Supporting Information for

Trapping of Intermediates with Substrate Analog HBOCoA in the Polymerizations Catalyzed by Class III Polyhydroxybutyrate (PHB) Synthase from *Allochromatium Vinosum*

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Table of Contents

1. General information

All chemicals were purchased at the highest purity grade. All solvents were anhydrous. All reactions were performed under argon atmosphere unless otherwise specified. Thin layer chromatography (TLC) was performed using 60 mesh silica gel plates and visualization was performed using short wavelength UV light (254 nm) and basic $KMnO_4$ staining. NMR spectra were recorded on a Varian 400 MHz spectrometer. Chemical shifts of proton (^{1}H NMR) and carbon (^{13}C NMR) were reported in ppm relative to the residual solvent peaks except that methanol was employed as the external reference for ^{13}C NMR when D_2O was used. Chemical shifts of phosphorus (^{31}P NMR) were reported in ppm relative to the external reference of 85% H_3PO_4. MALDI-TOF and high-resolution mass spectroscopies were recorded on Bruker Ultra Flex II and Q-Star Elite spectrometers, respectively. Radioactivity was recorded on a Beckman LS 6500 scintillation counter using Emulsifier-Safe (PerkinElmer) as the liquid scintillation counting cocktail.

2. Protein purification

The synthase *wt*-PhaEC$_{Av}$[1] and CoA biosynthetic enzymes *Sa*PanK,[2] *Ec*CoaD,[3] and *Ec*CoaE[4] were purified according to the reported procedures. The activity assay of *wt*-PhaEC$_{Av}$ was performed by a continuous method as previously described[5] except that 0.20 mg/mL bovine serum albumin (BSA) was used. The specific activity (SA) was determined to be 294 μmol•min^{-1}•mg^{-1} at 30°C. The SAs of *Sa*PanK, *Ec*CoaD, and *Ec*CoaE were measured as previously reported[1-3] and were at 45.0, 27.0, and 20.0 μmol•min^{-1}•mg^{-1} at 25°C, respectively.

2. Synthesis of oxo analogs

(4R)-N-(3-((2-hydroxyethyl)amino)-3-oxopropyl)-2-(4-methoxyphenyl)-5,5-dimethyl-1,3-dioxane-4-carboxamide ***6***: A PMP-group protected D-pantothenic acid **5**[6] (4.0 g, 11.8 mmol) in dry CH_2Cl_2 (150 mL) was treated with ethyl chloroformate (1.42 g, 13.0 mmol) for 10 min. Then 2-amino ethanol (0.87 g, 14.2 mmol) and pyridine (1.62 g, 16.5 mmol) were added and the mixture was stirred overnight at room temperature. The organic layer was washed with brine (3×50 mL), dried with Na_2SO_4 and concentrated under a reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc = 6/1 to 3/1) to give **6** as a white solid (3.40 g, 75.8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43 (d, 2H, J = 8.0 Hz), 7.03 (br, 1H), 6.93 (d,

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2H, *J* = 8.0 Hz), 6.47 (br, 1H), 5.46 (s, 1H), 4.08 (s, 1H), 3.82 (s, 3H), 3.72 (d, 1H, *J* = 12.0 Hz), 3.69 (d, 1H, *J* = 12.0 Hz), 3.64 (m, 2H), 3.55 (dt, 2H, *J* = 12.0, 8.0 Hz), 3.40-3.34 (m, 2H), 2.85 (t, 1H, *J* = 4.0 Hz), 2.45 (t, 2H, *J* = 4.0 Hz), 1.11 (s, 3H), 1.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.91, 170.01, 160.43, 130.21, 127.67, 113.91, 101.50, 83.95, 78.57, 62.15, 55.48, 42.58, 36.45, 35.23, 33.23, 21.98, 19.26.

2-(3-((4R)-2-(4-methoxyphenyl)-5,5-dimethyl-1,3-dioxane-4-carboxamido)propanamido)ethyl (3R)-3-(benzyloxy)butanoate ***8a***: To a solution of carboxylic acid **7a**[7] (0.60 g, 3.09 mmol) in dry CH_2Cl_2 (25 mL) were added DCC (0.93 g, 4.63 mmol) and catalytic DMAP. After 10 min, alcohol **6** (1.17g, 3.09 mmol) in dry CH_2Cl_2 (5 mL) was added and the mixture was stirred overnight at room temperature. The organic layer was washed with brine (3×10 mL), dried with anhydrous Na_2SO_4 and concentrated under a reduced pressure. The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH = 100/1 to 20/1) to give **8a** as a colorless oil (1.46 g, 84.9%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42 (d, 2H, *J* = 8.0 Hz), 7.30 (m, 5H), 7.01 (t, 1H, *J* = 4.0 Hz), 6.92 (d, 2H, *J* = 8.0 Hz), 6.01 (t, 1H, *J* = 4.0 Hz), 5.45 (s, 1H), 4.58 (d, 1H, *J* = 12.0 Hz), 4.46 (d, 1H, *J* = 12.0 Hz), 4.13 (m, 2H), 4.11 (s, 1H), 4.06 (m, 1H), 3.81 (s, 3H), 3.68 (dt, 2H, *J* = 24.0, 12.0 Hz), 3.45 (m, 4H), 2.61 (m, 1H), 2.50 (m, 1H), 2.25 (m, 2H), 1.28 (d, 3H, *J* = 8.0 Hz), 1.09 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.48, 171.08, 169.53, 160.35, 138.40, 130.29, 128.56, 127.87, 127.81, 127.62, 113.86, 101.43, 83.95, 78.61, 72.21, 71.93, 63.33, 55.45, 41.94, 38.61, 35.85, 34.92, 33.19, 21.97, 19.81, 19.25.

(2R)-4-(2-(3-((4R)-2-(4-methoxyphenyl)-5,5-dimethyl-1,3-dioxane-4-carboxamido)propan-amido)ethoxy)-4-oxobutan-2-yl (3R)-3-(benzyloxy)butanoate ***8b***: To a solution of acid **7b**[2] (0.13 g, 0.48 mmol) in dry CH_2Cl_2 (20 mL) was added oxalyl chloride (0.12 g, 0.96 mmol) at room temperature. After 10 min, a drop of DMF was added to initiate the reaction. The mixture was stirred for 2 hr. and then concentrated under a reduced pressure. The residue was re-dissolved in dry CH_2Cl_2 (5 mL) and added through cannulation to a mixture of alcohol **6** (0.21 g, 0.55 mmol) and pyridine (0.11 g, 1.40 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C. The reaction was stirred overnight at room temperature. The organic layer was washed with saturated brine, dried with Na_2SO_4 and concentrated under a reduced pressure. The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH = 100/1 to 20/1) to give **8b** as a white solid (0.28 g, 90.8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43 (d, 2H, *J* = 8.0 Hz), 7.32-7.27 (m, 5H), 7.06 (t, 1H, *J* = 4.0 Hz),

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6.92 (d, 2H, *J* = 12.0 Hz), 6.30 (t, 1H, *J* = 4.0 Hz), 5.45 (s, 1H), 5.34 (m, 1H), 4.56 (d, 1H, *J* = 12.0 Hz), 4.47 (d, 1H, *J* = 12.0 Hz), 4.09 (m, 1H), 4.07 (s, 1H), 4.02-3.98 (m, 2H), 3.82 (s, 3H), 3.68 (dd, 2H, *J* = 24.0, 12.0 Hz), 3.52 (m, 2H), 3.34 (m, 2H), 2.61-2.51 (m, 3H), 2.45-2.39 (m, 3H), 1.27 (d, 3H, *J* = 8.0 Hz), 1.26 (d, 3H, *J* = 8.0 Hz), 1.10 (s, 3H), 1.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.00, 170.47, 169.74, 169.02, 159.85, 138.03, 129.89, 128.05, 127.40, 127.32, 127.25, 113.37, 100.89, 83.46, 78.04, 71.64, 70.48, 67.09, 63.00, 54.97, 41.79, 40.55, 37.95, 35.18, 34.59, 32.72, 21.58, 19.77, 19.39, 18.8.

(12R,16R)-1-((4R)-2-(4-methoxyphenyl)-5,5-dimethyl-1,3-dioxan-4-yl)-12-methyl-1,5,10,14-tetraoxo-9,13-dioxa-2,6-diazaheptadecan-16-yl (3R)-3-(benzyloxy)butanoate ***8c****:* The reaction and silica gel chromatography were performed in the same way as **8b** using acid **7c**[2] (0.30 g, 0.82 mmol) and alcohol **6** (0.32 g, 0.84 mmol) to yield **8c** as a white solid (0.49 g, 82.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43 (d, 2H, *J* = 8.0 Hz), 7.32-7.27 (m, 5H), 7.07 (t, 1H, *J* = 4.0 Hz), 6.92 (d, 2H, *J* = 8.0 Hz), 6.38 (t, 1H, *J* = 4.0 Hz), 5.46 (s, 1H), 5.32 (m, 1H), 5.25 (q, 1H, *J* = 8.0 Hz), 4.56 (d, 1H, *J* = 12.0 Hz), 4.49 (d, 1H, *J* = 12.0 Hz), 4.11 (m, 2H), 4.07 (s, 1H), 3.99 (q, 1H, *J* = 8.0 Hz), 3.81 (s, 3H), 3.68 (q, 2H, *J* = 12.0 Hz), 3.57-3.52 (m, 2H), 3.50-3.44 (m, 2H), 2.64-2.55 (m, 3H), 2.51-2.38 (m, 5H), 1.27 (d, 3H, *J* = 4.0 Hz), 1.25 (d, 3H, *J* = 4.0 Hz), 1.24 (d, 3H, *J* = 4.0 Hz), 1.10 (s, 3H), 1.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.22, 170.62, 170.00, 169.64, 169.37, 160.17, 138.42, 130.14, 128.33, 127.62, 127.56, 127.50, 113.70, 101.25, 83.80, 78.44, 71.86, 70.77, 67.69, 67.30, 63.51, 55.32, 42.07, 40.98, 40.89, 38.35, 35.66, 34.83, 33.06, 21.86, 20.07, 19.84, 19.79, 19.14.

(2R)-4-(2-(3-((4R)-2-(4-methoxyphenyl)-5,5-dimethyl-1,3-dioxane-4-carboxamido)propan-amido)ethoxy)-4-oxobutan-2-yl (3R)-3-(butyryloxy)butanoate ***8d****:* The reaction and silica gel chromatography were performed in the same way as **8b** using acid **7d**[2] (0.30 g, 1.15 mmol) and alcohol **6** (0.42 g, 1.10 mmol) to yield **8d** as a white solid (0.53 g, 77.5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41 (d, 2H, *J* = 8.0 Hz), 7.09 (t, 1H, *J* = 4.0 Hz), 6.90 (d, 1H, *J* = 8.0 Hz), 6.59 (t, 1H, *J* = 4.0 Hz), 5.44 (s, 1H), 5.30 (m, 1H), 5.25 (q, 1H, *J* = 8.0 Hz), 4.10 (dd, 2H, *J* = 12.0, 4.0 Hz), 4.05 (s, 1H), 3.80 (s, 3H), 3.69 (d, 1H, *J* = 12.0 Hz), 3.64 (d, 1H, *J* = 12.0 Hz), 3.56-3.50 (m, 2H), 3.48-3.42 (m, 2H), 2.61-2.55 (m, 2H), 2.51-2.46 (m, 2H), 2.43 (t, 2H, *J* = 8.0 Hz), 2.22 (t, 2H, *J* = 8.0 Hz), 1.60 (sextet, 2H, *J* = 8.0 Hz), 1.26 (d, 3H, *J* = 8.0 Hz), 1.25 (d, 3H, *J* = 4.0 Hz), 1.08 (s, 3H), 1.07 (s, 3H), 0.91 (t, 2H, *J* = 8.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.02, 171.36, 170.10, 169.84, 169.58, 160.30, 130.25, 127.58, 113.81, 101.38, 83.92, 78.55, 67.78, 67.02,

63.65, 55.41, 41.19, 41.05, 38.51, 36.37, 35.84, 35.05, 33.15, 21.93, 20.16, 20.00, 19.21, 18.47, 13.71.

*(12R,16R)-1-((4R)-2-(4-methoxyphenyl)-5,5-dimethyl-1,3-dioxan-4-yl)-12-methyl-1,5,10,14-tetraoxo-9,13-dioxa-2,6-diazaheptadecan-16-yl (3R)-3-(butyryloxy)butanoate **8e**:* The reaction and silica gel chromatography were performed in the same way as **8b** using acid **7e**[2] (0.30 g, 0.87 mmol) and alcohol **6** (0.32 g, 0.84 mmol) to yield **8e** as a white solid (0.47 g, 79.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43 (d, 2H, *J* = 8.0 Hz), 7.07 (t, 1H, *J* = 4.0 Hz), 6.92 (d, 2H, *J* = 8.0 Hz), 6.43 (t, 1H, *J* = 4.0 Hz), 5.46 (s, 1H), 5.34-5.22 (m, 3H), 4.12 (dd, 2H, *J* = 12.0, 4.0 Hz), 4.07 (s, 1H), 3.82 (s, 3H), 3.68 (dd, 2H, *J* = 20.0, 8.0 Hz), 3.54 (m, 2H), 3.48 (m, 2H), 2.63-2.57 (m, 3H), 2.53-2.42 (m, 5H), 2.23 (t, 2H, *J* = 8.0 Hz), 1.62 (sextet, 2H, *J* = 8.0 Hz), 1.29 (d, 3H, *J* = 4.0 Hz), 1.28 (d, 3H, *J* = 4.0 Hz), 1.26 (d, 3H, *J* = 4.0 Hz), 1.10 (s, 3H), 1.09 (s, 3H), 0.93 (t, 3H, *J* = 8.0 Hz),; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172.93, 171.32, 170.05, 169.64, 169.52, 160.30, 130.27, 127.58, 113.81, 101.39, 83.93, 78.57, 67.82, 67.60, 67.06, 63.60, 55.41, 41.06, 41.03, 41.00, 38.50, 36.40, 35.84, 34.97, 33.15, 21.94, 20.15, 19.98, 19.89, 19.22, 18.50, 13.71.

*(R)-4-(2-(3-((R)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethoxy)-4-oxobutan-2-yl (R)-3-hydroxybutanoate **9a**:* In the presence of H_2 and $Pd(OH)_2$/C (20 mg), ester **8a** (0.20 g, 0.36 mmol) in MeOH (20 mL) was stirred vigorously for 24 hours at room temperature. The $Pd(OH)_2$/C powder was removed by filtration and the solvent was removed under a reduced pressure. The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH= 20/1 to 10/1) to give **9a** as colorless oil (0.12 g, 95.7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41 (t, 1H, *J* =4.0 Hz), 6.78 (t, 1H, *J* = 4.0 Hz), 4.34 (d, 1H, *J* = 4.0 Hz), 4.32-4.23 (m, 2H), 4.17 (dq, 1H, *J* = 12.0, 4.0 Hz), 4.00 (d, 1H, *J* = 4.0 Hz), 3.93 (d, 1H, *J* = 4.0 Hz), 3.72-3.56 (m, 3H), 3.52-3.44 (m, 3H), 3.40-3.33 (m, 1H), 3.99 (d, 1H, J=4.0 Hz), 3.93 (s, 1H), 3.72-3.56 (m, 3H), 3.52-3.43 (m 2H), 3.40-3.32 (m, 1H), 2.53-2.36 (m, 4H), 1.26 (d, 3H, *J* = 8.0 Hz), 1.01 (s, 3H), 0.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.56, 172.58, 172.09, 77.72, 70.98, 65.01, 63.43, 43.97, 39.47, 38.76, 36.05, 35.61, 23.23, 21.77, 20.48.

*(R)-4-(2-(3-((R)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethoxy)-4-oxobutan-2-yl (R)-3-hydroxybutanoate **9b**:* The reaction and silica gel chromatography were performed in the same way as **9a** using ester **8b** (0.12 g, 0.19 mmol) to yield **9b** as colorless oil (0.07 g, 92.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.43 (t, 1H, *J* = 4.0 Hz), 6.89 (t, 1H, *J* = 4.0 Hz), 5.36 (m, 1H), 4.36 (d, 1H, *J* = 8.0 Hz), 4.21 (m, 1H), 4.19-4.15 (m, 2H), 3.98 (d, 1H, *J* = 4.0 Hz), 3.82 (t, 1H, *J*

= 8.0 Hz), 3.69 (d, 1H, *J* = 4.0 Hz), 3.57-3.52 (m, 2H), 3.49-3.41 (m, 4H), 2.58 (m, 2H), 2.46 (t, 2H, *J* = 8.0 Hz), 2.42 (m, 2H), 1.31 (d, 3H, *J* = 4.0 Hz), 1.23 (d, 3H, *J* = 8.0 Hz), 0.99 (s, 3H), 0.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.08, 171.97, 171.57, 170.33, 76.82, 70.27, 67.37, 64.28, 63.14, 43.54, 40.75, 39.10, 38.38, 35.47, 35.25, 22.80, 20.93, 20.41, 19.93.

(3R,15R,19R)-1,3-dihydroxy-2,2,15-trimethyl-4,8,13,17-tetraoxo-12,16-dioxa-5,9-diazaicosan-19-yl (R)-3-hydroxybutanoate ***9c****:* The reaction and silica gel chromatography were performed in the same way as **9a** using ester **8c** (0.15 g, 0.20 mmol) to yield **9c** as colorless oil (0.10 g, 96.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.47 (t, 1H, *J* = 4.0 Hz), 6.92 (t, 1H, *J* = 4.0 Hz), 5.28 (m, 2H), 4.49 (d, 1H, *J* = 4.0 Hz), 4.18 (nonet, 1H, *J* = 8.0 Hz), 4.16-4.13 (m, 2H), 3.96 (d, 1H, *J* = 4.0 Hz), 3.67 (br, 1H), 3.54 (q, 2H, *J* = 4.0 Hz), 3.48-3.40 (m, 4H), 2.63-2.57 (m, 2H), 2.52 (dd, 2H, *J* = 12.0, 4.0 Hz), 2.45 (t, 2H, *J* = 8.0 Hz), 2.40 (t, 2H, *J* = 4.0 Hz), 1.29 (d, 3H, *J* = 4.0 Hz), 1.28 (d, 3H, *J* = 4.0 Hz), 1.21 (d, 3H, *J* = 4.0 Hz), 0.97 (s, 3H), 0.89 (s, 3H); ^{13}C NMR δ: 174.13, 172.05, 171.81, 170.31, 170.06, 77.51, 70.62, 67.93, 67.53, 64.40, 63.37, 43.54, 40.90, 39.29, 38.47, 35.65, 35.32, 22.77, 21.27, 20.50, 20.08, 19.95.

(R)-4-(2-(3-((R)-2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)ethoxy)-4-oxobutan-2-yl (R)-3-(butyryloxy)butanoate ***9d****:* The reaction was performed in the same way as **9a** using ester **8d** (0.30 g, 0.48 mmol). The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH= 20/1) to give **9d** as colorless oil (0.22 g, 90.9%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.41 (t, 1H, *J* = 4.0 Hz), 6.72 (t, 1H, *J* = 4.0 Hz), 5.31 (m, 1H), 5.23 (m, 1H), 4.23 (d, 1H, *J* = 4.0 Hz), 4.16 (dt, 2H, *J* = 8.0, 4.0 Hz), 3.98 (t, 1H, *J* = 4.0 Hz), 3.73 (br, 1H), 3.53 (m, 2H), 3.50-3.40 (m, 4H), 2.24 (t, 2H, *J* = 8.0 Hz), 1.62 (q, 2H, *J* = 4.0 Hz), 3.48-3.40 (m, 4H), 2.62-2.49 (m, 4H), 2.45 (t, 2H, *J* = 8.0 Hz), 2.24 (t, 2H, *J* = 8.0 Hz), 1.61 (sextet, 2H, *J* = 8.0 Hz), 1.28 (d, 3H, *J* = 8.0 Hz), 1.26 (d, 3H, *J* = 8.0 Hz), 0.99 (s, 3H), 0.92 (t, 3H, *J* = 8.0 Hz), 0.90 (s, 3H); ^{13}C NMR δ: 173.76, 173.24, 171.97, 170.36, 170.11, 77.70, 71.01, 67.94, 67.12, 63.64, 41.24, 39.46, 38.76, 36.43, 35.70, 35.29, 21.68, 20.49, 20.30, 20.07, 18.52, 13.75.

(3R,15R,19R)-1,3-dihydroxy-2,2,15-trimethyl-4,8,13,17-tetraoxo-12,16-dioxa-5,9-diazaicosan-19-yl (R)-3-(butyryloxy)butanoate ***9e****:* The reaction and silica gel chromatography were performed in the same way as **9d** using ester **8e** (0.40 g, 0.56 mmol) to yield **9e** as colorless oil (0.30 g, 90.8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.36 (br, 1H), 6.62 (br, 1H), 5.36-5.20 (m, 3H), 4.17 (dt, 1H, *J* = 20.0, 8.0 Hz), 3.98 (s, 1H), 3.63-3.32 (m, 8H), 2.24 (t, 2H, *J* = 8.0 Hz), 1.62 (q, 2H, *J* = 4.0 Hz), 3.48-3.40 (m, 4H), 2.63-2.57 (m, 2H), 2.52 (dd, 2H, *J* = 12.0, 4.0 Hz), 2.45

(sextet, 2H, *J* = 8.0 Hz), 1.28 (q, 9H, *J* = 8.0 Hz), 1.02 (s, 3H), 0.93 (t, 3H, *J* = 8.0 Hz), 0.92 (s, 3H); ^{13}C NMR δ: 173.87, 173.07, 171.96, 170.28, 169.85, 169.67, 77.53, 70.90, 67.92, 67.67, 67.09, 63.50, 41.02, 39.39, 38.64, 36.40, 35.68, 35.28, 21.52, 20.46, 20.17, 19.98, 19.90, 18.48, 13.69.

*(R)-2,4-dihydroxy-N-(3-((2-hydroxyethyl)amino)-3-oxopropyl)-3,3-dimethylbutanamide **10**:* The reaction was performed in the same way as **9a** using alcohol **6** (0.20 g, 0.52 mmol). The residue was purified by silica gel chromatography (CH_2Cl_2/MeOH= 20/1 to 5/1) to give **10** as colorless oil (0.13 g, 95.3%). ^{1}H NMR (400 MHz, D_2O) δ: 3.99 (s, 1H), 3.66 (t, 2H, *J* = 4.0 Hz), 3.53 (t, 2H, *J* = 8.0 Hz), 3.52 (d, 1H, *J* = 8.0 Hz), 3.40 (d, 1H, *J* = 8.0 Hz), 3.33 (t, 2H, *J* = 8.0 Hz), 2.53 (t, 2H, *J* = 8.0 Hz), 0.93 (s, 3H), 0.90 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ: 176.30, 175.34, 76.94, 69.51, 61.05, 42.63, 39.73, 36.62, 36.45, 21.63, 20.22.

*Enzymatic Synthesis of HBOCoA **1***: A 2-mL reaction mixture consisted of precursor **9a** (20.0 mM), ATP (50.0 mM), $MgCl_2$ (10.0 mM), *Sa*CoaA (80.0 μg), *Ec*CoaD (80.0 μg) and *Ec*CoaE (80.0 μg) in 100 mM Tris-HCl (pH 7.60). The reaction was initiated by addition of the enzymes and incubated at 37 °C for 3 h. The reaction was stopped by heating the reaction mixture in a 95 °C water bath for 5 min, and the precipitated protein was removed by centrifugation (14,000 rpm × 5 min). The supernatant was loaded onto a semi-preparative HPLC column (Luna C18-2, 5 μm, 10 mm × 250 mm) that was eluted at 3.00 mL/min using a linear gradient from 5 to 90% methanol in 10.0 mM ammonium acetate (pH 5.00) over 60 min. The fractions containing the product were pooled, concentrated, and lyophilized to give a white powder (17 mg, 50.8% yield based on UV absorbance, HPLC: t_R = 15.1 min); ^{1}H NMR (400MHz, D_2O) δ:8.56 (s, 1H), 8.28 (s, 1H), 6.20 (d, 2H, *J* = 8.0 Hz), 4.62 (m, 1H), 4.28-4.23 (m, 3H), 4.21 (t, 2H, *J* = 8.0 Hz), 4.03 (s, 1H), 3.86 (d, 1H, *J* = 8.0 Hz), 3.60 (d, 1H, *J* = 8.0 Hz), 3.53-3.46 (m, 4H), 2.64-2.55 (m, 2H), 2.50 (t, 2H, *J* = 8.0 Hz), 1.26 (d, 3H, *J* = 4.0 Hz), 0.91 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ: 174.84, 174.26, 174.03, 155.64, 152.93, 149.43, 140.04, 118.76, 86.72, 83.68, 74.30, 74.01, 72.02, 65.65, 64.71, 63.56, 43.27, 38.41, 35.60, 35.48, 22.15, 20.98, 18.33; ^{31}P NMR (161 MHz, D_2O) δ: 2.13 (s, 1P), −11.31 (d, 1P, *J* = 19.3 Hz), −11.88 (d, 1P, *J* = 19.3 Hz); HRMS: calc. for $C_{25}H_{41}N_7O_{19}P_3^-$ [M−H]$^-$: 836.1676, found: 836.1710.

*Enzymatic Synthesis of (HB)$_2$OCoA **2a***: The reaction and HPLC were performed in the same way as **1** using precursor **9b** to give product **2a** (18 mg, 48.7% yield based on UV absorbance, t_R =

26.4 min). ^{1}H NMR (400 MHz, D_2O) δ: 8.54, (s, 1H), 8.26 (d, 1H, *J* = 4.0 Hz), 6.18 (d, 1H, *J* = 8.0 Hz), 5.27 (dq, 2H, *J* = 12.0, 4.0 Hz), 4.87 (q, 1H, *J* = 4.0 Hz), 4.61 (m, 1H), 4.27 (m, 2H), 4.21 (m, 1H), 4.16 (t, 2H, *J* = 4.0 Hz) 4.02 (s, 1H), 3.86 (d, 1H, *J* = 8.0 Hz), 3.59 (d, 1H, *J* = 8.0 Hz), 3.48 (q, 2H, *J* = 8.0 Hz), 3.44 (t, 2H, *J* = 8.0 Hz), 2.71 (d, 2H, *J* = 8.0 Hz), 2.56-2.45 (m, 4H), 1.30 (d, 3H, *J* = 4.0 Hz), 1.22 (d, 3H, *J* = 4.0 Hz), 0.91 (s, 3H), 0.77 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ: 174.86, 174.21, 173.35, 172.98, 155.21, 152.39, 149.31, 140.14, 118.69, 86.67, 83.59, 83.54, 74.28, 74.23, 74.19, 73.94, 73.89, 72.08, 72.02, 68.79, 65.51, 64.78, 64.65, 63.71, 43.58, 40.37, 38.51, 38.43, 38.34, 35.58, 35.47, 22.08, 20.98, 19.11, 18.30; ^{31}P NMR (161 MHz, D_2O) δ: 0.51 (s, 1P), −10.71 (d, 1P, *J* = 25.8 Hz), −11.36 (d, 1P, *J* = 24.2 Hz); HRMS: calc. for $C_{29}H_{47}N_7O_{21}P_3^-$ [M−H]$^-$: 922.2043, found: 922.2076.

Enzymatic Synthesis of (HB)$_3$OCoA ***2b***: The reaction and HPLC were performed in the same way as **1** using precursor **9c** to give product **2b** (19 mg, 47.1% yield based on UV absorbance, t_R = 36.5 min. ^{1}H NMR (400 MHz, D_2O) δ: 8.57 (s, 1H), 8.26 (s, 1H), 6.17 (d, 1H, *J* = 4.0 Hz), 5.26 (dq, 2H, *J* = 12.0, 4.0 Hz), 4.87 (m, 1H), 4.62 (m, 1H), 4.28 (m, 2H), 4.22 (m, 1H), 4.17 (t, 2H, *J* = 4.0 Hz) 4.03 (s, 1H), 3.86 (d, 1H, *J* = 8.0 Hz), 3.60 (d, 1H, *J* = 8.0 Hz), 3.49 (m, 2H), 3.45 (t, 1H, *J* = 4.0 Hz), 2.74-2.66 (m, 4H), 2.54-2.47 (m, 4H), 1.27 (d, 6H, *J* = 4.0 Hz), 1.24 (d, 3H, *J* = 8.0 Hz), 0.92 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ: 174.99, 174.31, 173.40, 173.01, 172.42, 155.32, 152.48, 140.38, 86.89, 83.71, 74.43, 74.36, 74.12, 72.22, 69.18, 68.85, 65.66, 64.74, 63.85, 43.72, 40.80, 40.43, 38.59, 38.50, 35.74, 35.64, 22.28, 21.15, 19.25, 19.22, 18.47; ^{31}P NMR (161 MHz, D_2O) δ: 0.85 (s, 1P), −10.06 (d, 1P, *J* = 17.7 Hz), −10.66 (d, 1P, *J* = 17.7 Hz); HRMS: calc. for $C_{33}H_{53}N_7O_{23}P_3^-$ [M−H]$^-$: 1008.2411, found: 1008.2398.

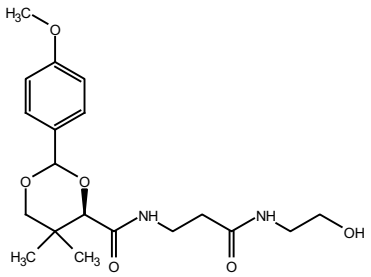
Enzymatic Synthesis of sT-O-CoA ***3a***: The reaction and HPLC were performed in the same way as **1** using precursor **9d** to give product **3a** (16 mg, 40.8% yield based on UV absorbance, t_R = 43.2 min. ^{1}H NMR (400 MHz, D_2O) δ: 8.56 (s, 1H), 8.29 (s, 1H), 6.19 (d, 1H, *J* = 8.0 Hz), 5.24 (dq, 2H, *J* = 12.0, 4.0 Hz), 4.86 (m, 1H), 4.61 (m, 1H), 4.26 (m, 2H), 4.17 (t, 2H, *J* = 4.0 Hz) 4.03 (s, 1H), 3.85 (d, 1H, *J* = 8.0 Hz), 3.57 (d, 1H, *J* = 8.0 Hz), 3.49 (m, 2H), 3.45 (t, 1H, *J* = 8.0 Hz), 2.71-2.65 (m, 4H), 2.49 (t, 1H, *J* = 8.0 Hz), 2.32 (t, 1H, *J* = 8.0 Hz), 1.59 (sextet, 2H, *J* = 8.0 Hz), 1.28 (d, 6H, *J* = 4.0 Hz), 0.91 (t, 3H, *J* = 6.0 Hz), 0.90 (s, 3H), 0.77 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ: 176.26, 174.86, 174.16, 172.86, 172.34, 155.46, 152.68, 149.40, 140.10, 118.75, 86.71, 83.58, 74.28, 74.02, 72.10, 69.05, 68.45, 65.54, 63.72, 40.75, 40.33, 38.45, 38.37, 36.24, 35.61, 35.51, 21.03, 19.17, 19.13, 18.31, 18.21, 13.06; ^{31}P NMR (161 MHz, D_2O) δ: 1.07

(s, 1P), −10.46 (d, 1P, *J* = 16.1 Hz), −11.07 (d, 1P, *J* = 16.1 Hz); HRMS: calc. for $C_{33}H_{53}N_7O_{22}P_3^-$ [M−H]$^-$: 992.2462, found: 992.2440.

