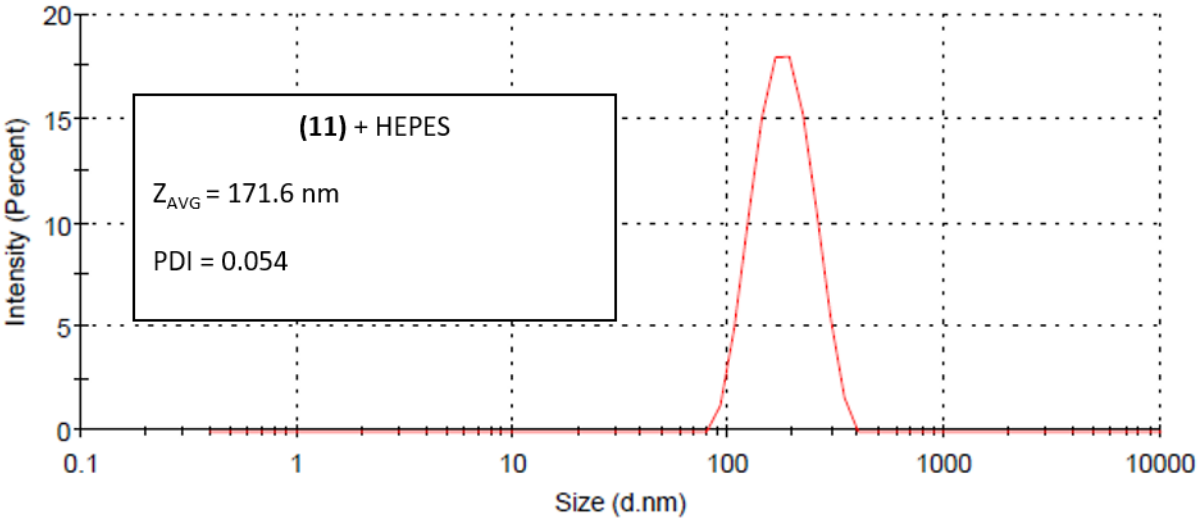

(14)
Z_{AVG} = 135.2 nm
PDI = 0.194
Intensity (Percent)
Size (d.nm)
0
2
4
6
8
10
12
14
0.1
1
10
100
1000
10000


(14)
Z_{AVG} = 135.2 nm
PDI = 0.177
Intensity (Percent)
Size (d.nm)
0
2
4
6
8
10
12
0.1
1
10
100
1000
10000


(14)
Z_{AVG} = 137.6 nm
PDI = 0.200
Intensity (Percent)
Size (d.nm)
0
2
4
6
8
10
0.1
1
10
100
1000
10000

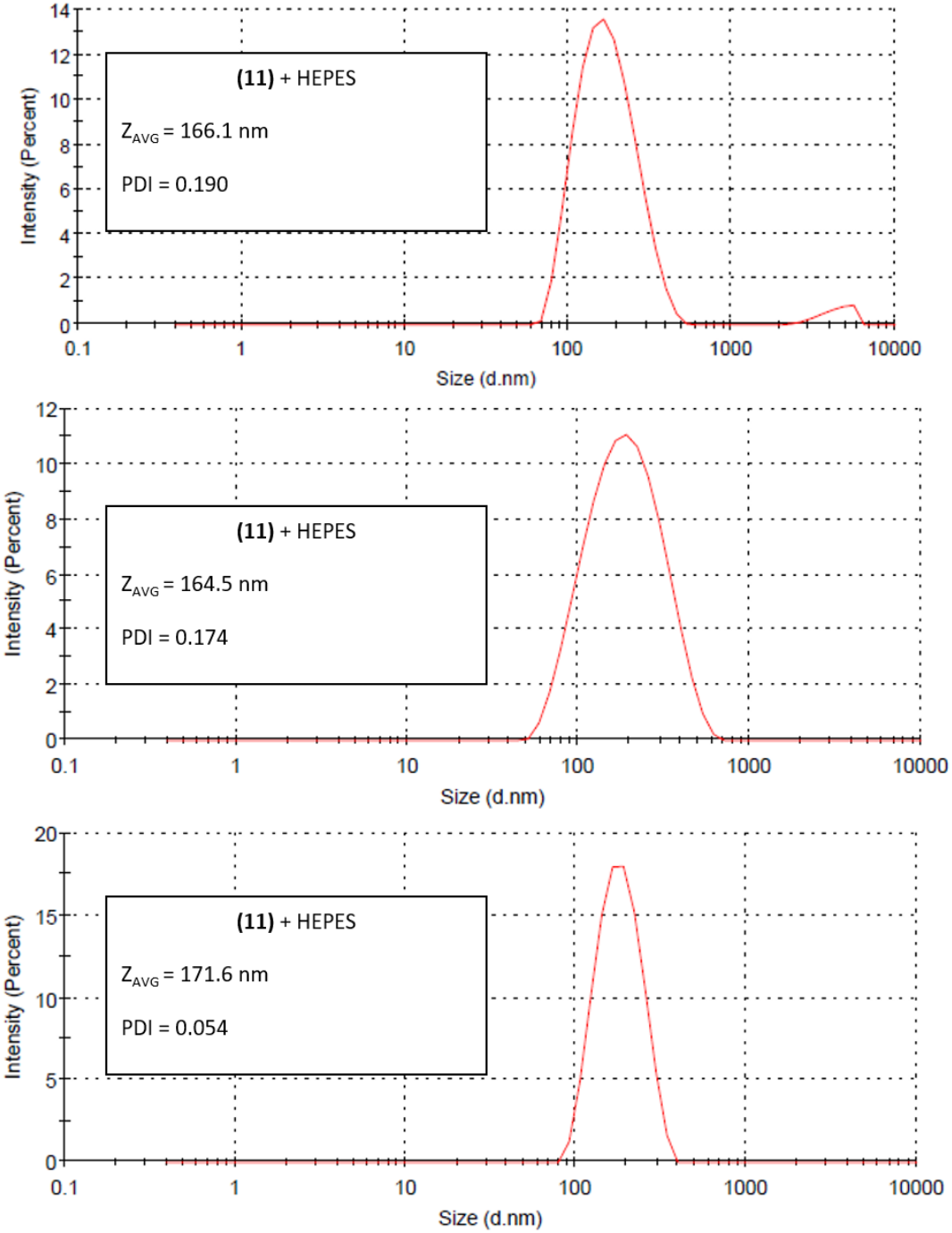

(11) + HEPES
Z_{AVG} = 166.1 nm
PDI = 0.190
Intensity (Percent)
Size (d.nm)

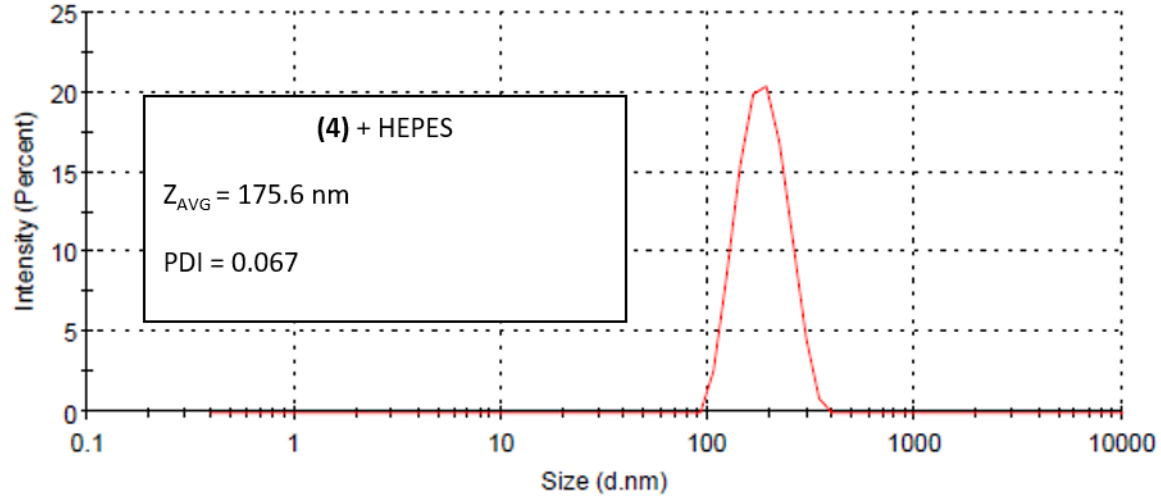

(11) + HEPES
Z_{AVG} = 164.5 nm
PDI = 0.174
Intensity (Percent)
Size (d.nm)

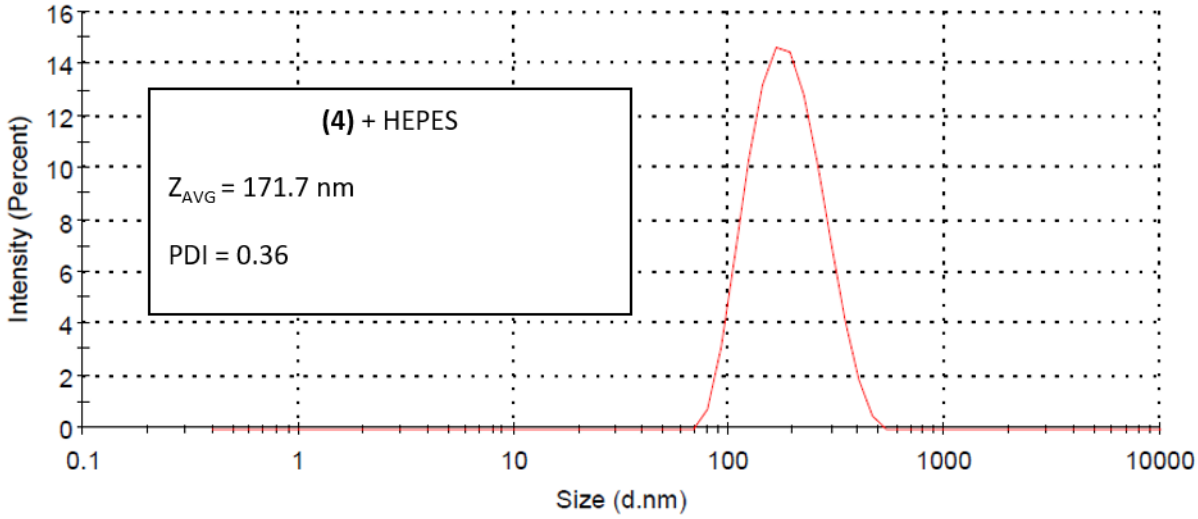

(11) + HEPES
Z_{AVG} = 171.6 nm
PDI = 0.054
Intensity (Percent)
Size (d.nm)

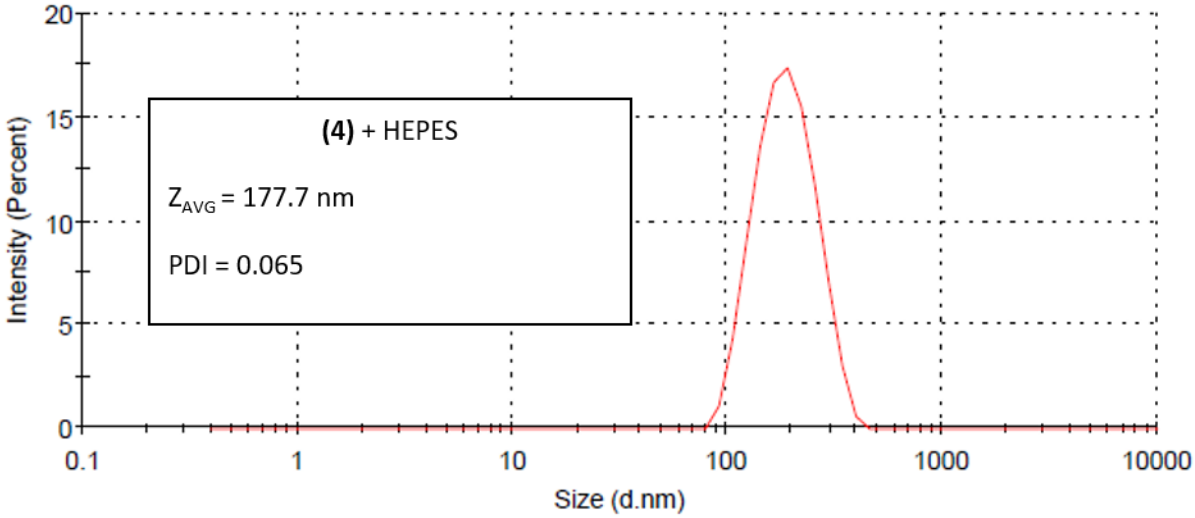
- DLS spectra of neoglycolipids with Concanavalin A

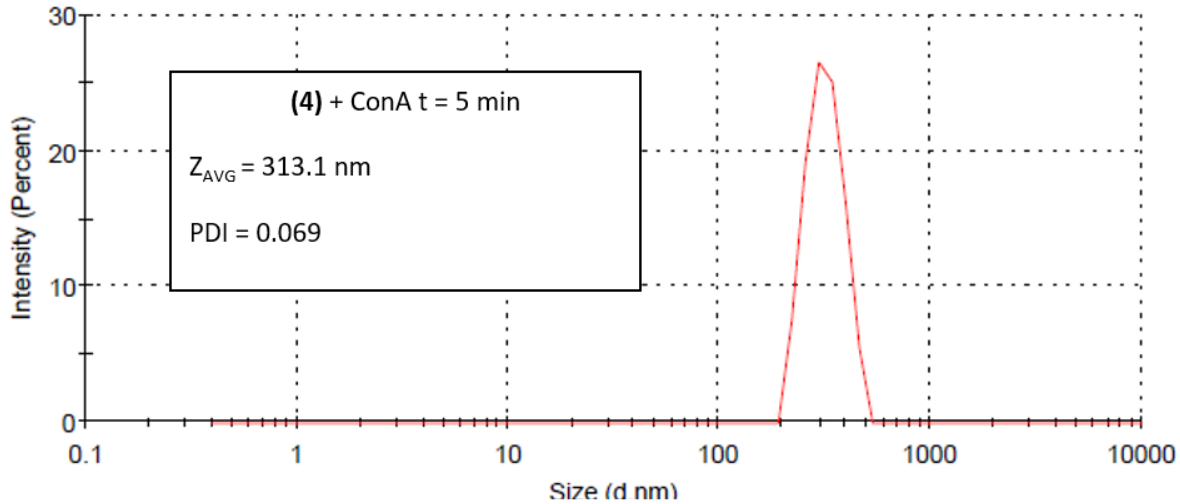
Vesicles were prepared by injection of 50 µL of a 5 mg/mL solution of the neoglycolipid in THF in 1 mL of nanopure water. 100 µ: of a solution of Concanavalin A (1 mg/mL) in HEPES buffer (20 mM in nanopure water) containing $CaCl_2$ (1 mM) and $MnCl_2$ (1 mM) was injected, followed by 5 seconds of vortexing. DLS spectra were recorded after 5, 20 and 30 minutes. Negative controls were recorded using a solution of the HEPES buffer without lectin

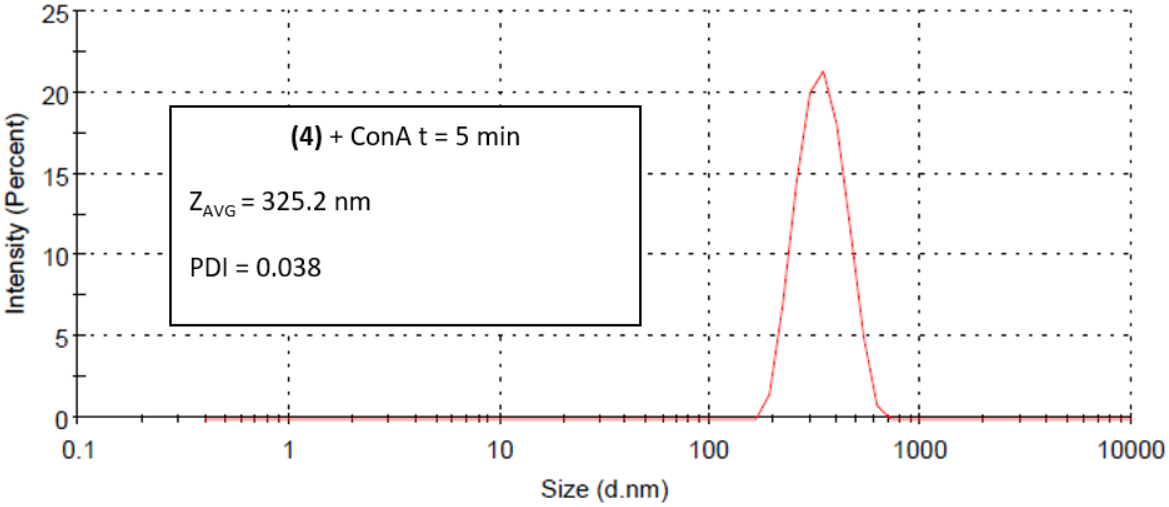


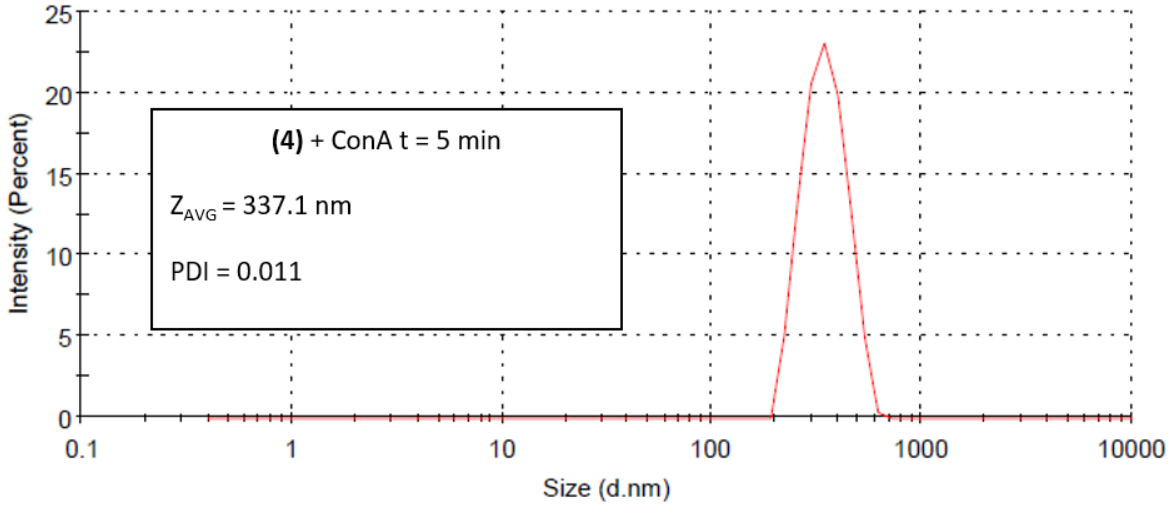

(4) + HEPES
Z_{AVG} = 175.6 nm
PDI = 0.067
Intensity (Percent)
Size (d.nm)

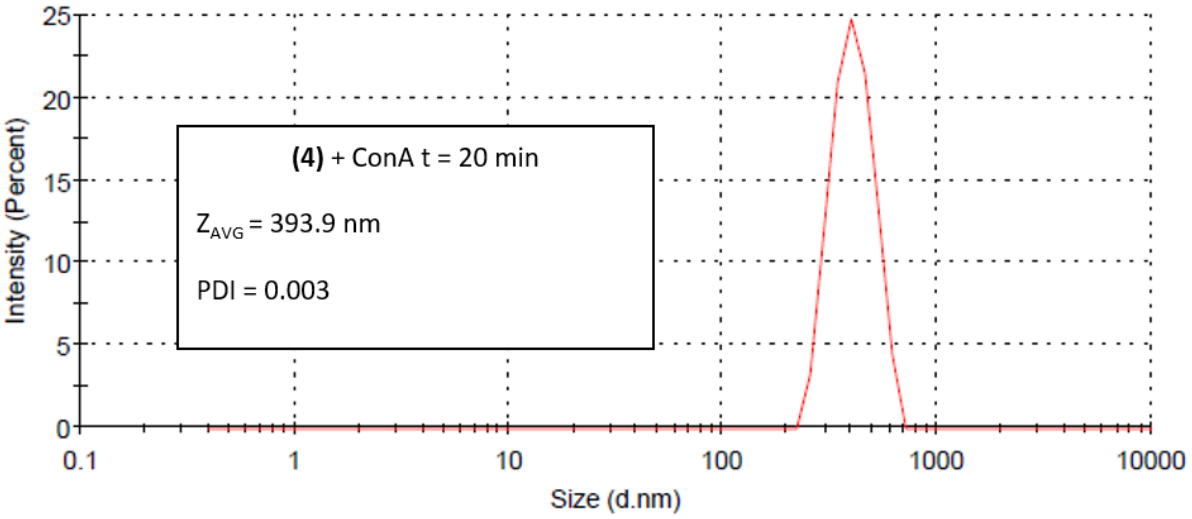

(4) + HEPES
Z_{AVG} = 171.7 nm
PDI = 0.36
Intensity (Percent)
Size (d.nm)

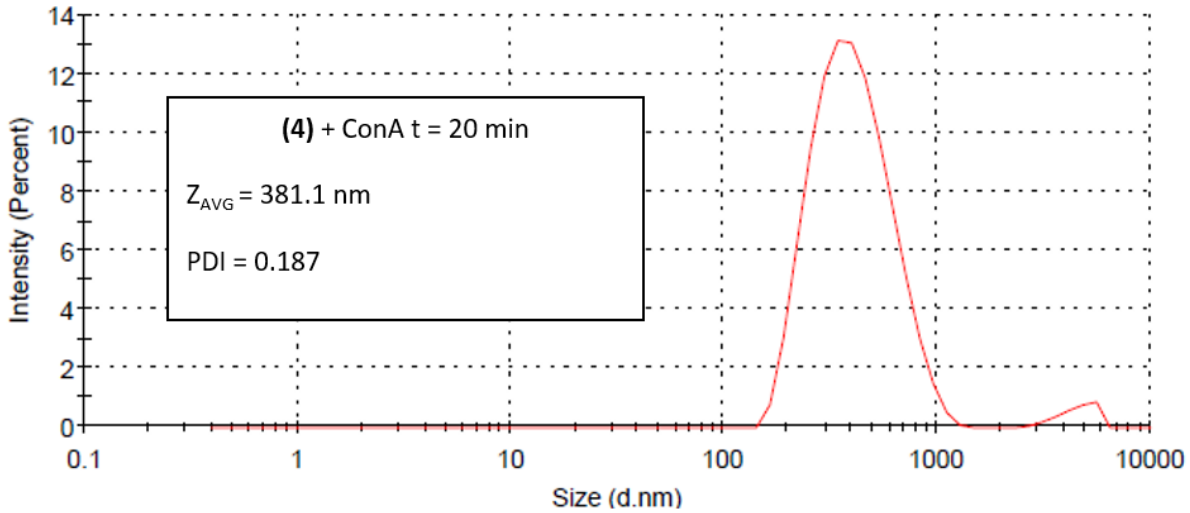

(4) + HEPES
Z_{AVG} = 177.7 nm
PDI = 0.065
Intensity (Percent)
Size (d.nm)

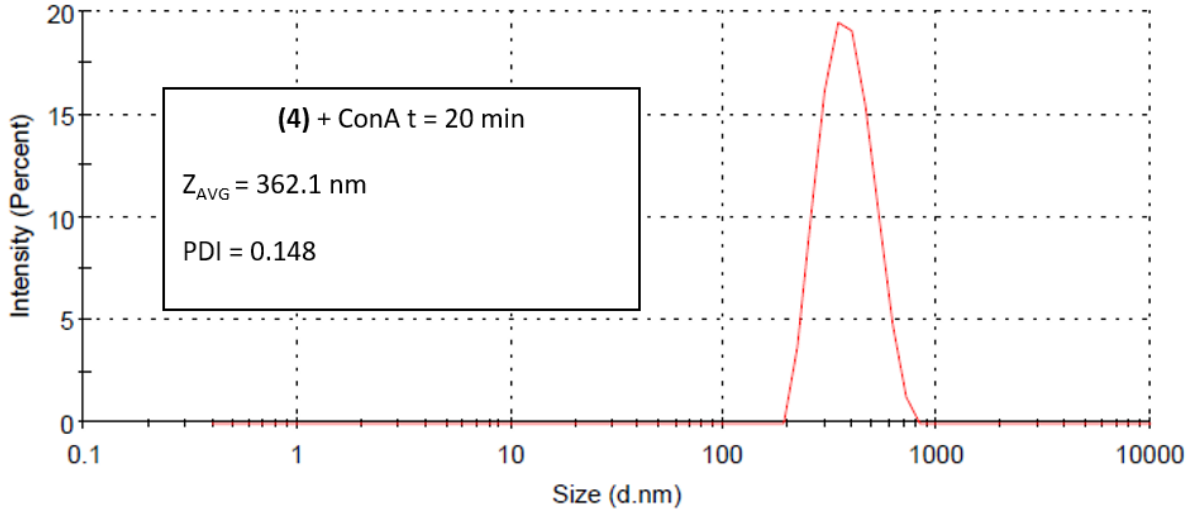

(4) + ConA t = 5 min
Z_{AVG} = 313.1 nm
PDI = 0.069
Intensity (Percent)
Size (d.nm)

