

## Rapid Access to Phospholipid Analogues Using Thiol-Yne Chemistry

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## 1. Characterization Data for Dithioether Phosphatidylcholine 2-5

**1.2 Synthesis of C14:0 dithioether PC 2:** 11 mg of the starting material prop-2-ynyle phosphatidylcholine<sup>1</sup> yields 32 mg **2** as a white solid, in 95% yield. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 4.32 (s, 2H), 4.09 (s, 1H), 3.92 (s, 1H), 3.81 (s, 2H), 3.37 (s, 9H), 2.98 – 2.91 (m, 1H), 2.90 – 2.83 (m, 1H), 2.80 – 2.73 (m, 1H), 2.55 (dd, *J* = 15.0, 7.2 Hz, 4H), 1.63 – 1.49 (m, 4H), 1.34 (s, 4H), 1.24 (s, 40H), 0.87 (t, *J* = 6.8 Hz, 6H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 76.70, 66.48, 59.45, 54.53, 54.53, 54.53, 46.47, 34.52, 33.24, 32.07, 32.07, 31.70, 30.14, 29.97, 29.88, 29.88, 29.88, 29.88, 29.85, 29.85, 29.83, 29.83, 29.80, 29.80, 29.52, 29.52, 29.52, 29.52, 29.23, 29.18, 22.84, 22.84, 14.28, 14.28. HRMS [M+Na]<sup>+</sup> *m/z* calcd. for [C<sub>36</sub>H<sub>76</sub>NO<sub>4</sub>PS<sub>2</sub>Na]<sup>+</sup> 704.4846, found 704.4848.

**1.3 Synthesis of C16:0 dithioether PC 3:** 11 mg of the starting material prop-2-ynyle phosphatidylcholine yields 37 mg **3** as a white solid, in 97 % yield. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 4.31 (s, 2H), 4.07 (s, 1H), 3.91 (s, 1H), 3.81 (s, 2H), 3.38 (s, 9H), 2.97 – 2.89 (m, 1H), 2.88 – 2.83 (m, 1H), 2.79 – 2.73 (m, 1H), 2.53 (dd, *J* = 15.7, 8.0 Hz, 4H), 1.60 – 1.46 (m, 4H), 1.34 (s, 4H), 1.24 (s, 48H), 0.86 (t, *J* = 7.0 Hz, 6H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 76.70, 66.47, 59.45, 54.53, 54.53, 54.53, 46.44, 34.47, 33.21, 32.07, 32.07, 31.70, 30.15, 29.98, 29.91, 29.91, 29.91, 29.91, 29.91, 29.89, 29.89, 29.89, 29.89, 29.89, 29.89, 29.89, 29.83, 29.83, 29.83, 29.83, 29.54, 29.54, 29.53, 29.53, 29.25, 29.21, 22.84, 22.84, 14.28, 14.28. HRMS [M+Na]<sup>+</sup> *m/z* calcd. for [C<sub>40</sub>H<sub>84</sub>NO<sub>4</sub>PS<sub>2</sub>Na]<sup>+</sup> 760.5472, found 760.5473.

**1.4 Synthesis of C18:0 dithioether PC 4:** 25 mg of the starting material prop-2-ynyle phosphatidylcholine yields 83 mg **4** as a white solid, in 91% yield. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 4.30 (s, 2H), 4.08 (s, 1H), 3.96 (s, 1H), 3.79 (s, 2H), 3.37 (s, 9H), 2.98 – 2.89 (m, 1H), 2.88 – 2.83 (m, 1H), 2.79 – 2.73 (m, 1H), 2.61 – 2.45 (m, 4H), 1.60 – 1.46 (m, 4H), 1.34 (s, 4H), 1.24 (s, 56H), 0.86 (t, *J* = 7.0 Hz, 6H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 76.70, 66.50, 59.44, 54.52, 54.52, 54.52, 46.50, 34.56, 33.28, 32.06, 32.06, 31.69, 30.13, 29.97, 29.89, 29.89, 29.89, 29.89, 29.89, 29.89, 29.89, 29.86, 29.86, 29.86, 29.86, 29.86, 29.86, 29.81, 29.81, 29.81, 29.81, 29.80, 29.80, 29.51, 29.51, 29.51, 29.51, 29.22, 29.17, 22.83, 22.83, 14.27, 14.27. HRMS [M+Na]<sup>+</sup> *m/z* calcd. for [C<sub>44</sub>H<sub>92</sub>NO<sub>4</sub>PS<sub>2</sub>Na]<sup>+</sup> 816.6098, found 816.6099.

**1.5 Synthesis of C18:1 dithioether PC 5:** 8 mg of the starting material prop-2-ynyle phosphatidylcholine yields 17.6 mg **5** as a waxy solid, in 61 % yield. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 5.37 (s, 3H), 5.34 (s, 1H), 4.34 (s, 2H), 4.08 (s, 1H), 3.94 (s, 1H), 3.81 (s, 2H), 3.39 (s, 9H), 3.01 – 2.93 (m, 1H), 2.89 (dd, *J* = 13.1, 6.6 Hz, 1H), 2.78 (dd, *J* = 13.2, 6.0 Hz, 1H), 2.54 (dd, *J* = 15.8, 8.2 Hz, 4H), 1.97 (dt, *J* = 11.7, 6.6 Hz, 8H), 1.58 – 1.51 (m, 4H), 1.34 – 1.21 (m, 44H), 0.87 (t, *J* = 7.0 Hz, 6H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>) δ 130.55, 130.37, 130.09, 129.90, 76.72, 66.66, 59.40, 54.71, 54.71, 54.71, 46.58, 34.67, 33.37, 32.78, 32.78, 32.05, 32.05, 31.71, 31.13, 30.12, 29.97, 29.94, 29.92, 29.83, 29.83, 29.81, 29.81, 29.68, 29.65, 29.65, 29.63, 29.48, 29.48, 29.48, 29.48, 29.46, 29.46, 29.36, 29.36, 29.34, 29.34, 29.22, 29.16, 27.37, 22.83, 22.83, 14.27, 14.27. HRMS [M+Na]<sup>+</sup> *m/z* calcd. for [C<sub>44</sub>H<sub>88</sub>NO<sub>4</sub>PS<sub>2</sub>Na]<sup>+</sup> 812.5785, found 812.5784.

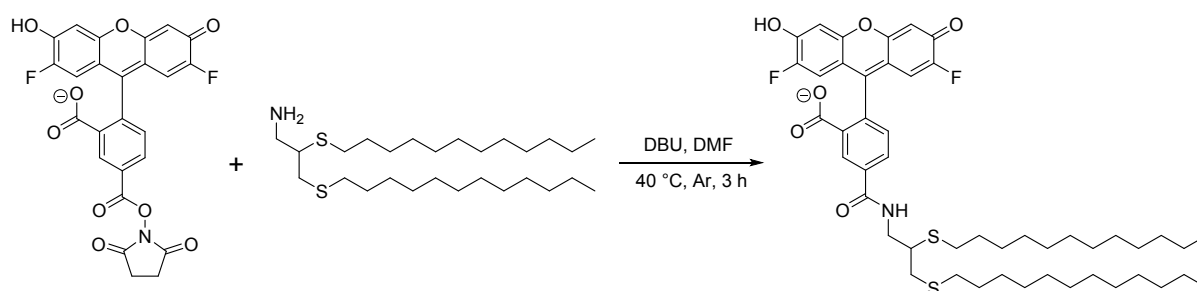
## 2. General Procedure for Synthesis of Dithioether Glucopyranosyl Lipid

In a 10 mL microwave reaction tube, the alkyne-modified glucose and alkylthiol were sonicated under N<sub>2</sub> protection for 60 min to dissolve in 3 mL DMF. The mixture was then transferred to a 3500  $\mu$ L macro fluorescence cuvette and 2,2-dimethoxy-2-phenylacetophenone (2 mg, 0.008 mmol) was added. The reaction proceeded under UV (354 nm) for 10 minutes. The solvent was removed and the residue was purified by column chromatography.

**2.1** Synthesis of Acetyl-Protected Glucopyranosyl Lipid **6**: 40 mg of the starting material 2-propynyl-tetra-*O*-acetyl- $\beta$ -D-glucopyranoside (Sigma-Aldrich, St. Louis, MO) affords 78 mg **6** as white solid after silica column chromatography (DCM : MeOH = 20 : 1). Yield: 79%. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  5.19 (td,  $J$  = 9.5, 3.2 Hz, 1H), 5.07 (td,  $J$  = 9.7, 4.0 Hz, 1H), 4.99 (ddd,  $J$  = 9.7, 8.0, 6.4 Hz, 1H), 4.52 (d,  $J$  = 8.0 Hz, 1H), 4.26 (dd,  $J$  = 12.3, 4.7 Hz, 1H), 4.15 – 4.03 (m, 2H), 3.73 – 3.57 (m, 2H), 2.91 (ddd,  $J$  = 13.3, 7.2, 5.2 Hz, 1H), 2.77 (dtd,  $J$  = 17.8, 13.3, 6.7 Hz, 2H), 2.56 – 2.48 (m, 4H), 2.08 (s, 3H), 2.05 (d,  $J$  = 2.6 Hz, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.57 – 1.52 (m, 4H), 1.37 – 1.32 (m, 4H), 1.22 (d,  $J$  = 18.3 Hz, 56H), 0.86 (t,  $J$  = 6.9 Hz, 6H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  179.80, 170.79, 170.42, 170.39, 169.54, 169.52, 169.43, 169.38, 101.38, 101.05, 72.87, 72.76, 71.92, 71.87, 71.59, 71.24, 71.23, 68.43, 61.99, 45.76, 45.24, 34.60, 34.58, 33.54, 33.21, 32.05, 32.05, 32.02, 31.74, 30.03, 30.00, 29.87, 29.83, 29.83, 29.83, 29.83, 29.83, 29.83, 29.83, 29.83, 29.83, 29.81, 29.81, 29.79, 29.79, 29.76, 29.75, 29.69, 29.69, 29.67, 29.49, 29.49, 29.42, 29.40, 29.39, 29.07, 29.03, 29.03, 22.82, 22.82, 20.91, 20.89, 20.75, 20.73, 14.27, 14.27. HRMS [M+Na]<sup>+</sup>  $m/z$  calcd. for [C<sub>53</sub>H<sub>98</sub>O<sub>10</sub>S<sub>2</sub>Na]<sup>+</sup> 981.6494, found 981.6488.

