Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

A Mechanism-Based Fluorescence Transfer Assay for Examining Ketosynthase Selectivity

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Methods and Materials:

HPLC was performed with a Waters 1525 system. The gradient employed was A = water + 0.1% formic acid, B = acetonitrile + 0.1% formic acid, 5–95% B over 60 min with a Waters XBridge C18 5u column (4.6 × 100 mm). Mass spectra were acquired with a Waters Micromass ZQ mass detector in EI+mode: Capillary voltage = 3.50 kV, cone voltage = 30 V, extractor = 3 V, RF lens = 0.0 V, source T = 100 °C, desolvation T = 200 °C, desolvation gas = 300 L h−1, esolvation gas = 0.0 L h−1 The system was operated by and spectra were processed using the Waters Empower software suite. Perkin Elmer Spectrum 100 FTIR instrument was used for IR measurements.

General method for preparation of all N-acetylcysteamine (SNAc) thioester derivatives. To a solution of triethylamine (2.80mmol) in dichlromethane (10mL) was added the appropriate acid (1.40mmol), (3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.40mmol), 1-Hydroxybenzotriazole (HOBt) (1.40mmol) and N-acetylcysteamine (SNAc) (1.35mmol) under Argon. The reaction mixture was stirred overnight. The organic layer was washed with saturated $NaHCO_3$ solution, 0.1 N HCl solution and brine. It was then dried over anhydrous sodium sulfate, concentrated under vacuum, and purified by flash column to provide the final product in pure form.

Propionyl-SNAc (1) Pale yellow oil (74%). ^{1}H NMR (400 MHz, CDCl3) δ ppm 0.86(t,J=7.58Hz, 3H) 1.69 (s, 3H) 2.28 (q, J=7.4Hz, 2H) 2.72(t, J=6.8Hz, 2H) 3.07 (q, J=6.6Hz, 2H) 7.39 (br s, 1H). ^{13}C NMR (400 MHz, CDCl3) δ ppm 199.4, 170.6, 39.0, 36.8, 28.0, 22.6, 9.4. IR: ν 3282, 2979,1690,1650,1546,1373,1288,1090,935 cm^{-1}.LRMS (ESI+) m/z calcd. for $C_7H_{13}NO_2S$ [M+H]+ 176.073, found 176.1.

Butyroyl-SNAc (2) Pale yellow solid (71%)^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.92-0.96(t, J=, 3H), 1.67-1.69(m, 2H), 1.95(s, 3H), 2.52-2.56(t,2H),2.99-3.00(t, 2H), 3.40-3.42(q, 2H), 6.12(s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm 11.54, 17.17, 21.27, 26.55, 37.64, 44.12, 168.64, 197.97. IR: ν 3291.13, 2967.08, 1651.12, 1547.47, 1436.23, 1370.50,1284.55, 1112.64, 988.76, 905.33, 723.31 cm^{-1} LRMS (ESI+) m/z calcd. for $C_8H_{15}NO_2S$ [M+H]+ 190.089, found 190.0.

Pentanolyl-SNAc (3) Pale yellow solid (82%). 1H NMR (400 MHz, $CDCl_3$) δ ppm 0.87-0.91 (t, J=6.6 Hz, 3H), 1.3-1.36(m, J=6.6 Hz, 2H), 1-58-1.66(m, J=6.6 Hz, 2H), 1.95 (s, 3H), 2.53-2.57 (t, J=6.6 Hz, 2H), 2.98-3.02 (t, J=6.6 Hz, 2H), 3.37-3.42 (q, J=6.5 Hz, 2H), 6.22 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm 11.71, 20.08, 21.10, 25.69, 26.38, 37.58, 41.83, 168.64, 197.92. IR: ν 3288.16, 3077.00, 2959.74, 2933.28, 2873.68, 1651.25, 1546.86, 1287.53, 1016.73, 731.82 cm^{-1} LRMS (ESI+) m/z calcd. for $C_9H_{17}NO_2S$ [M+H]+ 204.105, found 204.1.

Hexanoyl-SNAc (4) Colorless solid (73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.84-0.87 (t, J=6.3 Hz, 3H), 1.25-1.31 (m, J=6.3 Hz, 4H), 1.58-1.66(m, J=6.3 Hz, 2H), 1.93 (s, 3H), 2.51-2.54 (t, J=6.6 Hz, 2H), 2.97-3.00 (t, J=6.6 Hz, 2H), 3.36-3.40 (q, J=6.3 Hz, 2H), 6.33 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm: 11.93, 20.35, 21.25, 23.41, 26.48, 29.13, 37.77, 42.16, 168.45, 198.24. IR: ν 3293.04, 3093.17, 2952.97, 2930.26, 2870.06, 1641.09, 1551.33, 1408.42, 1358.88, 1292.89, 970.52, 745.99 cm^{-1} LRMS (ESI+) m/z calcd. for $C_{10}H_{19}NO_2S$ [M+H]+ 218.120, found 218.0.

Heptanoyl-SNAc (5) Pale yellow solid (60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.83-0.87 (t, J=6.3 Hz, 3H), 1.23-1.33 (m, J=6.3 Hz, 6H), 1.58-1.66(m, J=7.5 Hz, 2H), 1.93 (s, 3H), 2.51-2.55 (t, J=6.6 Hz, 2H), 2.97-3.01 (t, J=6.4 Hz, 2H), 3.36-3.41 (q, J=6.6 Hz, 2H), 6.28 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm : 12.02, 20.44, 21. 10, 23.61, 26.38, 26.59, 29.41, 37.61, 42.12, 168.59, 197.94. IR: ν 3290.00, 3103.58, 2954.74, 2929.89, 2871.55, 1681.0, 1635.24, 1557.27, 1442.49, 1370.78, 1293.34, 1045.19, 971.98, 760.76 cm^{-1} LRMS (ESI+) m/z calcd. for $C_{11}H_{21}NO_2S$ [M+H]+ 232.136, found 232.1.

Octanoyl-SNAc (6) Colorless solid (84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.84-0.87 (t, J=6.3 Hz, 3H), 1.20-1.30 (m, J=6.3 Hz, 9H), 1.58-1.66(m, J=7.4 Hz, 2H), 1.94 (s, 3H), 2.52-2.55 (t, J=6.6 Hz, 2H), 2.97-3.01 (t, J=6.6 Hz, 2H), 3.37-3.42 (q, J=6.3 Hz, 2H), 6.27 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm 12.12, 20.64, 21.23, 23.72, 26.46, 26.94, 29.66, 37.76, 42.19, 168.47, 198.21. IR: ν 3291.33, 3101.19, 2922.78, 2848.61, 1682.4, 1637.18, 1555.34, 1294.29, 1178.47, 1045.81, 967.51, 728.99 cm^{-1} LRMS (ESI+) m/z calcd. for $C_{12}H_{23}NO_2S$ [M+H]+ 246.1522, found 246.1.

3-Methylbutanoyl-SNAc (7) Pale yellow oil (76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.90-0.92 (d, J=6.6 Hz, 6H), 1.92 (s, 3H), 2.07-2.14 (dt, J=13.6, 6.8 Hz, 1H), 2.39-2.41 (d, J=7.1 Hz, 2H), 2.96-3.00 (t, J=6.6 Hz, 2H), 3.55-3.39 (q, J=6.3, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm 22.19, 23.08, 26.44, 28.34, 39.64, 52.81, 170.43, 199.38. IR: ν 3284.67, 3078.14, 2959.39, 2872.19, 1686.32, 1693.00, 1547.45, 1284.34, 1133.59, 10007.92, 752.91 cm^{-1} LRMS (ESI+) m/z calcd. for $C_9H_{17}NO_2S$ [M+H]+ 204.105, found 204.1.

3,3'-Dimethylbutanoyl-SNAc (8) Pale yellow oil (68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.97 (s, 9H), 1.92 (s, 3H), 2.40 (s, 2H), 2.94-2.98 (t, J=6.6 Hz, 2H), 3.34-3.38 (q, J=6.6 Hz, 2H), 6.33 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm 21.13, 26.65, 27.71, 29.65, 37.67, 54.82, 168.62, 196.33. IR: ν 3285.15, 3080.63, 2956.84, 2870.18, 1685.76, 1615.23, 1548.26, 1367.02, 1058.89, 1006.31, 907.46, 756.66 cm^{-1} LRMS (ESI+) m/z calcd. for $C_{10}H_{19}NO_2S$ [M+H]+ 218.1209, found 218.1.

2-Methylpropanoyl-SNAc (9) Pale yellow oil(64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.16-.1.18 (dt, J=6.9, 0.7 Hz, 6H), 1.94 (s, 3H), 2.70-2.77 (m, J=6.6 Hz, 1H), 2.97-3.00 (t, J=6.6 Hz, 2H), 3.37-3.42 (q, J=6.2 Hz, 2H), 6.22 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm 19.34, 23.14, 28.11, 39.70, 43.12, 170.38, 204.72. IR: ν 3285.37, 3082.62, 2972.41, 2933.13 1682.23, 1650.32, 1548.77, 1438,83, 1372.86, 1288.66, 1095.01, 1037.19, 893.45, 860.88, 701.62 cm^{-1} LRMS (ESI+) m/z calcd. for $C_8H_{15}NO_2S$ [M+H]+ 190.089, found 190.1.

Cyclopropanoyl-SNAc (10) Pale yellow oil (60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.88-0.93 (m, 2H), 1.06-1.10 (m, 2H), 1.90 (s, 3H), 1.94-2.00 (m, 2H), 2.95-2.98 (t, J=6.4 Hz, 2H), 3.31-3.36 (q, J=6.4 Hz, 2H), 6.56 (s, 1H).^{13}C NMR (400 MHz, $CDCl_3$) δ ppm 9.07, 20.73, 21.11, 26.49, 37.66, 168.67, 197.59. IR: ν 3285.27, 3082.12, 3009.99, 2932.76, 1651.90, 1544.26, 1367.08, 1039.33, 995.09. 713.10 cm^{-1} LRMS (ESI+) m/z calcd. for $C_8H_{13}NO_2S$ [M+H]+ 188.0739, found 188.1.

Cyclopentanoyl-SNAc (11) Pale yellow oil (73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.56-.1.88 (m, J=6.3 Hz, 8H), 1.93 (s, 3H), 2.94-3.00 (t, J=6.6 Hz, 2H), 3.36-3.40 (q, J=6.1 Hz, 2H), 6.27 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm 21.22, 23.95, 26.41, 28.7, 37.81, 51.31, 168.51, 201.65. IR: ν 3289.90, 2960.05, 2870.94, 1654.20, 1547.67, 1287.90, 997.65, 730.27 cm^{-1} LRMS (ESI+) m/z calcd. for $C_{10}H_{17}NO_2S$ [M+H]+ 216.105, found 216.1.

Cyclohexanoyl-SNAc (12) Pale yellow oil (78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.15-1.29 (m, 4H), 1.29-1.46 (qd, J=12(3), 2.8 Hz, 2H), 1.61-1.89(m, 5H),1.93 (s, 3H), 2.44-2.50 (m, 1H), 2.95-3.00 (t, J=6.6 Hz, 2H), 3.36-3.40 (q, J=6.3 Hz, 2H), 6.33 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm 21.25, 23.52, 23.63, 26.10, 27.62, 37.83, 50.78, 168.44, 201.75. IR: ν 3286.02, 3079.68, 2931.37, 2855.68, 1651.63, 1547.25, 1448.78, 1288.56, 1050.97, 968.48, 731.10 cm^{-1} LRMS (ESI+) m/z calcd. for $C_{11}H_{19}NO_2S$ [M+H]+ 230.120, found 230.1.

Benzoyl-SNAc (13) Yellow oil (75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.93 (s, 3H), 3.22-3.25 (t, J=6.6 Hz, 2H), 3.51-3.57 (q, J=6.6 Hz, 2H), 6.18 (s, 1H), 7.45-7.48 (t, 2H), 7.58-7.62 (t, 1H), 7.95-7.98 (d, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm 21.32, 26.67, 37.76, 125.39, 126.81, 131.81, 134.78, 168.58, 190.33. IR: ν 3299.96, 3085.64, 2929.32, 1655.48, 1545.73, 1446.18, 1400,27, 1359.73, 1295.83, 1203.59, 910.55, 768.55, 725.93, 658.99 cm^{-1} LRMS (ESI+) m/z calcd. for $C_{11}H_{13}NO_2S$ [M+H]+ 224.073, found 224.1.

4-Hydroxybenzoyl-SNAc (14) Colorless gelatinous solid (84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.81 (s, 3H), 3.05-3.08 (t, J=6.6 Hz, 2H), 3.23-3.27 (q, J=6.6 Hz, 2H), 6.87-6.89-7.48 (d, 2H), 7.80-7.82 (d, 2H), 8.10-8.13 (t, J=5.6 Hz, 1H), 10.55 (s, 1H).^{13}C NMR (400 MHz, $CDCl_3$) δ ppm 21.09, 26.51, 36.96, 37.61, 37.81, 38.02, 38.23, 38.65, 114.13, 126.14, 127.94, 161.31, 167.95, 187.45. IR: ν 3436.12, 2251.86, 1655.24, 1215.62, 1023.59, 821.43, 758.88 cm^{-1} LRMS (ESI+) m/z calcd. for $C_{11}H_{13}NO_3S$ [M+H]+ 240.068, found 240.1.

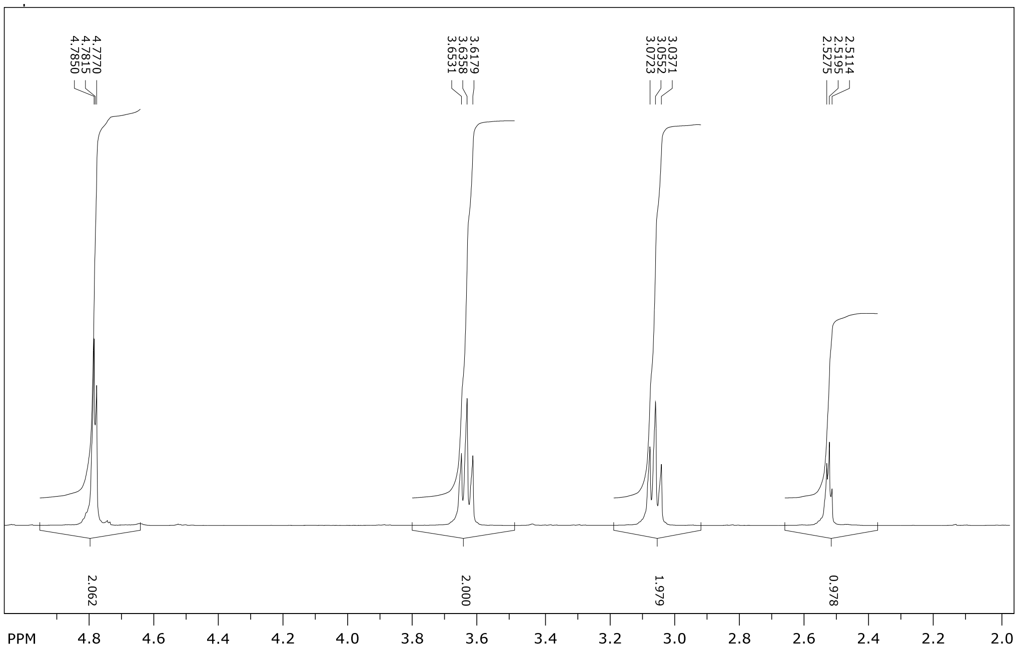
4-Aminobenzoyl-SNAc (15) Pale yellow solid (71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.97 (s, 3H), 3.18-3.21 (t, J=6.6 Hz, 2H), 3.52-3.53 (q, J=6.6 Hz, 2H), 4.23 (s, 2H), 6.04 (s, 1H), 6.64-6.66 (d, 2H), 7.81-7.83 (d, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm: 21.31, 26.27, 38.23, 111.83, 124.76, 127.88, 149.92, 168.58, 188.66. IR: ν 3435.45, 3337.65, 3214.55, 2926.30, 1734.94, 1627.62, 1566.86, 1512.79, 1166.68, 908.76, 830.93 cm^{-1}. LRMS (ESI+) m/z calcd. for $C_{11}H_{14}N_2O_2S$ [M+H]+ 239.084, found 239.1.

4-Nitrobenzoyl-SNAc (16) White solid (74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.00 (s, 3H), 3.28-3.32 (t, J=6.6 Hz, 2H), 3.54-3.59 (q, J=6.6 Hz, 2H), 6.05 (s, 1H), 8.11-8.14 (d, 2H), 8.30-8.34 (d, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm: 21.22, 27.25, 37.19, 122.00, 126.37, 139.34, 148.61, 168.79, 188.44. IR: ν 3286.71, 3079. 65, 2931.34, 1648.22, 1547.62, 1518.41, 1374.59, 1348.69, 1194.19, 919.25,848.83, 718.01 cm^{-1} LRMS (ESI+) m/z calcd. for $C_{11}H_{12}N_2O_4S$ [M+H]+ 269.059, found 269.0.