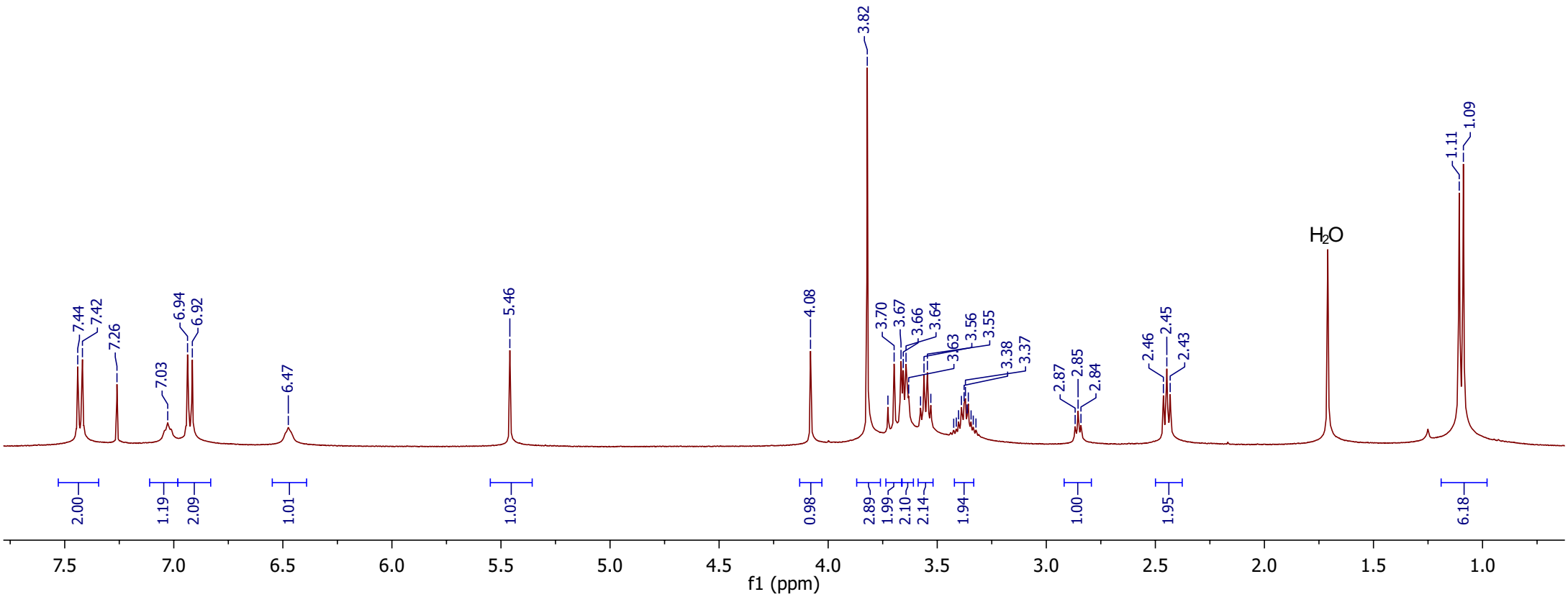
Enzymatic Synthesis of sTet-O-CoA ***3b***: The reaction and HPLC were performed in the same way as **1** using precursor **9e** to give product **3b** (19 mg, 44.0% yield based on UV absorbance, t_R = 46.7 min. ^{1}H NMR (400 MHz, D_2O) δ: 8.57 (s, 1H), 8.29 (s, 1H), 6.20 (d, 1H, *J* = 4.0 Hz), 5.26 (m, 3H), 4.91 (m, 1H), 4.61 (m, 1H), 4.27 (m, 2H), 4.18 (t, 2H, *J* = 4.0 Hz) 4.04 (s, 1H), 3.87 (d, 1H, *J* = 4.0 Hz), 3.58 (d, 1H, *J* = 4.0 Hz), 3.51-3.41 (m, 4H), 2.74-2.61 (m, 6H), 2.50 (t, 2H, *J* = 8.0 Hz), 2.33 (t, 2H, *J* = 8.0 Hz), 1.60 (sextet, 2H, *J* = 8.0 Hz), 1.29 (d, 6H, *J* = 8.0 Hz), 1.27 (d, 3H, *J* = 8.0 Hz), 0.92 (t, 3H, *J* = 6.0 Hz), 0.91 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ: 176.28, 174.88, 174.15, 172.82, 172.28, 172.17, 155.66, 152.91, 149.52, 140.07, 86.65, 83.71, 74.26, 74.20, 74.06, 72.09, 68.99, 68.39, 65.56, 63.71, 40.66, 40.30, 38.53, 38.45, 38.36, 36.25, 35.60, 35.52, 21.03, 19.15, 19.11, 19.04, 18.28, 18.22, 13.04; ^{31}P NMR (161 MHz, D_2O) δ: 1.28 (s, 1P), −10.47 (br, 1P), −11.00 (br, 1P); HRMS: calc. for $C_{37}H_{59}N_7O_{24}P_3^-$ [M−H]$^-$: 1078.2830, found: 1078.2873.

Enzymatic Synthesis of CoAOH ***4***: The reaction was performed in the same way as **1** using precursor **10**. HPLC purification was carried out in the same way as **1** except that a linear gradient from 5 to 90% methanol in 10.0 mM ammonium acetate (pH 5.00) over 60 min was used. The product **4** (16 mg, 53.3% yield based on UV absorbance) was eluted at 8.70 min. ^{1}H NMR (400 MHz, D_2O) δ: 8.50 (s, 1H), 8.18 (s, 1H), 6.14 (d, 1H, *J* = 8.0 Hz), 4.59 (m, 1H), 4.25 (m, 2H), 4.00 (s, 1H), 3.83 (m, 1H), 3.62 (t, 2H, *J* = 4.0 Hz), 3.57 (m, 1H), 3.46 (t, 2H, *J* = 4.0 Hz), 3.28 (t, 2H, *J* = 4.0 Hz), 2.46 (t, 2H, *J* = 8.0 Hz), 0.88 (s, 3H), 0.76 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ: 174.83, 174.25, 155.25, 152.51, 149.20, 139.98, 118.54, 86.59, 83.60, 83.54, 74.30, 74.12, 73.93, 73.89, 72.02, 71.96, 65.48, 60.01, 41.58, 38.45, 38.37, 35.58, 35.50, 20.91, 18.29; ^{31}P NMR (161 MHz, D_2O) δ: 1.45 (s, 1P), −10.09 (d, 1P, *J* = 19.3 Hz), −10.70 (d, 1P, *J* = 19.3 Hz); HRMS: calc. for $C_{21}H_{35}N_7O_{17}P_3^-$ [M−H]$^-$: 750.1308, found: 750.1287.

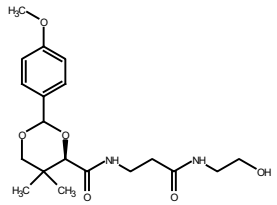
^{1}H NMR, 400 MHz, $CDCl_3$



6

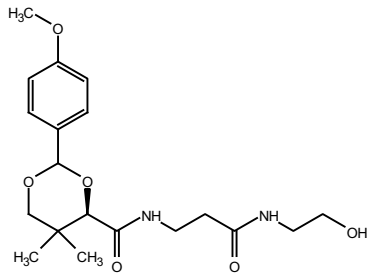


gCOSY NMR, 400 MHz, $CDCl_3$



6

^{13}C NMR, 100 MHz, $CDCl_3$



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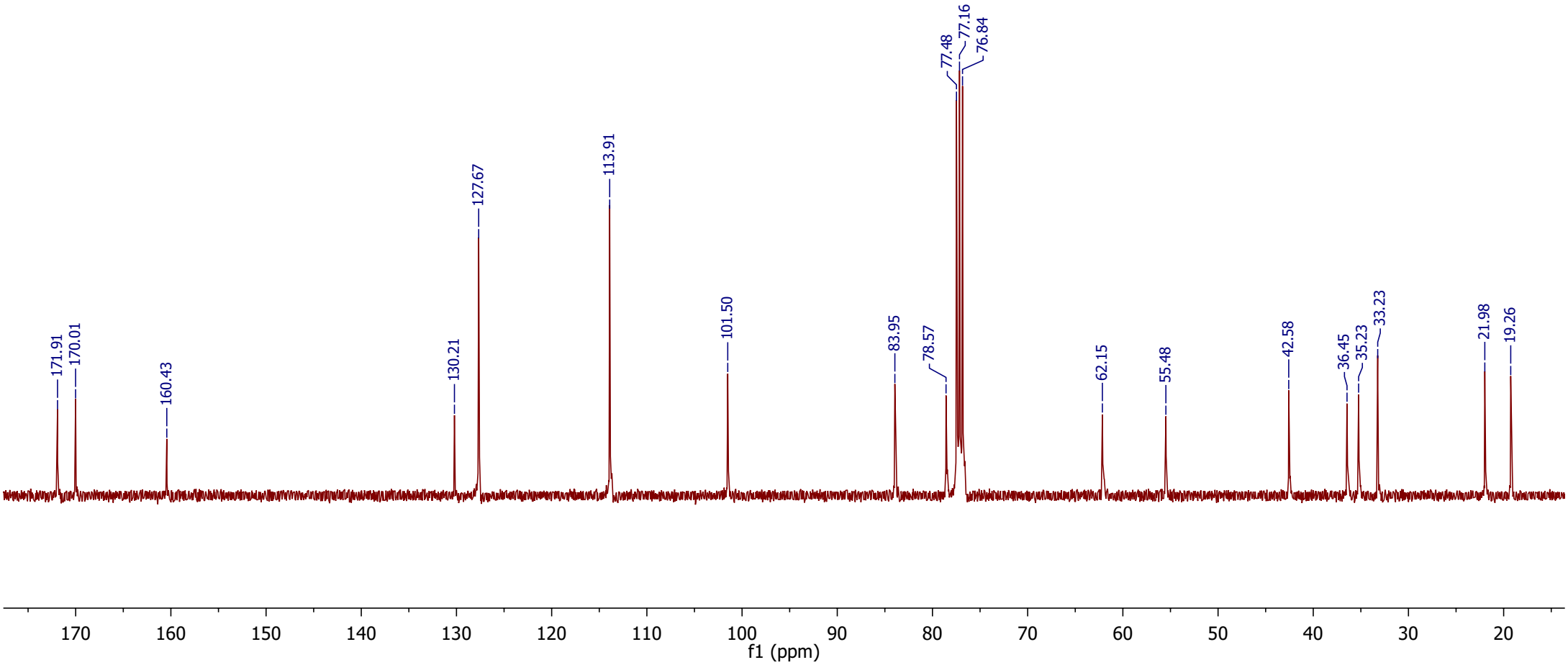
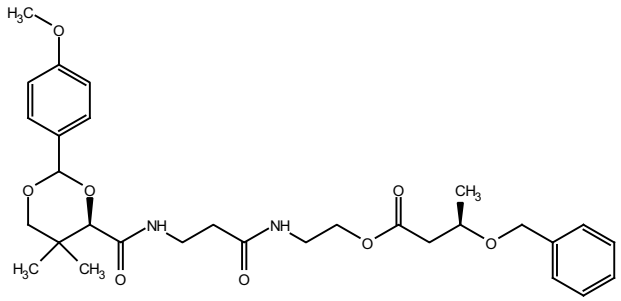
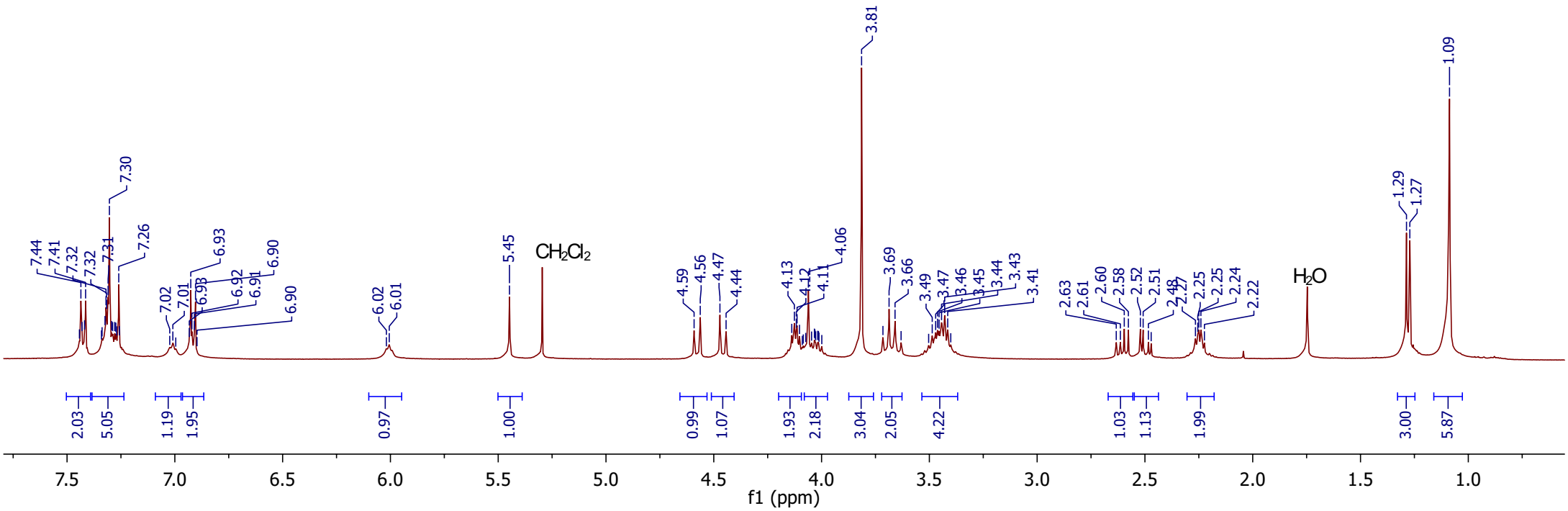


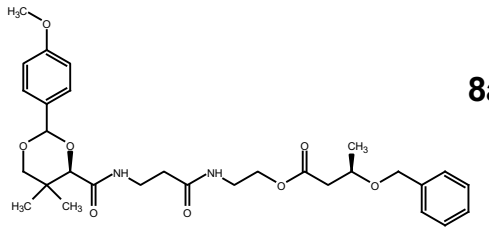
^{1}H NMR, 400 MHz, $CDCl_3$



8a



^{1}H NMR, 400 MHz, $CDCl_3$



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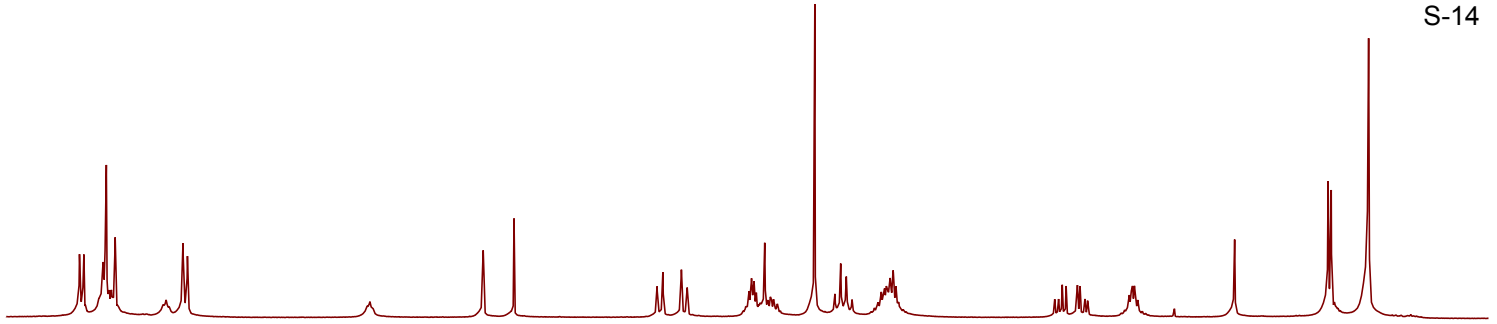
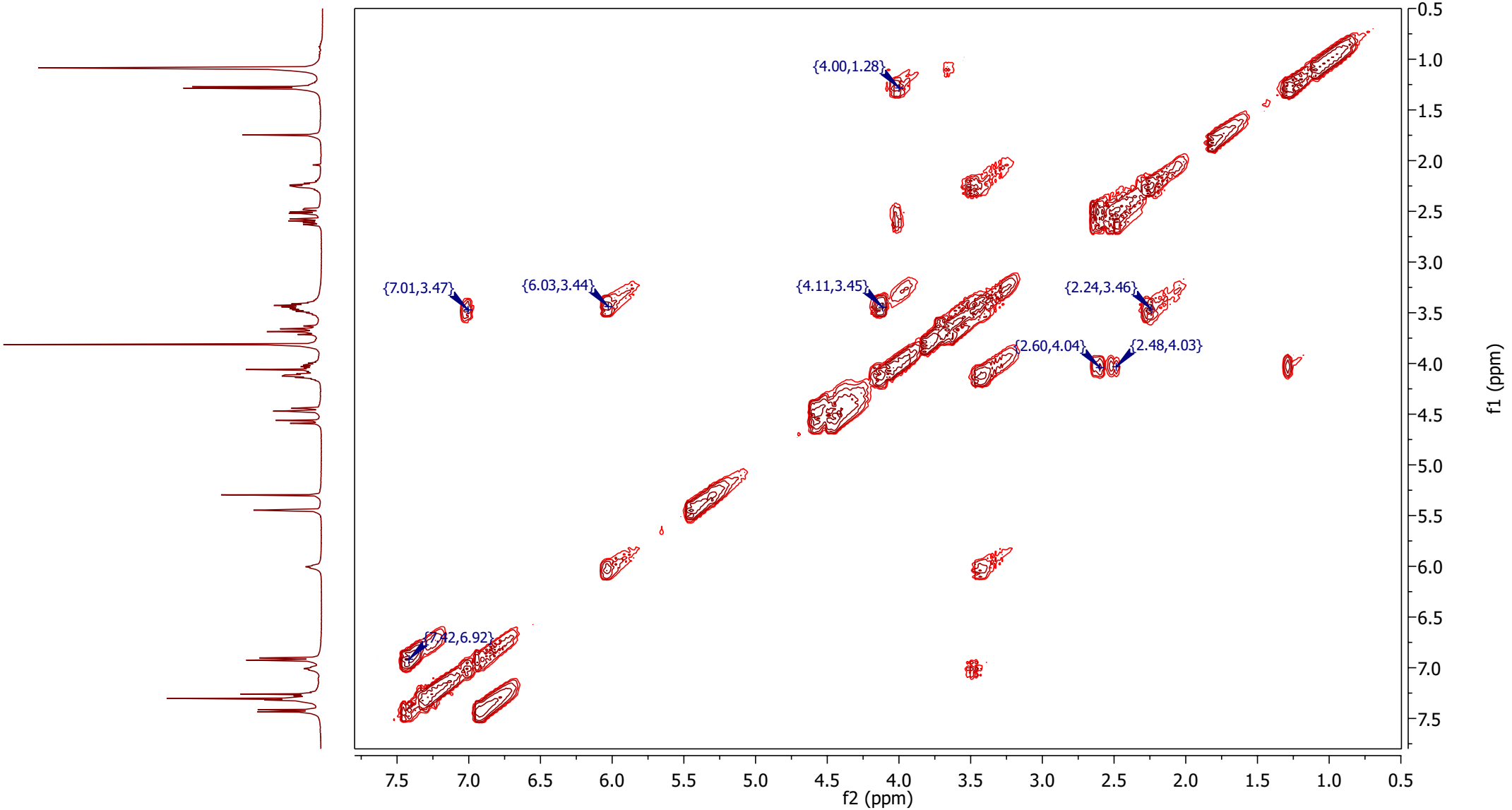
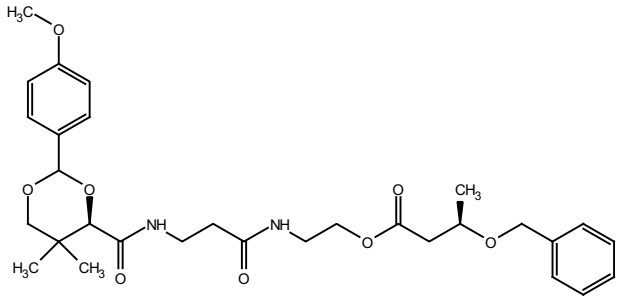
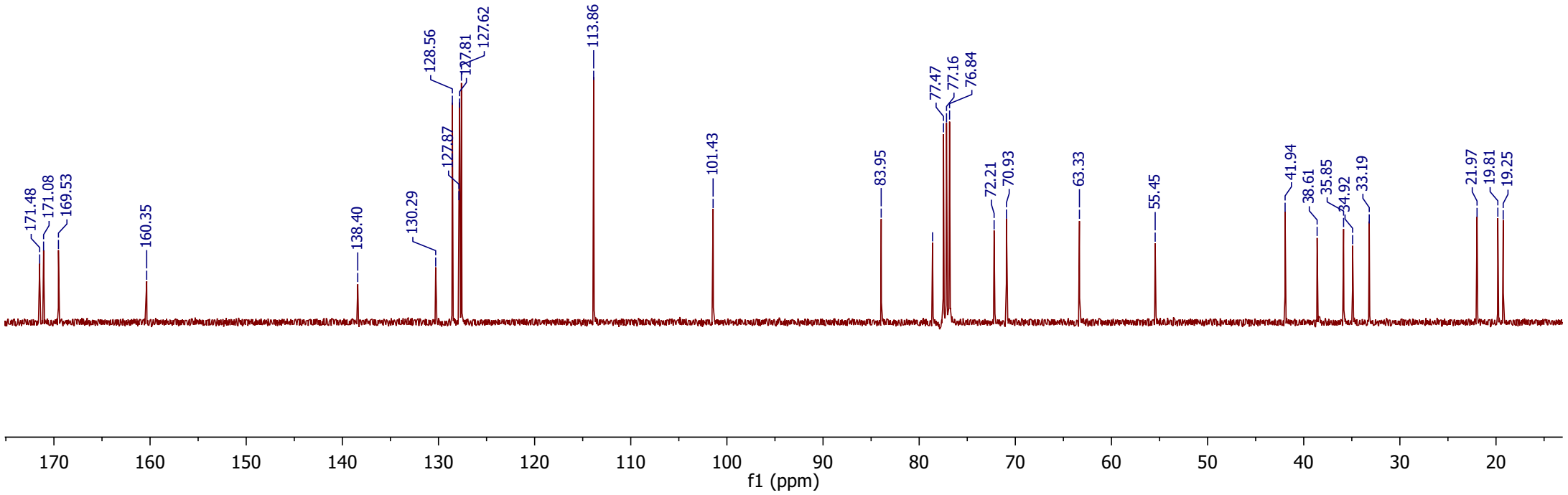




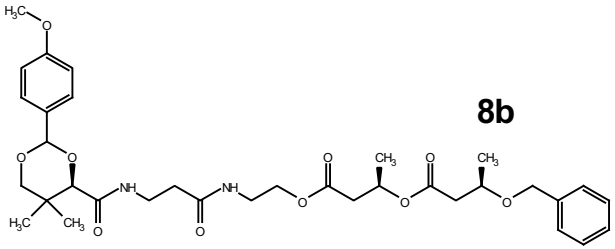
^{13}C NMR, 100 MHz, $CDCl_3$



8a

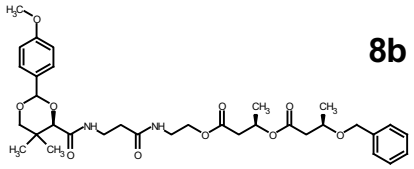


^{1}H NMR, 400 MHz, $CDCl_3$

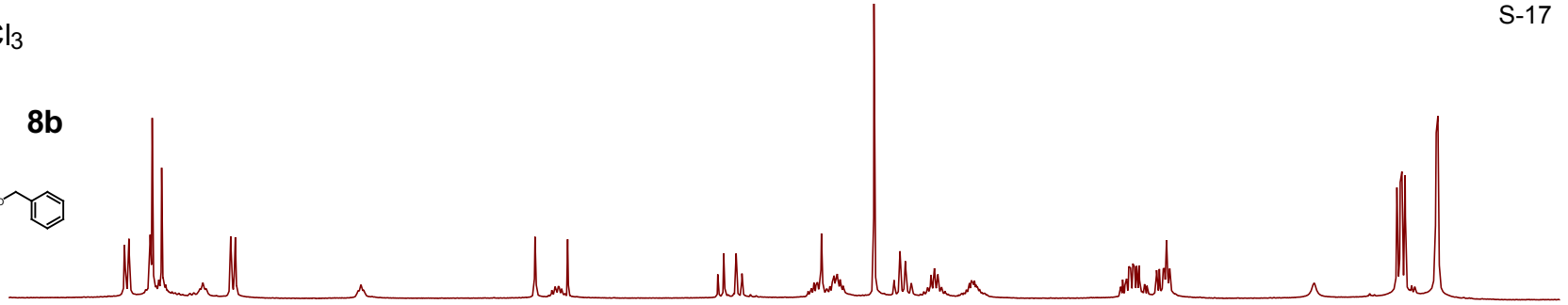


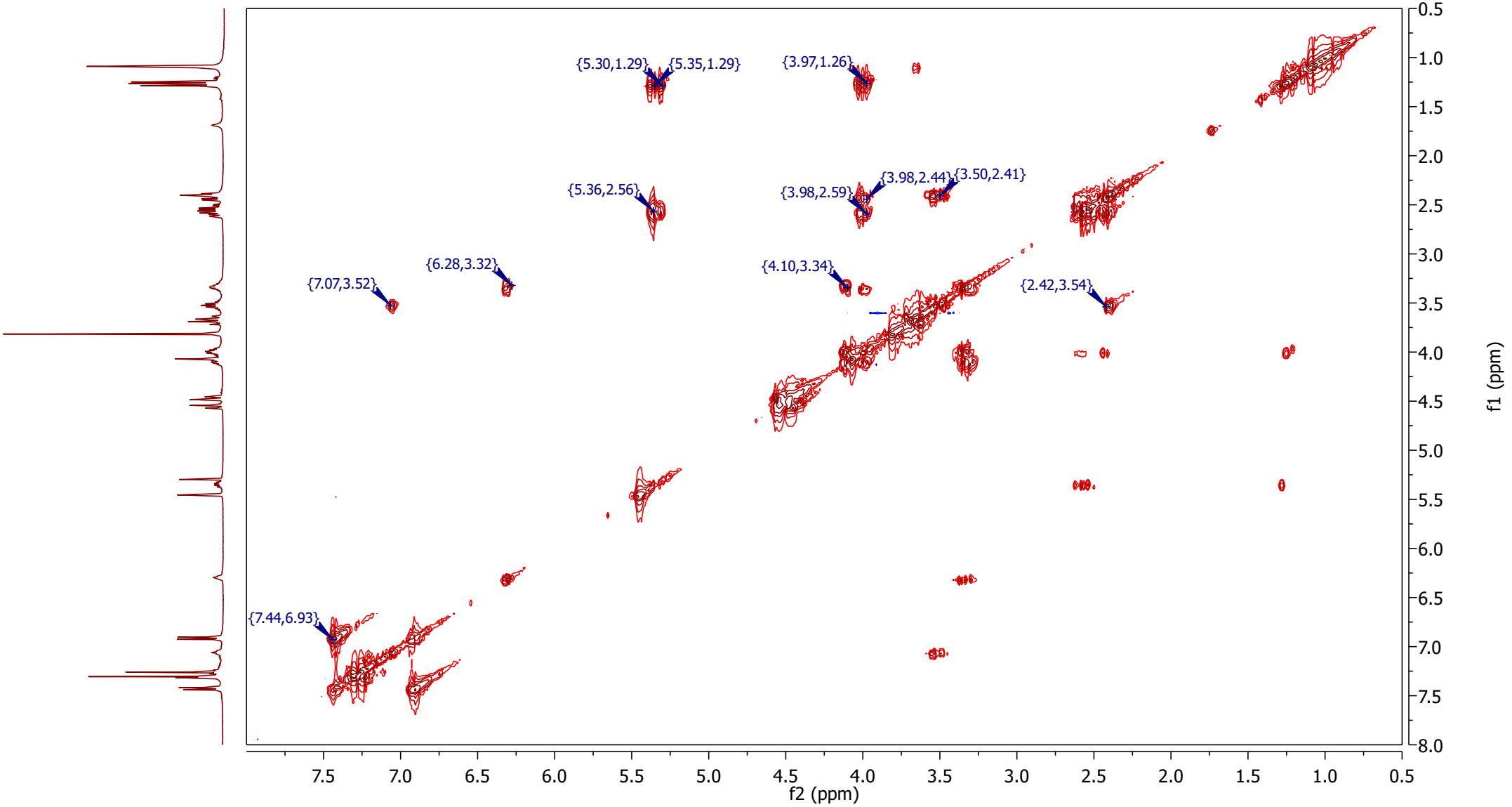
8b

gCOSY, 400 MHz, $CDCl_3$

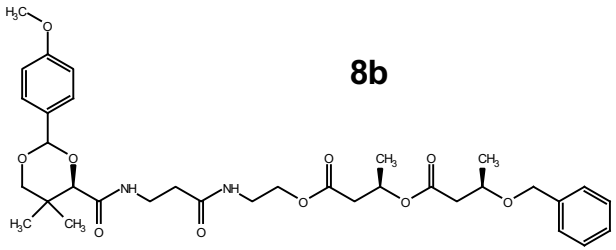


8b





^{13}C NMR, 100 MHz, $CDCl_3$



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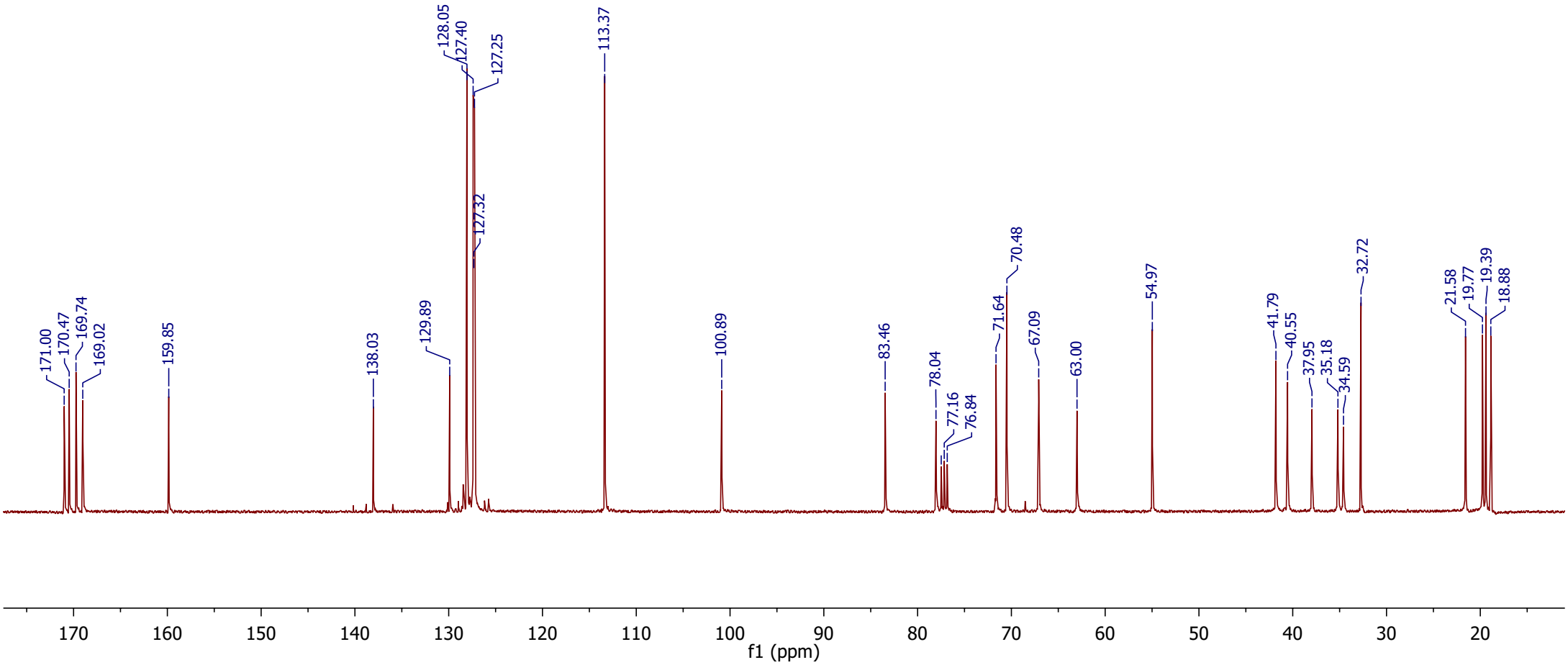
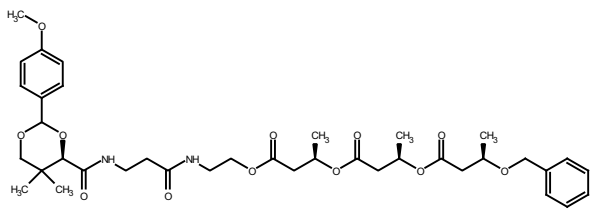