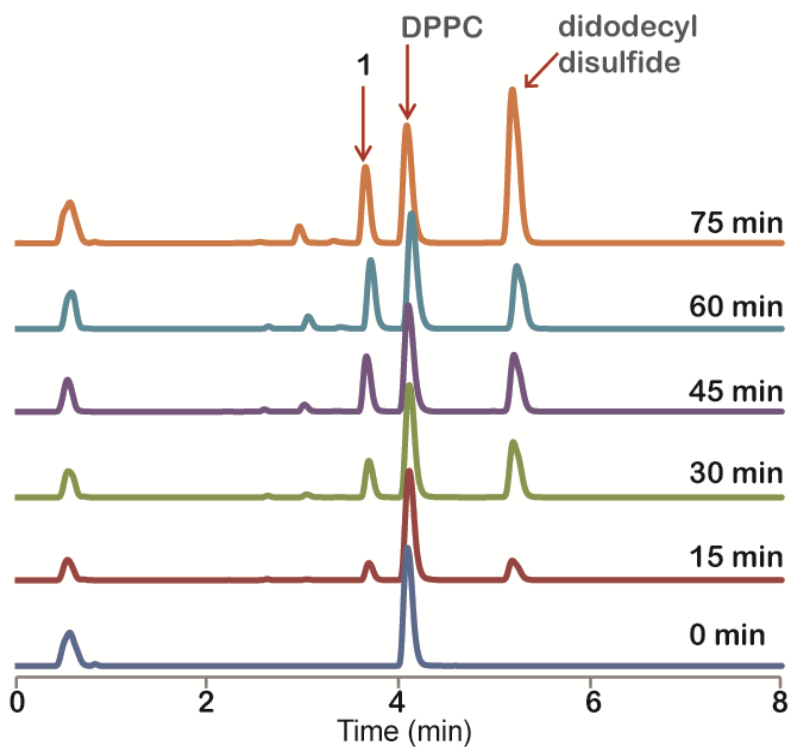
**2.2** Synthesis of Protection-Free Glucopyranosyl Lipid **7**: 11 mg of the starting material 2-propynyl- $\beta$ -D-glucopyranoside<sup>2</sup> affords 28.8 mg **7** as white solid after silica column chromatography (DCM : MeOH : H<sub>2</sub>O = 20 : 1 : 0.1). Yield: 85%. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  4.87 (s, 1H), 4.67 (s, 1H), 4.33 (d,  $J$  = 7.5 Hz, 1H), 4.07 (ddd,  $J$  = 16.4, 10.3, 5.3 Hz, 1H), 3.84 (s, 2H), 3.74 (ddd,  $J$  = 17.0, 10.3, 5.9 Hz, 1H), 3.64 – 3.51 (m, 2H), 3.44 – 3.38 (m, 1H), 3.31 (dd,  $J$  = 9.0, 2.8 Hz, 1H), 3.01 – 2.96 (m, 1H), 2.92 – 2.76 (m, 2H), 2.56 (dt,  $J$  = 14.9, 7.5 Hz, 4H), 2.45 (s, 1H), 1.57 (dt,  $J$  = 15.0, 7.4 Hz, 4H), 1.35 (s, 4H), 1.31 – 1.19 (m, 40H), 0.87 (t,  $J$  = 6.9 Hz, 6H). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>)  $\delta$  103.43;103.21, 76.34;76.29, 75.76, 73.53;73.47, 71.41;71.13, 69.60;69.52, 61.71;61.62, 45.76;45.63, 36.70, 34.82;34.69, 33.40;33.38, 32.08, 32.08, 31.64;31.62, 30.03;30.01, 29.89, 29.89, 29.89, 29.89, 29.89, 29.86, 29.86, 29.84, 29.84, 29.79, 29.79, 29.54, 29.54, 29.52, 29.52, 29.23;29.21, 29.16, 22.85, 22.85, 14.29, 14.29. HRMS [M-H]<sup>-</sup>  $m/z$  calcd. for [C<sub>37</sub>H<sub>74</sub>O<sub>6</sub>S<sub>2</sub>]<sup>-</sup> 677.4854, found 677.4858.

### 3. Synthesis of Oregon Green C12:0 Dithioether Lipid



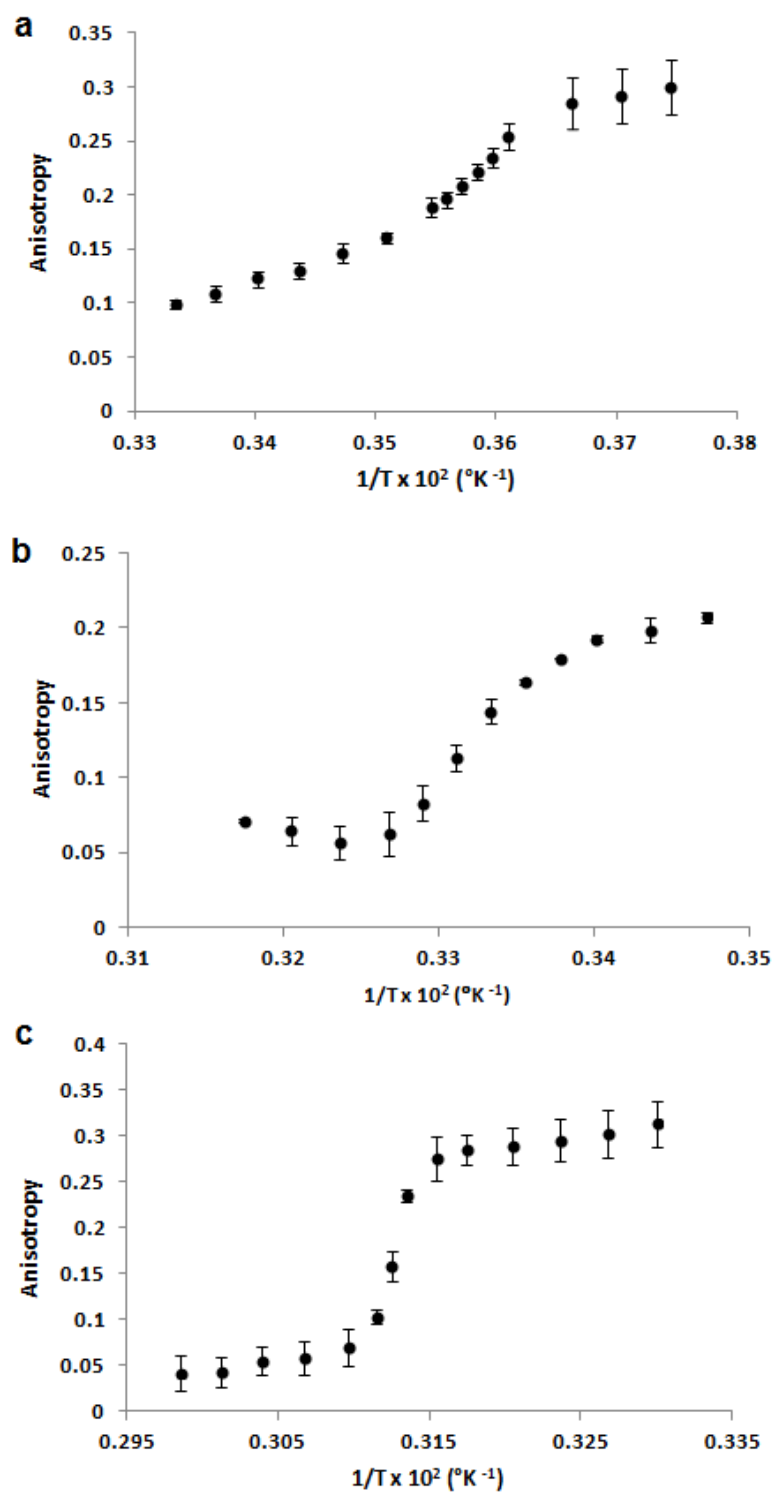
Synthesis of 5-((2,3-bis(dodecylthio)propyl)carbamoyl)-2-(2,7-difluoro-3-hydroxy-6-oxo-6,9a-dihydro-1H-xanthen-9-yl)benzoic acid **8**: 2,3-bis(dodecylthio)propan-1-amine<sup>3</sup> (2.3 mg, 5  $\mu$ mol) was dissolved in 50  $\mu$ L anhydrous DMF. DBU (1.2 mg, 7.5  $\mu$ mol) was added to the mixture and allowed to stir at room temperature for 10 min. A solution of 50  $\mu$ L of 50 mM Oregon Green 488 succinimidyl ester (Molecular Probes, Eugene, OR) (1.3 mg, 2.5  $\mu$ mol) in DMF was added. The mixture was stirred at 40 °C for 3 h under argon. DMF was removed *in vacuo* and the residue was purified by HPLC. 1.8 mg of **8** was isolated as an orange solid in 84% yield. <sup>1</sup>H NMR (500 MHz, Methanol-*d*<sub>4</sub>)  $\delta$  8.48 (s, 1H), 8.22 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 6.84 (d, *J* = 7.4 Hz, 2H), 6.46 (d, *J* = 10.9 Hz, 2H), 3.78 (dd, *J* = 13.7, 6.5 Hz, 1H), 3.56 (dd, *J* = 13.7, 7.4 Hz, 1H), 3.16 (p, *J* = 6.9 Hz, 1H), 2.90 – 2.77 (m, 2H), 2.65 (td, *J* = 7.2, 2.8 Hz, 2H), 2.60 (t, *J* = 7.3 Hz, 2H), 1.65 – 1.55 (m, 4H), 1.46 – 1.34 (m, 4H), 1.25 (d, *J* = 6.5 Hz, 32H), 0.87 (td, *J* = 7.0, 2.6 Hz, 6H). HRMS [M-H]<sup>-</sup> *m/z* calcd. for [C<sub>48</sub>H<sub>64</sub>F<sub>2</sub>NO<sub>6</sub>S<sub>2</sub>]<sup>-</sup> 852.4149, found 852.4144. HPLC gradient: 0 min – 2.5 min 85% Phase B in Phase A, 2.5 min – 18 min 85% Phase B in Phase A to 100% Phase B, 18 min – 28 min 100% Phase B, 28 min – 32 min 85% Phase B in Phase A (Phase A: H<sub>2</sub>O with 0.1% formic acid, Phase B: MeOH with 0.1% formic acid).

#### 4. HPLC Traces for Lipid Driven Formation of Dithioether PC



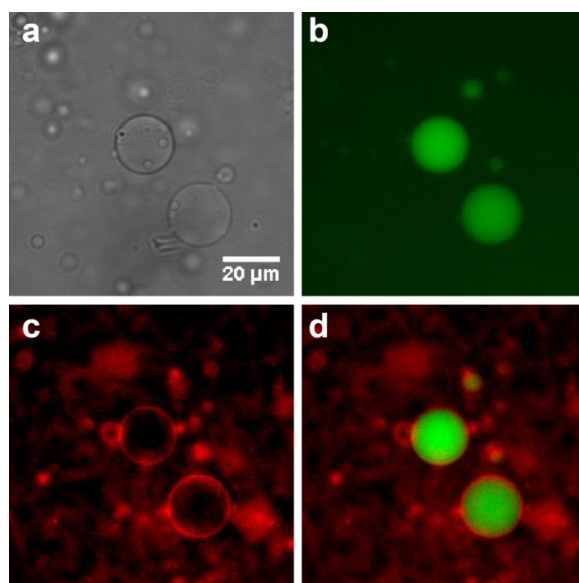
**Figure S1.** Progression of lipid **1** formation in 35 mM DPPC solution monitored by HPLC-ELSD. Concentration of **1** calculated by known standard calibration. The amount of DPPC addition is crucial for high conversion of dithioether phospholipid. When the DPPC concentration is lower than 25 mM, the conversion is slow. Prolonged exposure to the UV light generates disulfide byproduct thus halting the reaction. When the DPPC concentration is higher than 50 mM, the solution is crowded with DPPC thus limiting conversion. We have also observed addition of TCEP, a reducing agent, prevents this reaction.

#### 4. Dithioether PC Anisotropy Measurements

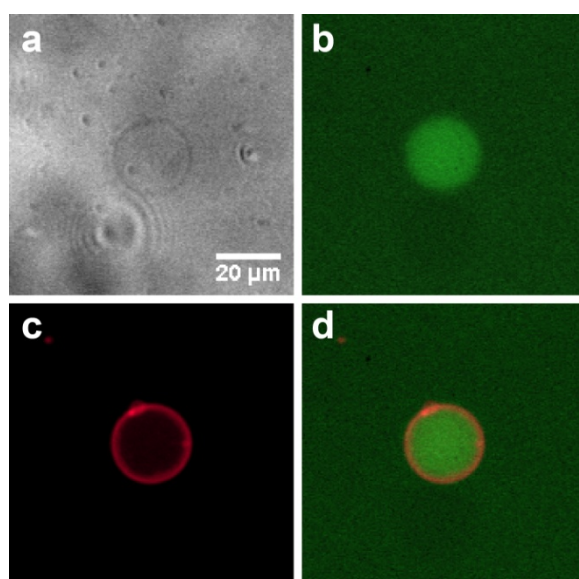


**Figure S2.** Steady-state fluorescence anisotropy curve as a function of temperature for saturated dithioether PCs. a) C12:0 lipid **1** b) C14:0 lipid **2** and c) C16:0 lipid **3**. Error bars denote SD of three measurements.

## 5. Fluorescence Microscopy Images of Giant Unilamellar Vesicles Formation



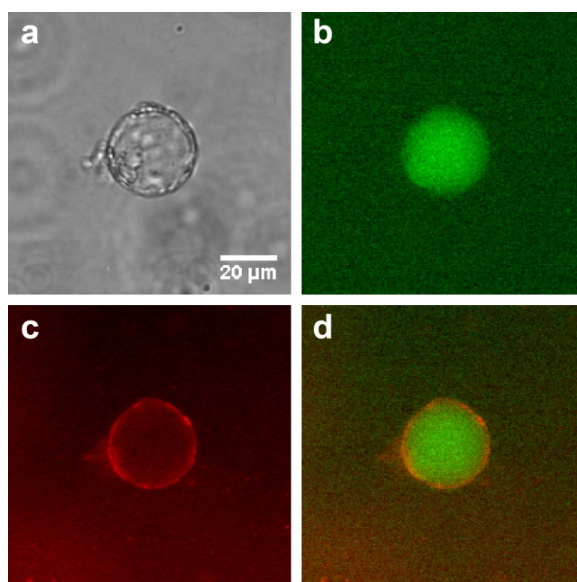
**Figure S3.** Fluorescence microscopy images of the GUVs made of C12:0 dithioether PC **1**. a) Brightfield. b) GFP channel illustrating HPTS encapsulation in the GUVs. c) DesRed channel illustrating the staining of the lipid membrane with Texas Red DHPE. d) Merge of GFP and DesRed channels.