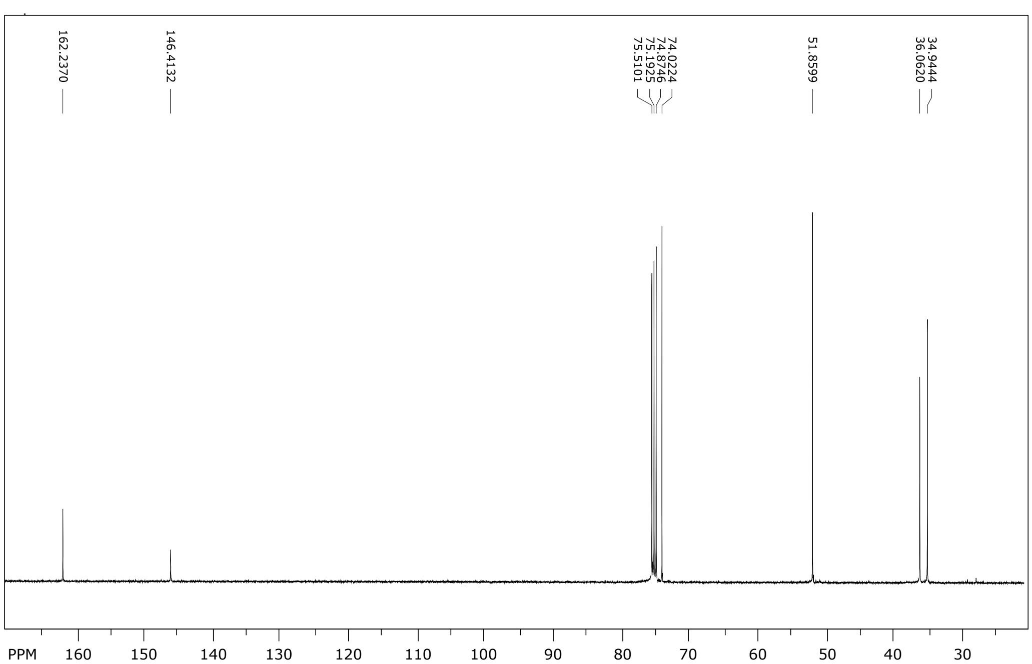
4-Methylbenzoyl-SNAc (17) White solid (67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.96 (s, 3H), 2.39 (s, 3H), 3.18-3.22 (t, J=6.6 Hz, 2H), 3.48-3.53 (q, J=6.6 Hz, 2H), 6.36 (s, 1H), 7.22-7.24 (d, 2H), 7.83-7.85 (d, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm 19.78, 21.26, 26.54, 37.76, 125.42, 127.43, 132.26, 142.71, 168.64, 189.81. IR: ν 3305.41, 3076.62, 230.59, 1649.03, 1543.57, 1362.91, 1290.17, 1206.37, 1173.56, 913.35, 815.70 cm^{-1} LRMS (ESI+) m/z calcd. for $C_{12}H_{15}NO_2S$ [M+H]+ 238.089, found 238.1.

4-Methoxybenzoyl-SNAc (18) Pale yellow oil (64%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.98 (s, 3H), 3.20-3.23 (t, 2H), 3.51-3.55(m, J=6.1 Hz, 2H), 3.88 (s, 3H), 6.10 (s, 1H), 6.93-6.95 (d, J=8.6 Hz, 2H), 7.94-7.96 (d, J=8.8 Hz, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm: 21.31, 26.50, 37.92, 53.64, 111.95, 127.60, 162.11, 168.54, 188.77. IR: ν 3303.88, 3075.41, 2935.79, 2836.53, 1650.92, 1600.77, 1575.18, 1542.59, 1506.15, 1363.23, 1223.16, 1028.35, 733.44 cm^{-1} LRMS (ESI+) m/z calcd. for $C_{12}H_{15}NO_3S$ [M+H]+ 254.083, found 254.0.

^{1}H NMR of Poc β-lactam



^{13}C NMR of Poc β-lactam



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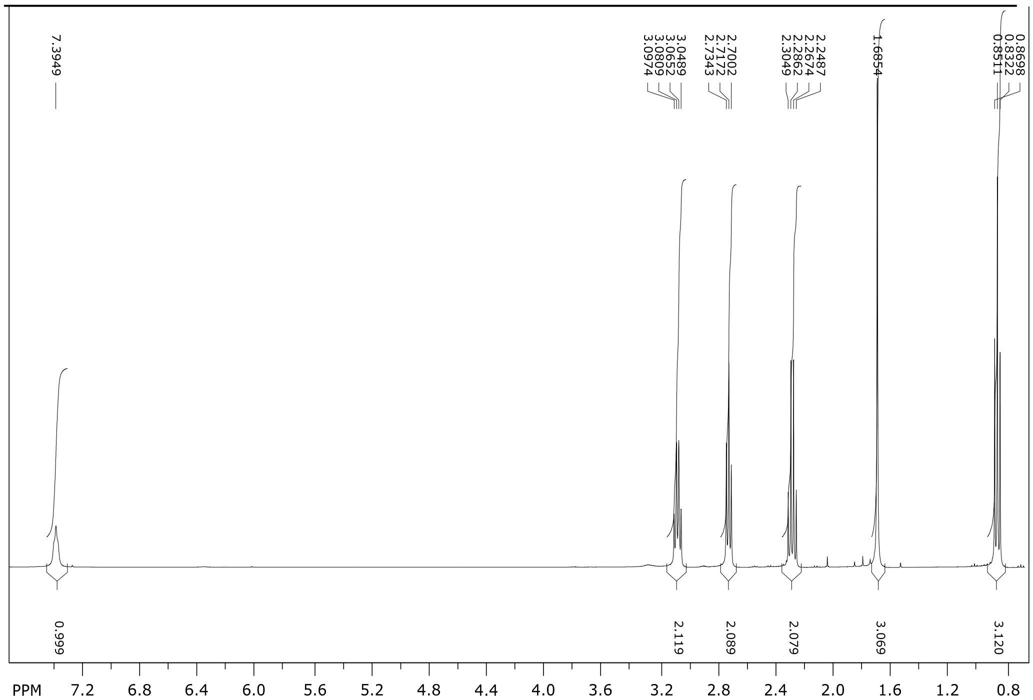

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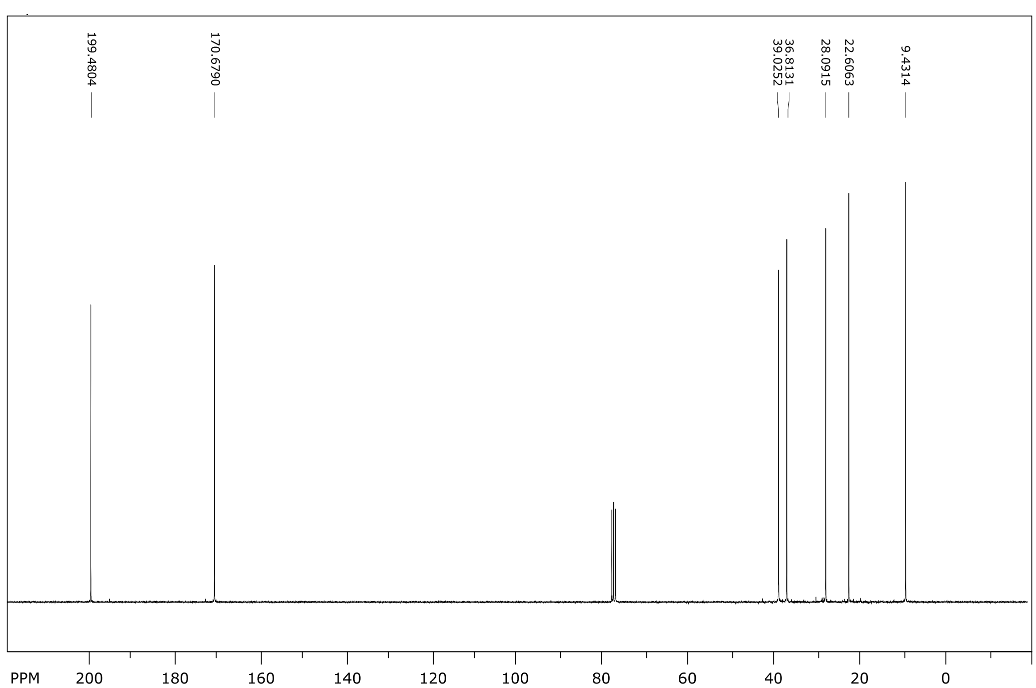

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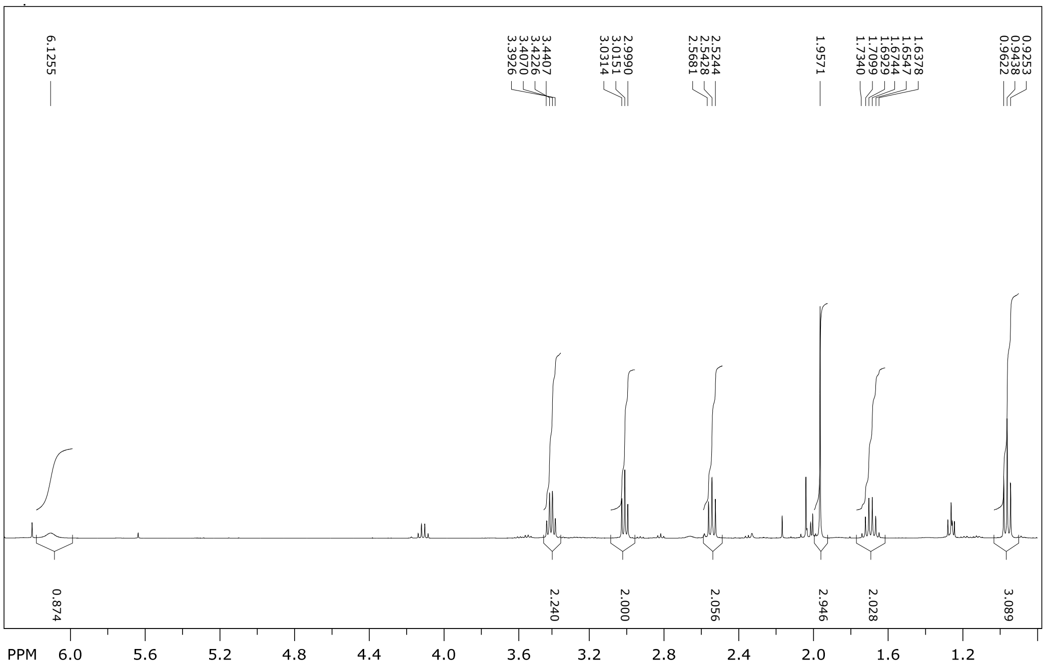

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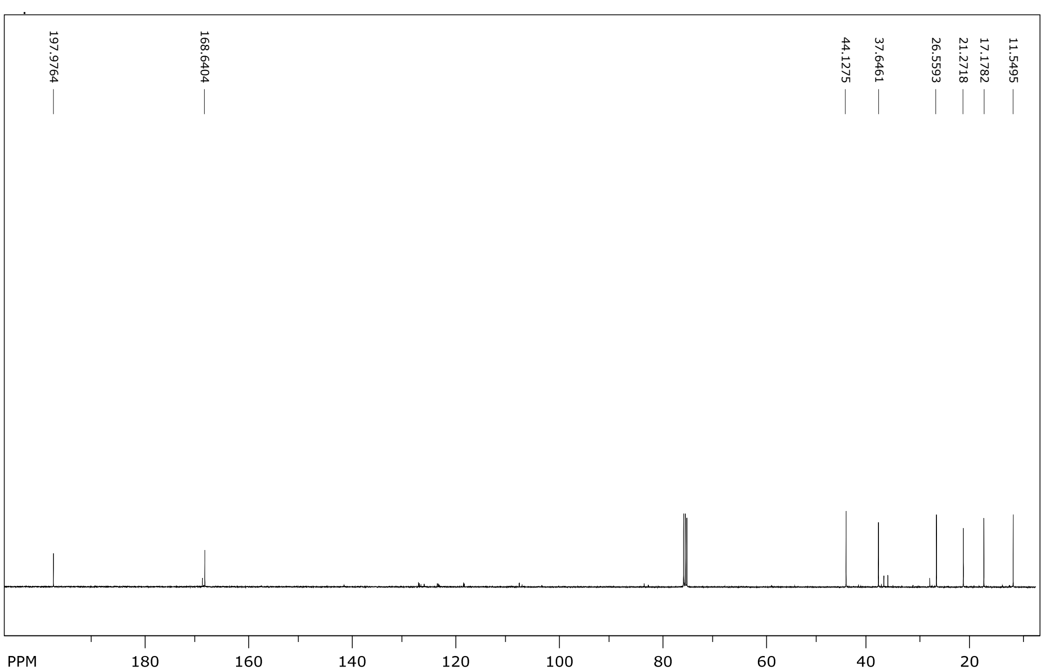

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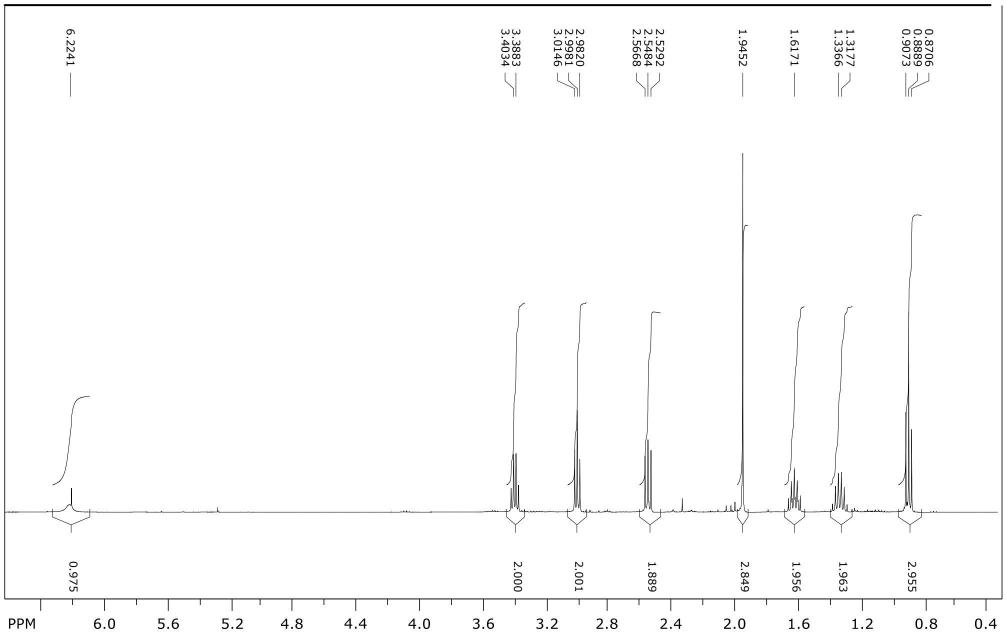

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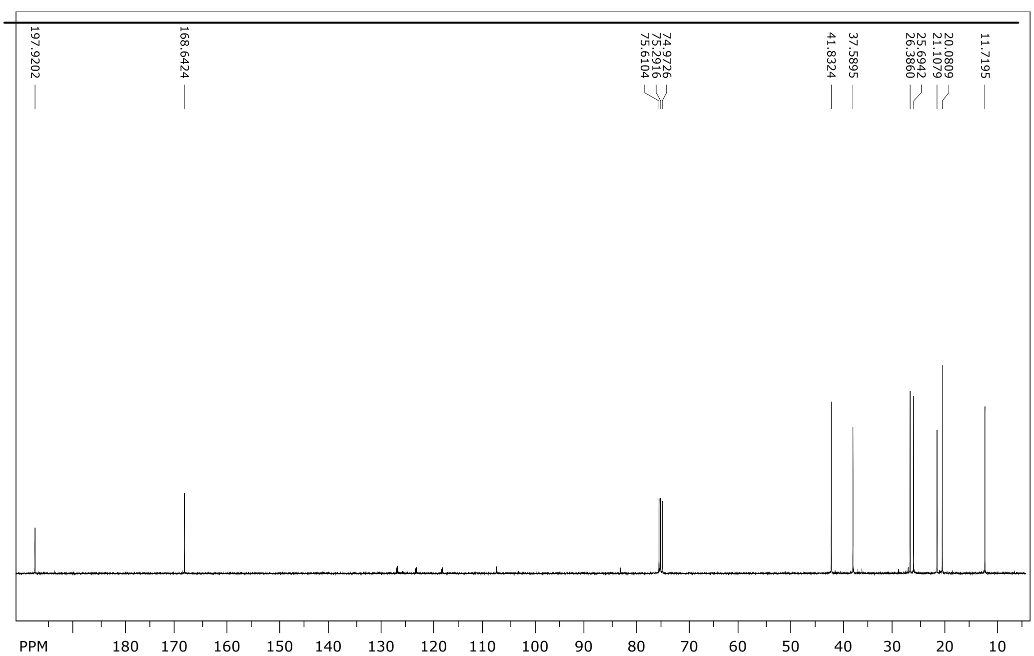

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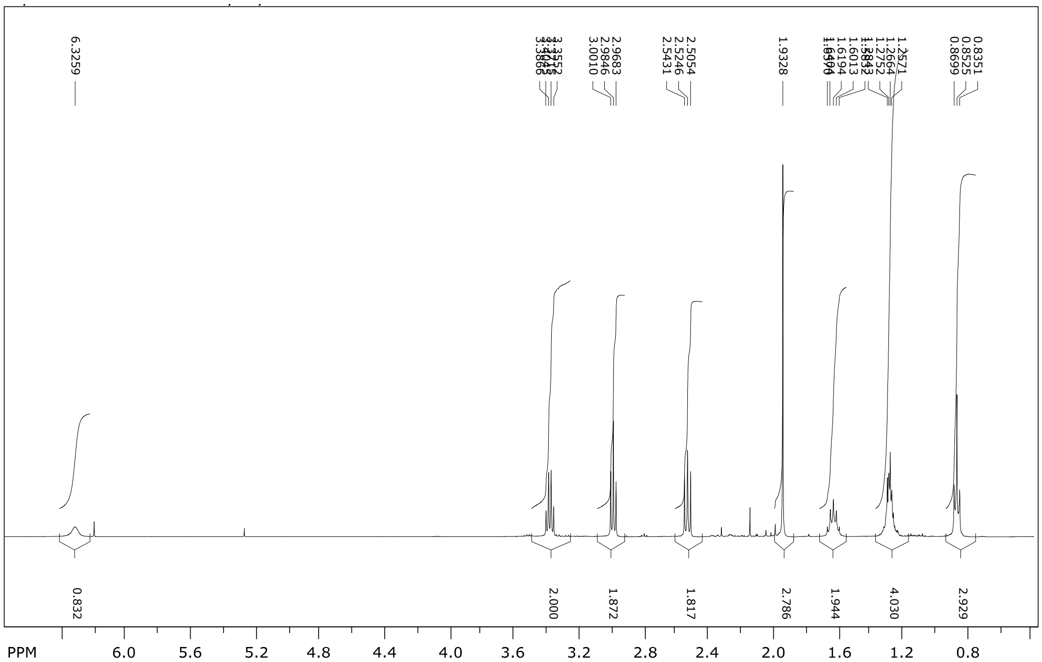

