Mimics of Small Ribozymes Utilizing a Supramolecular Scaffold

Supporting Information

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Contents

***N*-[2-(*N*^{4},*N*^{7},*N*^{10}-Tri-*tert*-butoxycarbonyl-1,4,7,10-tetraazacyclododecan-1-yl)ethyl]-4-nitrobenzenesulfonamide (5).** 4-Nitrobenzenesulfonyl chloride (6,45 g, 29.1 mmol) was dissolved in a mixture of dry CH_2Cl_2 (6 mL) and pyridine (3 mL) and ethanolamine (0.586 mL, 9.71 mmol) was added dropwise at 0 °C (reacted vigorously). The reaction mixture was allowed to warm up to room temperature and stirred for 2,5 h, after which CH_2Cl_2 (50 mL) was added and the resulting solution washed with 2 M aq. HCl (50 mL). The organic phase was filtered and the precipitated light brown solid suspended in CH_2Cl_2 (100 mL) The mixture was washed with 2 M aq. KOH (2 × 100 mL), the organic phase dried with Na_2SO_4 and evaporated to a light brown powder. The crude *N*-(4-nitrobenzenesulfonyl)aziridine thus obtained (4, 1.00 g, 4.38 mmol) and 1,4,7-tris(*tert*-butoxycarbonyl)cyclen (**3**, 2.05 g, 4.33 mmol) were dissolved in MeCN (24 mL). The reaction mixture was stirred at room temperature for 168 h, after which it was evaporated to dryness. The residue was dissolved in CH_2Cl_2 and washed with saturated aq. $NaHCO_3$. The organic phase was dried with Na_2SO_4 and evaporated to dryness. The residue was purified on a silica gel column eluting with a mixture of EtOAc and petroleum ether (70:30, *v*/*v*). Yield 2.14 g (70%) of pale yellow foam. TLC: R_f (MeOH:CH_2Cl_2, 1:9, *v*/*v*) = 0.78; R_f (EtOAc:hexane, 7:3, *v*/*v*) = 0.62. ^{1}H NMR (δ_H)(500 MHz, $CDCl_3$): 8.36 (d, 2H, *J* = 8.8 Hz), 8.10 (d, 2H, *J* = 8.5 Hz), 3.47 (m, 12H), 3.09 (dd, 2H, *J* = 4.8 Hz), 2.59 (br, 6H), 1.48 (s, 27H). HRMS (ESI$^+$): *m*/*z* calcd 701.3544 obsd 701.3588 $[M+H]^+$.

1,4,7-tris(*tert*-butoxycarbonyl)-10-(2-aminoethyl)cyclen (6). Compound **5** (2.00 g, 2.85 mmol) was dissolved in DMF (10.0 mL). Thioglycolic acid (0.456 mL) and DBU (4.0 mL) were added and the resulting mixture was stirred at room temperature for 2,5 h. Ethyl acetate (300mL) was added and the mixture was washed with saturated aq. $NaHCO_3$ (5 × 40 mL). The organic phase was dried with Na_2SO_4 and evaporated to yield 1.44 g (98%) of **6** as a yellowish glass. ^{1}H NMR (δ_H)(500 MHz, $CDCl_3$): 3.42 (m, 12H), 2.87 (t, 2H, *J* = 7.1 Hz), 2.67 (m, 6H), 1.47 (s, 9H), 1.45 (s, 18H). HRMS (ESI$^+$): *m*/*z* calcd 516.3756 obsd 516.3722 $[M+H]^+$.

6-Chloro-N^2,N^4-bis-[2-($N^{4'}$,$N^{7'}$,$N^{10'}$-Tri-*tert*-butoxycarbonyl-1,4,7,10-tetraazacyclododecan-1-yl)ethyl]-1,3,5-triazine-2,4-diamine (7). Compound **6** (1.44 g, 2.79 mmol), cyanuric chloride (0.210 g, 1.139 mmol) and DIPEA (2.5 mL, 14.35 mmol) were dissolved in MeCN (25 mL). The mixture was stirred at room temperature for 4 h, after which it was evaporated to dryness, the residue was dissolved in CH_2Cl_2 (50 mL) and washed with saturated aq. $NaHCO_3$ (2 × 50 mL). The organic phase was dried with Na_2SO_4 and evaporated to dryness and the residue was purified on a silica gel column eluting with a mixture of Et_3N, MeOH and CH_2Cl_2 (1:5:94, *v*/*v*). Yield 1.23 g (95%). ^{1}H NMR (δ_H)(500 MHz, $CDCl_3$): 3.70 – 3.20 (m, 28H), 2.90 – 2.50 (m, 12H), 1.47 (s, 36H), 1.45 (s, 18H). HRMS (ESI$^+$): *m*/*z* calcd 1142.7063 obsd 1142.6998 $[M+H]^+$.

N^2,N^4-bis-[2-(1,4,7,10-tetraazacyclododecan-1-yl)ethyl]-1,3,5-triazine-2,4-diamine (2h). Compound **6** (0.220 g, 0.427 mmol), 2,4-dichloro-1,3,5-triazine (0.0276 g, 0.175 mmol) and DIPEA (0.4 mL, 2,3 mmol) were dissolved in MeCN (4.0 mL). The mixture was stirred at 50 °C for 216 h after which it was evaporated to dryness. The residue was dissolved in CH_2Cl_2 (20 mL) and washed with saturated aq. $NaHCO_3$ (2 × 20 mL). The organic phase was dried with Na_2SO_4 and evaporated to dryness and the residue was purified twice on a silica gel column eluting with a mixture of Et_3N, MeOH and CH_2Cl_2 (1:10:89 and 1:5:94, *v*/*v*, respectively). The product (0.0673 g, 0.0607 mmol) was dissolved in a mixture of TFA (2.0 mL), CH_2Cl_2 (1.6 mL) and MeOH (0.4 mL) and the resulting solution was stirred at room temperature for 4 h, after which it was evaporated to dryness. The residue was passed through Dowex 1x2 (HO^-) resin to yield 21.6 mg (24%) of the desired product as a free amine. ^{1}H NMR (δ_H)(500 MHz, D_2O): 8.35 (br, 1H), 3.13 – 3.00 (m, 12H), 2.99 – 2.85 (m, 28H). ^{13}C NMR (δ_C)(125 MHz, D_2O): 172.1, 164.7, 53.6, 51.7, 51.5, 45.9, 45.1, 44.4. HRMS (ESI$^+$): *m*/*z* calcd 508.4307 obsd 508.4310 $[M+H]^+$.

N^2,N^4-bis-[2-(1,4,7,10-tetraazacyclododecan-1-yl)ethyl]-N^6-[2-(1H-imidazol-5-yl)ethyl]melamine (2i). Compound **7** (0.33 g, 0.289 mmol), histamine (0.32 g, 2.88 mmol) and DIPEA (2 mL) were dissolved in MeCN (8 mL). The resulting mixture was stirred at 55 °C for 96 h, after which it was evaporated to dryness. The residue was purified on a silica gel column (300 mL SiO_2) eluting with a mixture of MeOH and CH_2Cl_2 (15:85, *v*/*v*). The product (0.255 g, 0.2094 mmol) was dissolved in a mixture of TFA (5.0 mL), MeOH (1.0 mL) and CH_2Cl_2 (4.0 mL) and the resulting solution

was stirred at room temperature for 4 h, after which it was evaporated to dryness. The residue was passed through Dowex 1x2 (HO^-) resin to yield 86.3 mg (67%) of the desired product as a free amine. ^{1}H NMR (δ_H)(500 MHz, D_2O): 7.52 (s, 1H), 6.77 (s, 1H), 3.44 (br, 2H), 3.20 (br, 4H), 2.70 (m, 2H), 2.55 – 2.25 (m, 36H). ^{13}C NMR (δ_C)(125 MHz, D_2O): 177.2, 165.2, 135.6, 134.8, 116.9, 53.4, 51.1, 45.0, 44.4, 43.5, 40.0, 38.5, 26.5. HRMS (ESI^+): *m*/*z* calcd 309.2510 obsd 309.2520 $[M+2H]^{2+}$.

***N*^{2},*N*^{4}-bis-[2-(1,4,7,10-tetraazacyclododecan-1-yl)ethyl]-*N*^{6}-methylmelamine (2n).** Compound **7** (0.100 g, 0.087 mmol) was dissolved in 30% solution of methylamine in ethanol (1.0 mL). After being stirred at room temperature for 16 h the reaction mixture was evaporated to dryness and the residue was purified on a silica gel column eluting with a mixture of Et_3N, MeOH and CH_2Cl_2 (1:5:94, *v*/*v*). The product (0.090 g, 0.079 mmol) was dissolved in a mixture of TFA (2.5 mL), CH_2Cl_2 (2.0 mL) and MeOH (0.5 mL) and the resulting solution was stirred at room temperature for 4,5 h, after which it was evaporated to dryness. The residue was passed through Dowex 1x2 (HO^-) resin to yield 40.4 mg (86%) of the desired product as a free amine. ^{1}H NMR (δ_H)(500 MHz, D_2O): 3.28 (br, 4H), 2.75 (br, 3H), 2.48 (m, 36H). ^{13}C NMR (δ_C)(125 MHz, D_2O): 165.4, 53.5, 51.2, 44.9, 44.3, 43.4, 38.6, 26.9. HRMS (ESI^+): *m*/*z* calcd 537.4572 obsd 537.4613 $[M+H]^+$.

6-Methoxy-*N*^{2},*N*^{4}-bis-[2-(1,4,7,10-tetraazacyclododecan-1-yl)ethyl]-1,3,5-triazine-2,4-diamine (2o). Compound **7** (0.167 g, 0.146 mmol) was dissolved in 0.1 M methanolic sodium methoxide (10 mL). After being stirred at 50 °C for 16 h the reaction mixture was neutralized with 1.0 M acetic acid in methanol and evaporated to dryness. The residue was dissolved in CH_2Cl_2 (50 mL) and washed with saturated aq. $NaHCO_3$ (80 mL). The organic phase was dried with Na_2SO_4 and evaporated to dryness and the residue was purified twice on a silica gel column eluting with a mixture of Et_3N, MeOH and CH_2Cl_2 (1:10:89 and 1:5:94, *v*/*v*, respectively). The product (0.068 g, 0.0597 mmol) was dissolved in a mixture of TFA (2.0 mL), CH_2Cl_2 (1.6 mL) and MeOH (0.4 mL) and the resulting solution was stirred at room temperature for 4 h, after which it was evaporated to dryness. The residue was passed through Dowex 1x2 (HO^-) resin to yield 26.0 mg (33%) of the desired product as a free amine. ^{1}H NMR (δ_H)(500

MHz, D_2O): 3.80 (br, 3H), 3.50 – 3.15 (m, 6H), 2.85 – 2.35 (m, 34H). ^{13}C NMR (δ_C)(125 MHz, D_2O): 170.7, 165.9, 54.2, 50.4, 44.6, 43.3, 42.8, 38.6, 38.4. HRMS (ESI^+): *m*/*z* calcd 538.4412 obsd 538.4404 $[M+H]^+$.

2′-*O*-Methyluridylyl-3′,5′-uridine (8). 5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-methyluridine-3′-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite (1.00 g, 1.314 mmol) and 2′,3′-*O*-isopropylideneuridine (0.448 g, 1.576 mmol) were dissolved in anhydrous MeCN (10 mL). 0.45 mol L^{-1} 1*H*-tetrazole in MeCN (3.5 mL, 1.575 mmol) was added and the resulting mixture was stirred at room temperature for 2 h. A solution of I_2 (0.43 g, 1.69 mmol) in a mixture of THF (8 mL), water (4 mL) and 2,6-lutidine (2 mL) was added and the stirring was continued for 1 h, after which the reaction mixture was concentrated under reduced pressure, the residue was dissolved in CH_2Cl_2 (100 mL) and washed with 5% aq. $NaHSO_3$ (100 mL). The organic phase was dried with Na_2SO_4 and evaporated to dryness. The residue was purified on a silica gel column (550 mL SiO_2) eluting with a mixture of Et_3N, MeOH and CH_2Cl_2 (1:9:90, *v*/*v*). The product (1.0302 g, 1.073 mmol) was then dissolved in saturated methanolic ammonia (10 mL) and the mixture was stirred at room temperature for 1 h, after which it was evaporated to dryness. The residue was dissolved in water (10 mL), 37% aq. HCl (0.5 mL) was added and the resulting mixture stirred at room temperature for 6 h, washed with CH_2Cl_2 (6 × 20 mL) and evaporated to dryness. The residue was passed through Dowex 50Wx8 (Na^+) resin and evaporated to dryness, yielding 0.5939 (94%) of **8** as the sodium salt. ^1H NMR (δ_H)(500 MHz, D_2O): 7.88 (d, 1H, J = 8.2 Hz), 7.86 (d, 1H, J = 8.1 Hz), 5.88 (d, 1H, J = 3.9 Hz), 5.88 (d, 1H, J = 3.3 Hz), 5.80 (d, 1H, J = 8.2 Hz), 5.79 (d, 1H, J = 8.1 Hz), 4.58 (ddd, 1H, J_1 = 6.7 Hz, J_2 = 5.6 Hz, J_3 = 2.7 Hz), 4.26 (d, 1H, J = 3.6 Hz), 4.26 (m, 1H), 4.21 (m, 2H), 4.15 (ddd, 1H, J_1 = 11.8 Hz, J_2 = 4.1 Hz, J_3 = 2.7 Hz), 4.08 (m, 2H), 3.87 (dd, 1H, J_1 = 13.1 Hz, J_2 = 2.5 Hz), 3.76 (dd, 1H, J_1 = 13.1 Hz, J_2 = 3.8 Hz), 3.47 (s, 3H). ^{13}C NMR (δ_C)(125 MHz, D_2O): 166.0, 165.9, 151.6, 151.3, 141.4, 141.3, 102.4, 102.2, 89.0, 87.3, 83.3, 82.6, 81.5, 73.8, 71.4, 69.3, 64.5, 59.9, 57.9. ^{31}P NMR (δ_P)(202 MHz, D_2O): -0.89 (s, 1P). HRMS (ESI^-): *m*/*z* calcd 563.1032 obsd 563.1146 $[M-H]^-$.

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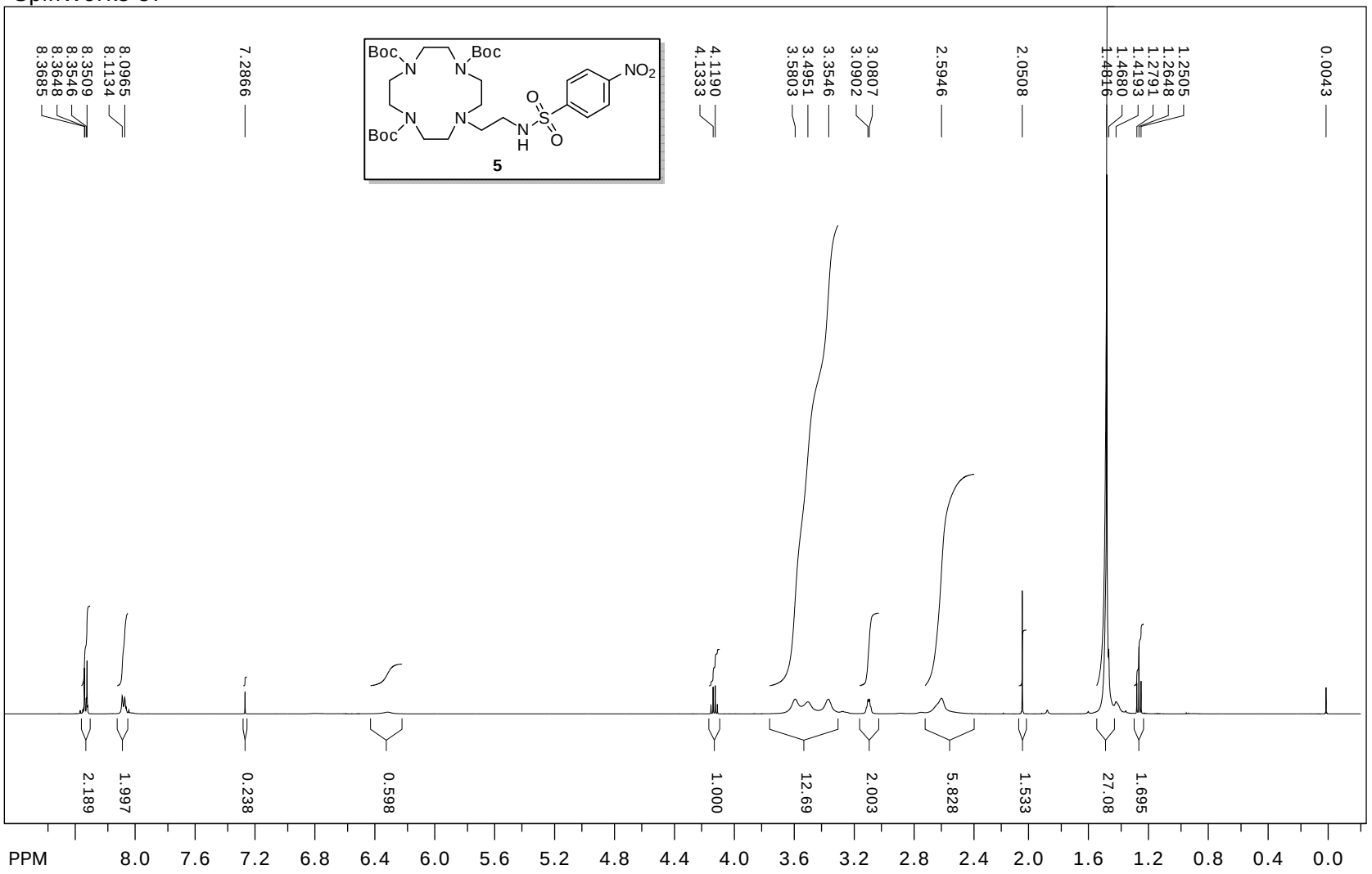

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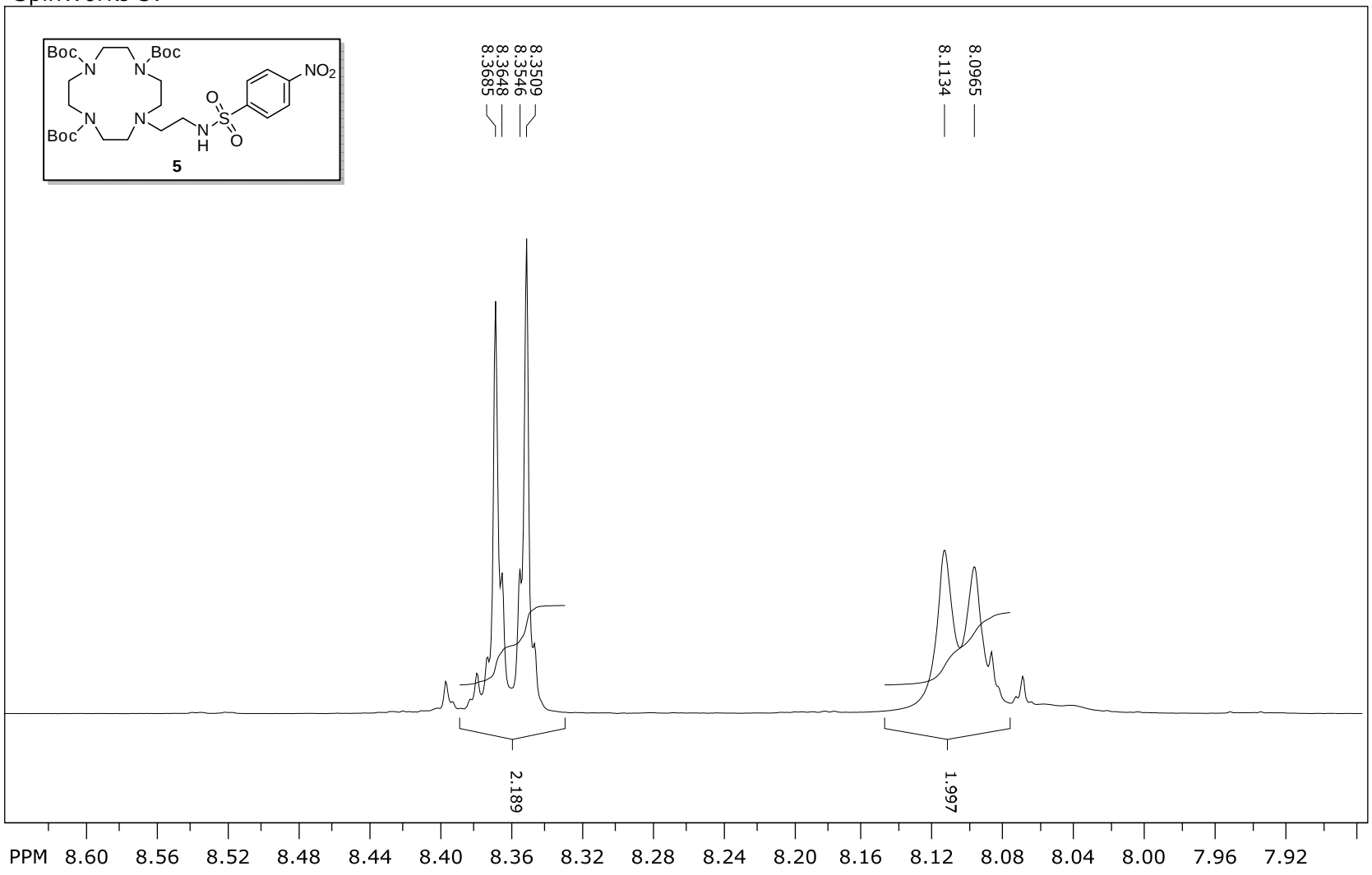