**Figure S4.** Fluorescence microscopy images of the GUVs made of C18:1 dithioether PC **5**. a) Brightfield. b) GFP channel illustrating HPTS encapsulation in the GUVs c) DesRed channel illustrating the staining of the lipid membrane with Texas Red DHPE. d) Merge of GFP and DesRed channels.

## 6. Cell-Free Expression of GFP in Dithioether PC GUVs

C18:0 dithioether PC 5 GUVs were formed with aforementioned procedure<sup>4</sup> In a 2 mL vial 40  $\mu\text{L}$  of 10 mM 5 solution in chloroform was dried under  $\text{N}_2$  to form a lipid film. 200  $\mu\text{L}$  of light mineral oil was added to the vial and the mixture was sonicated for 1 hour until the lipid was fully dissolved in the oil. In a 0.7 mL eppendorf tube, 1  $\mu\text{g}/50 \mu\text{L}$  pBEST-OR2-OR1-Pr-UTR1-deGFP-T500 Plasmid DNA<sup>5</sup> (Addgene, Cambridge, MA) in 10  $\mu\text{L}$  *E. coli* cell lysates, S30 T7 high yield protein expression system (Promega, Madison, WI) was added to 100  $\mu\text{L}$  of mineral oil containing lipid. The mixture was then flicked to form an emulsion. The emulsion was gently layered on top of 100  $\mu\text{L}$  of lower buffer (100 mM HEPES, 200 mM glucose, in  $\text{H}_2\text{O}$ , pH = 7.4) in a different tube and the mixture was centrifuged at 10,000 ref for 5 min. Light mineral oil was removed by vacuum suction and the vesicle solution was obtained. Upon the formation of GUVs, the sample was incubated at 37  $^\circ\text{C}$  for 4 h. 0.1  $\mu\text{L}$  of 100  $\mu\text{M}$  Texas Red DHPE solution in EtOH was added to 10  $\mu\text{L}$  vesicle solution prior to microscope observation.

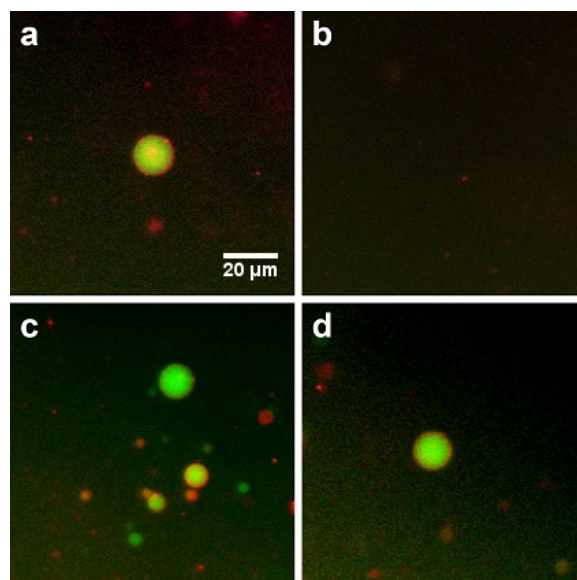


**Figure S5.** Fluorescence microscopy images of the GUVs made of C18:1 dithioether PC 5. (a) Brightfield (b) GFP channel illustrating GFP expression after 4 h of incubation. (c) DesRed channel illustrating the staining of the lipid membrane with Texas Red DHPE. (d) Merge of GFP and DesRed channels.



## 7. Dithioether PC GUV Stability against PLA<sub>2</sub>

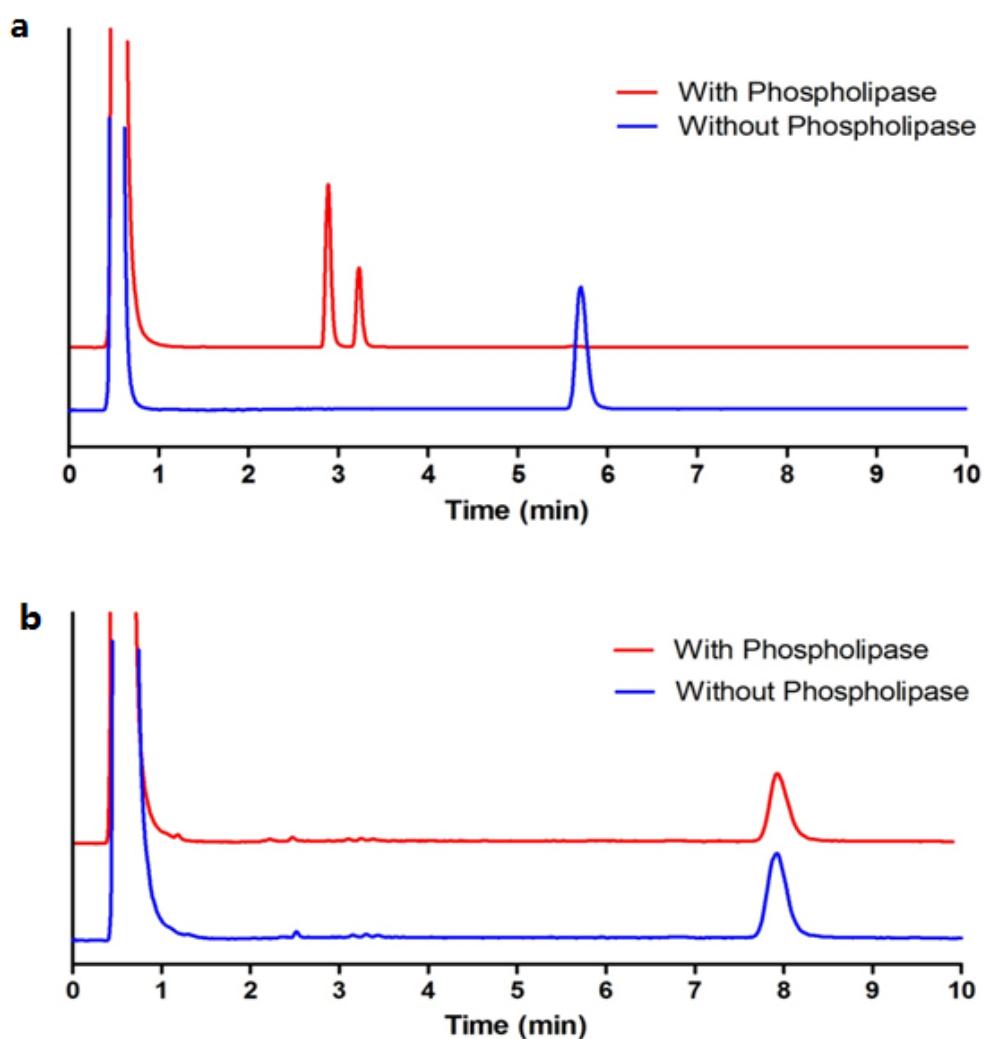
GUVs were formed and characterized using aforementioned procedure<sup>4</sup>. In a 2 mL vial 40  $\mu\text{L}$  of 10 mM lipid solution in chloroform was dried under N<sub>2</sub> to form a lipid film. 200  $\mu\text{L}$  of light mineral oil was added to the vial and the mixture was sonicated for 1 hour until the lipid was fully dissolved in the oil. In a 0.7 mL Eppendorf tube 10  $\mu\text{L}$  of the upper buffer (100 mM HEPES, 200 mM sucrose, 10 mM CaCl<sub>2</sub>, 35 mM KCl, 1 mM HPTS in H<sub>2</sub>O, pH = 7.4) was added to 100  $\mu\text{L}$  of mineral oil containing lipid. The mixture was then flicked to form an emulsion. The emulsion was gently layered on top of 100  $\mu\text{L}$  of lower buffer (100 mM HEPES, 200 mM glucose, 10 mM CaCl<sub>2</sub>, 35 mM KCl in H<sub>2</sub>O, pH = 7.4) in a different tube and the mixture was centrifuged at 10,000 rcf for 5 min. Light mineral oil was removed by vacuum suction and the vesicle solution was obtained. 0.1  $\mu\text{L}$  of 100  $\mu\text{M}$  Texas Red DHPE solution in EtOH was added to 10  $\mu\text{L}$  vesicle solution prior to microscope observation. Bee venom PLA<sub>2</sub> (Sigma-Aldrich, St. Louis, MO) was added to the samples to give a final concentration of 10  $\mu\text{g}/\text{mL}$ . Fluorescence microscopy images before and after the treatment of PLA<sub>2</sub> were obtained.



**Figure S6.** Dithioether PC GUVs shows resistance against PLA<sub>2</sub> hydrolysis, while DMPC GUVs were fully hydrolyzed after the treatment. Fluorescence microscopy images of merged DesRed and GFP channels illustrating DMPC GUVs after undergoing treatment with a) 0  $\mu\text{g}/\text{mL}$  PLA<sub>2</sub> and b) 10  $\mu\text{g}/\text{mL}$  PLA<sub>2</sub>. Fluorescence microscopy images of merged DesRed and GFP channels illustrating C14:0 dithioether phospholipid **2** GUVs after undergoing treatment with c) 0  $\mu\text{g}/\text{mL}$  PLA<sub>2</sub> and d) 10  $\mu\text{g}/\text{mL}$  PLA<sub>2</sub>.

## 8. HPLC Analysis of Dithioether PC Stability against PLA<sub>2</sub>

2 mM lipid (DOPC or **5**) was suspended in 100  $\mu$ L of 100 mM HEPES buffer (pH = 7.4) containing 10 mM CaCl<sub>2</sub> and 35 mM KCl. 10  $\mu$ g/mL PLA<sub>2</sub> was added and the mixture was gently shaken for 15 min. The mixture was diluted with methanol and subject to HPLC analysis. ELSD and MS signals were simultaneously monitored to confirm the identity of the compound. HPLC analysis was carried out on an Eclipse Plus C8 analytical column. HPLC gradient: 0 min – 1 min 50% Phase A in Phase B to 5% Phase A in Phase B, 1 min – 9 min, 5% Phase A in Phase B, 9 min – 10 min, 5% Phase A in Phase B to 50% Phase A in Phase B. (Phase A: H<sub>2</sub>O with 0.1% formic acid, Phase B: MeOH with 0.1% formic acid).