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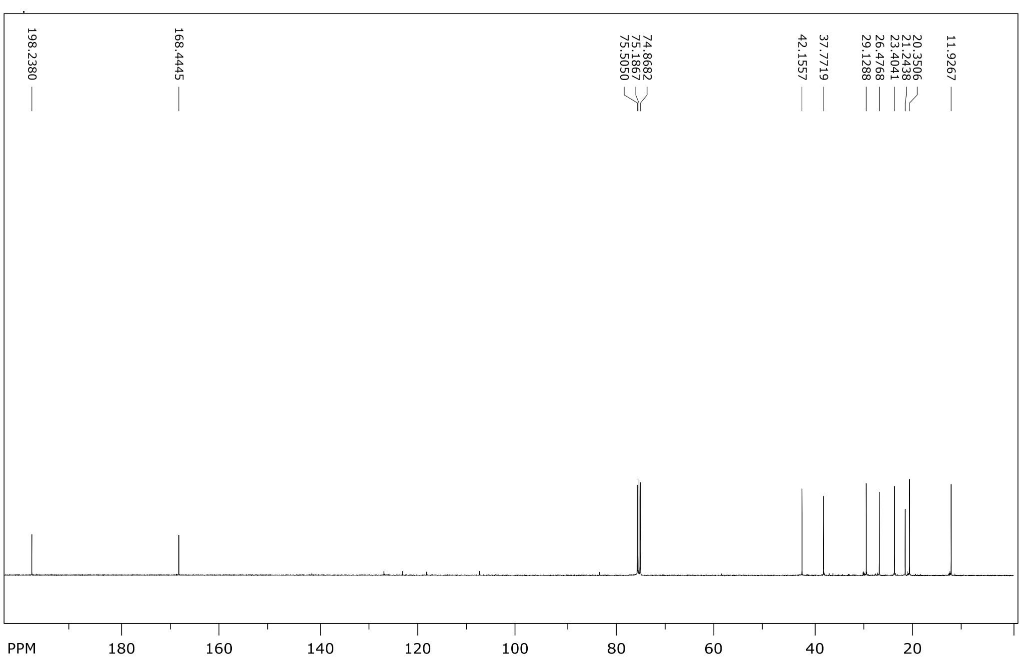

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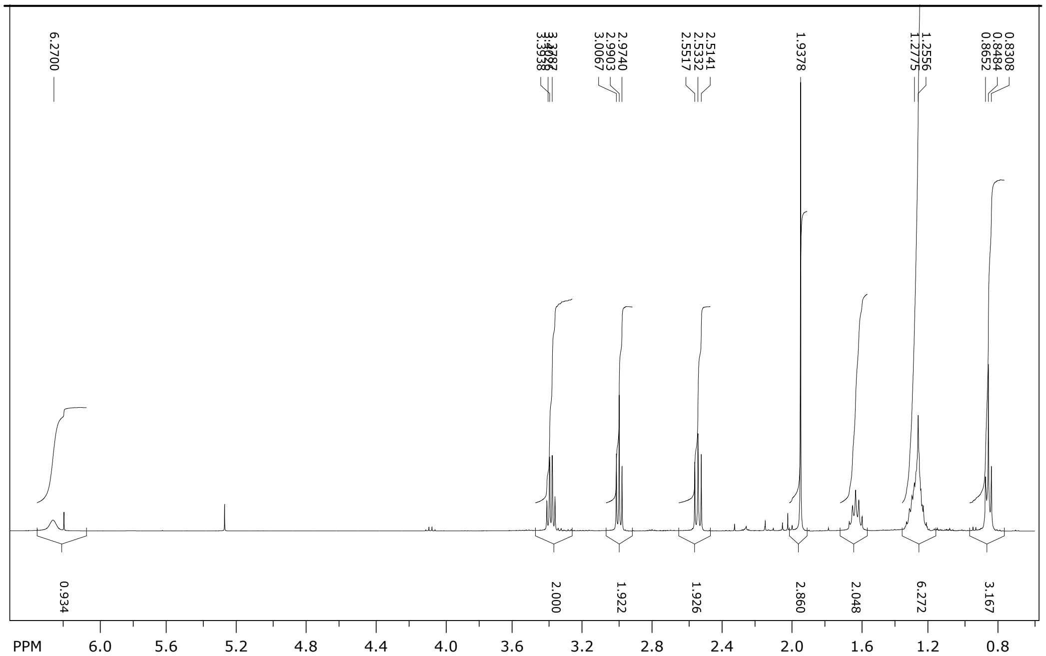

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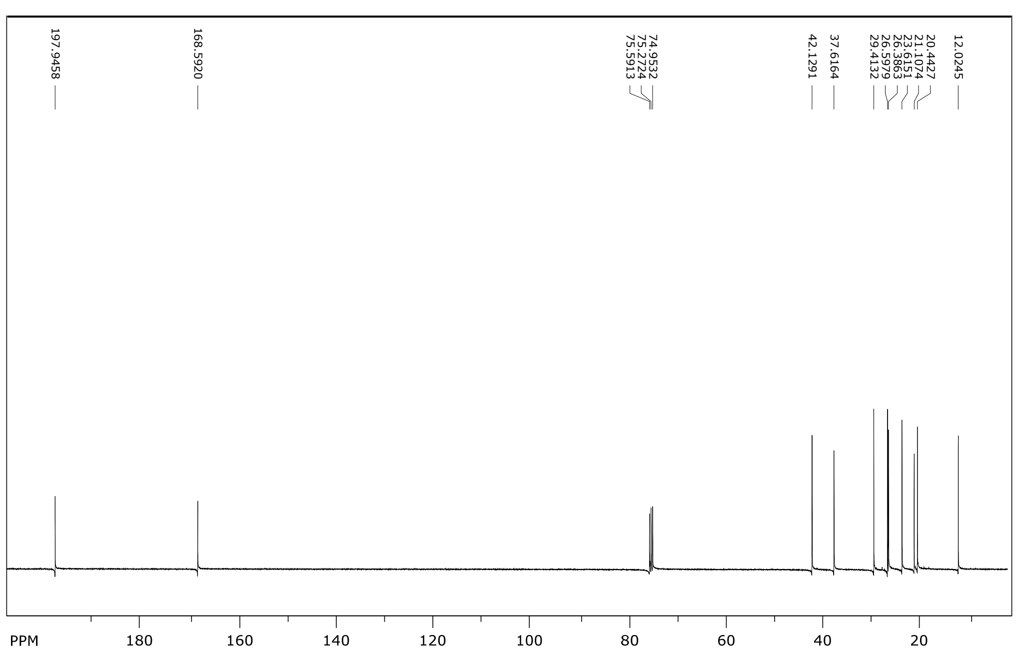

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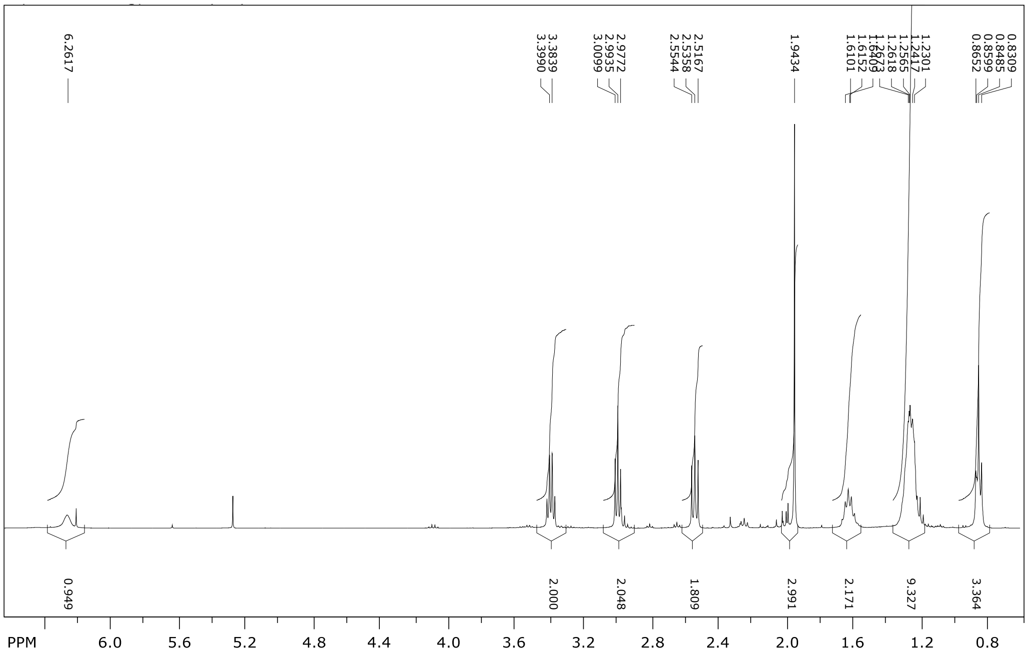

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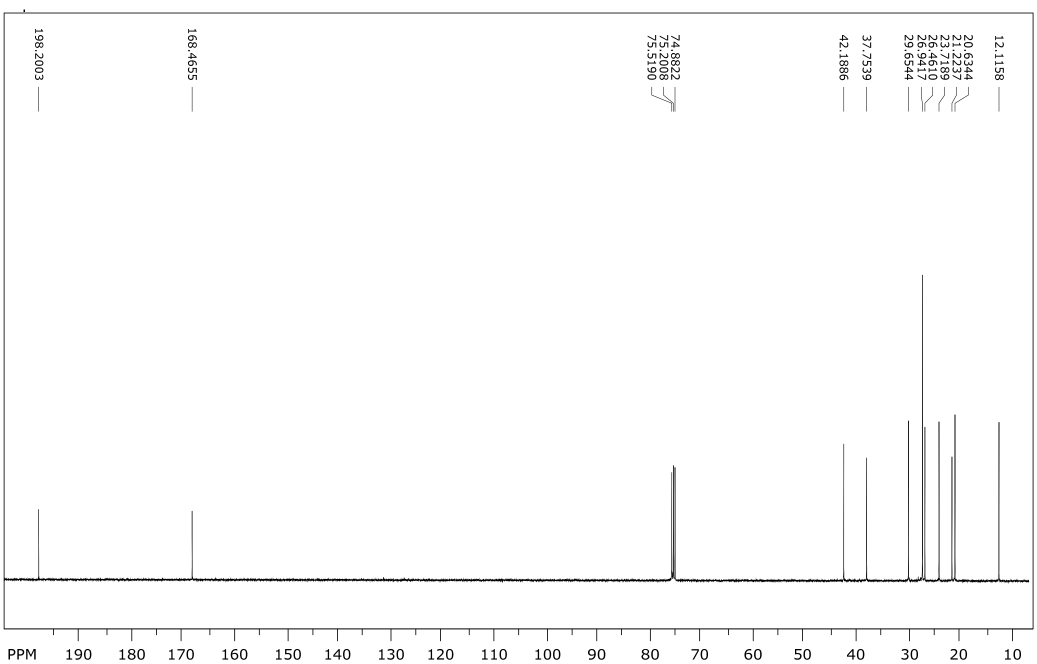

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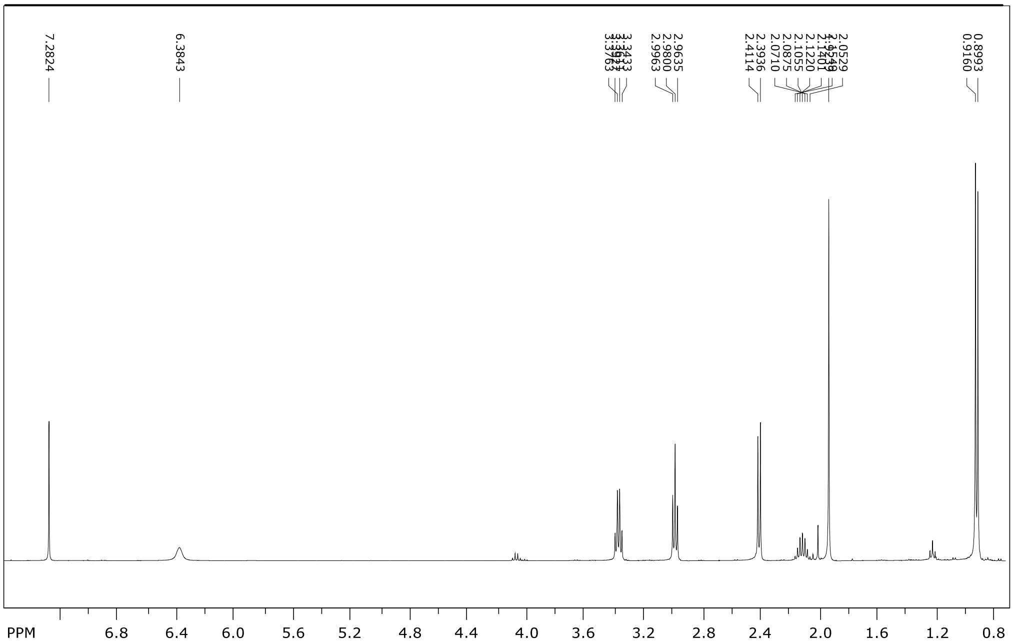
^{1}H NMR 7



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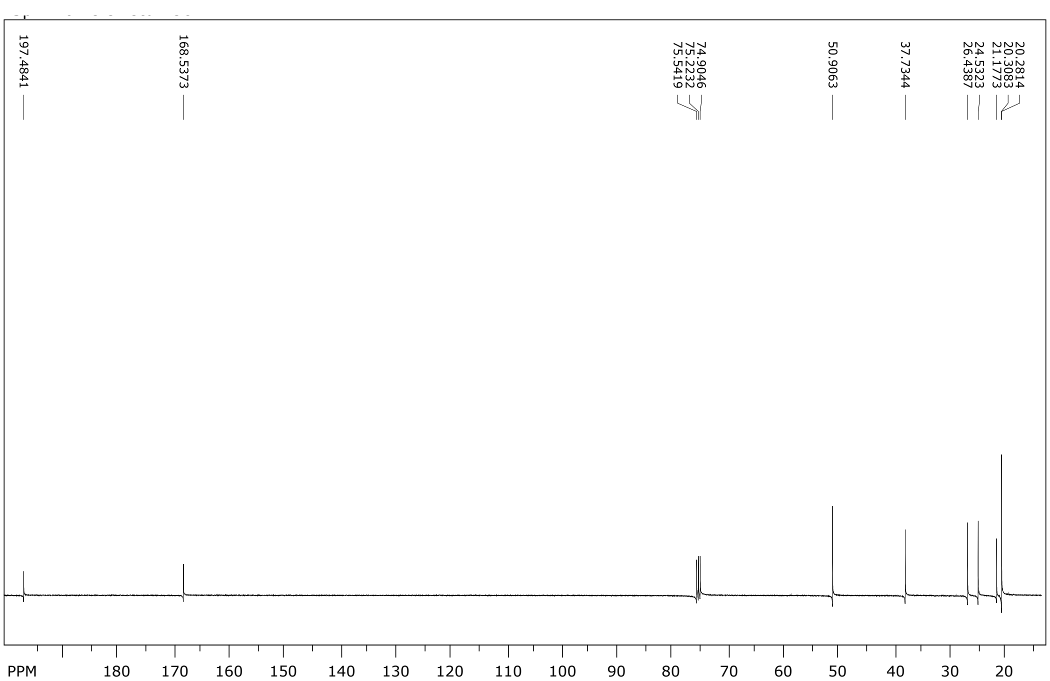

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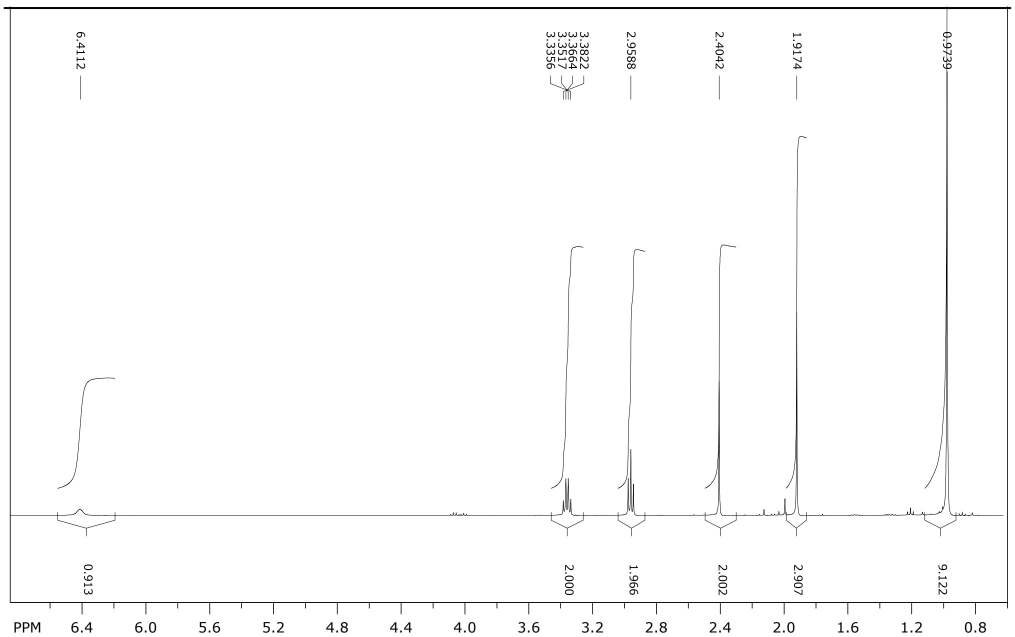