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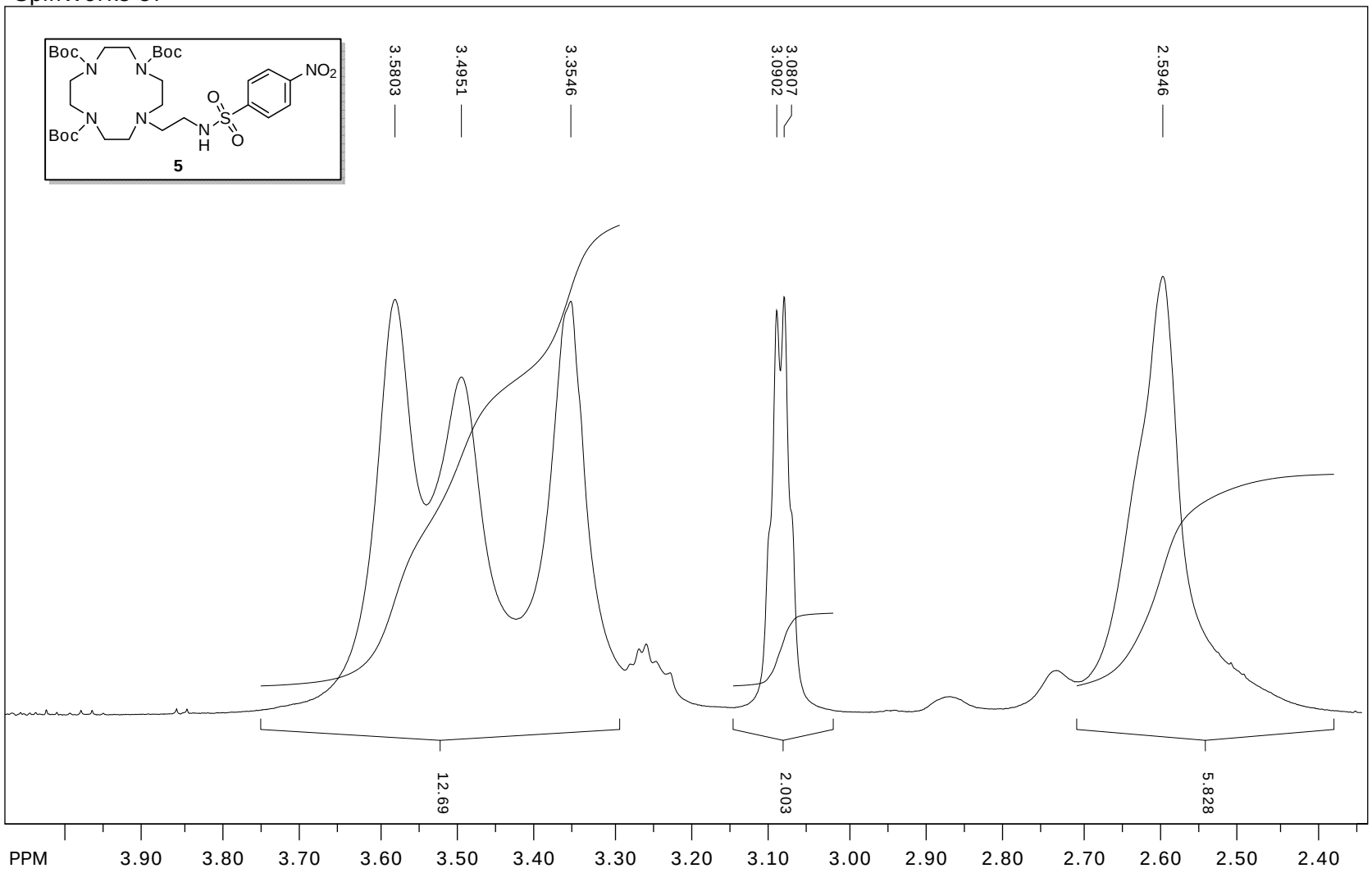

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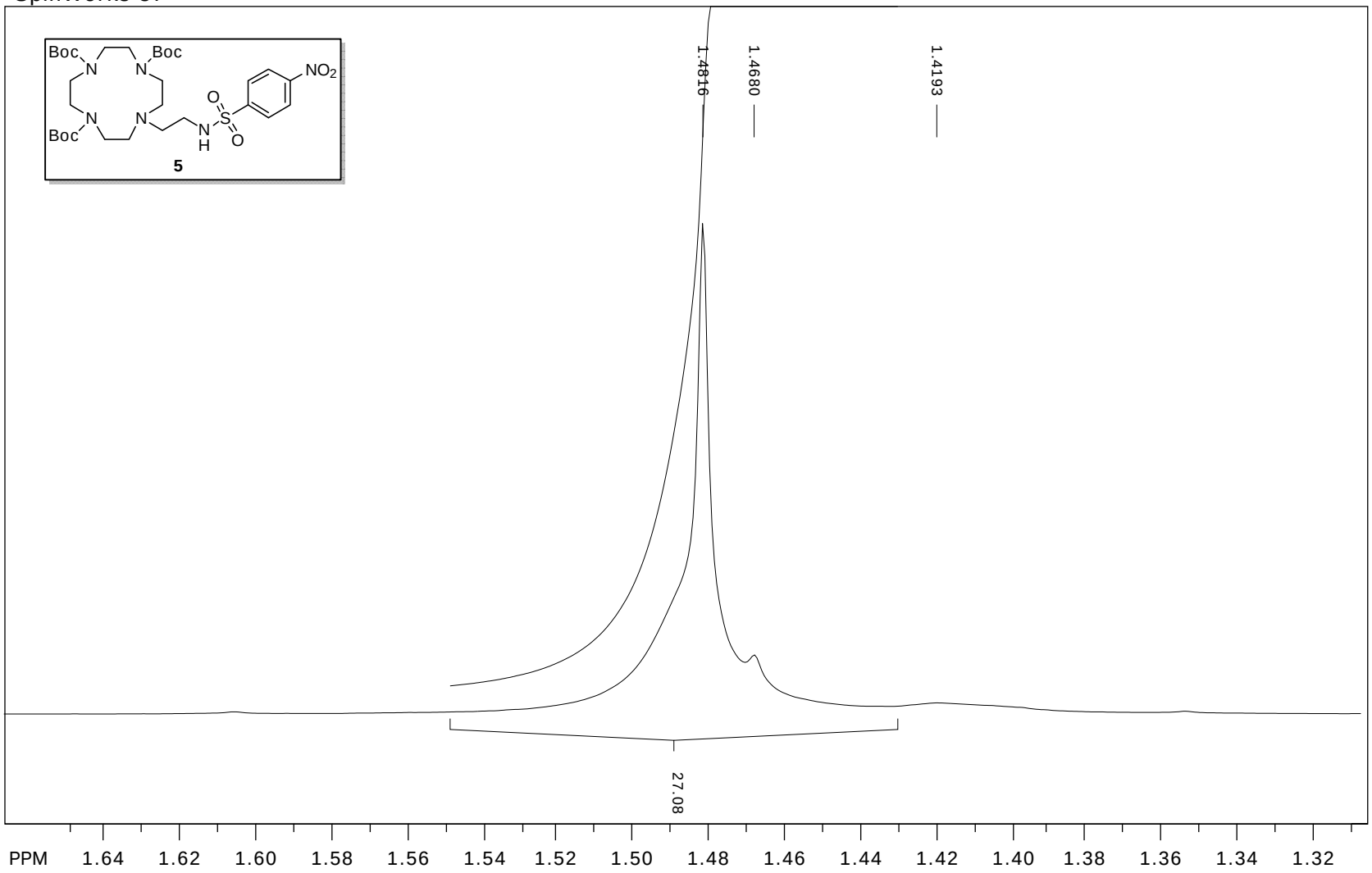

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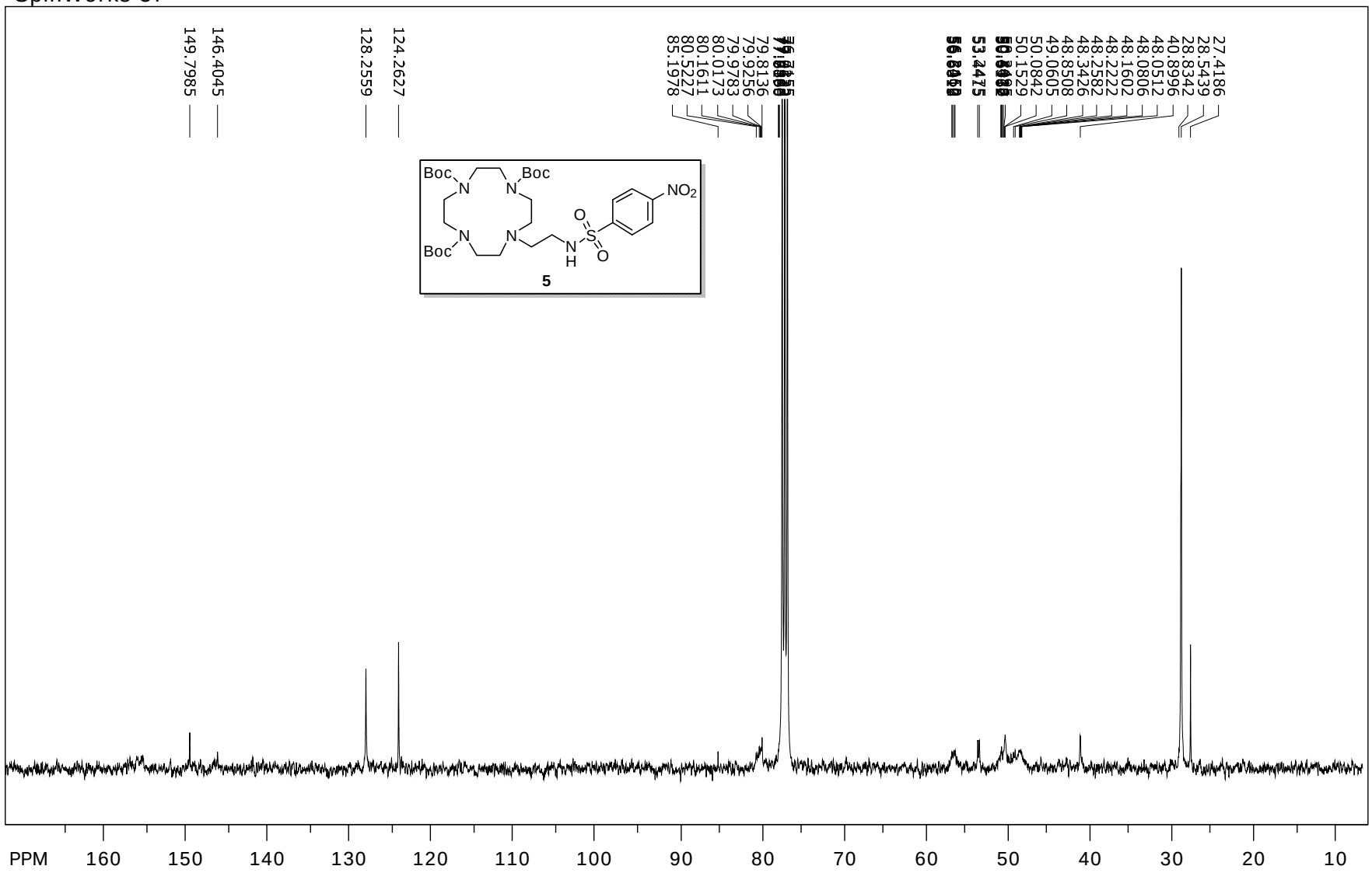

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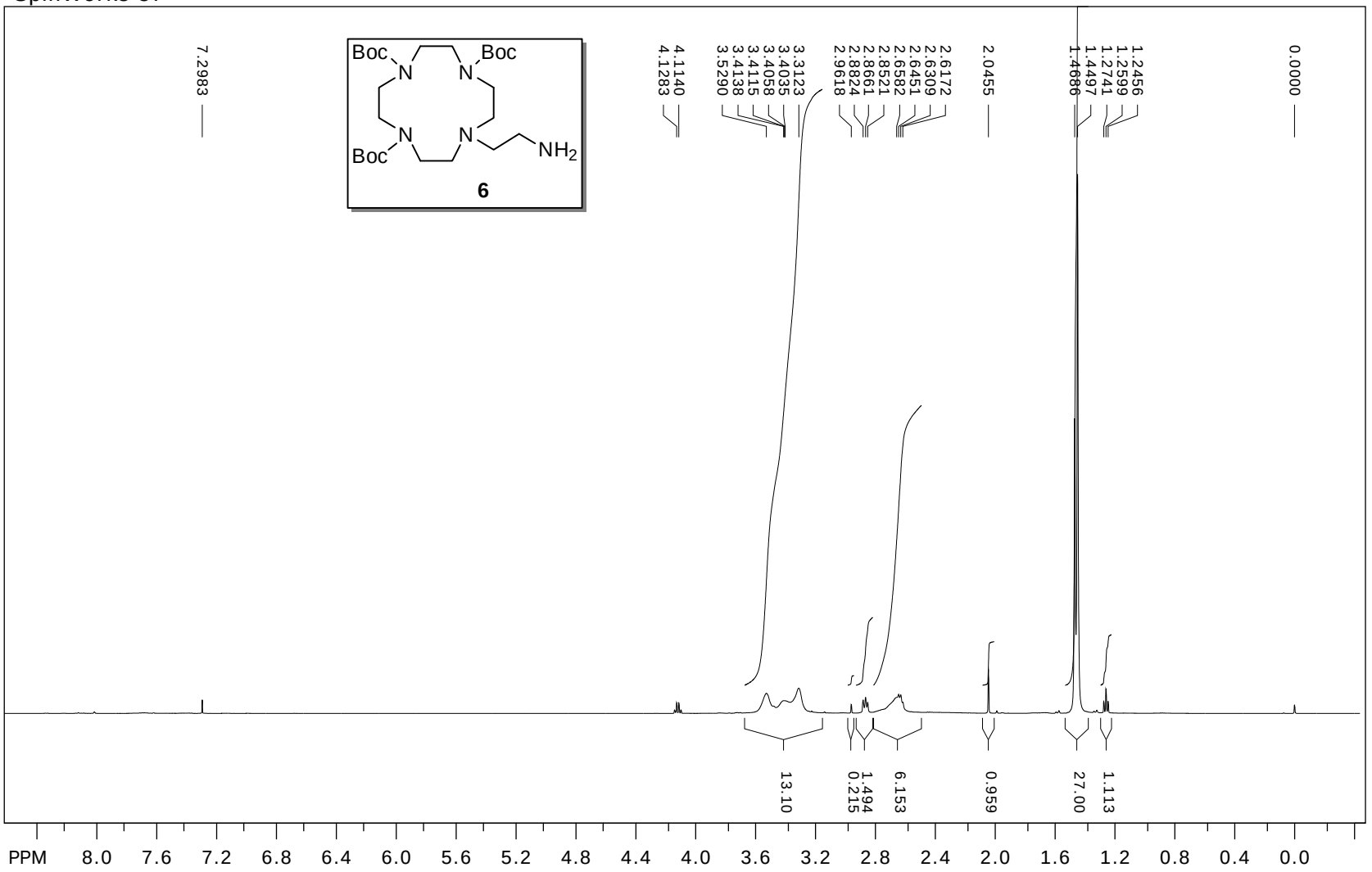

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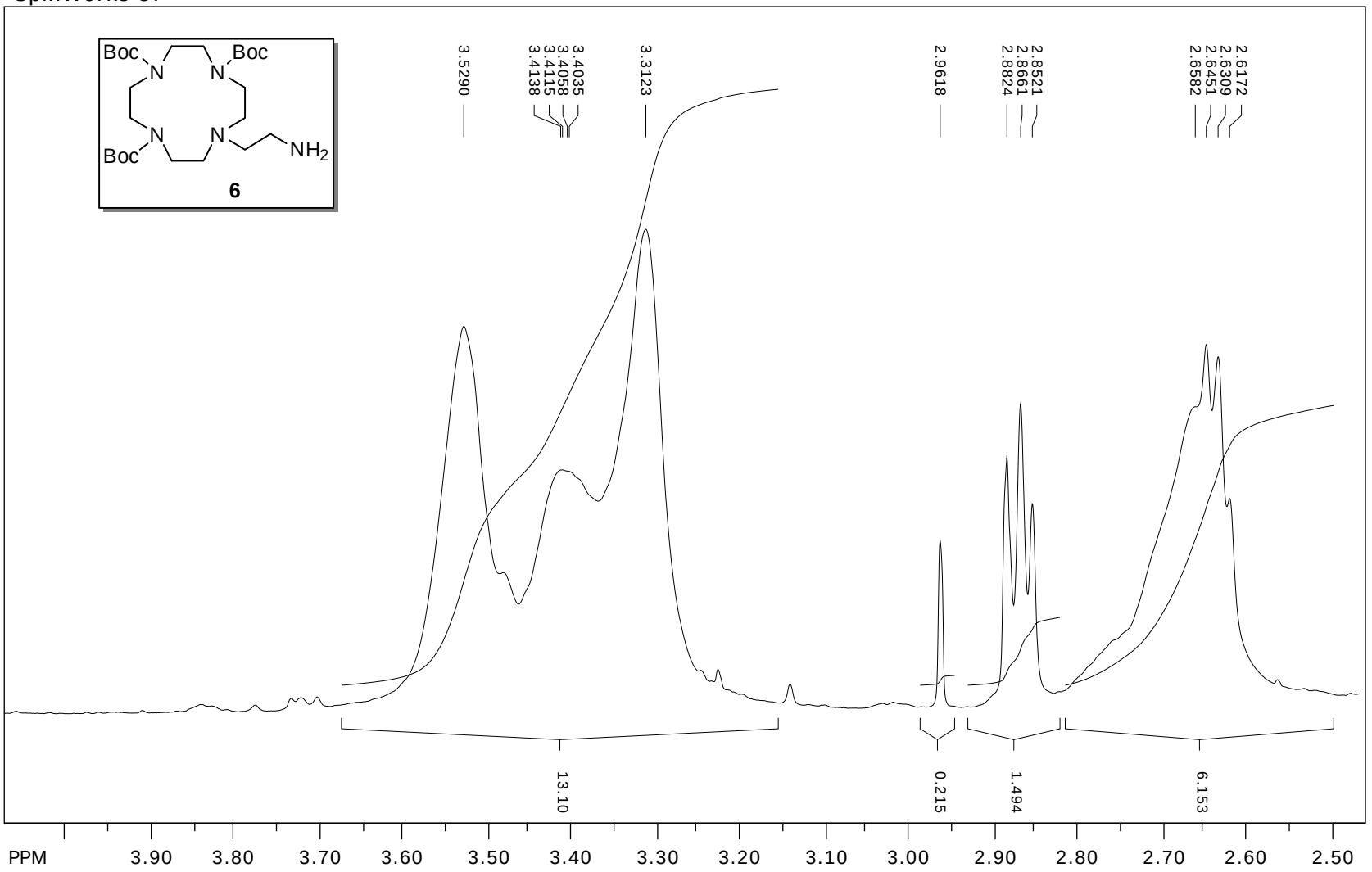

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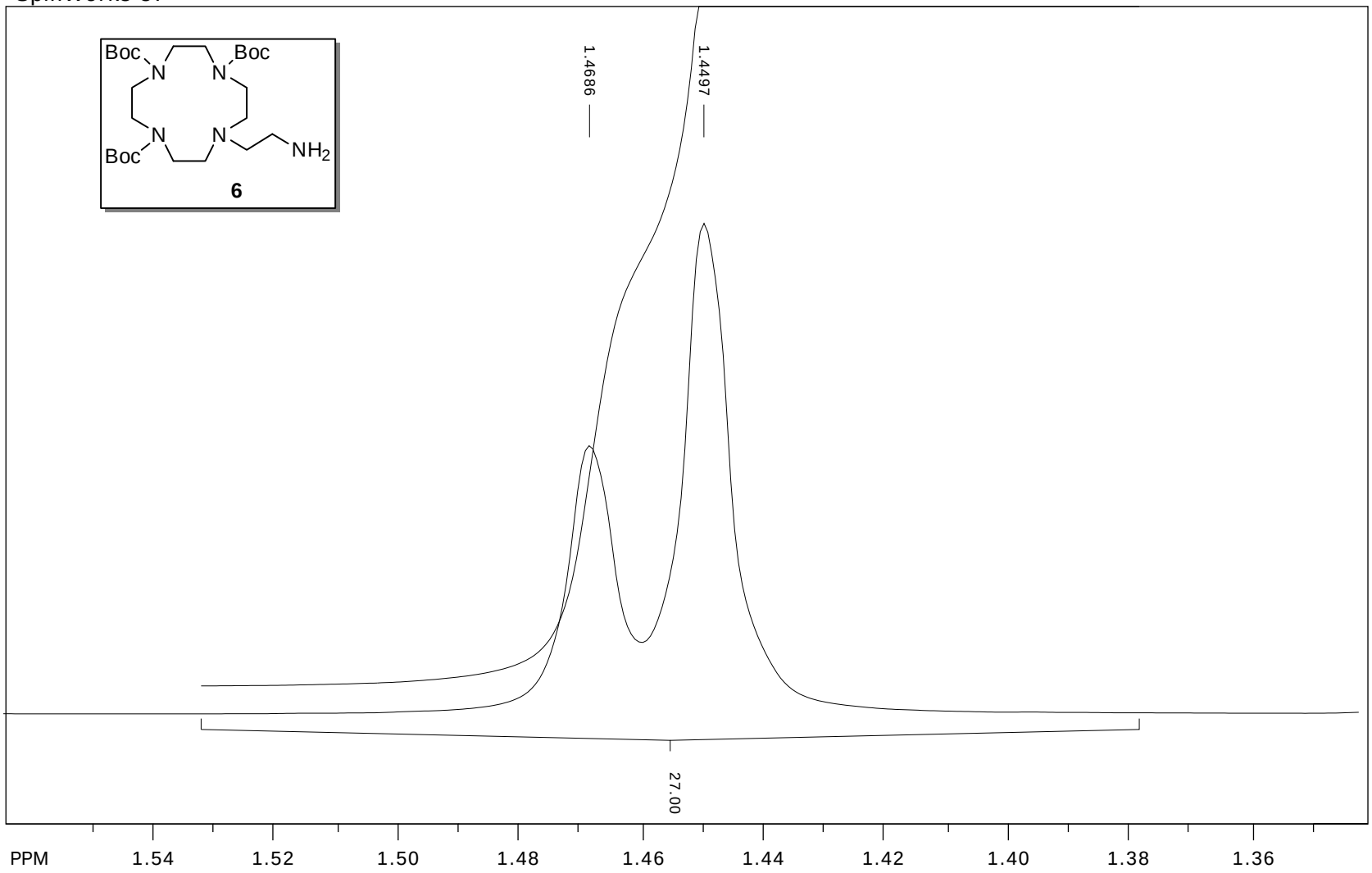

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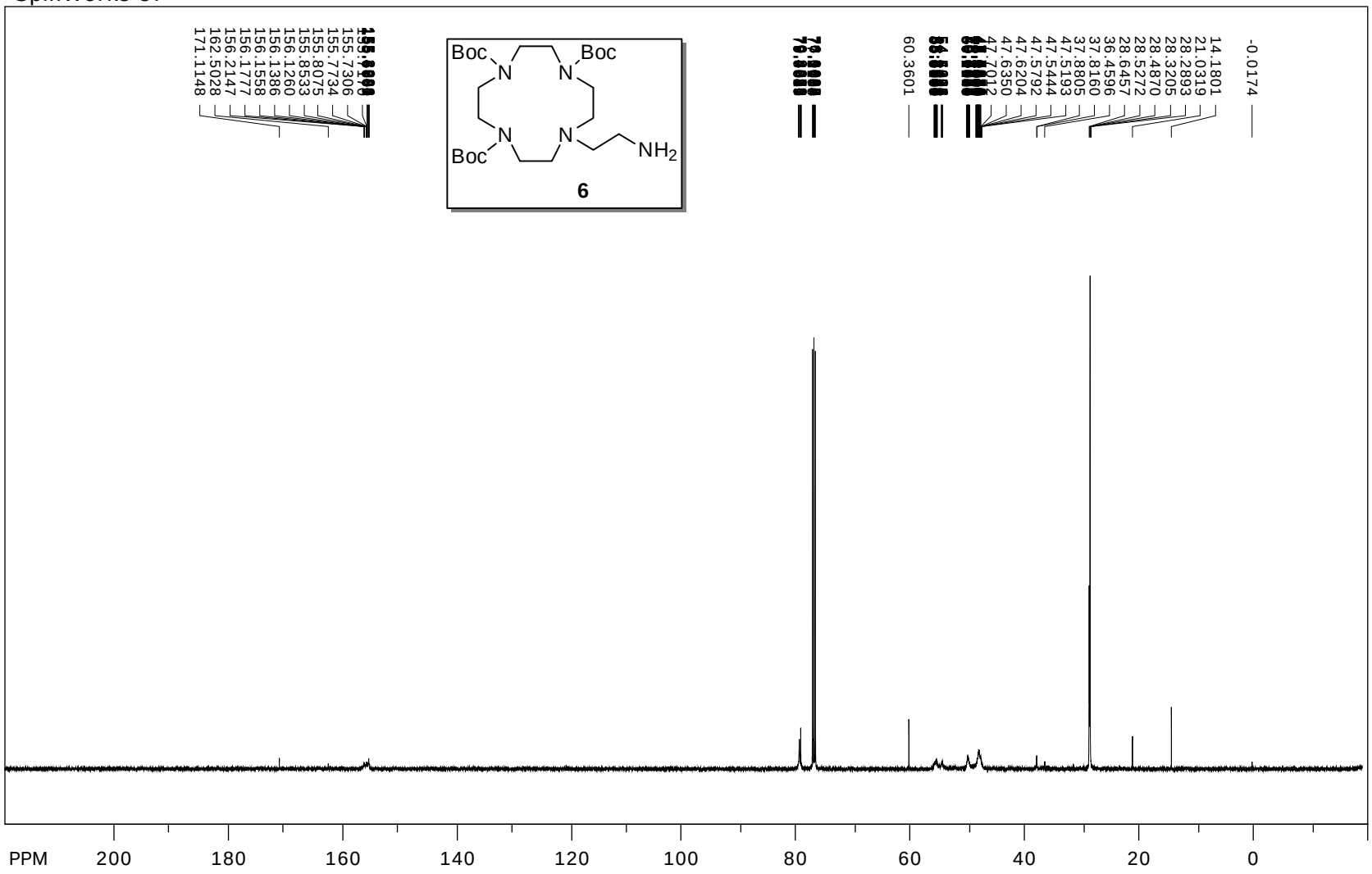