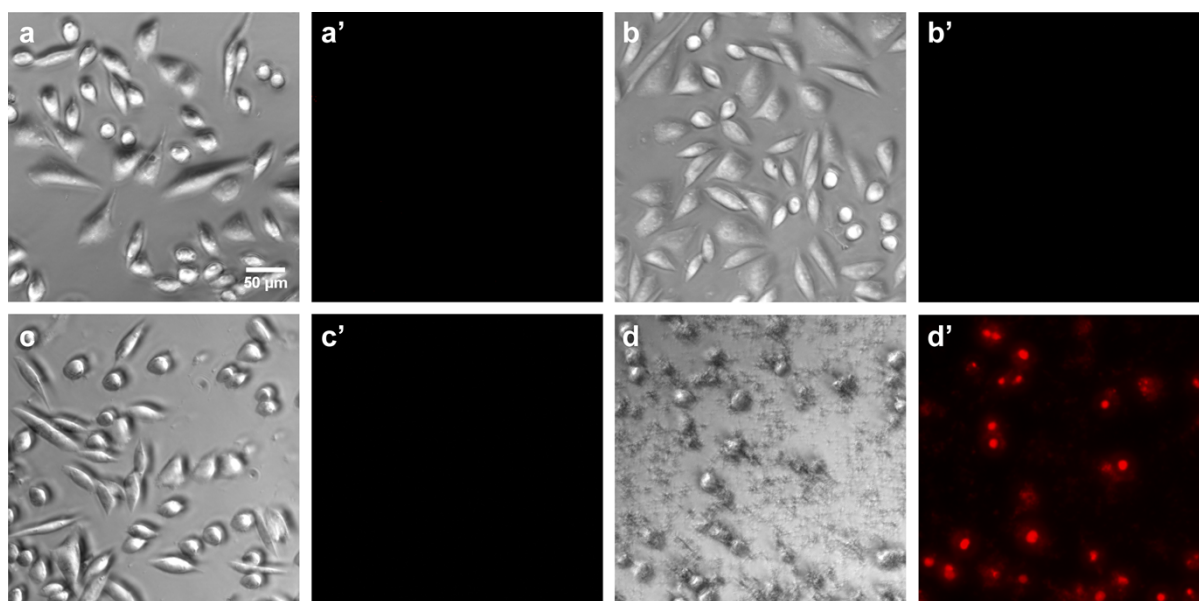


**Figure S7.** HPLC-ELSD signals for PLA<sub>2</sub> treatment of DOPC and C18:1 dithioether PC **5**. a) DOPC before (blue) and after (red) the treatment of 10  $\mu$ g/ml PLA<sub>2</sub>. Retention time for DOPC is 5.7 min. The hydrolysis of DOPC by PLA<sub>2</sub> afford C18:1 lyso PC (2.9 min) and oleic acid (3.2 min). b) **5** before (blue) and after (red) the treatment of 10  $\mu$ g/ml PLA<sub>2</sub>. No hydrolysis was observed. Retention time for C18:1 dithioether PC **5** is 7.9 min.

## 10. Live Cell Imaging

Chinese hamster ovary (CHO) cells were incubated in a 96 well tissue culture plate in cDMEM medium (10% fetal bovine serum, 1% penicillin/streptomycin) at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub> for 24 h to adhere to the plate. After allowing the cells to adhere, the media was aspirated. The cells were incubated in cDMEM and 5 μM Texas Red DHPE and 5 μM **8** for 1 h at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub> protected from light. The media was aspirated, and the cells were washed twice with HBSS (Invitrogen, Carlsbad, CA) before imaging.

## 11. Cell Viability Study



**Figure S8.** Membrane integrity assay by DNA staining dye propidium iodide. CHO cells were incubated with different additives for 24 h, followed by 1 h incubation with propidium iodide. The brightfield images indicated that the cells with a) no additives as control, b) 500 μg/ml POPC and c) 500 μg/ml **5** were adherent to the surface. The cells treated with d) Lipofectamine 2000 were fully ruptured. Images to the right of each brightfield image **a'-d'** are the same samples in the DesRed channel visualizing propidium iodide stain. Cells with no fluorescent stains in **a'**, **b'** and **c'** suggest that they are fully alive. Conversely, the cells in **d'** are fluorescent indicating that they are dead.

## **12. Supplementary Videos**

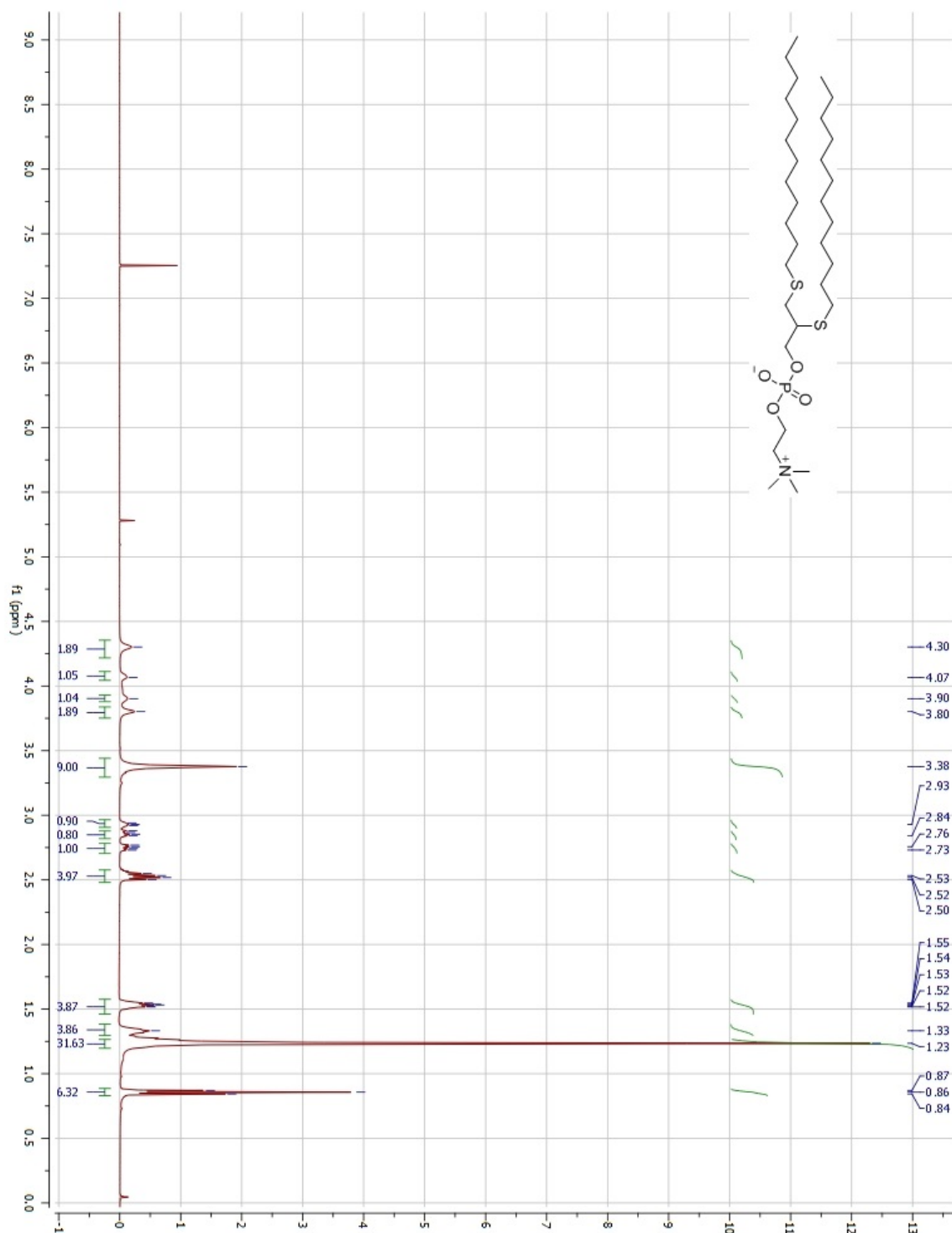
**12.1 Video 1. Shrinkage of a GUV encapsulated with GFP.** A GUV shrinkage experiment was performed as described in the experimental section. Fluorescence microscopy imaging demonstrates that after treating the vesicles with PLA<sub>2</sub>, the GUV begins to shrink as the DOPC is hydrolyzed and removed from membrane. GFP readily leaks out of the GUV. The real-time duration captured in the video recording is 21.3 min.

**12.2 Video 2. Shrinkage of a GUV encapsulated with 100 nm red fluorescent sulfate-modified polystyrene nanospheres.** A GUV shrinkage experiment was performed as described in the experimental section. Fluorescence microscopy imaging demonstrates that after treating the vesicles with PLA<sub>2</sub>, the GUV begins to shrink as the DOPC is hydrolyzed and removed from membrane. 100 nm red fluorescent sulfate-modified polystyrene nanospheres are contained and concentrated in the GUV. The real-time duration captured in the video recording is 17 min.

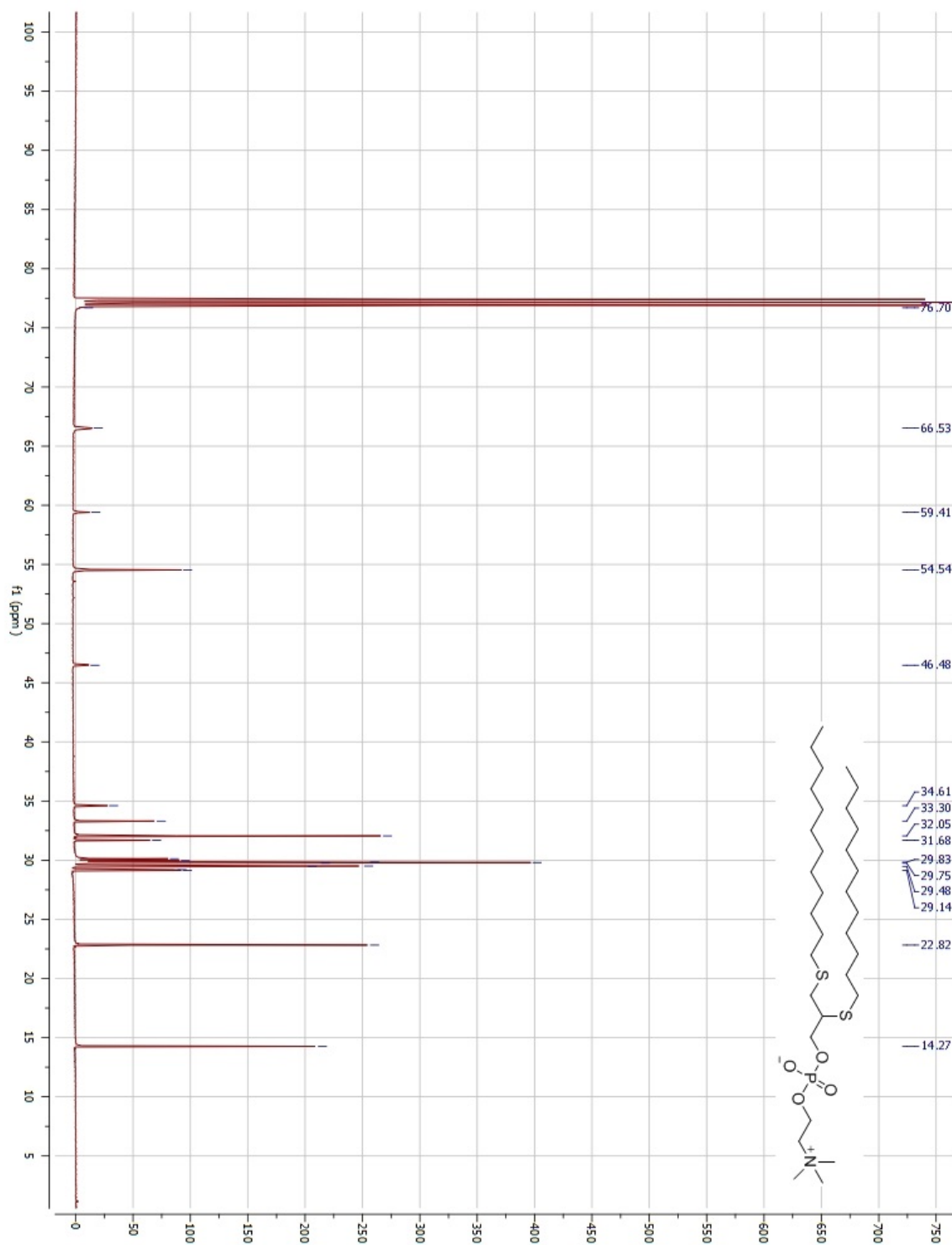
### 13. NMR Spectra for Synthesized Material