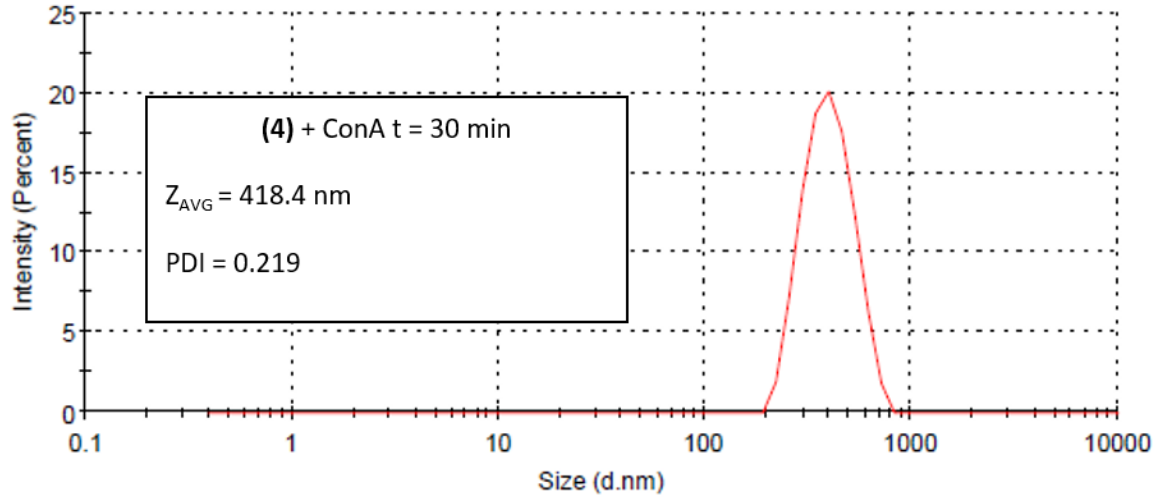

(4) + ConA t = 5 min
Z_{AVG} = 325.2 nm
PDI = 0.038
Intensity (Percent)
Size (d.nm)

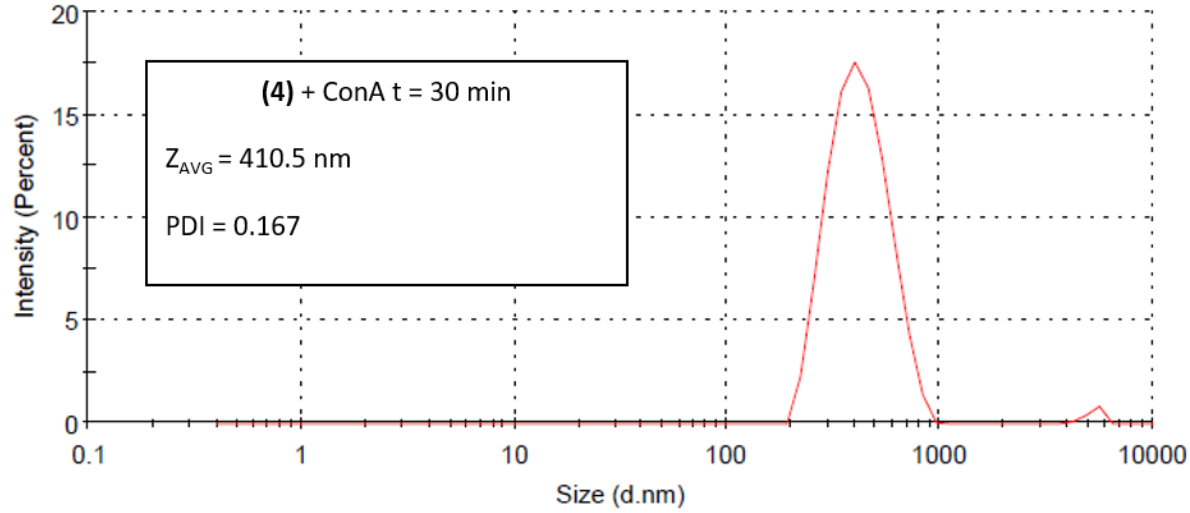

(4) + ConA t = 5 min
Z_{AVG} = 337.1 nm
PDI = 0.011
Intensity (Percent)
Size (d.nm)

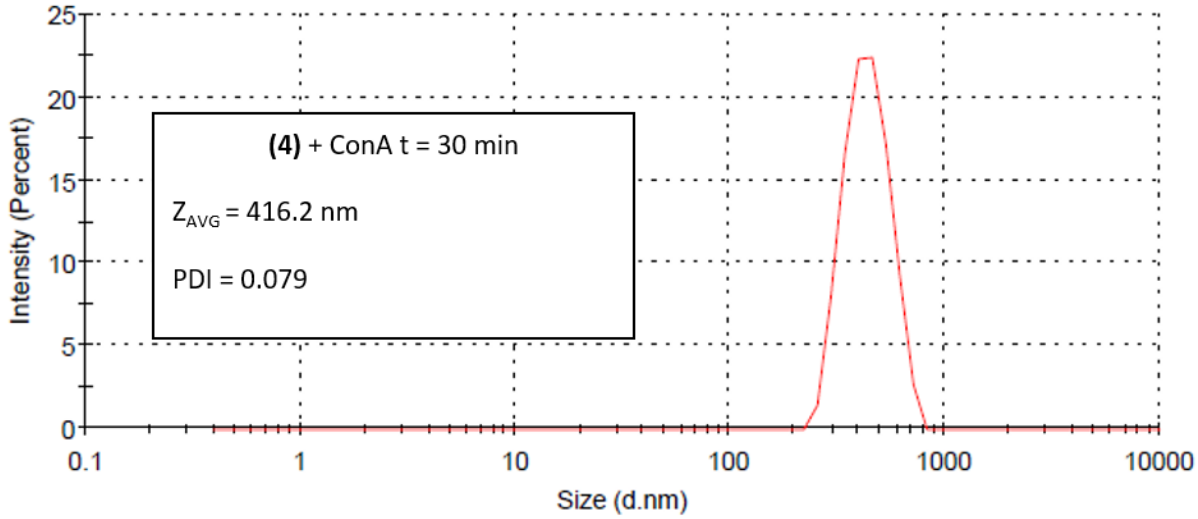

(4) + ConA t = 20 min
Z_{AVG} = 393.9 nm
PDI = 0.003
Intensity (Percent)
Size (d.nm)
0
5
10
15
20
25
0.1
1
10
100
1000
10000

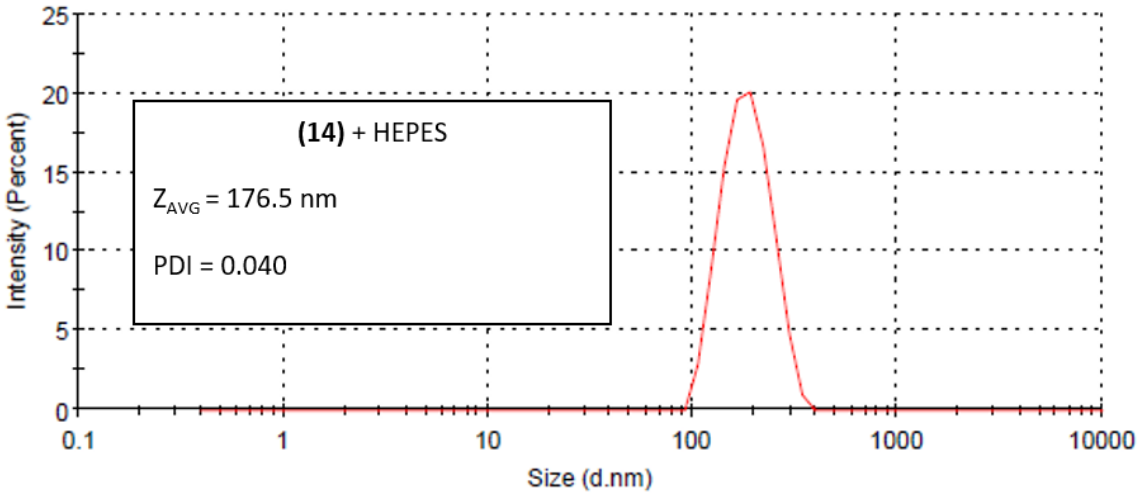

(4) + ConA t = 20 min
Z_{AVG} = 381.1 nm
PDI = 0.187
Intensity (Percent)
Size (d.nm)
0
2
4
6
8
10
12
14
0.1
1
10
100
1000
10000

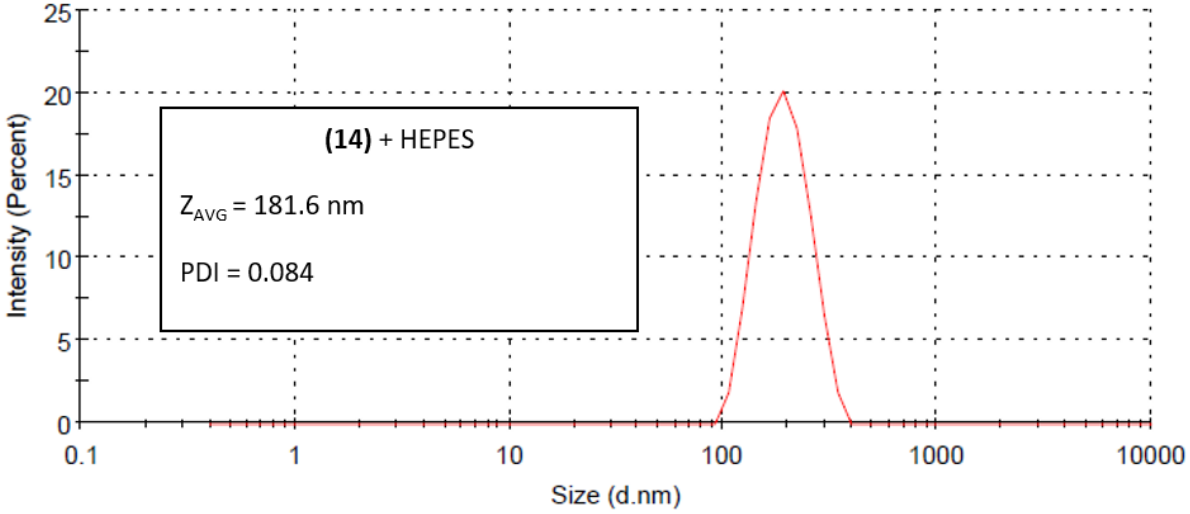

(4) + ConA t = 20 min
Z_{AVG} = 362.1 nm
PDI = 0.148
Intensity (Percent)
Size (d.nm)
0
5
10
15
20
0.1
1
10
100
1000
10000

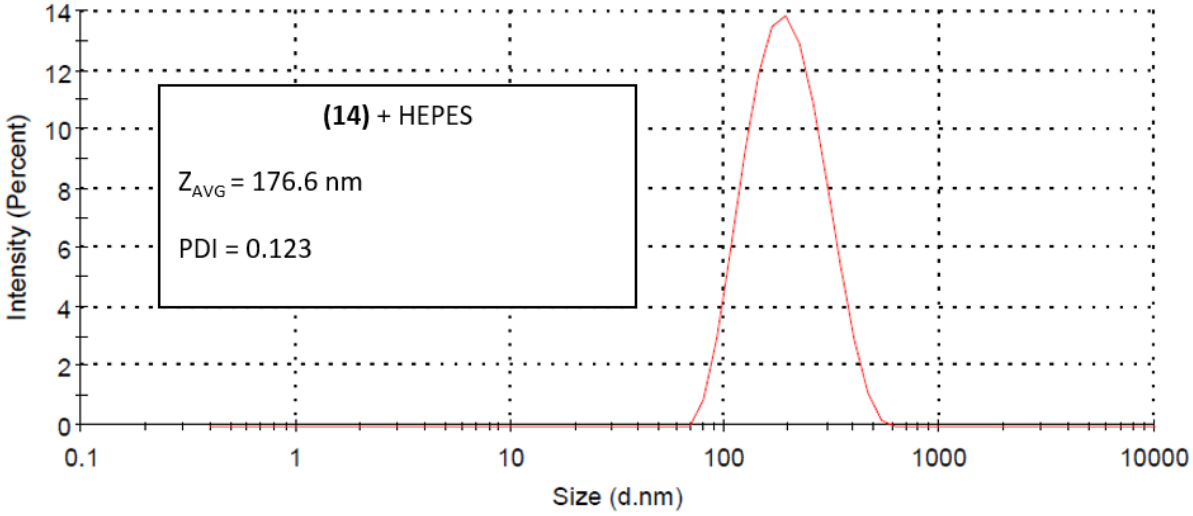

(4) + ConA t = 30 min
Z_{AVG} = 418.4 nm
PDI = 0.219
Intensity (Percent)
Size (d.nm)
0
5
10
15
20
25
0.1
1
10
100
1000
10000

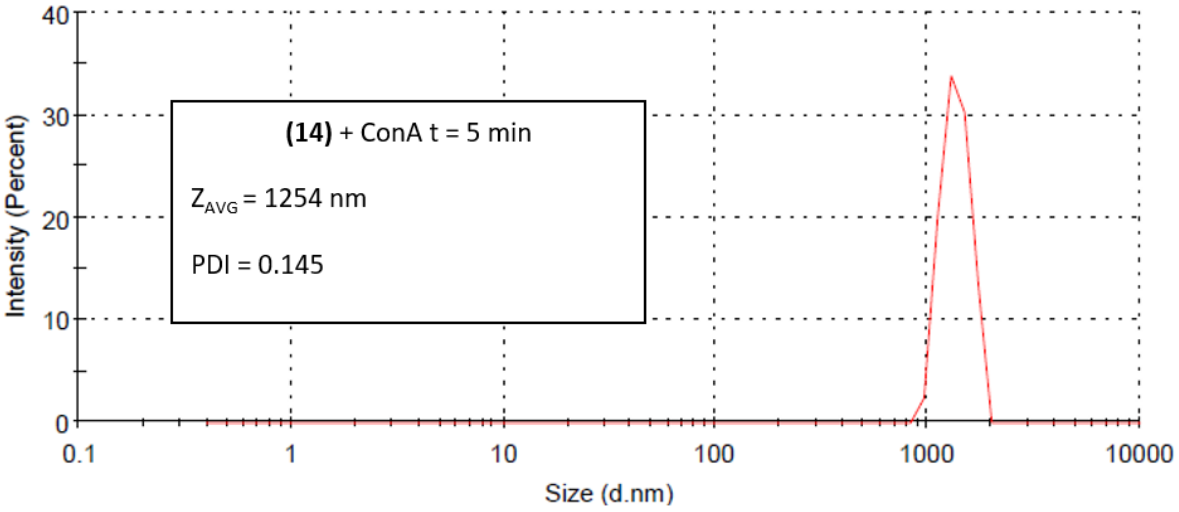

(4) + ConA t = 30 min
Z_{AVG} = 410.5 nm
PDI = 0.167
Intensity (Percent)
Size (d.nm)
0
5
10
15
20
0.1
1
10
100
1000
10000

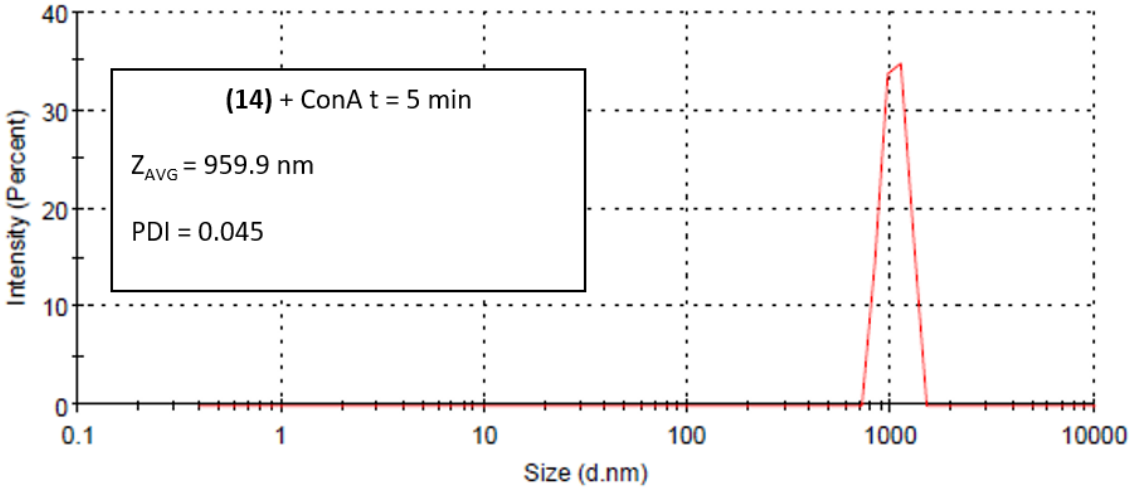

(4) + ConA t = 30 min
Z_{AVG} = 416.2 nm
PDI = 0.079
Intensity (Percent)
Size (d.nm)
0
5
10
15
20
25
0.1
1
10
100
1000
10000

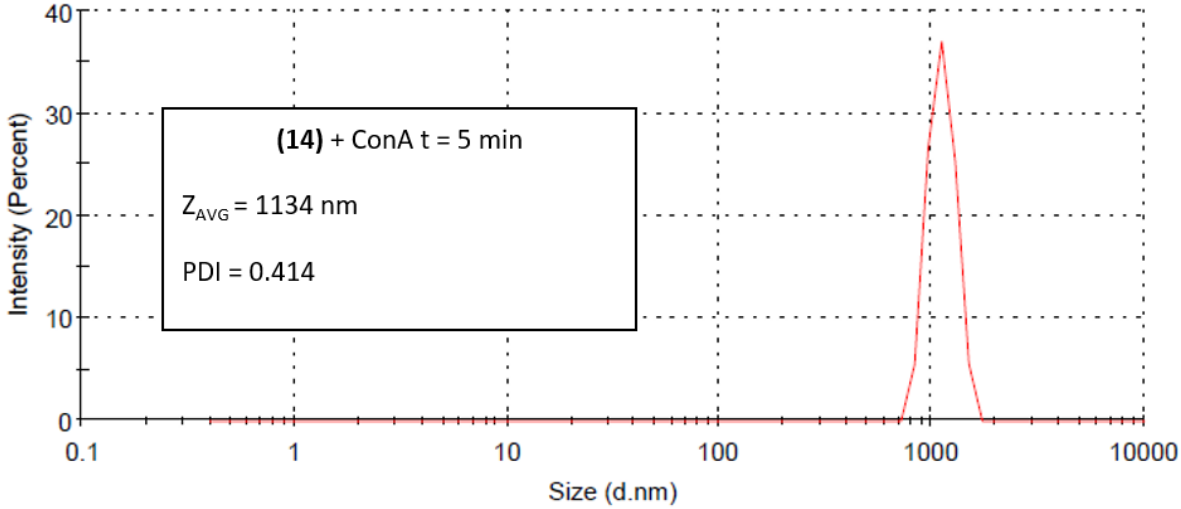

(14) + HEPES
Z_{AVG} = 176.5 nm
PDI = 0.040
Intensity (Percent)
Size (d.nm)
0
5
10
15
20
25
0.1
1
10
100
1000
10000

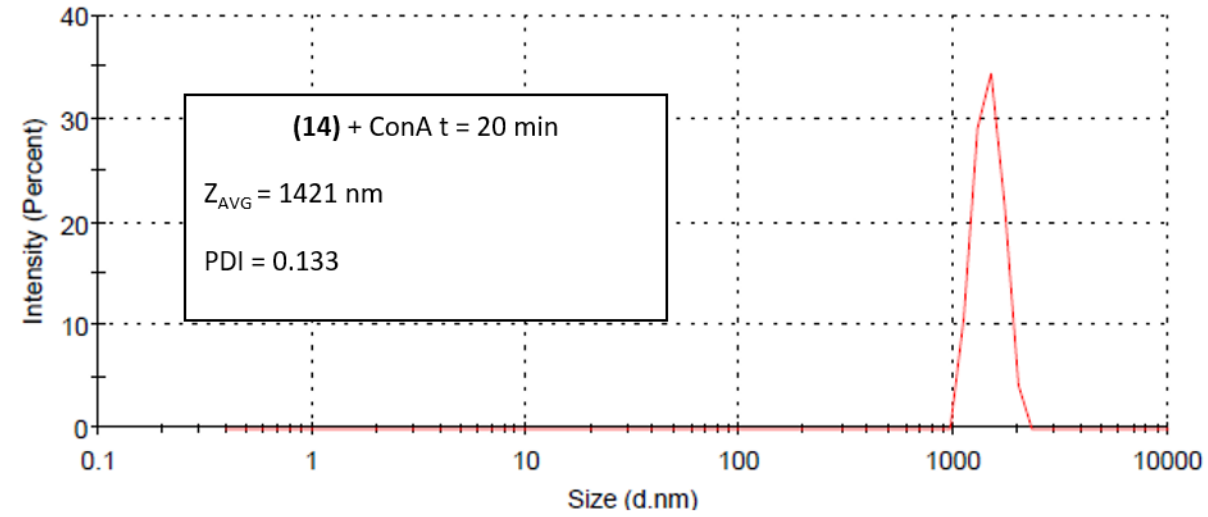

(14) + HEPES
Z_{AVG} = 181.6 nm
PDI = 0.084
Intensity (Percent)
Size (d.nm)
0
5
10
15
20
25
0.1
1
10
100
1000
10000

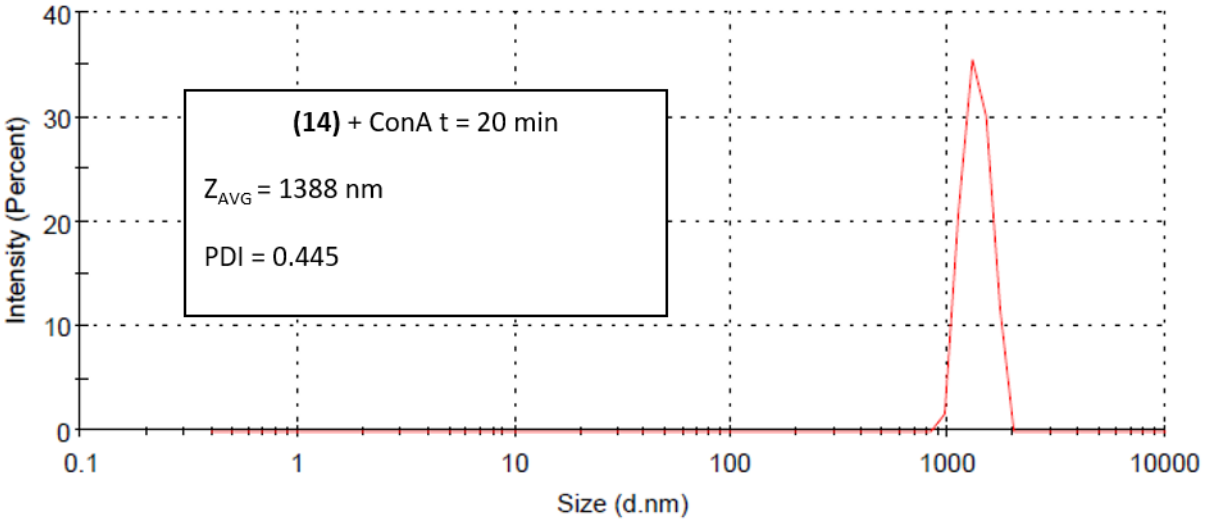

(14) + HEPES
Z_{AVG} = 176.6 nm
PDI = 0.123
Intensity (Percent)
Size (d.nm)
0
2
4
6
8
10
12
14
0.1
1
10
100
1000
10000

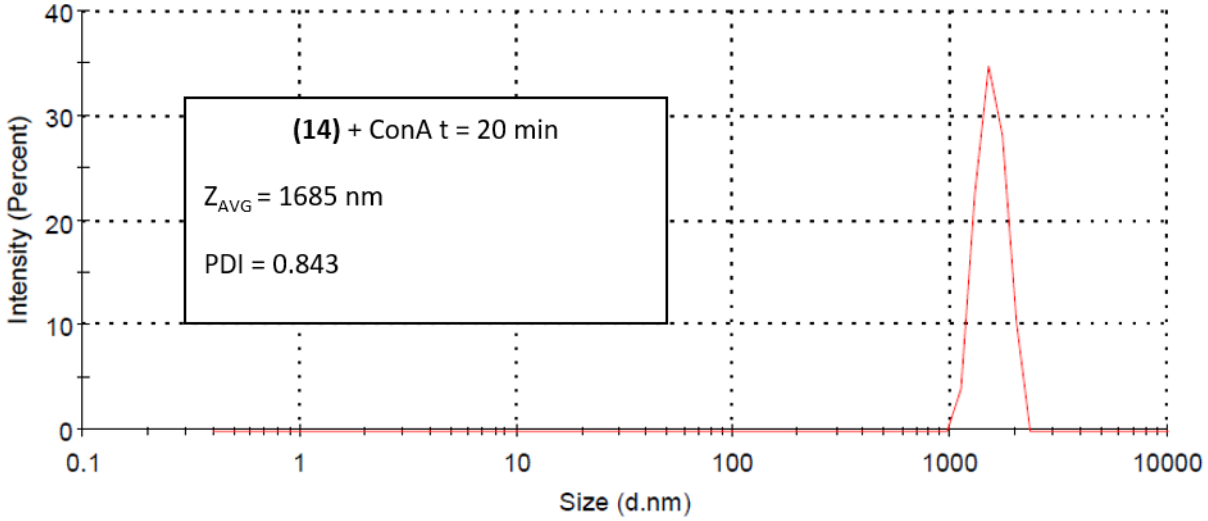

(14) + ConA t = 5 min
Z_{AVG} = 1254 nm
PDI = 0.145
Intensity (Percent)
Size (d.nm)
0
10
20
30
40
0.1
1
10
100
1000
10000