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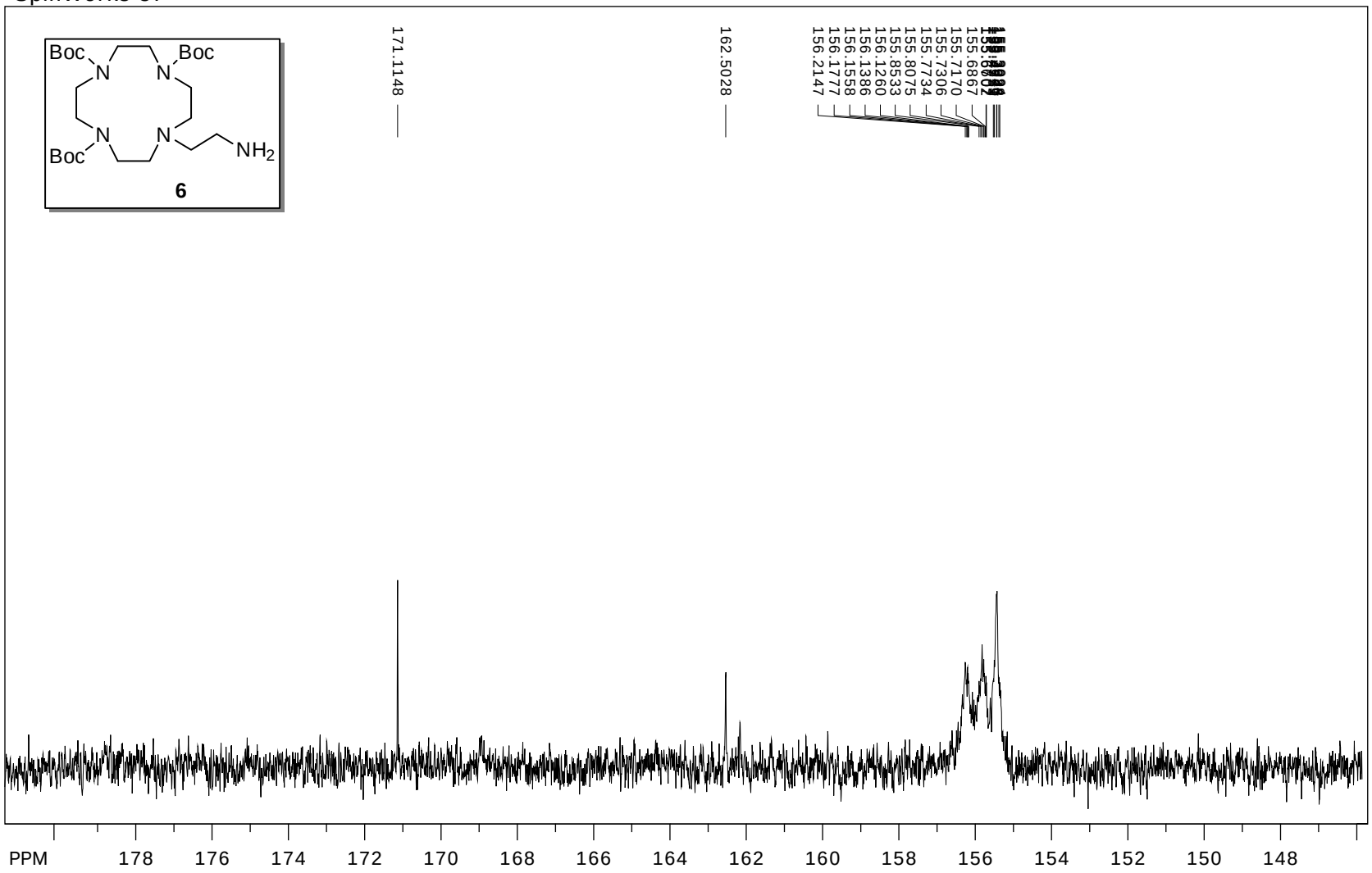

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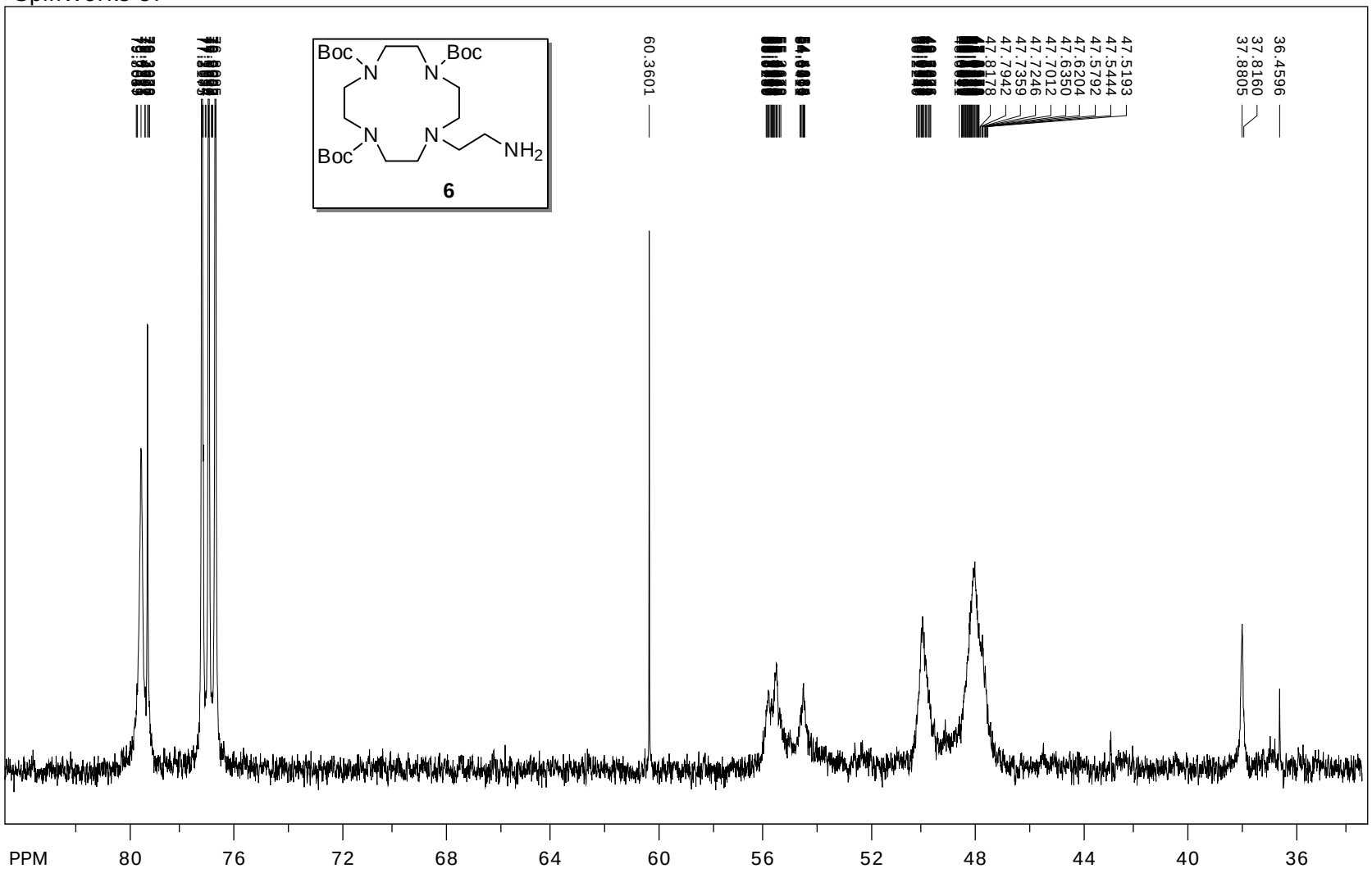

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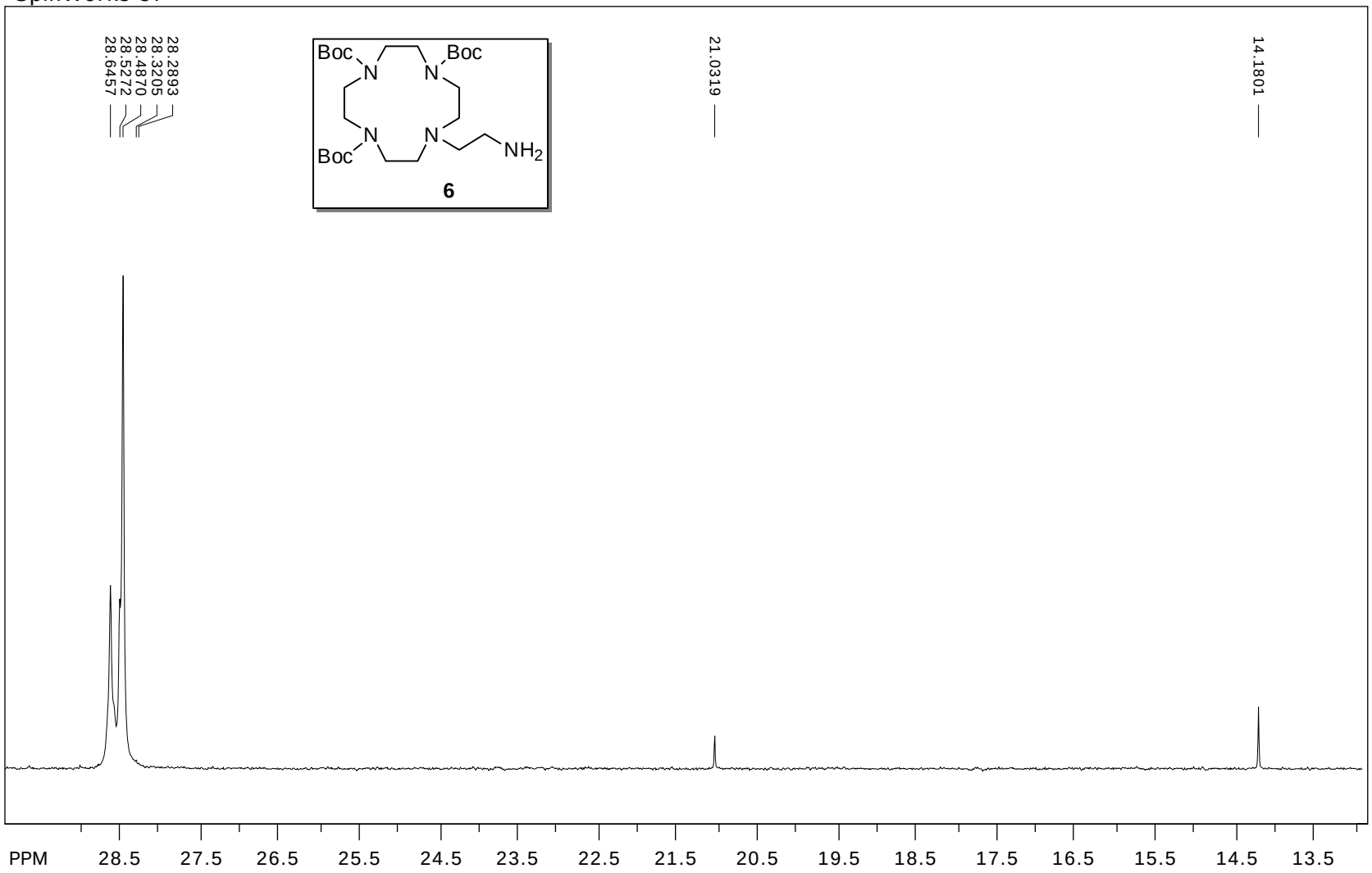

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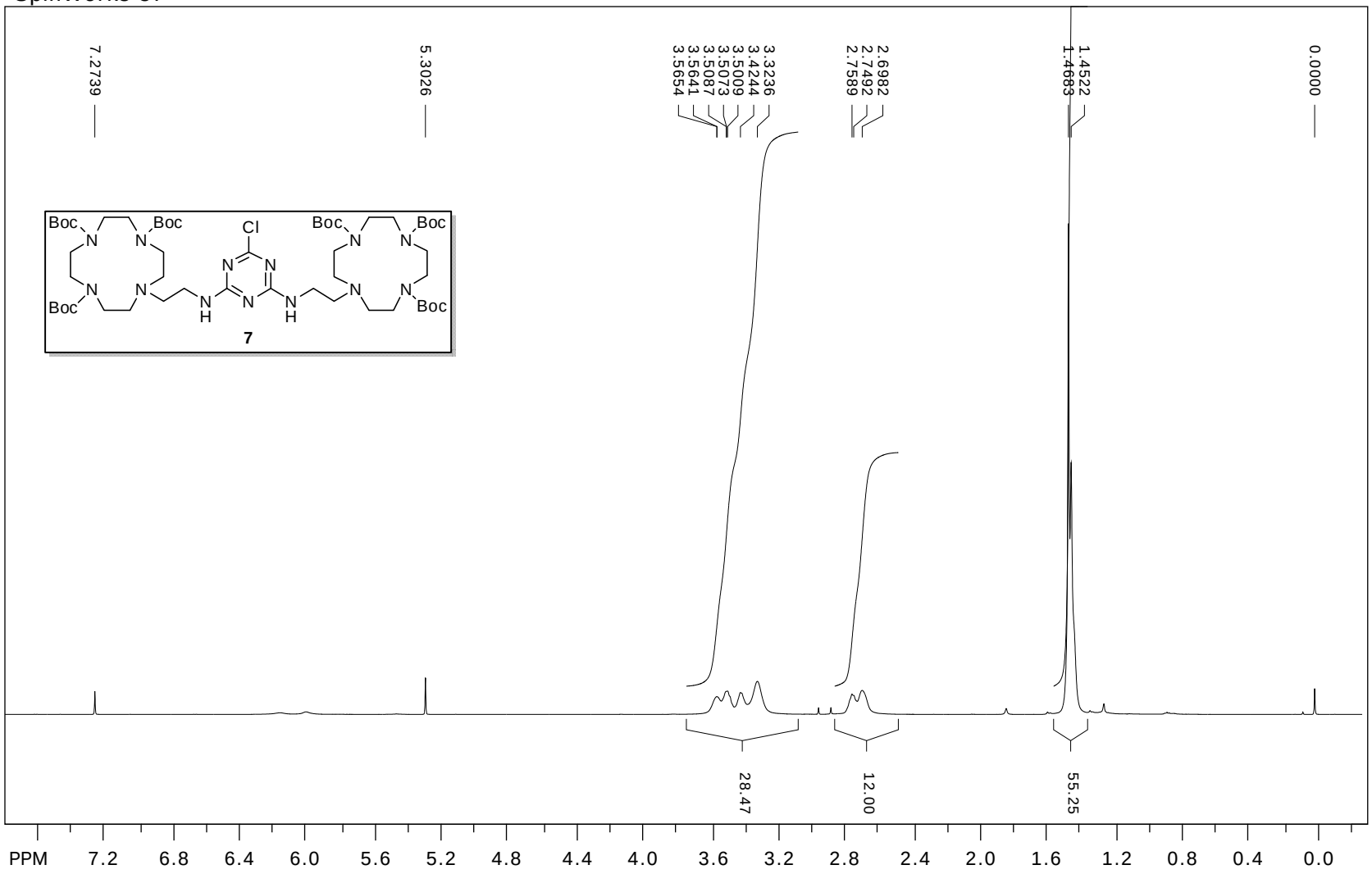

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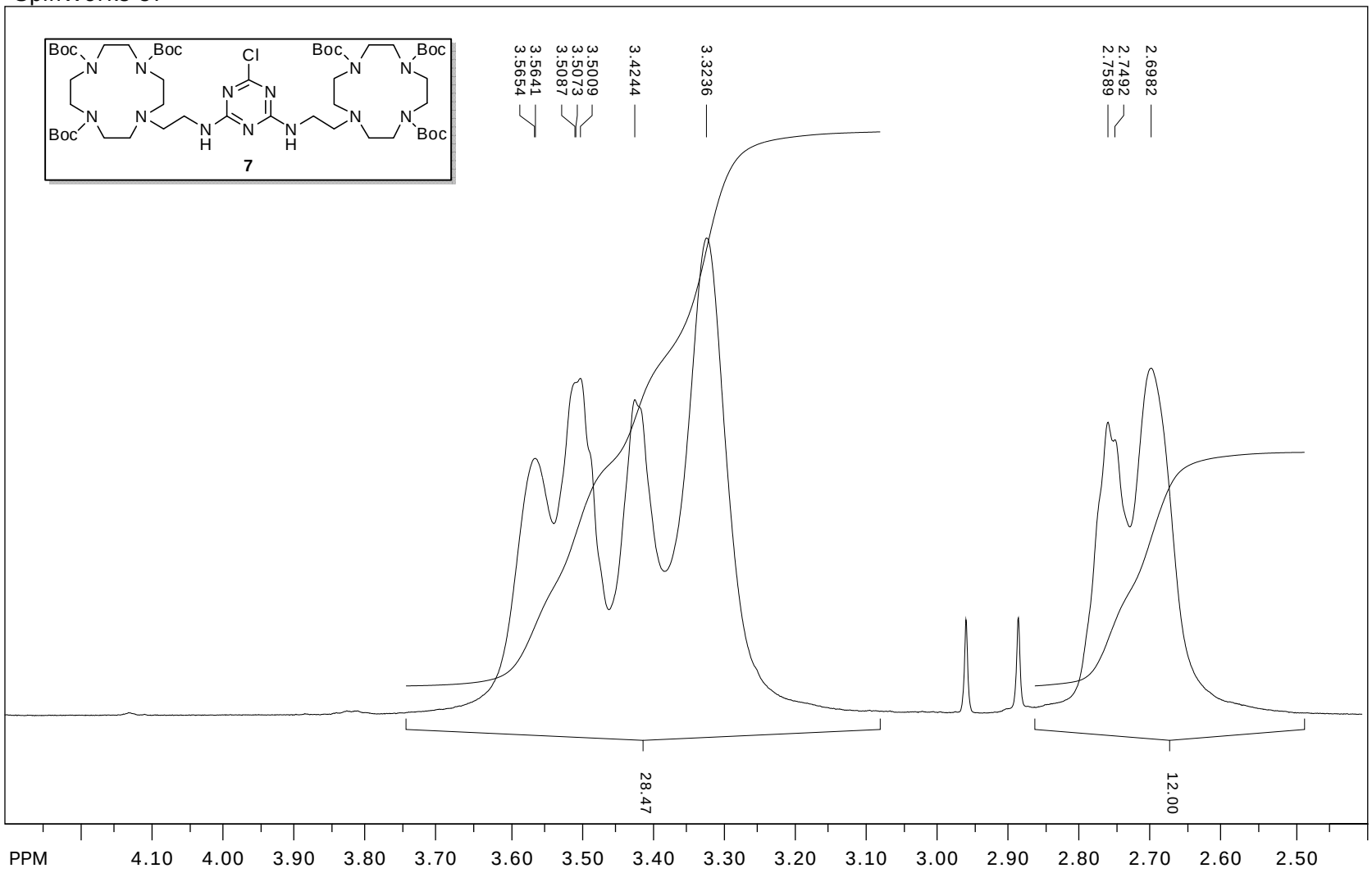