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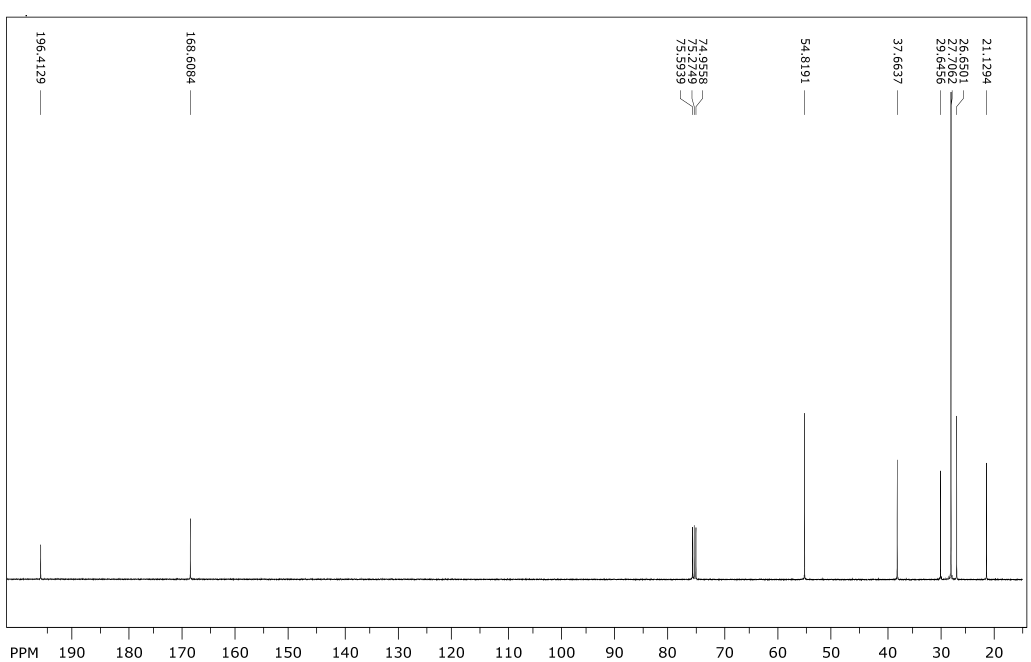
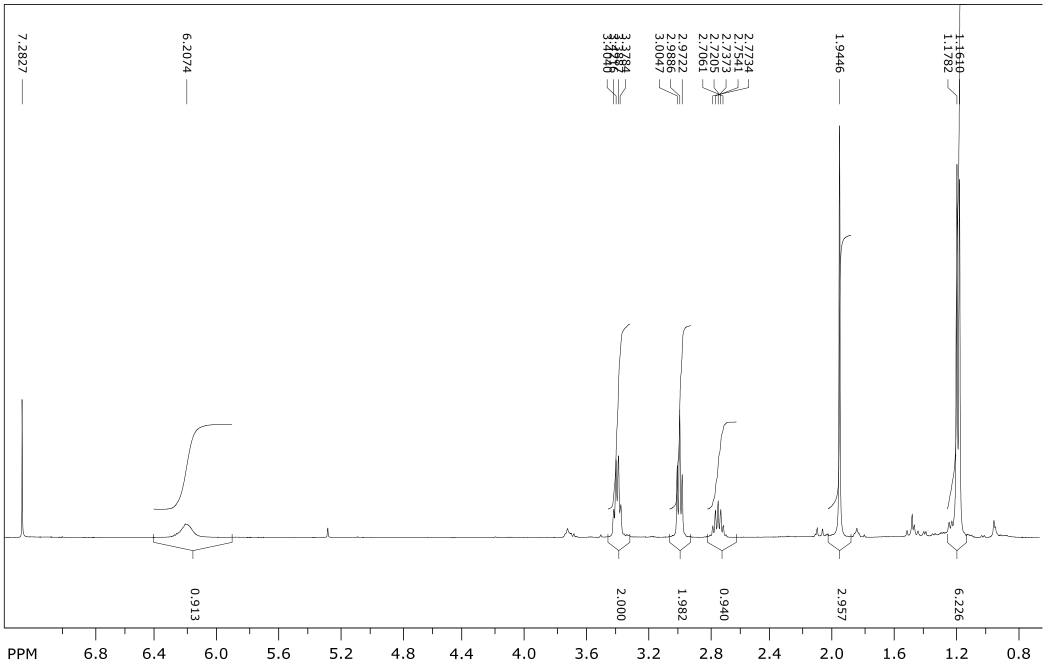
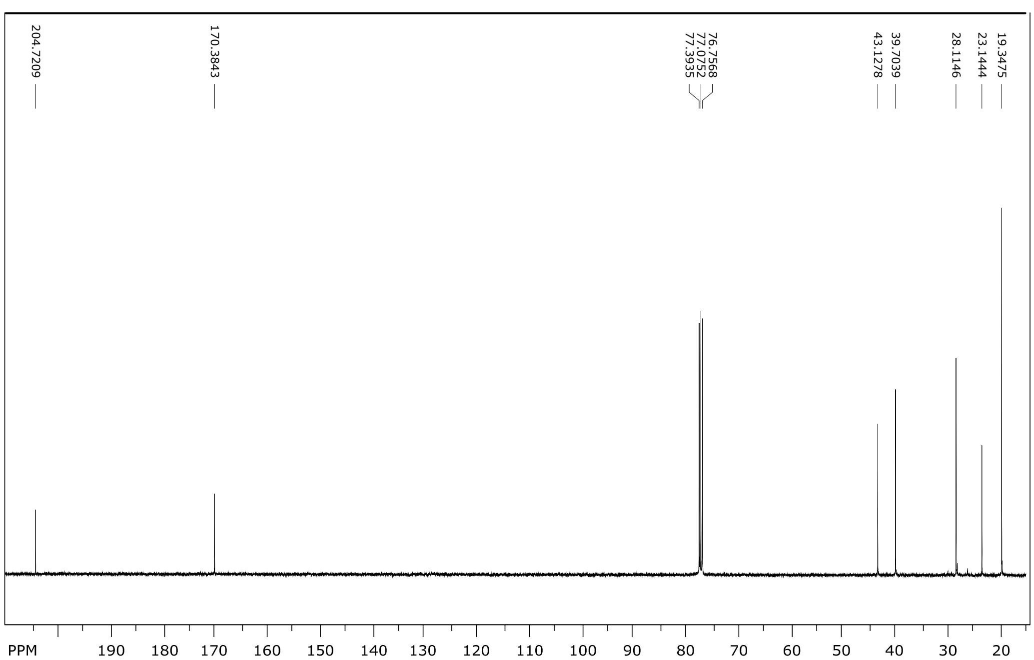