^{1}H NMR, 400 MHz, $CDCl_3$



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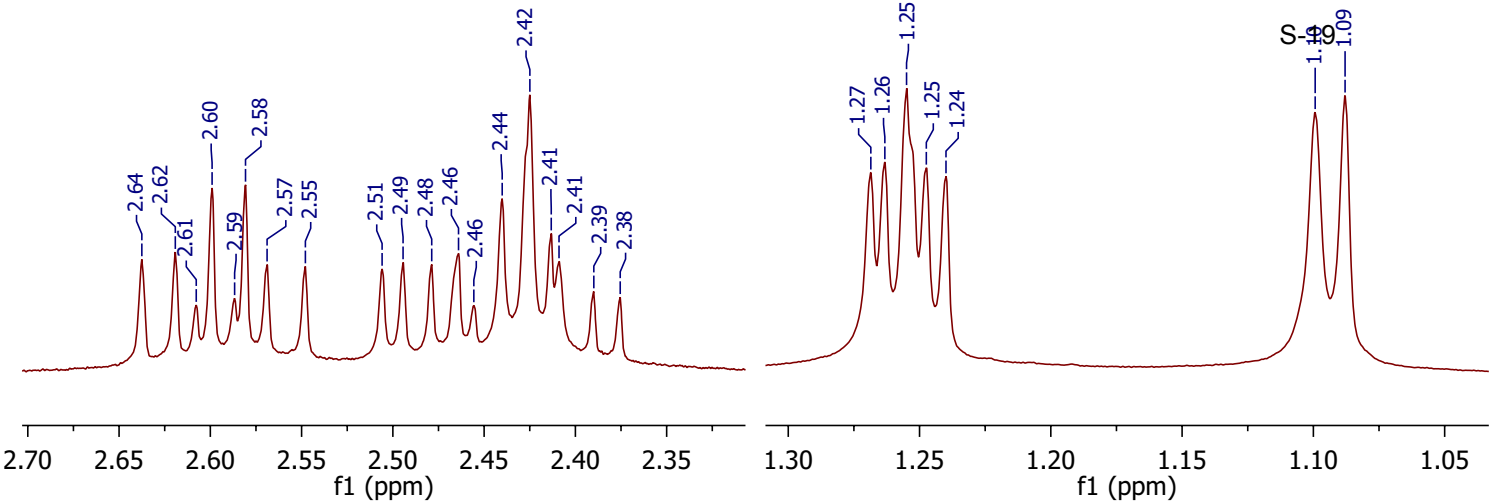
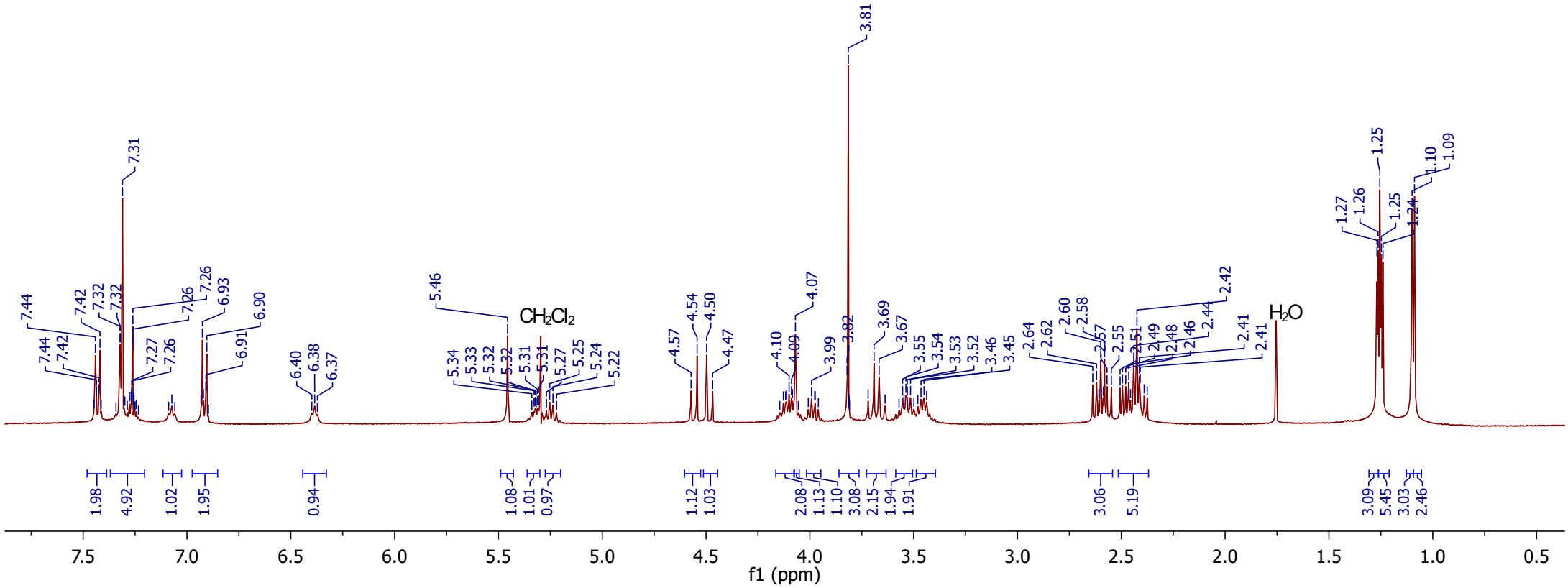
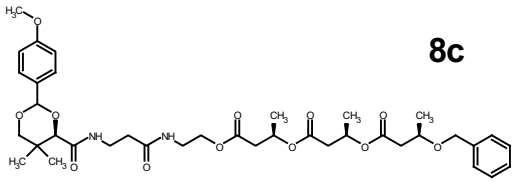




gCOSY, 400 MHz, $CDCl_3$



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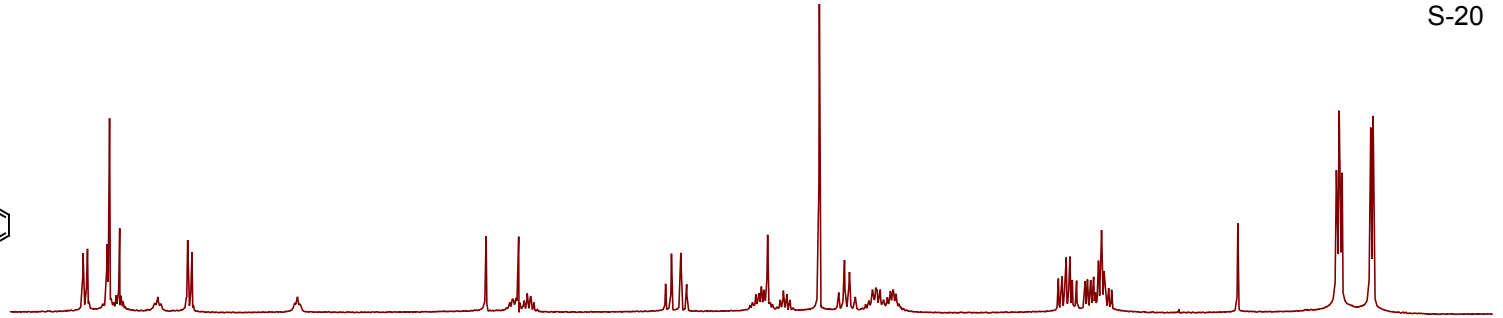
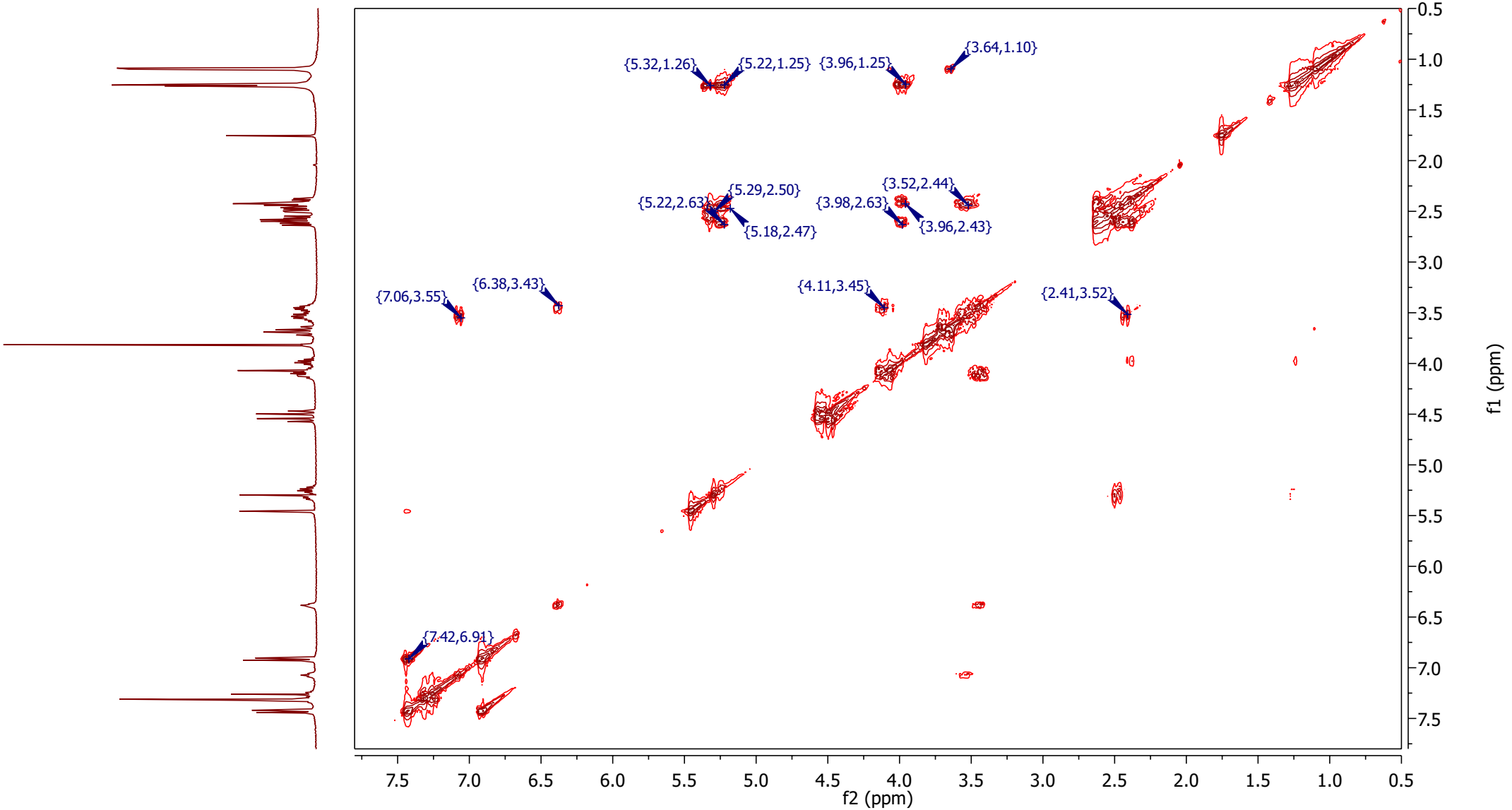
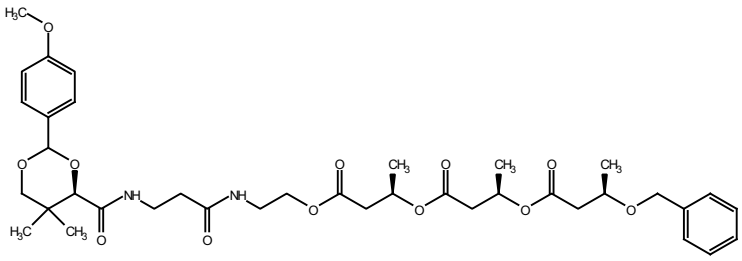




^{13}C NMR, 100 MHz, $CDCl_3$



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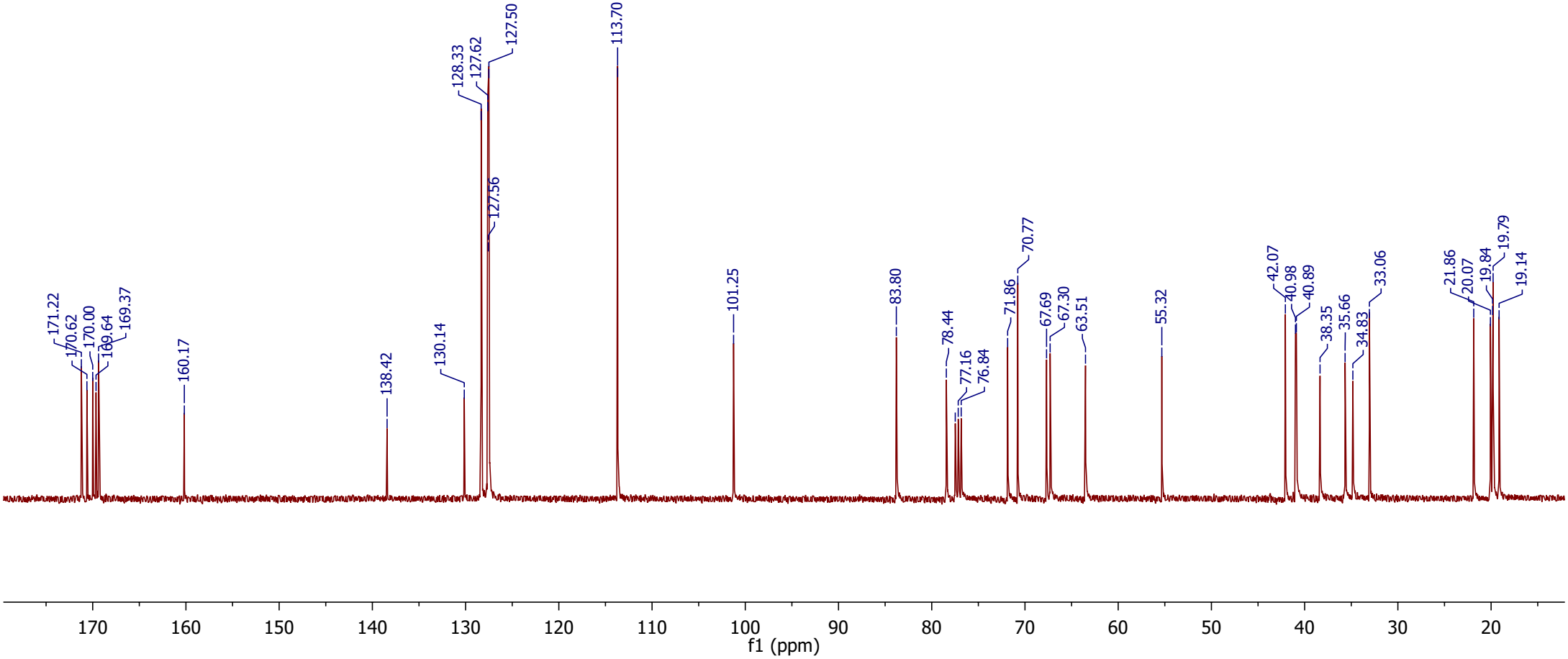
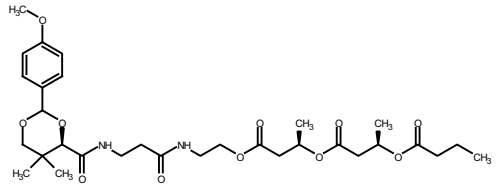


^{1}H NMR, 400 MHz, $CDCl_3$



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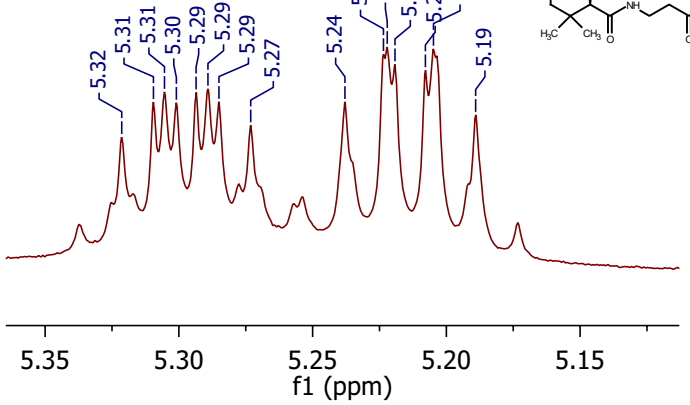
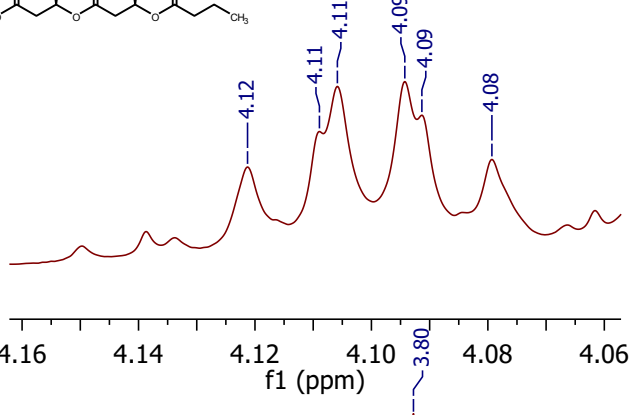
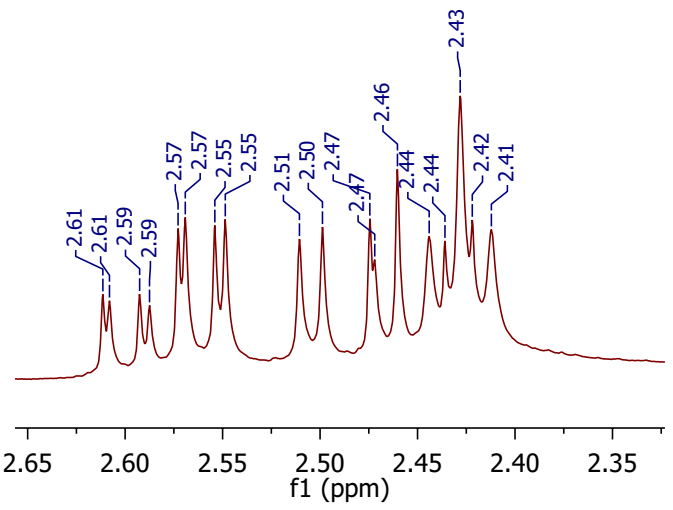
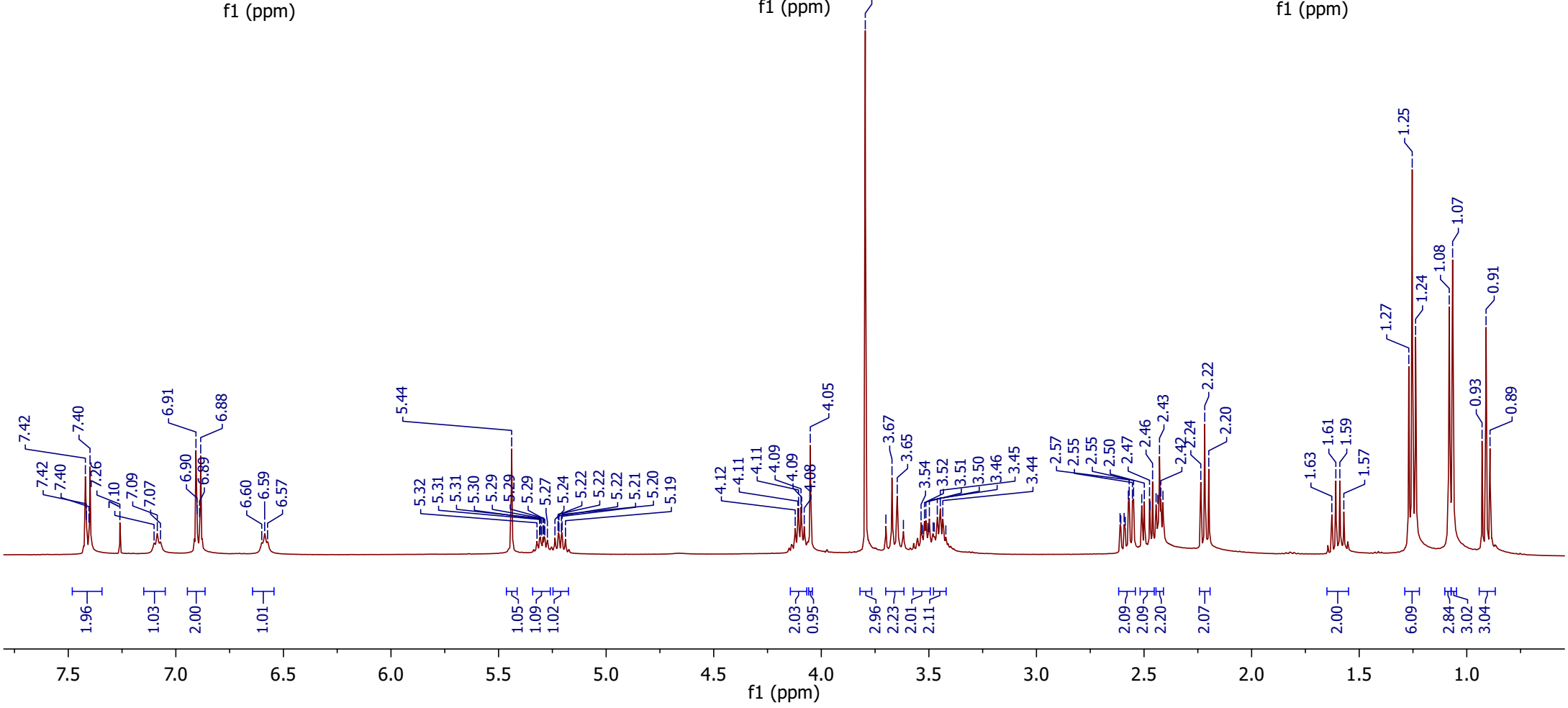
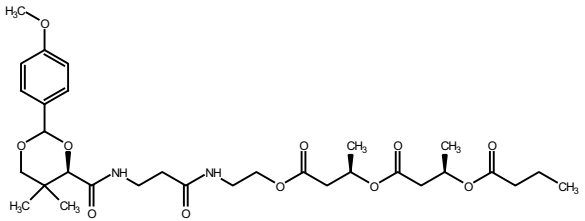








gCOSY, 400 MHz, $CDCl_3$



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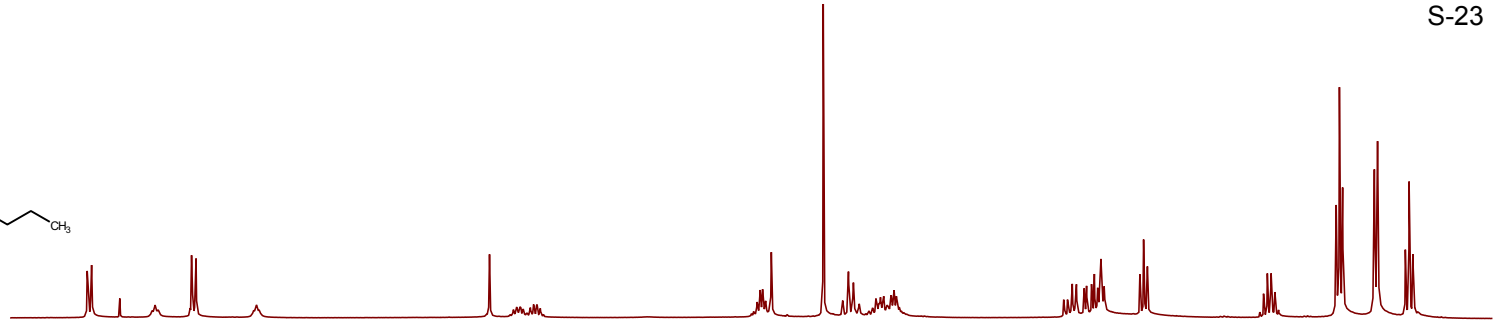
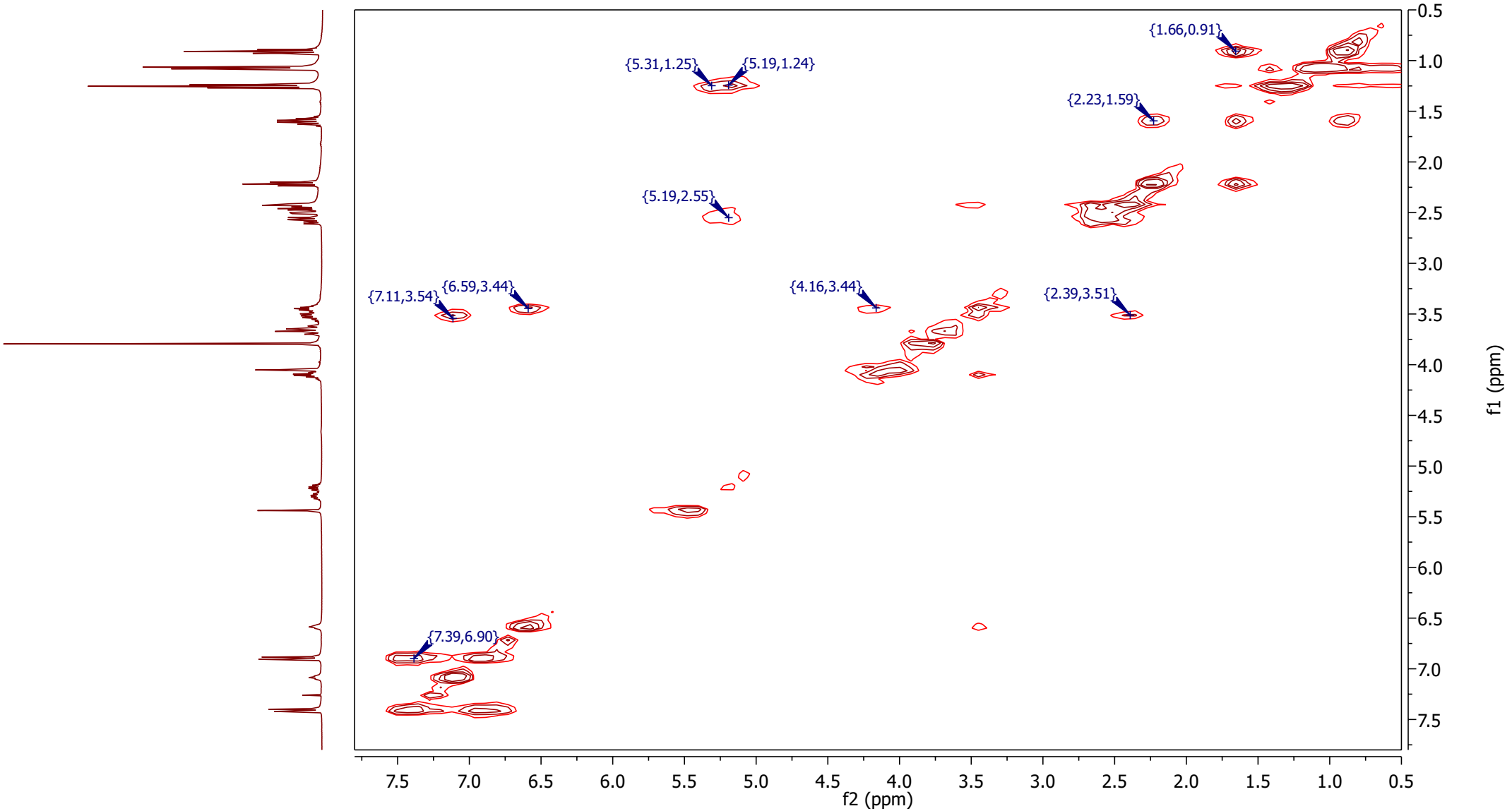
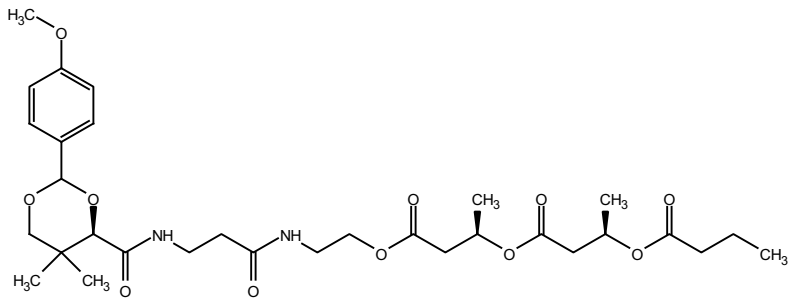




^{13}C NMR, 100 MHz, $CDCl_3$



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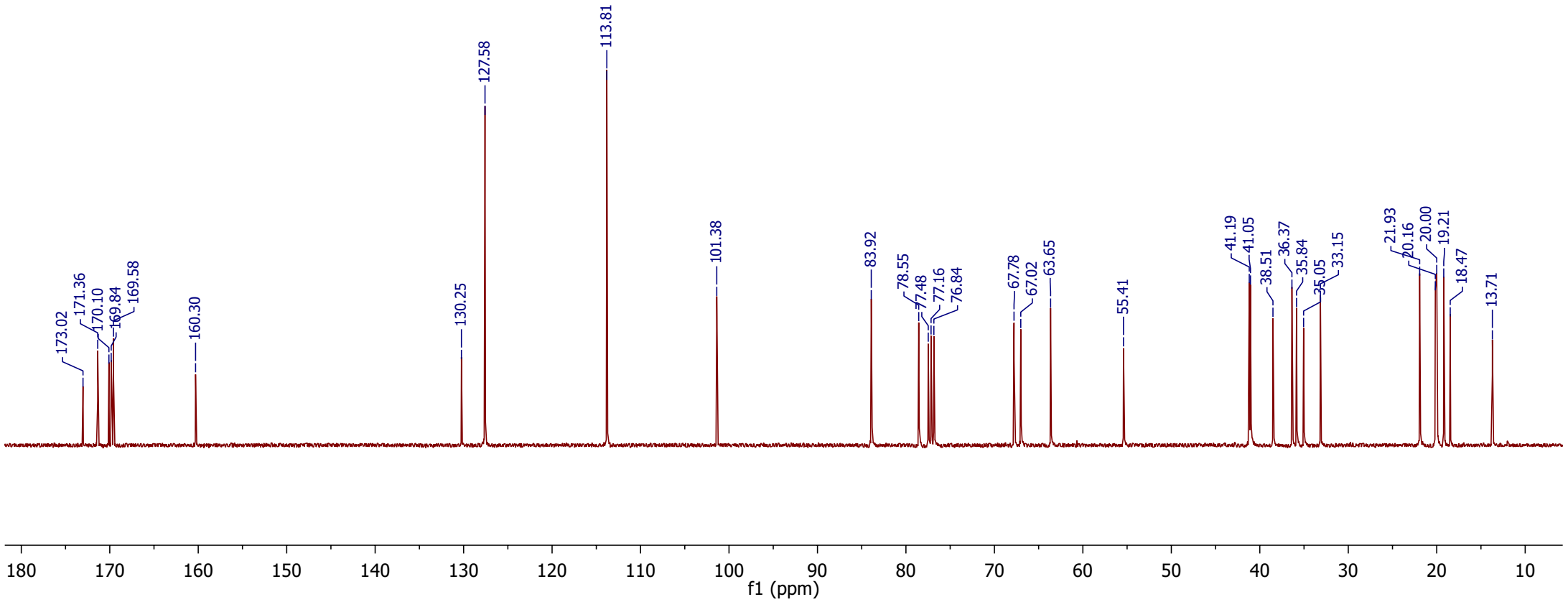
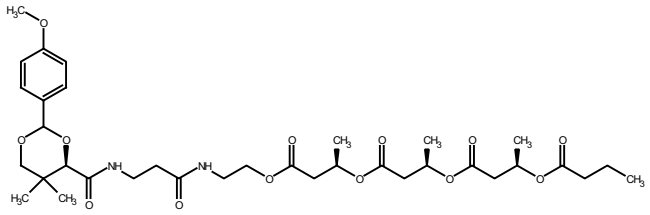