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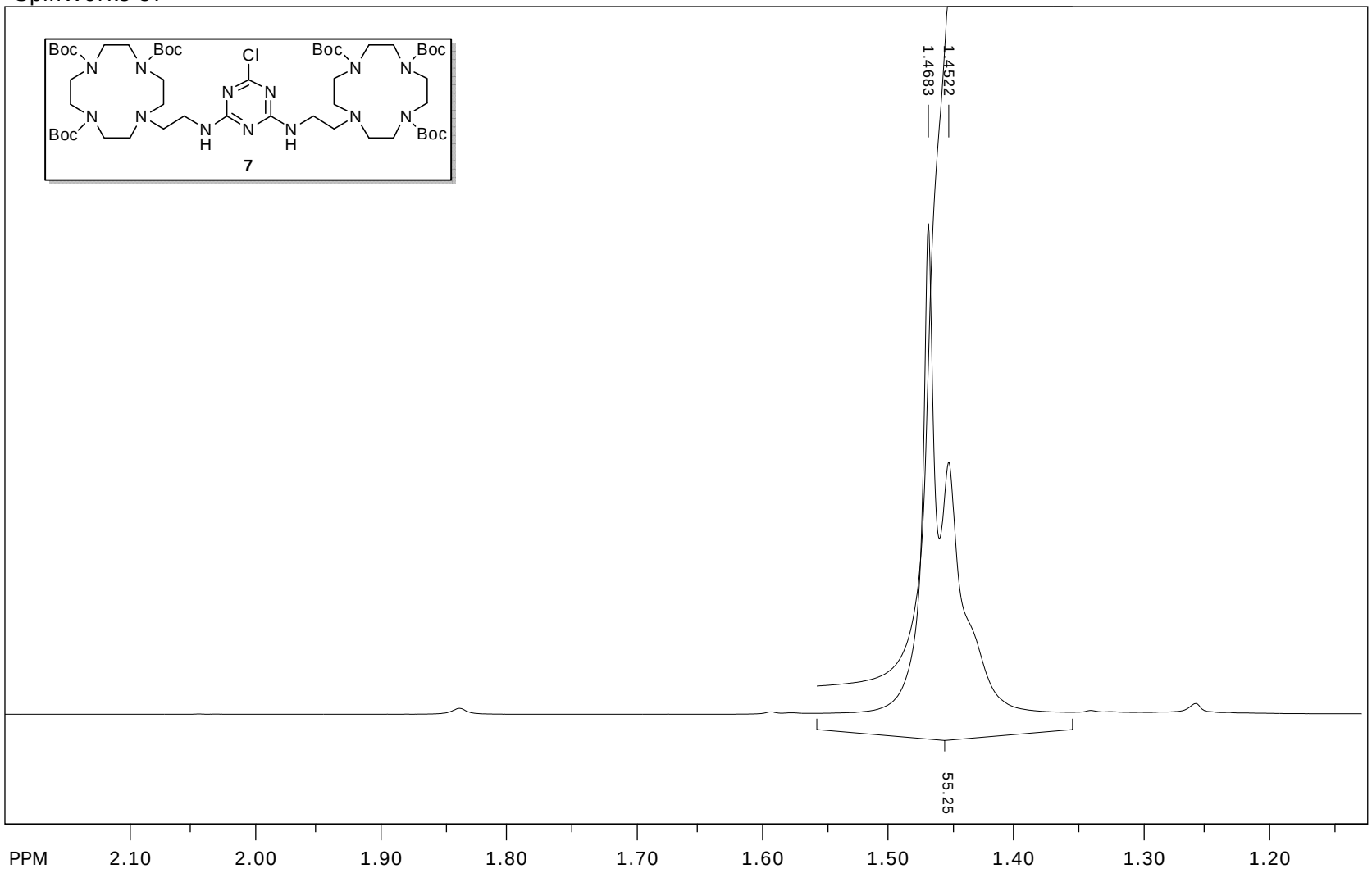
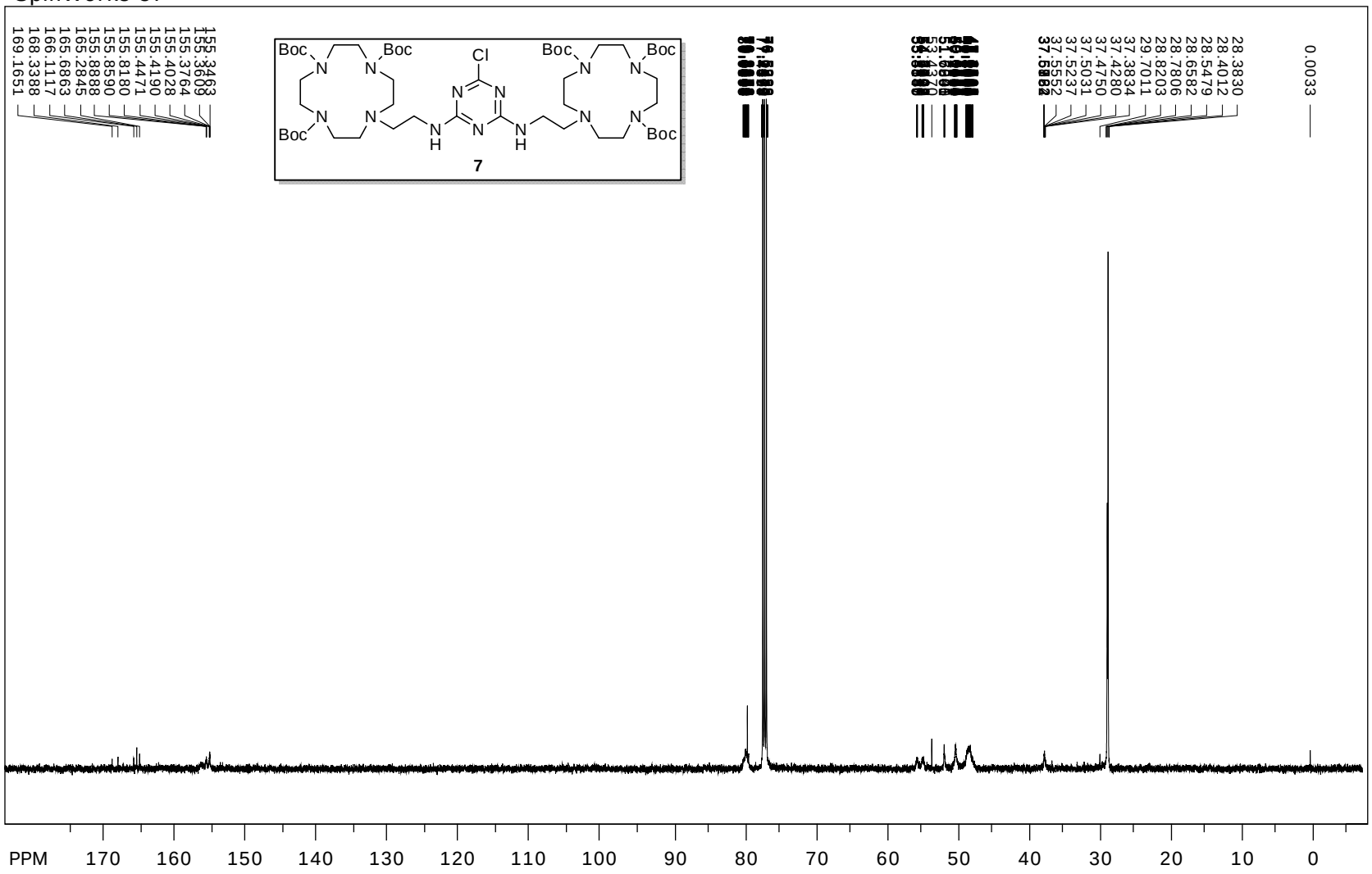


file: ...uments\NMR Data\TL141\TL141_1H\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.129809 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 21.484 ppm/cm: 0.04296

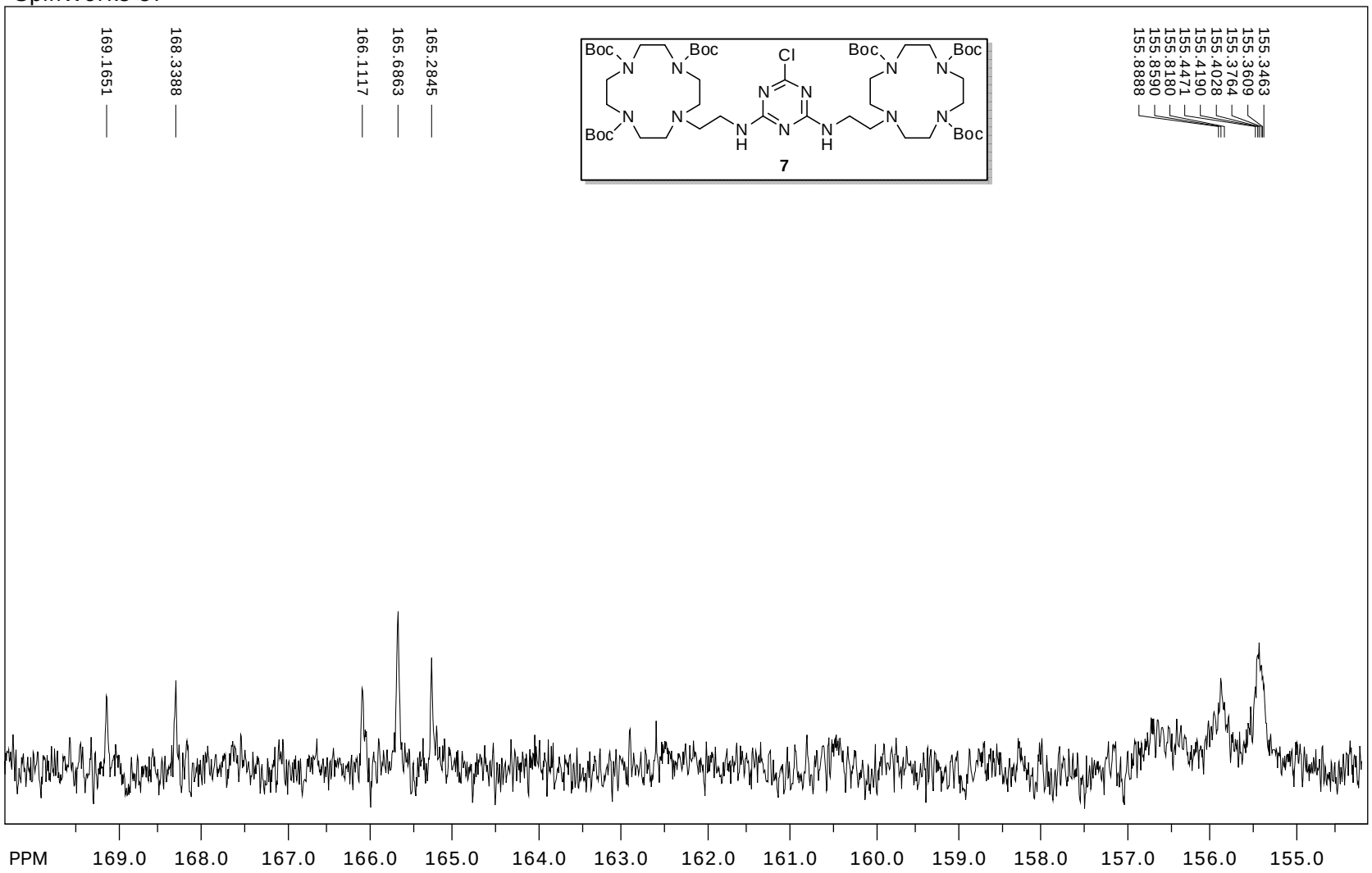
SpinWorks 3:



file: ...ments\NMR Data\TL141\TL141_13C\fid expt: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 30030.03 Hz = 238.7687 ppm = 0.458222 Hz/pt
number of scans: 1471

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 964.039 ppm/cm: 7.66507

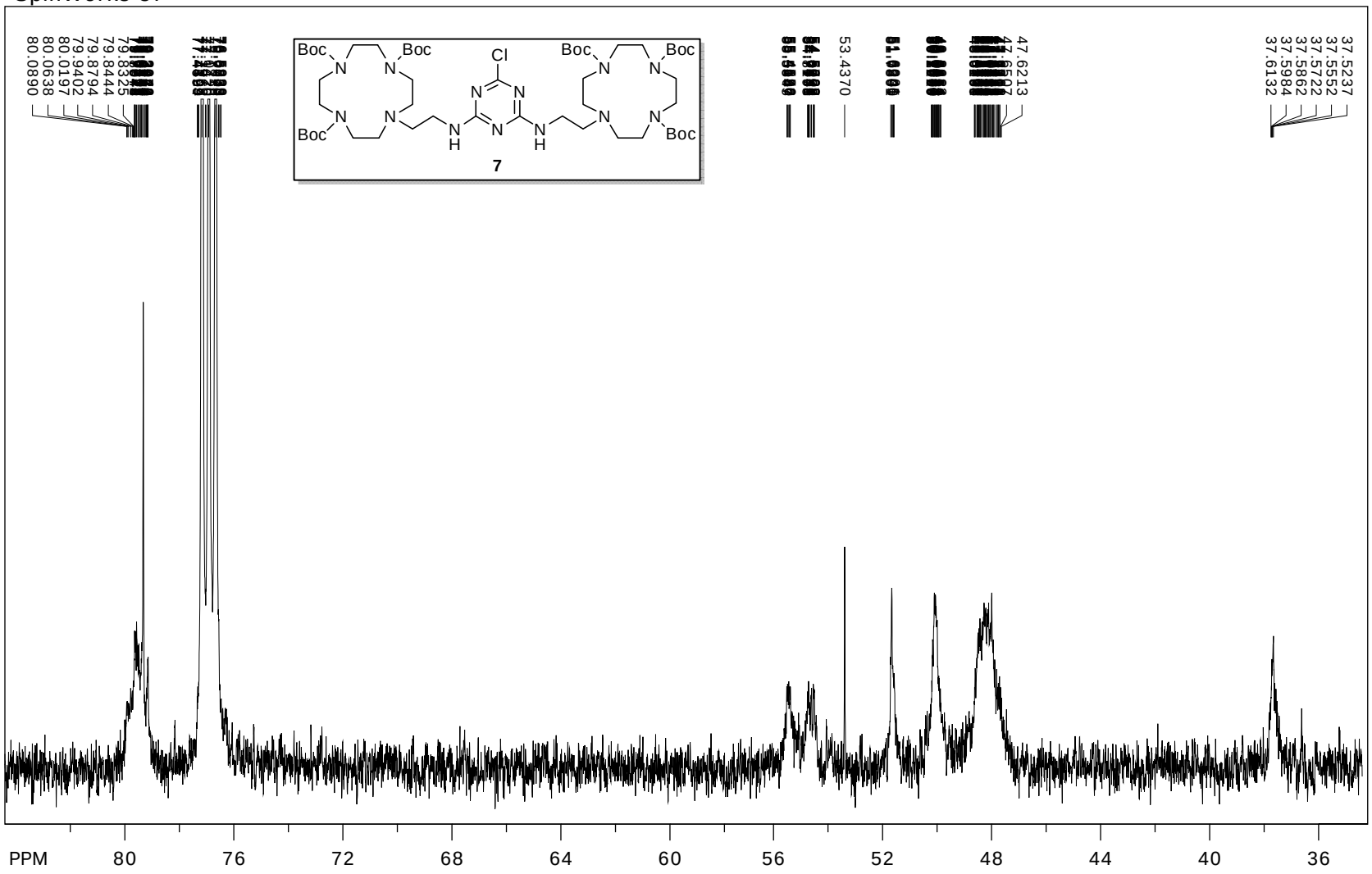
SpinWorks 3:



file: ...ments\NMR Data\TL141\TL141_13C\fid expt: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 30030.03 Hz = 238.7687 ppm = 0.458222 Hz/pt
number of scans: 1471

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 81.538 ppm/cm: 0.64831

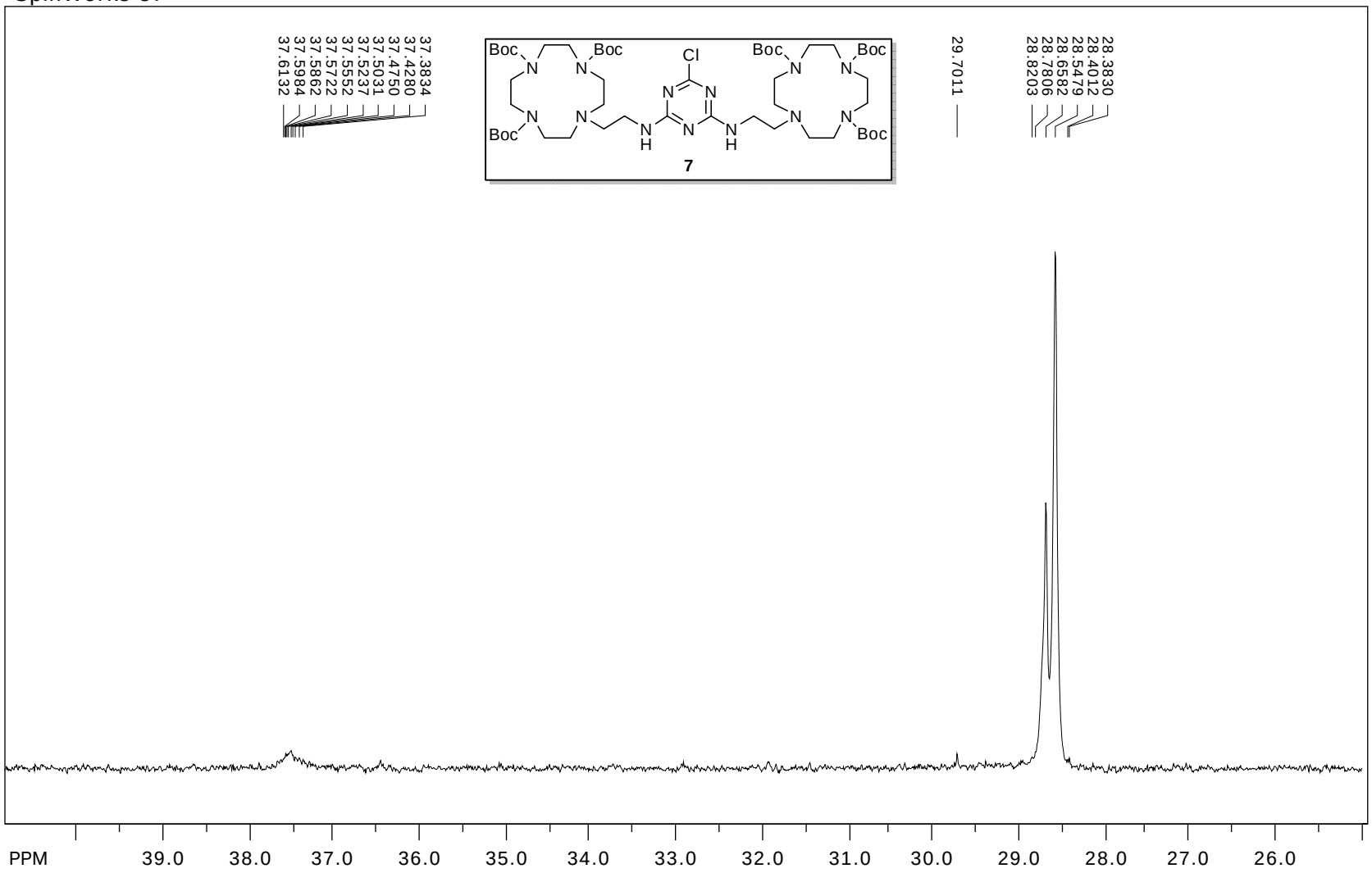
SpinWorks 3:



file: ...ments\NMR Data\TL141\TL141_13C\fid expt: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 30030.03 Hz = 238.7687 ppm = 0.458222 Hz/pt
number of scans: 1471

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 253.456 ppm/cm: 2.01523

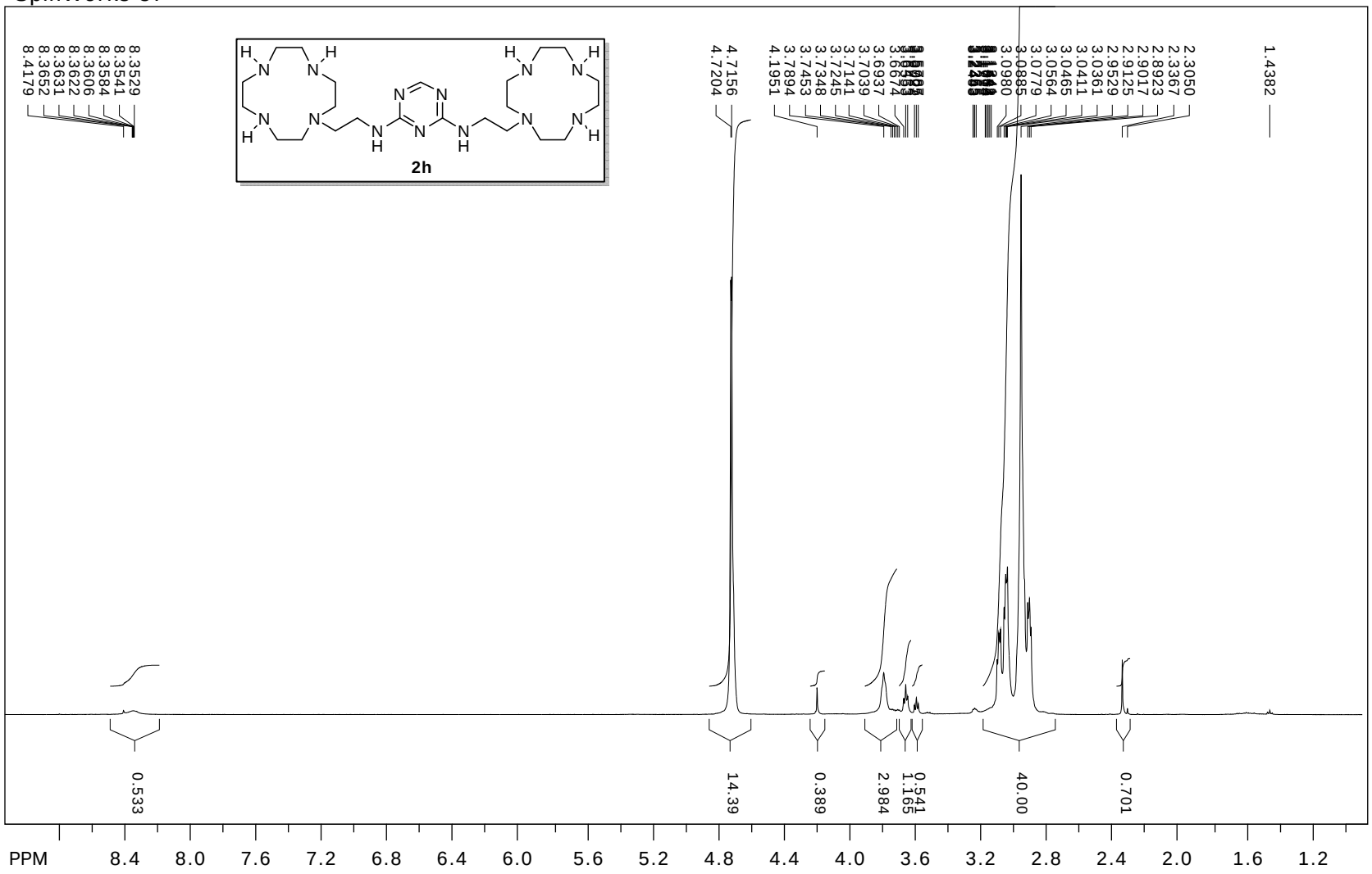
SpinWorks 3:



file: ...ments\NMR Data\TL141\TL141_13C\fid expt: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 30030.03 Hz = 238.7687 ppm = 0.458222 Hz/pt
number of scans: 1471

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 80.195 ppm/cm: 0.63763

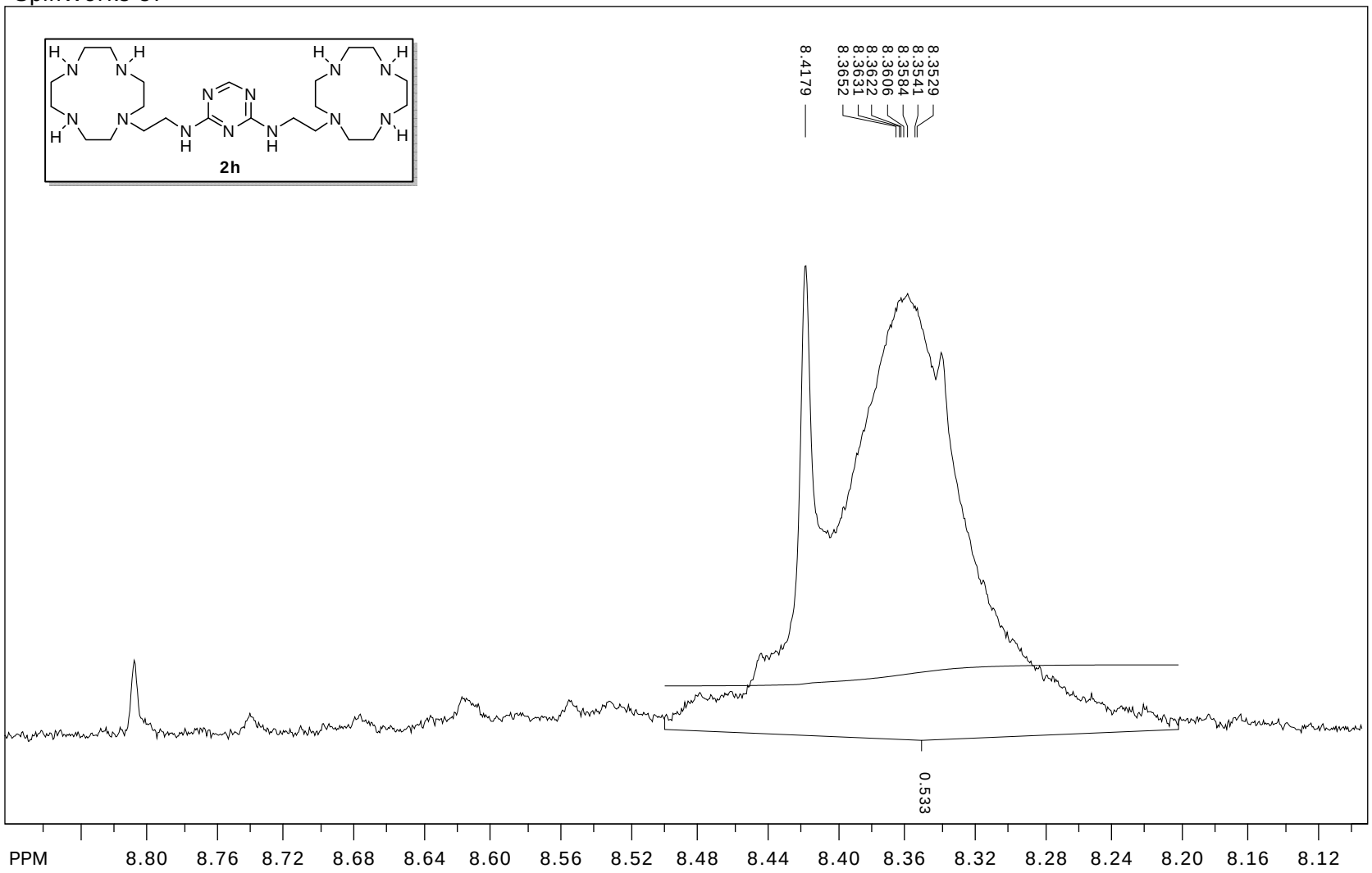
SpinWorks 3:



file: ...ents\NMR Data\TL_166_batch2\60\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130000 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 165.352 ppm/cm: 0.33062