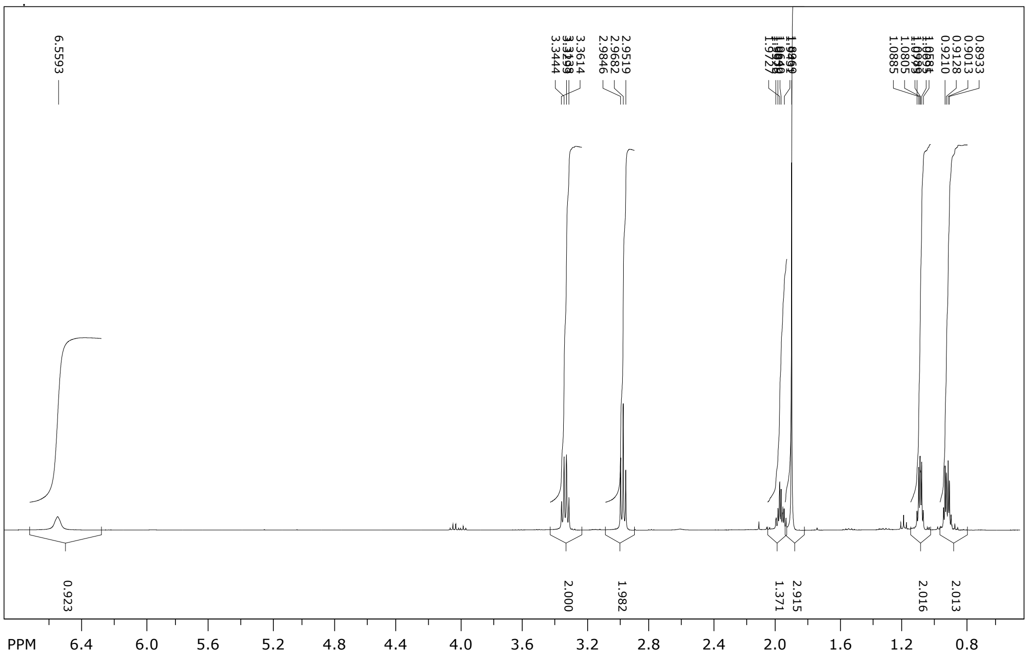
^{1}H NMR 9



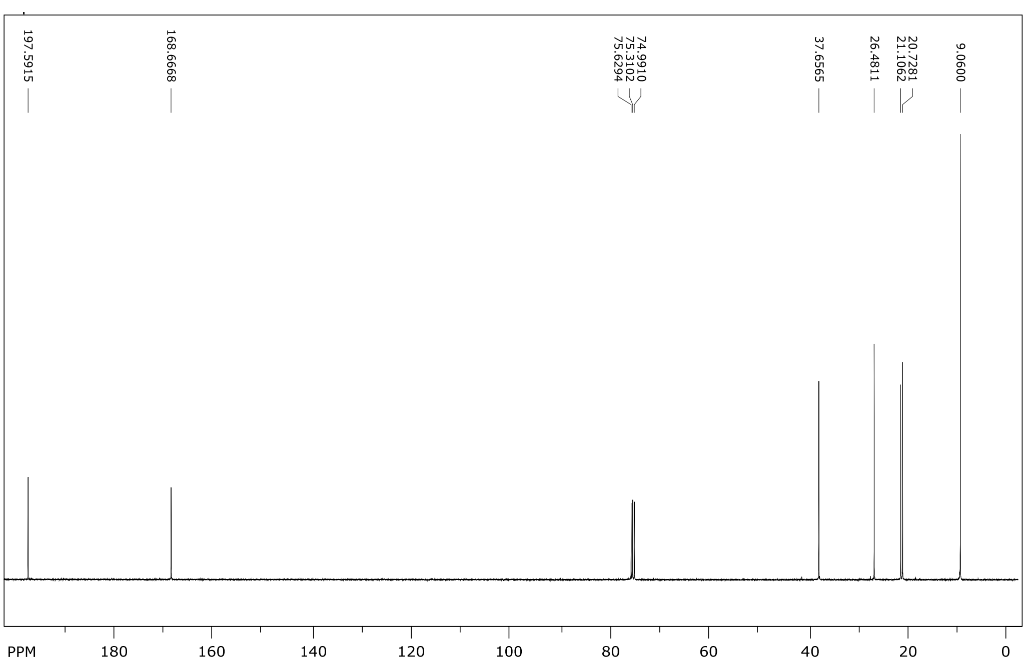
^{13}C NMR 9



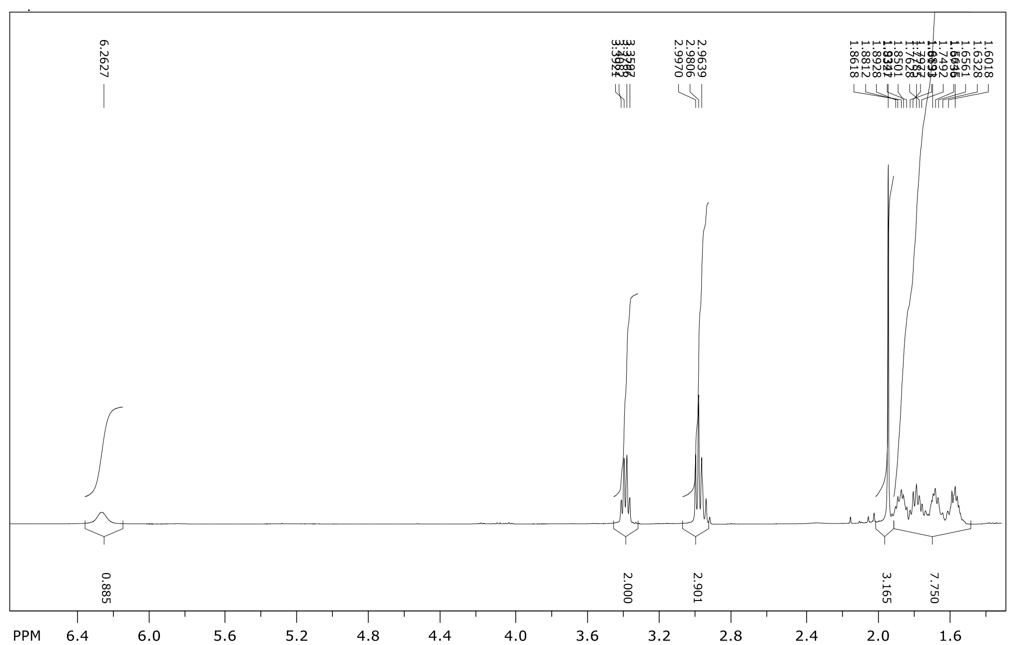
[1]H NMR 10



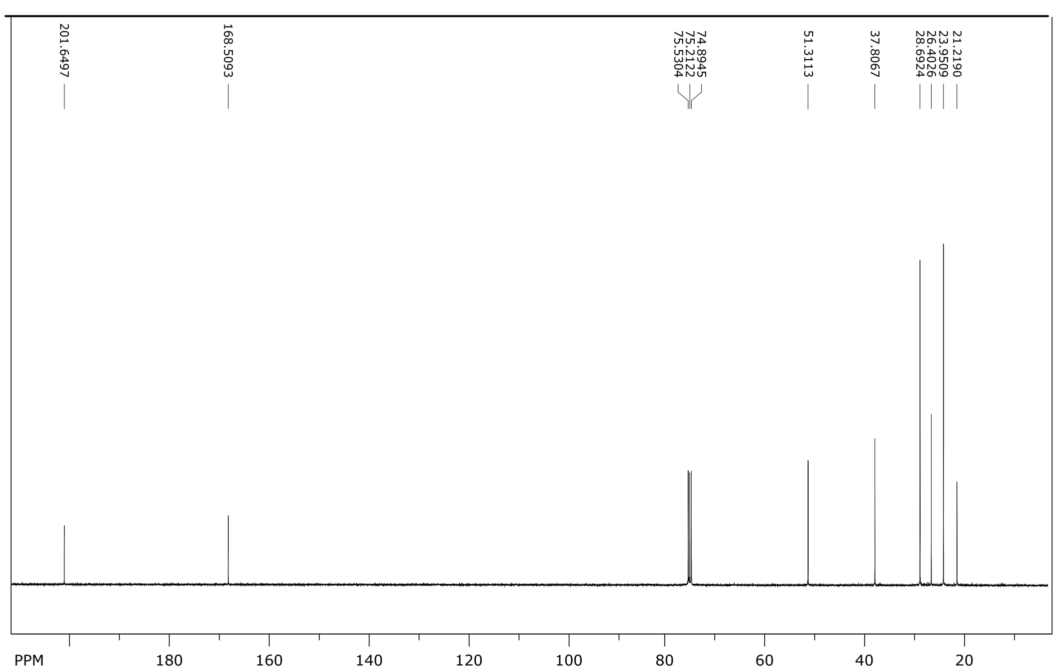
[13]C NMR 10



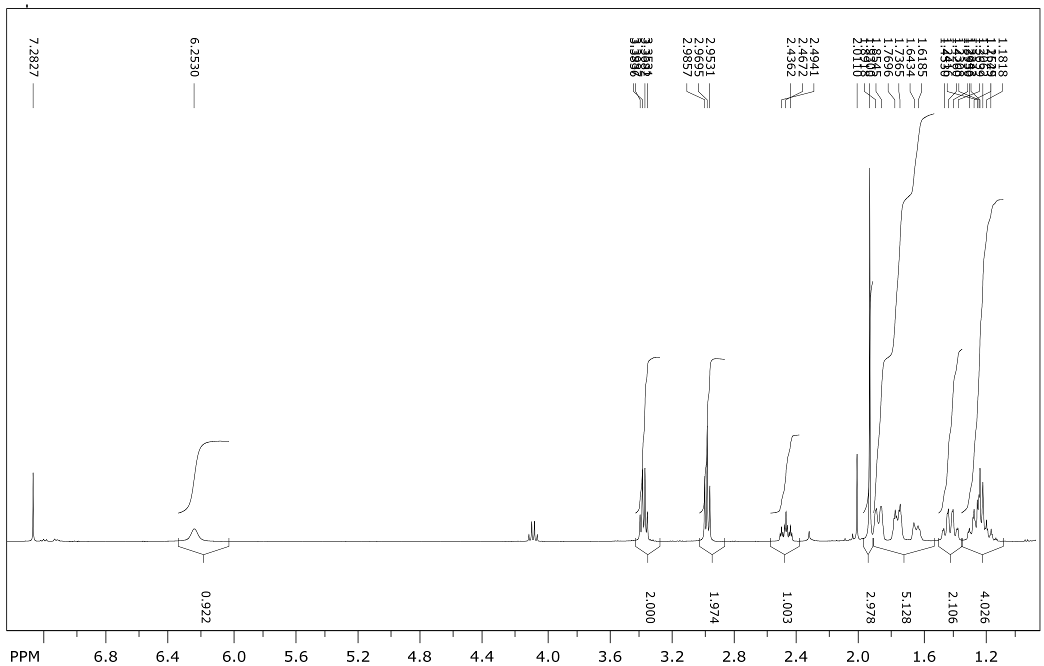
^{1}H NMR 11



^{13}C NMR 11



^{1}H NMR 12



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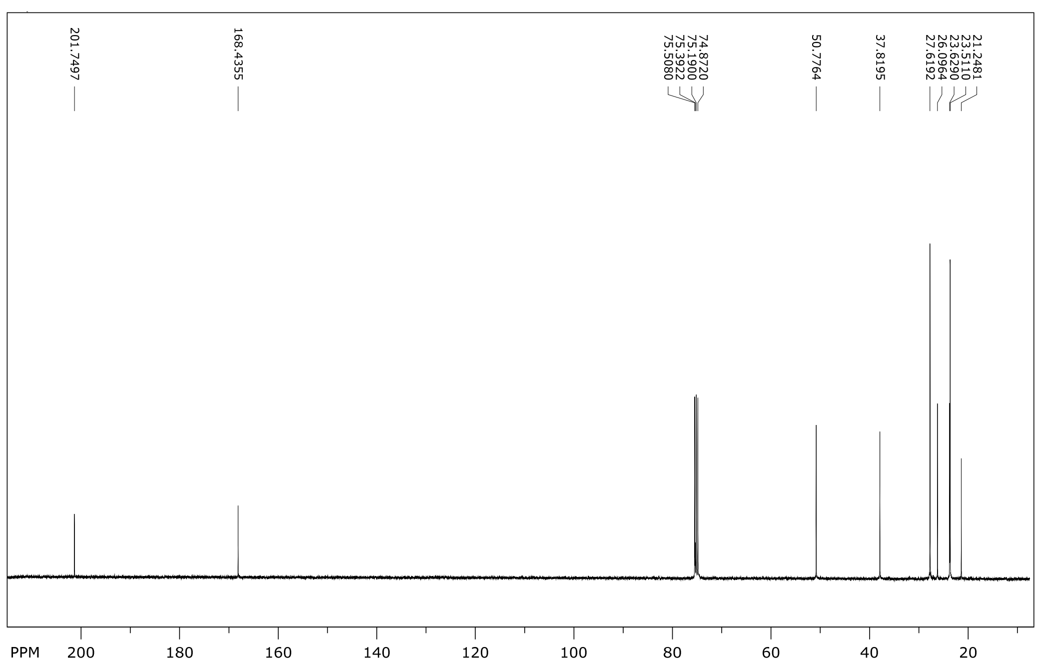

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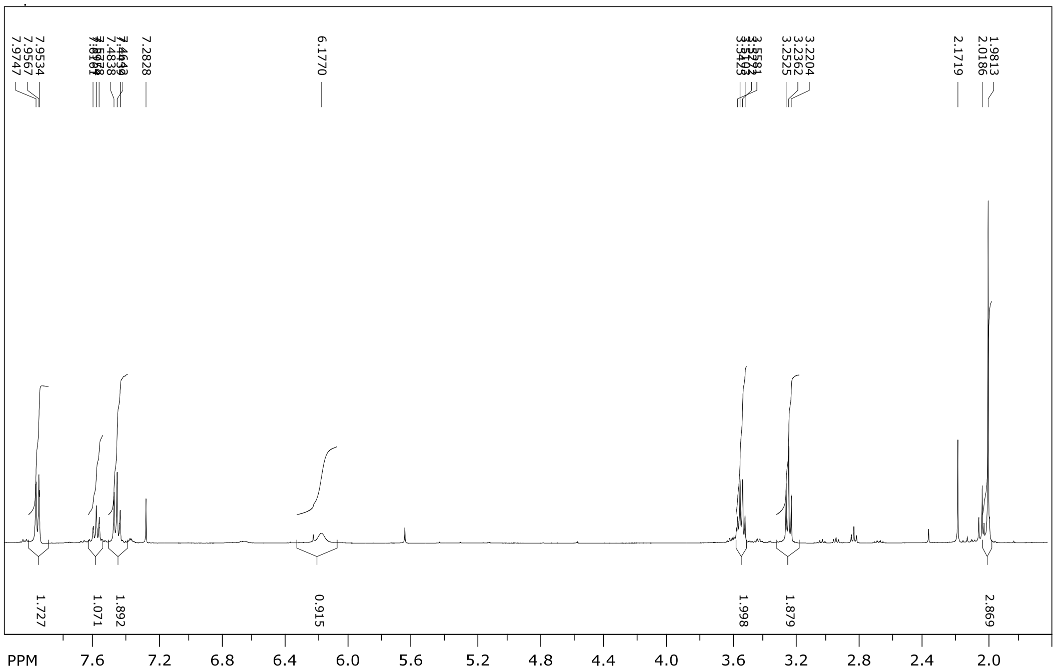
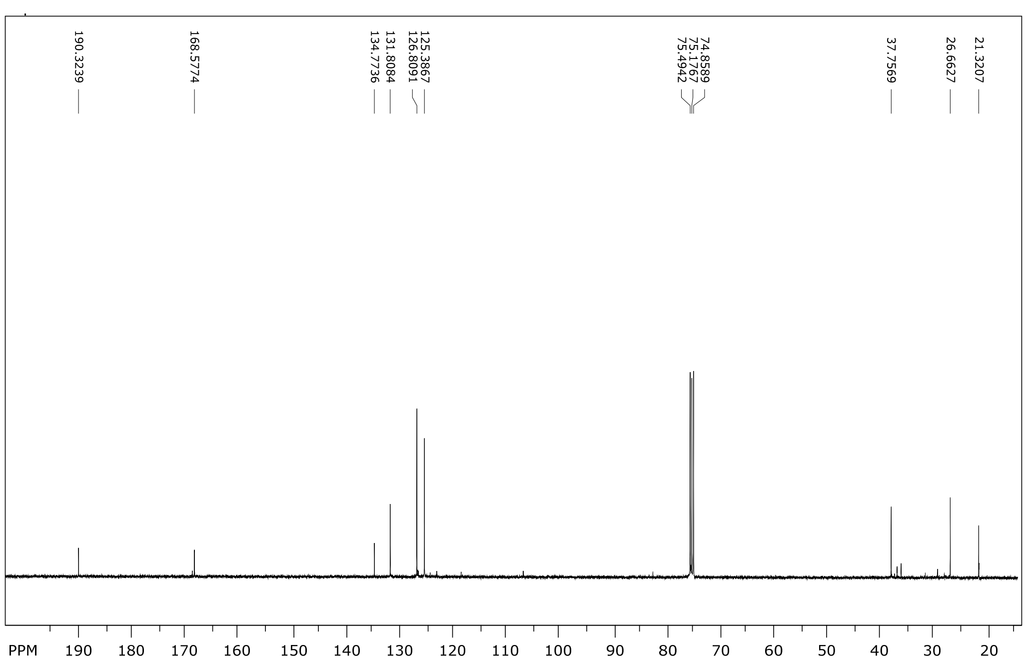
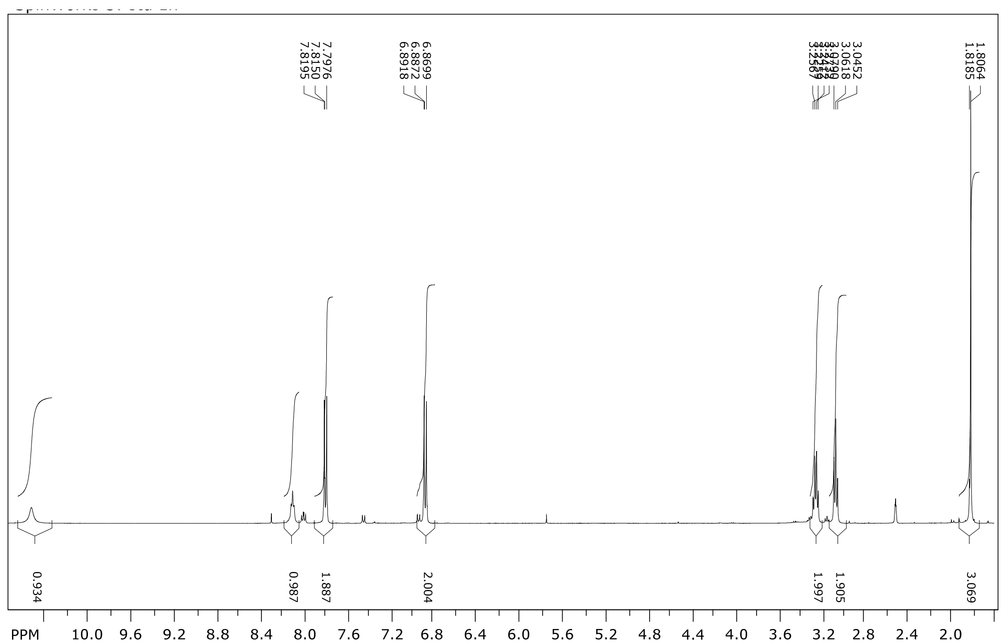