(14) + ConA t = 5 min
Z_{AVG} = 959.9 nm
PDI = 0.045
Intensity (Percent)
Size (d.nm)
0
10
20
30
40
0.1
1
10
100
1000
10000

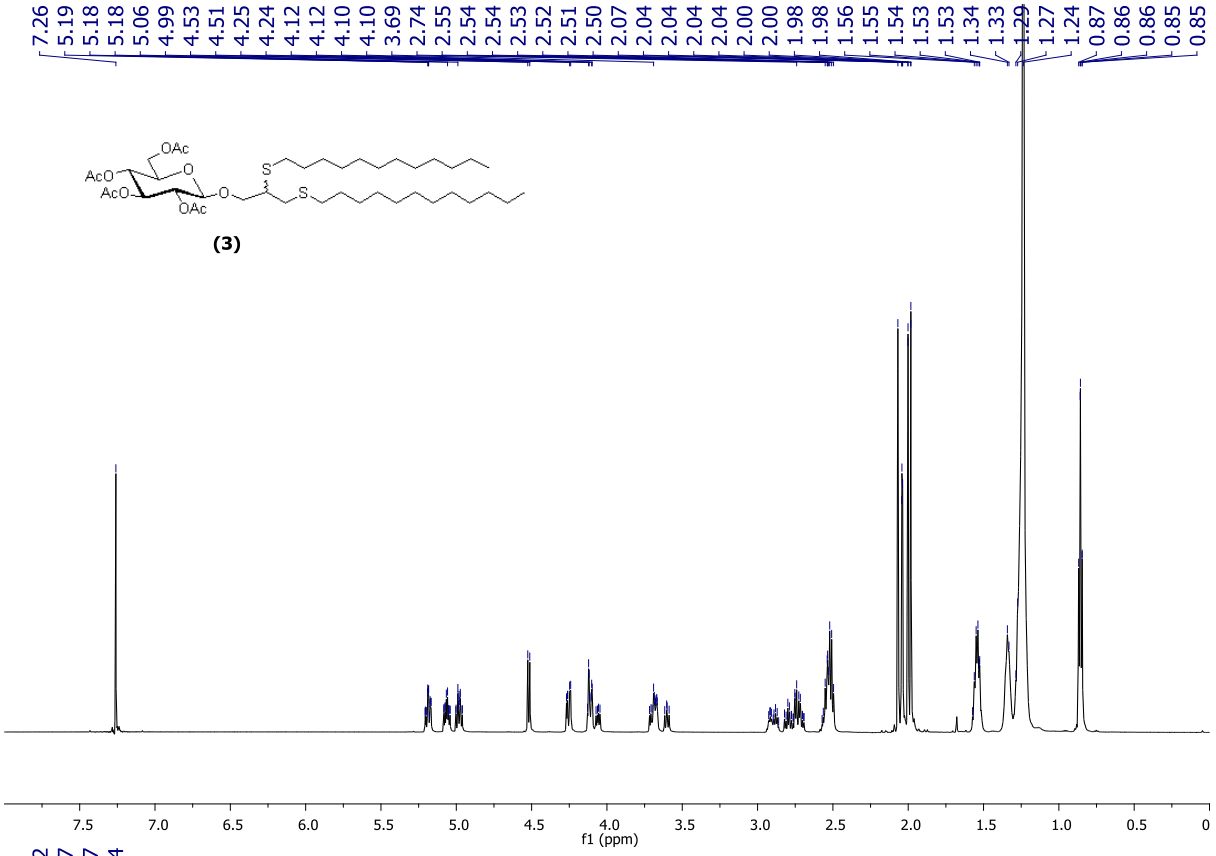

(14) + ConA t = 5 min
Z_{AVG} = 1134 nm
PDI = 0.414
Intensity (Percent)
Size (d.nm)
0
10
20
30
40
0.1
1
10
100
1000
10000

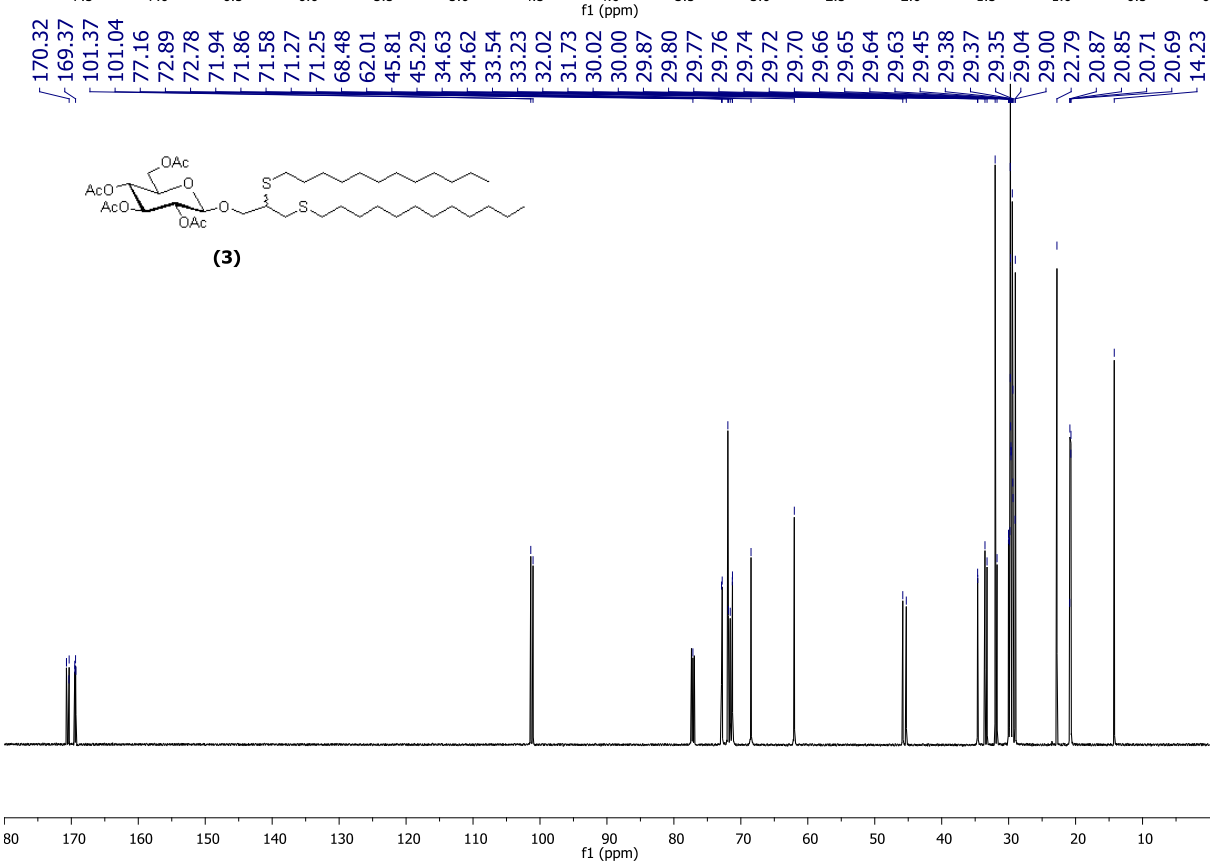

(14) + ConA t = 20 min
Z_{AVG} = 1421 nm
PDI = 0.133
Intensity (Percent)
Size (d.nm)
0
10
20
30
40
0.1
1
10
100
1000
10000

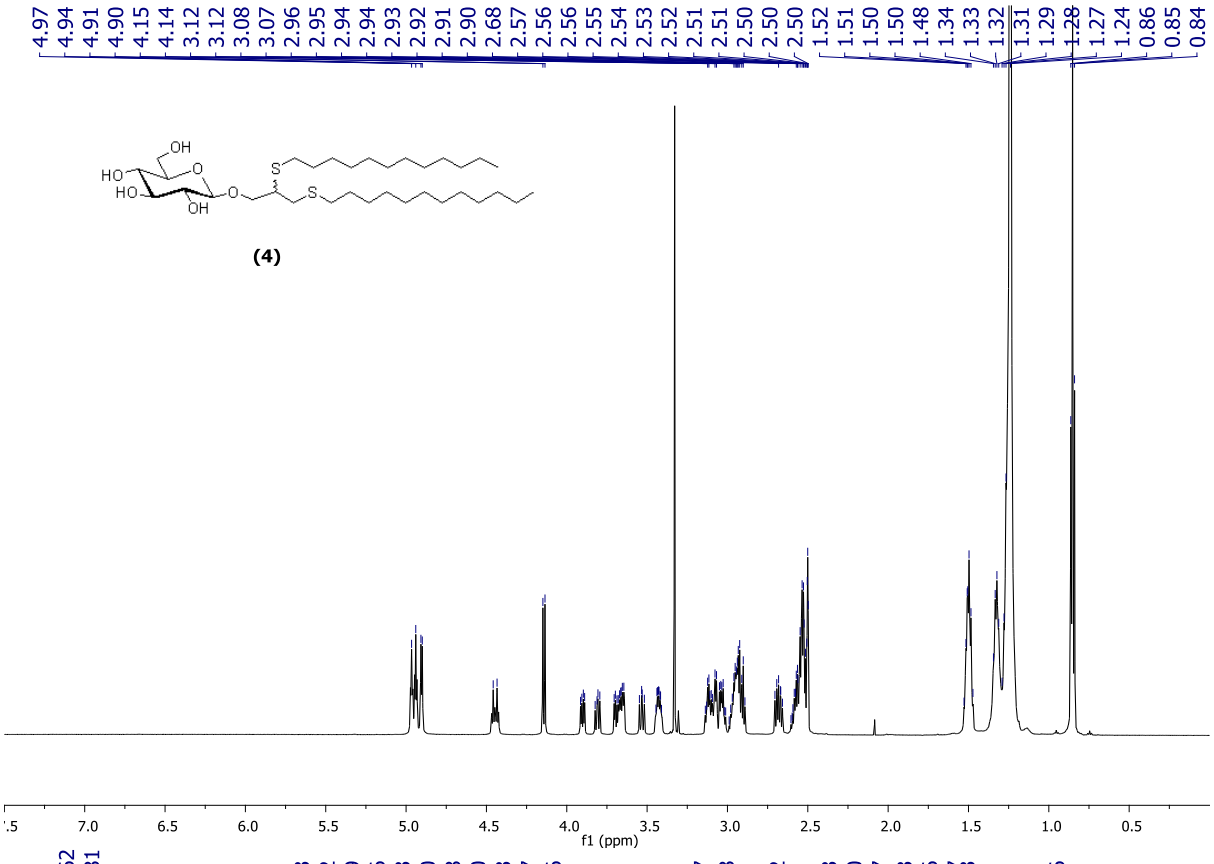

(14) + ConA t = 20 min
Z_{AVG} = 1388 nm
PDI = 0.445
Intensity (Percent)
Size (d.nm)
0
10
20
30
40
0.1
1
10
100
1000
10000

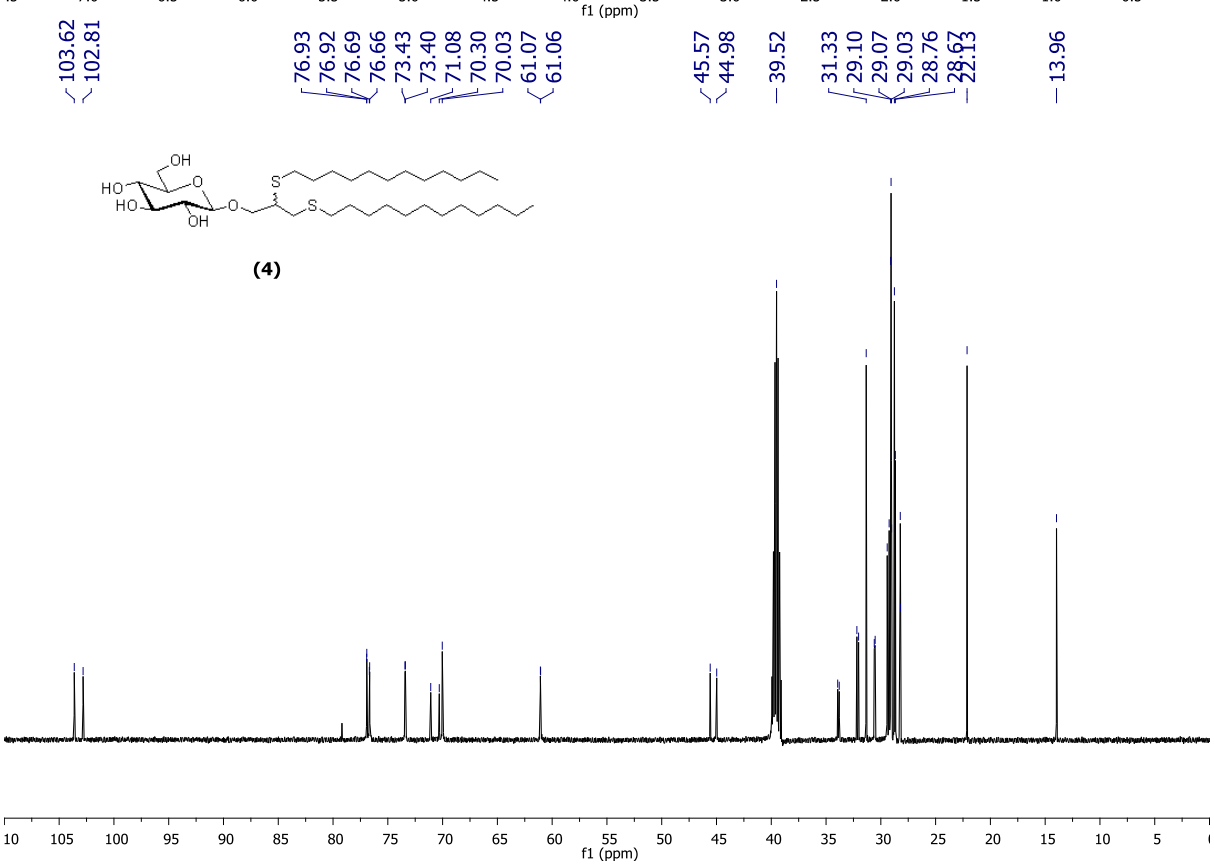

(14) + ConA t = 20 min
Z_{AVG} = 1685 nm
PDI = 0.843
Intensity (Percent)
Size (d.nm)
0
10
20
30
40
0.1
1
10
100
1000
10000