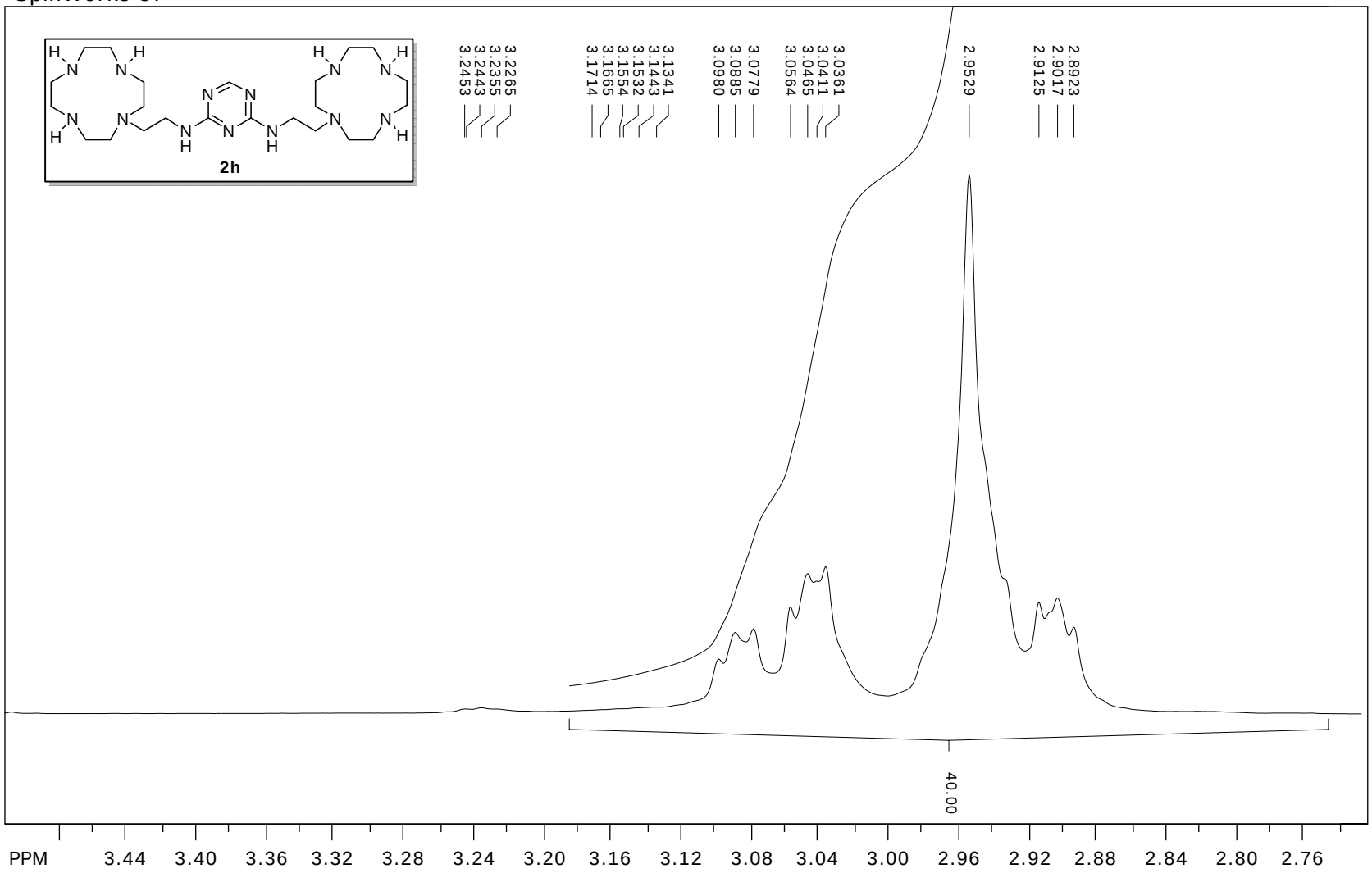
SpinWorks 3:



file: ...ents\NMR Data\TL_166_batch2\60\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130000 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 15.825 ppm/cm: 0.03164

SpinWorks 3:



file: ...ents\NMR Data\TL_166_batch2\60\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130000 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 15.739 ppm/cm: 0.03147

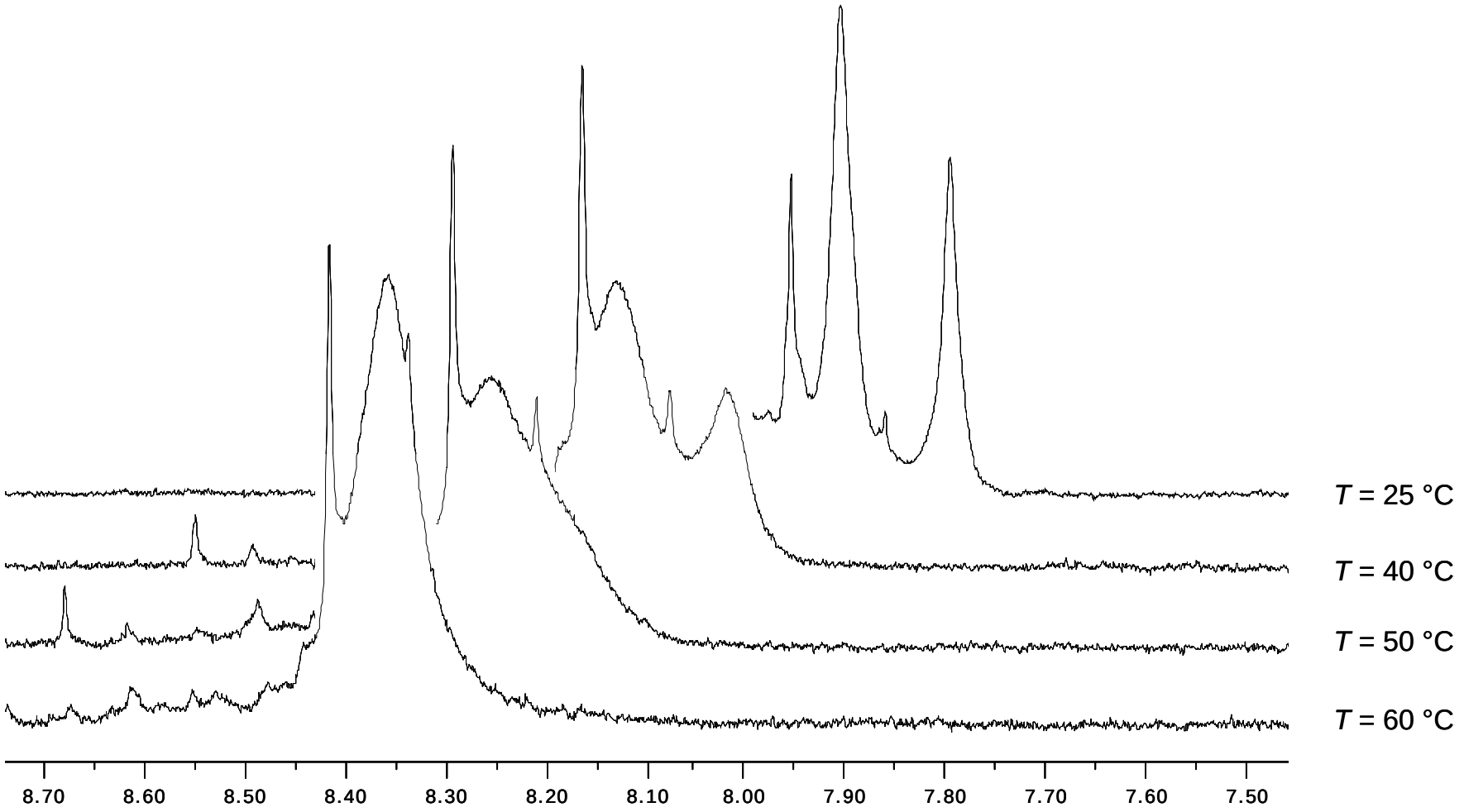
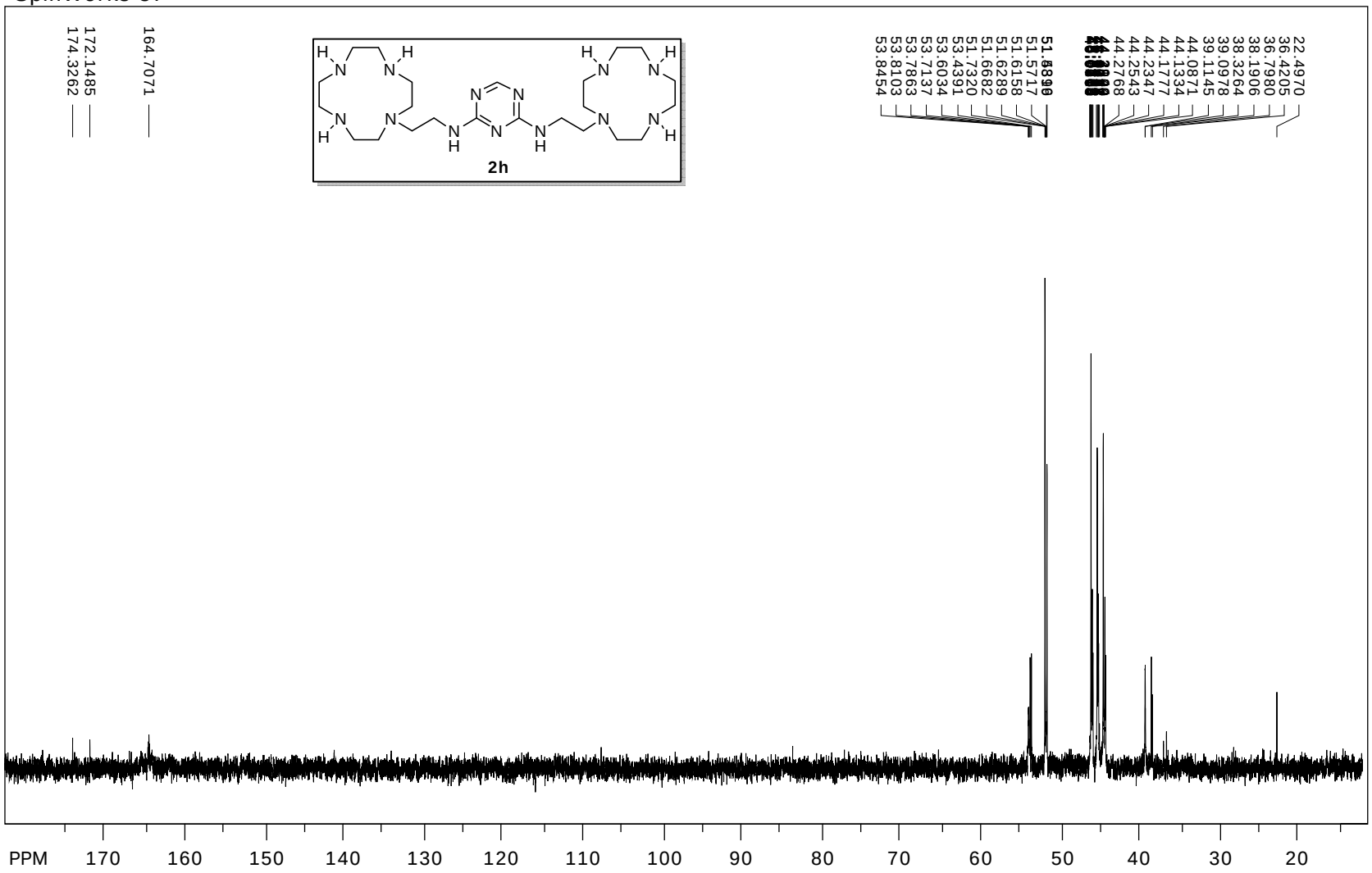


Figure S1. The ^{1}H NMR signal of the sole aromatic proton of **2h** measured at different temperatures, illustrating the fact that at room temperature equilibrium between the various conformers is slow on NMR time scale.

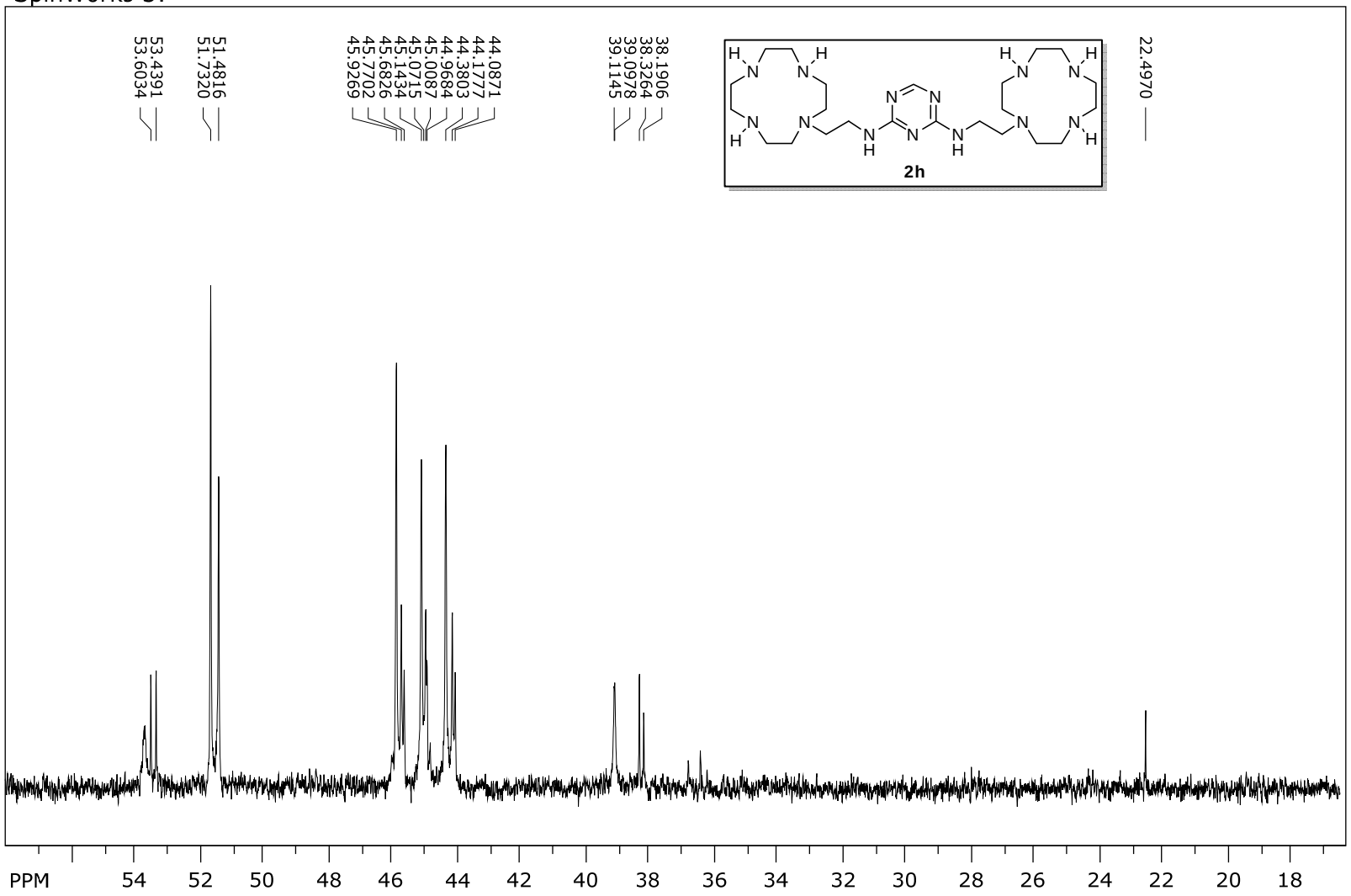
SpinWorks 3:



file: ...ents\NMR Data\TL_166_batch2\61\fid expt: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 30030.03 Hz = 238.7687 ppm = 0.458222 Hz/pt
number of scans: 765

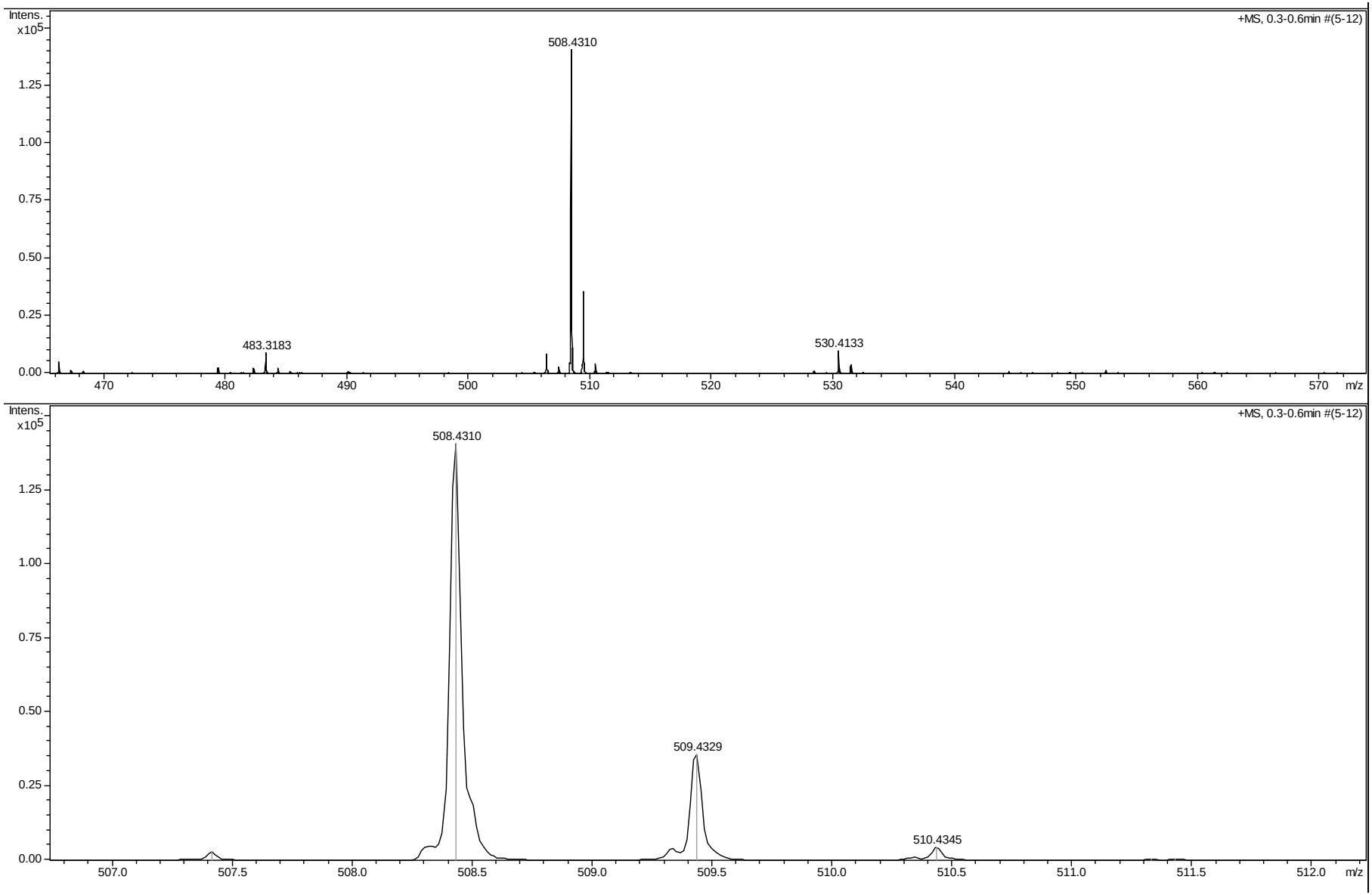
freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 860.846 ppm/cm: 6.84458

SpinWorks 3:

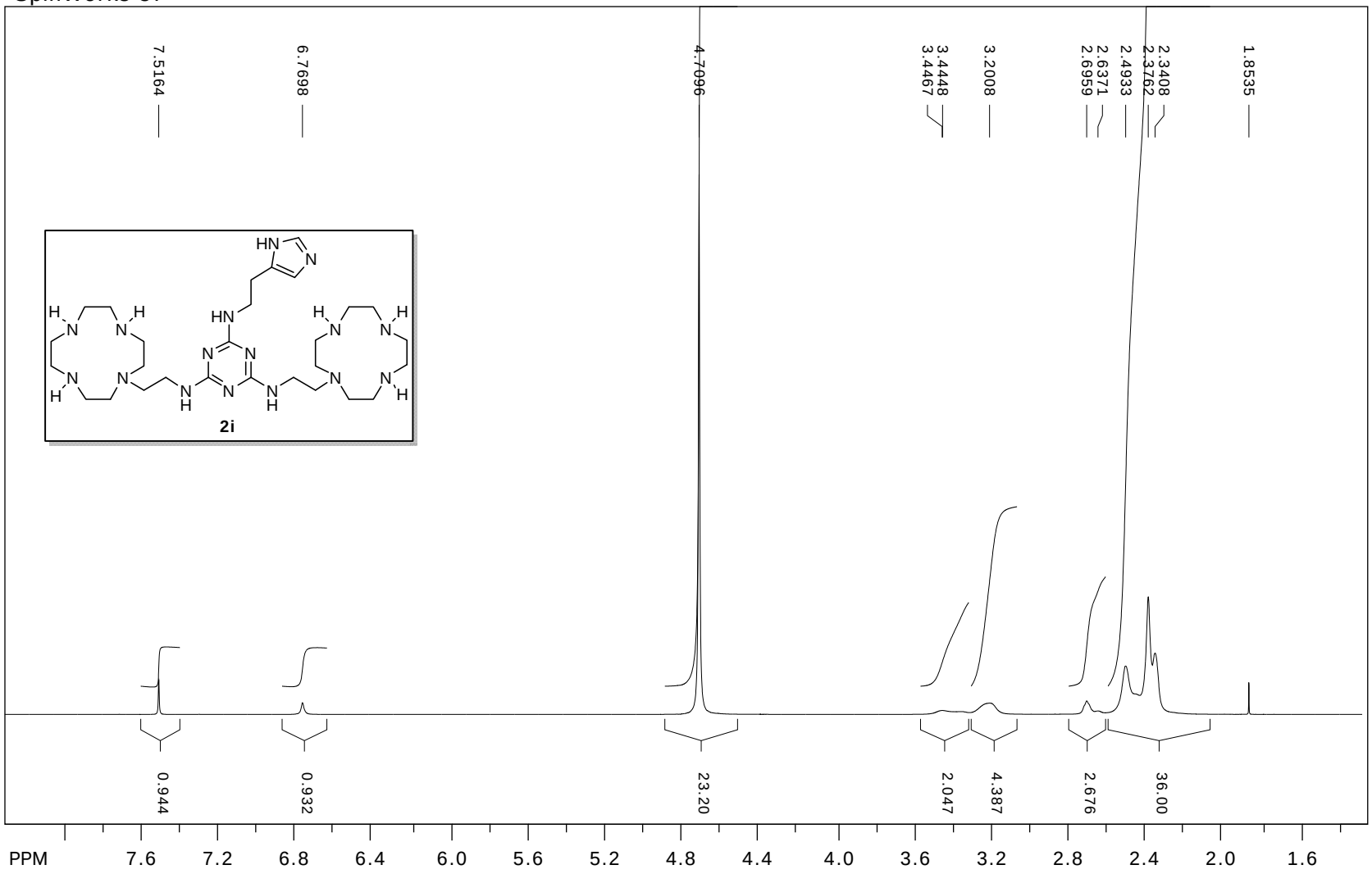


file: ... Data\TL_166_batch2\61\spectrum.dx expt: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 30030.03 Hz = 238.7687 ppm = 0.458222 Hz/pt
number of scans: 0

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 218.757 ppm/cm: 1.73934



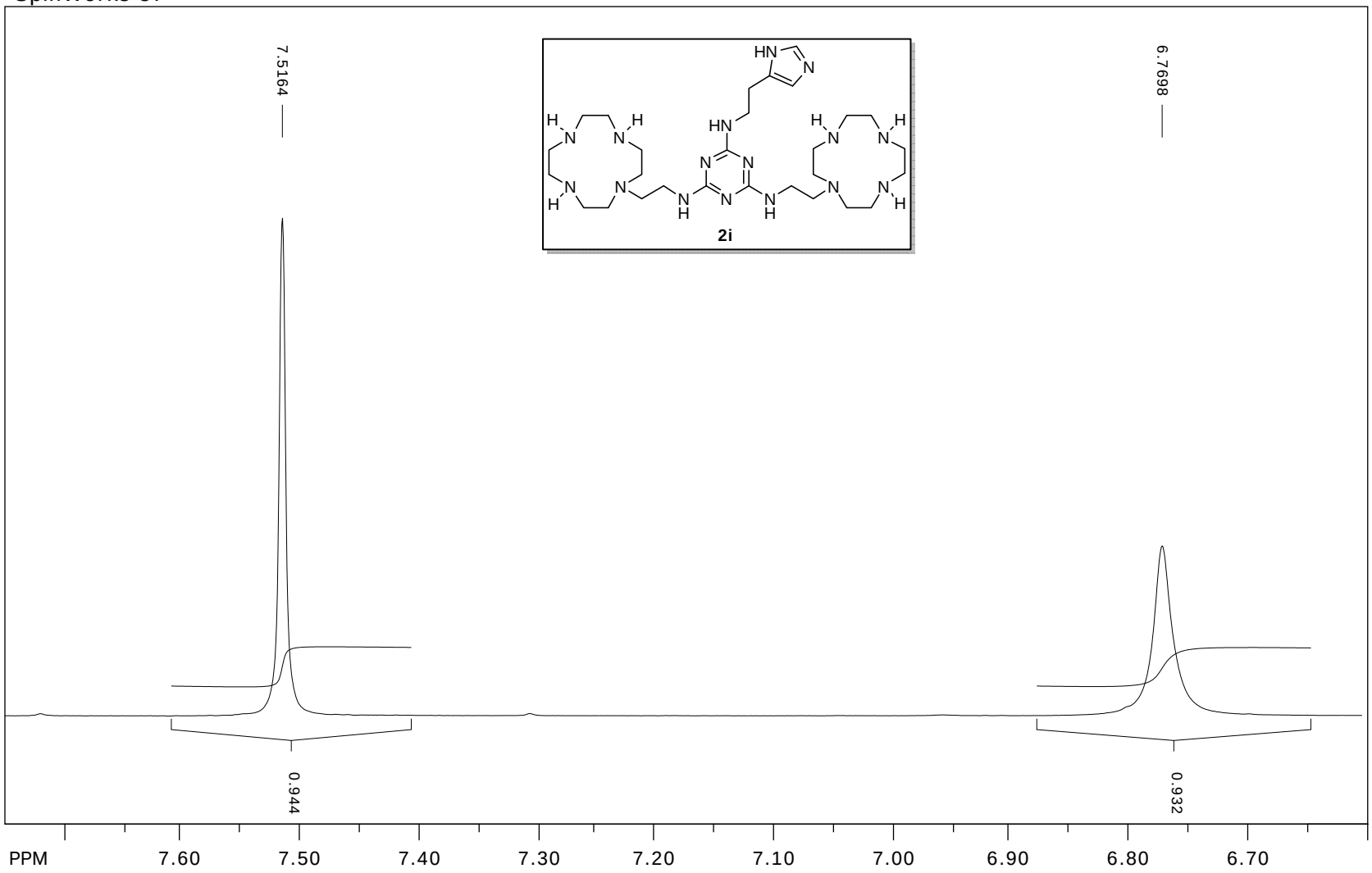
SpinWorks 3:



file: ...uments\NMR Data\TL160\TL160_1H\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130000 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 141.063 ppm/cm: 0.28205

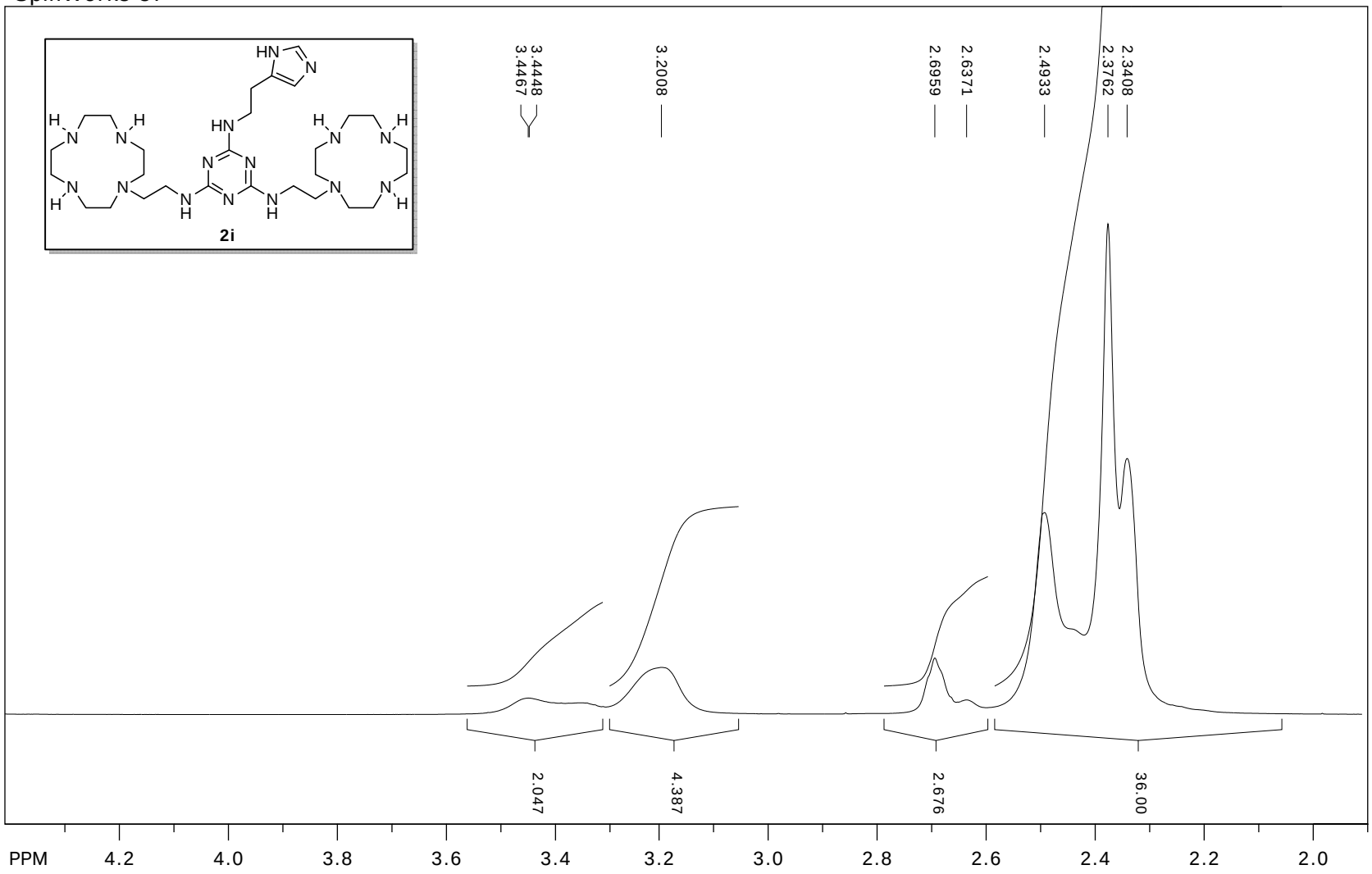
SpinWorks 3:



file: ...uments\NMR Data\TL160\TL160_1H\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130000 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 23.041 ppm/cm: 0.04607

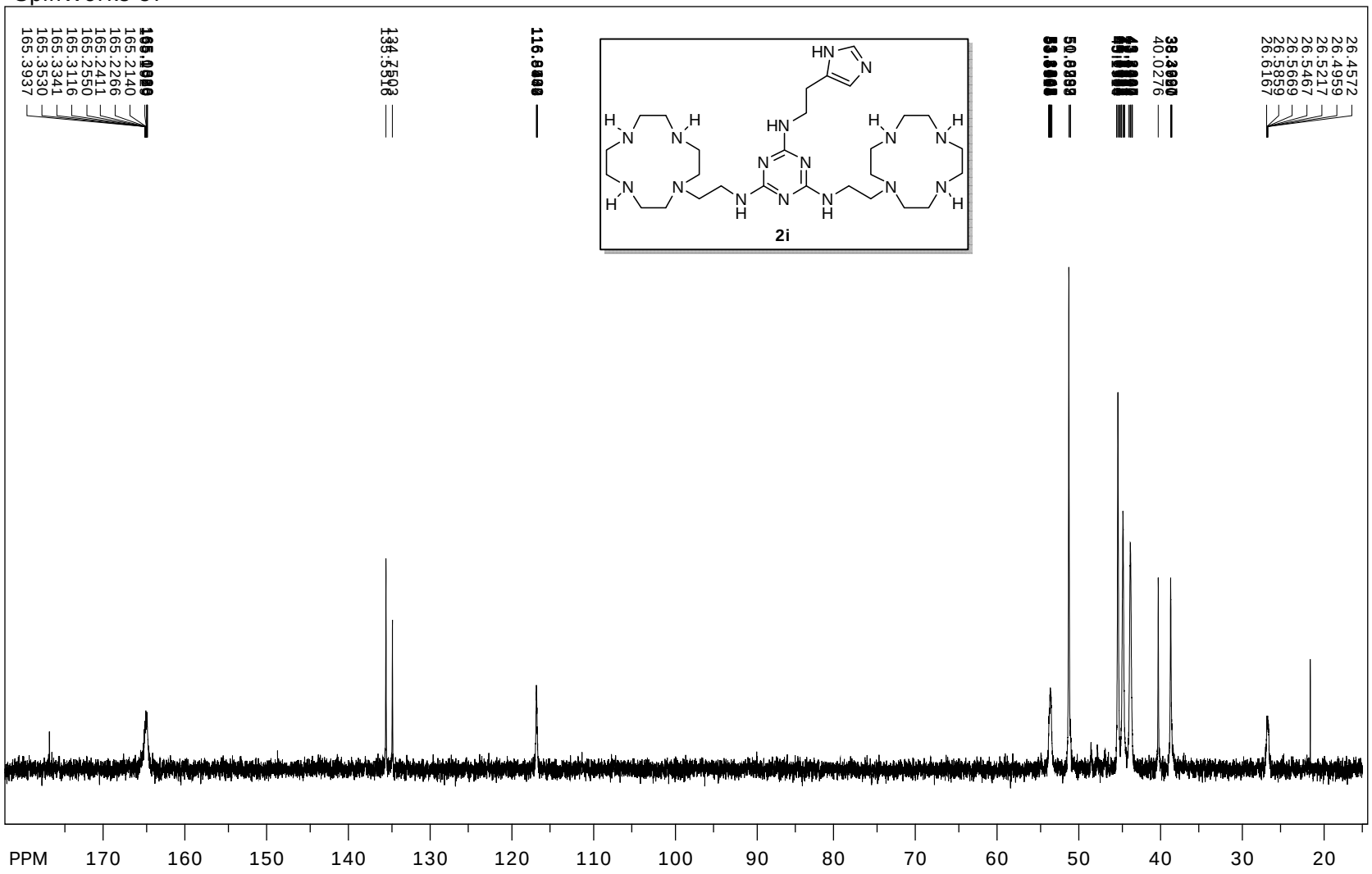
SpinWorks 3:



file: ...uments\NMR Data\TL160\TL160_1H\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130000 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 50.135 ppm/cm: 0.10024

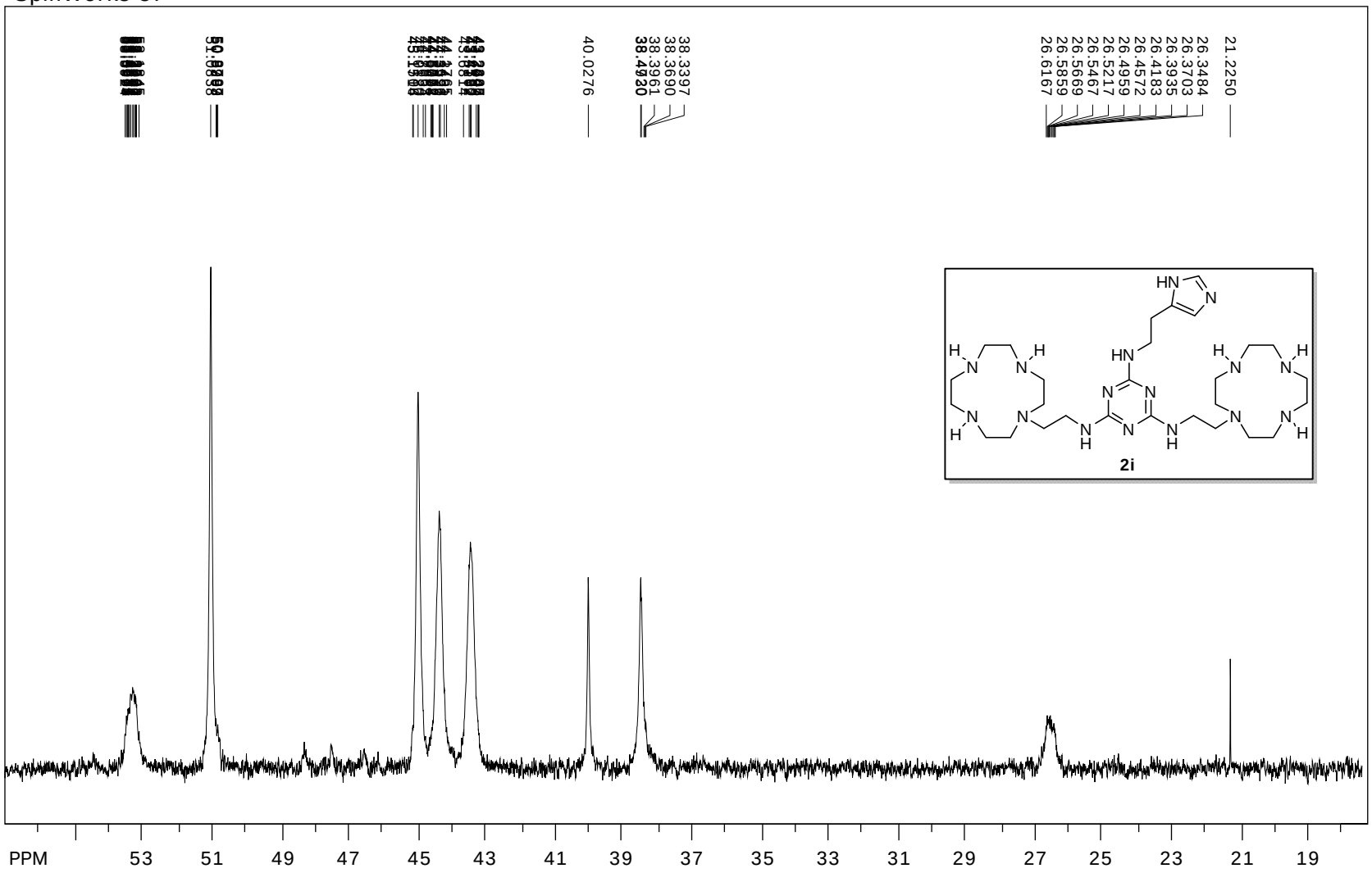
SpinWorks 3:



file: ...ments\NMR Data\TL160\TL160_13C\fid expt: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 30030.03 Hz = 238.7687 ppm = 0.458222 Hz/pt
number of scans: 727

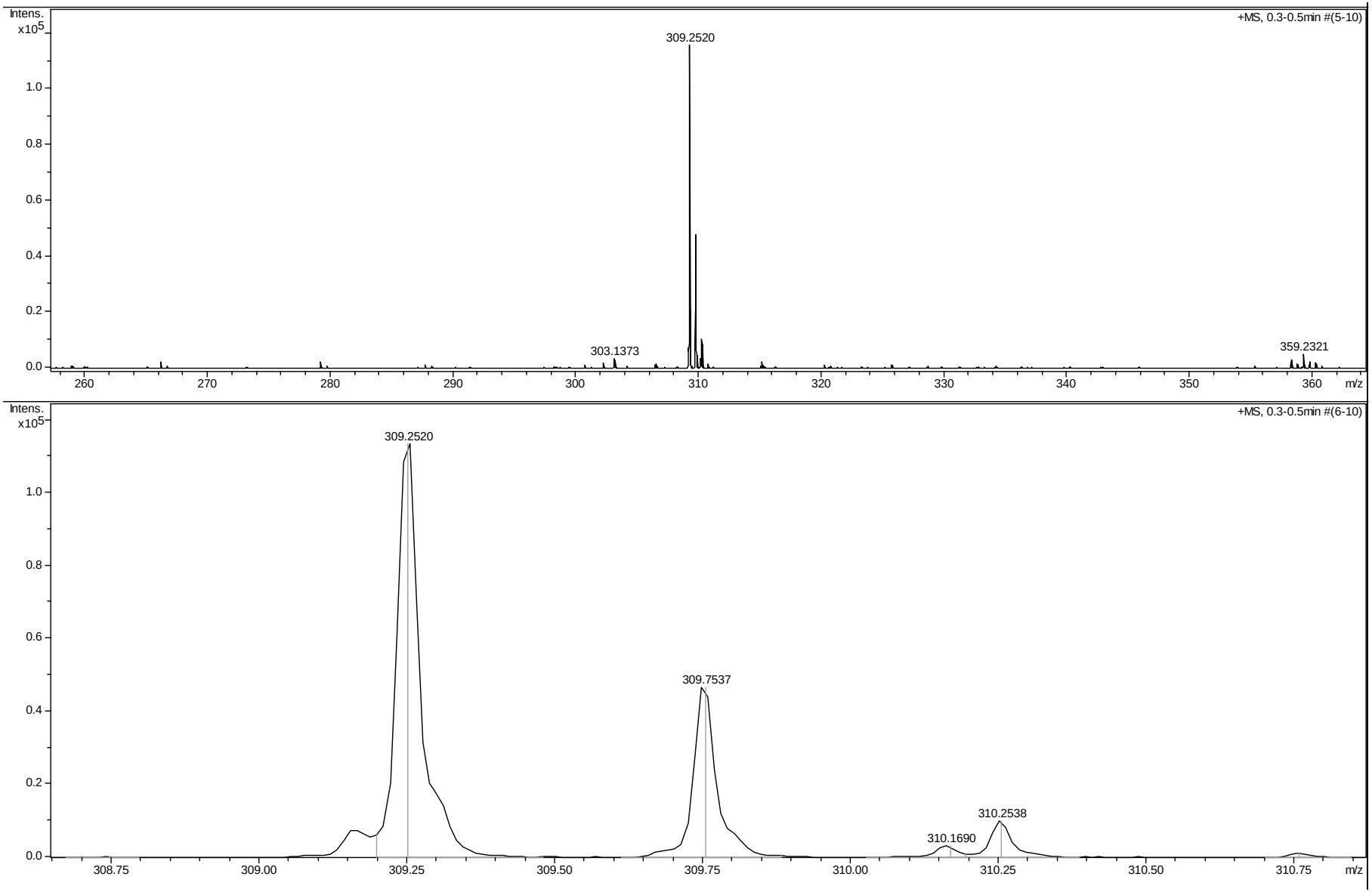
freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 844.552 ppm/cm: 6.71503

SpinWorks 3:

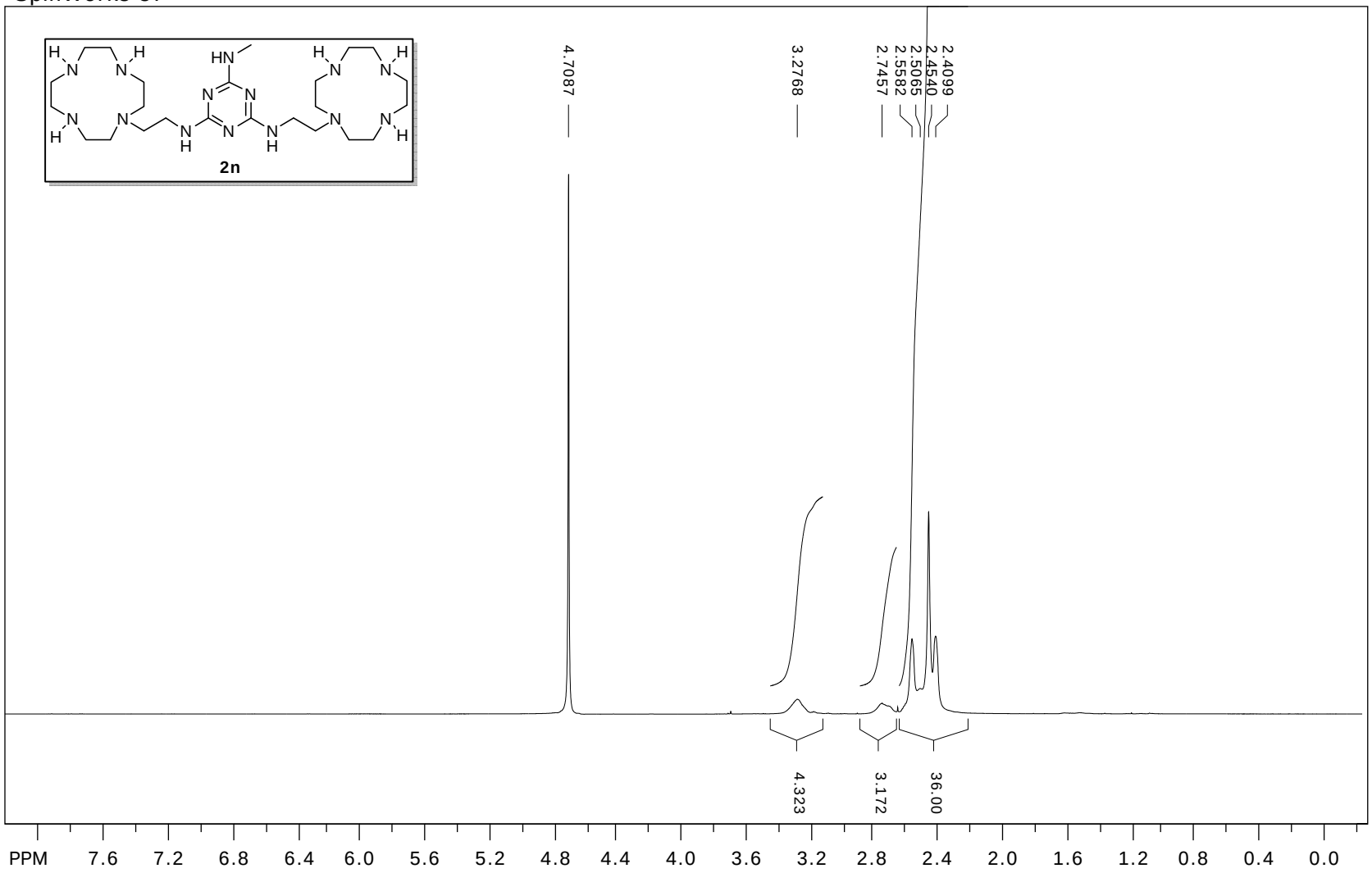


file: ...ments\NMR Data\TL160\TL160_13C\fid expt: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 30030.03 Hz = 238.7687 ppm = 0.458222 Hz/pt
number of scans: 727

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 199.969 ppm/cm: 1.58995


Intens.
x10^5
+MS, 0.3-0.5min #(5-10)
309.2520
303.1373
359.2321
m/z
Intens.
x10^5
+MS, 0.3-0.5min #(6-10)
309.2520
309.7537
310.2538
310.1690
m/z

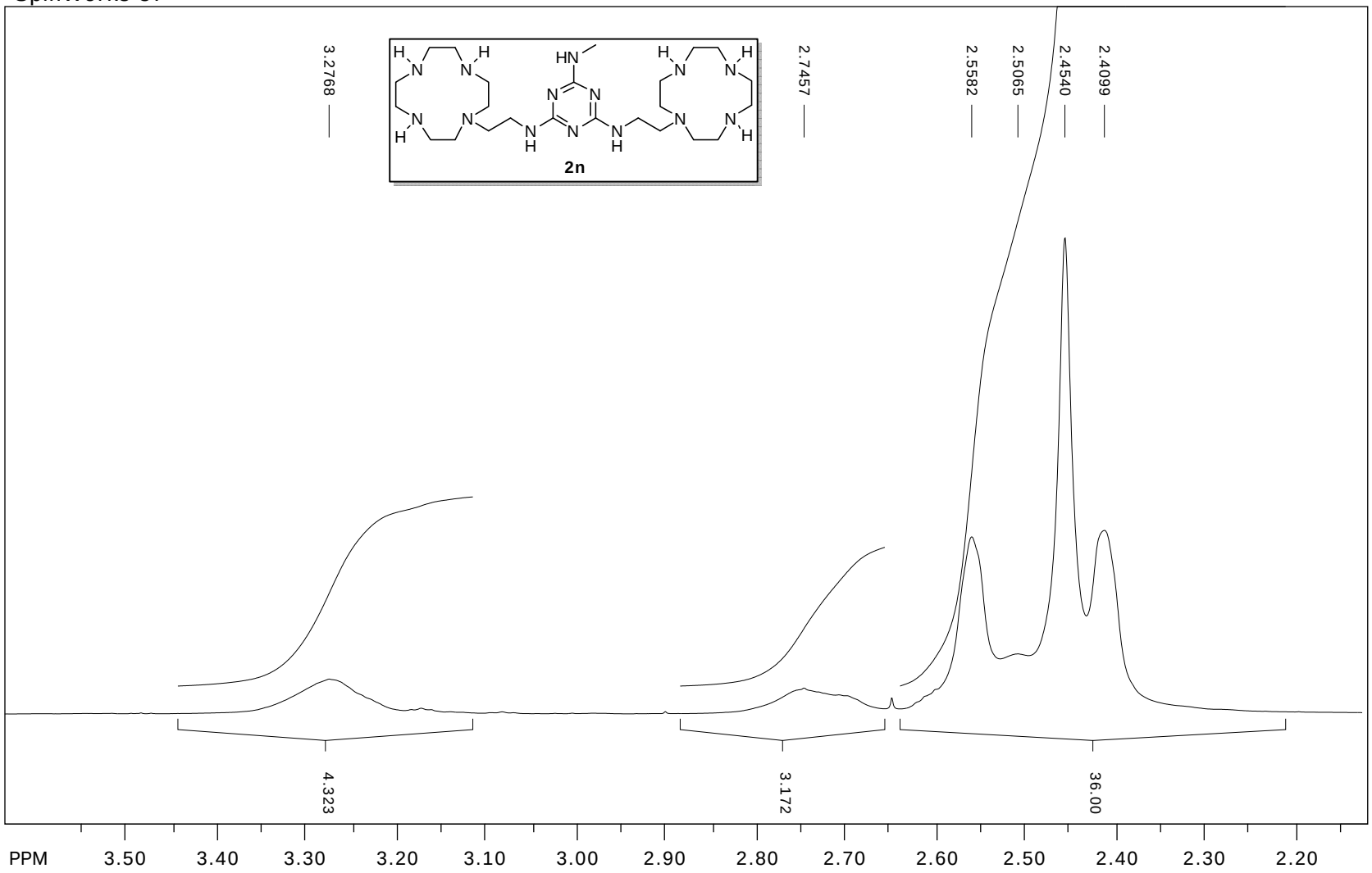
SpinWorks 3:



file: ...uments\NMR Data\TL153\TL153_1H\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130000 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 170.022 ppm/cm: 0.33995

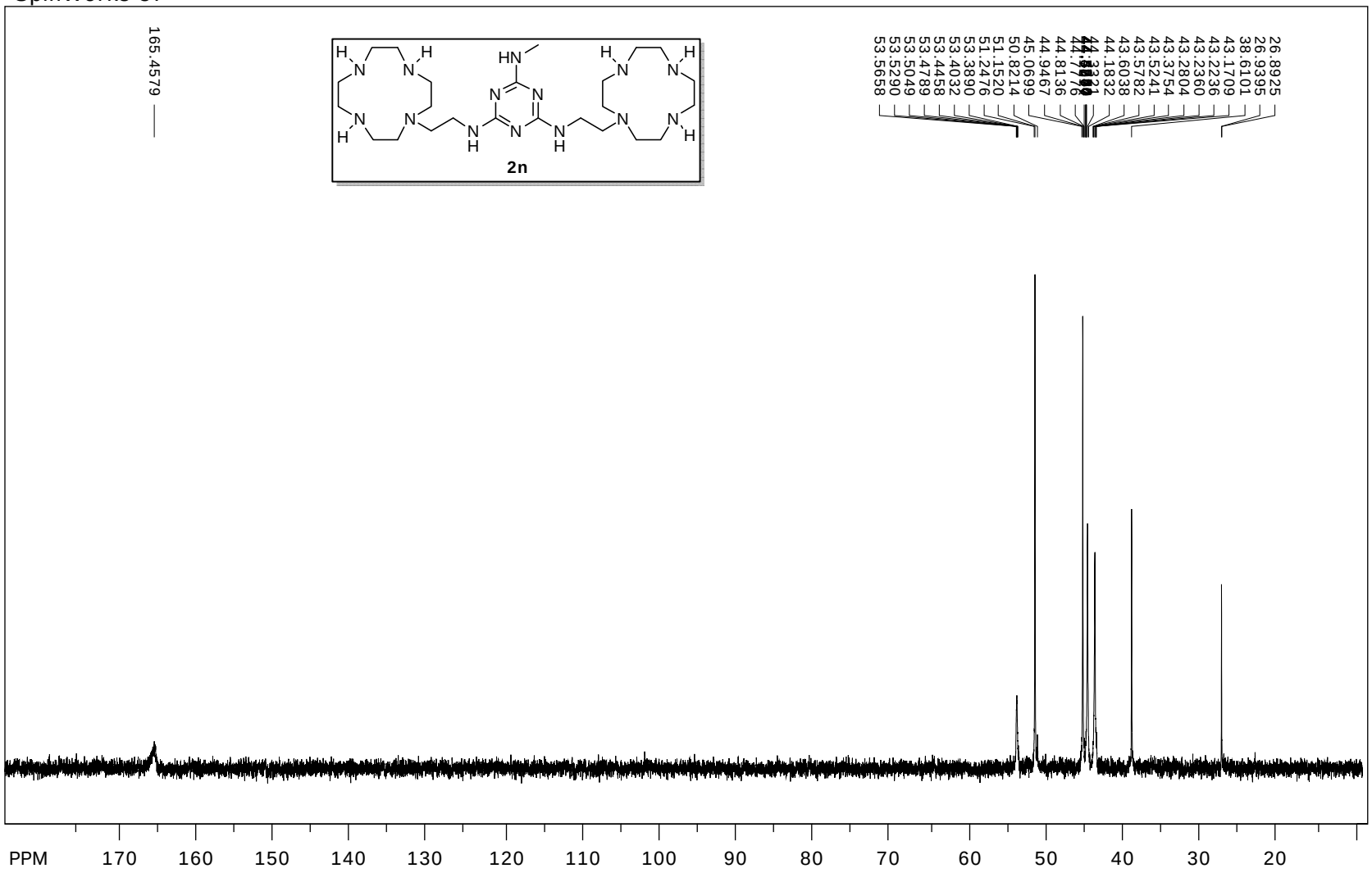
SpinWorks 3:



file: ...uments\NMR Data\TL153\TL153_1H\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130000 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 30.366 ppm/cm: 0.06072

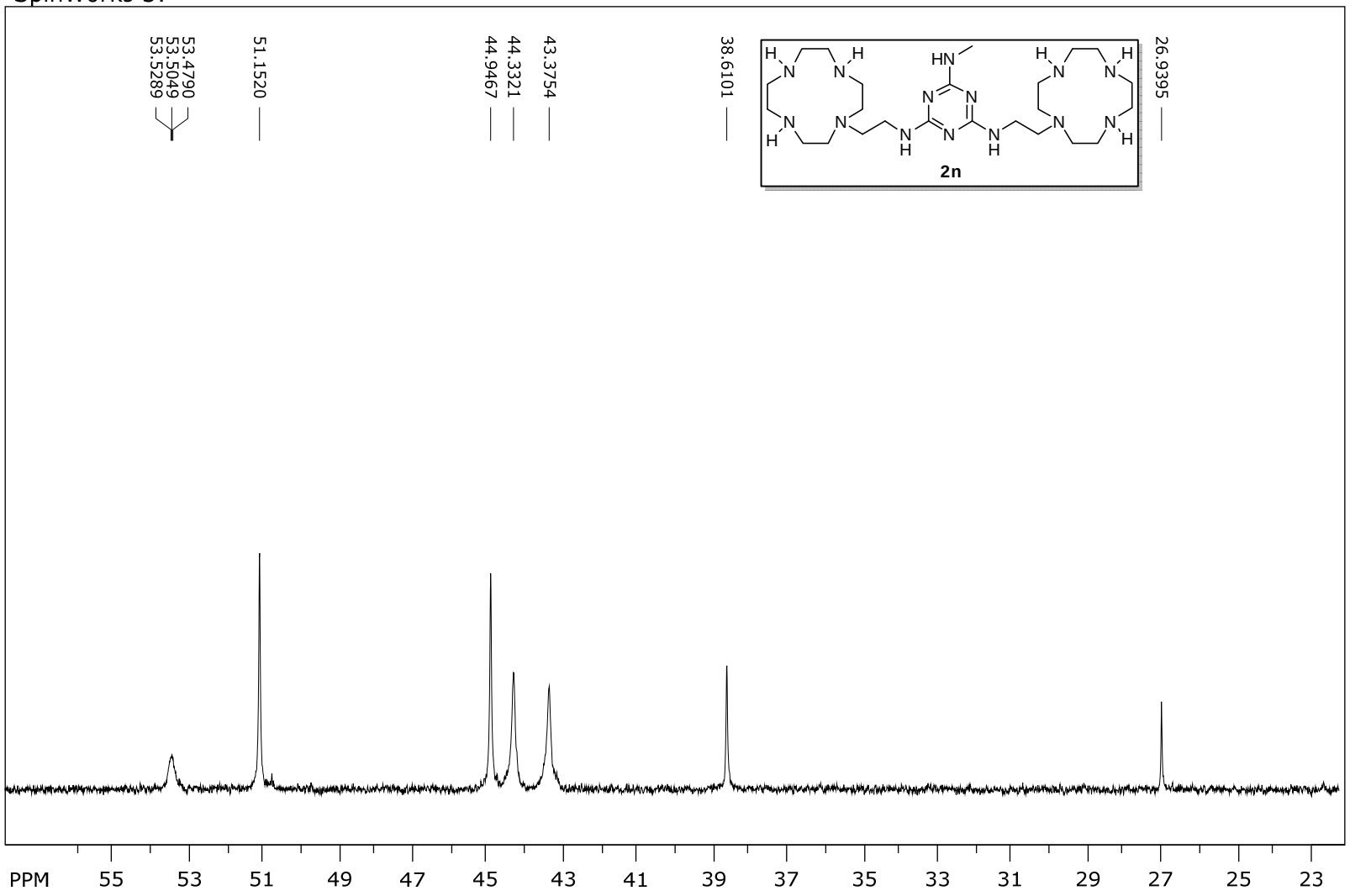
SpinWorks 3:



file: ...ments\NMR Data\TL153\TL153_13C\fid expt: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 30030.03 Hz = 238.7687 ppm = 0.458222 Hz/pt
number of scans: 647

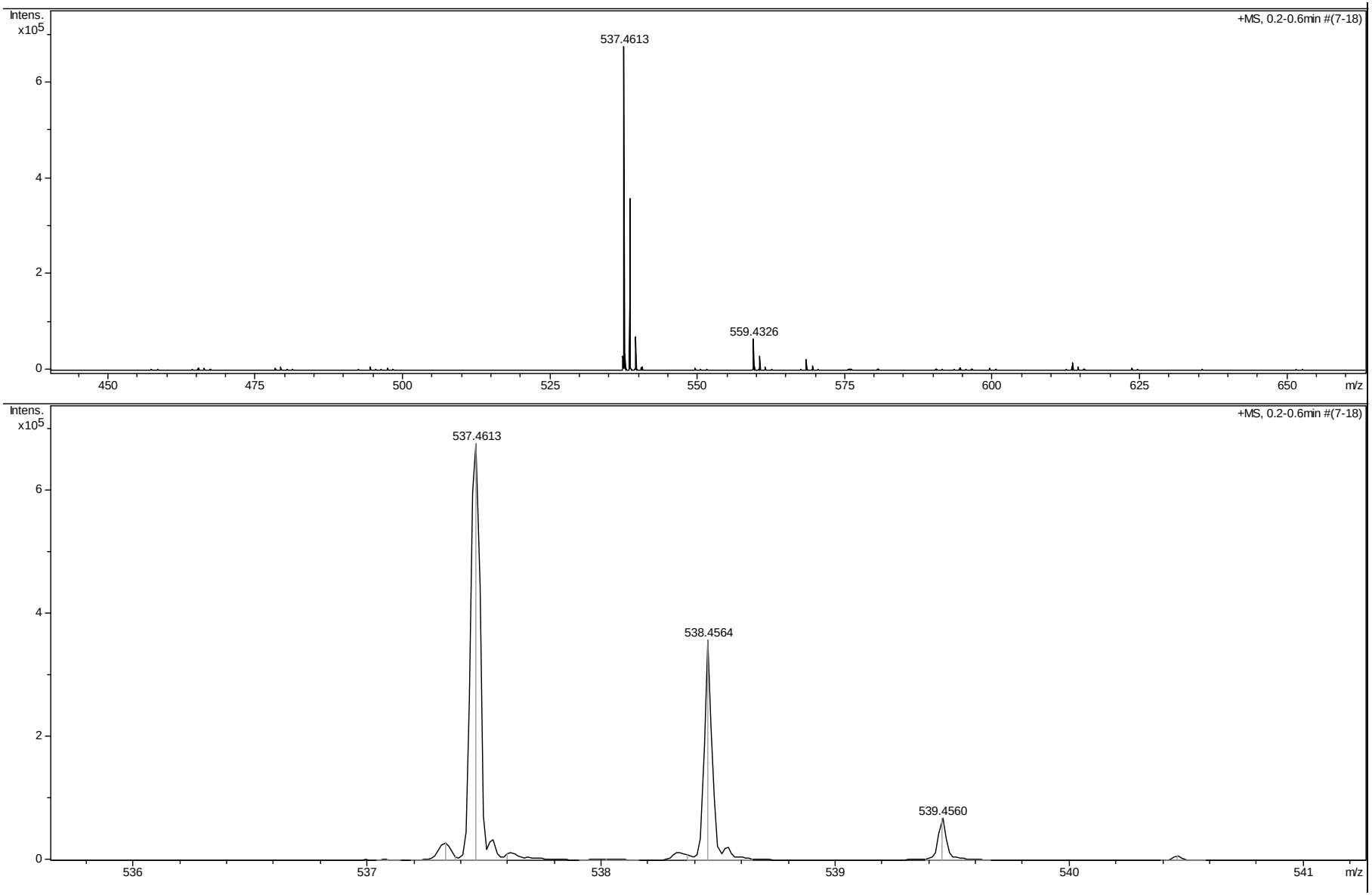
freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 886.191 ppm/cm: 7.04611

SpinWorks 3:

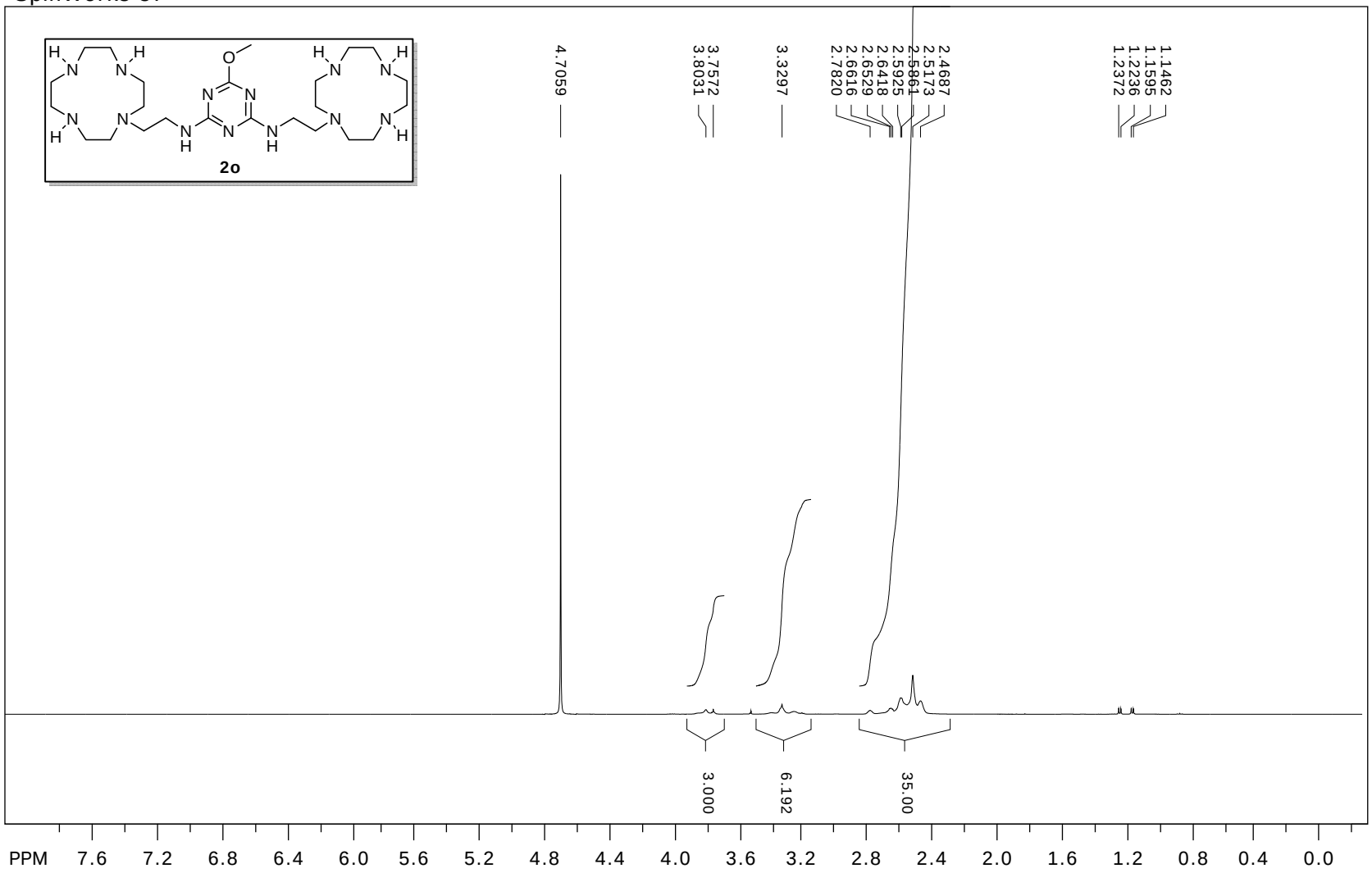


file: ...R Data\TL153\TL153_13C\spectrum.dx expt: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 30030.03 Hz = 238.7687 ppm = 0.458222 Hz/pt
number of scans: 0

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 0.000 GF: 0.0000
Hz/cm: 187.641 ppm/cm: 1.49193