#### 13.1 C12:0 dithioether PC1

<sup>1</sup>H NMR

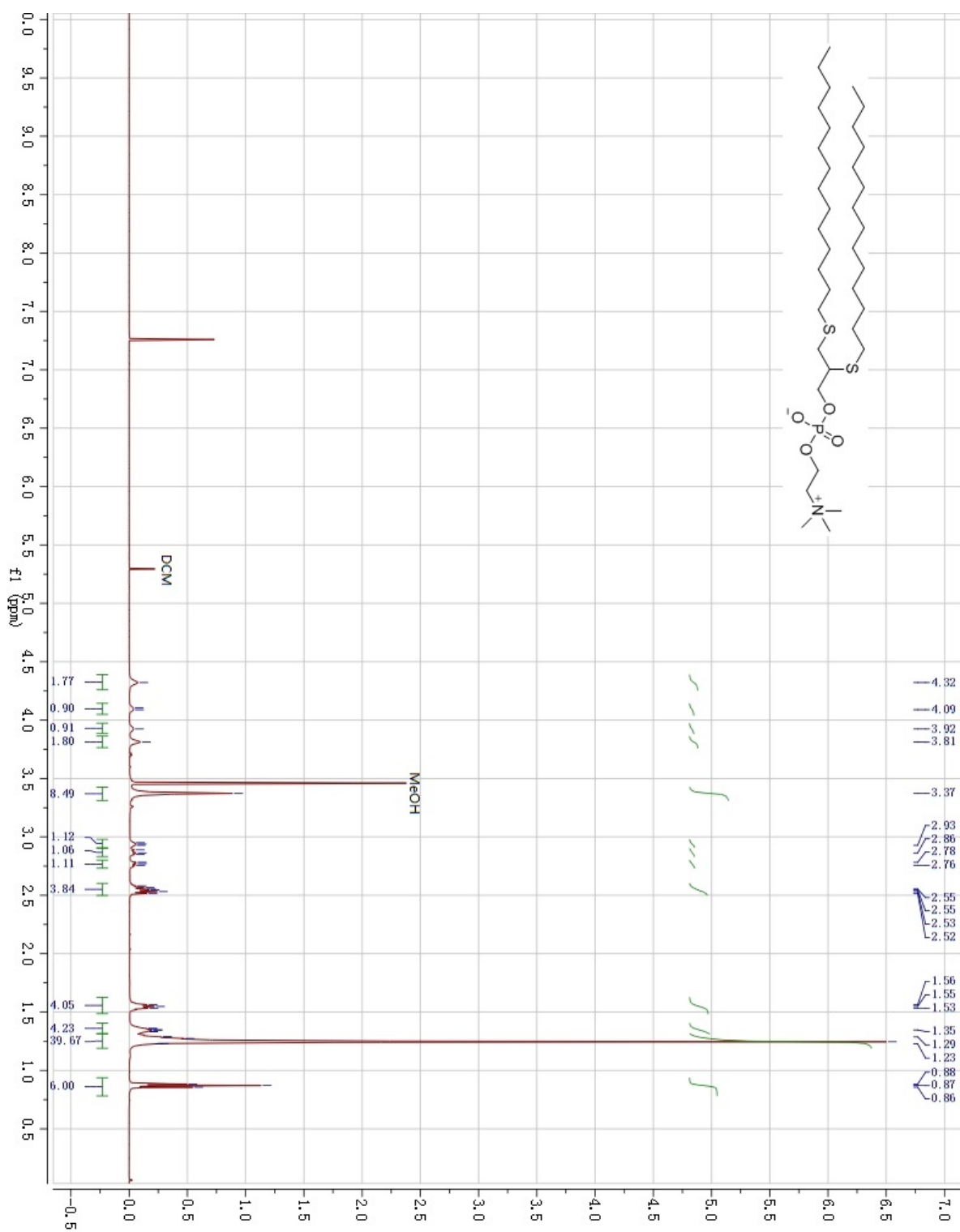


$^{13}\text{C}$  NMR

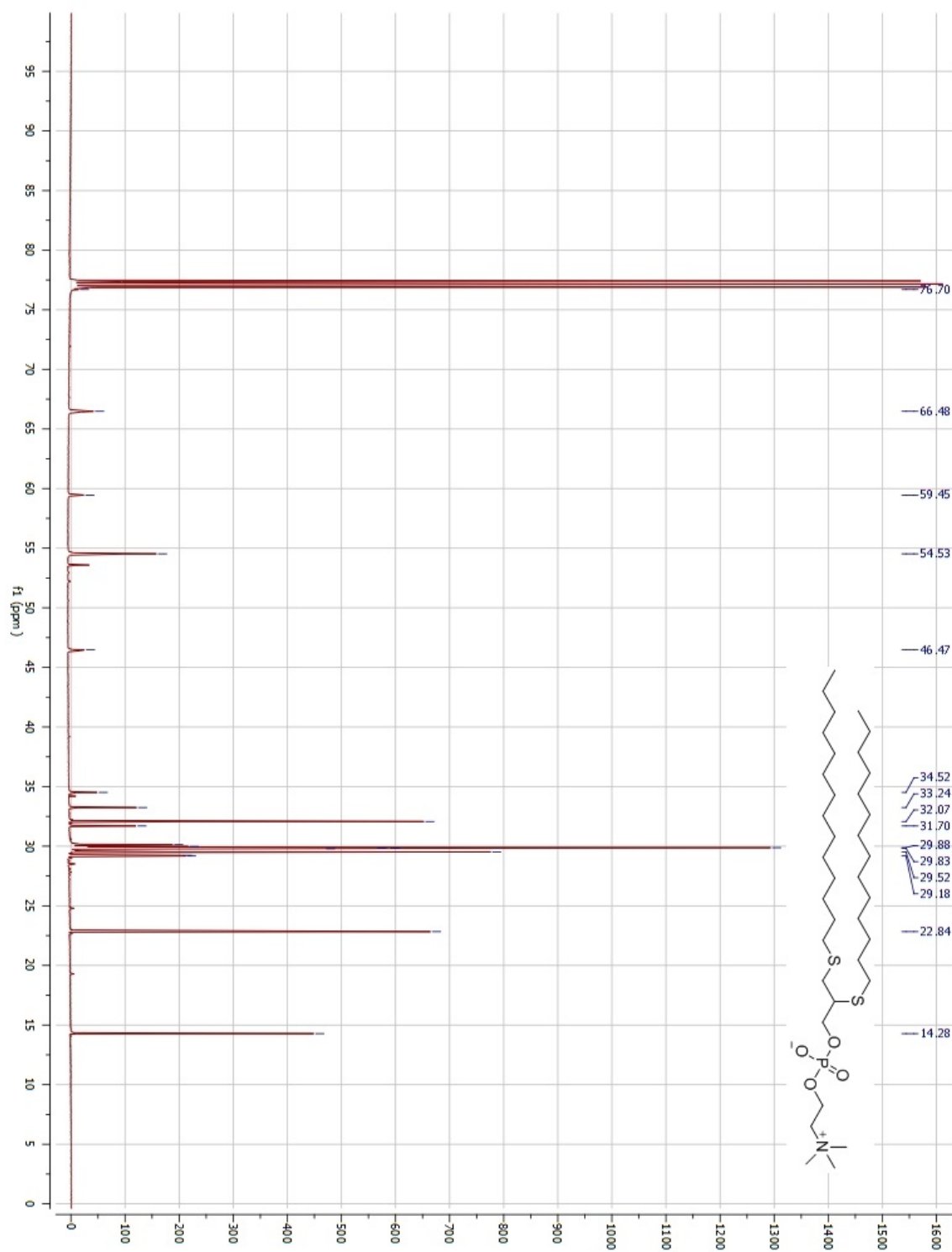


### 13.2 C14:0 dithioether PC 2

<sup>1</sup>H NMR



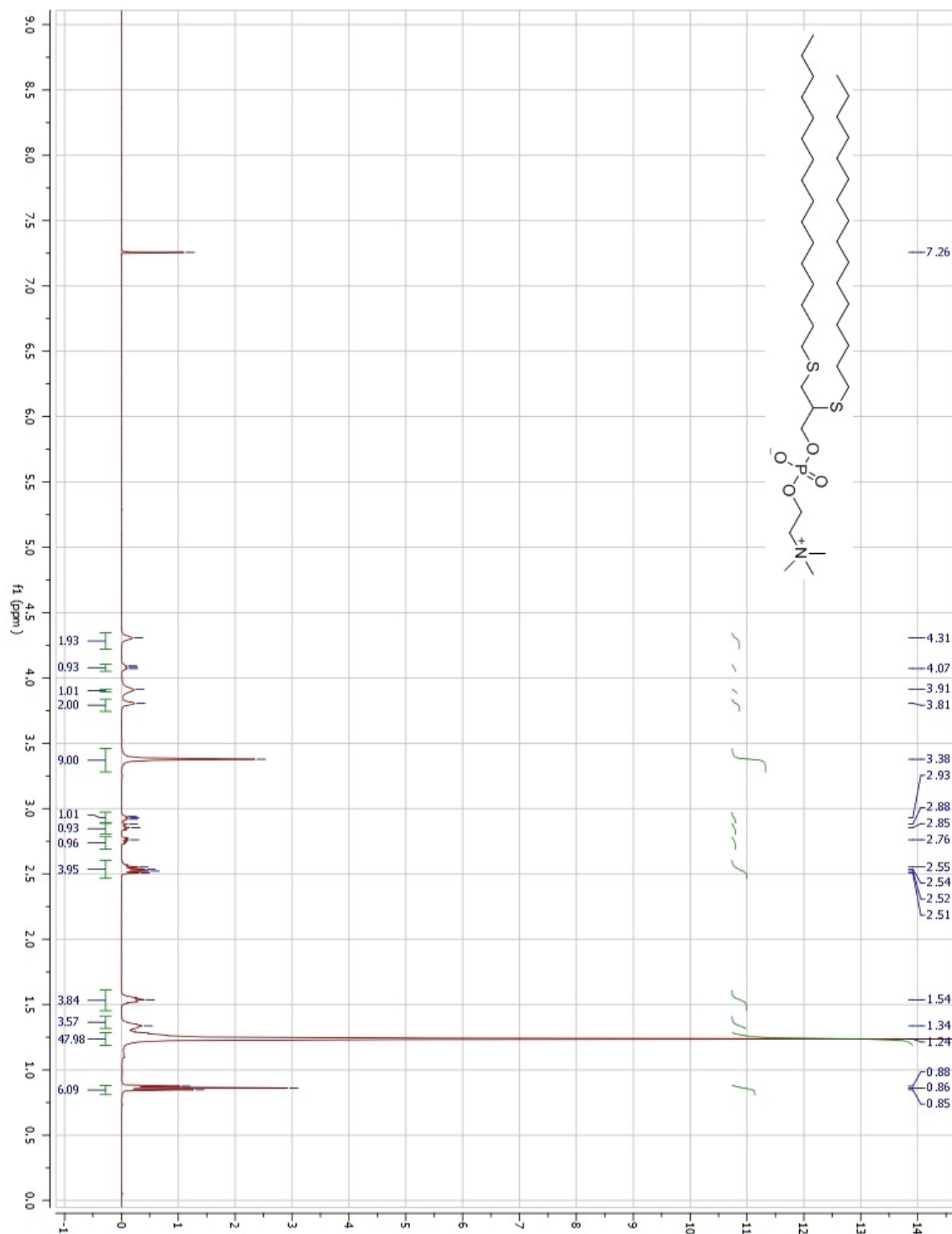
$^{13}\text{C}$  NMR



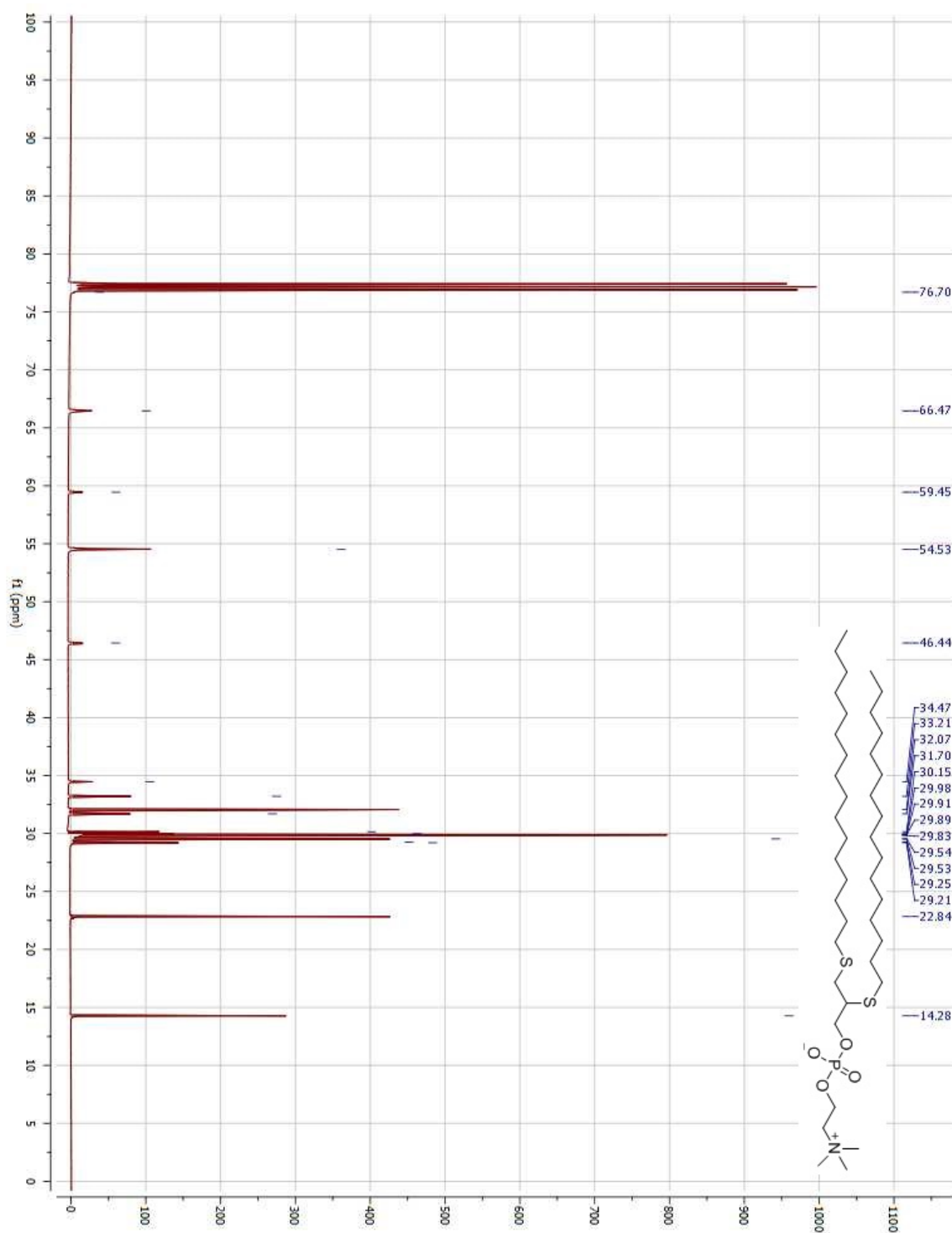