^{1}H NMR, 400 MHz, $CDCl_3$



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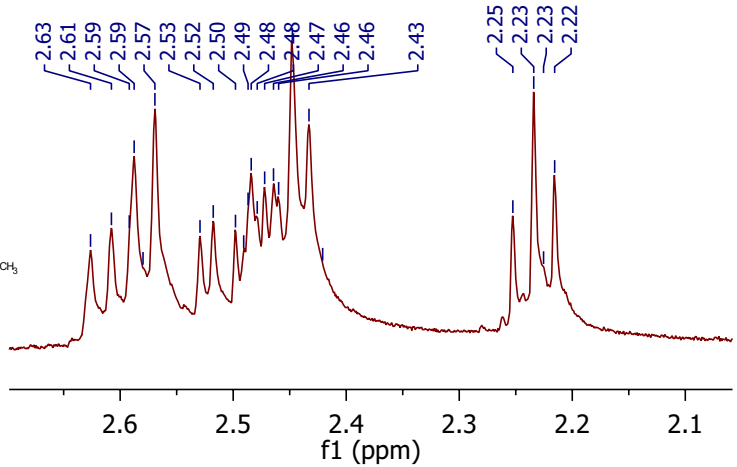
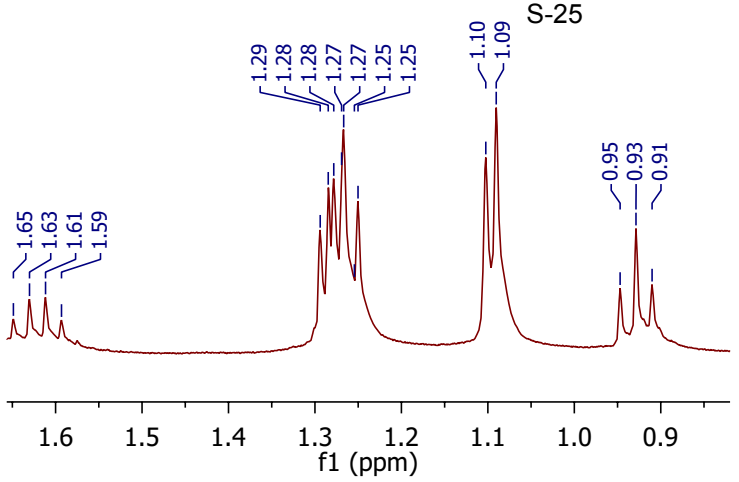
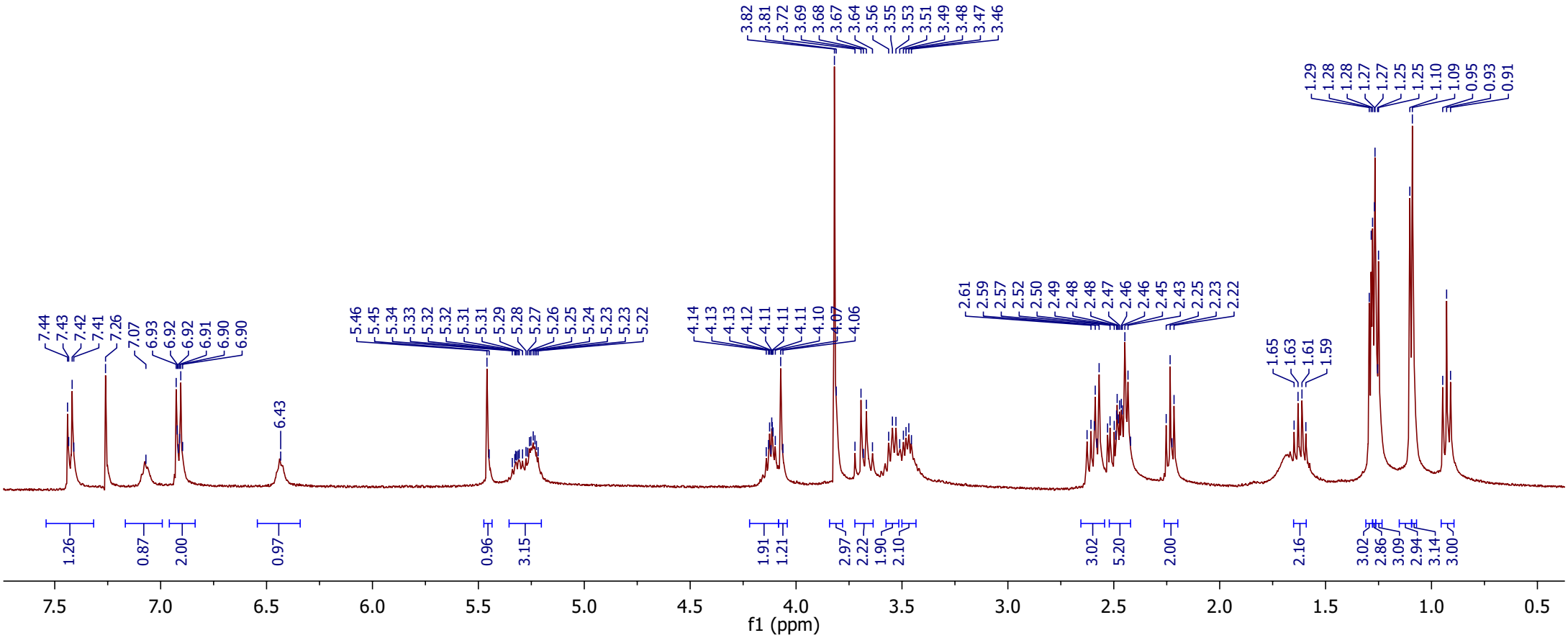
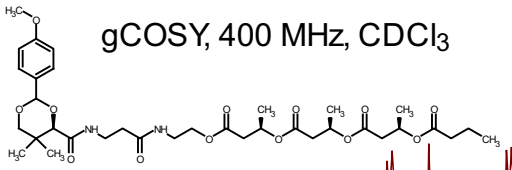




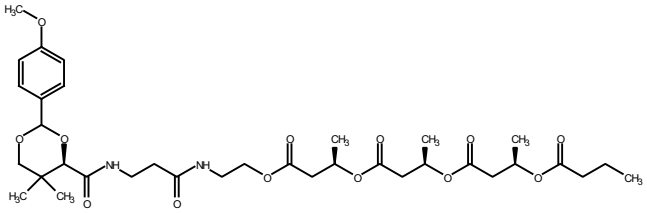


gCOSY, 400 MHz, $CDCl_3$

8e



^{13}C NMR, 100 MHz, $CDCl_3$



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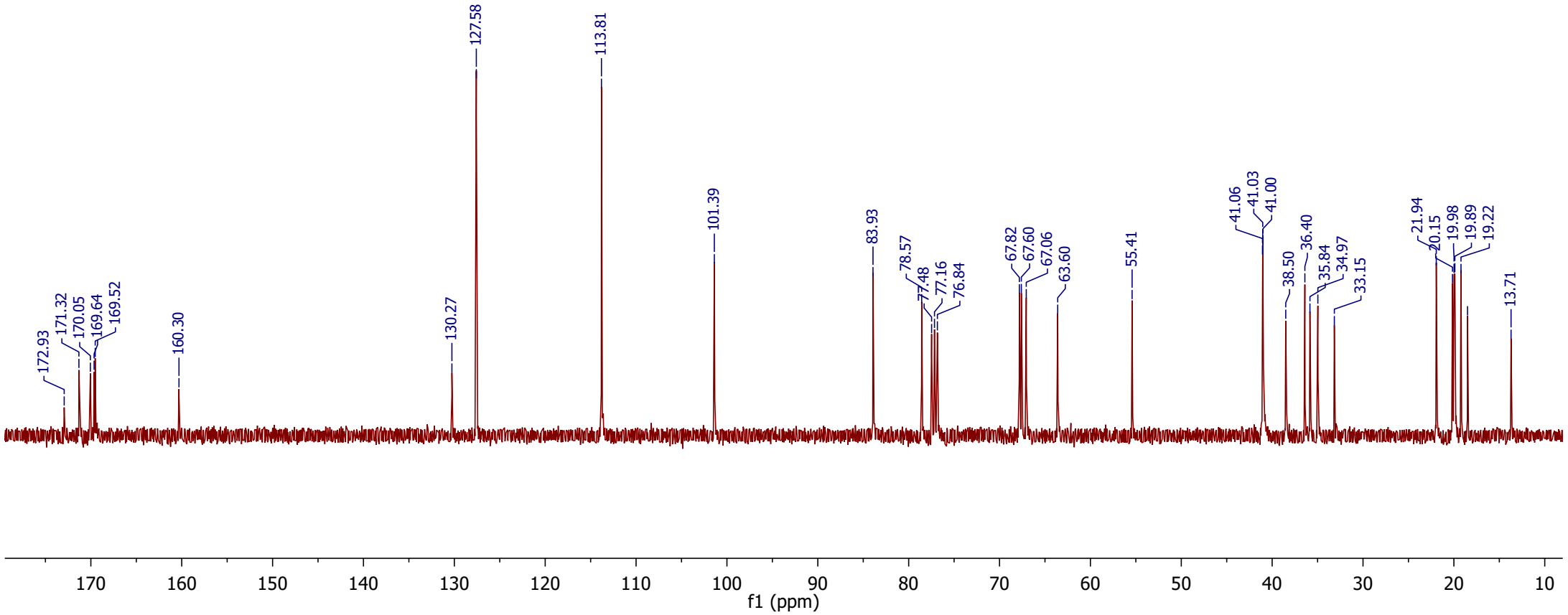
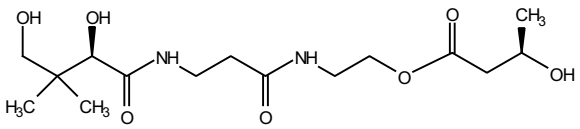


^{1}H NMR,400 MHz, $CDCl_3$



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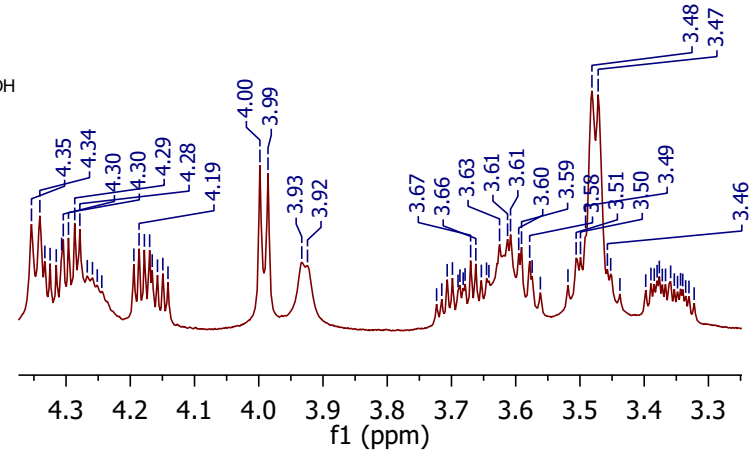
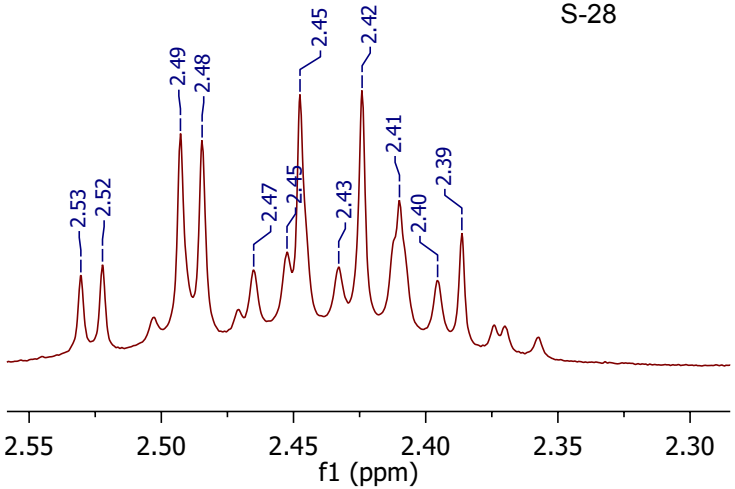
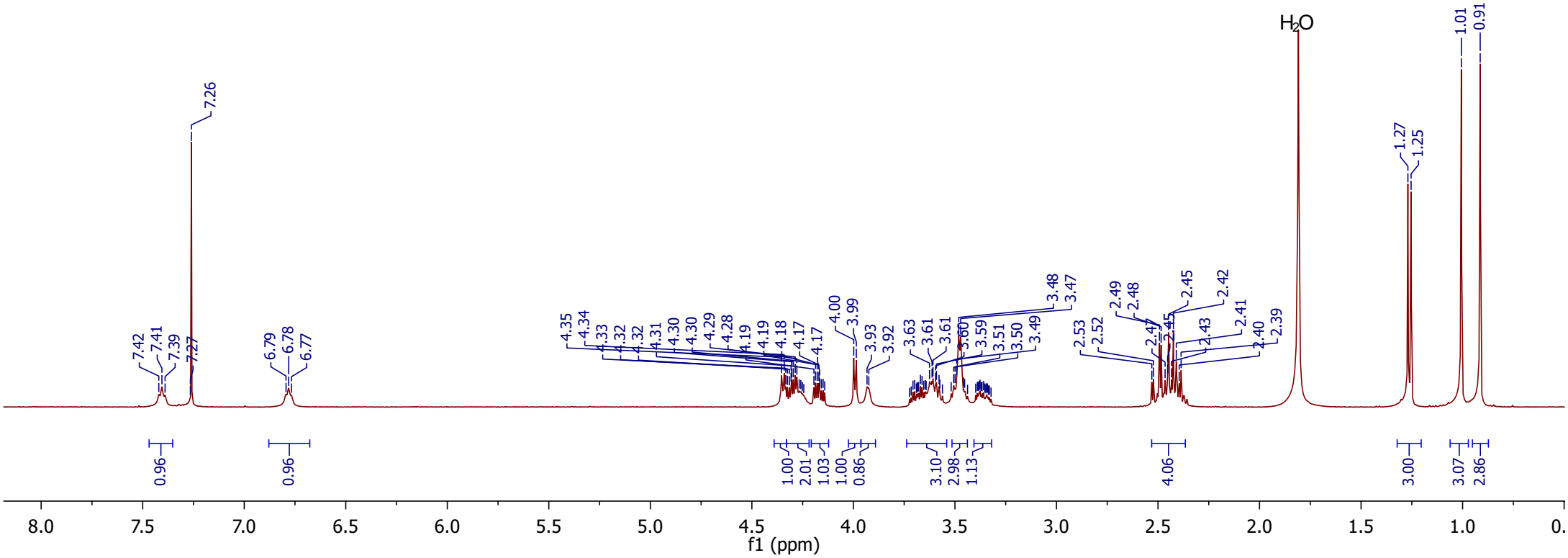
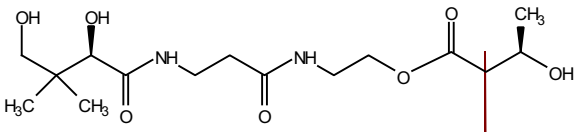




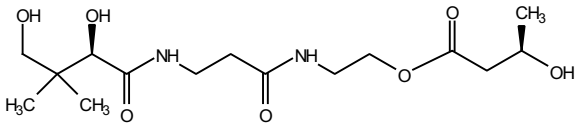


gCOSY,400 MHz, $CDCl_3$



9a

^{13}C NMR,100 MHz, $CDCl_3$



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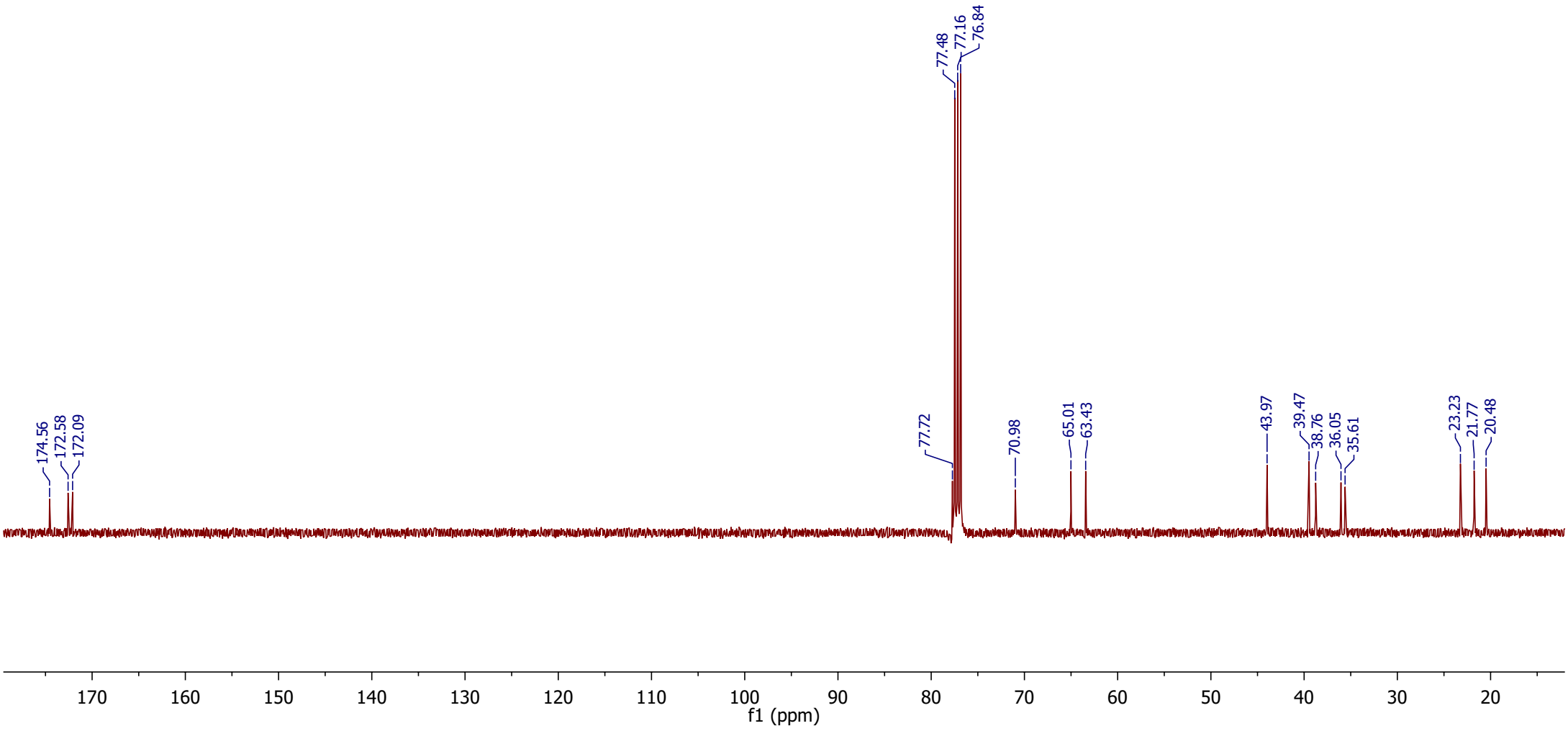
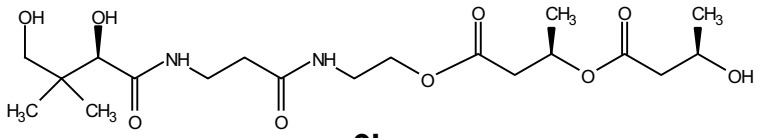


^{1}H NMR, 400 MHz, $CDCl_3$



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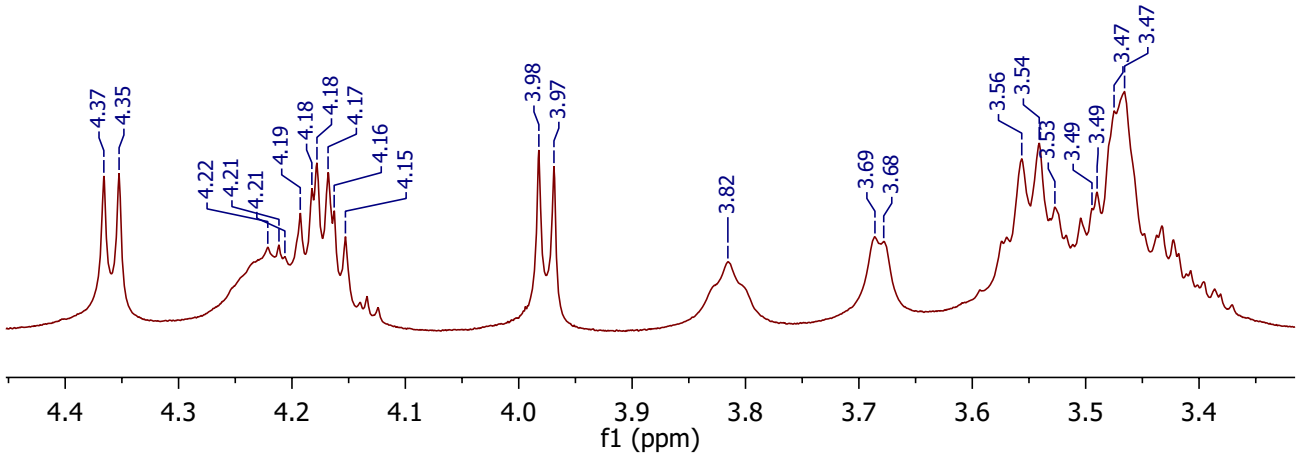
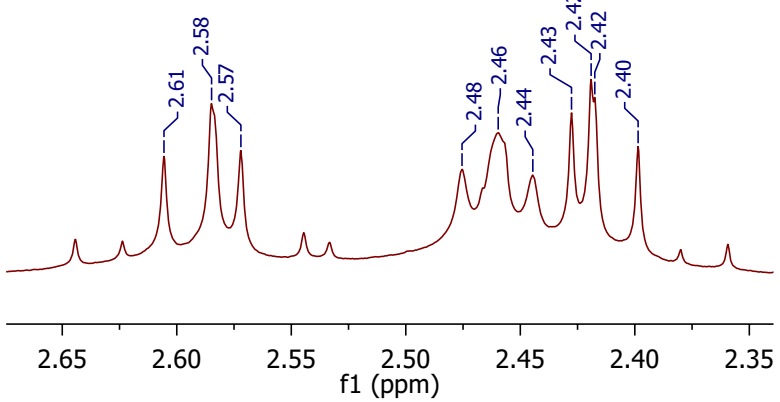
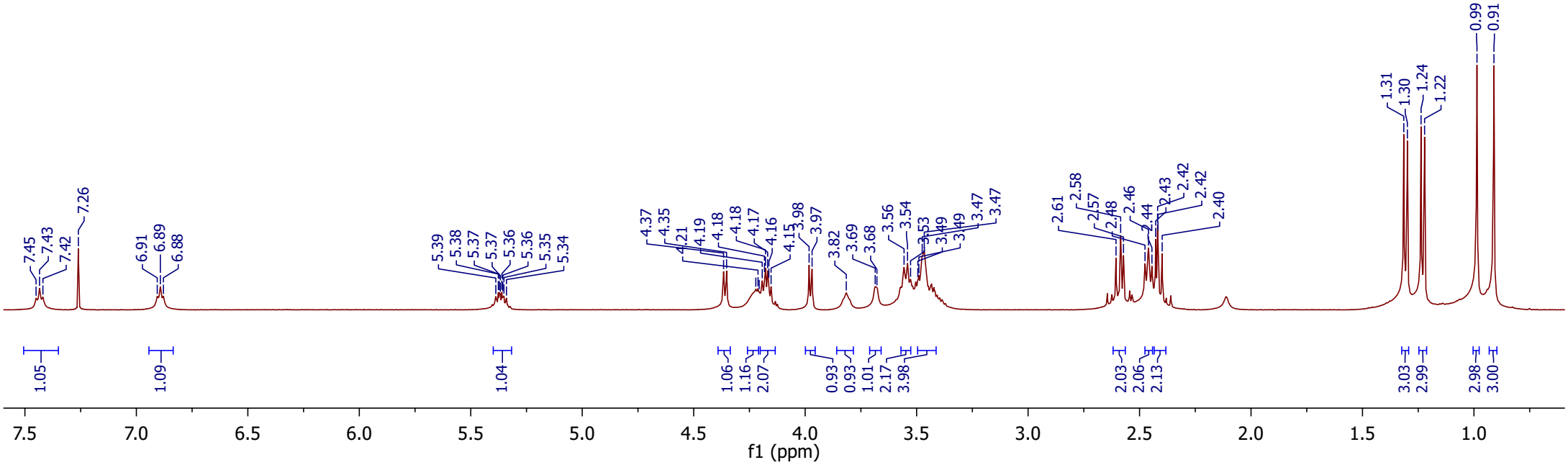
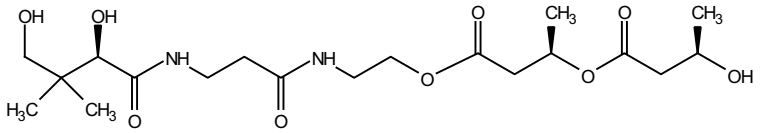






gCOSY, 400 MHz, $CDCl_3$



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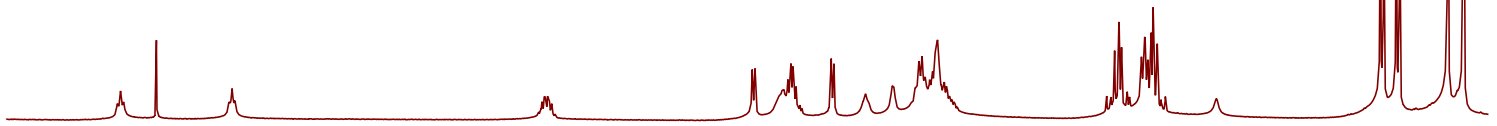
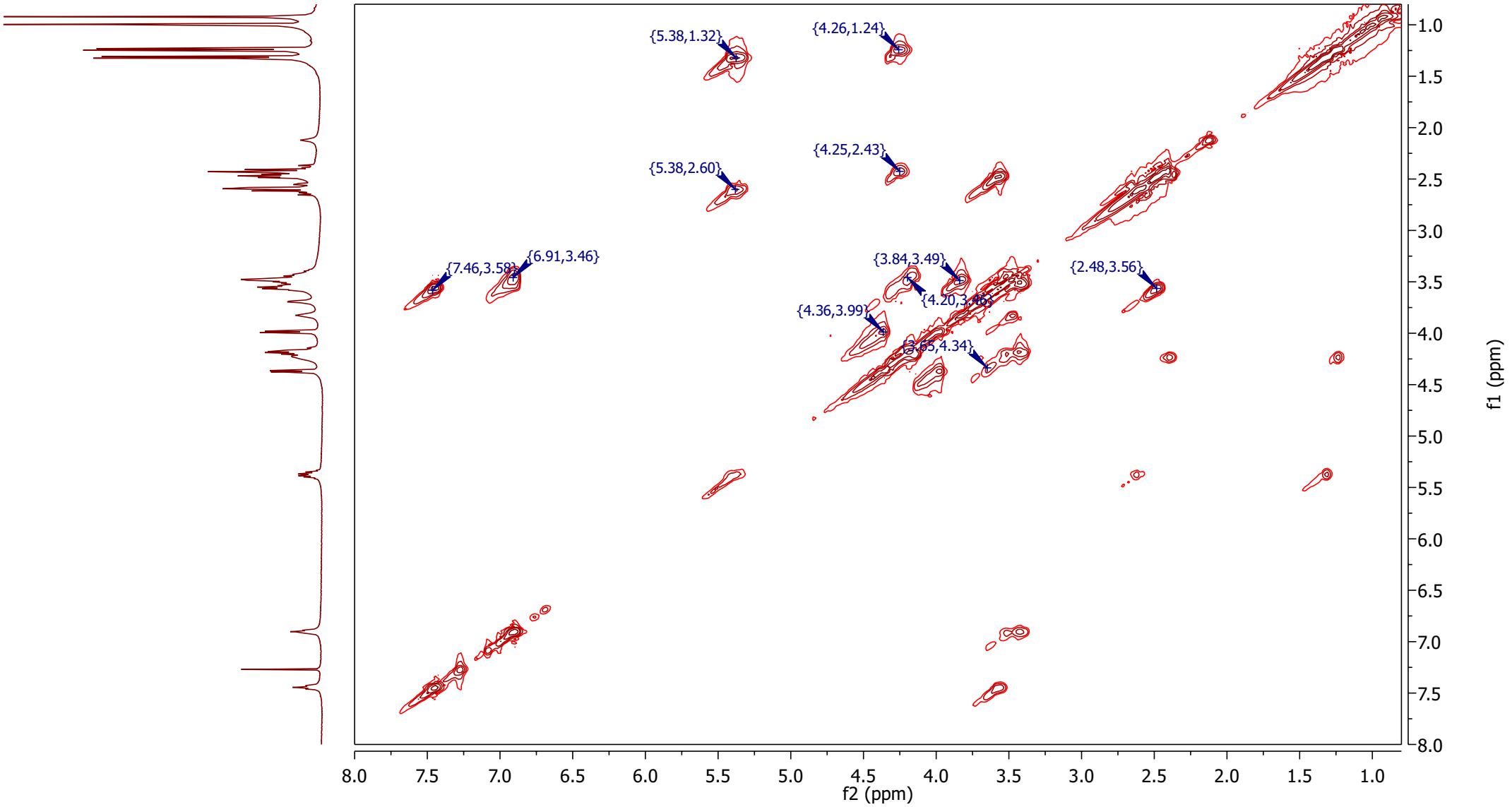
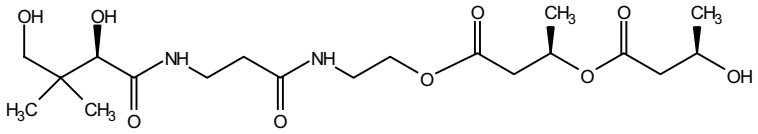