^{13}C NMR 13

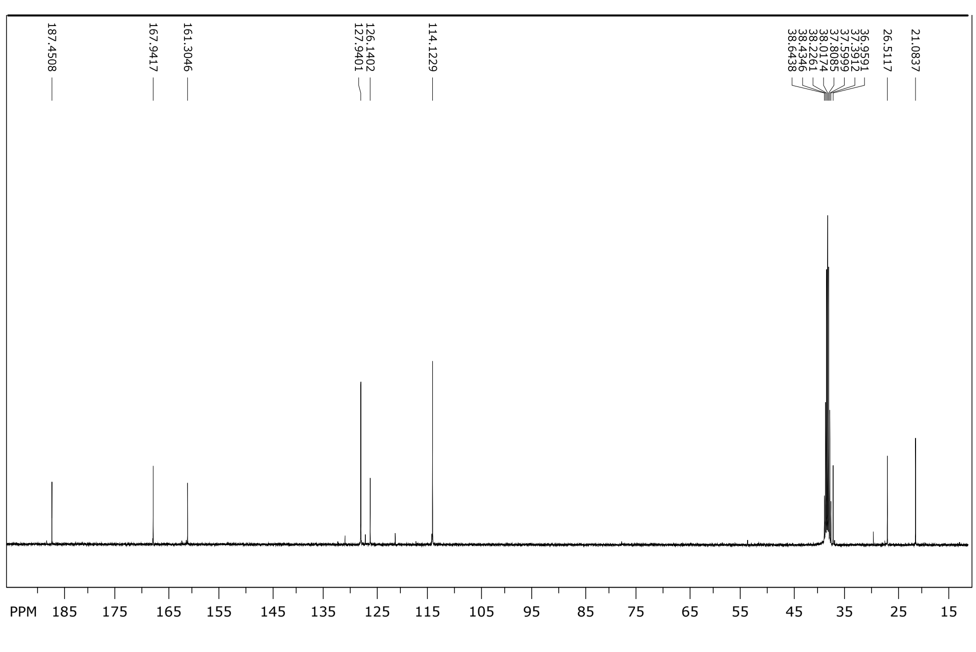


Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

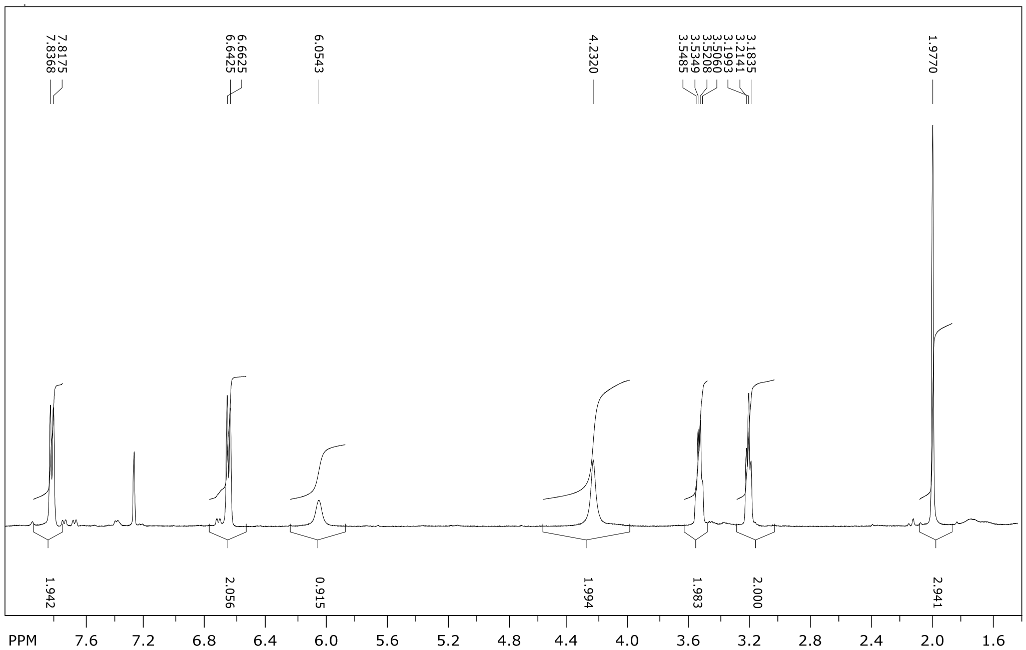
^{1}H NMR 14



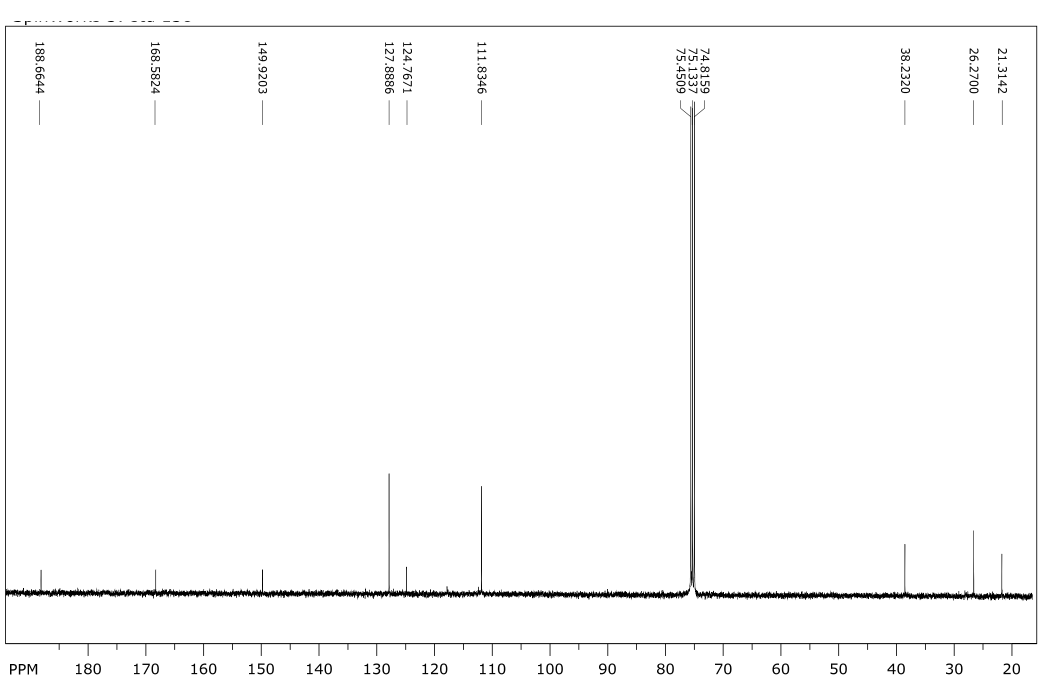
^{13}C NMR 14



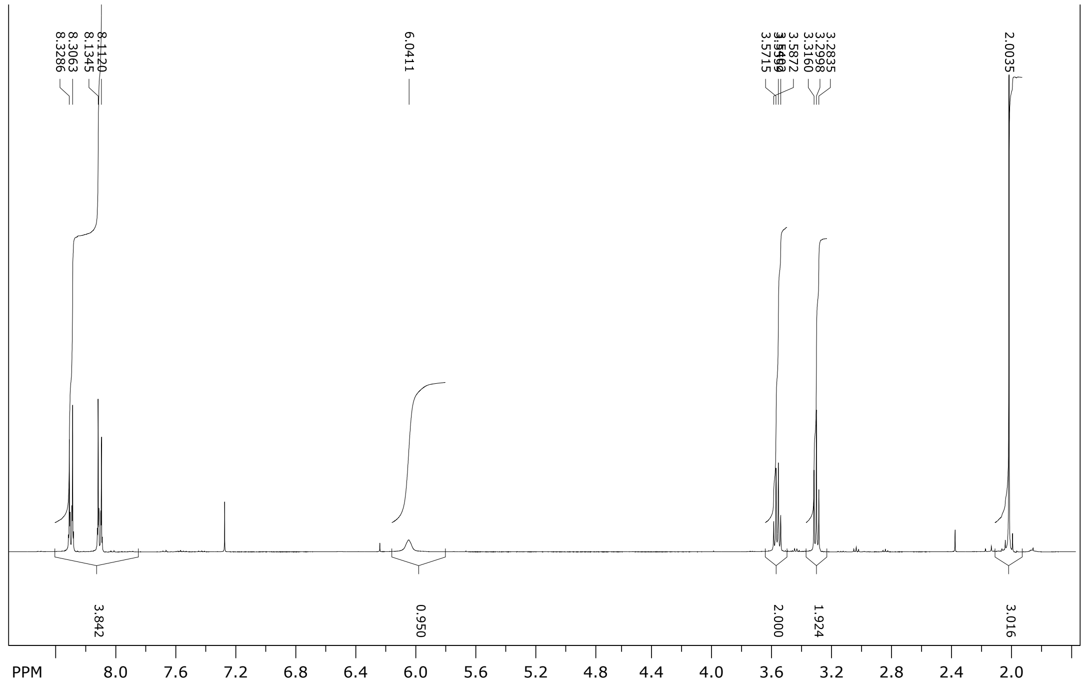
^{1}H NMR 15



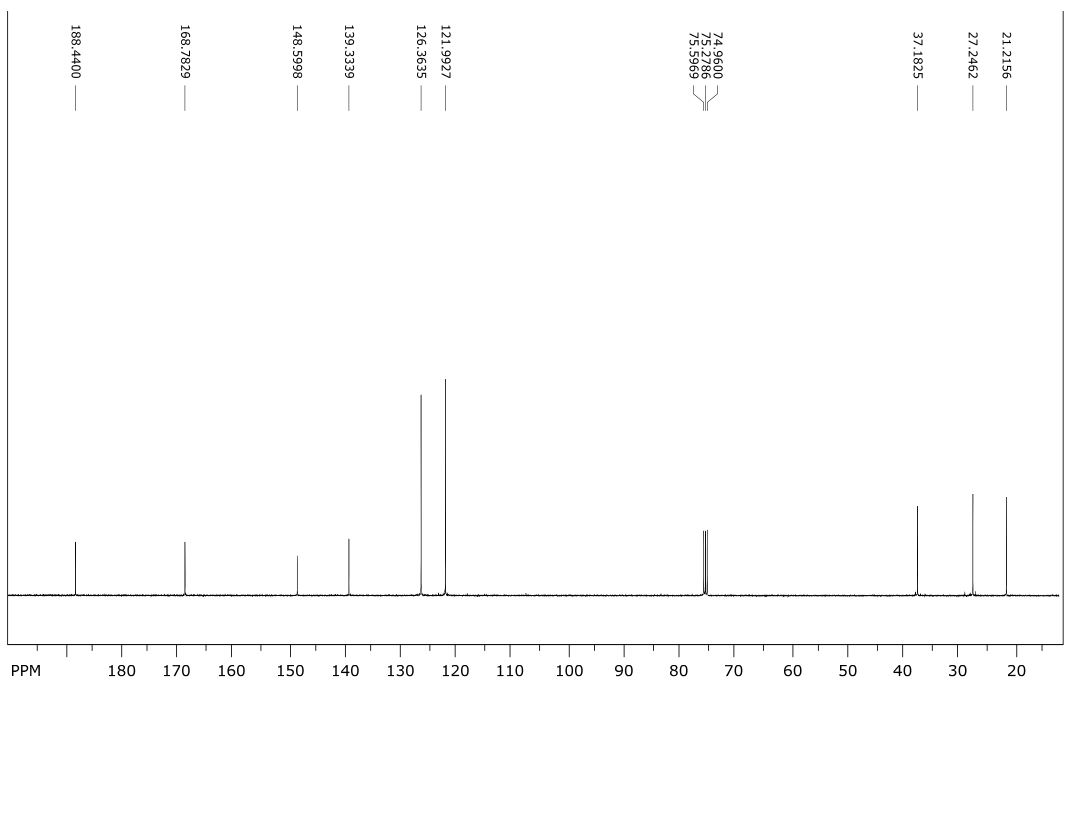
^{13}C NMR 15



^{1}H NMR 16

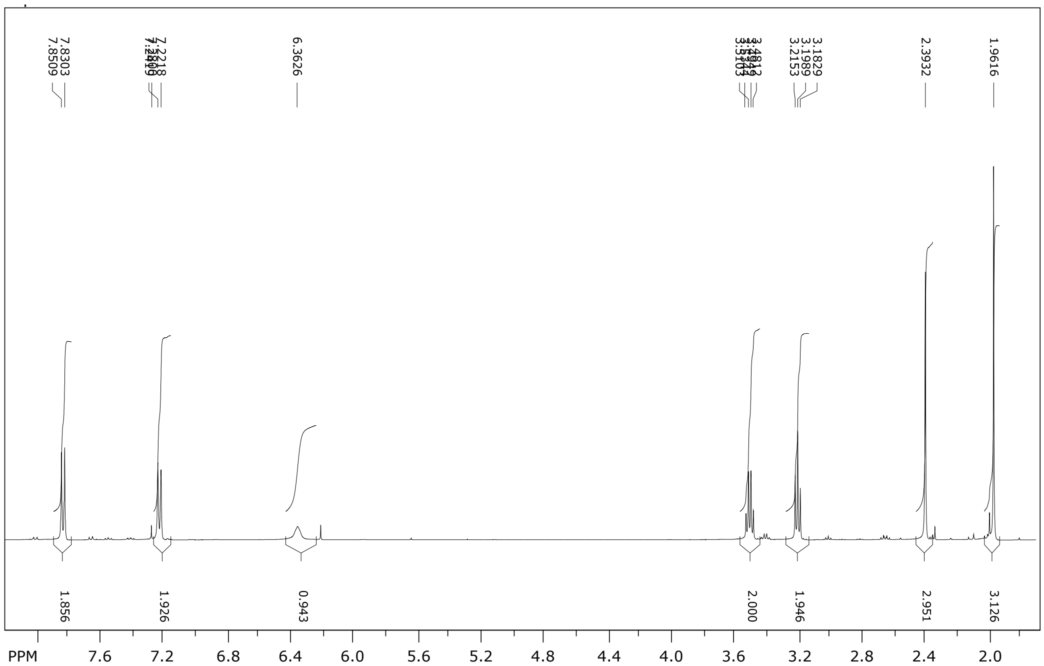


^{13}C NMR 16



Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

^{1}H NMR 17



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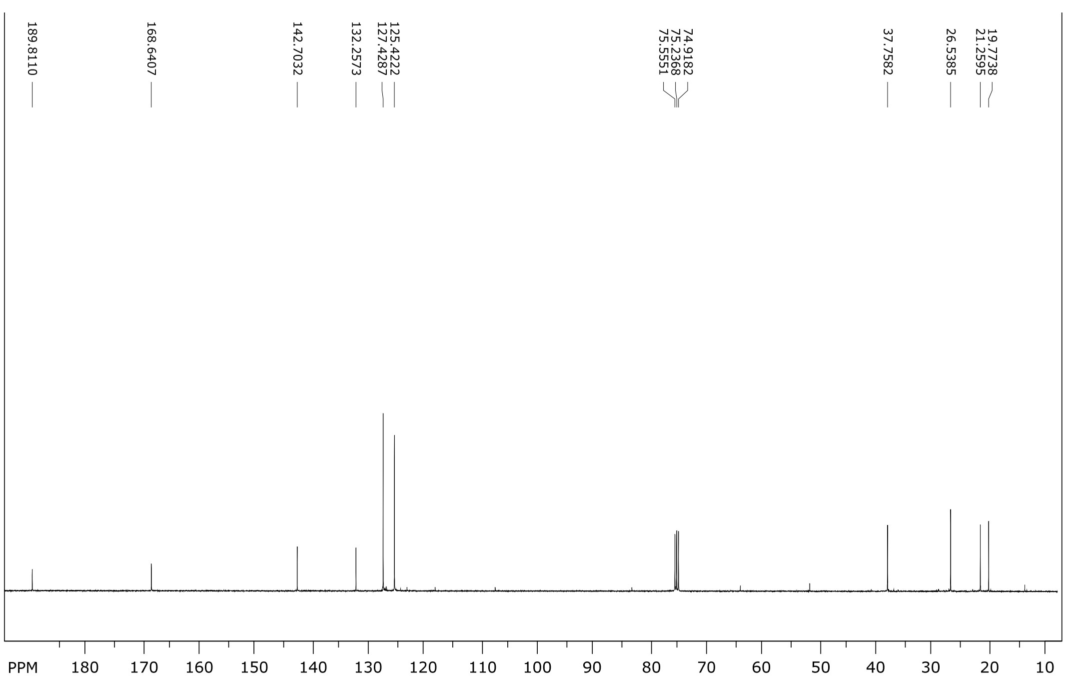
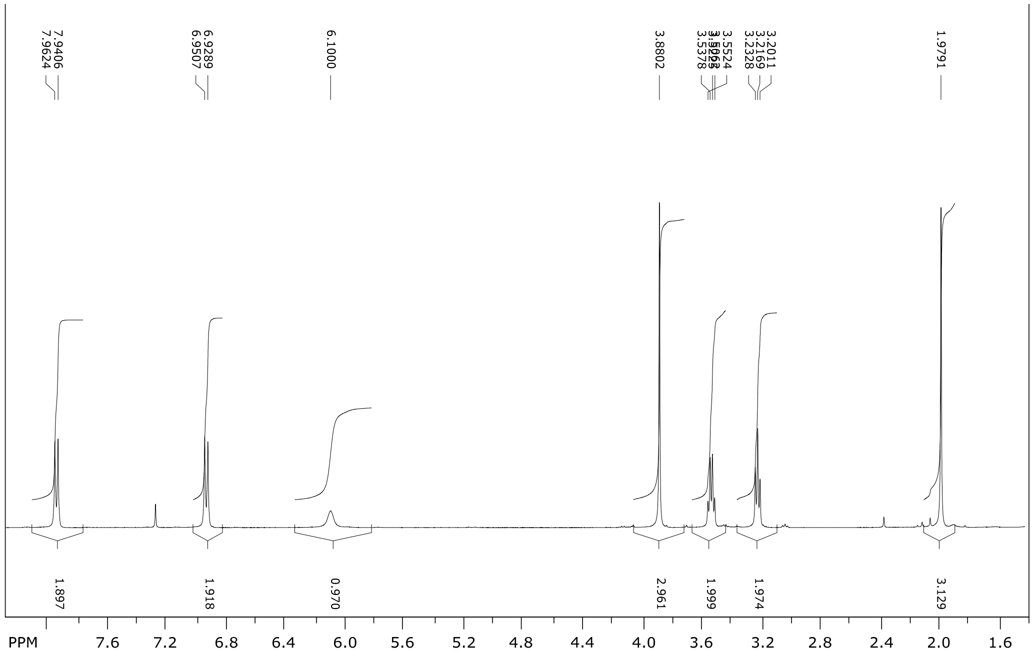
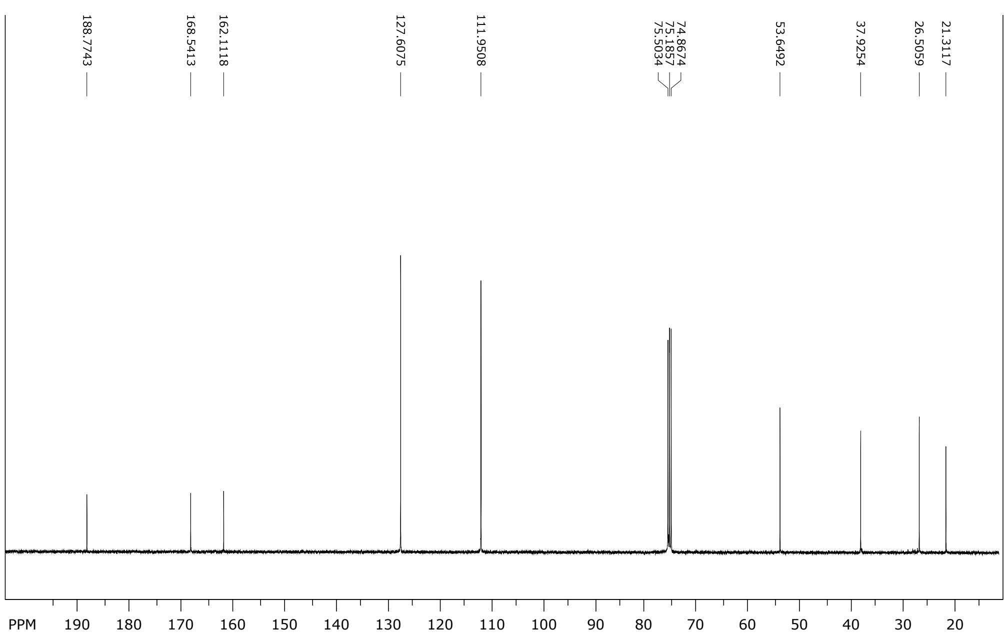


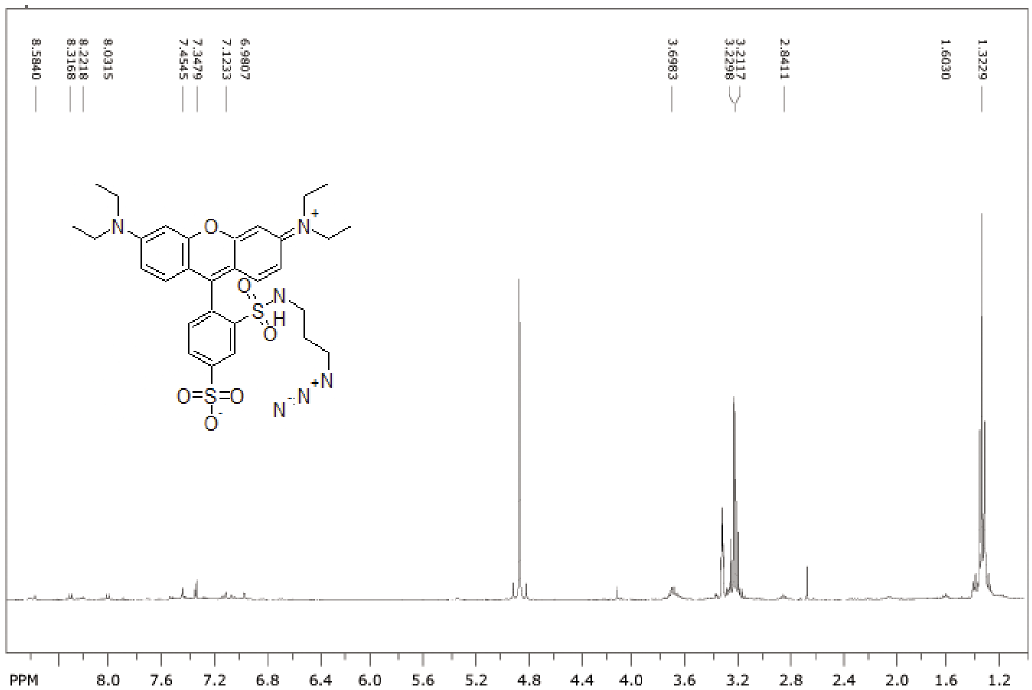
^{1}H NMR 18



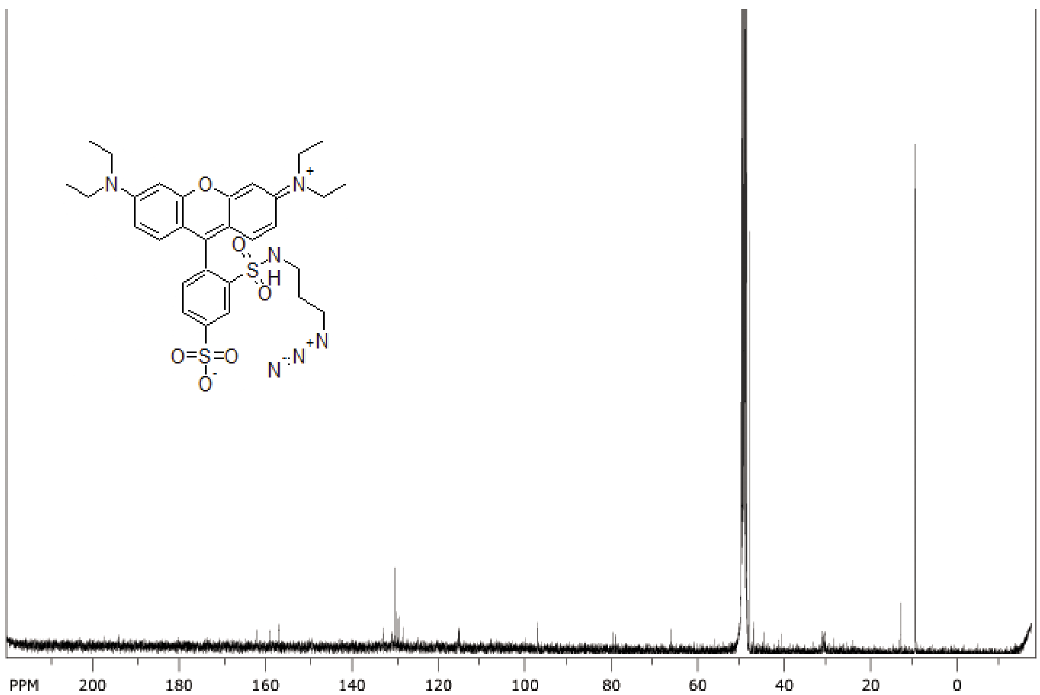
^{13}C NMR 18



^{1}H NMR of Rhodamine Azide

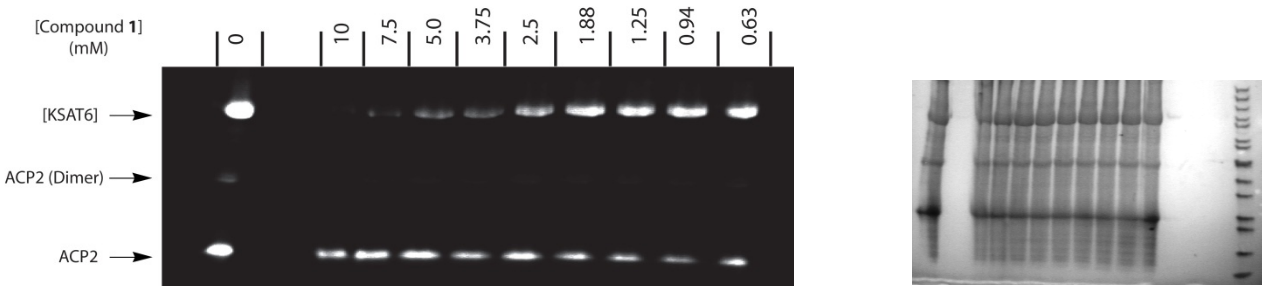


^{13}C NMR of Rhodamine Azide



Gel Stains: Full PAGE gels for fluorescence assays.