### 13.3 C16:0 dithioether PC 3

$^1\text{H NMR}$

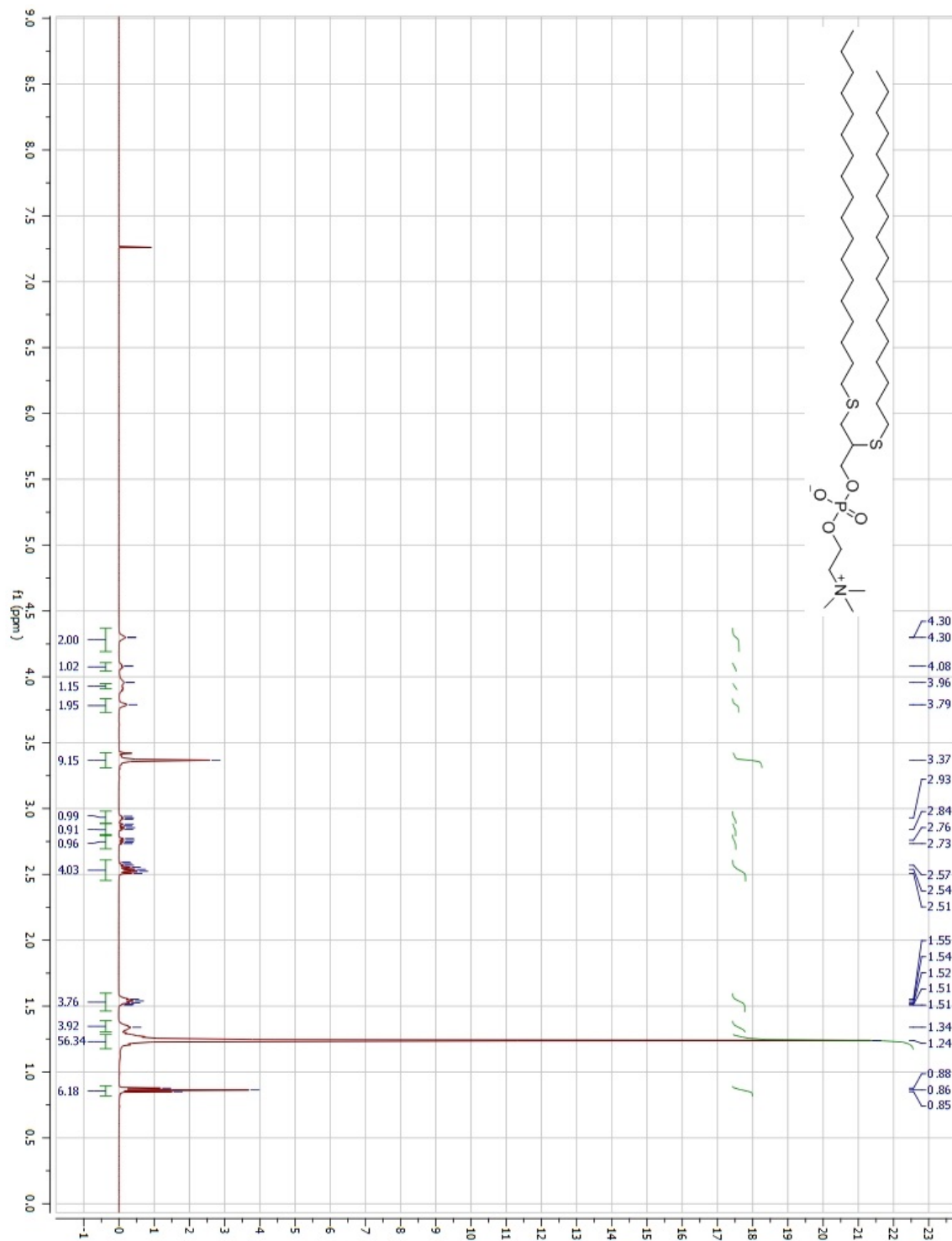


<sup>13</sup>C NMR

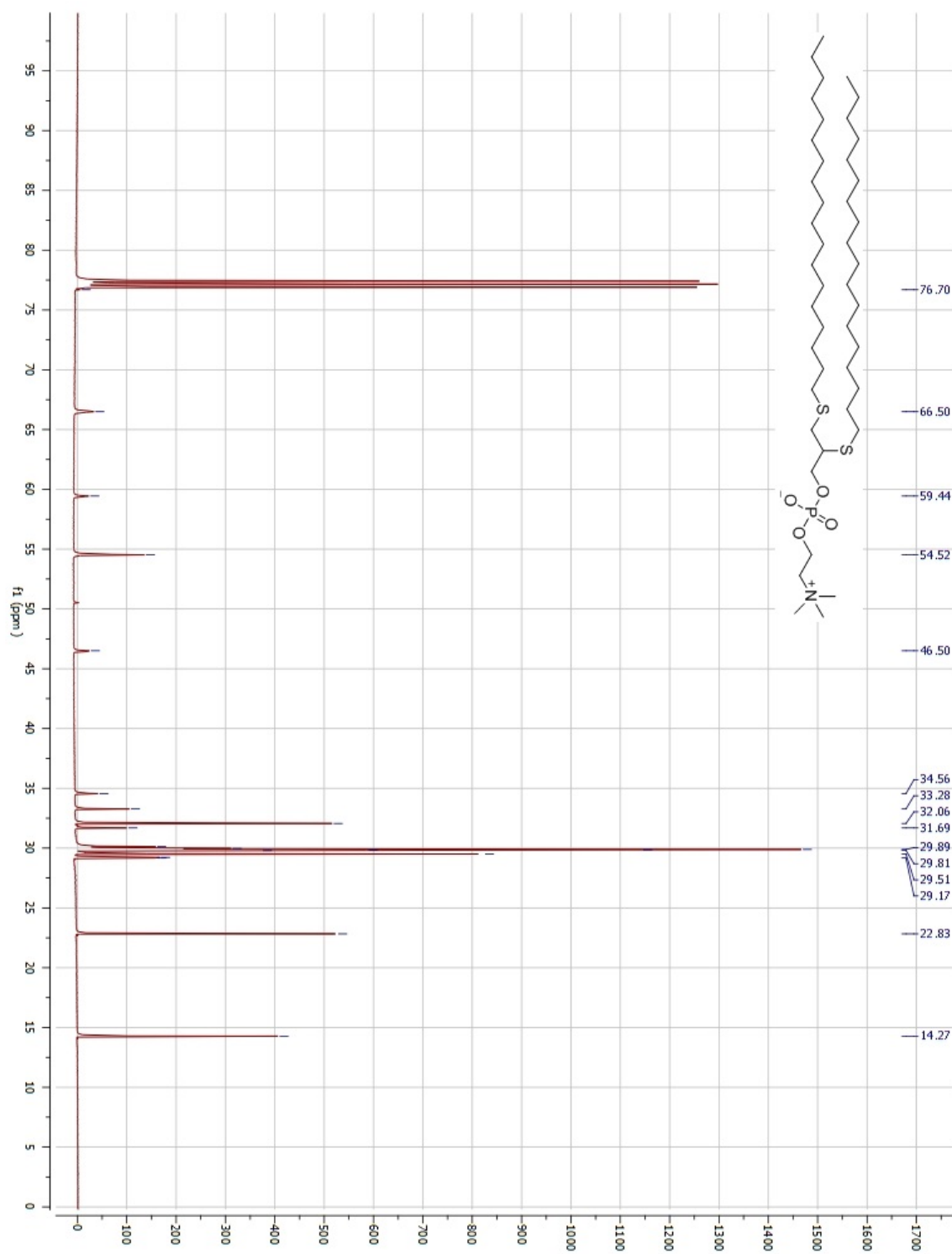


### 13.4 C18:0 dithioether PC 4

<sup>1</sup>H NMR

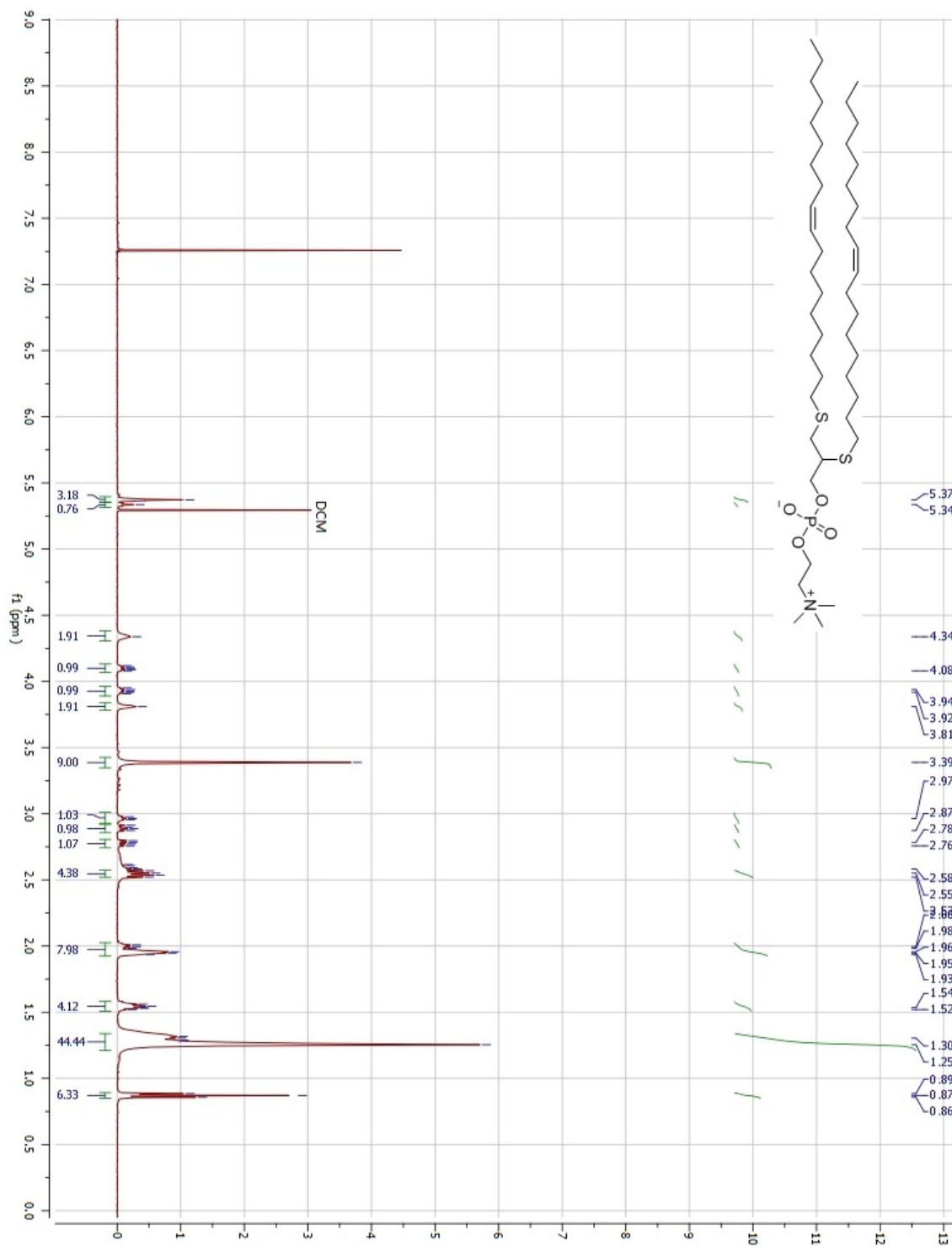


$^{13}\text{C}$  NMR

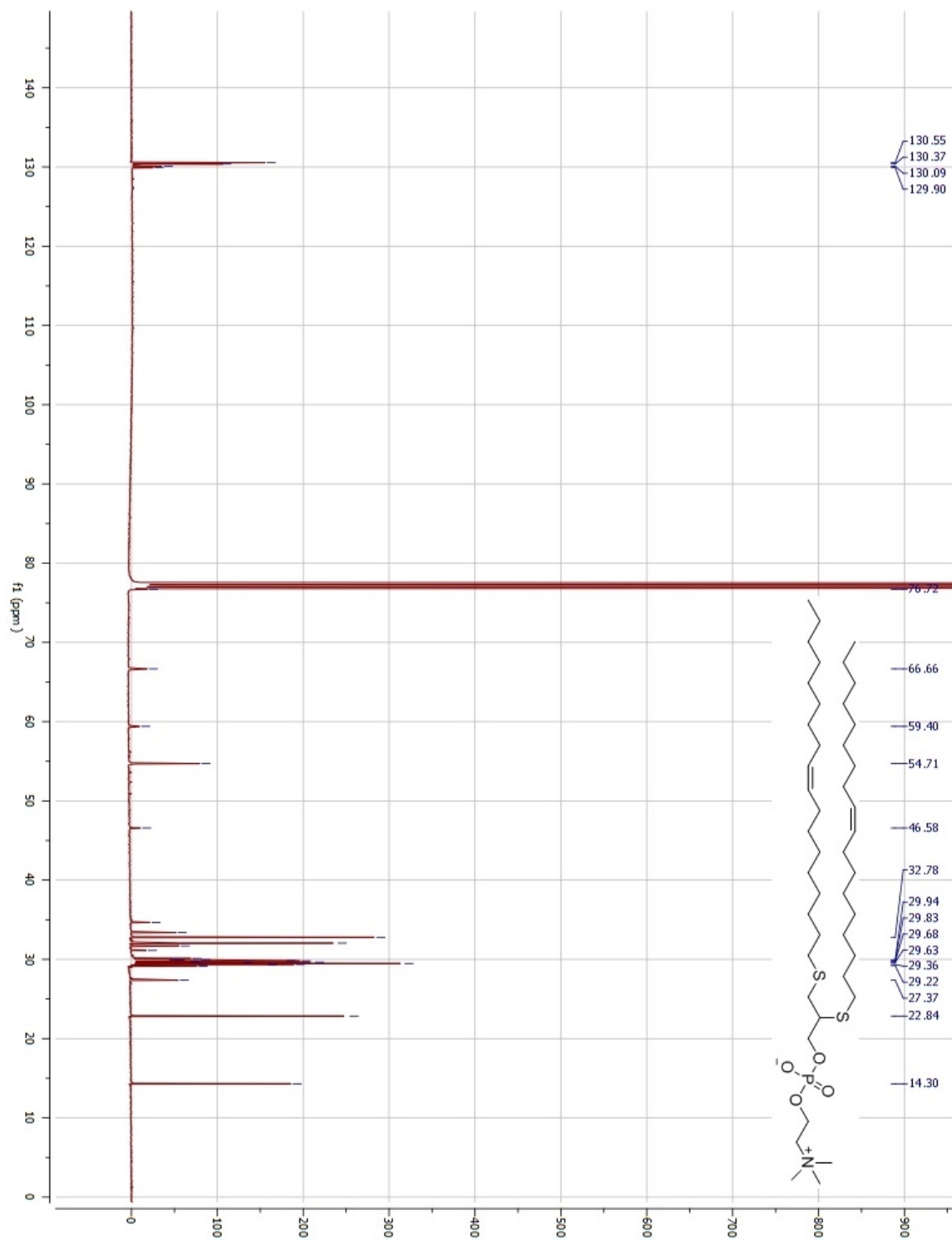