^{13}C NMR, 100 MHz, $CDCl_3$



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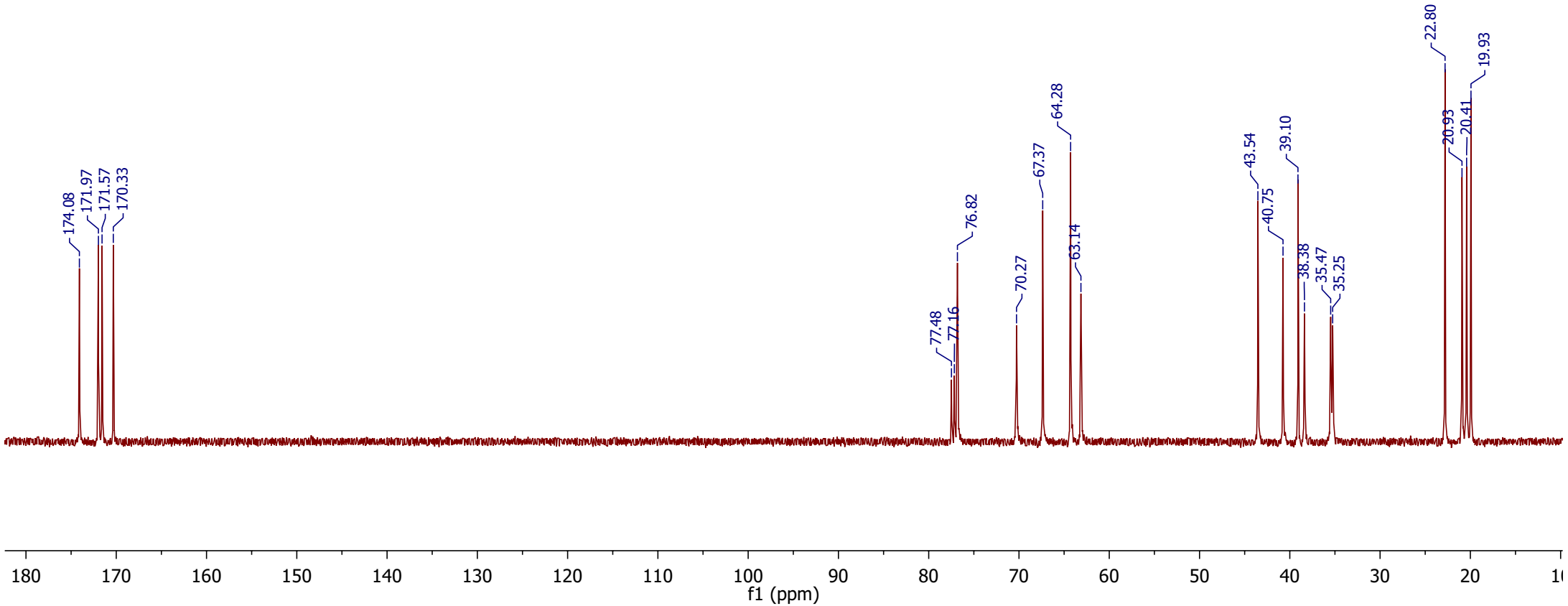

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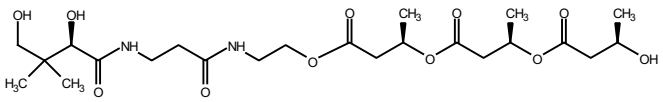

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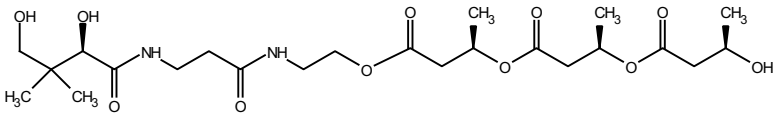
gCOSY, 400 MHz, $CDCl_3$



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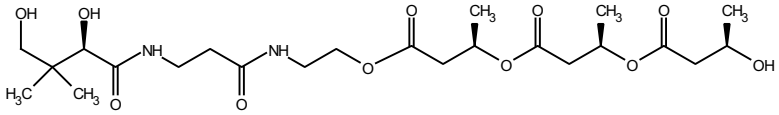

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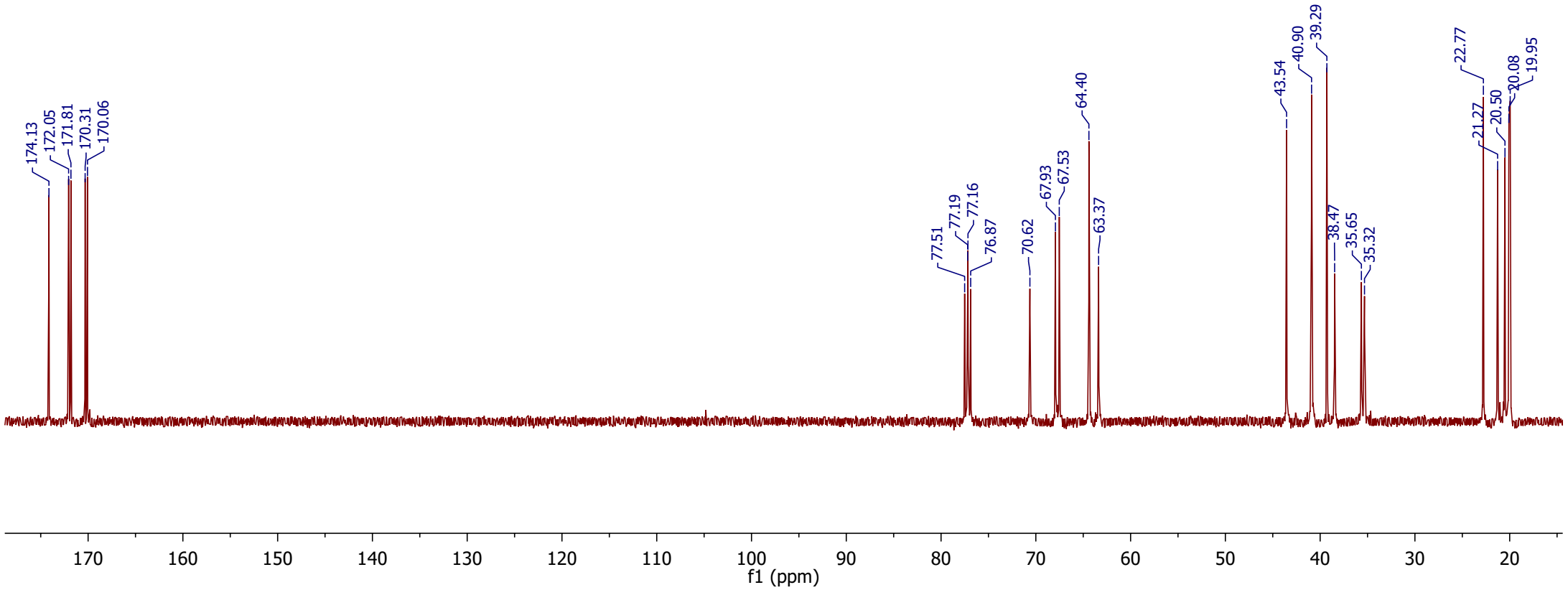
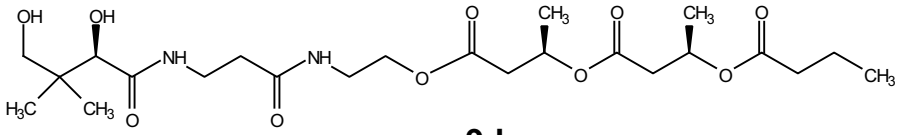


^{1}H NMR, 400 MHz, $CDCl_3$



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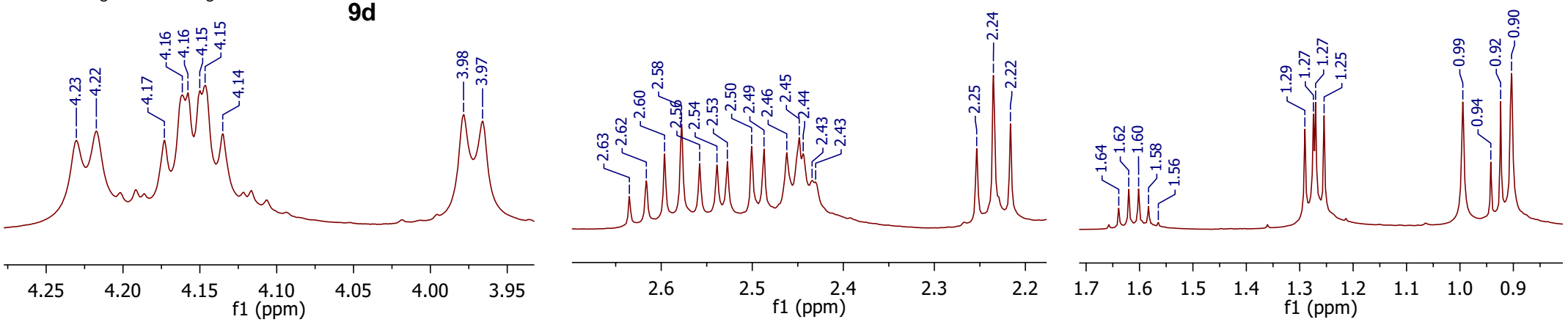
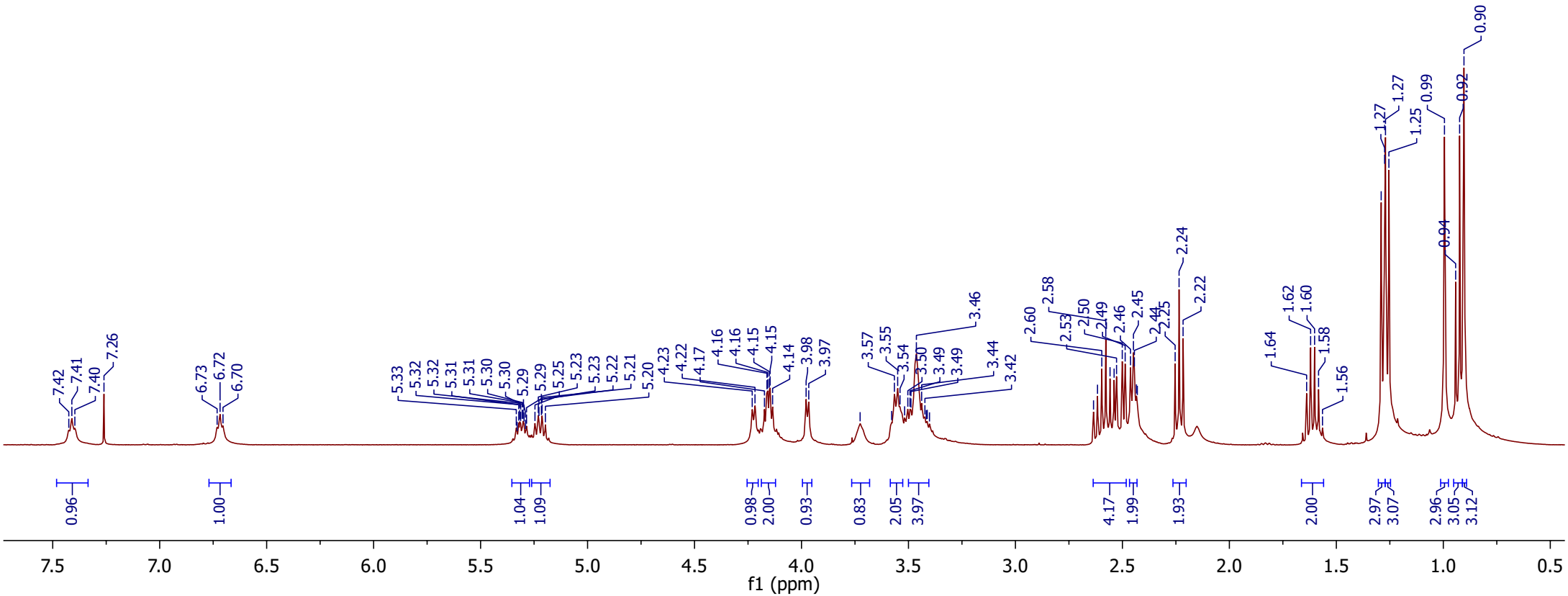
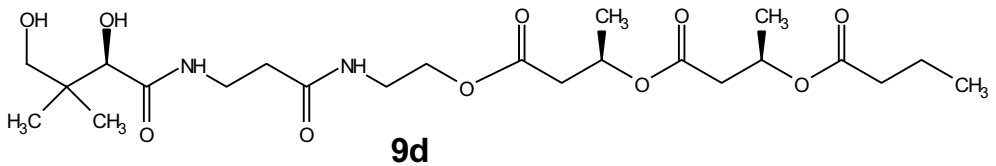




gCOSY, 400 MHz, $CDCl_3$



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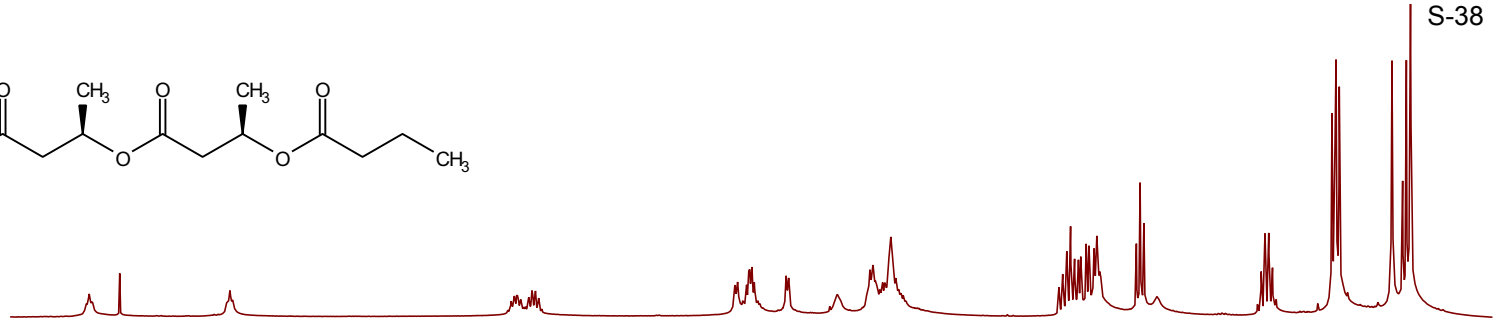
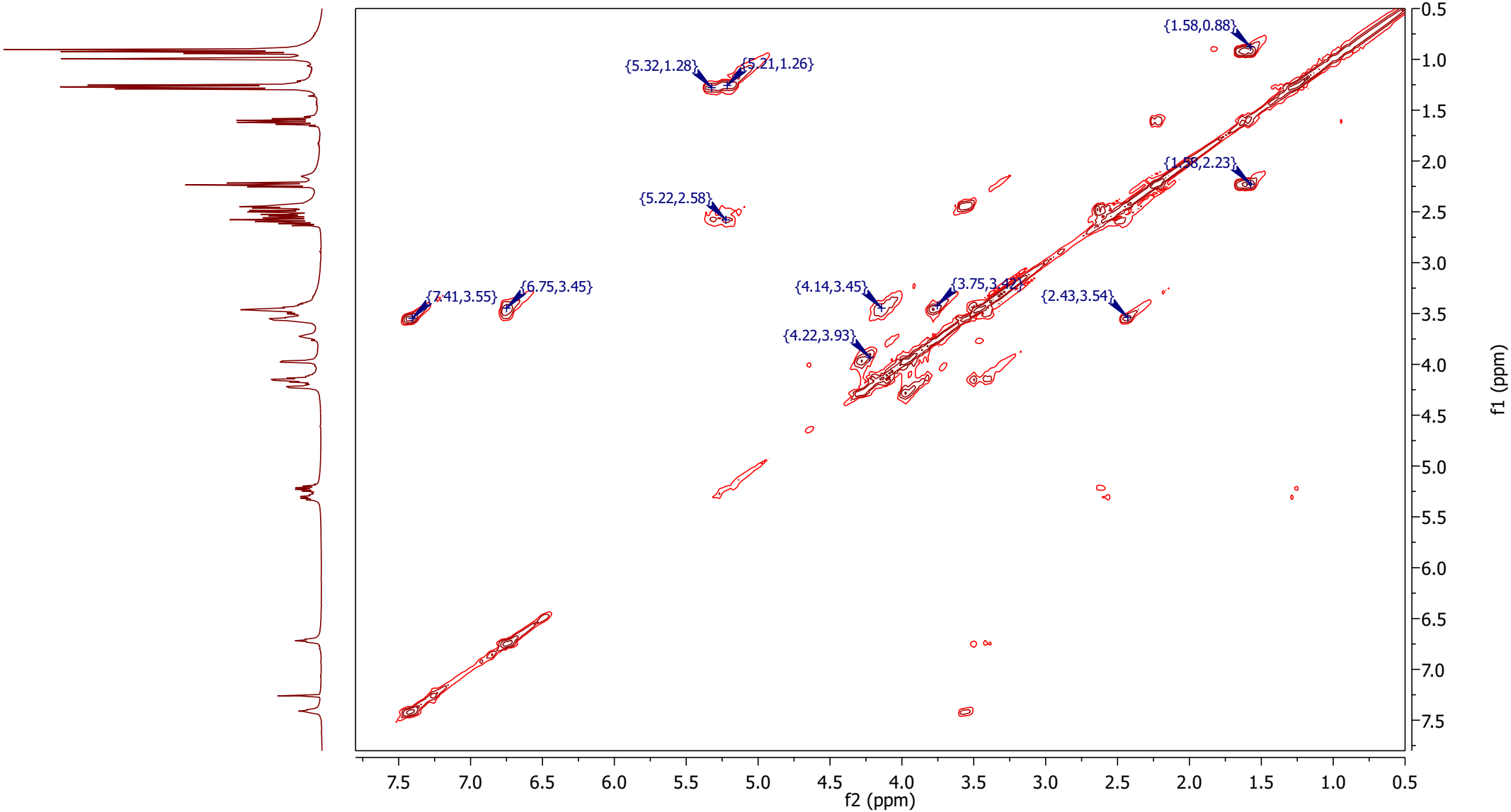
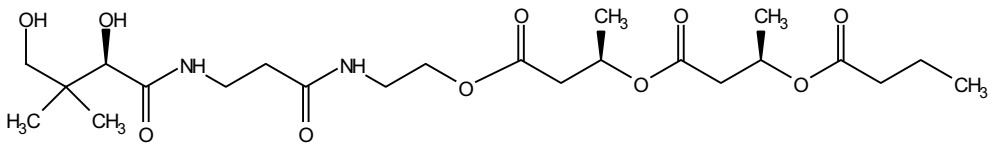




^{13}C NMR, 100 MHz, $CDCl_3$



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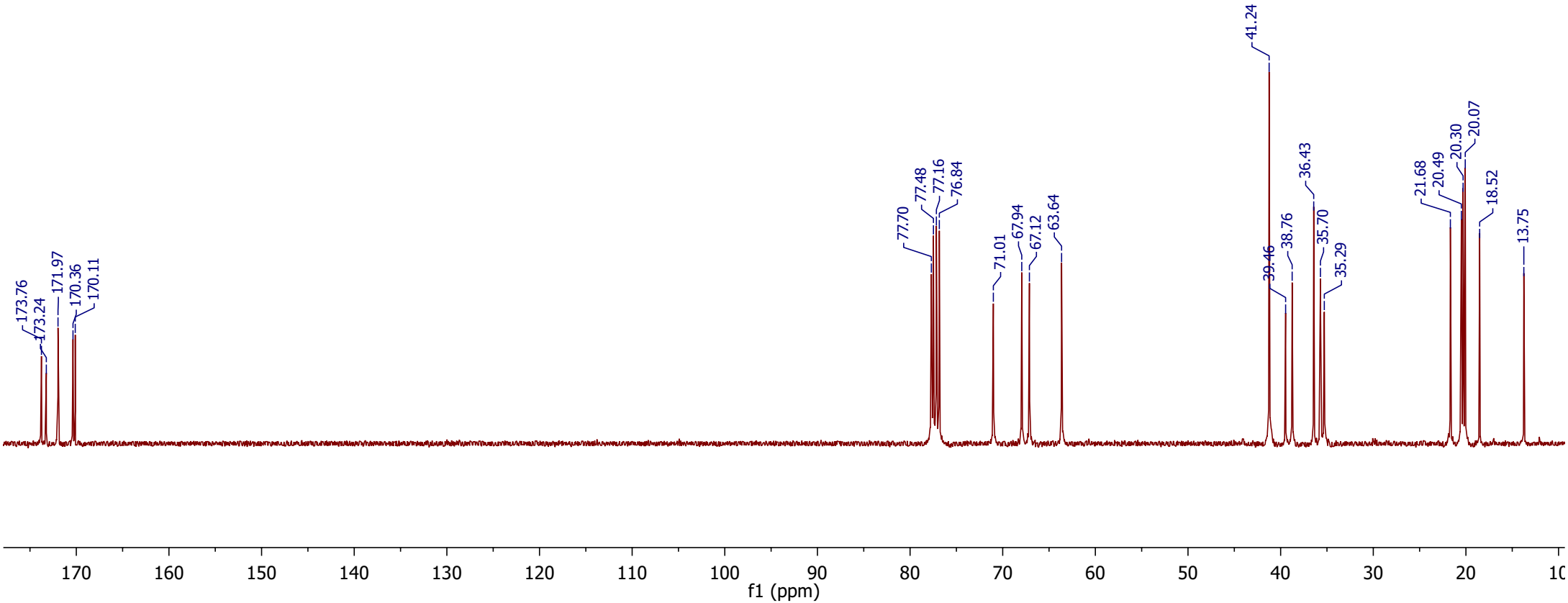
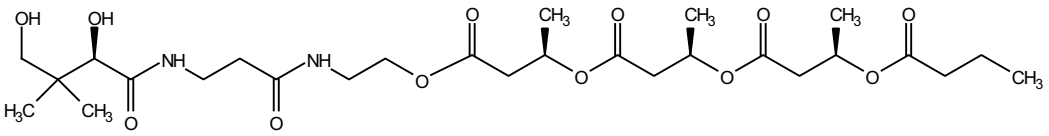


^{1}H NMR, 400 MHz, $CDCl_3$



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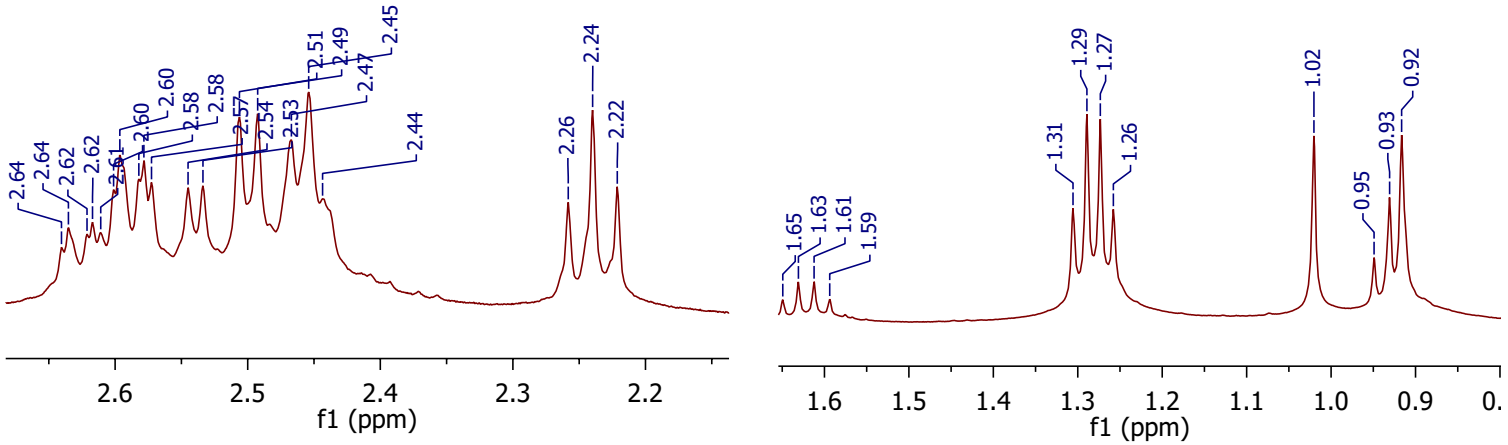
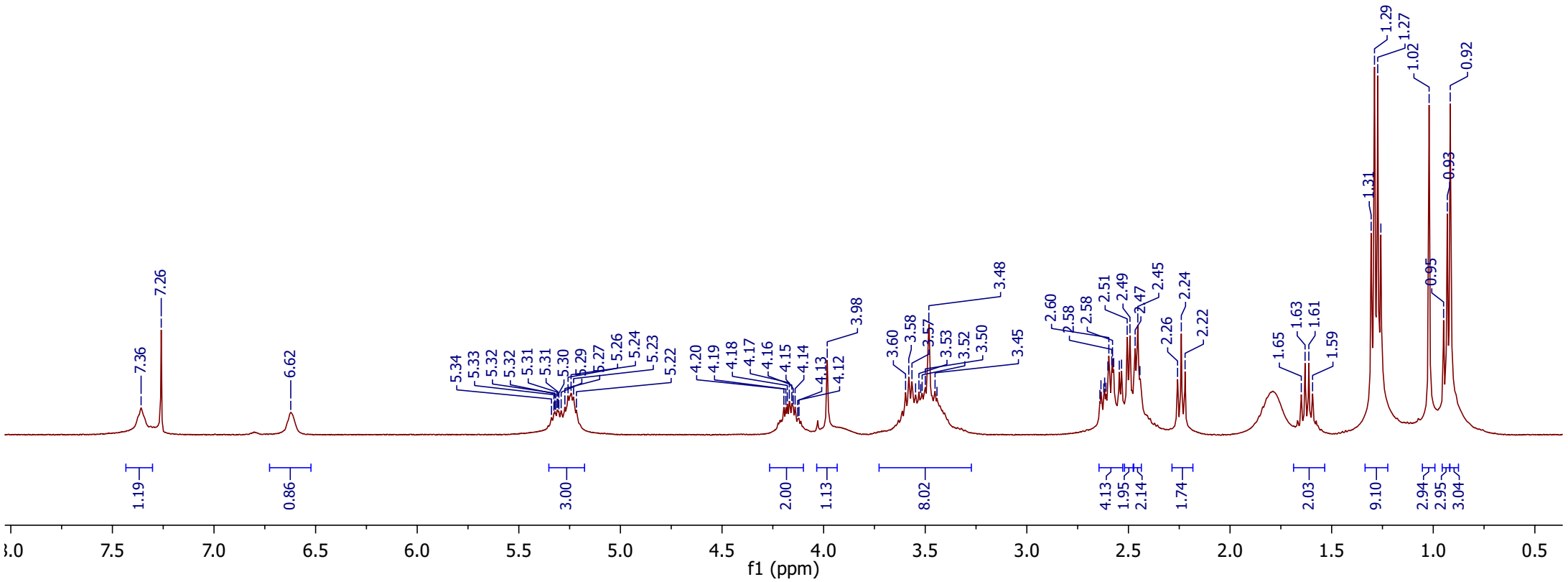
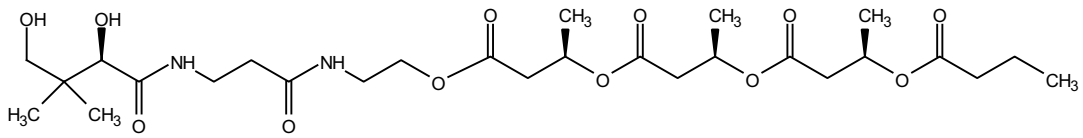


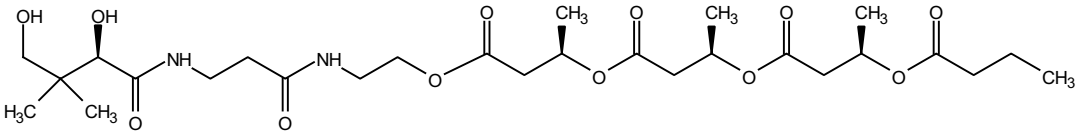


gCOSY, 100 MHz, $CDCl_3$



9e

^{13}C NMR, 100 MHz, $CDCl_3$



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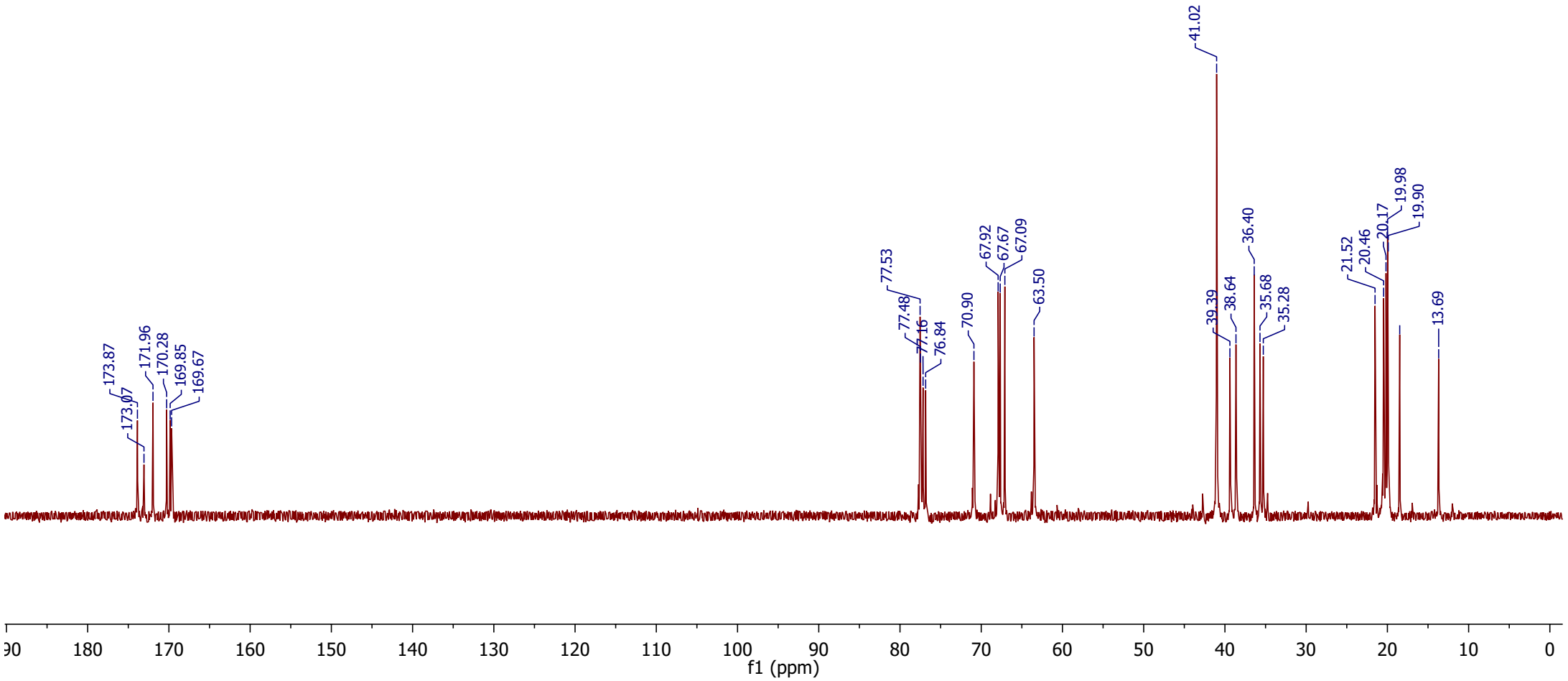
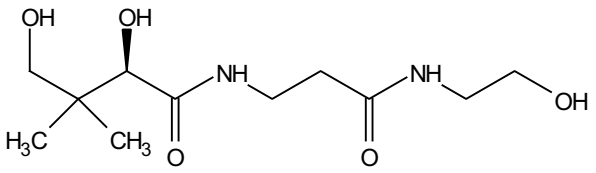