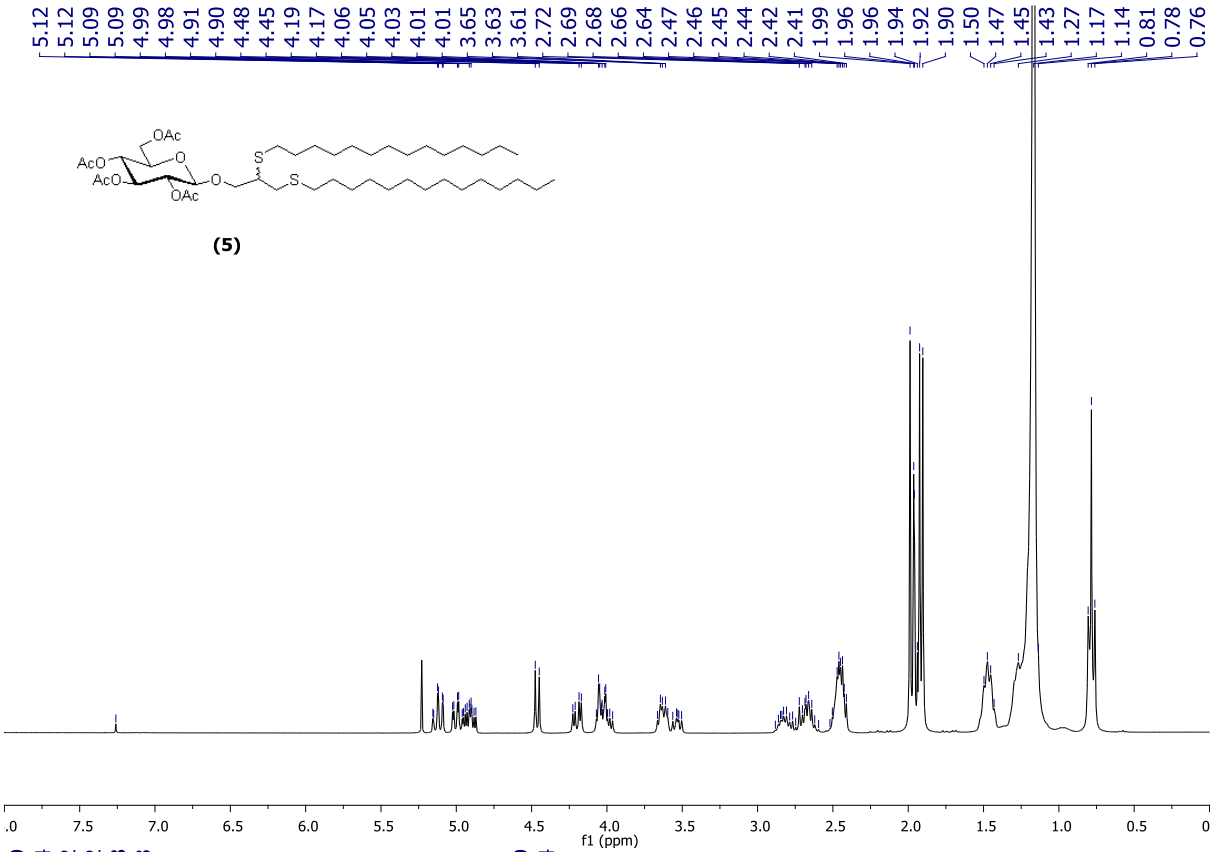
6 ^{1}H and ^{13}C NMR spectra

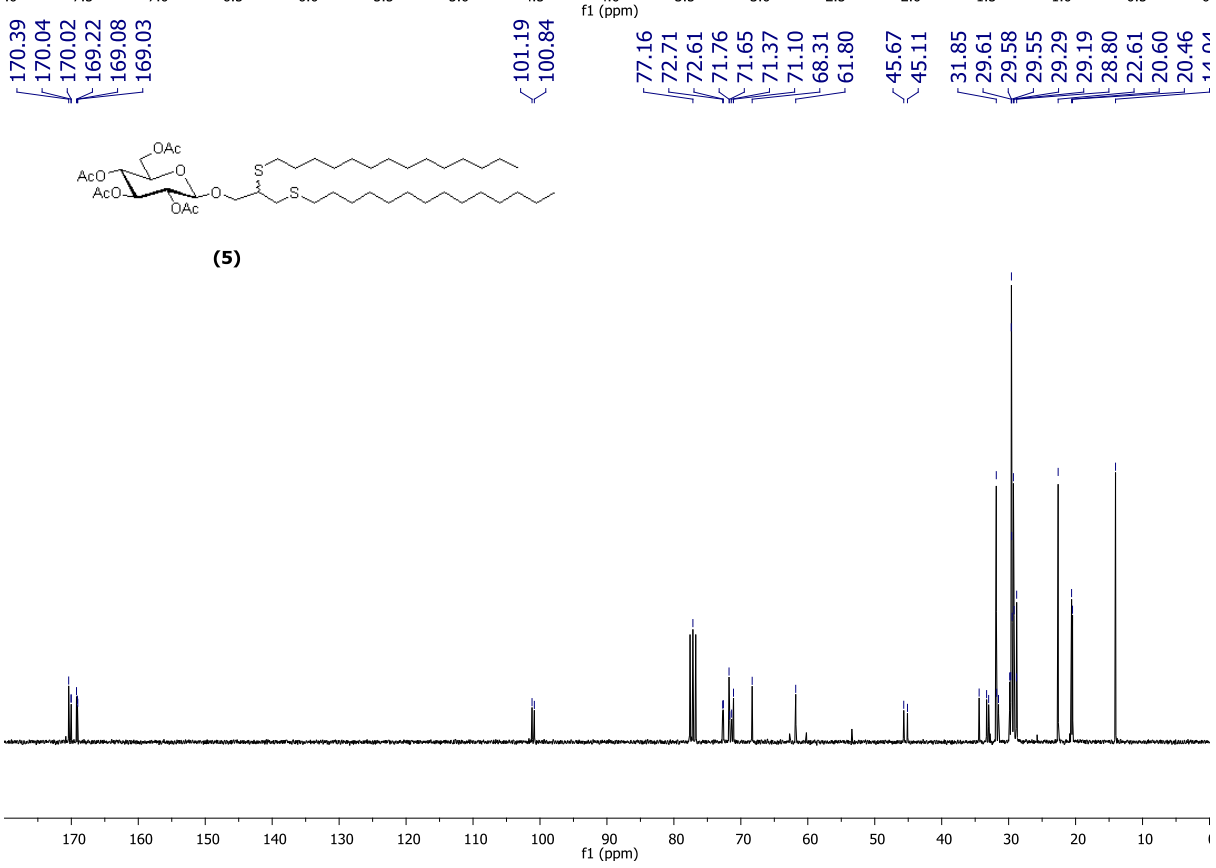


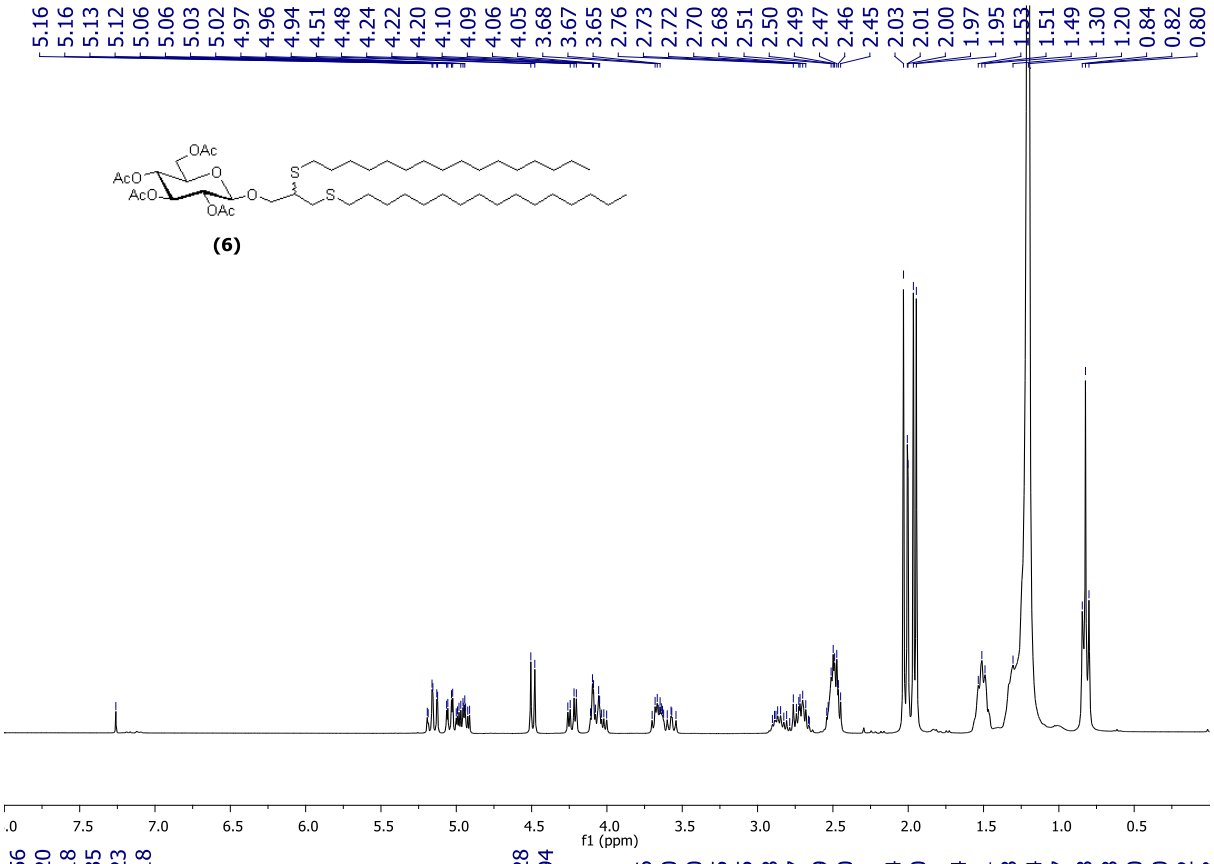


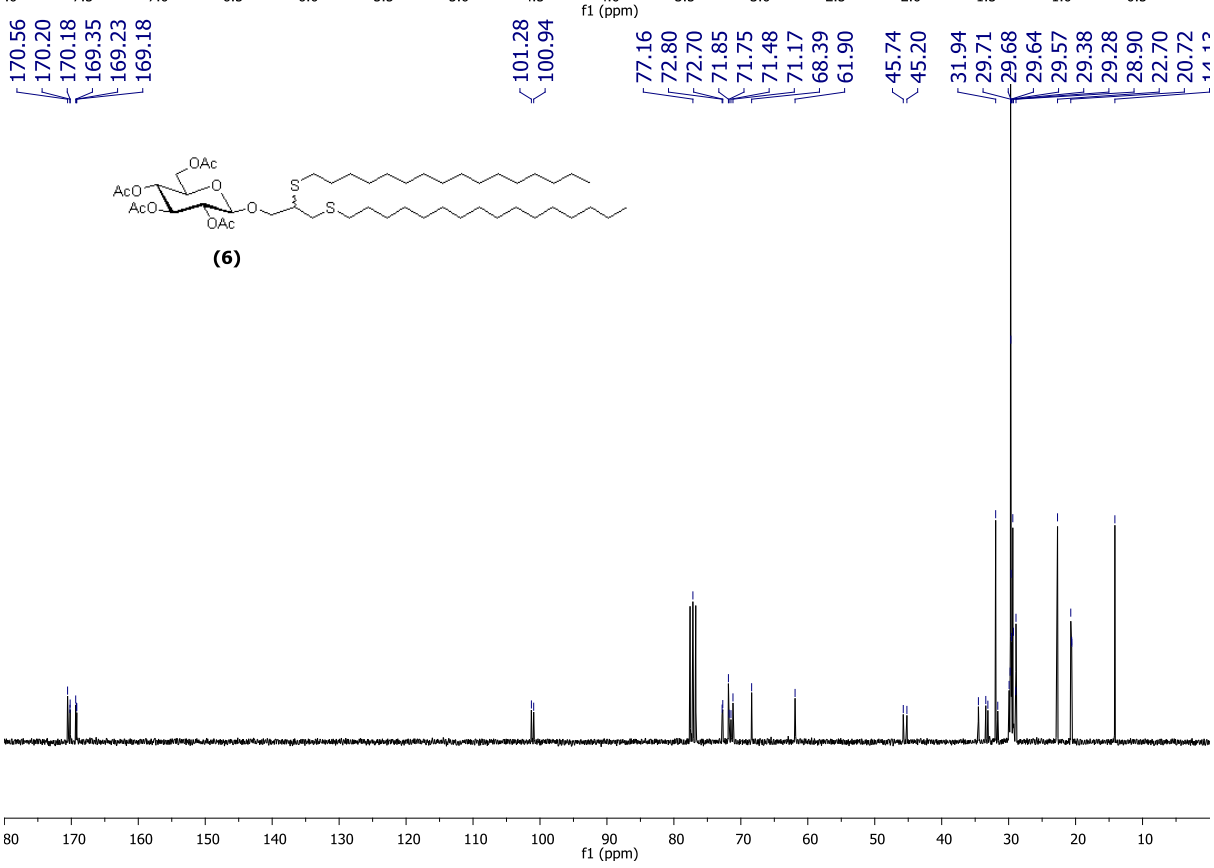

4.97 4.94 4.91 4.90 4.15 4.14 3.12 3.12 3.08 3.07 2.96 2.95 2.94 2.94 2.93 2.92 2.91 2.90 2.68 2.57 2.56 2.56 2.55 2.54 2.53 2.52 2.51 2.51 2.50 2.50 2.50 1.52 1.51 1.50 1.50 1.48 1.34 1.33 1.32 1.31 1.29 1.28 1.27 1.24 0.86 0.85 0.84
OH
HO
HO
O
OH
O
S
S
(4)
f1 (ppm)

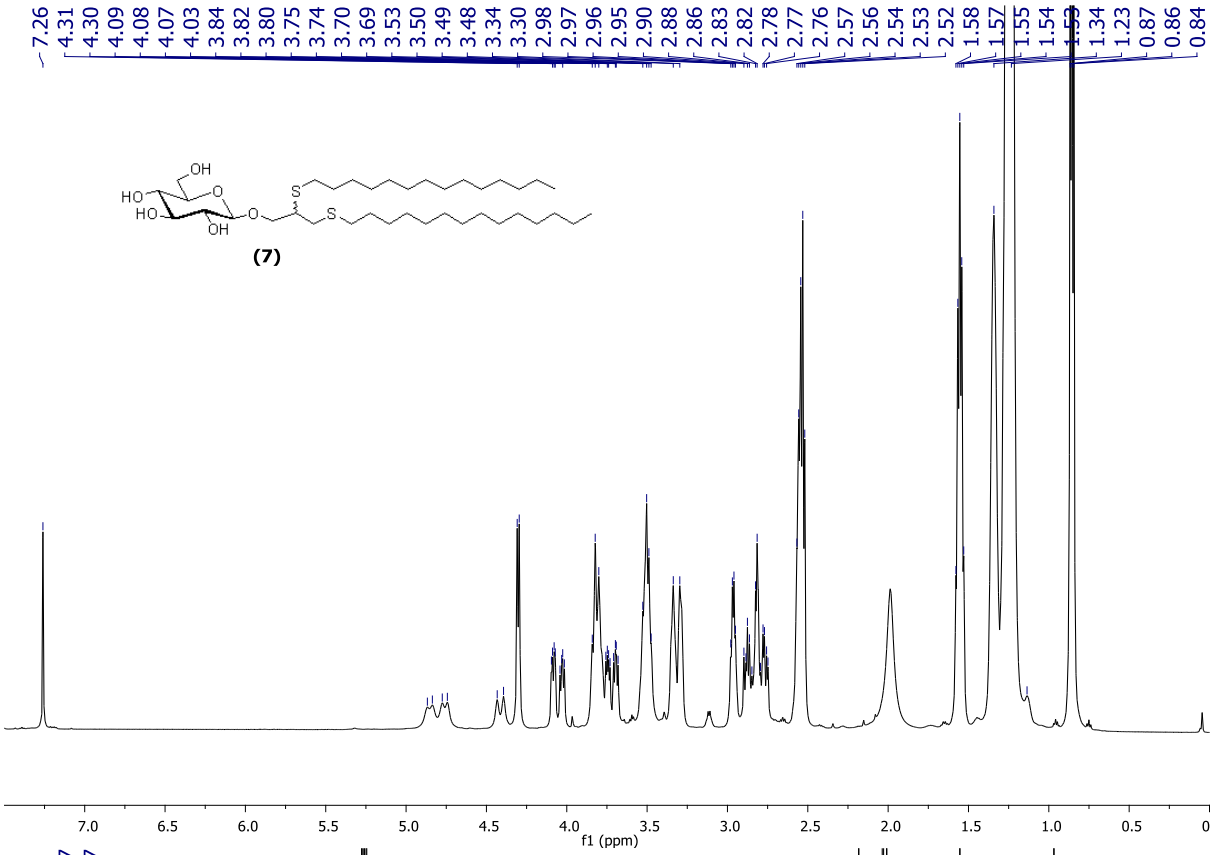

103.62 102.81 76.93 76.92 76.69 76.66 73.43 73.40 71.08 70.30 70.03 61.07 61.06 45.57 44.98 39.52 31.33 29.10 29.07 29.03 28.76 28.67 22.13 13.96
OH
HO
HO
O
OH
O
S
S
(4)
f1 (ppm)

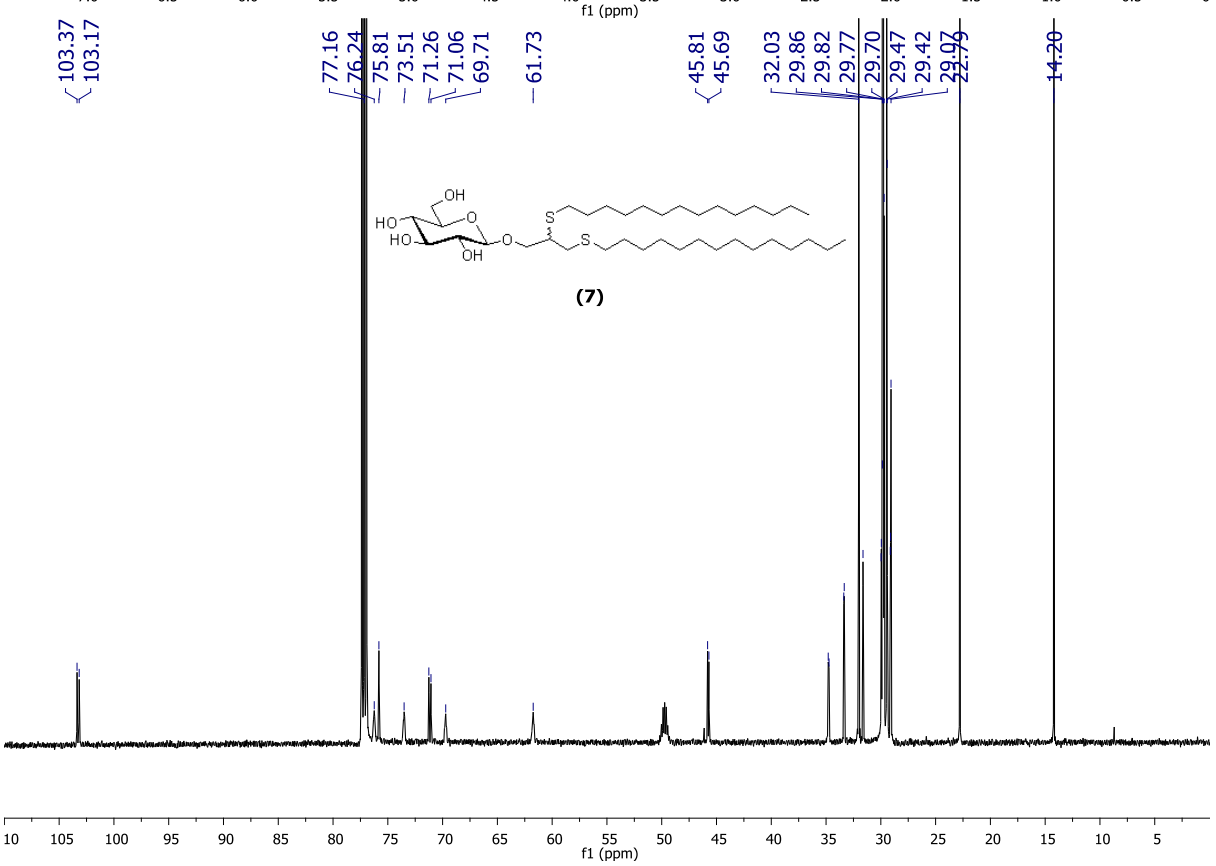

5.12 5.12 5.09 5.09 4.99 4.98 4.91 4.90 4.48 4.45 4.19 4.17 4.06 4.05 4.03 4.01 4.01 3.65 3.63 3.61 2.72 2.69 2.68 2.66 2.64 2.47 2.46 2.45 2.44 2.42 2.41 1.99 1.96 1.96 1.94 1.92 1.90 1.50 1.47 1.45 1.43 1.27 1.17 1.14 0.81 0.78 0.76
OAc
AcO
AcO
O
OAc
O
S
S
(5)
.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
f1 (ppm)

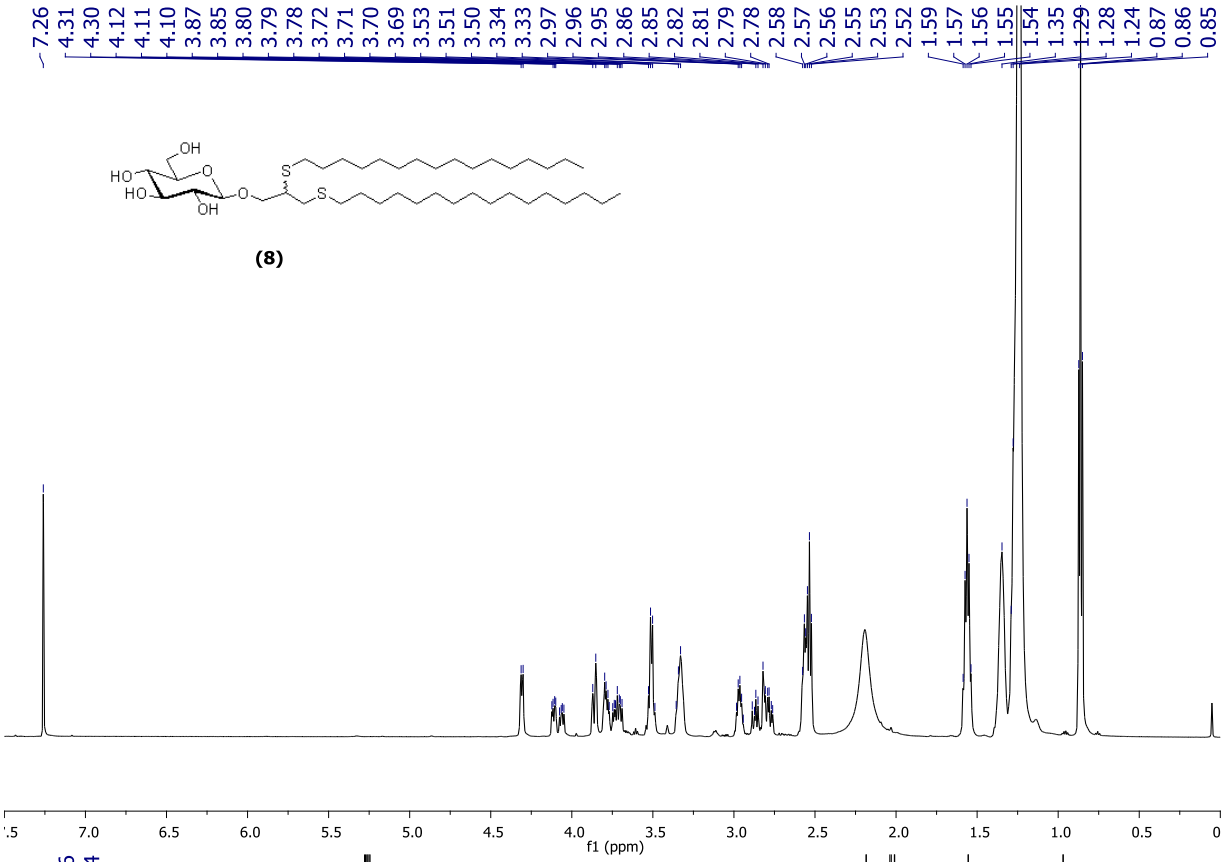

170.39 170.04 170.02 169.22 169.08 169.03
101.19 100.84
77.16 72.71 72.61 71.76 71.65 71.37 71.10 68.31 61.80
45.67 45.11
31.85 29.61 29.58 29.55 29.29 29.19 28.80 22.61 20.60 20.46
OAc
AcO
AcO
O
OAc
O
S
S
(5)
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

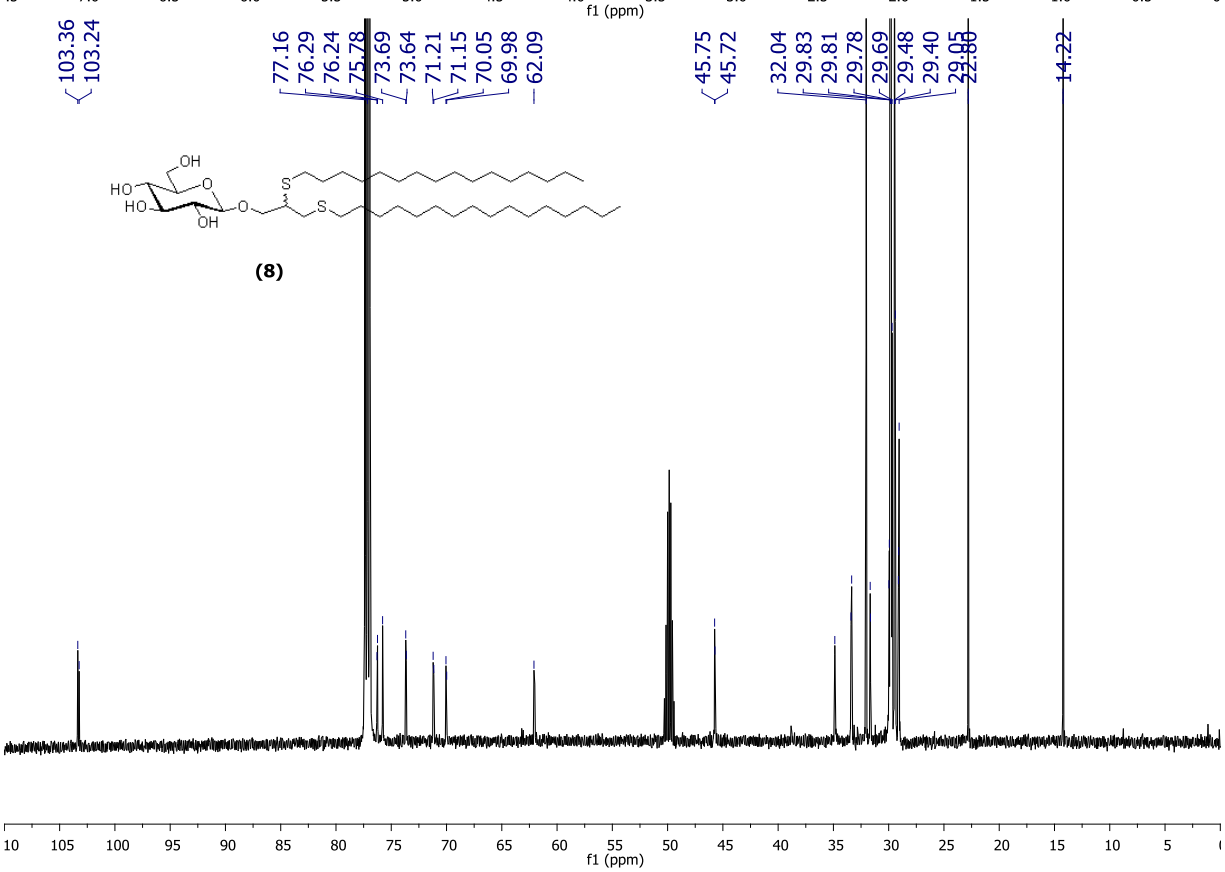

5.16
5.16
5.13
5.12
5.06
5.06
5.03
5.02
4.97
4.96
4.94
4.51
4.48
4.24
4.22
4.20
4.10
4.09
4.06
4.05
3.68
3.67
3.65
2.76
2.73
2.72
2.70
2.68
2.51
2.50
2.49
2.47
2.46
2.45
2.03
2.01
2.00
1.97
1.95
1.53
1.51
1.49
1.30
1.20
0.84
0.82
0.80
OAc
AcO
AcO
O
OAc
O
S
S
(6)
f1 (ppm)

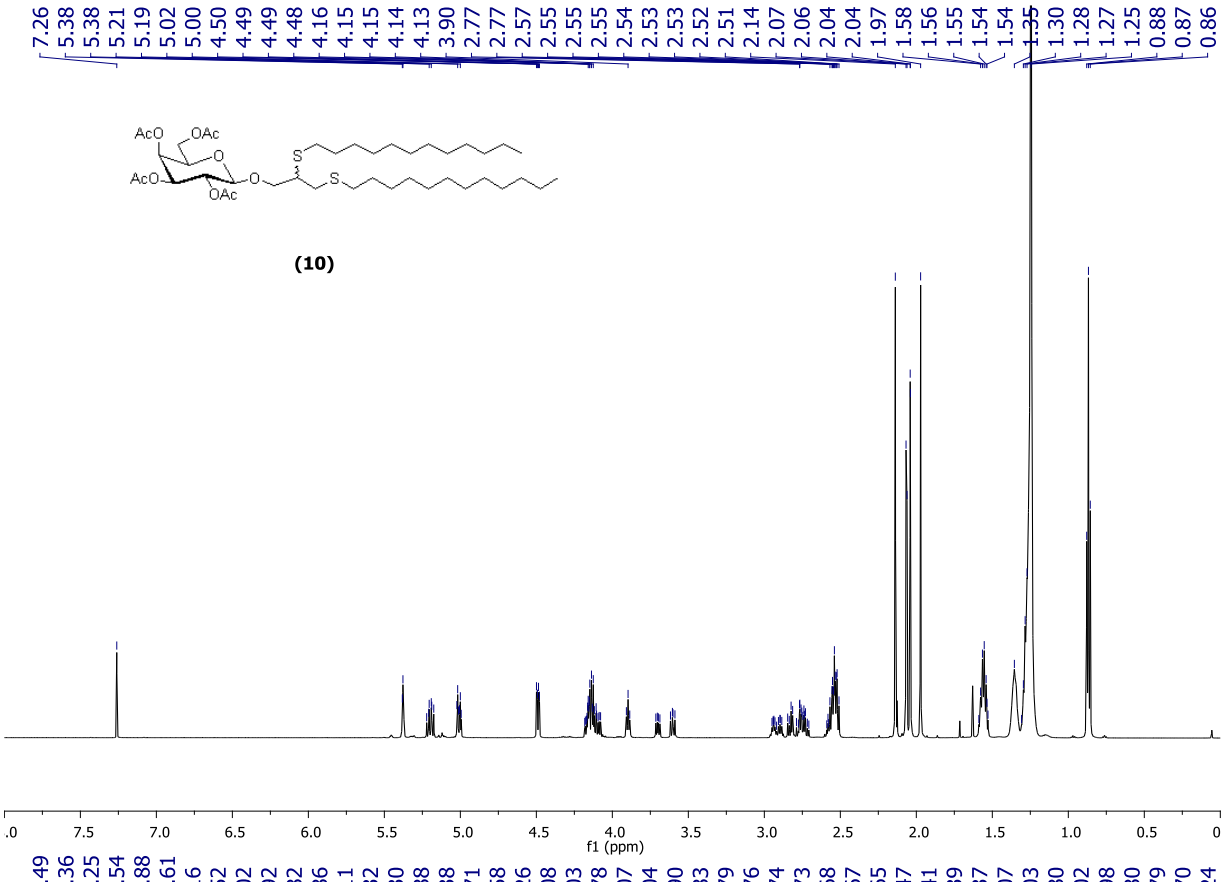

170.56
170.20
170.18
169.35
169.23
169.18
101.28
100.94
77.16
72.80
72.70
71.85
71.75
71.48
71.17
68.39
61.90
45.74
45.20
31.94
29.71
29.68
29.64
29.57
29.38
29.28
28.90
22.70
20.72
OAc
AcO
AcO
O
OAc
O
S
S
(6)
f1 (ppm)

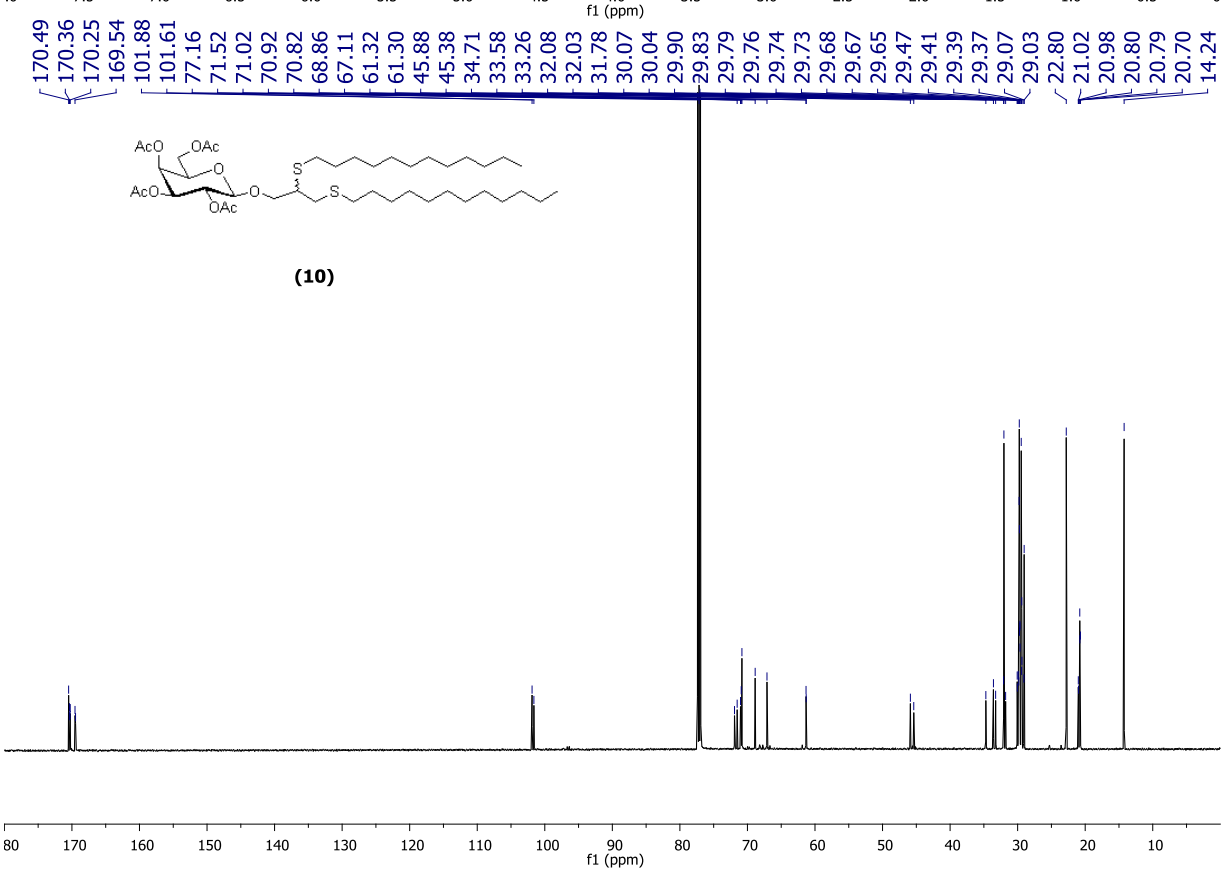

7.26
4.31
4.30
4.09
4.08
4.07
4.03
3.84
3.82
3.80
3.75
3.74
3.70
3.69
3.53
3.50
3.49
3.48
3.34
3.30
2.98
2.97
2.96
2.95
2.90
2.88
2.86
2.83
2.82
2.78
2.77
2.76
2.57
2.56
2.54
2.53
2.52
1.58
1.57
1.55
1.54
1.53
1.34
1.23
0.87
0.86
0.84
OH
HO
HO
O
OH
O
S
S
(7)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
f1 (ppm)