Fig. 5: SDS-PAGE analysis of fluorescence transfer from ACP2 to KSAT 6 pre-treated with various concentrations of compound 1



Fluorescence intensities for the above gel were obtained via fluorescence analysis using the ImageJ software. % KS acylation for each concentration of compound **1** assayed was calculated by subtracting the % of maximum fluorescence (determined by comparison to the control lane) for each band from 100%. A plot of % Acylation versus compound **1** concentration is shown below.

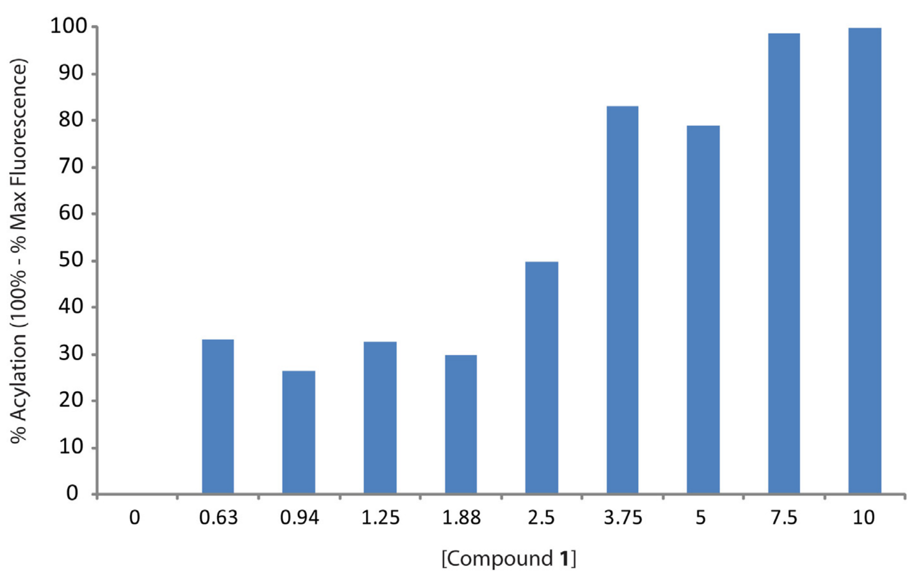


Fig. 6: SDS-PAGE analysis of fluorescence transfer from ACP2 to KSAT 6 pre-treated with compounds 1-6. Repeated runs for compounds 1-6.

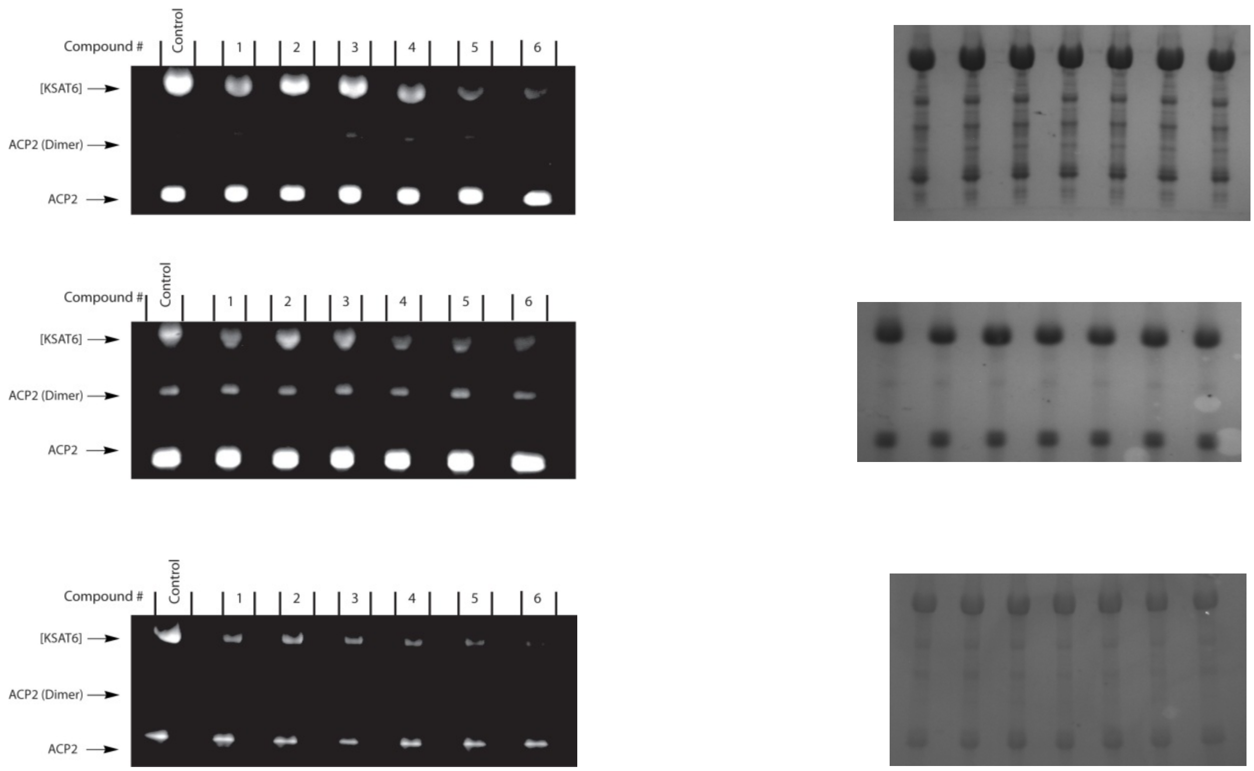


Fig. 8: SDS-PAGE analysis of fluorescence transfer from ACP2 to KSAT 6 pre-treated with compounds 7-12

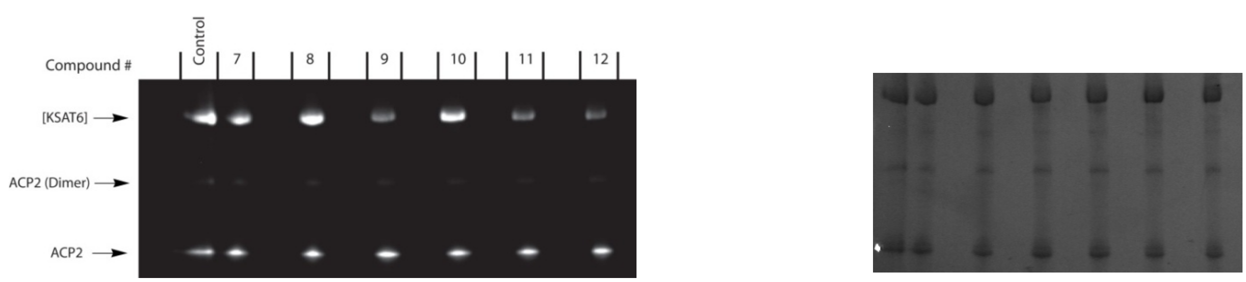


Fig. 10: SDS-PAGE analysis of fluorescence transfer from ACP2 to KSAT 6 pre-treated with compounds 13-18

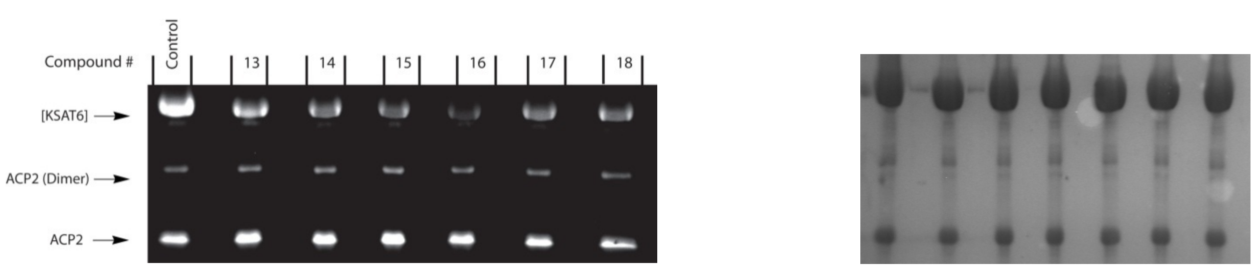
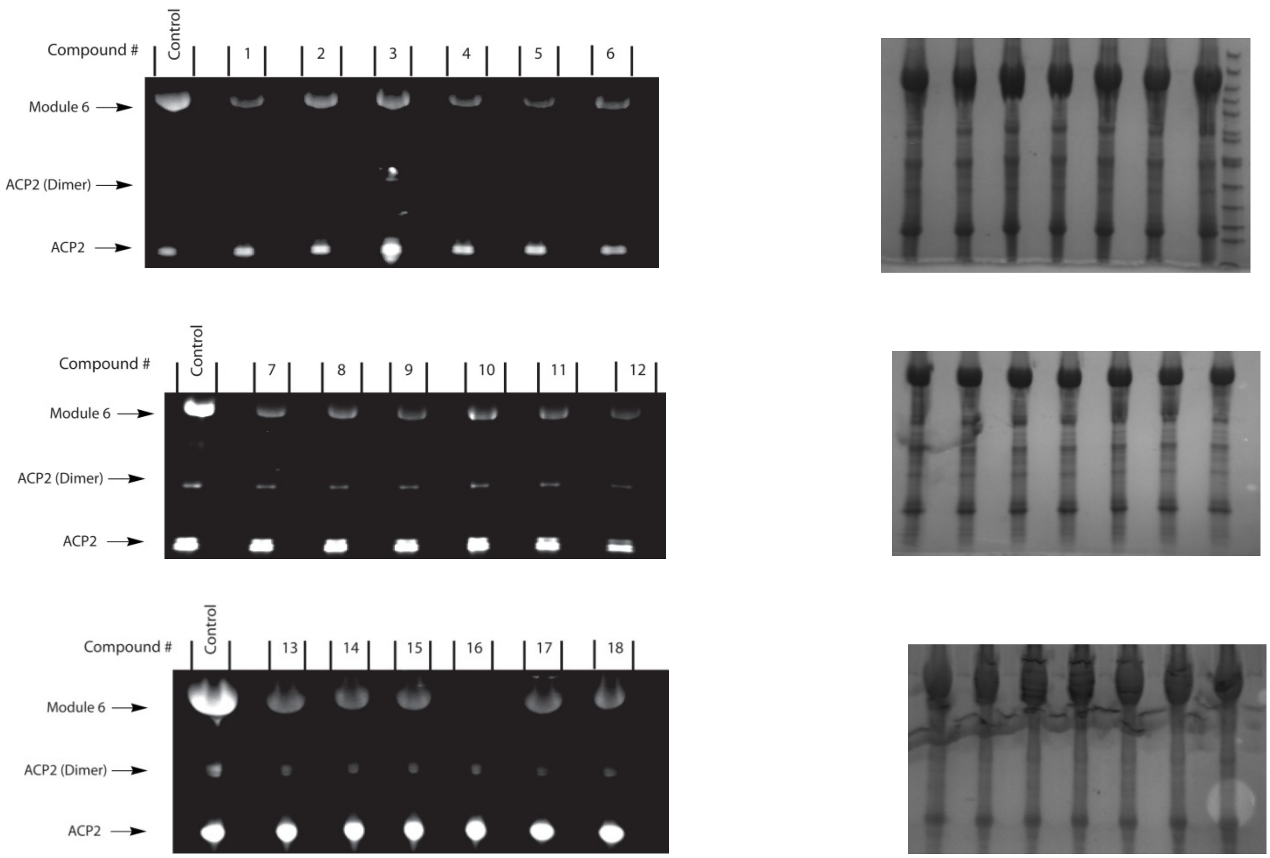
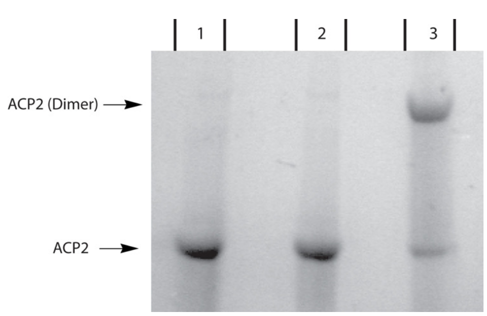


Fig. 11: SDS-PAGE analysis of fluorescence transfer from ACP2 to *apo*-module 6 pre-treated with compounds 1-18.

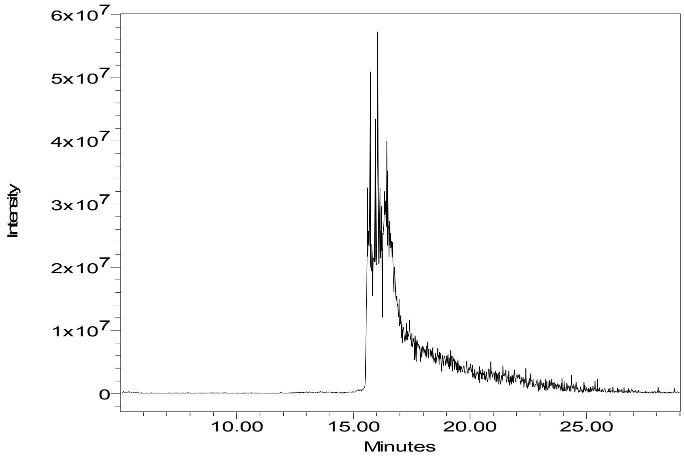


Dimerization of ACP2 is Copper dependent and random. It does not affect loading of the KSAT or the full module. The SDS-PAGE gel below demonstrates the copper-dependency of ACP2 dimer formation. Lane 1: ACP2 (25μM). Lane 2: ACP2 (25μM) + *N*-Poc-β-lactam (2.5mM). Lane 3: ACP2 (25μM) + Copper sulfate (1mM) + sodium ascorbate (1mM). All samples are in 100mM phosphate buffer (pH = 7.2) with 2.5mM TCEP.

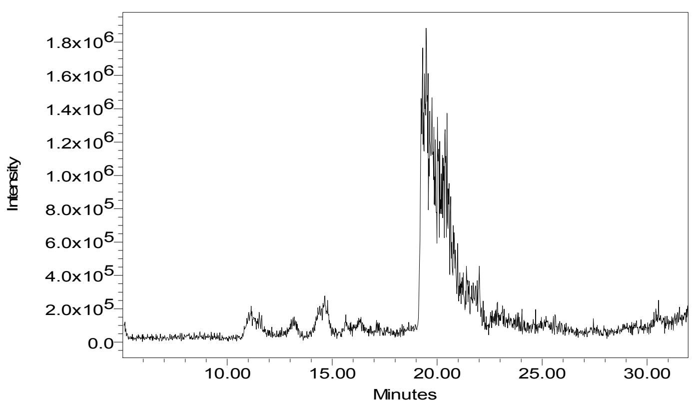


HPLC spectra (TIC)

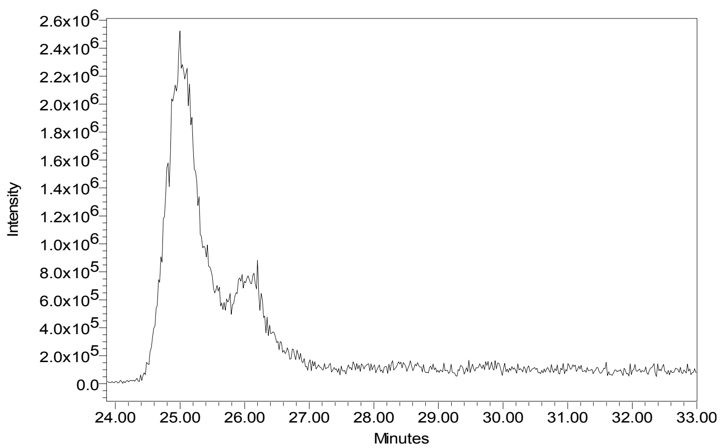
(1)



(2)



(3)