^{1}H NMR, 400 MHz, D_2O



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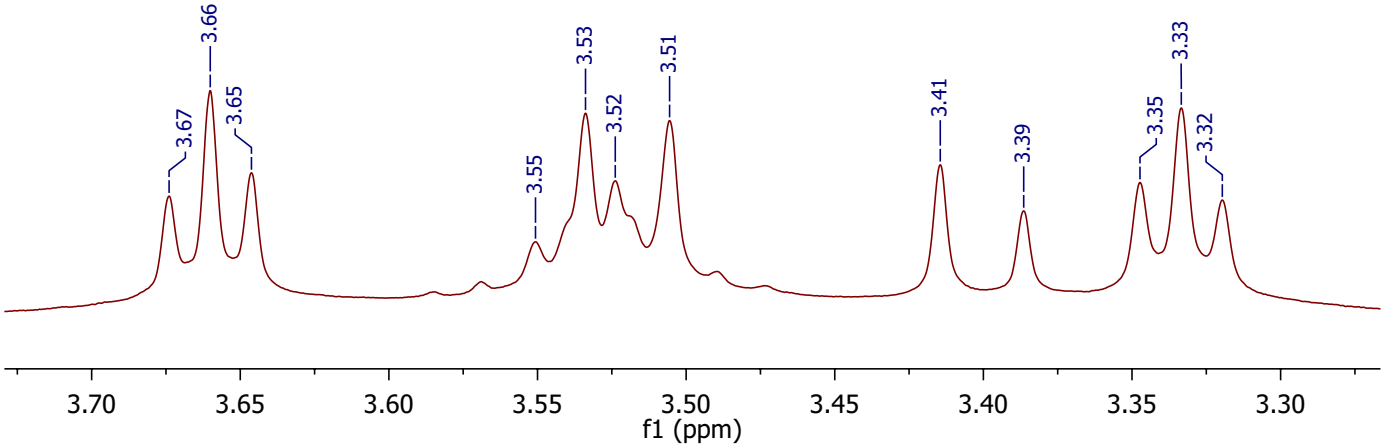
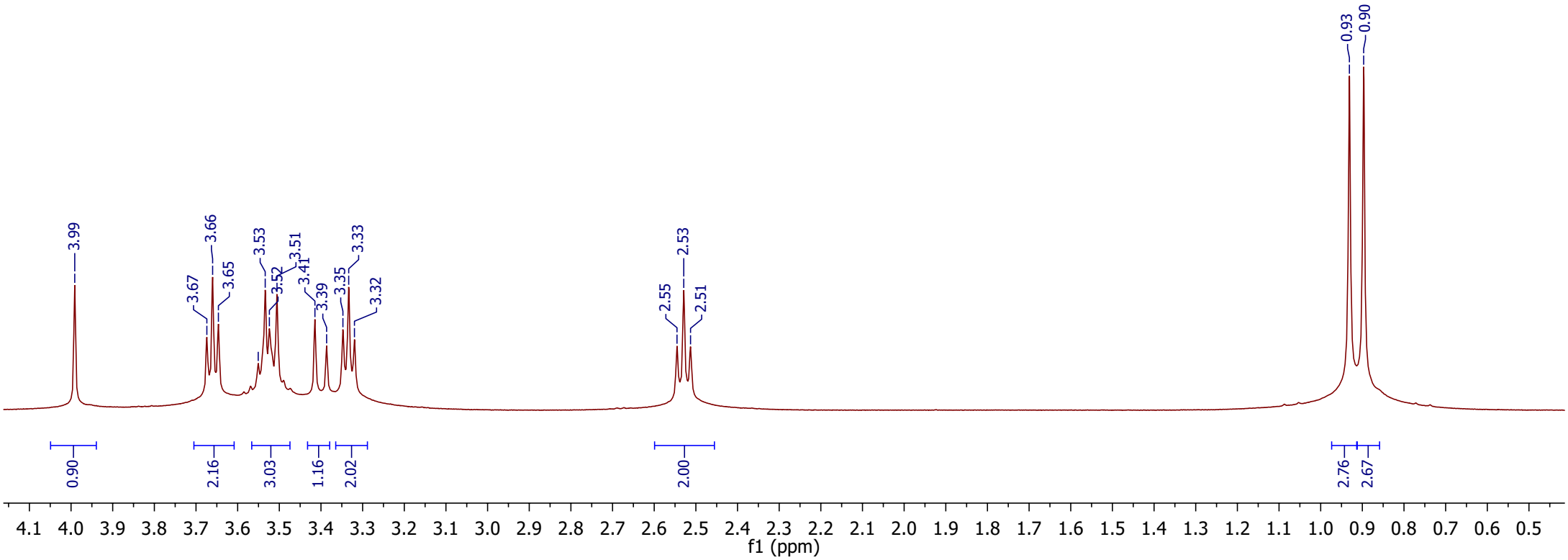
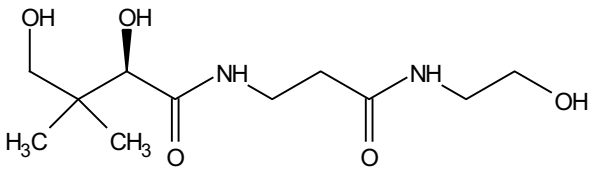




gCOSY, 400 MHz, D_2O



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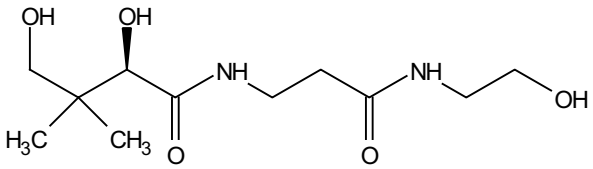
^{13}C NMR, 100 MHz, D_2O



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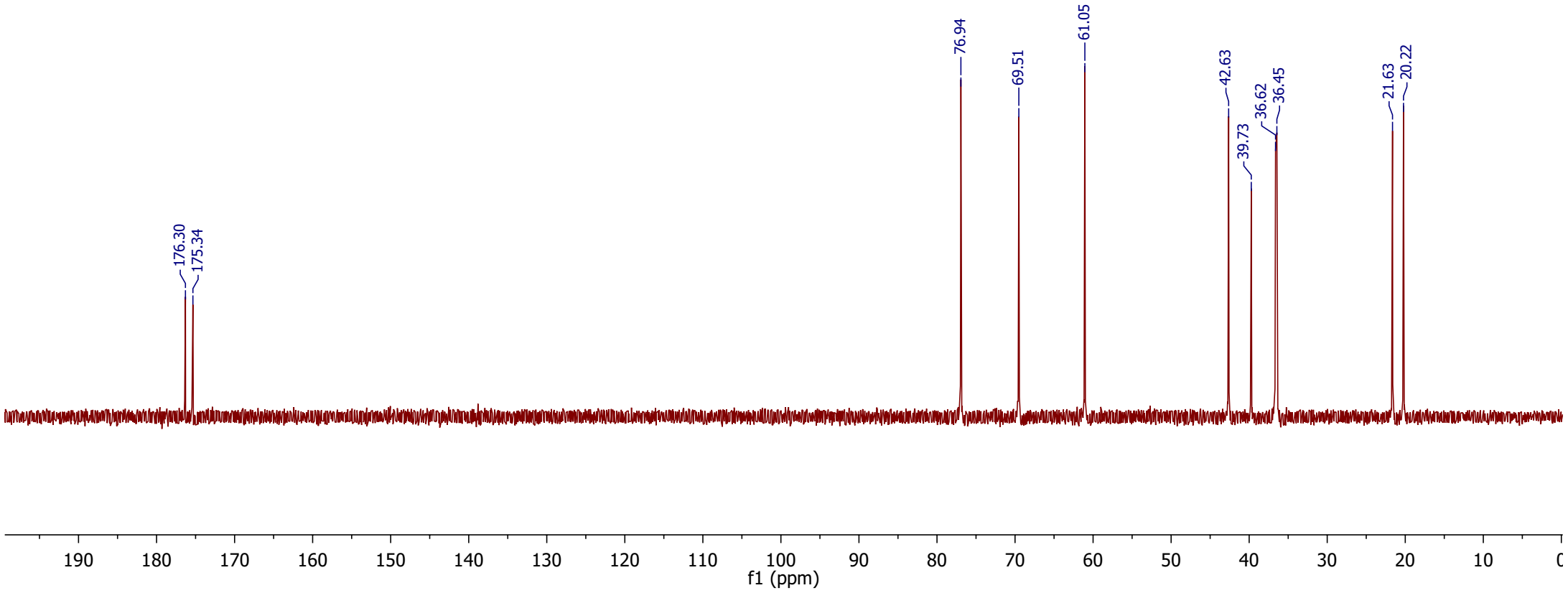
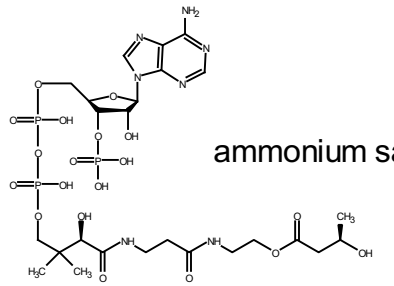
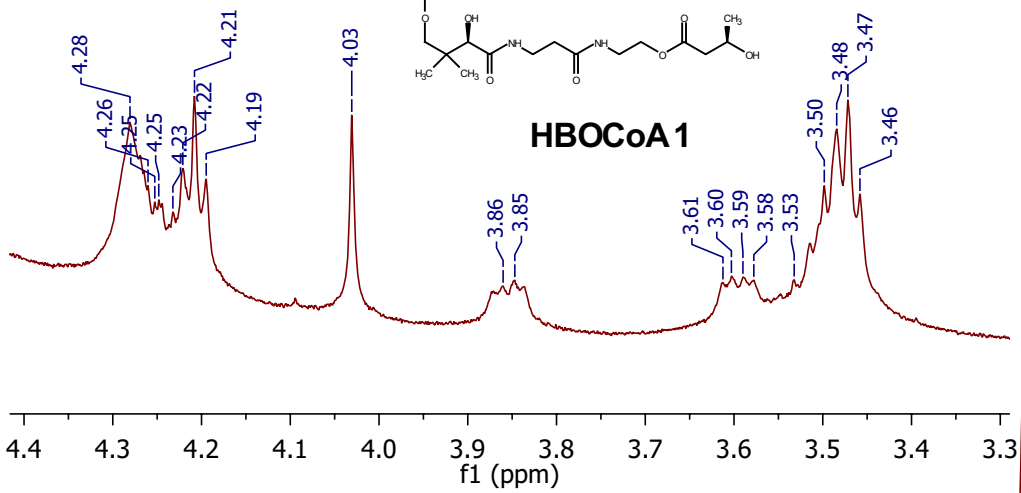