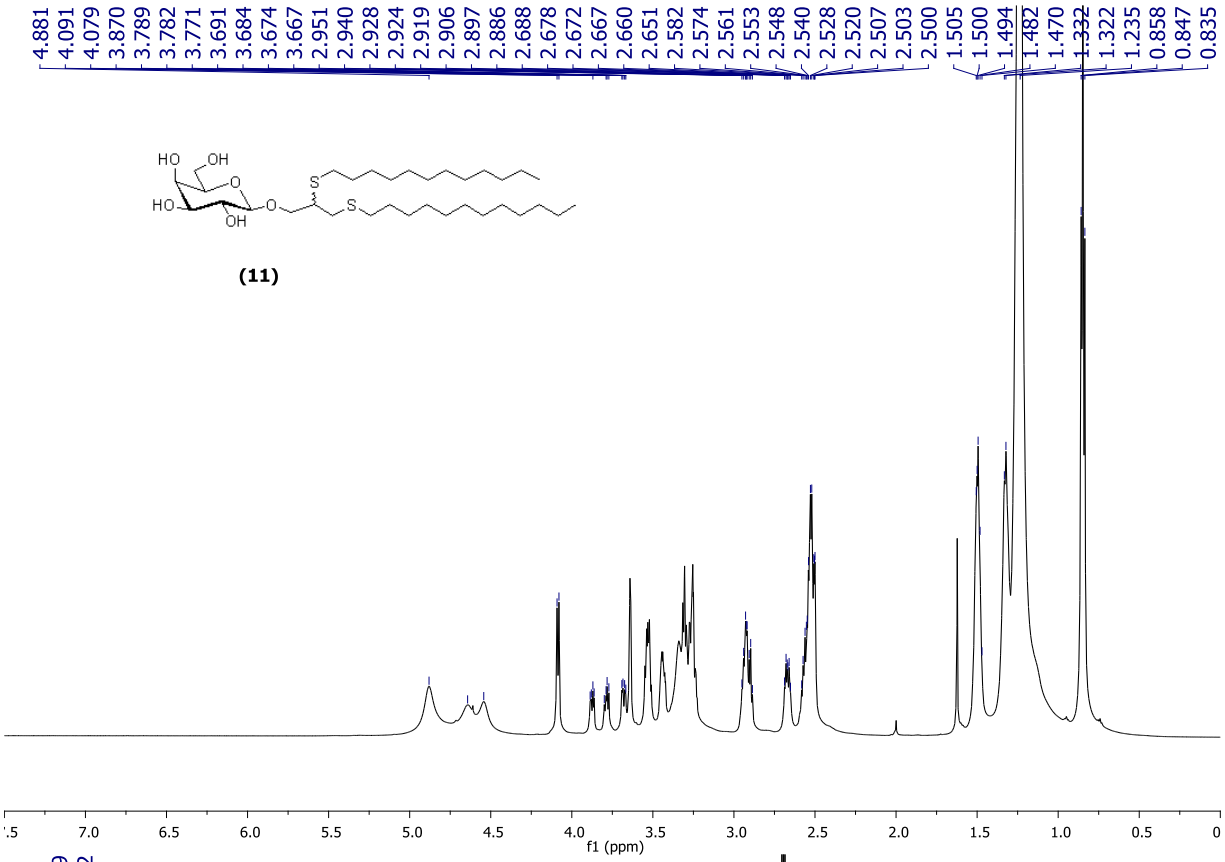

103.37
103.17
77.16
76.24
75.81
73.51
71.26
71.06
69.71
61.73
45.81
45.69
32.03
29.86
29.82
29.77
29.70
29.47
29.42
29.07
22.96
14.20
OH
HO
HO
O
OH
O
S
S
(7)
10
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
f1 (ppm)


7.26
4.31
4.30
4.12
4.11
4.10
3.87
3.85
3.80
3.79
3.78
3.72
3.71
3.70
3.69
3.53
3.51
3.50
3.34
3.33
2.97
2.96
2.95
2.86
2.85
2.82
2.81
2.79
2.78
2.58
2.57
2.56
2.55
2.53
2.52
1.59
1.57
1.56
1.55
1.54
1.35
1.29
1.28
1.24
0.87
0.86
0.85
OH
HO
HO
O
OH
O
S
S
(8)
f1 (ppm)

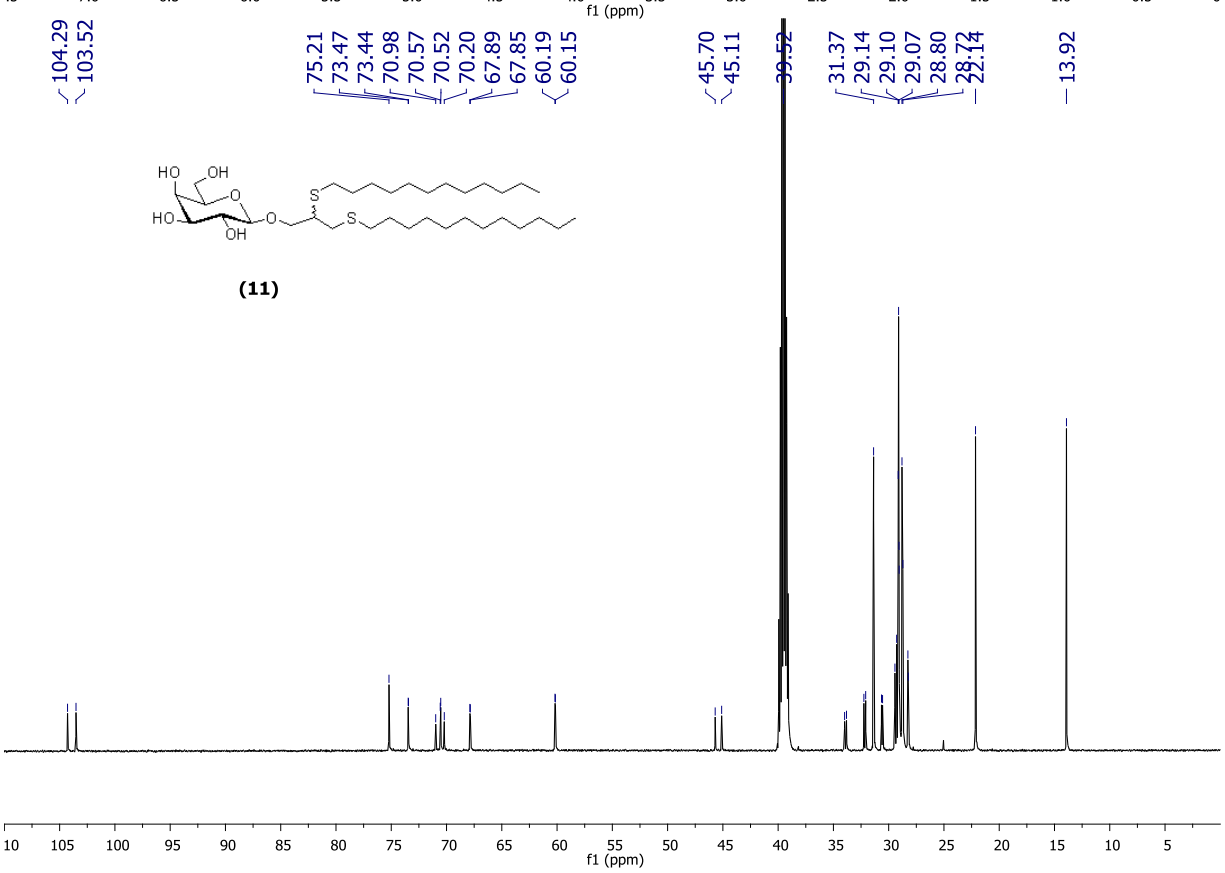

103.36
103.24
77.16
76.29
76.24
75.78
73.69
73.64
71.21
71.15
70.05
69.98
62.09
45.75
45.72
32.04
29.83
29.81
29.78
29.69
29.48
29.40
29.05
22.80
14.22
OH
HO
HO
O
OH
O
S
S
(8)
f1 (ppm)


7.26 5.38 5.38 5.21 5.19 5.02 5.00 4.50 4.49 4.49 4.48 4.16 4.15 4.15 4.14 4.13 3.90 2.77 2.77 2.57 2.55 2.55 2.55 2.54 2.53 2.53 2.52 2.51 2.14 2.07 2.06 2.04 2.04 1.97 1.58 1.56 1.55 1.54 1.54 1.35 1.30 1.28 1.27 1.25 0.88 0.87 0.86
AcO
OAc
O
AcO
OAc
O
S
S
(10)
.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
f1 (ppm)

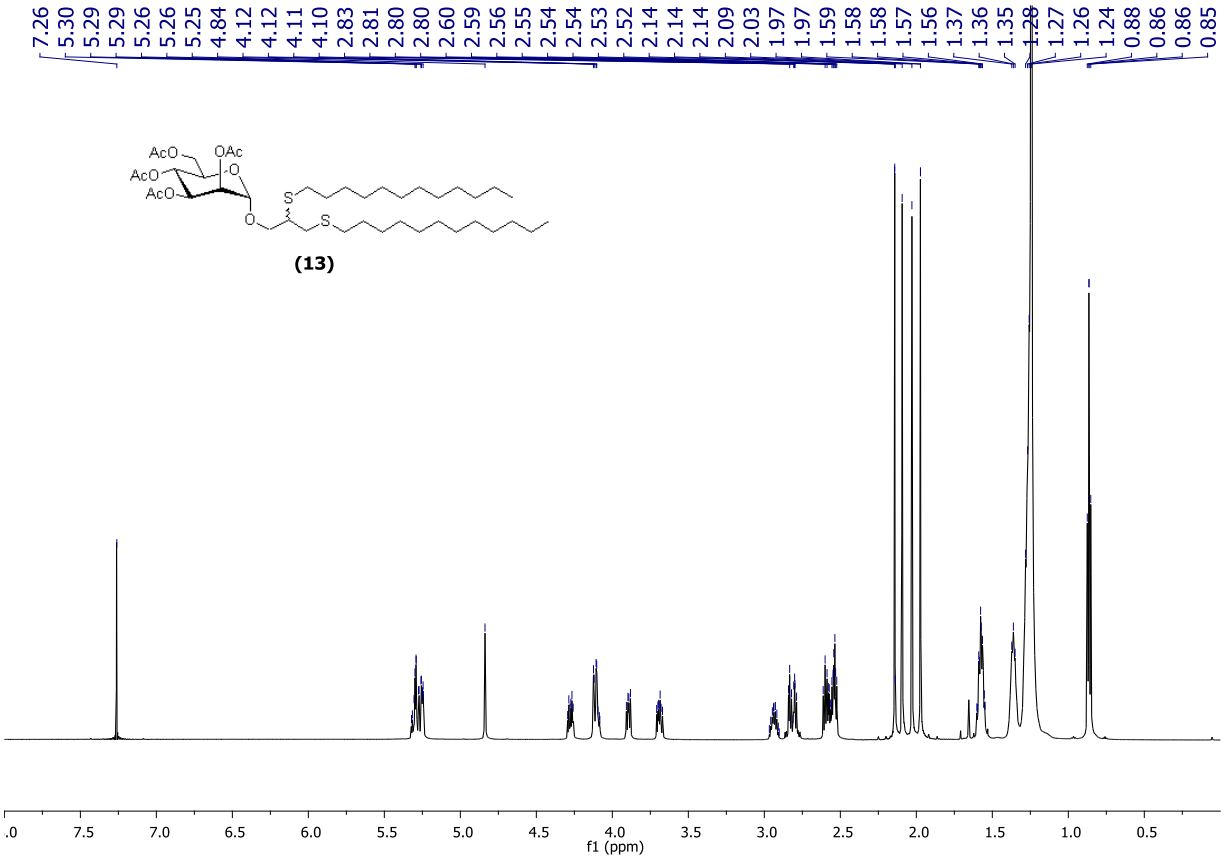

170.49 170.36 170.25 169.54 101.88 101.61 77.16 71.52 71.02 70.92 70.82 68.86 67.11 61.32 61.30 45.88 45.38 34.71 33.58 33.26 32.08 32.03 31.78 30.07 30.04 29.90 29.83 29.79 29.76 29.74 29.73 29.68 29.67 29.65 29.47 29.41 29.39 29.37 29.07 29.03 22.80 21.02 20.98 20.80 20.79 20.70 14.24
AcO
OAc
O
AcO
OAc
O
S
S
(10)
80 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

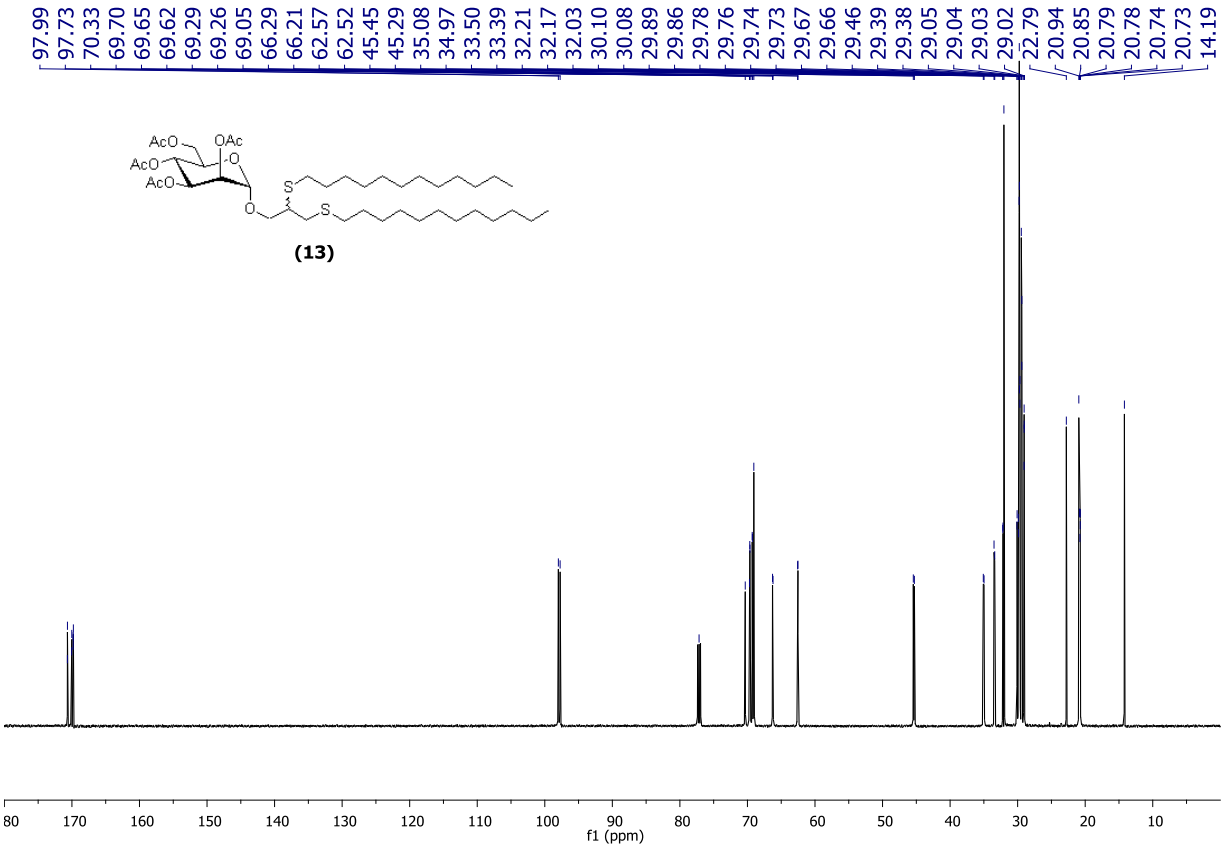

4.881
4.091
4.079
3.870
3.789
3.782
3.771
3.691
3.684
3.674
3.667
2.951
2.940
2.928
2.924
2.919
2.906
2.897
2.886
2.688
2.678
2.672
2.667
2.660
2.651
2.582
2.574
2.561
2.553
2.548
2.540
2.528
2.520
2.507
2.503
2.500
1.505
1.500
1.494
1.482
1.470
1.332
1.322
1.235
0.858
0.847
0.835
HO
OH
O
HO
O
S
S
OH
f1 (ppm)

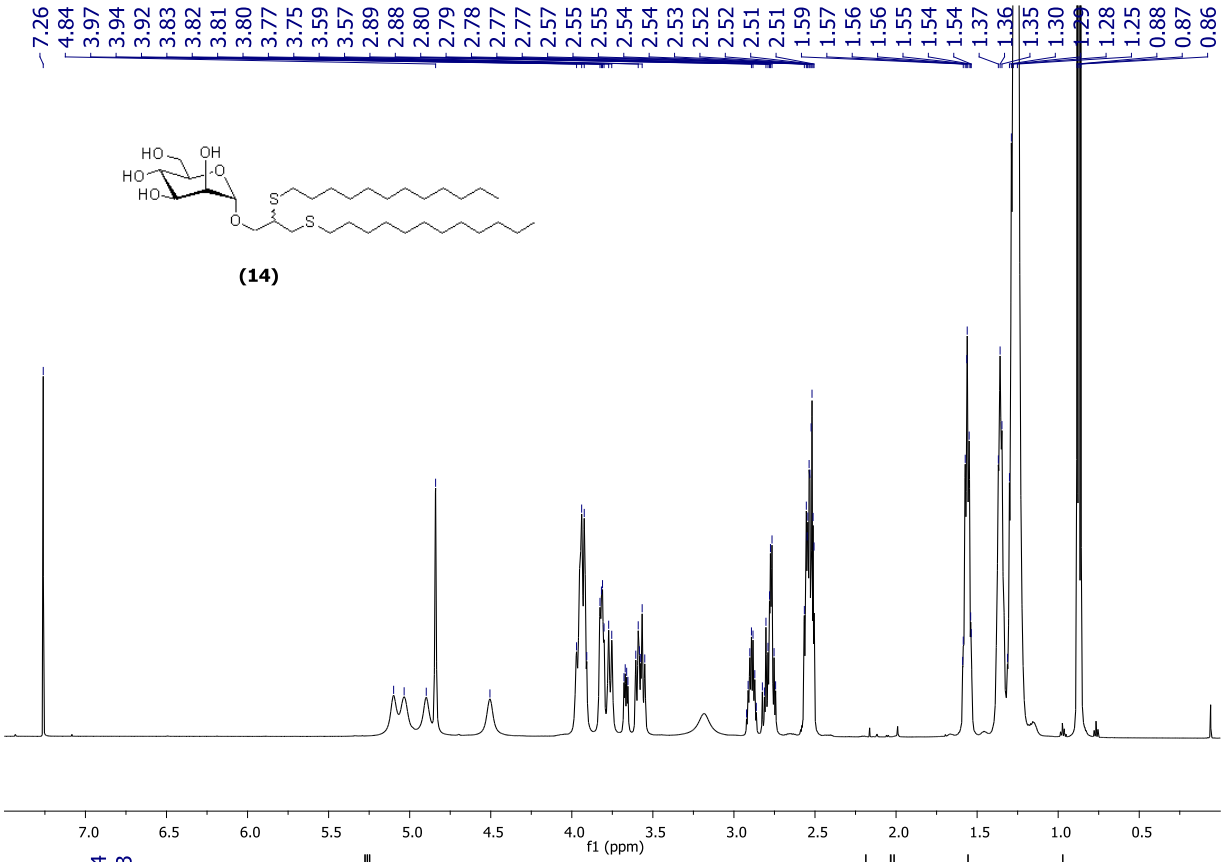
(11)

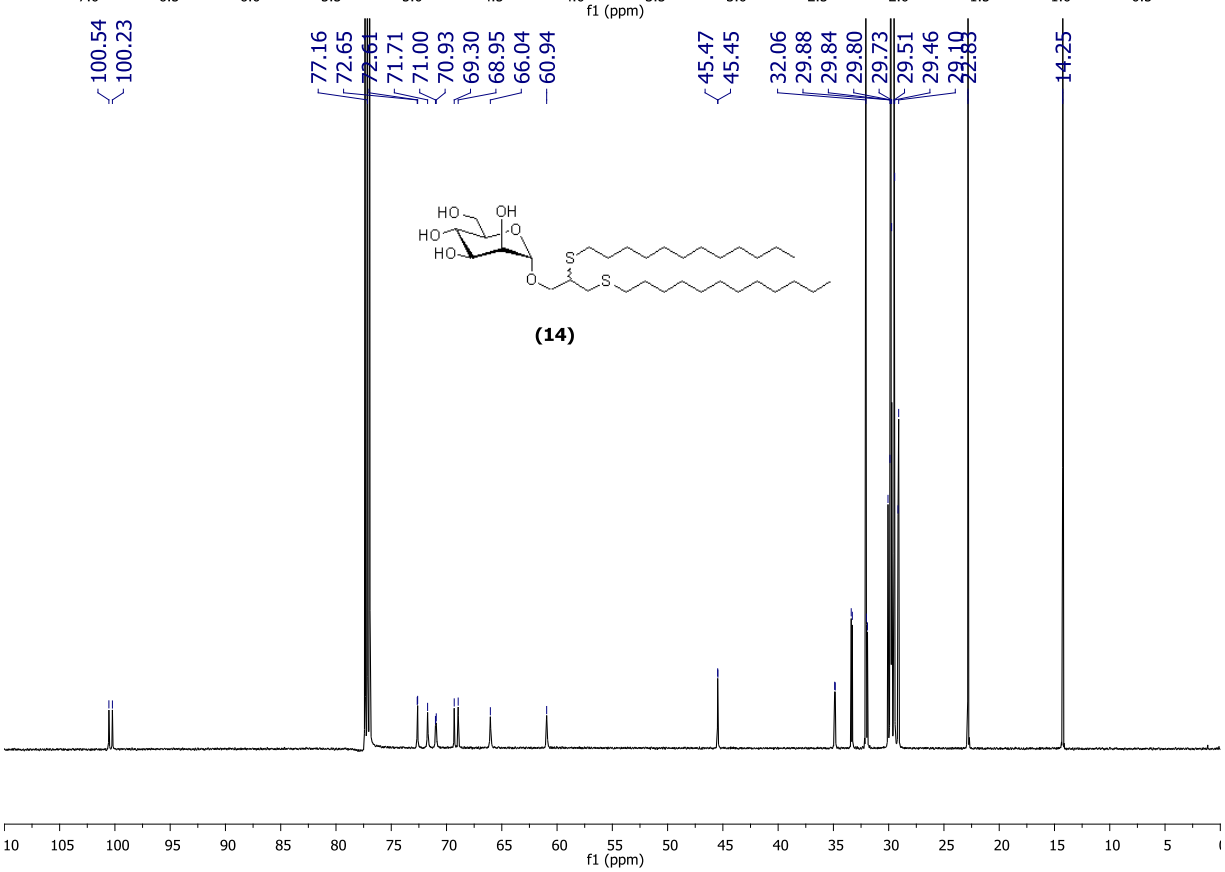

104.29
103.52
75.21
73.47
73.44
70.98
70.57
70.52
70.20
67.89
67.85
60.19
60.15
45.70
45.11
39.52
31.37
29.14
29.10
29.07
28.80
28.72
22.14
13.92
HO
OH
O
HO
O
S
S
OH
f1 (ppm)