### 13.5 C18:1 dithioether PC 5

<sup>1</sup>H NMR

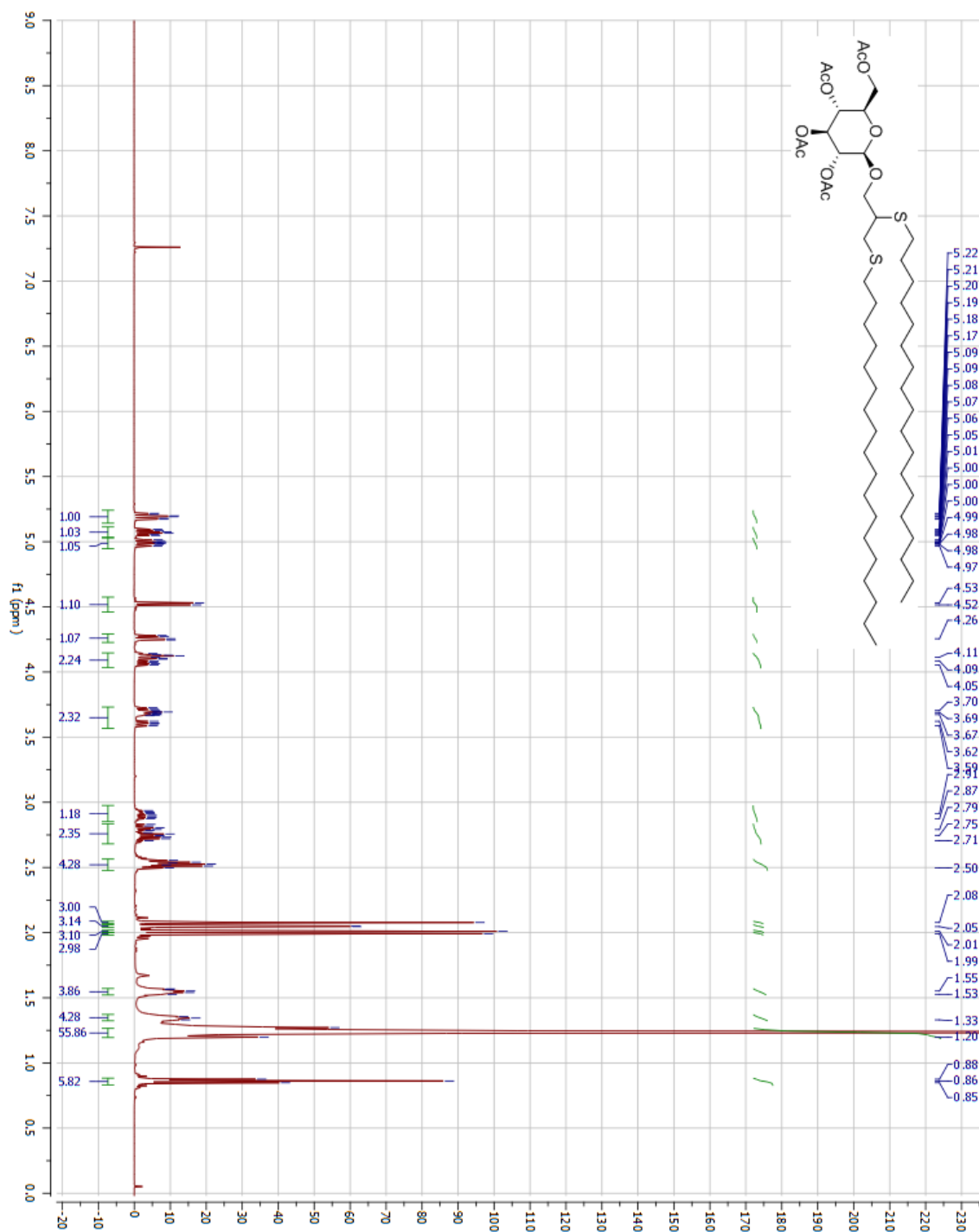


$^{13}\text{C}$  NMR

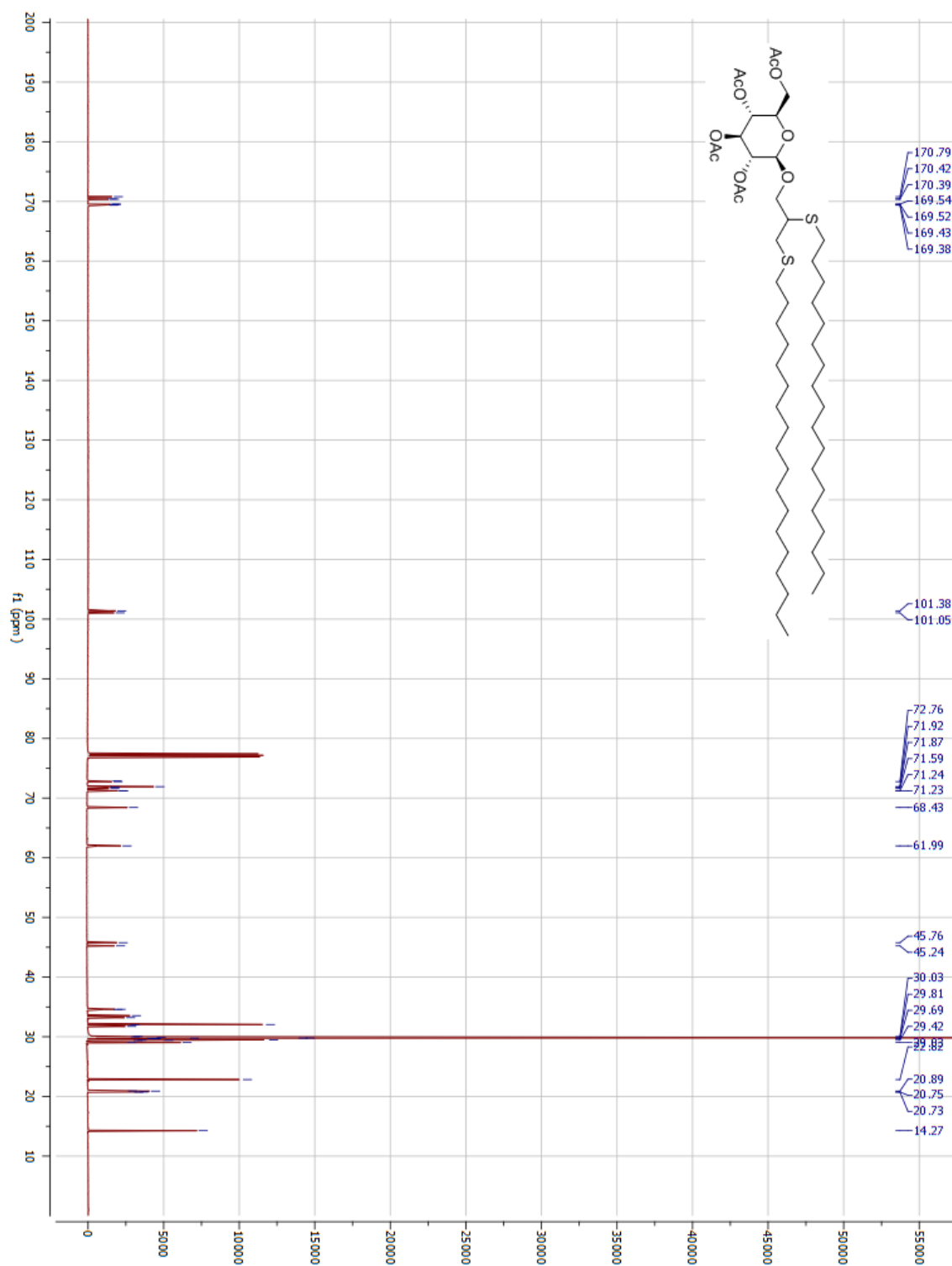


### 13.6 Acetyl-Protected Glucopyranosyl Lipid 6

<sup>1</sup>H NMR



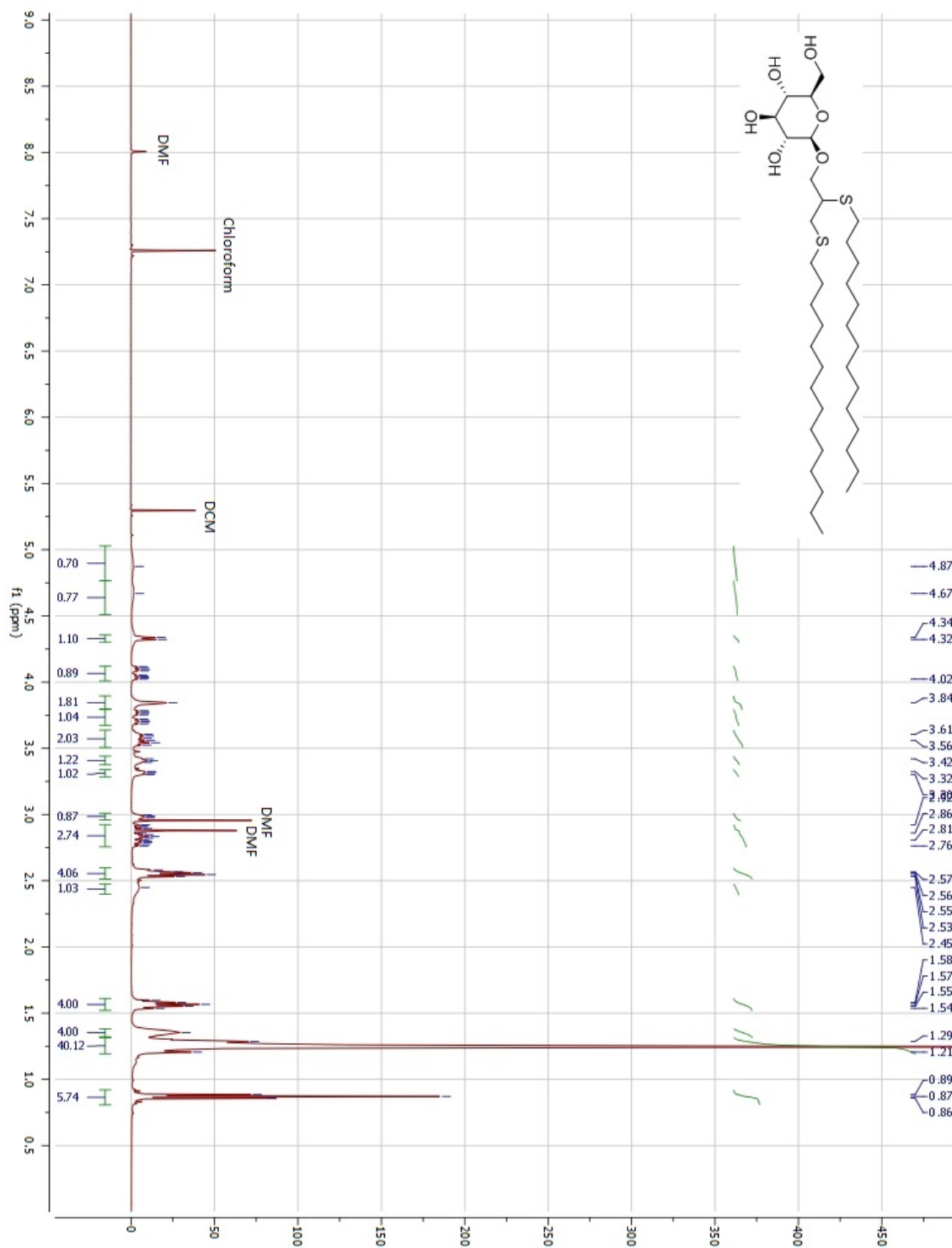
<sup>13</sup>C NMR



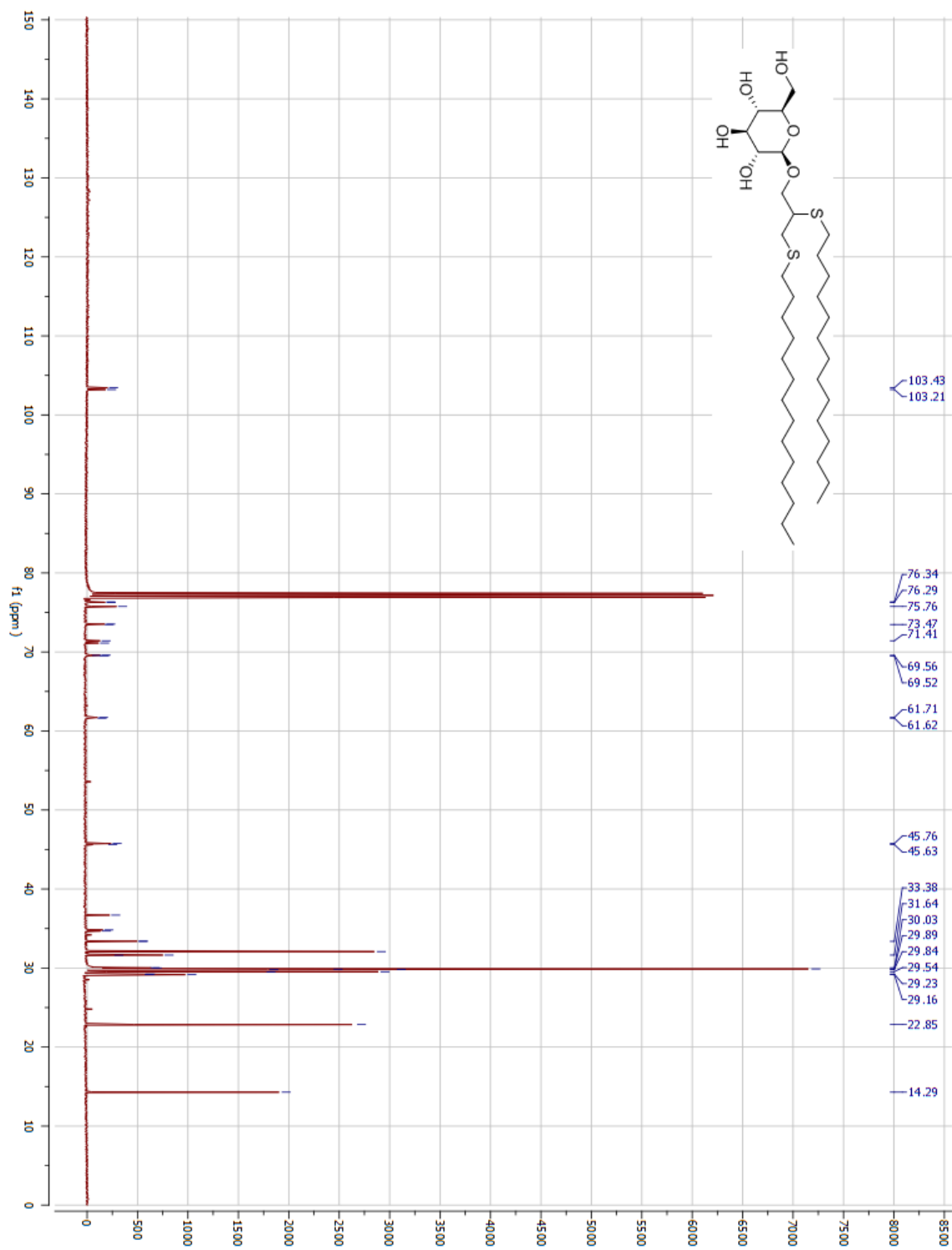