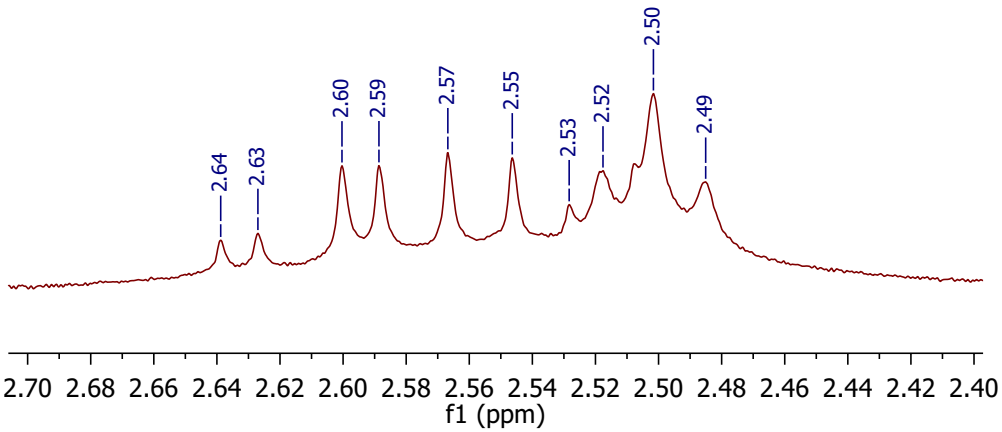
^{1}H NMR, 400 MHz, D_2O

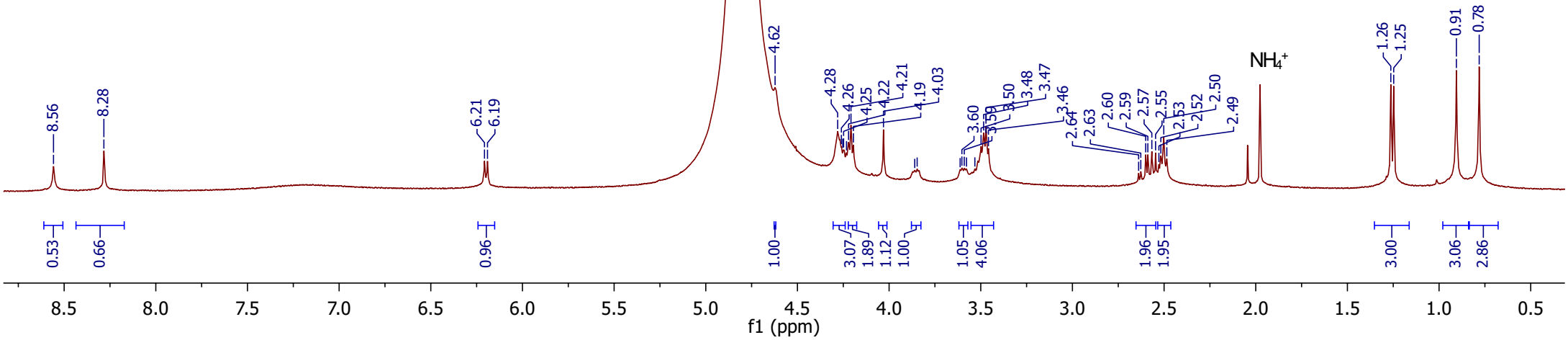


ammonium salt

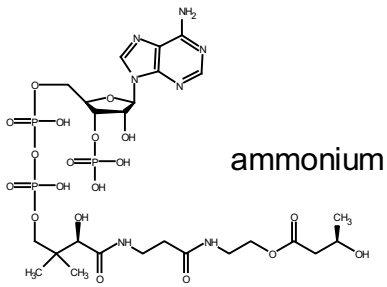
HBOCoA 1







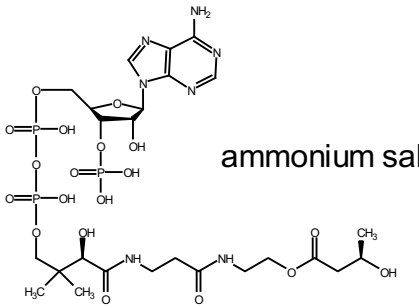
gCOSY NMR, 400 MHz, D_2O



ammonium salt

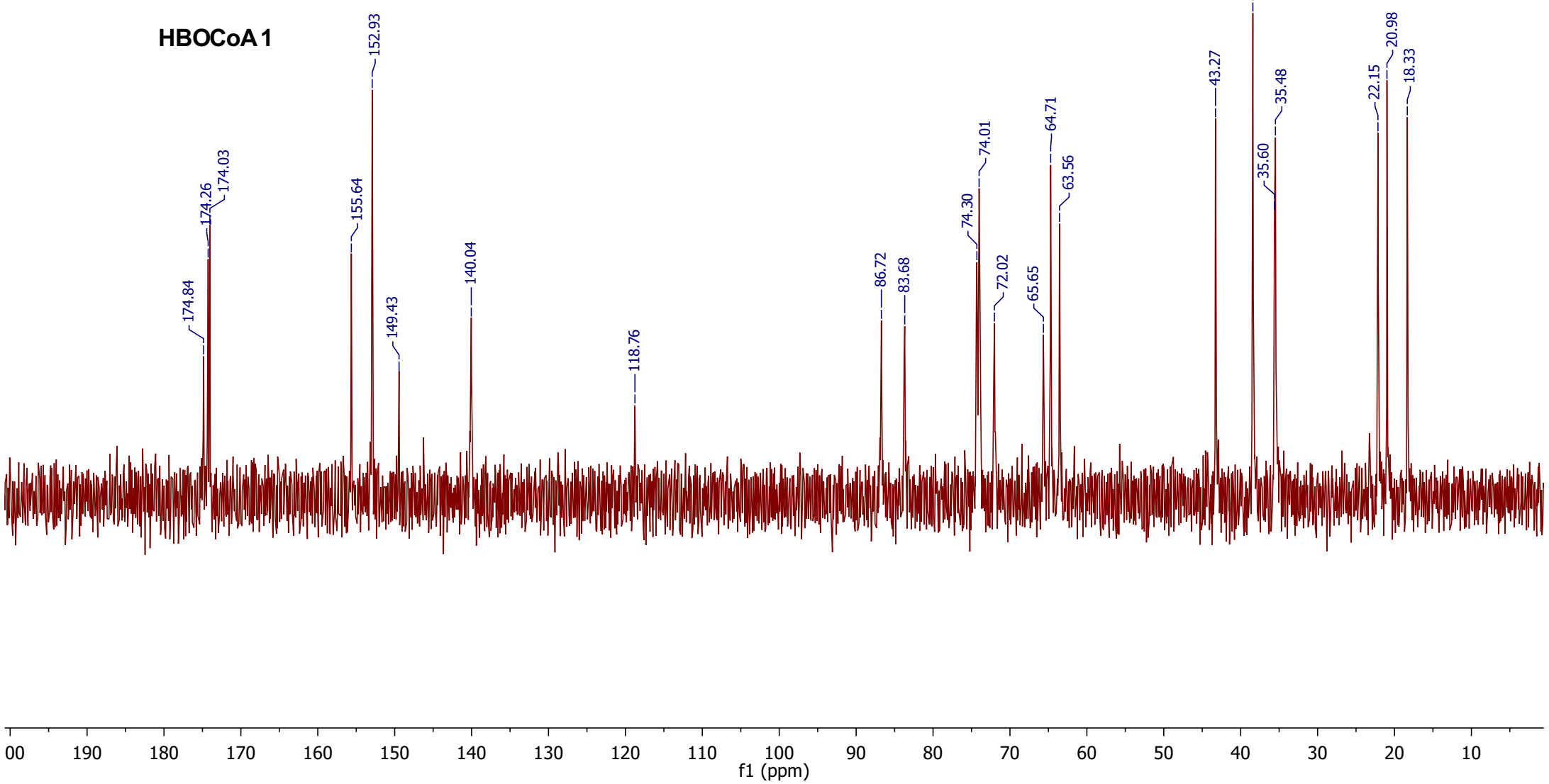
HBOCoA 1

^{13}C NMR, 100 MHz, D_2O

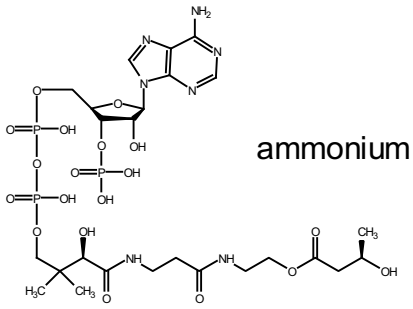


ammonium salt

HBOCoA 1

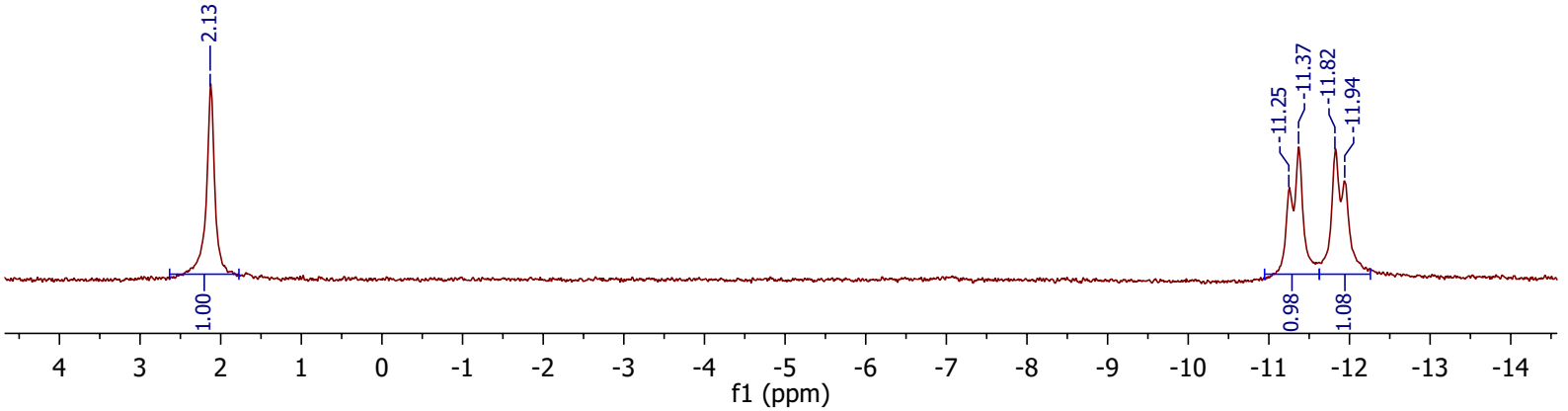


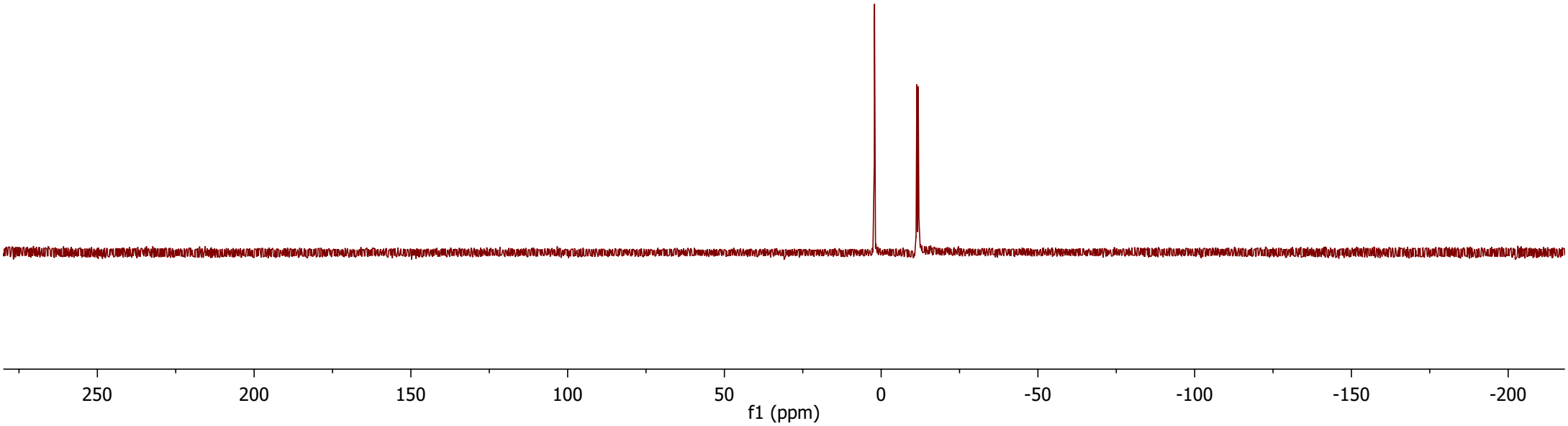
^{31}P NMR, 161 MHz, D_2O



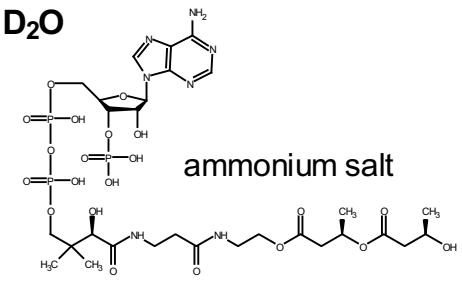
ammonium salt

HBOCoA 1



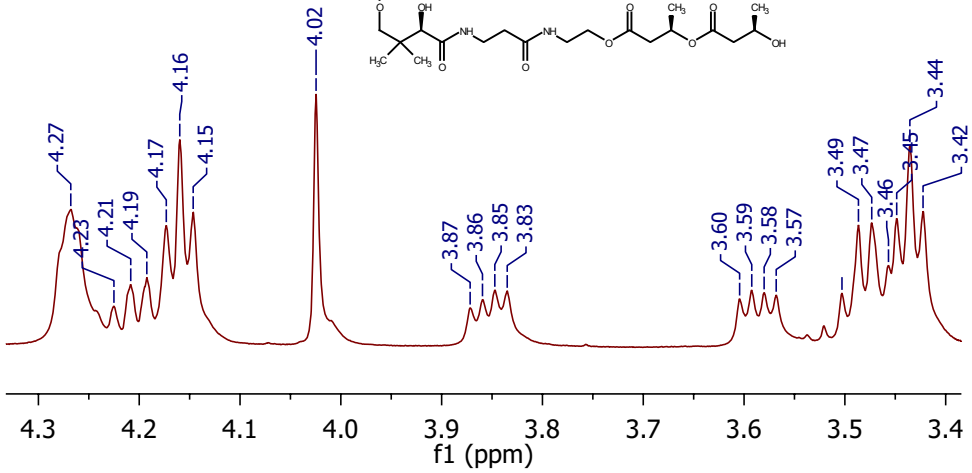


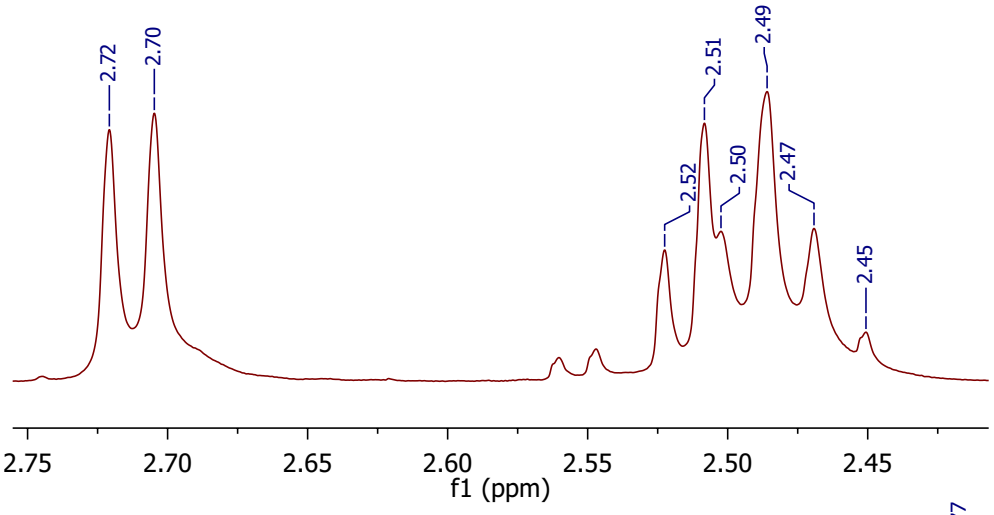
^{1}H NMR, 400 MHz, D_2O

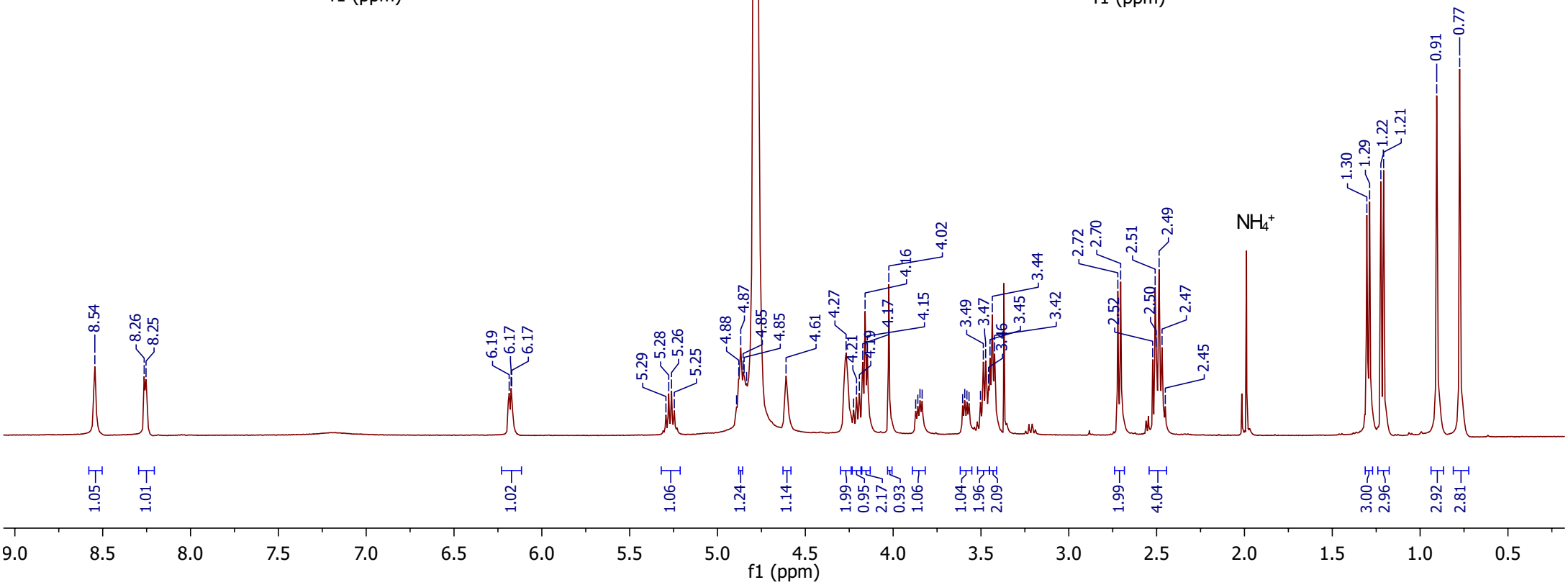


ammonium salt

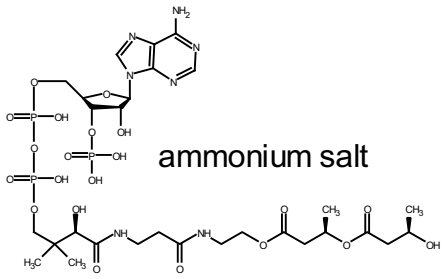
$(HB)_2$OCoA 2a





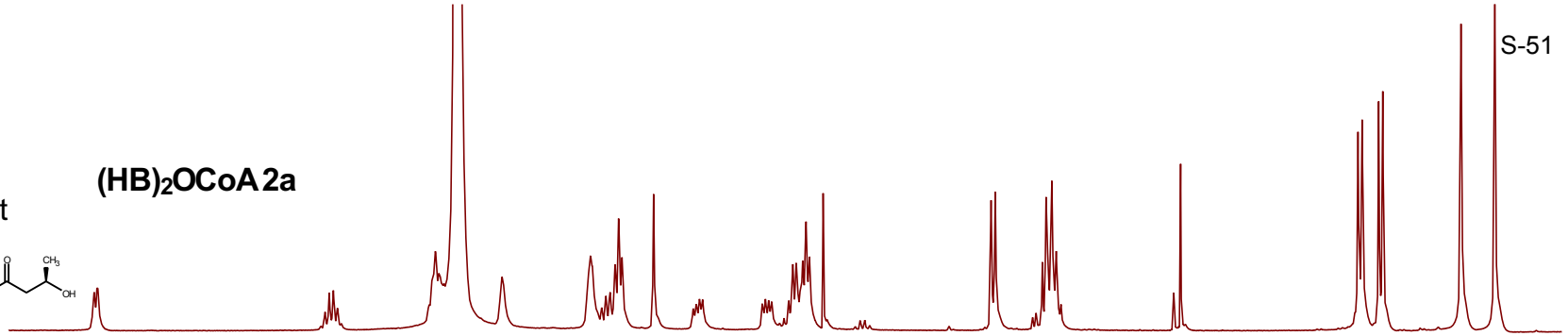


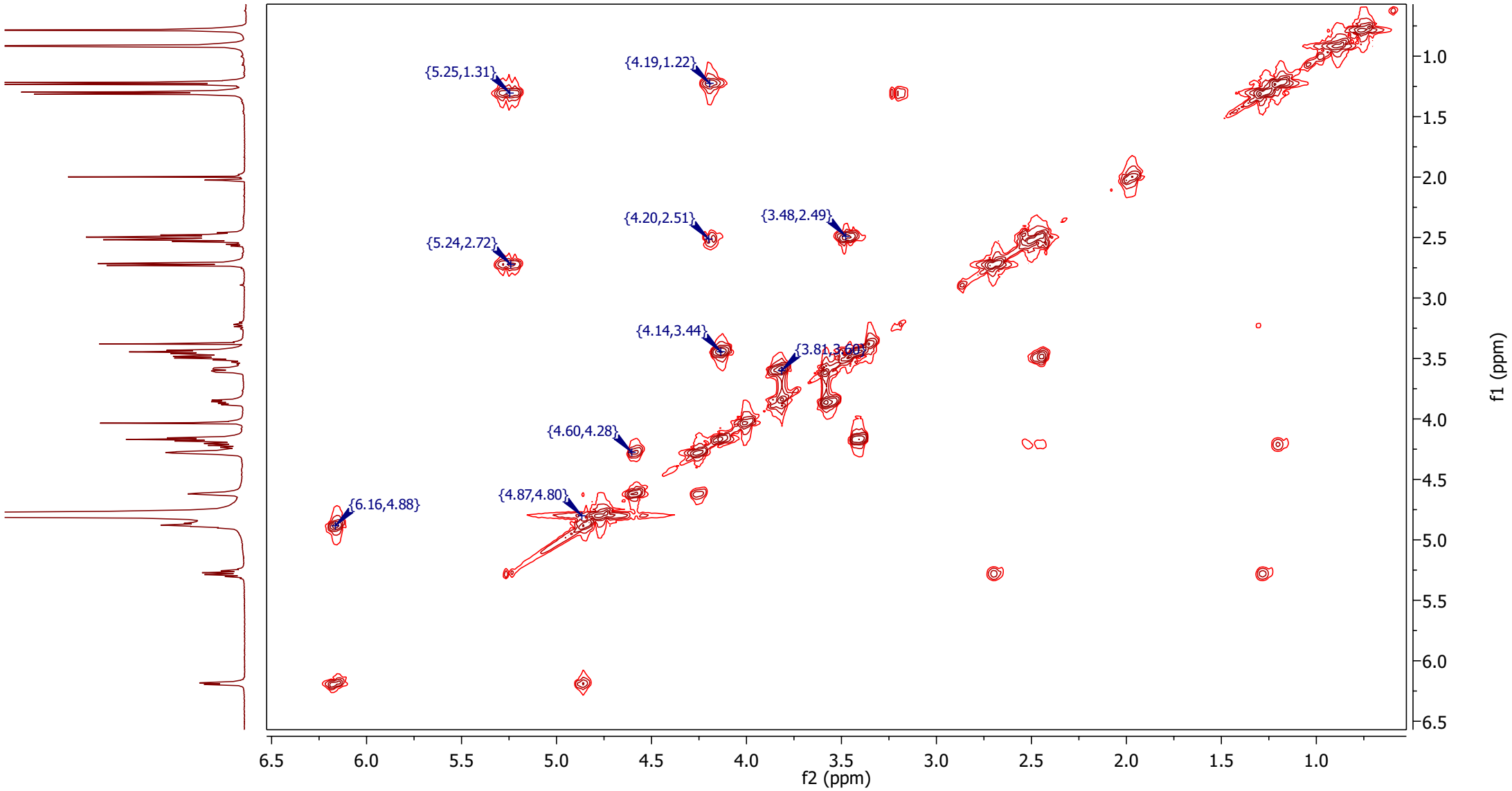
gCOSY, 400 MHz, D_2O



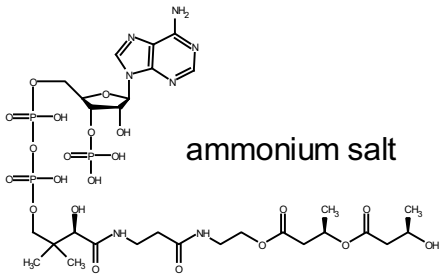
ammonium salt

$(HB)_2$OCoA 2a



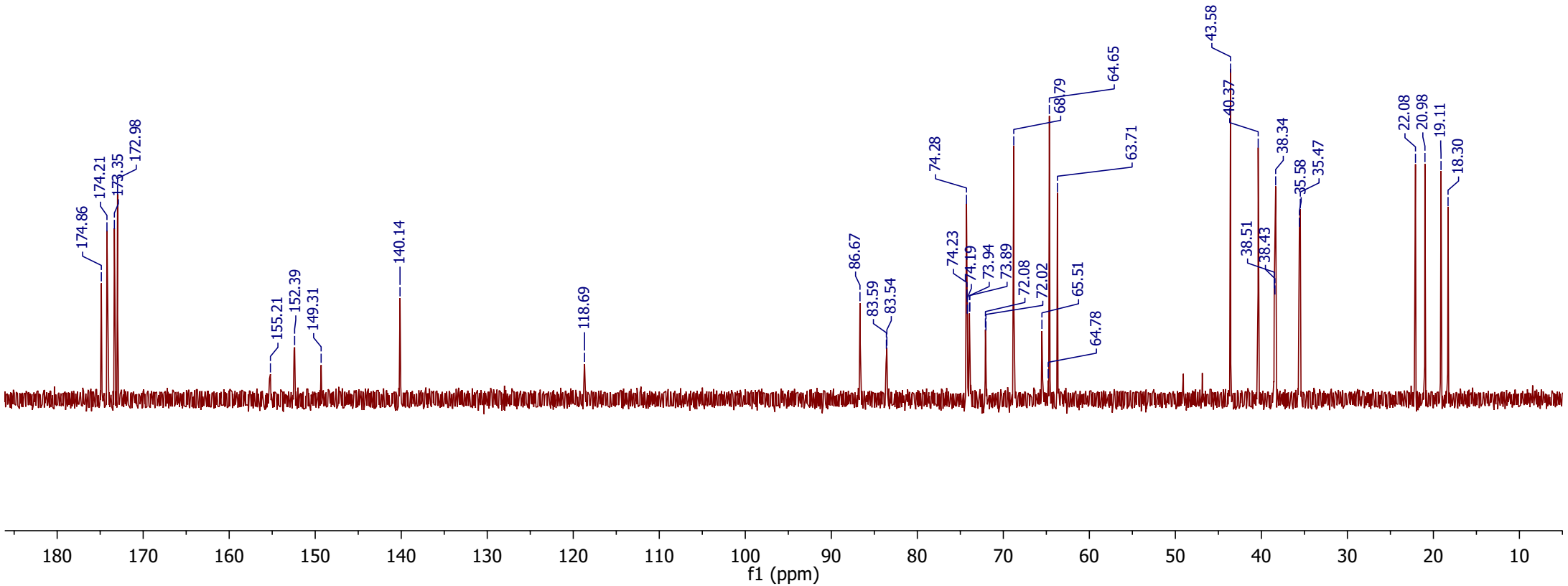


^{13}C NMR, 100 MHz, D_2O

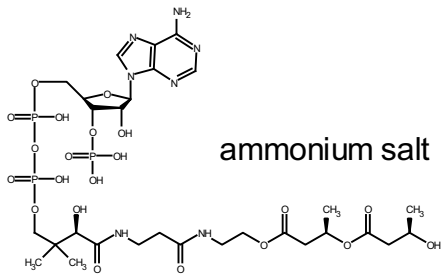


ammonium salt

$(HB)_2$OCoA 2a

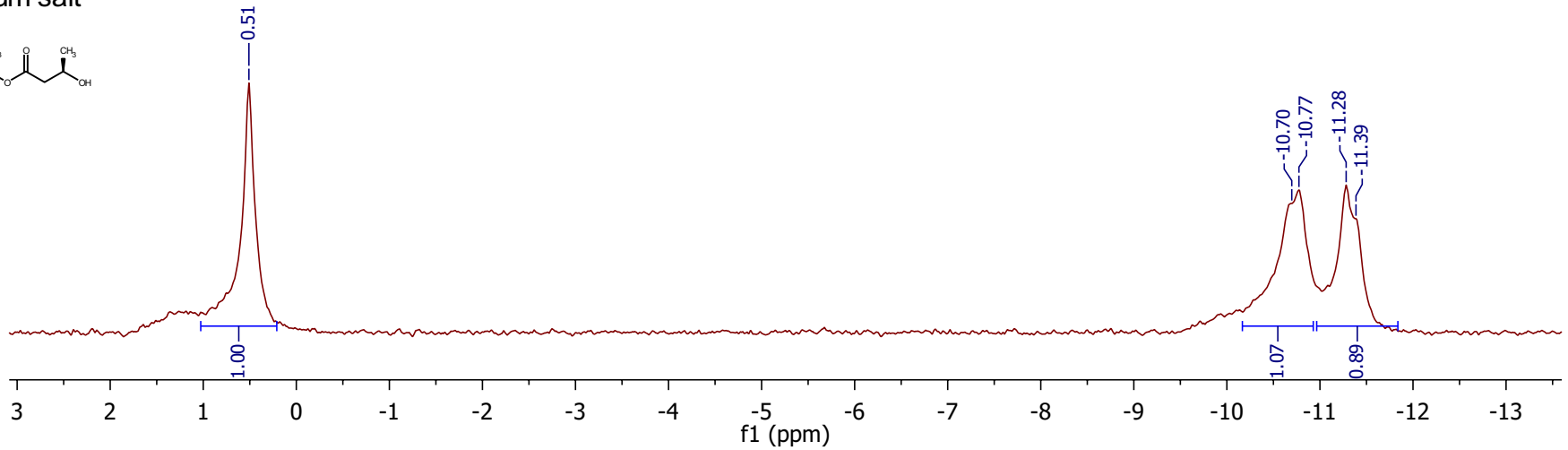


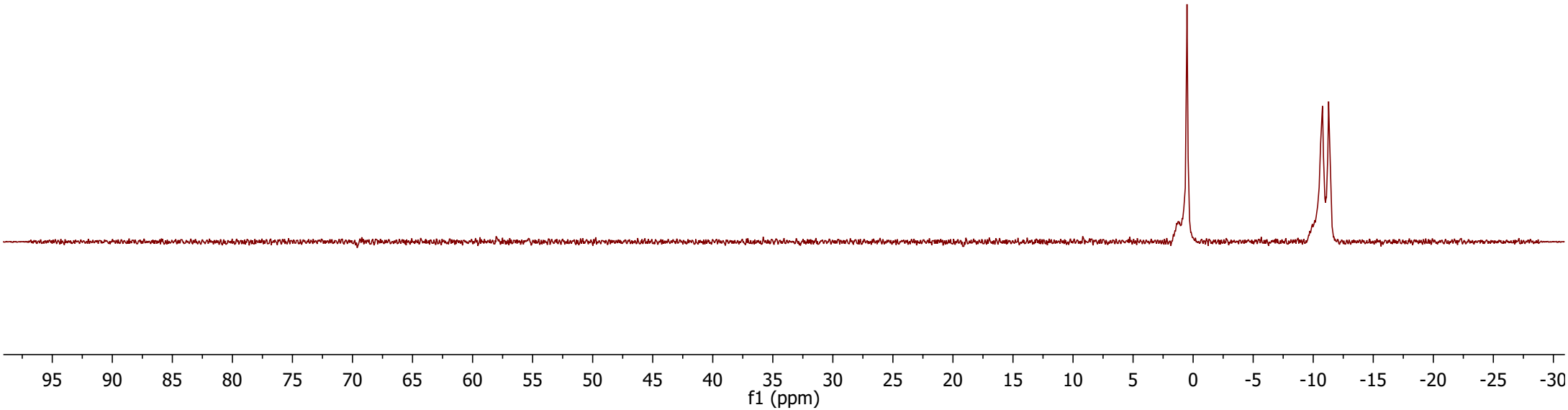
^{31}P NMR, 161 MHz, D_2O



ammonium salt

$(HB)_2$OCoA 2a

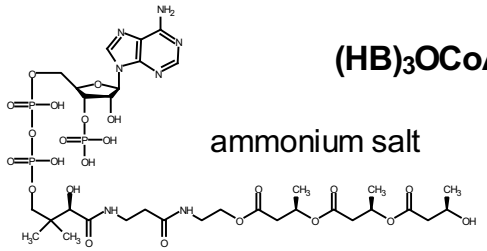




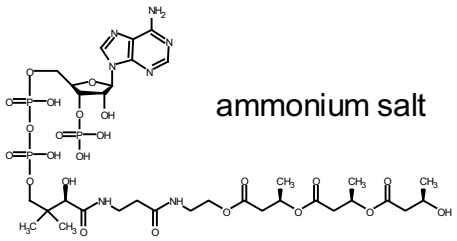
^{1}H NMR, 400 MHz, D_2O

$(HB)_3$OCoA 2b

ammonium salt

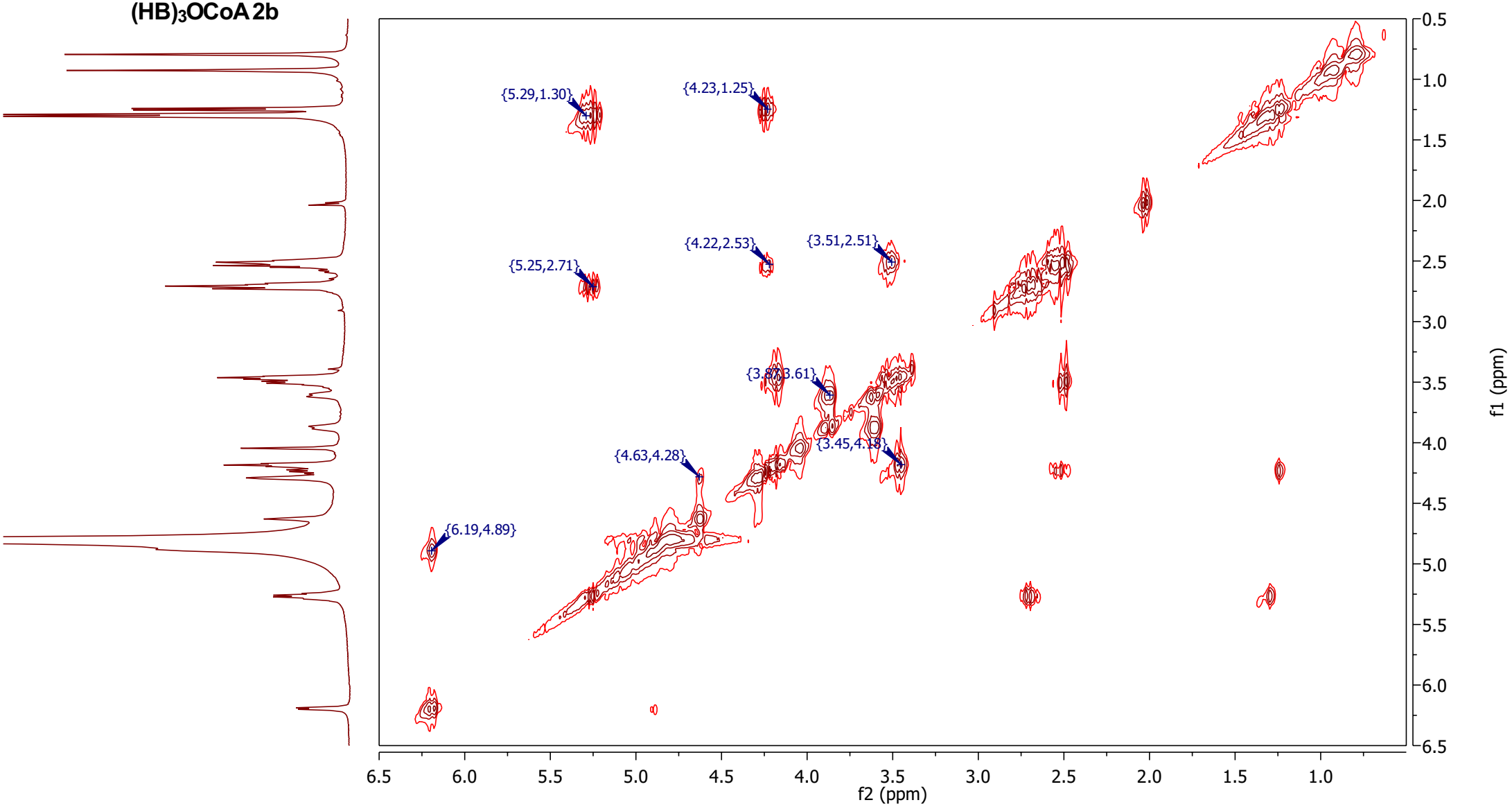


gCOSY, 400 MHz, D_2O

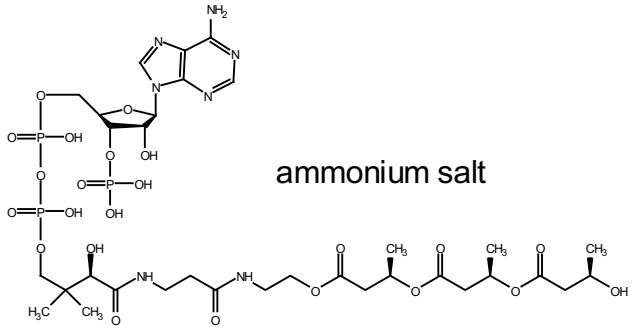


ammonium salt

$(HB)_3OCoA$ 2b

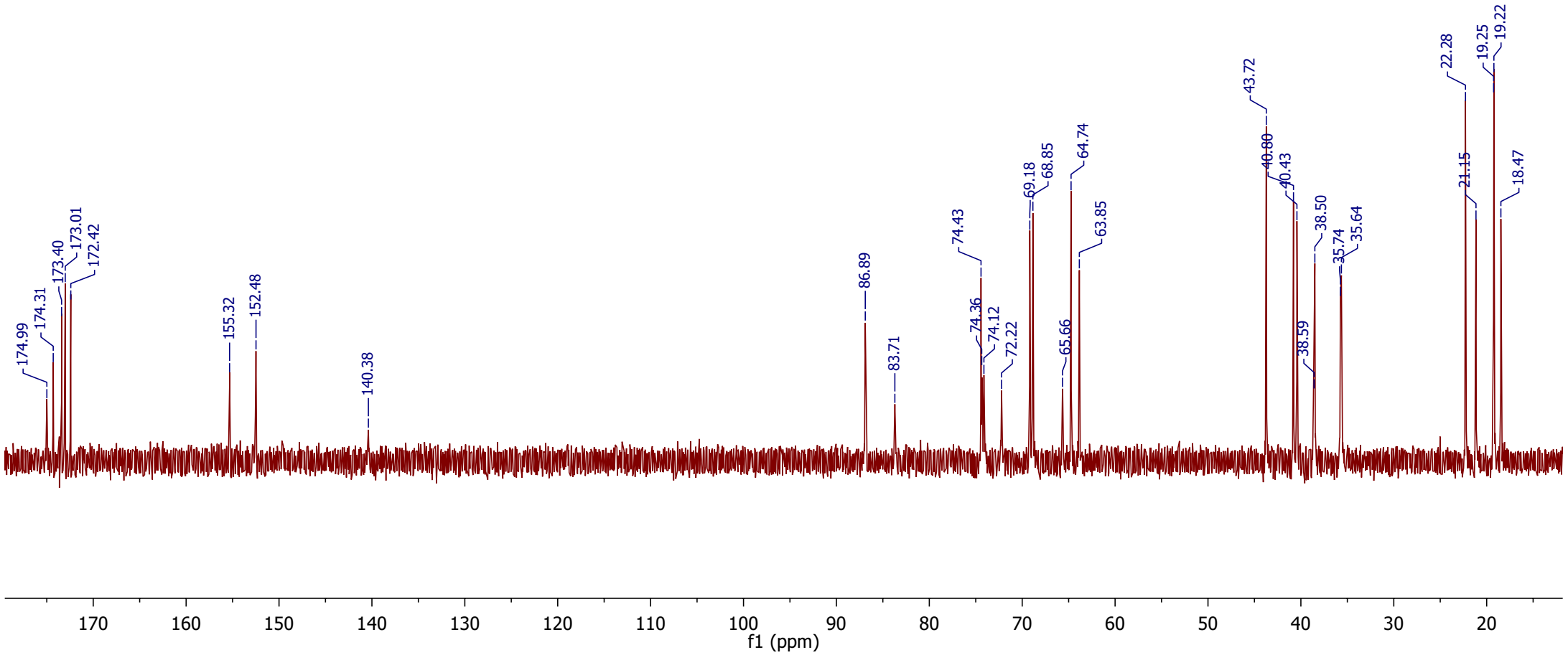


^{13}C NMR, 100 MHz, D_2O

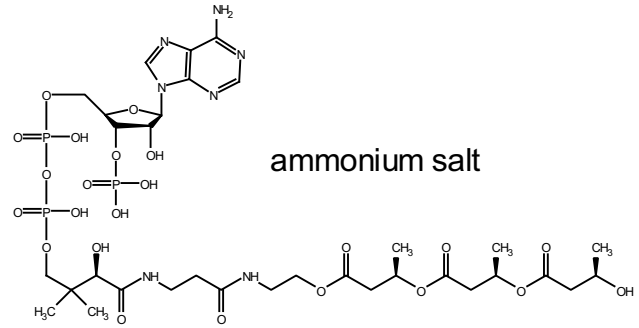


ammonium salt

$(HB)_3$OCoA 2b

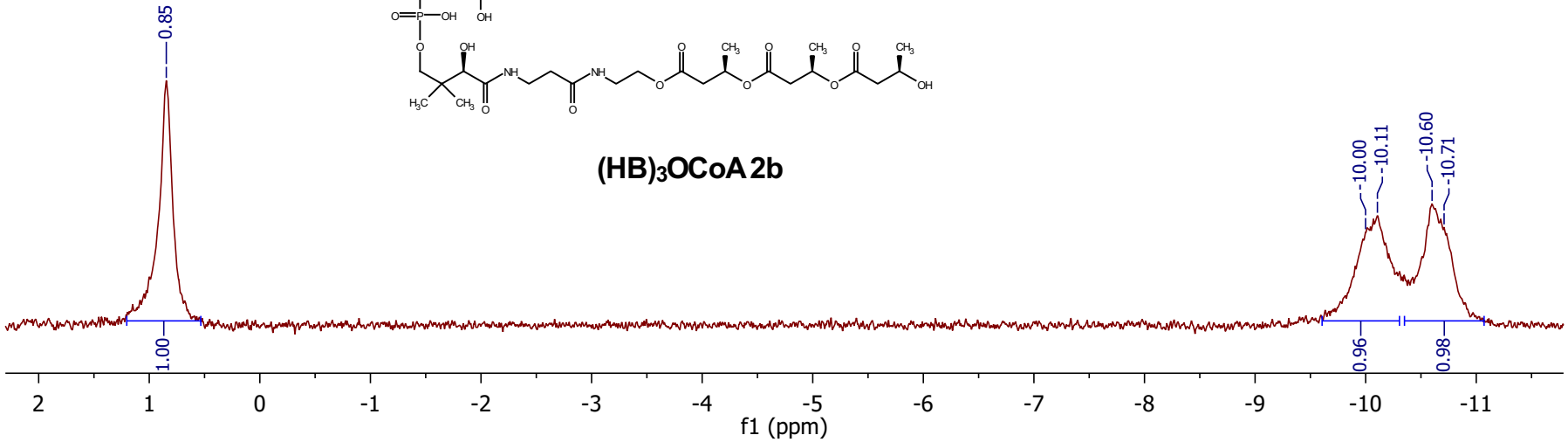


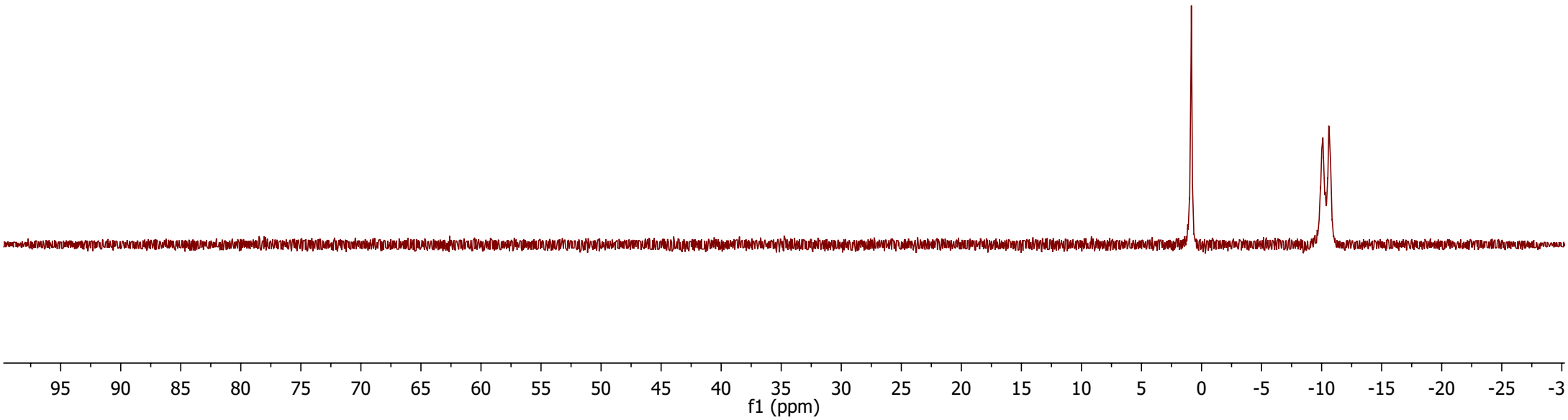
^{31}P NMR, 161 MHz, D_2O