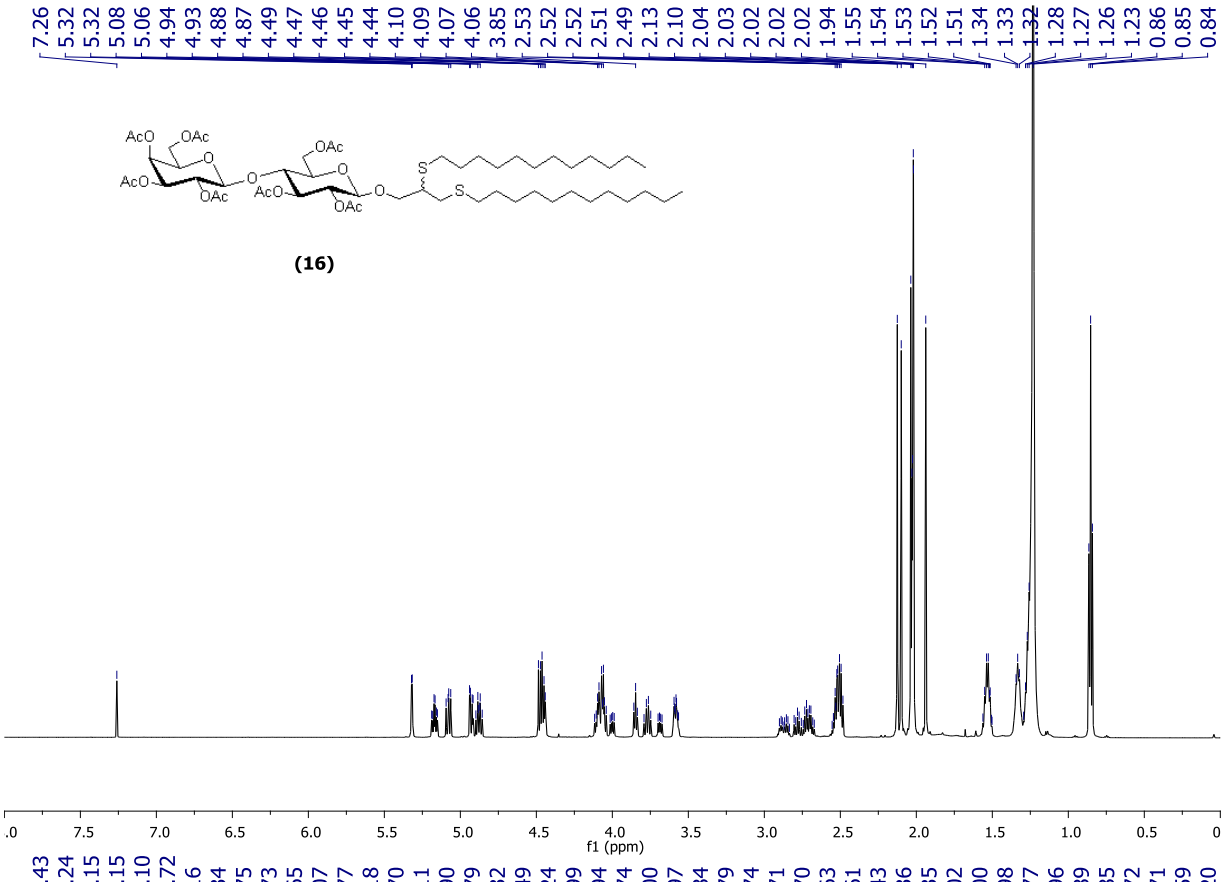
(11)

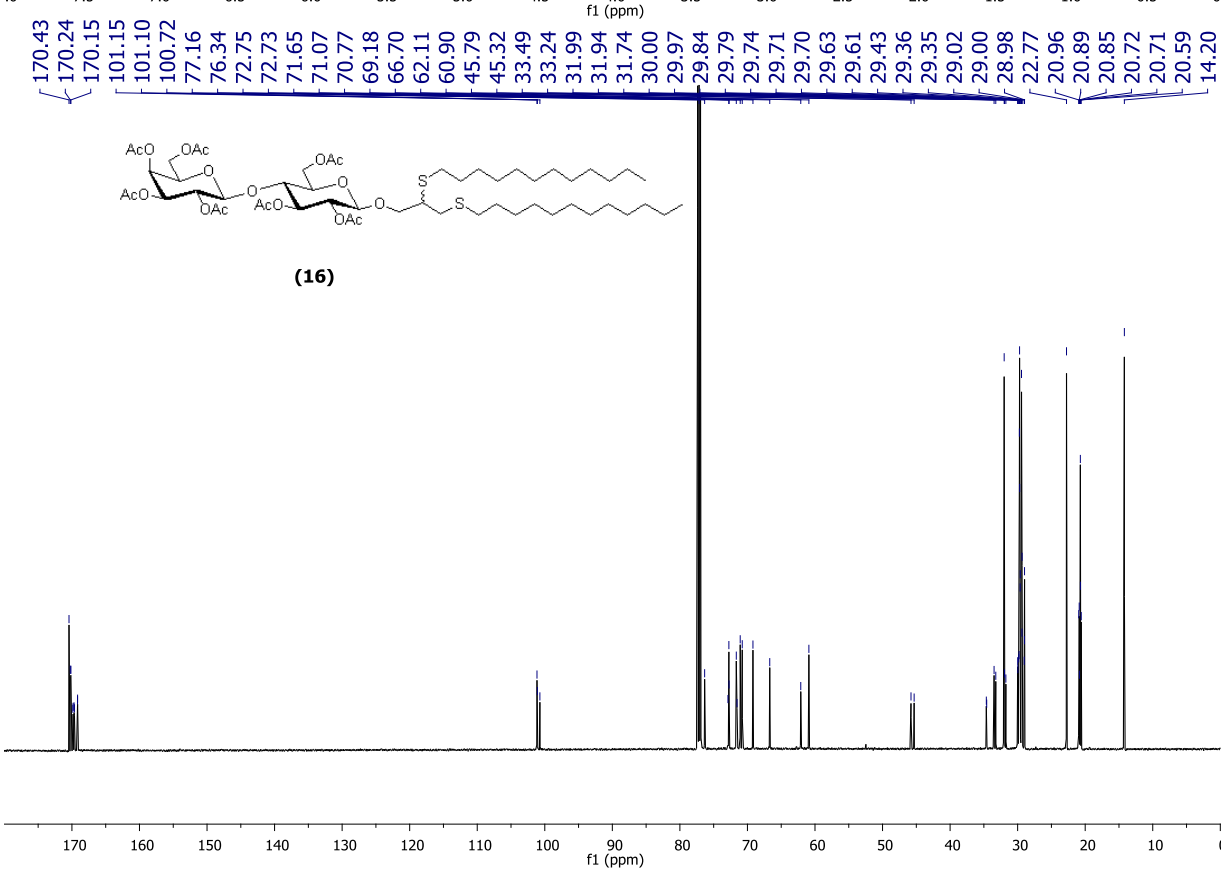

7.26 5.30 5.29 5.29 5.26 5.26 5.25 4.84 4.12 4.12 4.11 4.10 2.83 2.81 2.80 2.80 2.60 2.59 2.56 2.55 2.54 2.54 2.53 2.52 2.14 2.14 2.14 2.09 2.03 1.97 1.97 1.59 1.58 1.58 1.57 1.56 1.37 1.36 1.35 1.26 1.27 1.26 1.24 0.88 0.86 0.86 0.85
AcO
OAc
AcO
AcO
O
S
O
S
(13)
f1 (ppm)

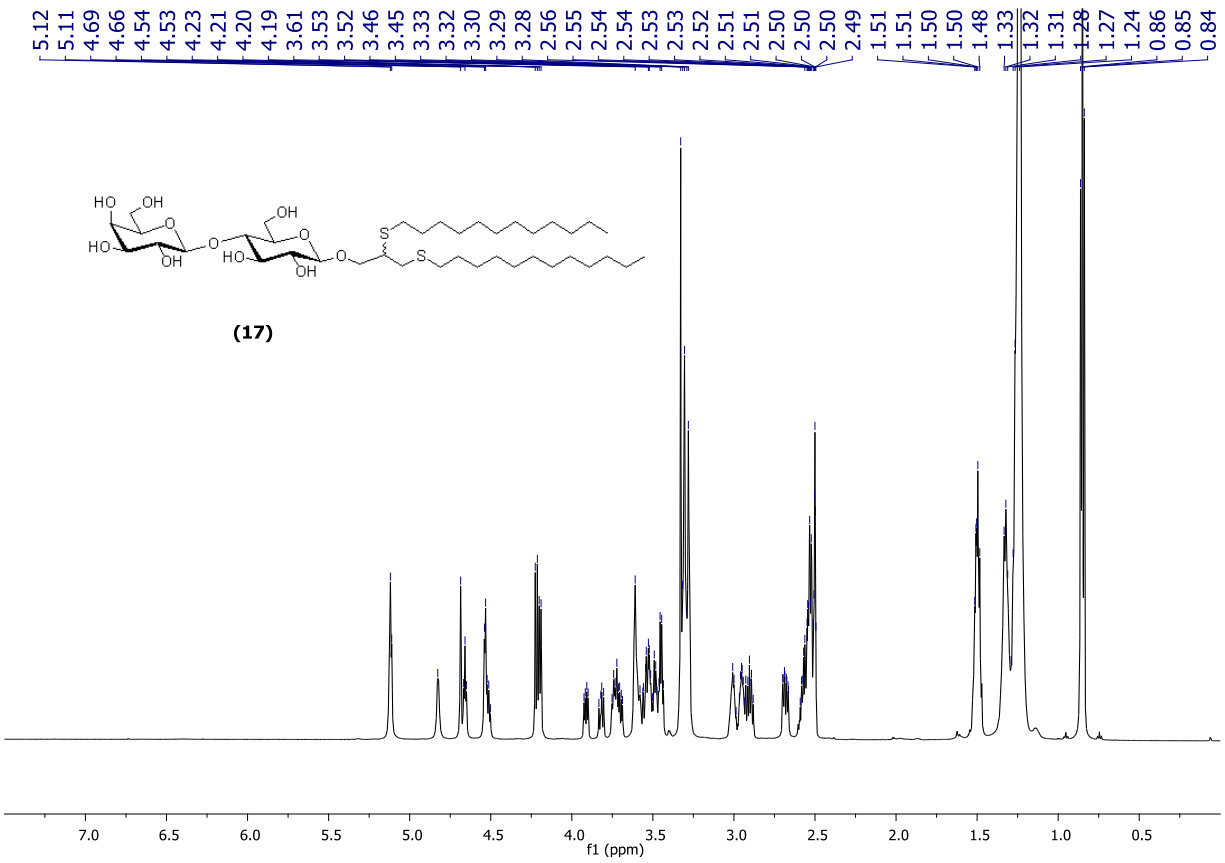

97.99 97.73 70.33 69.70 69.65 69.62 69.29 69.26 69.05 66.29 66.21 62.57 62.52 45.45 45.29 35.08 34.97 33.50 33.39 32.21 32.17 32.03 30.10 30.08 29.89 29.86 29.78 29.76 29.74 29.73 29.67 29.66 29.46 29.39 29.38 29.05 29.04 29.03 29.02 22.79 20.94 20.85 20.79 20.78 20.74 20.73 14.19
AcO
OAc
AcO
AcO
O
S
O
S
(13)
f1 (ppm)


7.26
4.84
3.97
3.94
3.92
3.83
3.82
3.81
3.80
3.77
3.75
3.59
3.57
2.89
2.88
2.80
2.79
2.78
2.77
2.77
2.57
2.55
2.55
2.54
2.54
2.53
2.52
2.52
2.51
2.51
1.59
1.57
1.56
1.56
1.55
1.54
1.54
1.37
1.36
1.35
1.30
1.29
1.28
1.25
0.88
0.87
0.86
HO
OH
HO
HO
O
S
S
O
(14)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

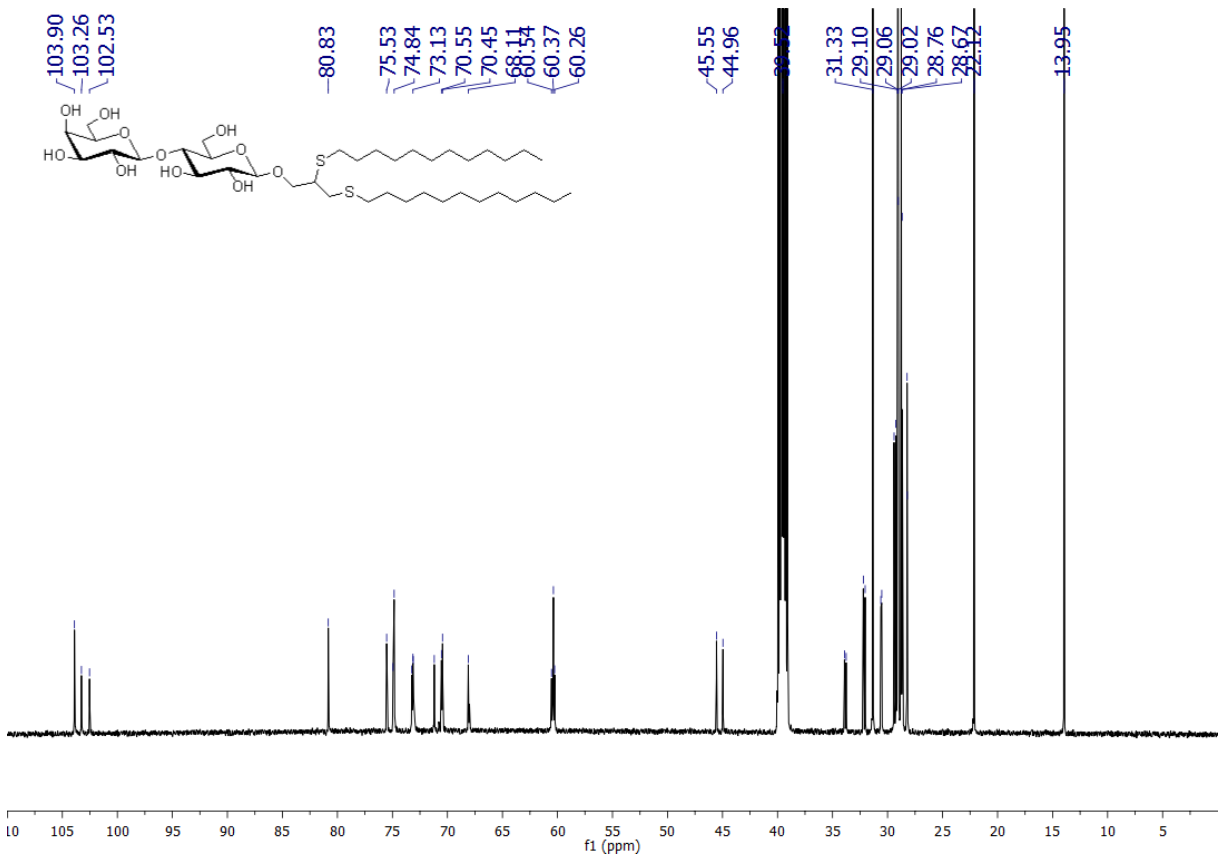

100.54
100.23
77.16
72.65
72.61
71.71
71.00
70.93
69.30
68.95
66.04
60.94
45.47
45.45
32.06
29.88
29.84
29.80
29.73
29.51
29.46
29.10
22.83
14.25
HO
OH
HO
HO
O
S
S
O
(14)
10
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
f1 (ppm)

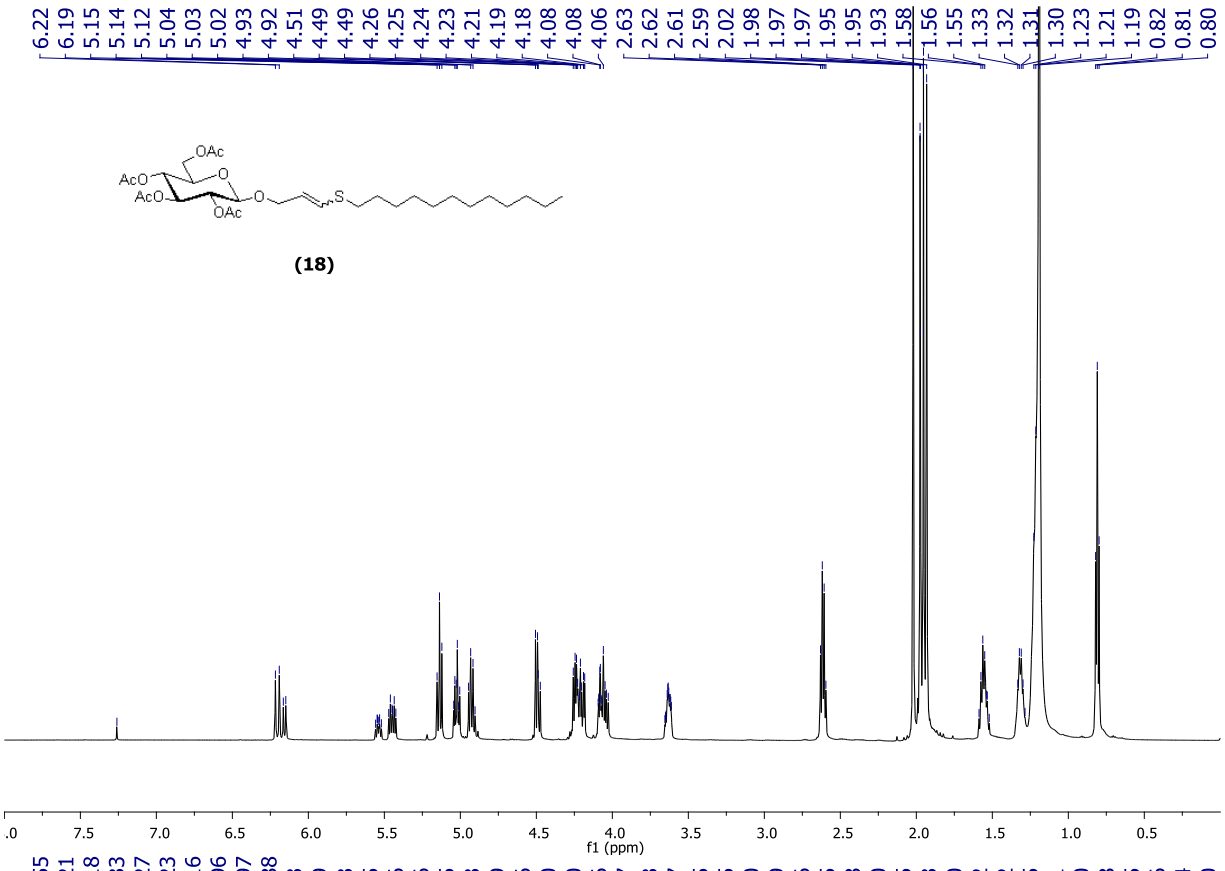

7.26
5.32
5.32
5.08
5.06
4.94
4.93
4.88
4.87
4.49
4.47
4.46
4.45
4.44
4.10
4.09
4.07
4.06
3.85
2.53
2.52
2.52
2.51
2.49
2.13
2.10
2.04
2.03
2.02
2.02
2.02
1.94
1.55
1.54
1.53
1.52
1.51
1.34
1.33
1.32
1.28
1.27
1.26
1.23
0.86
0.85
0.84
AcO
OAc
AcO
OAc
AcO
OAc
AcO
OAc
S
S
(16)
f1 (ppm)


170.43
170.24
170.15
101.15
101.10
100.72
77.16
76.34
72.75
72.73
71.65
71.07
70.77
69.18
66.70
62.11
60.90
45.79
45.32
33.49
33.24
31.99
31.94
31.74
30.00
29.97
29.84
29.79
29.74
29.71
29.70
29.63
29.61
29.43
29.36
29.35
29.02
29.00
28.98
22.77
20.96
20.89
20.85
20.72
20.71
20.59
14.20
AcO
OAc
AcO
OAc
AcO
OAc
AcO
OAc
S
S
(16)
f1 (ppm)

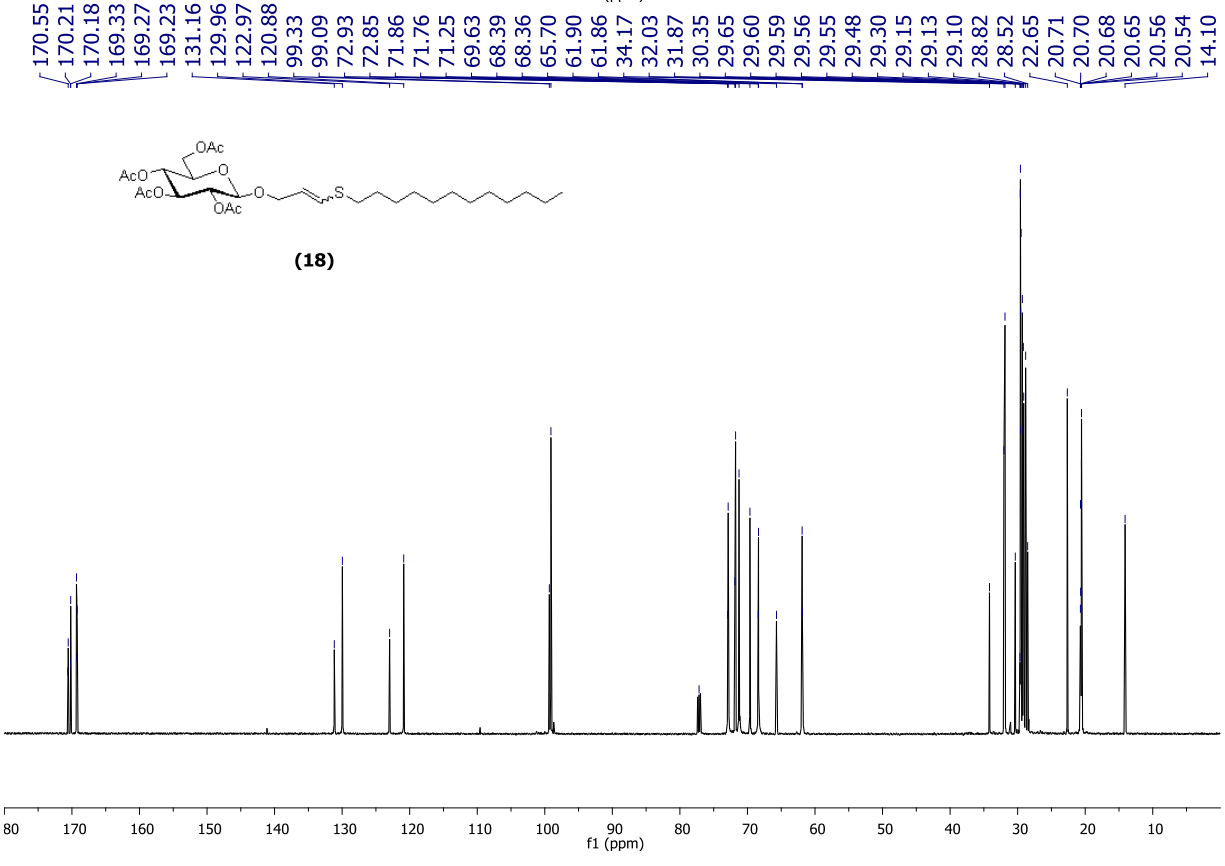

5.12
5.11
4.69
4.66
4.54
4.53
4.23
4.21
4.20
4.19
3.61
3.53
3.52
3.46
3.45
3.33
3.32
3.30
3.29
3.28
2.56
2.55
2.54
2.54
2.53
2.53
2.52
2.51
2.51
2.50
2.50
2.50
2.49
1.51
1.51
1.50
1.50
1.48
1.33
1.32
1.31
1.28
1.27
1.24
0.86
0.85
0.84
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

(17)