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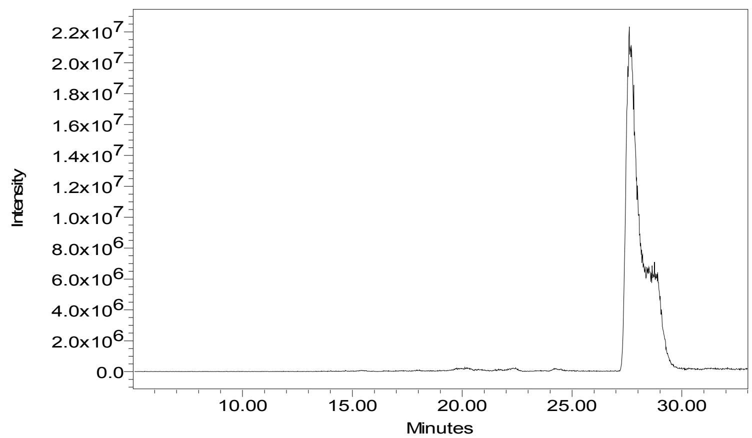
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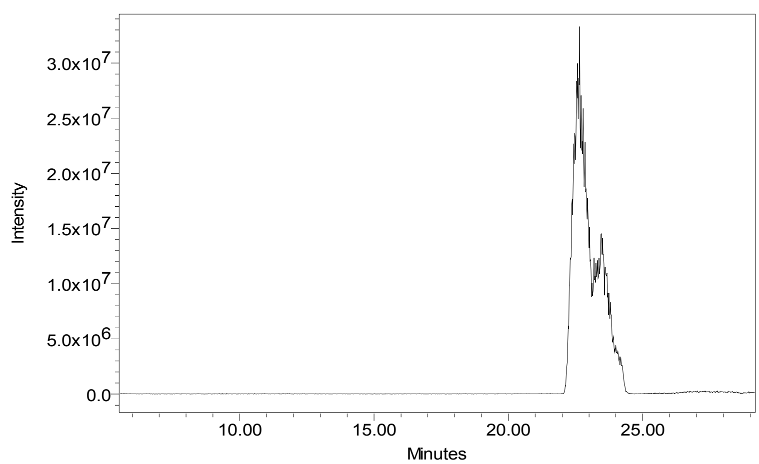
1.8x10^7
1.6x10^7
1.4x10^7
1.2x10^7
1.0x10^7
8.0x10^6
6.0x10^6
4.0x10^6
2.0x10^6
0.0
10.00
15.00
20.00
25.00
30.00
Minutes

(5)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2.0x10^7
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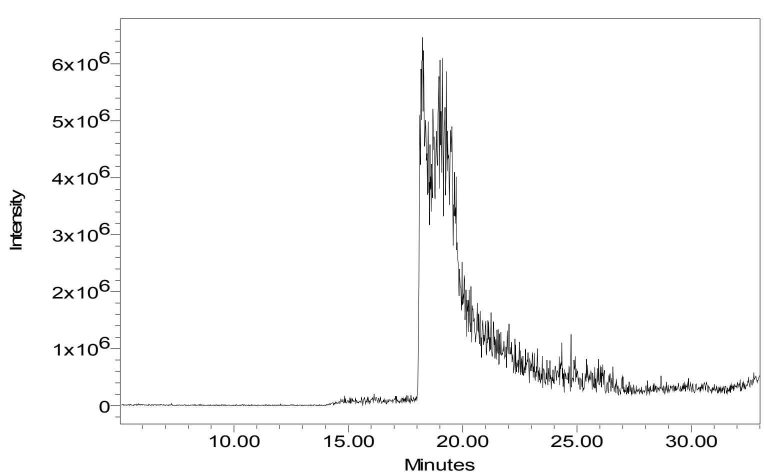
10.00
15.00
20.00
25.00
Minutes

(6)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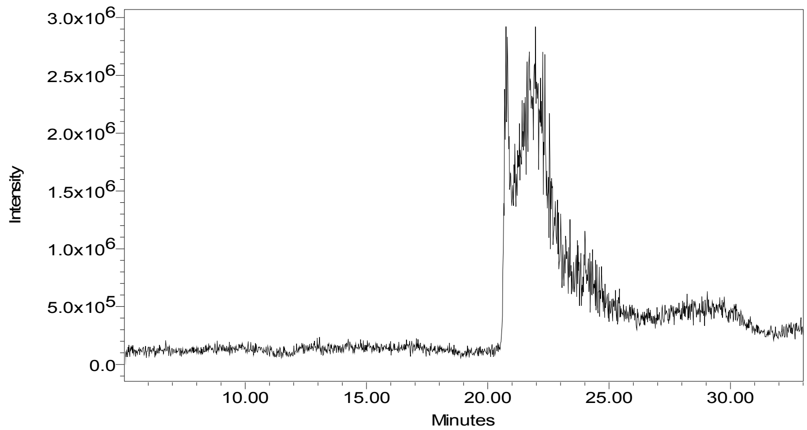
20.00
25.00
30.00
Minutes

(7)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2.0x10^6
1.5x10^6
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Intensity
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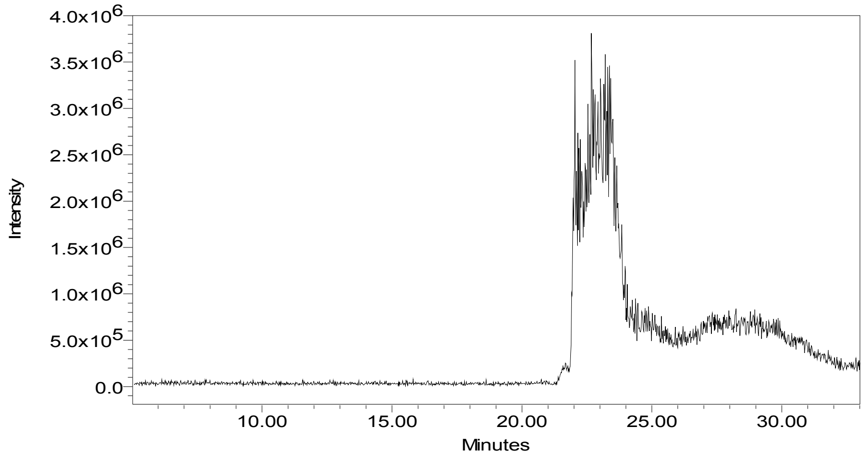
20.00
25.00
30.00
Minutes

(8)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3.0x10^6
2.5x10^6
2.0x10^6
1.5x10^6
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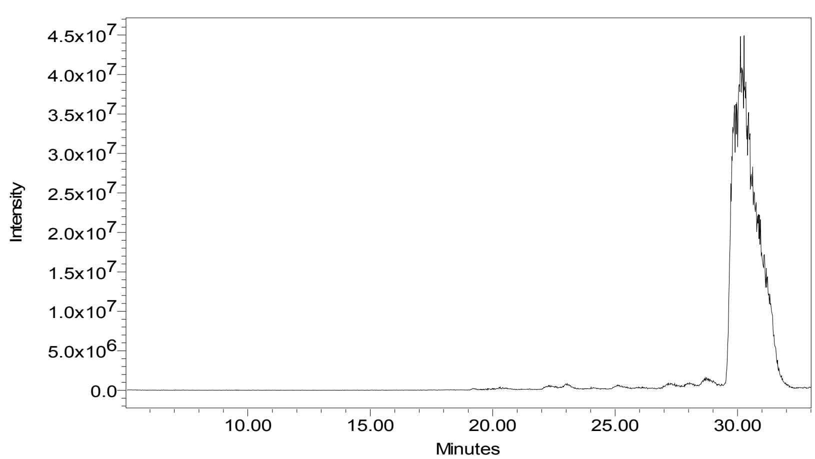
5.0x10^5
0.0
Intensity
10.00
15.00
20.00
25.00
30.00
Minutes

(9)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3.0x10^7
2.5x10^7
2.0x10^7
1.5x10^7
1.0x10^7
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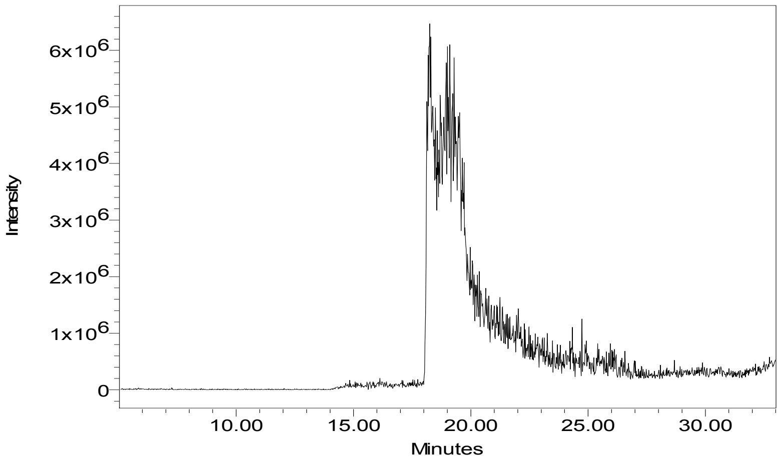
25.00
30.00
Minutes

(10)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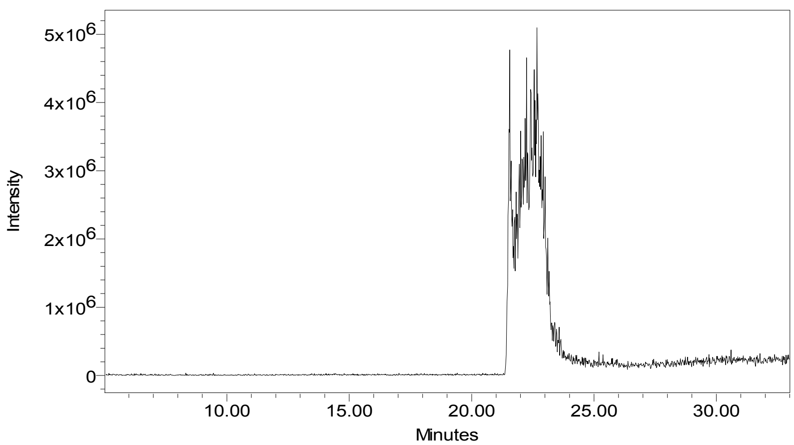
Minutes

(11)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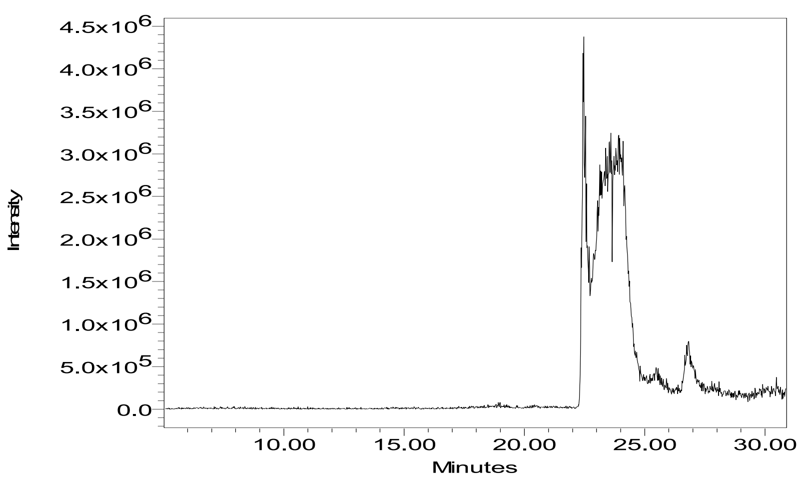
Minutes

(12)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1.0x10^6
5.0x10^5
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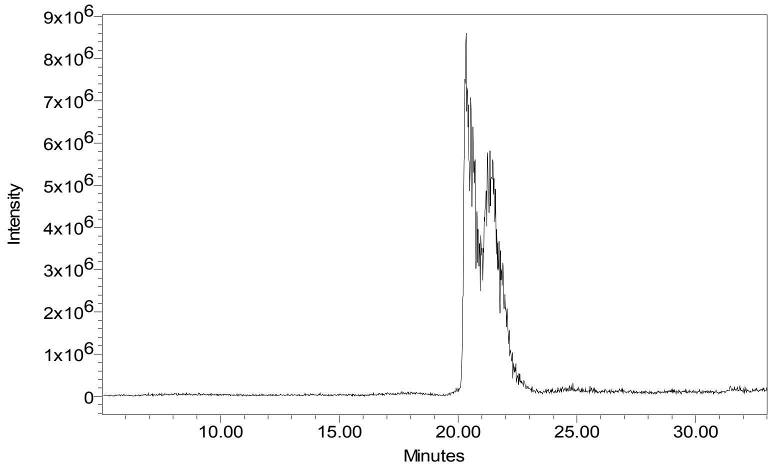
20.00
25.00
30.00
Minutes

(13)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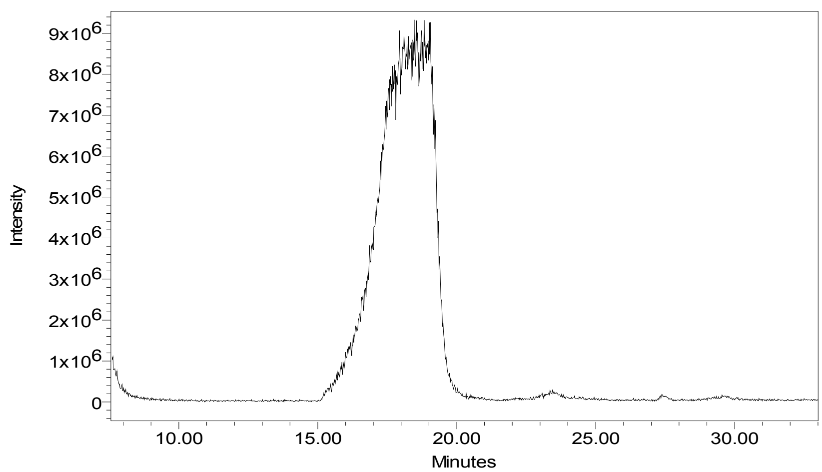
1x10^6
0
Intensity
10.00
15.00
20.00
25.00
30.00
Minutes

(14)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0
Intensity
10.00
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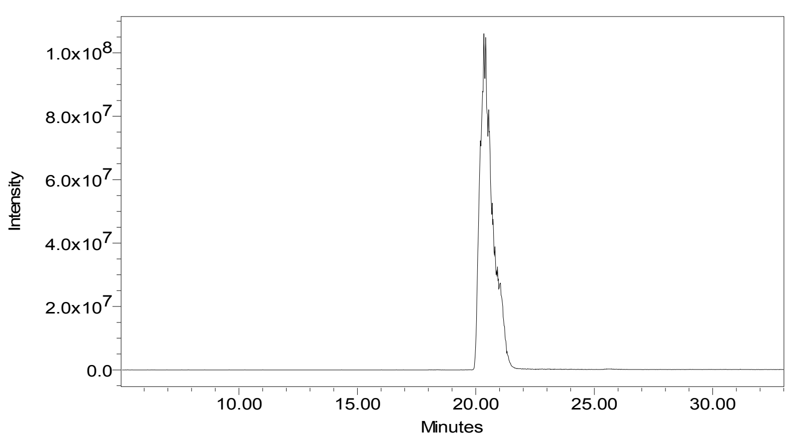
20.00
25.00
30.00
Minutes

(15)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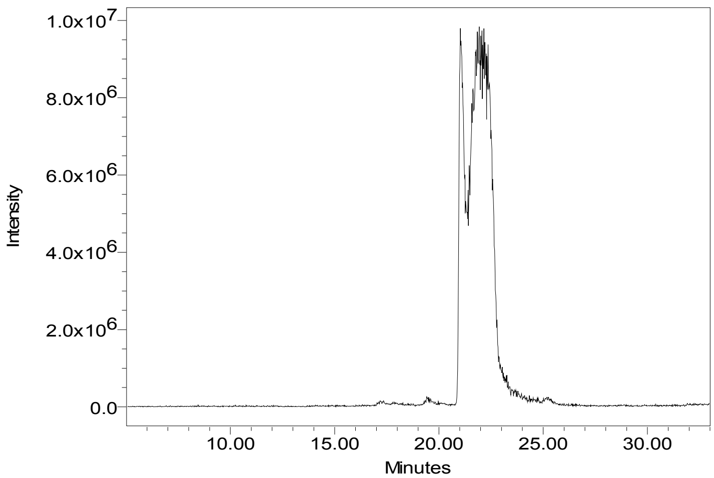
Minutes

(16)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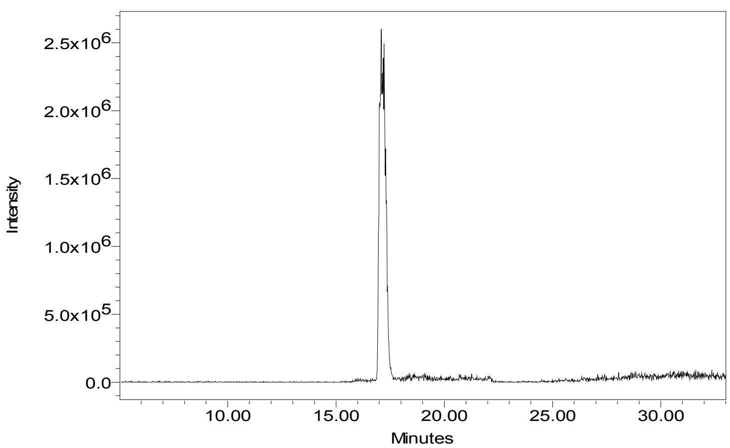
Minutes

(17)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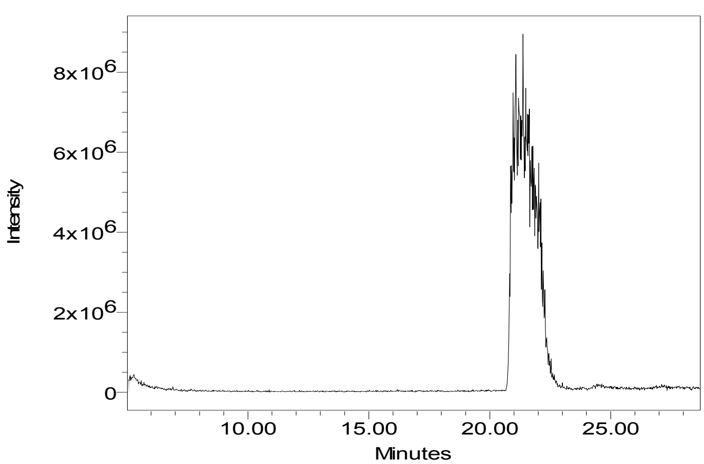
30.00
Minutes

(18)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Minutes