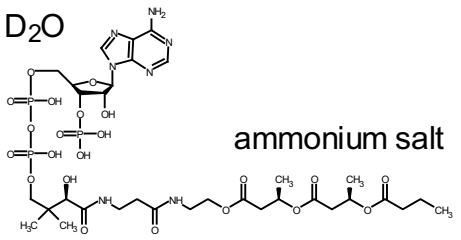
ammonium salt

$(HB)_3$OCoA 2b



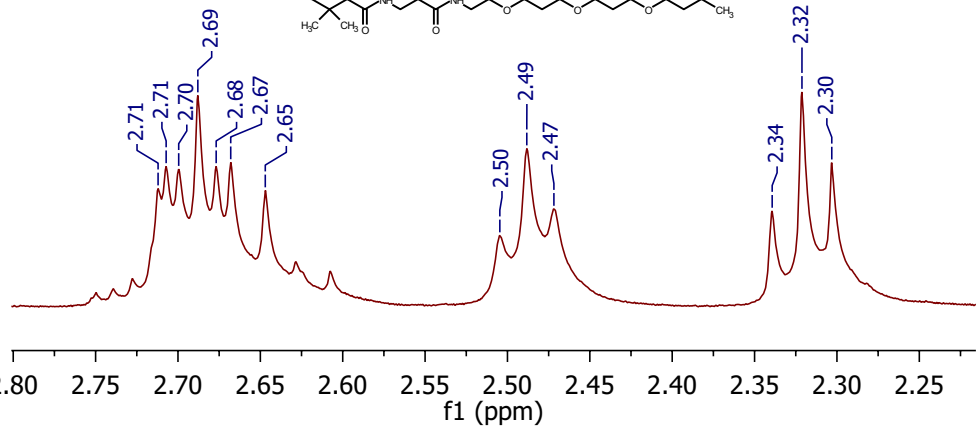


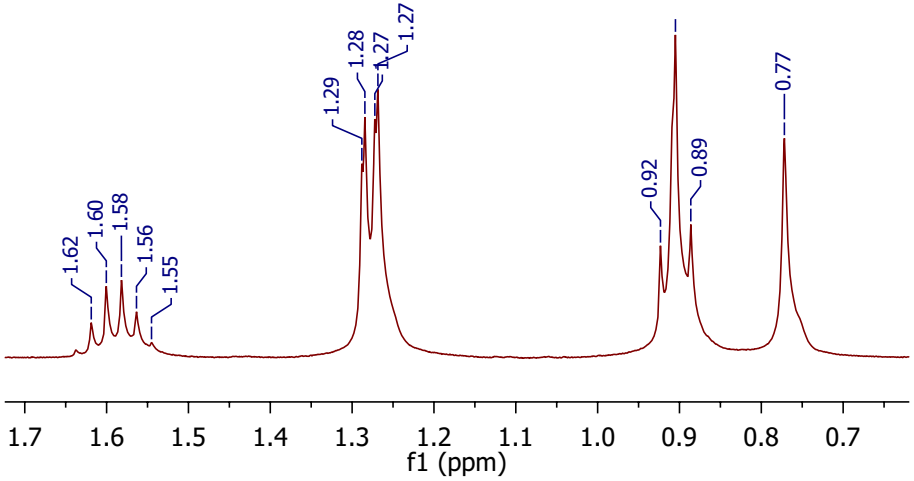
^{1}H NMR, 400 MHz, D_2O

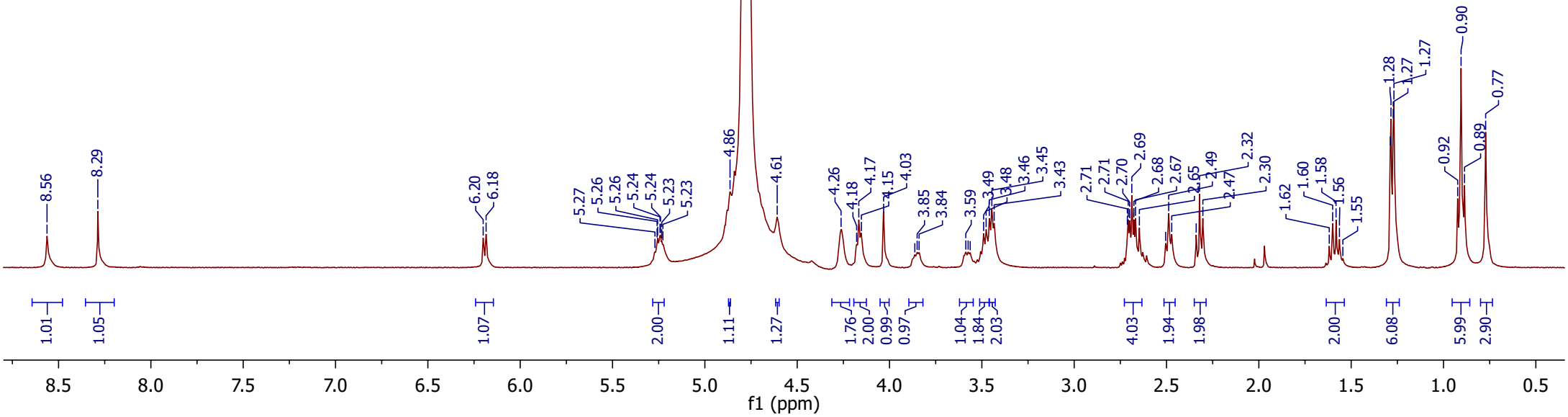


ammonium salt

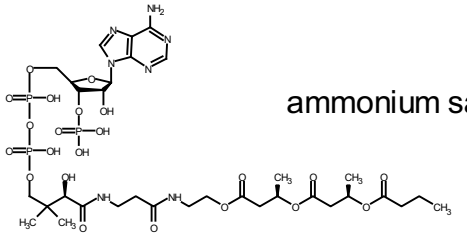
sT-O-CoA 3a







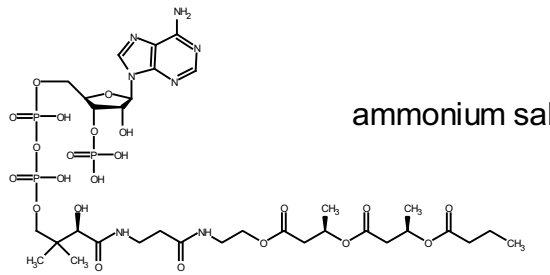
gCOSY, 400 MHz, D_2O



ammonium salt

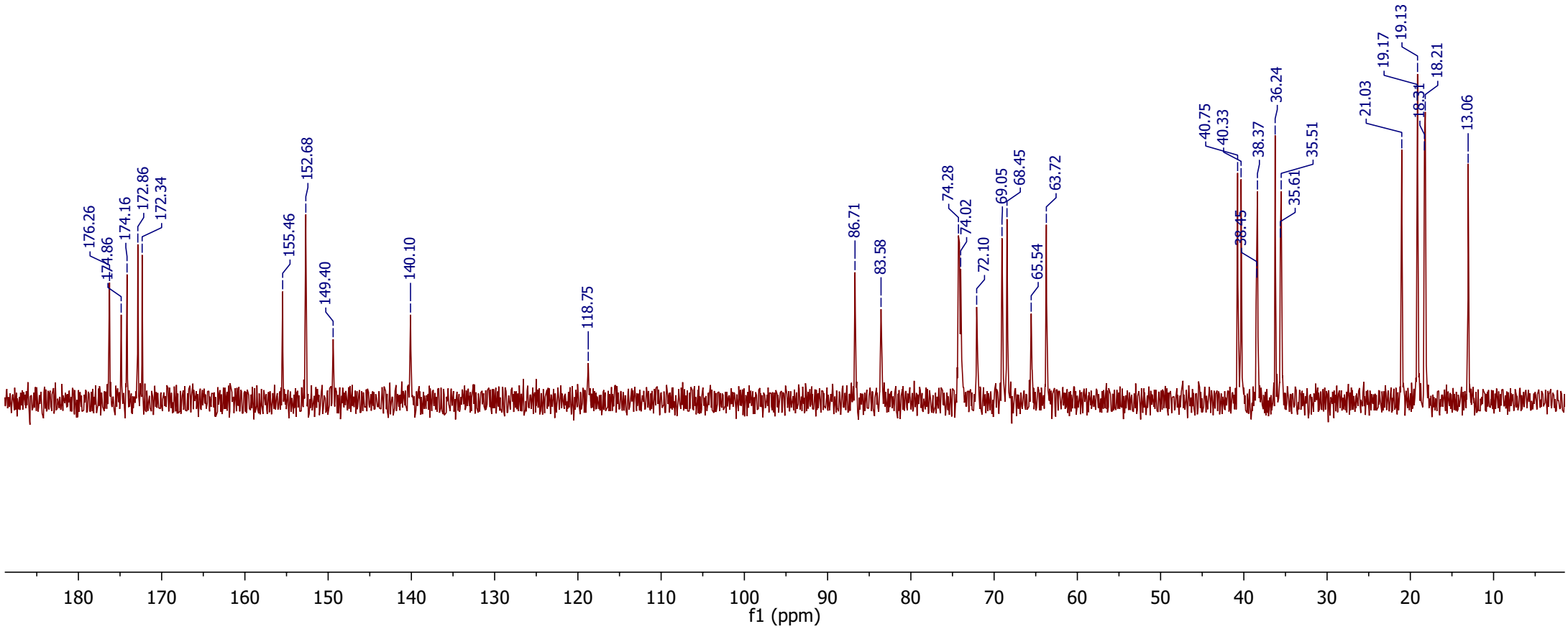
sT-O-CoA 3a

^{13}C NMR, 100 MHz, D_2O

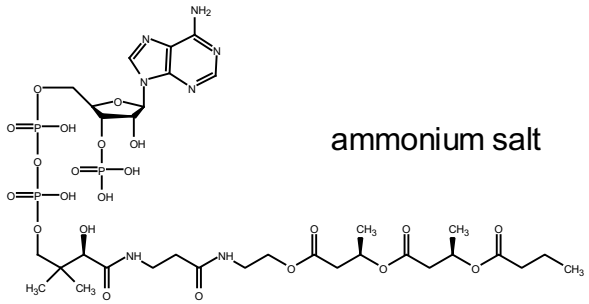


ammonium salt

sT-O-CoA 3a

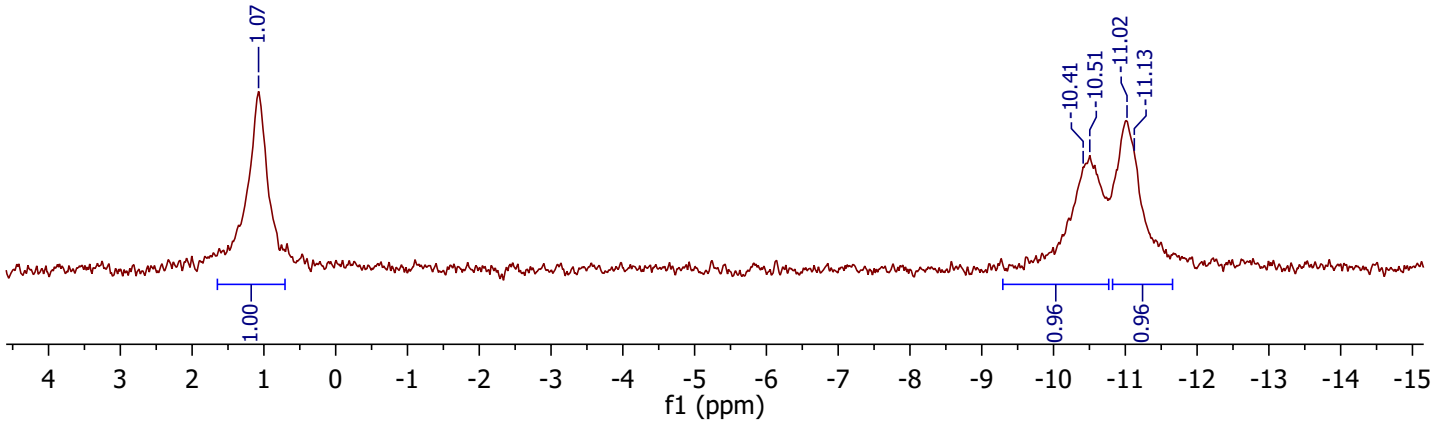


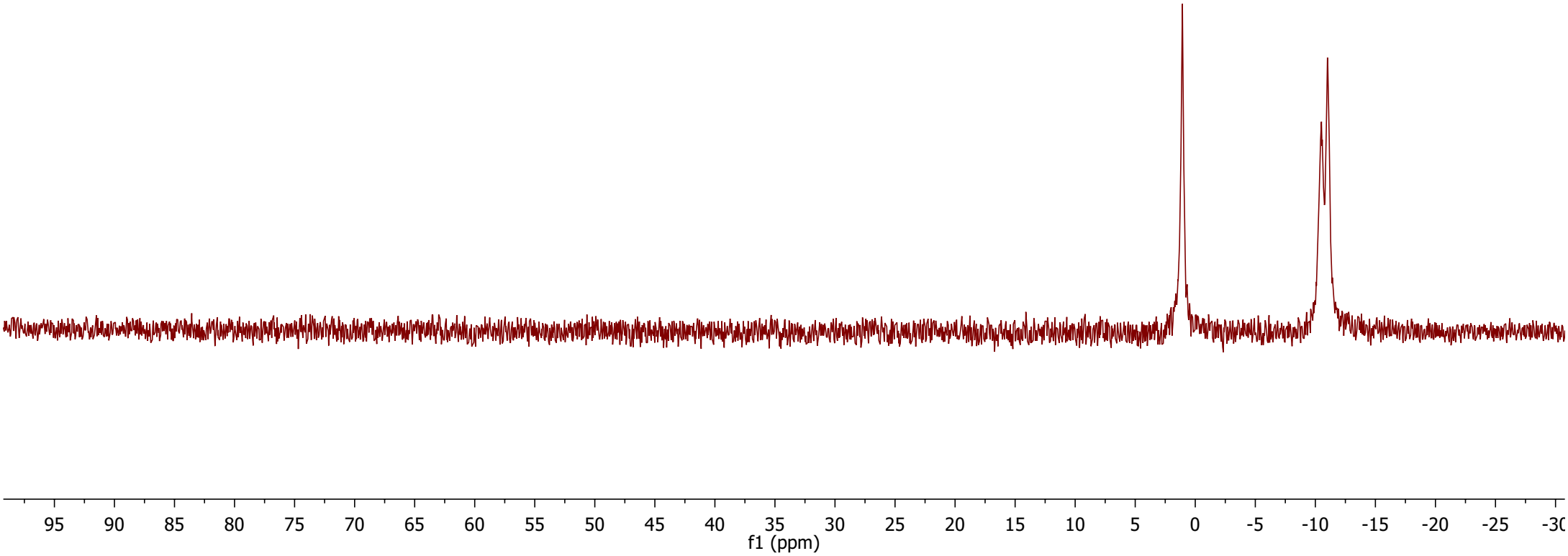
^{31}P NMR, 161 MHz, D_2O



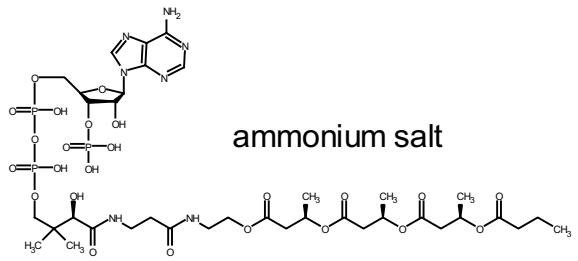
ammonium salt

sT-O-CoA 3a



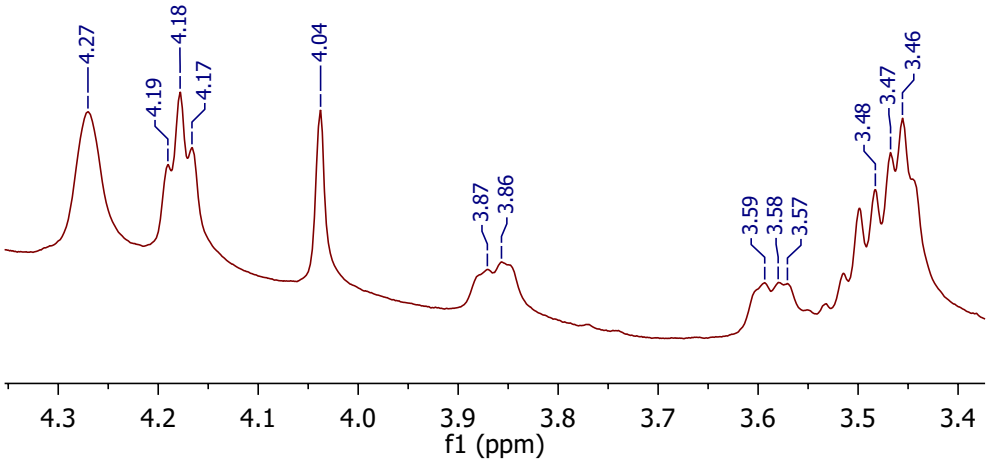


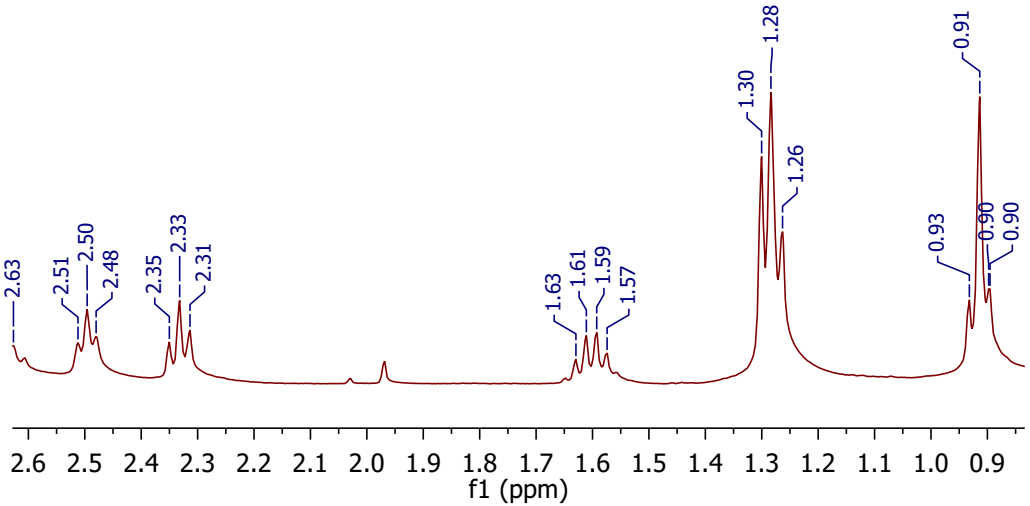
^{1}H NMR, 400 MHz, D_2O

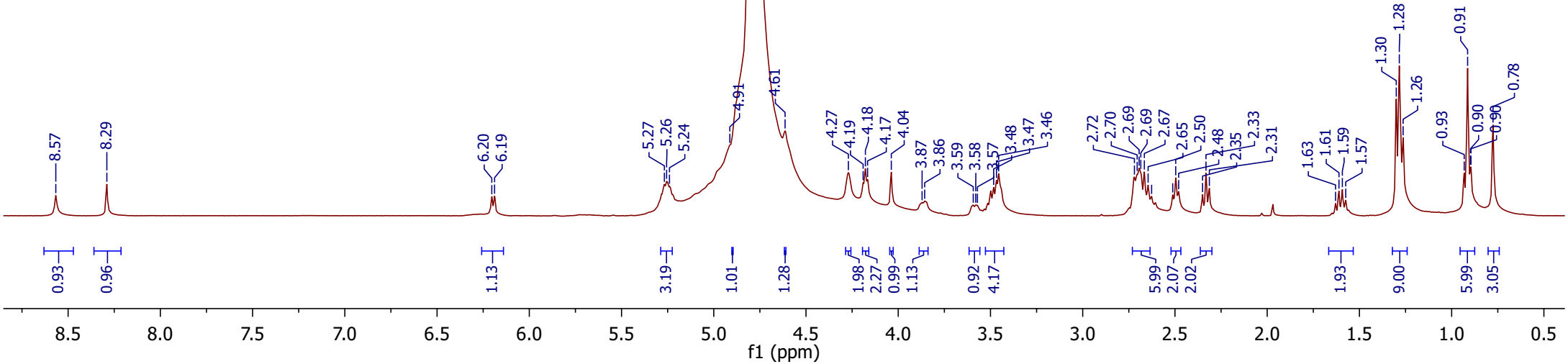


ammonium salt

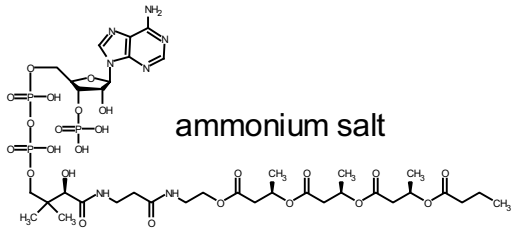
sTet-O-CoA 3b







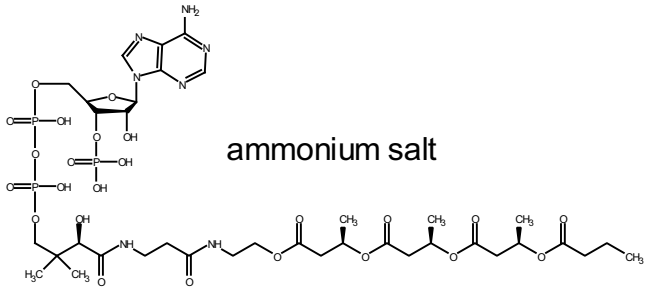
gCOSY, 400 MHz, D_2O



ammonium salt

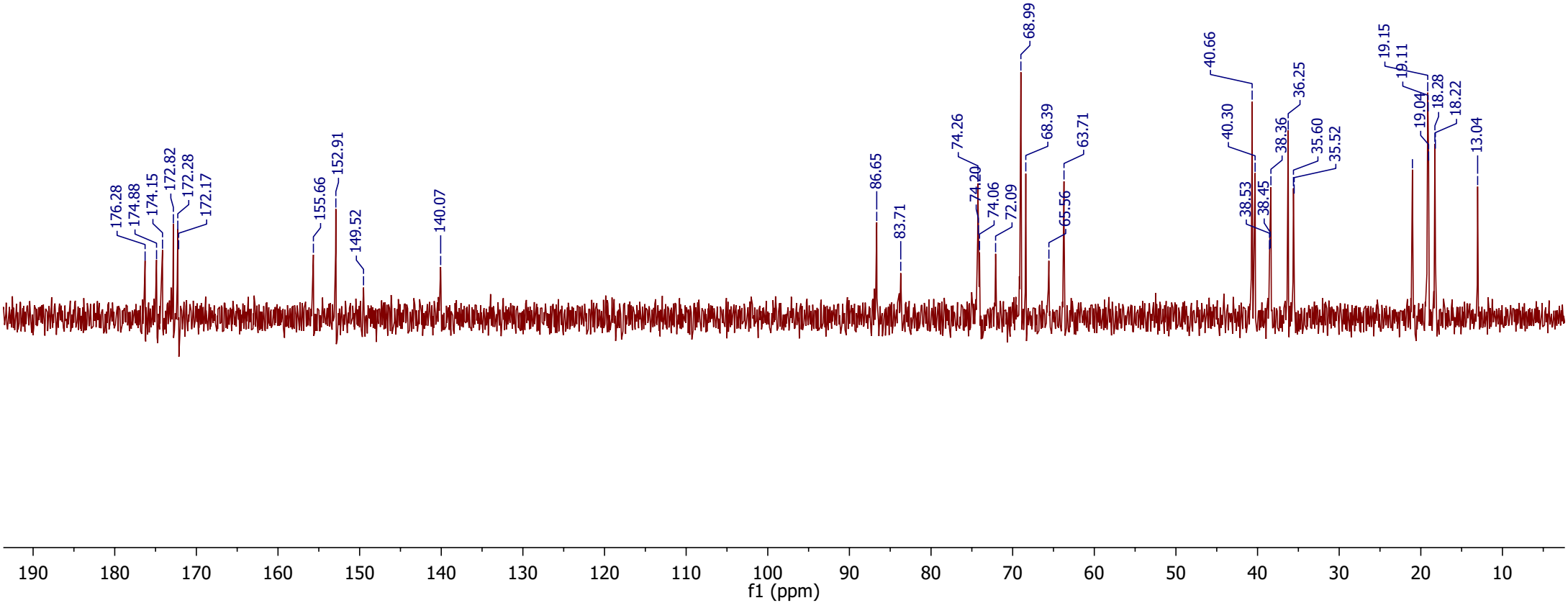
sTet-O-CoA 3b

^{13}C NMR, 100 MHz, D_2O

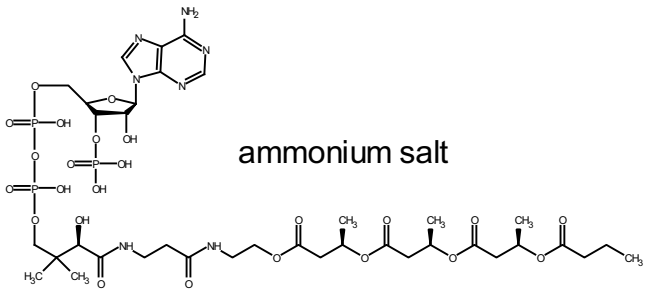


ammonium salt

sTet-O-CoA 3b

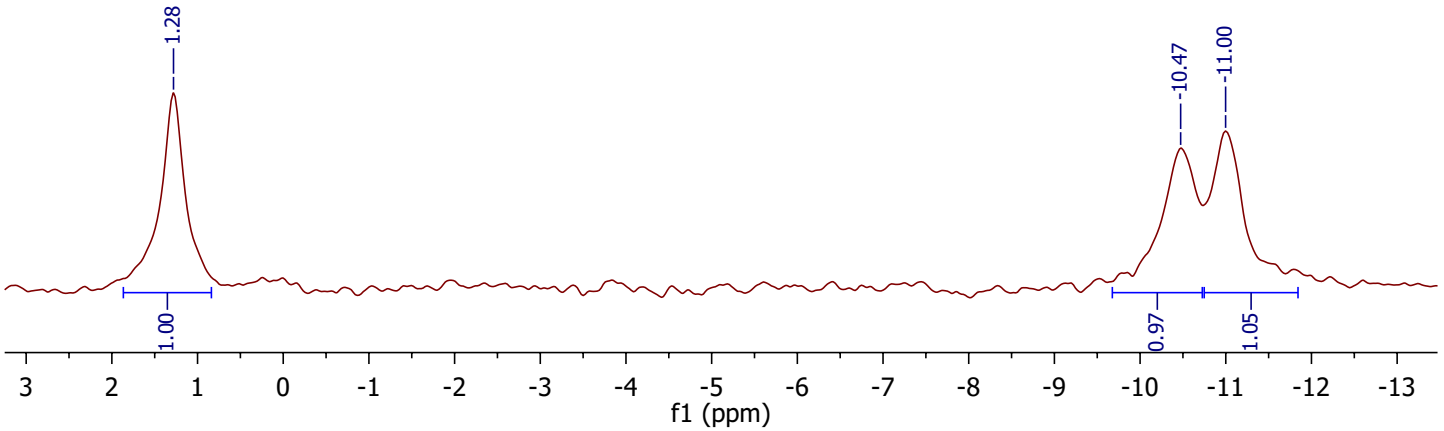


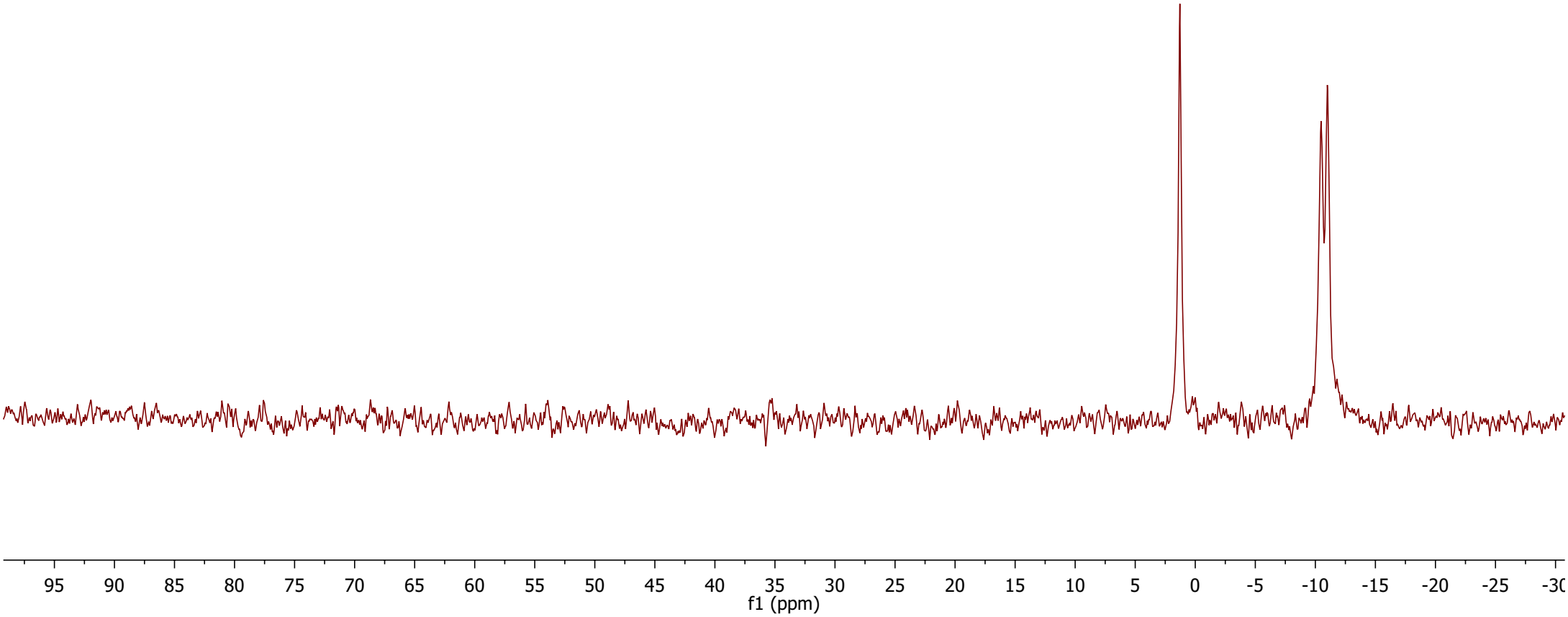
^{31}P NMR, 161 MHz, D_2O



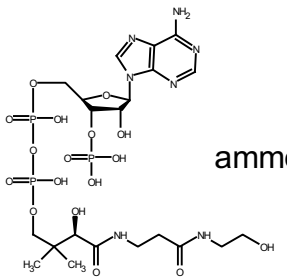
ammonium salt

sTet-O-CoA 3b



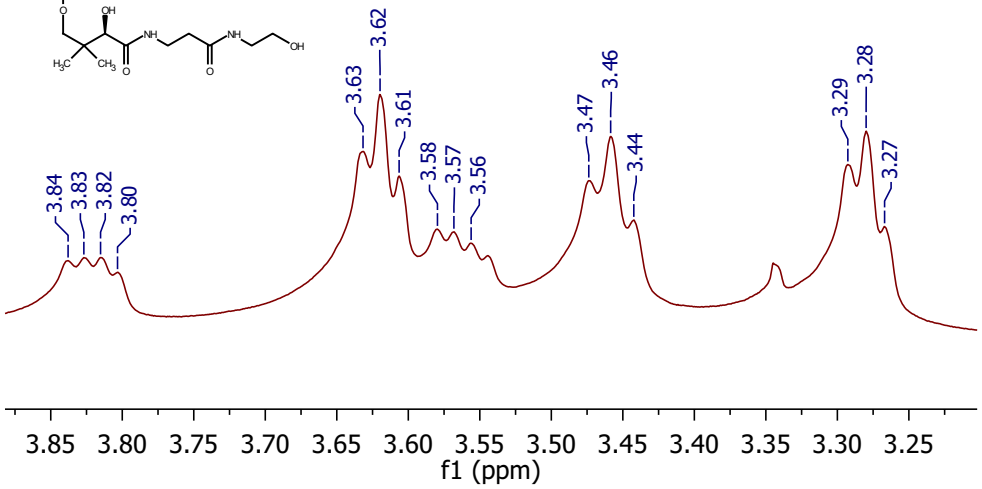


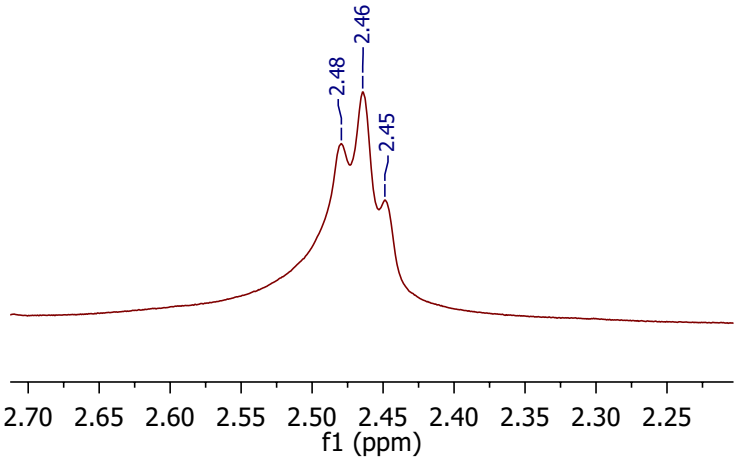
^{1}H NMR, 400 MHz, D_2O



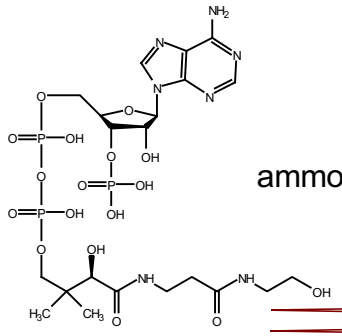
CoAOH 4

ammonium salt





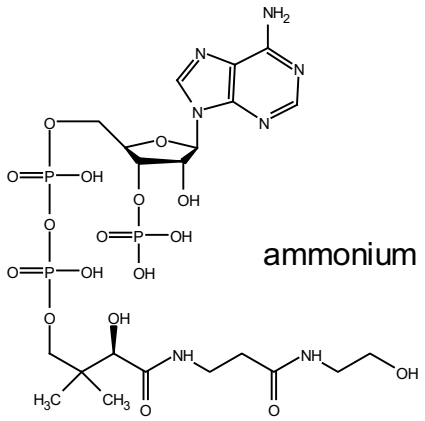
gCOSY, 400 MHz, D_2O



ammonium salt

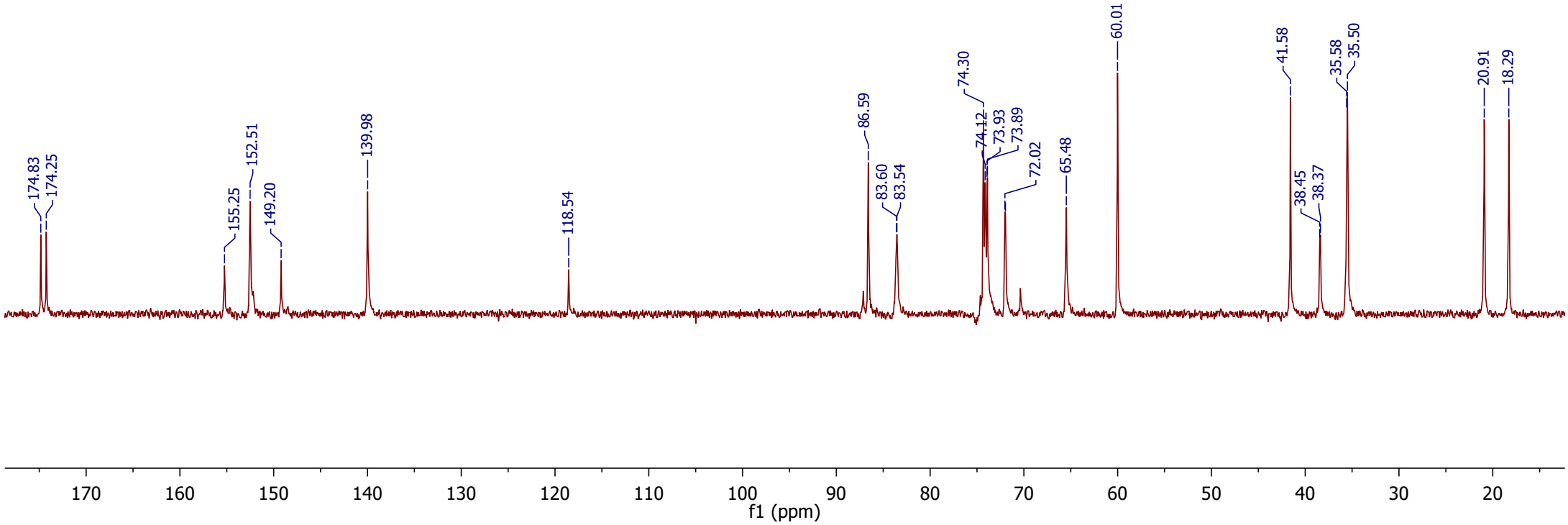
CoAOH 4

^{13}C NMR, 100 MHz, D_2O

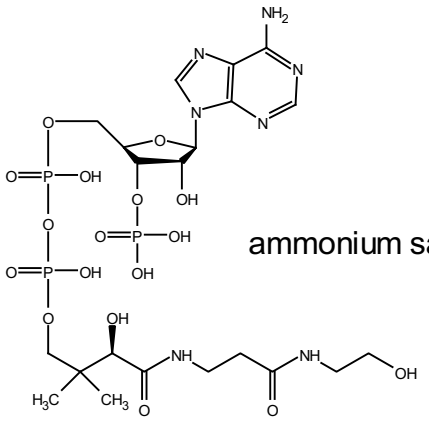


ammonium salt

CoAOH 4



^{31}P NMR, 161 MHz, D_2O



ammonium salt

CoAOH 4