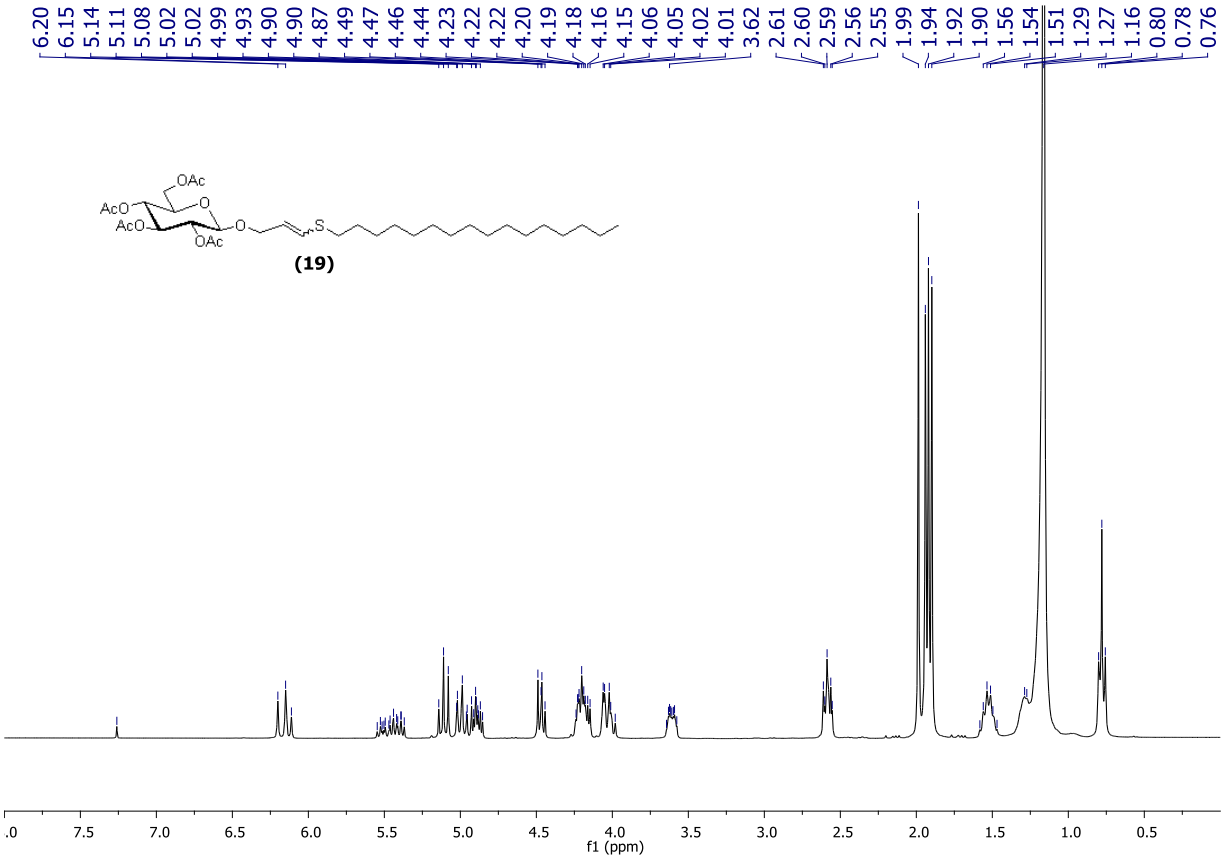

103.90
103.26
102.53
80.83
75.53
74.84
73.13
70.55
70.45
68.11
60.37
60.26
45.55
44.96
31.33
29.10
29.06
29.02
28.76
28.67
22.12
13.95
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
f1 (ppm)


6.22 6.19 5.15 5.14 5.12 5.04 5.03 5.02 4.93 4.92 4.51 4.49 4.49 4.26 4.25 4.24 4.23 4.21 4.19 4.18 4.08 4.08 4.06 2.63 2.62 2.61 2.59 2.02 1.98 1.97 1.97 1.95 1.95 1.93 1.58 1.56 1.55 1.33 1.32 1.31 1.30 1.23 1.21 1.19 0.82 0.81 0.80
OAc
AcO
AcO
O
OAc
O
S
f1 (ppm)

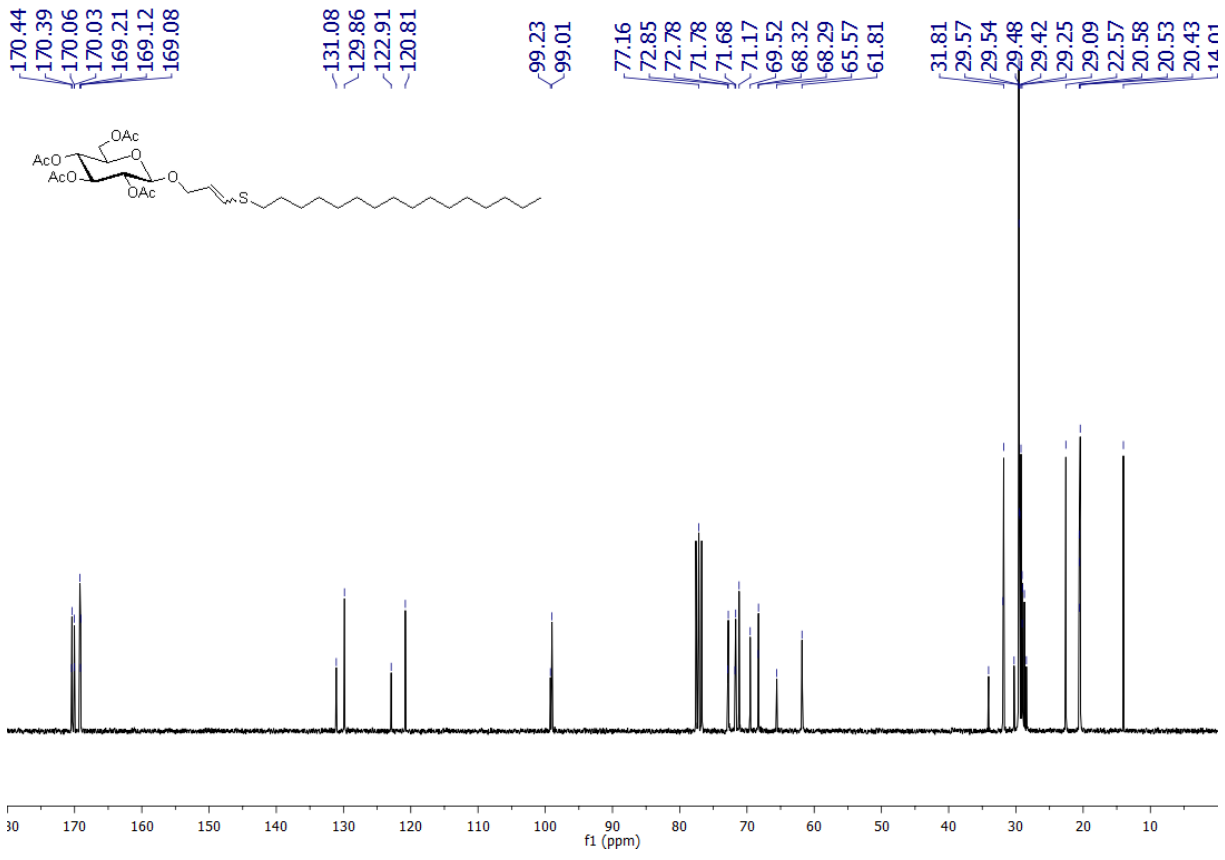
(18)

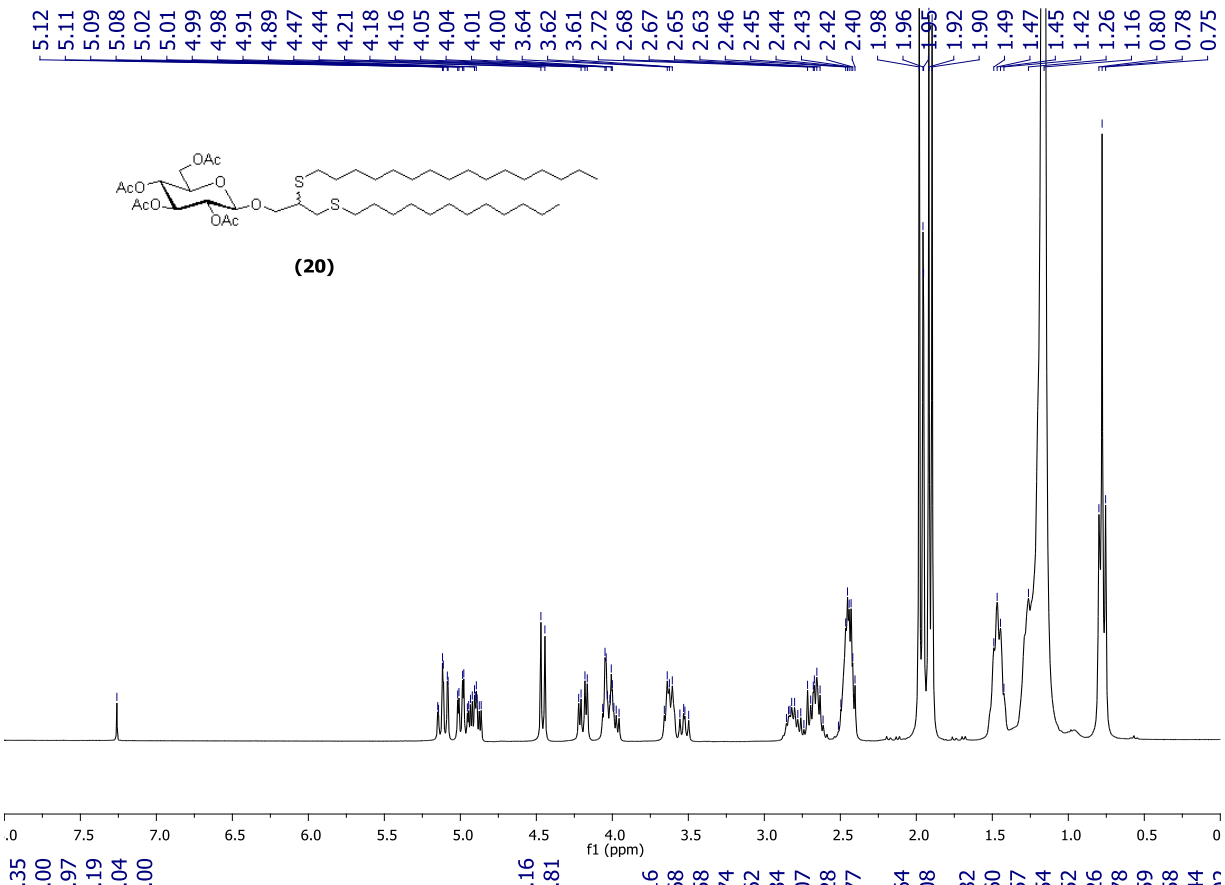

170.55 170.21 170.18 169.33 169.27 169.23 131.16 129.96 122.97 120.88 99.33 99.09 72.93 72.85 71.86 71.76 71.25 69.63 68.39 68.36 65.70 61.90 61.86 34.17 32.03 31.87 30.35 29.65 29.60 29.59 29.56 29.55 29.48 29.30 29.15 29.13 29.10 28.82 28.52 22.65 20.71 20.70 20.68 20.65 20.56 20.54 14.10
OAc
AcO
AcO
O
OAc
O
S
f1 (ppm)

(18)

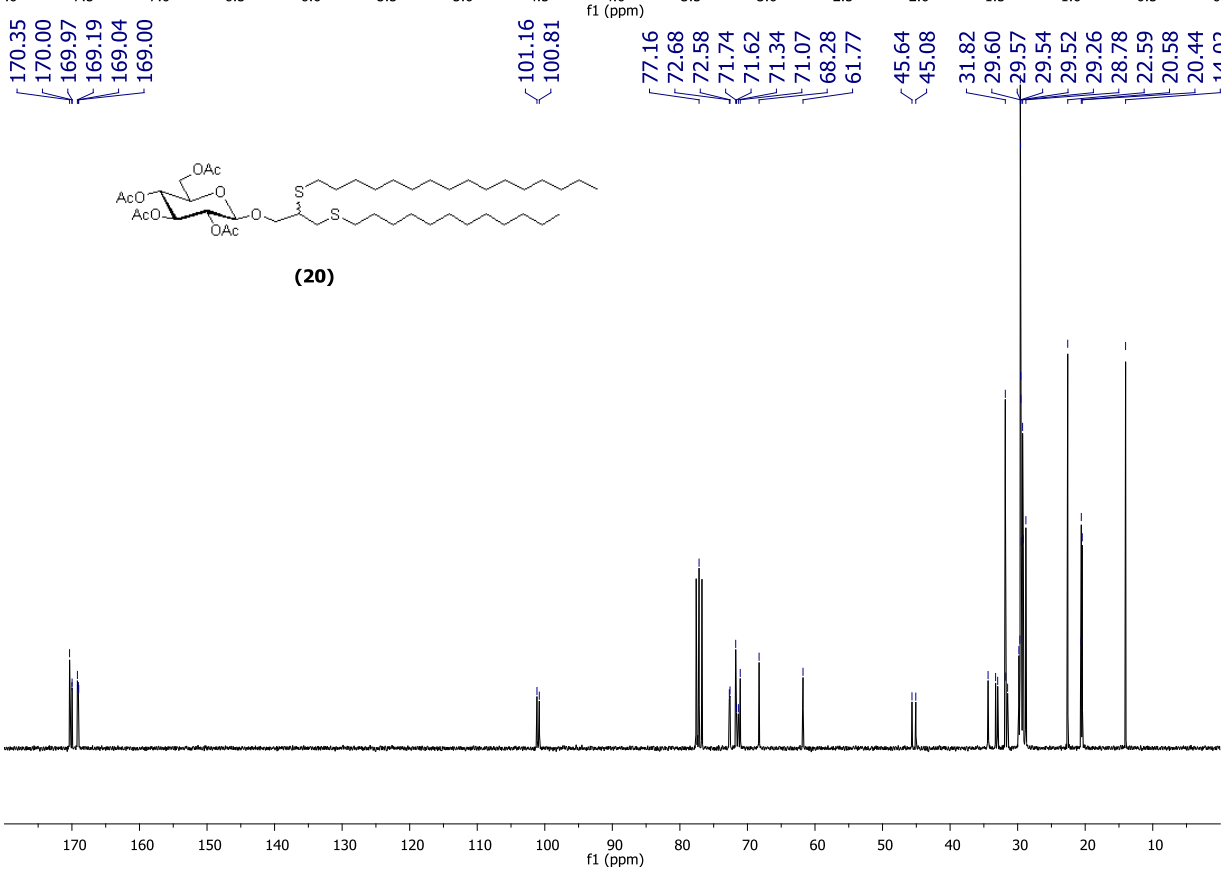

6.20
6.15
5.14
5.11
5.08
5.02
5.02
4.99
4.93
4.90
4.90
4.87
4.49
4.47
4.46
4.44
4.23
4.22
4.22
4.20
4.19
4.18
4.16
4.15
4.06
4.05
4.02
4.01
3.62
2.61
2.60
2.59
2.56
2.55
1.99
1.94
1.92
1.90
1.56
1.54
1.51
1.29
1.27
1.16
0.80
0.78
0.76
OAc
AcO
AcO
OAc
O
O
S
f1 (ppm)

(19)

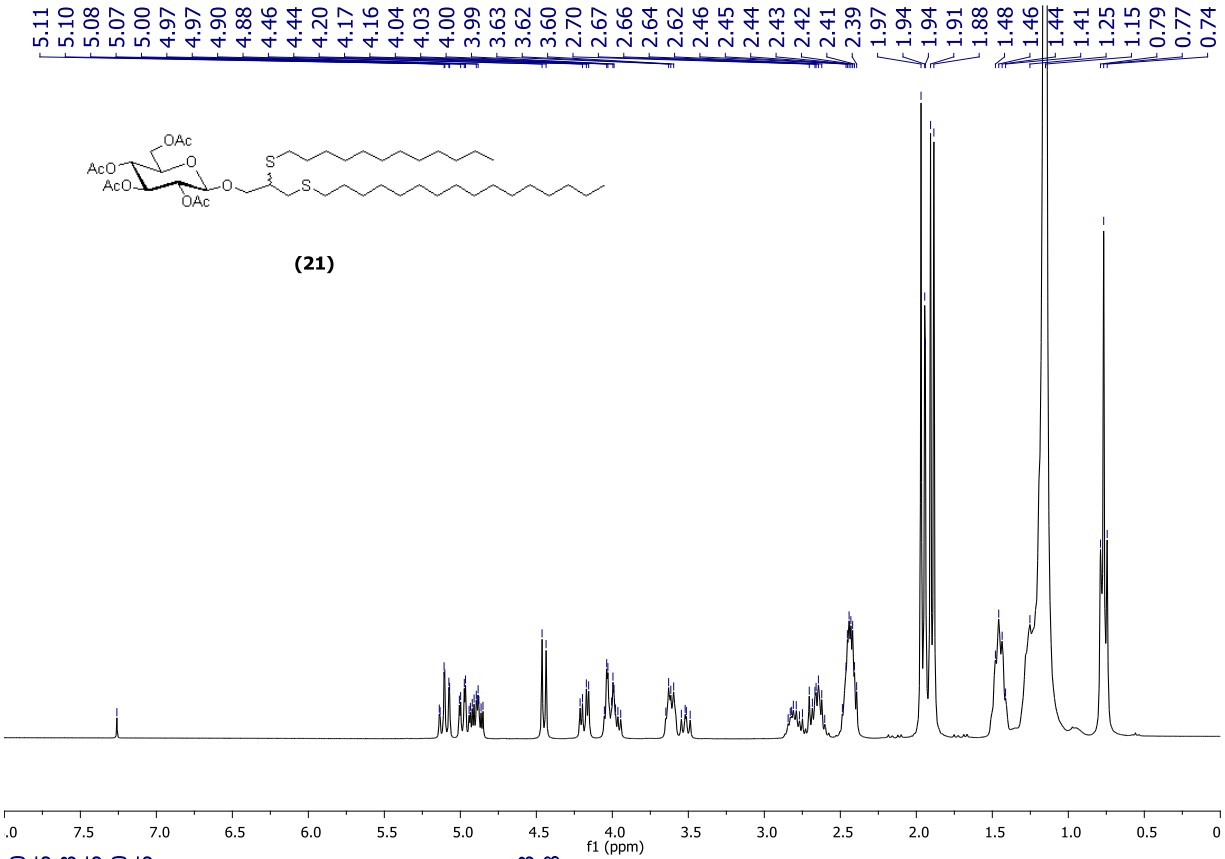

170.44
170.39
170.06
170.03
169.21
169.12
169.08
131.08
129.86
122.91
120.81
99.23
99.01
77.16
72.85
72.78
71.78
71.68
71.17
69.52
68.32
68.29
65.57
61.81
31.81
29.57
29.54
29.48
29.42
29.25
29.09
22.57
20.58
20.53
20.43
OAc
AcO
AcO
OAc
O
O
S
f1 (ppm)


5.12
5.11
5.09
5.08
5.02
5.01
4.99
4.98
4.91
4.89
4.47
4.44
4.21
4.18
4.16
4.05
4.04
4.01
4.00
3.64
3.62
3.61
2.72
2.68
2.67
2.65
2.63
2.46
2.45
2.44
2.43
2.42
2.40
1.98
1.96
1.95
1.92
1.90
1.49
1.47
1.45
1.42
1.26
1.16
0.80
0.78
0.75
OAc
AcO
AcO
OAc
O
O
S
S
f1 (ppm)

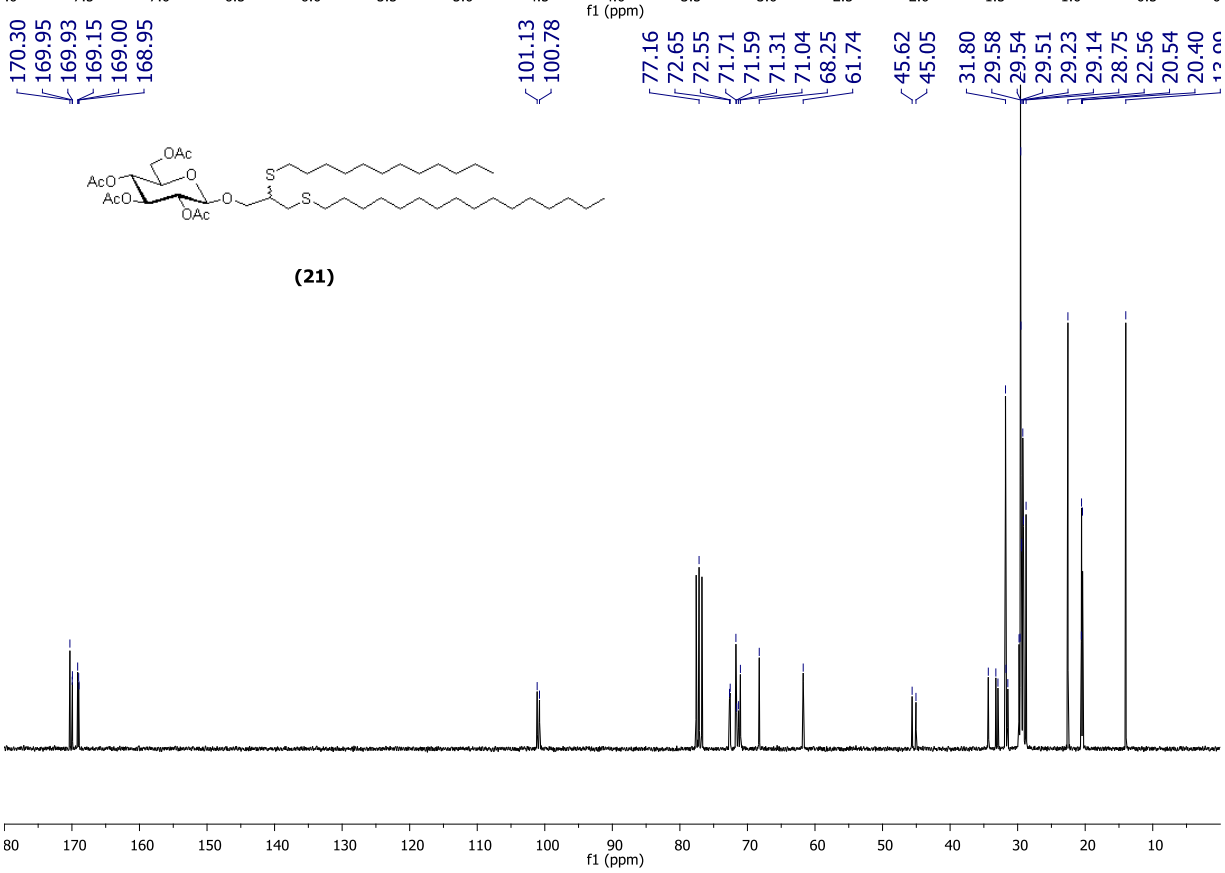
(20)


170.35
170.00
169.97
169.19
169.04
169.00
101.16
100.81
77.16
72.68
72.58
71.74
71.62
71.34
71.07
68.28
61.77
45.64
45.08
31.82
29.60
29.57
29.54
29.52
29.26
28.78
22.59
20.58
20.44
OAc
AcO
AcO
OAc
O
O
S
S
f1 (ppm)

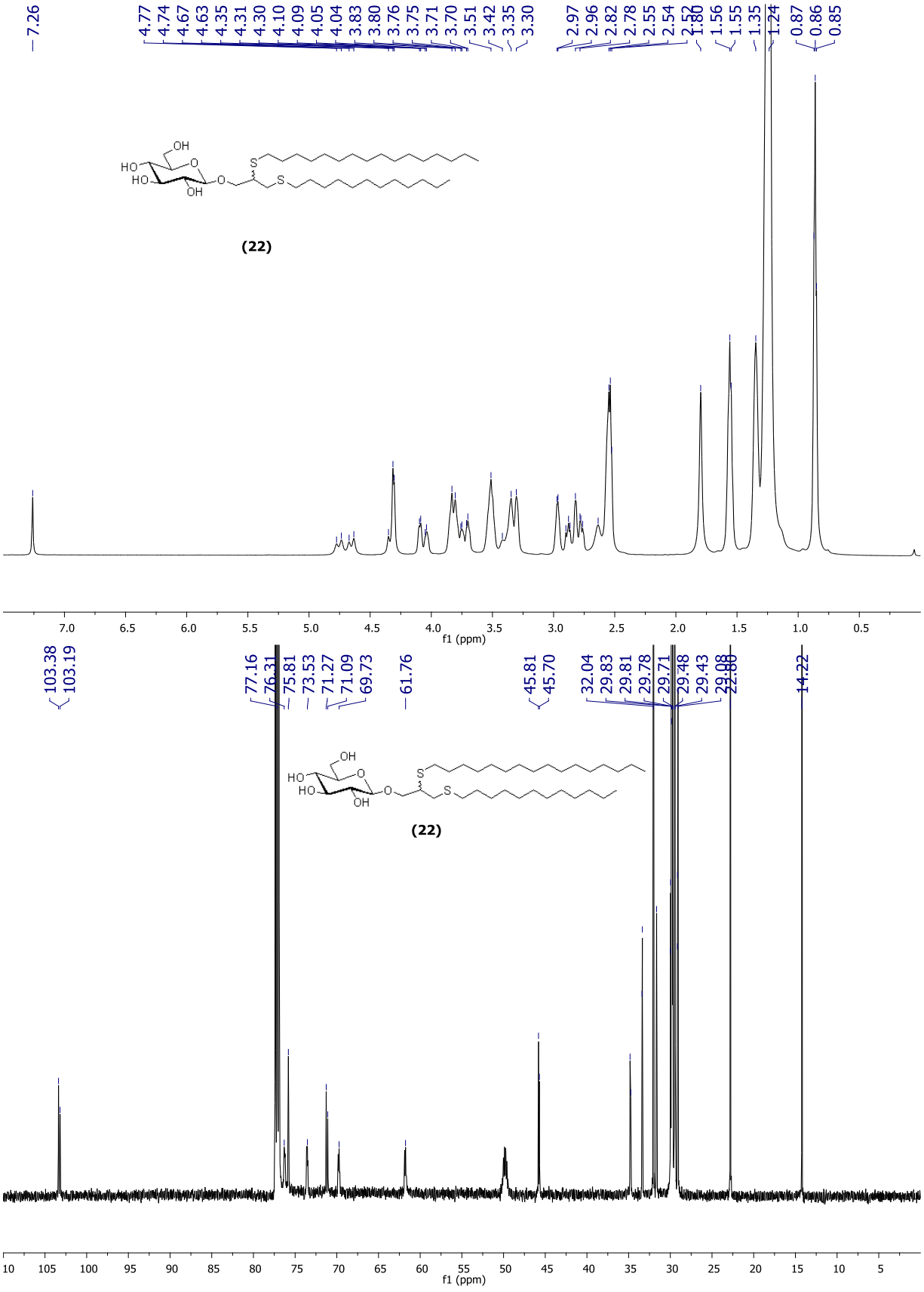
(20)