### 13.7 Protection-Free Glucopyranosyl Lipid 7

$^1\text{H}$  NMR

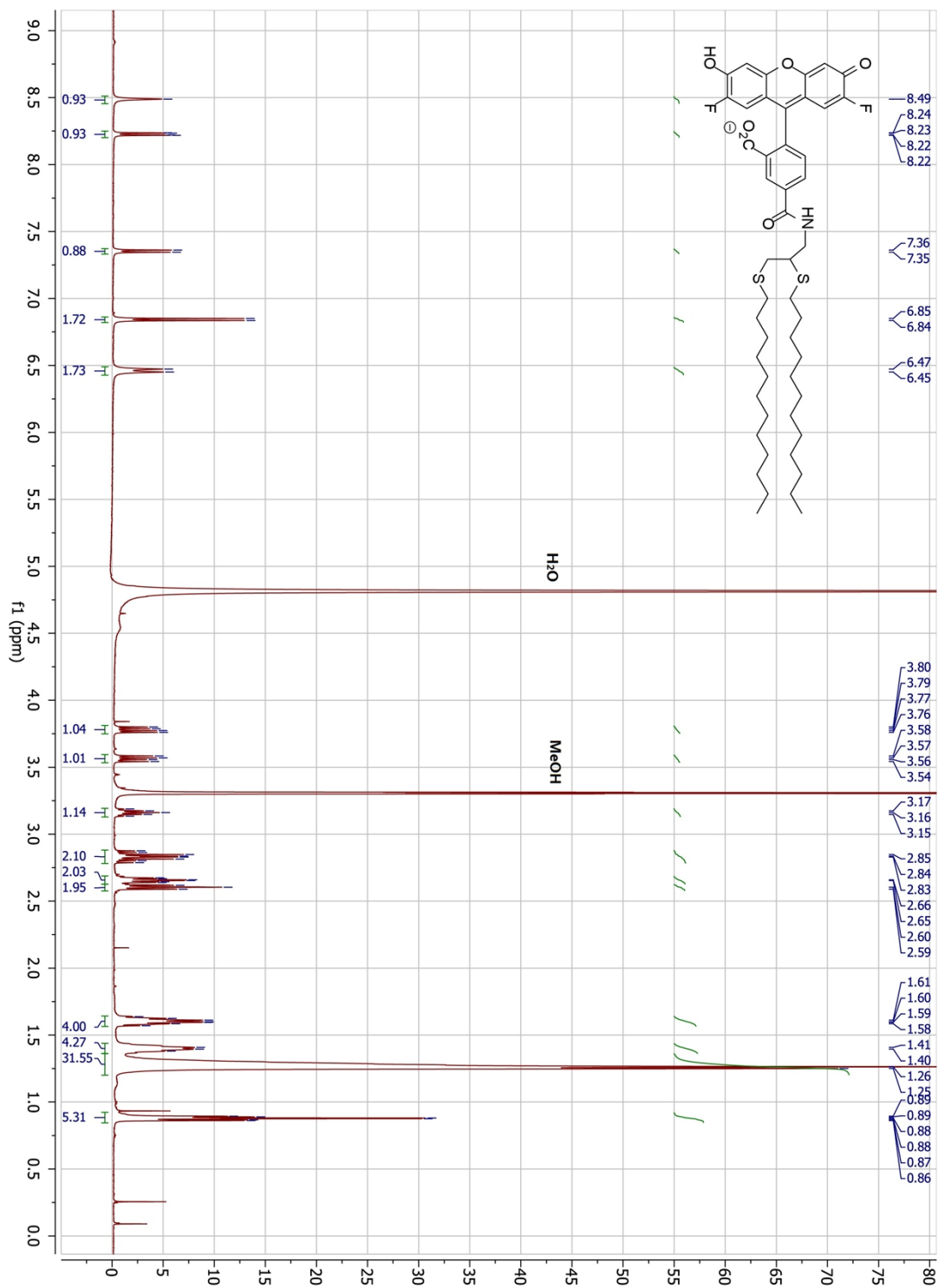


$^{13}\text{C}$  NMR



### 13.8 Oregon Green C12:0 Dithioether Lipid 8

<sup>1</sup>H NMR



#### 14. Supporting Information References:

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