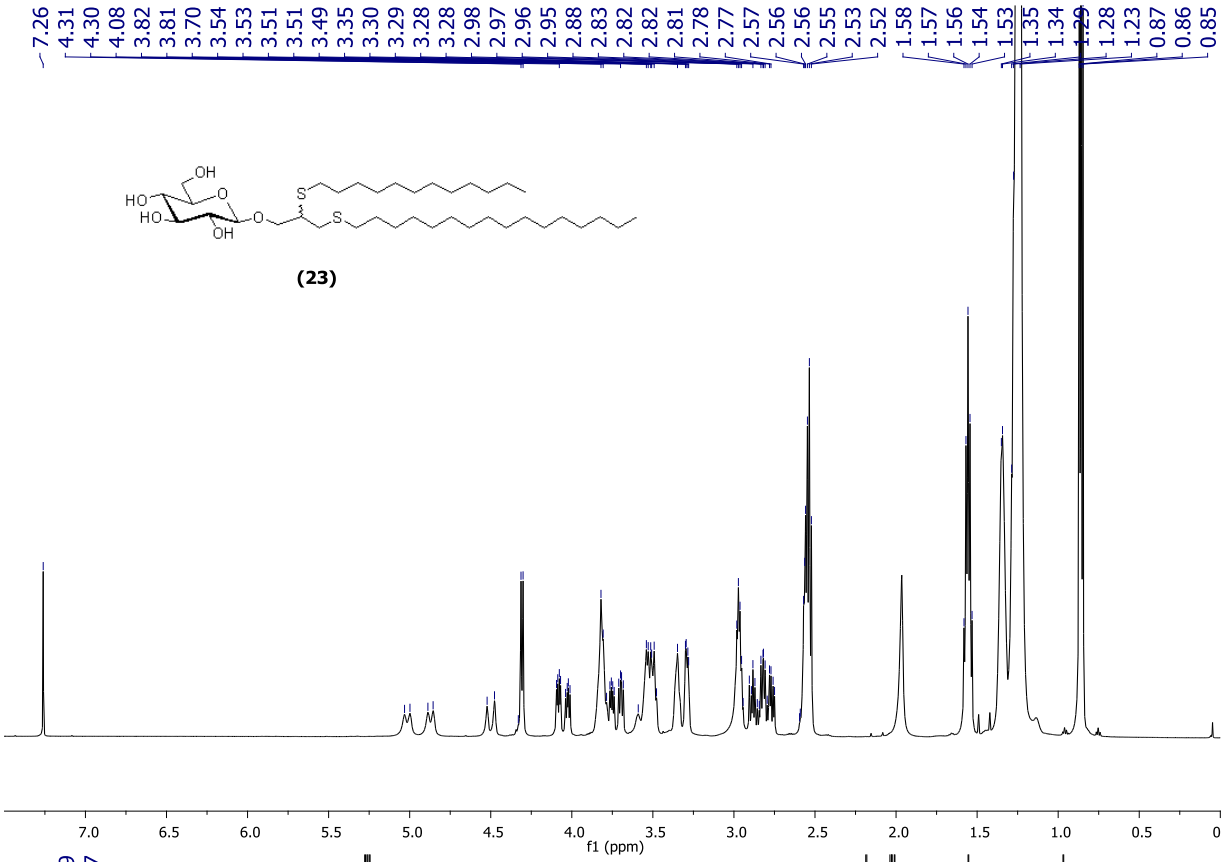

5.11
5.10
5.08
5.07
5.00
4.97
4.97
4.90
4.88
4.46
4.44
4.20
4.17
4.16
4.04
4.03
4.00
3.99
3.63
3.62
3.60
2.70
2.67
2.66
2.64
2.62
2.46
2.45
2.44
2.43
2.42
2.41
2.39
1.97
1.94
1.94
1.91
1.88
1.48
1.46
1.44
1.41
1.25
1.15
0.79
0.77
0.74
OAc
AcO
AcO
O
OAc
O
S
S
f1 (ppm)

(21)


170.30
169.95
169.93
169.15
169.00
168.95
101.13
100.78
77.16
72.65
72.55
71.71
71.59
71.31
71.04
68.25
61.74
45.62
45.05
31.80
29.58
29.54
29.51
29.23
29.14
28.75
22.56
20.54
20.40
OAc
AcO
AcO
O
OAc
O
S
S
f1 (ppm)

(21)


7.26
4.77
4.74
4.67
4.63
4.35
4.31
4.30
4.10
4.09
4.05
4.04
3.83
3.80
3.76
3.75
3.71
3.70
3.51
3.42
3.35
3.30
2.97
2.96
2.82
2.78
2.55
2.54
1.80
1.56
1.55
1.35
1.24
0.87
0.86
0.85
OH
HO
O
HO
OH
O
S
S
(22)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)
103.38
103.19
77.16
76.31
75.81
73.53
71.27
71.09
69.73
61.76
45.81
45.70
32.04
29.83
29.81
29.78
29.71
29.48
29.43
29.08
22.80
14.22
OH
HO
O
HO
OH
O
S
S
(22)
10
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
f1 (ppm)


7.26
4.31
4.30
4.08
3.82
3.81
3.70
3.54
3.53
3.51
3.51
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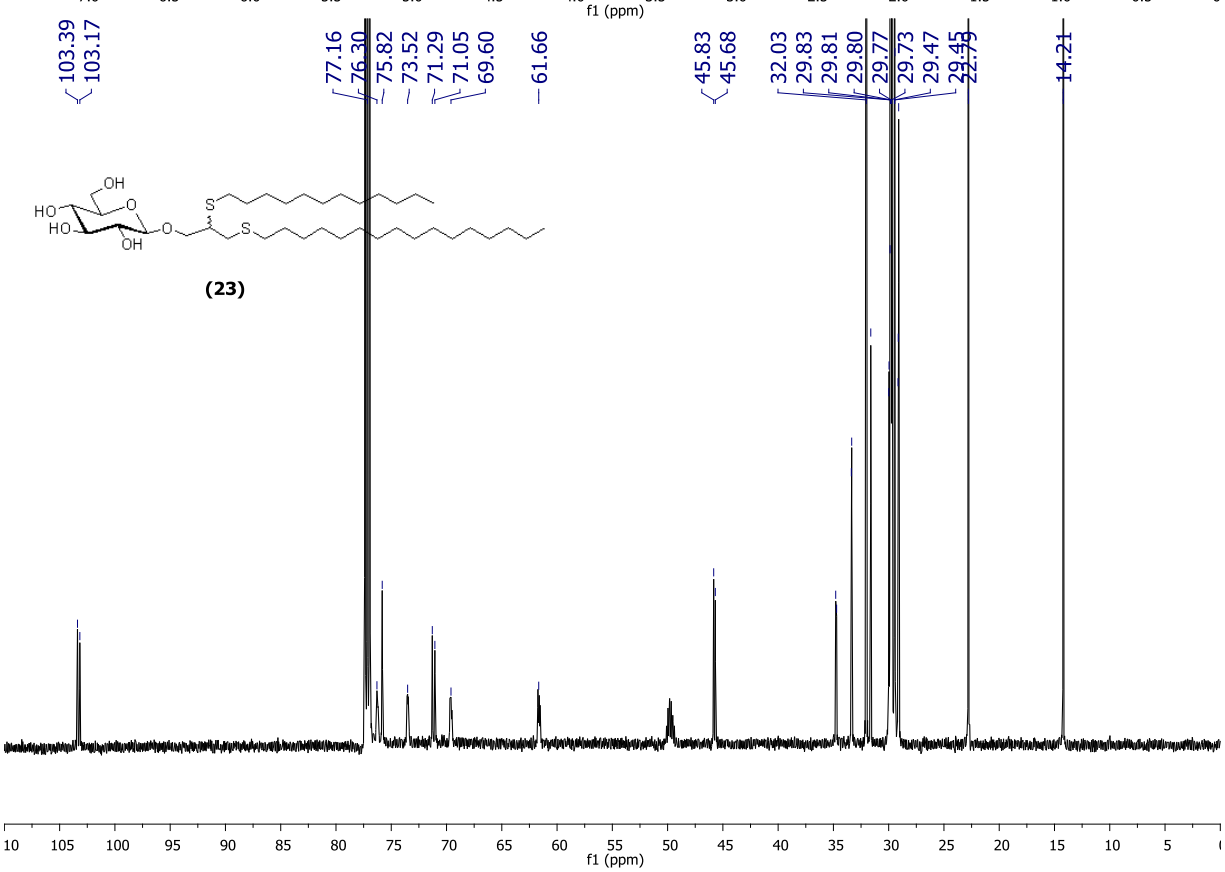
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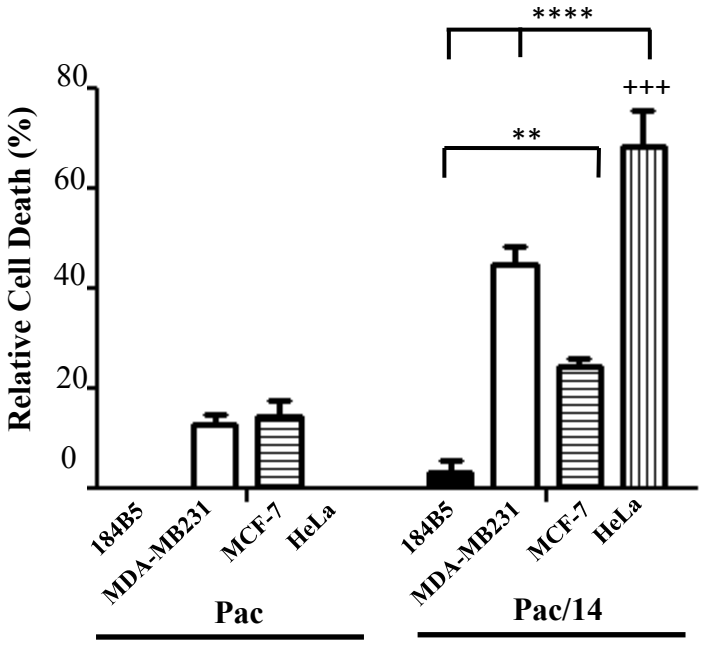

Figure S1. Formulation with the mannosylated lipid particle (compound 14) enhances killing effects of Paclitaxel toward cancer cells and increases anti-cancer cell selectivity. Both cancer (MDA-MB231, MCF-7 and HeLa) and non-cancer (184B5) cells were treated with Paclitaxel alone **(Pac**) or formulated Paclitaxel [Paclitaxel/compound 14 (1:4; w/w) (**Pac/14**)] at 6.25 nM for 72 hours. Relative cell death (%) was calculated as described in Materials and Methods. Comparisons between different groups were performed using both one - and two-way ANOVA methods. p values <0.05 was considered as significantly different. **: $p<0.01$, *** and +++: $p<0.001$, ****: $p<0.0001$

Materials and Methods

Cell culture

Human malignant cells were cultured in either RPMI-1640 (metastatic breast MDA-MB-231 cells and cervical cancer HeLa S3 cells) or DMEM (breast cancer MCF-7 cells) media. Non-malignant human mammary epithelial 184B5 cells were cultured DME/F12 media with an addition of growth factors. All media were supplemented with 10% FBS and antibiotics. Cells were maintained under a standard culture condition in a humidified incubator (5% CO_2) at37^0C

Sulforhodamine B (SRB) assay

$4x10^4$ cells per mL were plated in each well of a 96-well plate and incubated overnight before drug treatments as described previously (Hu *et al*., 2008; Skehan *et al*., 1990). Cells were then treated with different concentrations of paclitaxel in formulated or non-formulated form and incubated for additional 72 hours. Sham treated cells were used as a positive control and cells treated with 10% trichloroacetic acid (TCA) were used as a negative control. After treatment

period, cells were fixed with 10% TCA at 4^0C for 1 hour, followed by a staining step with 0.4 % (w/v) SRB solution (Sigma, Oakville, ON, Canada) for 30 minutes at room temperature. Finally, the cells were washed with 1% (v/v) acetic acid, air dried, and resuspended in 10mM Tris buffer, pH 10.5 (Thermo Fisher Scientific). Absorbance was determined at 540nm by an automated plate reader (SynergyH4 Hybrid Multi-Mode Microplate Reader - Bio-Tek, Winooski, VT). Relative cell growth was calculated using the following formula:

$$\text{Relative cell growth (\%)} = [(A_{treated} - A_{negative})/ (A_{sham} - A_{negative})] \times 100$$

Where, $A_{treated,}$ $A_{negative,}$ and A_{sham} are absorbance values of treated, negative control, sham-treated cells, respectively.

Relative cell death (%) = 100 – Relative cell growth. When percentage is ≥ 100, cell death is considered as 0%. Comparisons between the different cell line groups in the same treatment and between different treatments of the same cell line were made by p values using both two-way and one-way ANOVA. A p value of <0.05 was considered to be statistically significant

IC_{50} values were calculated using a sigmoidal dose-response curve (variable slope) in Graphpad Prism V 4.02 (Graph37 Pad Software, Inc. La Jolla, CA).

Results

Paclitaxel (USBiological, Cat#P1792A) has been well known in clinics for treatment of cancers, especially breast cancers. However, its side effects have also been well documented. Our goal is to develop a novel delivery system, compound **14**, which can increase paclitaxel's anti-tumor activity and reduce its toxic effects. As indicated in Fig. **S1**, our preliminary data show very promising results of the formulated vs. Paclitaxel alone at 6.25nM on tested cell lines:

i). Compound **14** significantly enhanced killing effects of paclitaxel in the human metastatic breast (MDA-MB231) and cervical (HeLa) cancer cells (p values <0.001). IC_{50} value determination in an experiment using two different cancer cell types, MDA-MB231 and HeLa, also indicated that IC_{50} of (Pac/14) were 2 and 3 times lower, i.e. 2 and 3 times more effective, than those of (Pac), respectively (data not shown). In MCF-7 cell treatment, (Pac/14) was 10% more effective than (Pac) in killing cells.

ii). More interestingly, when formulated with compound **14**, Pac significantly increased its anti-cancer selectivity toward MDA-MB231, MCF-7, and HeLa cell lines vs. non-cancer cells (184B5) ($p < 0.0001$, 0.01 and 0.0001, respectively).

iii). In the (Pac) group, 6.25 nM of (Pac) didn't cause any toxic effects on 184B5 and HeLa cells. Anti-tumor activity was also low in MDA-MB231 and MCF7 cell treatments (12.75% and 14.65%, respectively).

Altogether, our preliminary data suggested that compound **14** can be used as a promising delivery system for anti-cancer drugs, at least for paclitaxel at 6.25nM in this article's context. The mannosylated lipid particles may facilitate paclitaxel delivery to cells in a cancer-selective manner, thus increasing paclitaxel anti-tumor activity

The mechanism action of (Pac/14) among cancer cells and non-cancer cells will be further investigated and presented in a separate paper.

References

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Skehan, P. *et al*. New colorimetric cytotoxicity assay for anticancer-drug screening. *Journal of the National Cancer Institute* 1990;82:1107

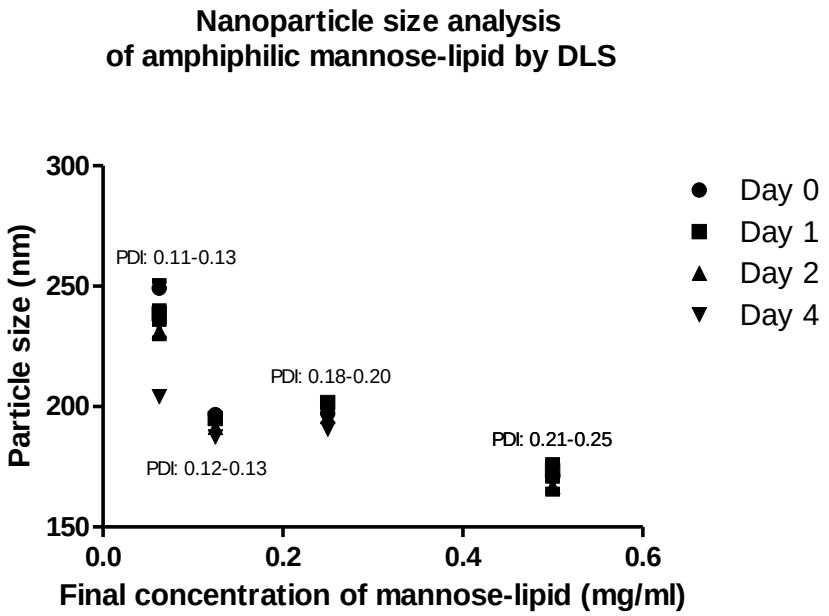

Figure S2. Stability of mannosylated lipid nanoparticle (compound 14) over time. The particle size was in the range of 170-200 nm using DLS measurements. The particles in this range were stable for at least 4 days at room temperature. Using a higher concentration of the particles (>0.5 mg/ml) resulted in aggregates and sizing could not be determined (Zetasizer ZS (Malvern) can measure only up to 6 micron particle size). When a lower concentration (<0.125 mg/ml) was used, particles were unstable and resulted in large variations in the particle size.

Dynamic Light Scattering (DLS) was performed with a Zetasizer NanoZS Malvern Instruments equipped with a 4 mW 633 nm He-Ne laser and avalanche photodiode positioned at 175° to the beam and temperature-controlled cuvette holder. Instrument parameters were determined automatically along with measurement times. Experiments were performed in triplicate.

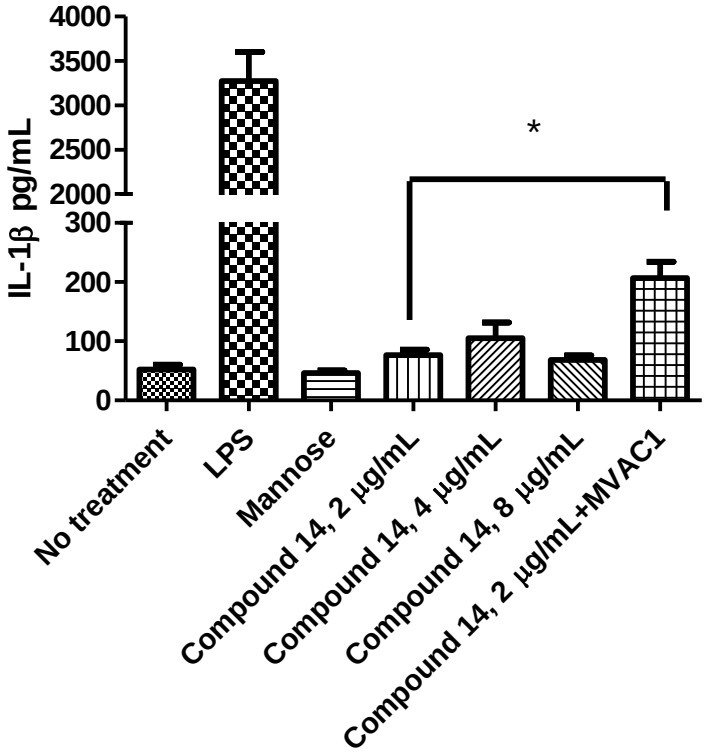

Figure S3. Level of IL-1β from stimulated JAWS II with compound 14 in presence of MVAC. JAWS II cells were grown to confluency in RPMI 1640 (Hyclone, GE) supplemented with 8% FBS (Gibco, Life Technologies), 1% penicillin/streptomycin and 5 ng/mL recombinant mouse GM-CSF (Gibco, Life Technologies). Cells were pulled and seeded on 12 well plates at a density of 500 000 cells/500 µL. Cells were then left unexposed, exposed to either mannose (10 µg/mL), or the Compound 14 with or without MVAC1 for 24 hours at 37°C, 5% CO_2. Cells were also exposed to 1 µg/mL LPS to ensure activation and production of IL-1β. After 24 hours the supernatants were pulled and immediately analyzed using the commercially available IL-1β ELISA Duo kit from R&D Systems (Fisher Scientific, Whitby ON). Compound **14** in the presence of MVAC1 induces significant levels of IL-1β production as compared to compound **14** alone (at the same concentration). MVAC1 has been previously tested in Dr. Le's lab at HSNRI in Sudbury, ON. We have demonstrated that the MVAC1 does not induce IL-1β production on its own (data not shown). Data are shown as ±SEM, n=3 for each group and are representative of multiple experiments. * p = 0.0107, unpaired T-test was performed using GraphPad Prism 5.

MVAC1 – Mucosal Vaccine Adjuvant Component 1 (Nicotine Vaccine Project supported by the Grand Challenges Canada (S4-0212-01) and the Northern Cancer Foundation for H.-T. Le)

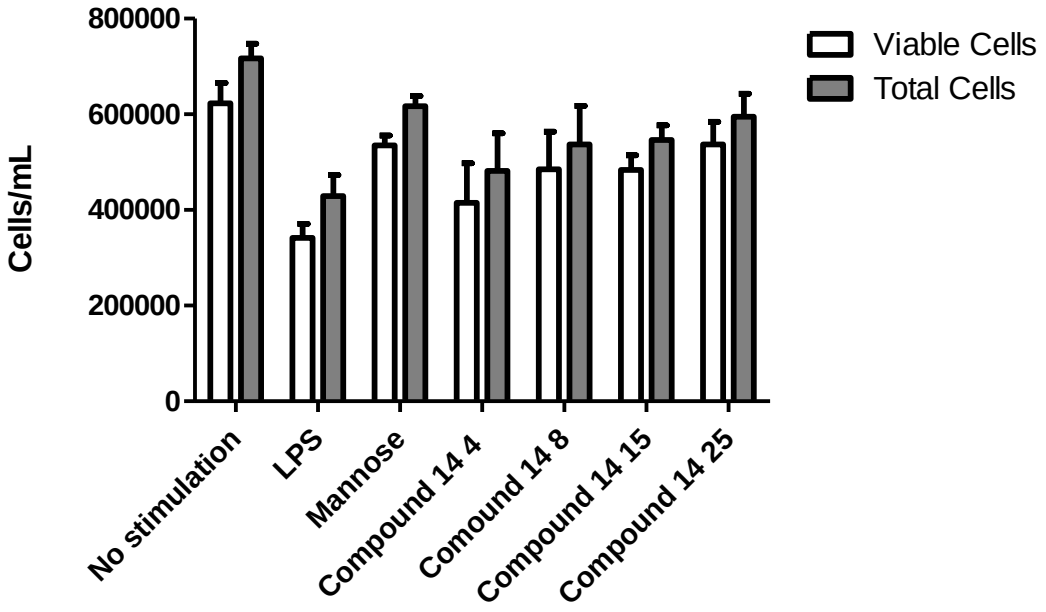

Figure S4. Viability of the JAWS II after 24 hours. JAWS II cells were seeded at a density of 5 x 10^5 cells/500 µL in triplicate using RPMI medium supplemented with 8% FBS, 1% penicillin/streptomycin and 5 ng/mL murine recombinant GM-CSF in 12 well plates. The cells were either unexposed (no stimulation) or exposed to LPS (1 µg/mL), mannose (10 µg/mL), or mannose-lipid at various concentrations (4-, 8-, 15- and 25 µg/mL). After 24 hours the cells were spun at 1200 rpm for 5 minutes to pull down the cells in suspension and the supernatants were collected and stored. The cells were then resuspended in PBS and viability was determined using Trypan Blue (Life Technologies) to discern between the viable and dead cells. Data are shown as ±SEM and are representative of 2 experiments.

Compound 14 was chosen in our test because it's among compound that produced the most promising results (Figure 4). Indeed there was some variability within the groups and between them, but there is no significant difference between the viabilities of the various concentrations of the compound and the unstimulated group as per an ANOVA with a Tukey HSD. There appears to be a trend towards fewer total cells collected 24 hours after the cells were treated and seeded which could be due to various factors. The compound could have activated some downstream signaling that inhibited cell growth as compared to the non-treated control. Some cells may have apoptosed during the 24 hours period which would have also decreased the total number of cells that would have been collected. Despite there being a trend towards fewer cells being collected, there is no change in terms of total viable cells as compared to total cells of the groups that were treated with compound **14** as compared to the untreated group.

7 Supporting references

(1) Mereyala, H. B.; Gurrala, S. R., *Carb. Res.* **1998,** *307*, 351-54.
(2) Wardrop, D. J.; Zhang, W.; Fritz, J., *Org. Lett.* **2002,** *4*, 489-92.