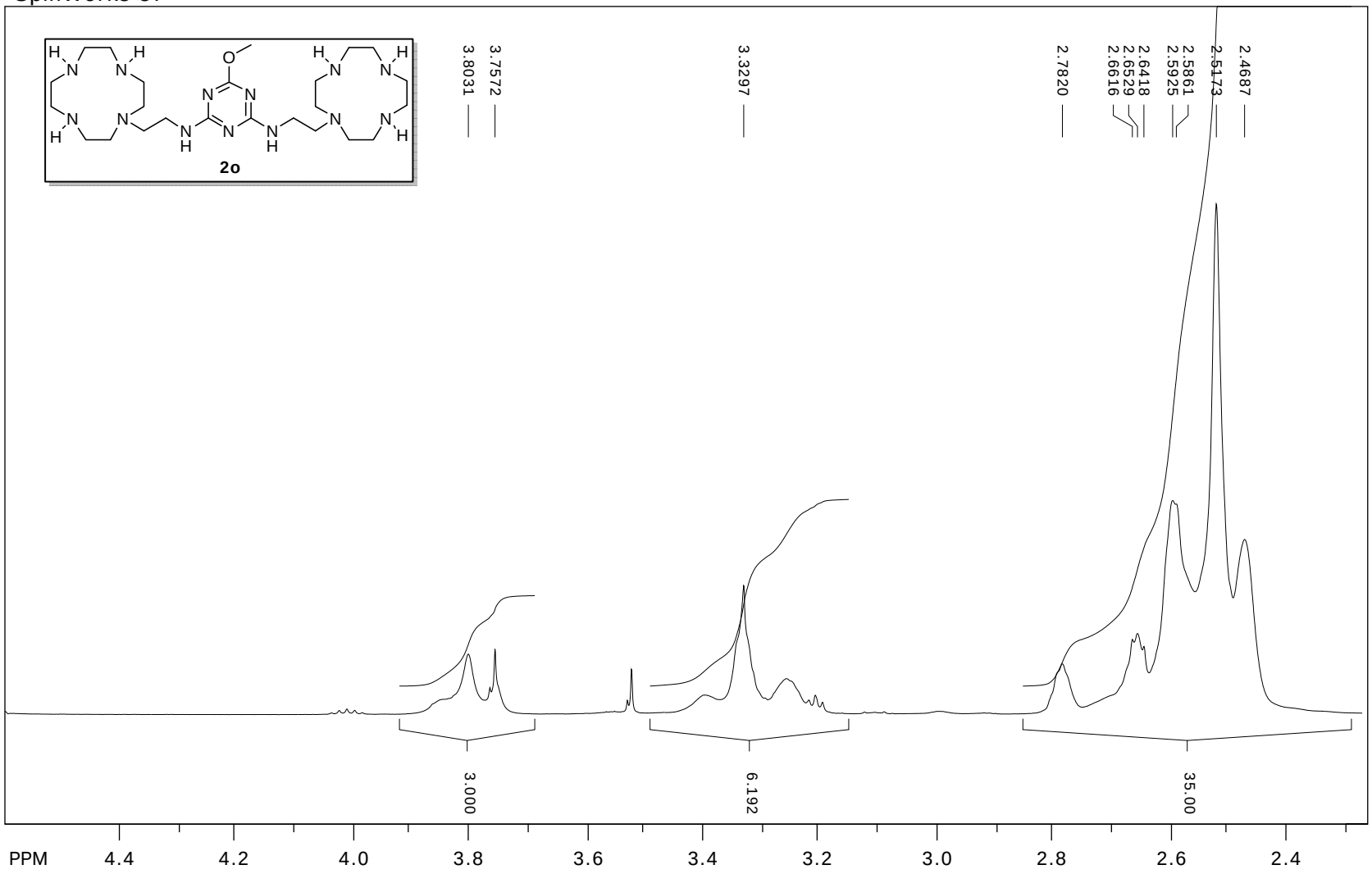
SpinWorks 3:



file: ...\My Documents\NMR Data\TL165\1\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130000 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 168.777 ppm/cm: 0.33746

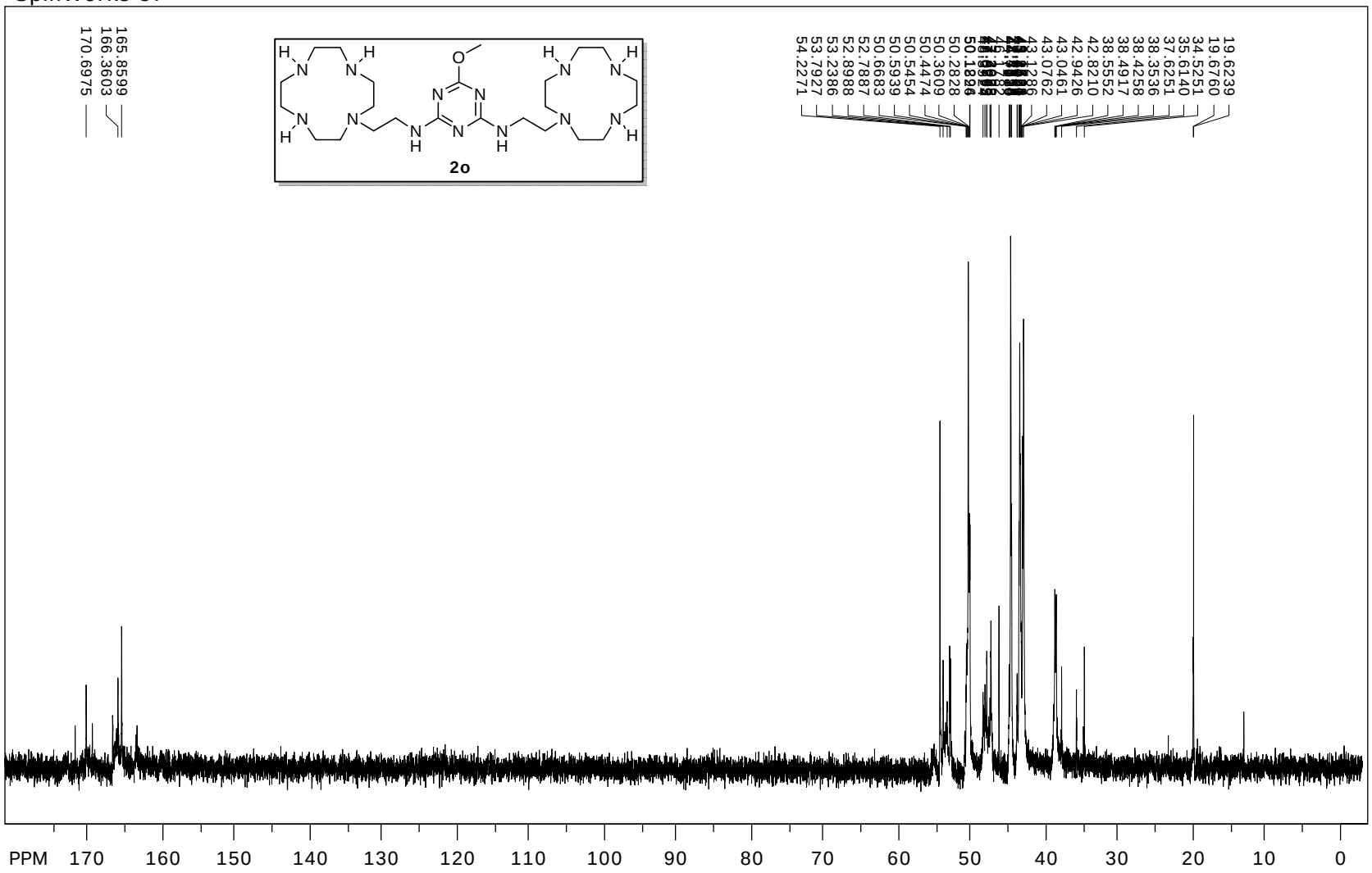
SpinWorks 3:



file: ...\My Documents\NMR Data\TL165\1\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130000 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 46.678 ppm/cm: 0.09333

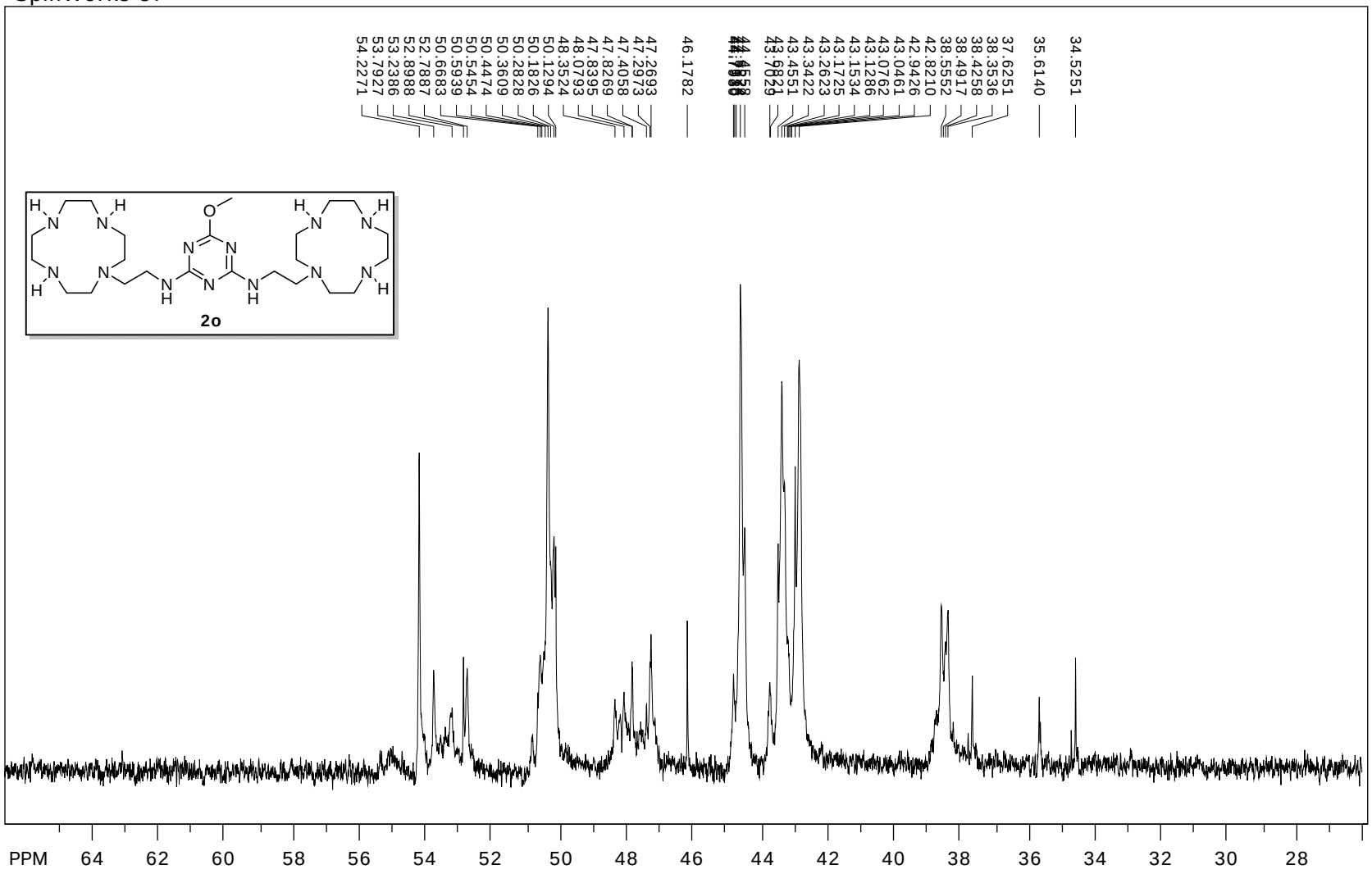
SpinWorks 3:



file: ...My Documents\NMR Data\TL165\13\fid expt: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 30030.03 Hz = 238.7687 ppm = 0.458222 Hz/pt
number of scans: 15000

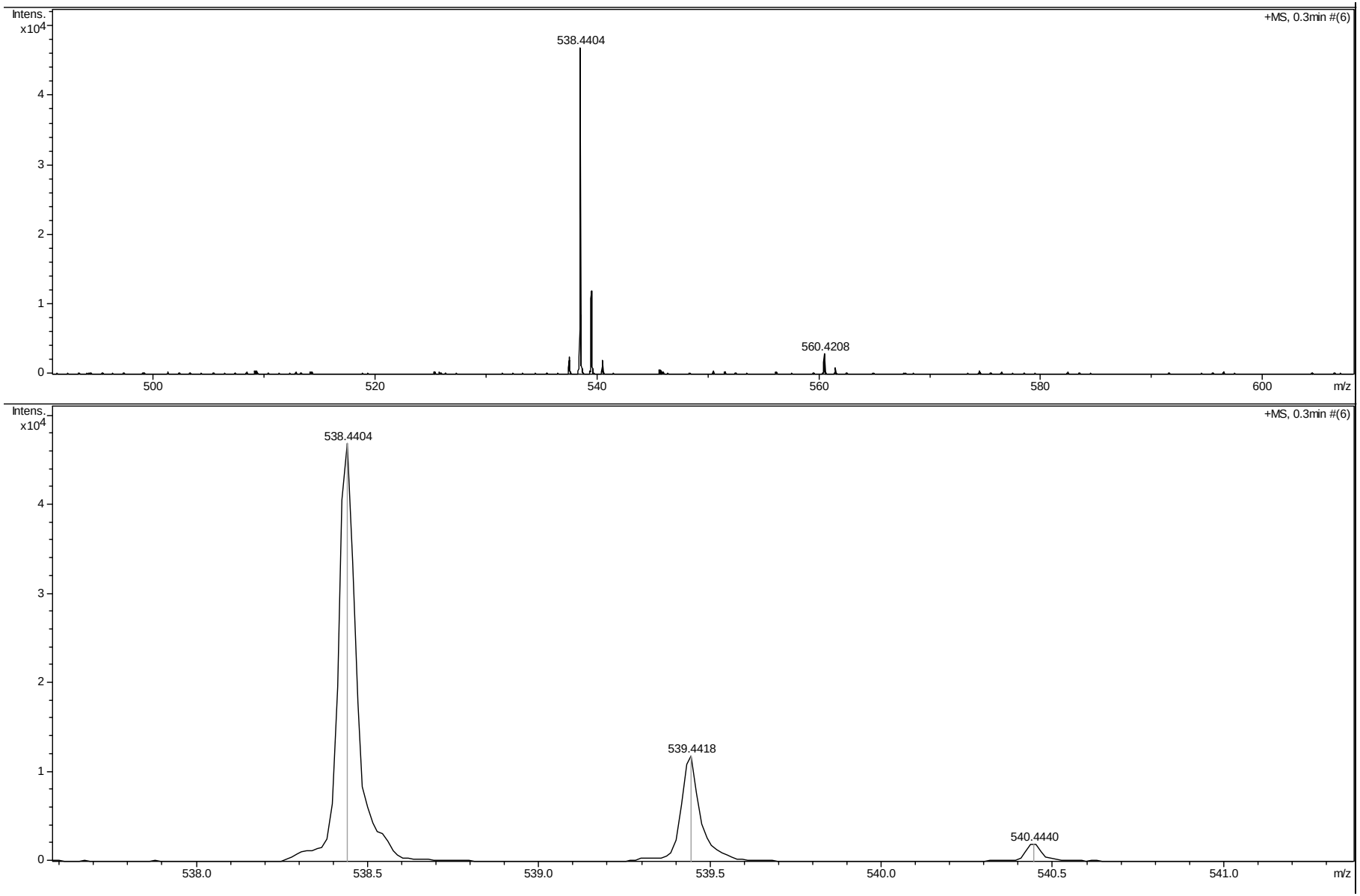
freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 931.451 ppm/cm: 7.40597

SpinWorks 3:

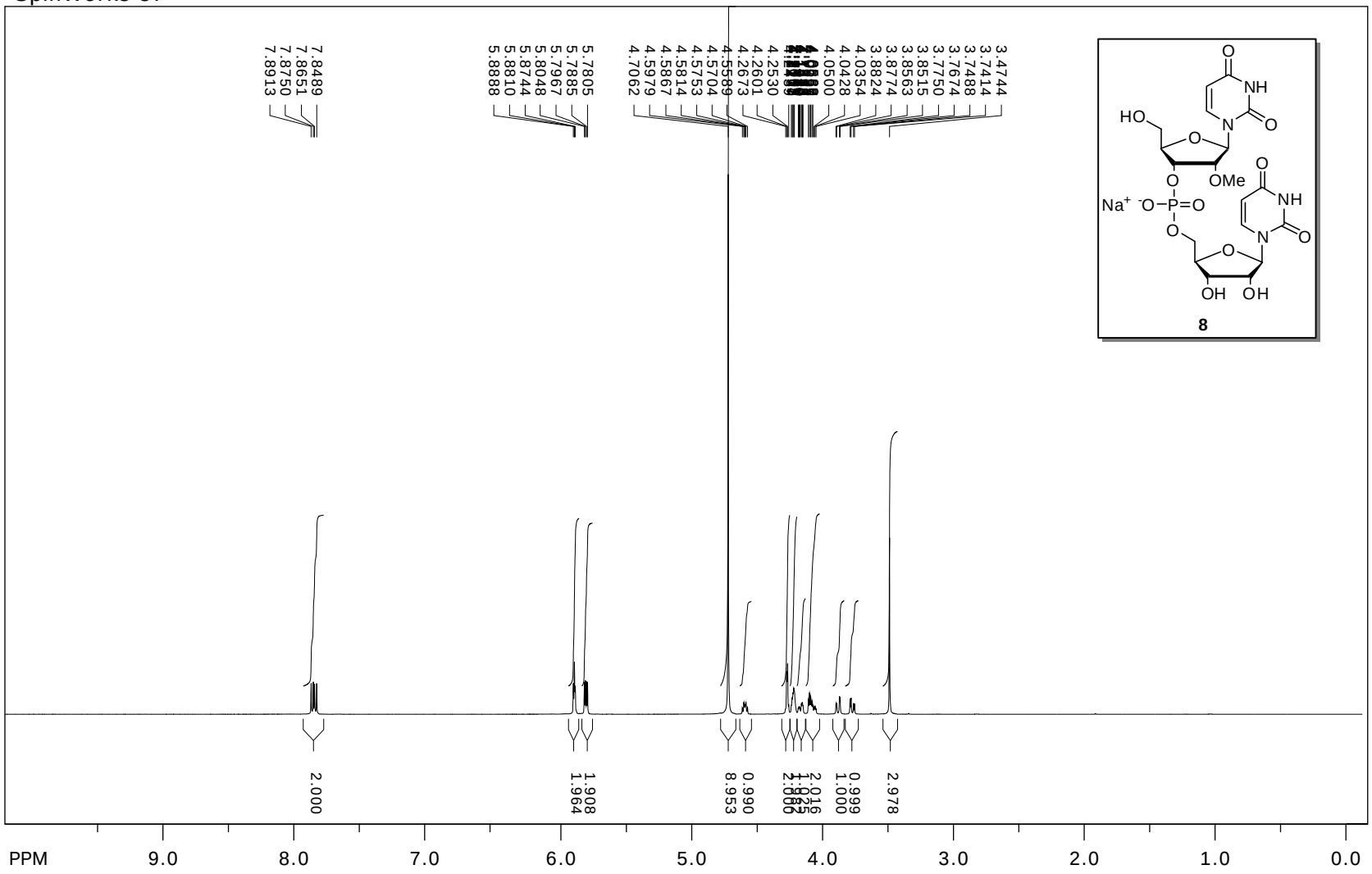


file: ...My Documents\NMR Data\TL165\13\fid expt: <zgpg30>
transmitter freq.: 125.770364 MHz
time domain size: 65536 points
width: 30030.03 Hz = 238.7687 ppm = 0.458222 Hz/pt
number of scans: 15000

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 204.961 ppm/cm: 1.62965


Intens.
x10^4
+MS, 0.3min #(6)
538.4404
560.4208
m/z
Intens.
x10^4
+MS, 0.3min #(6)
538.4404
539.4418
540.4440
m/z

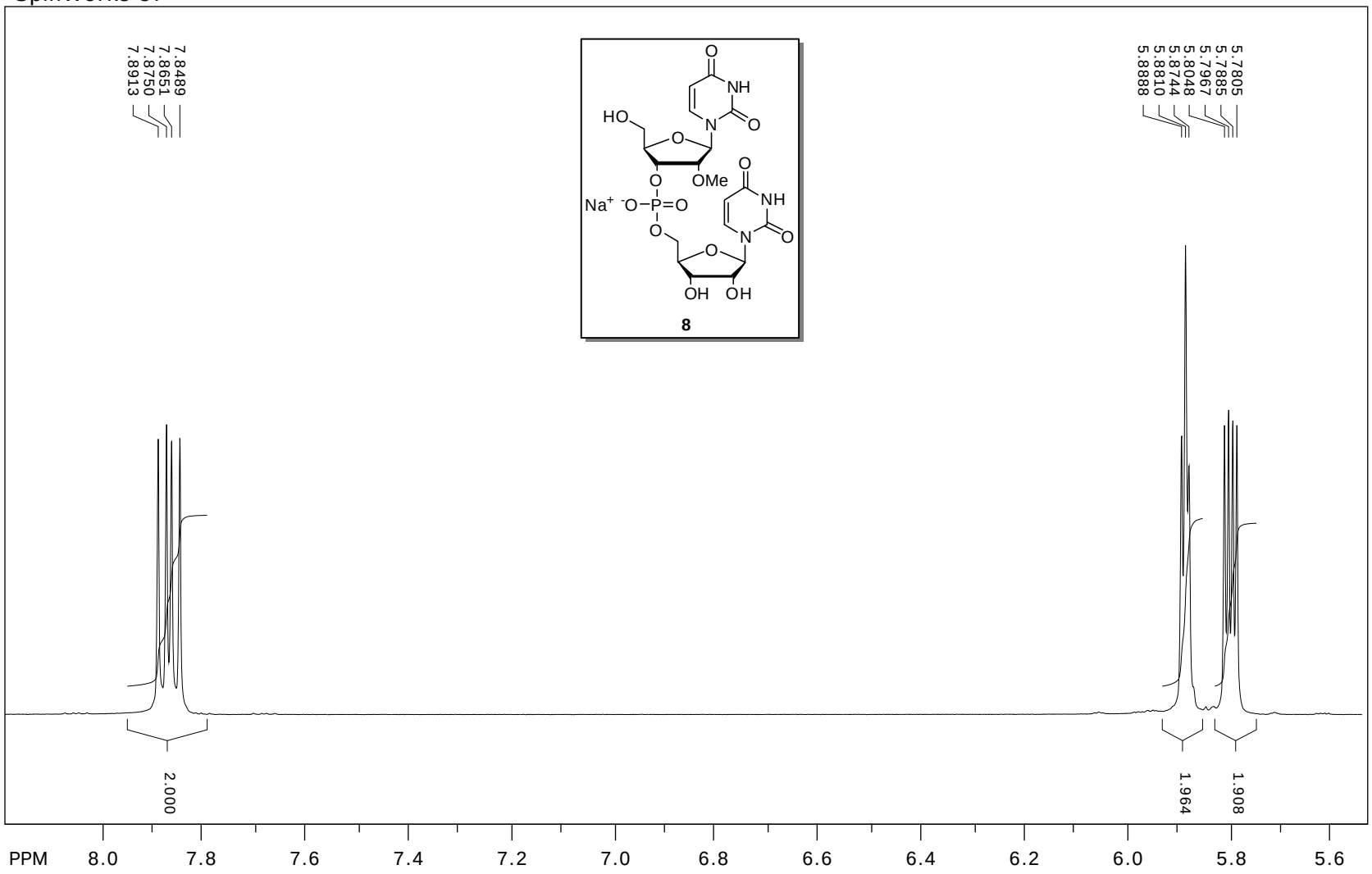
SpinWorks 3:



file: ...uments\NMR Data\TL159\TL159_1H\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130000 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 207.390 ppm/cm: 0.41467

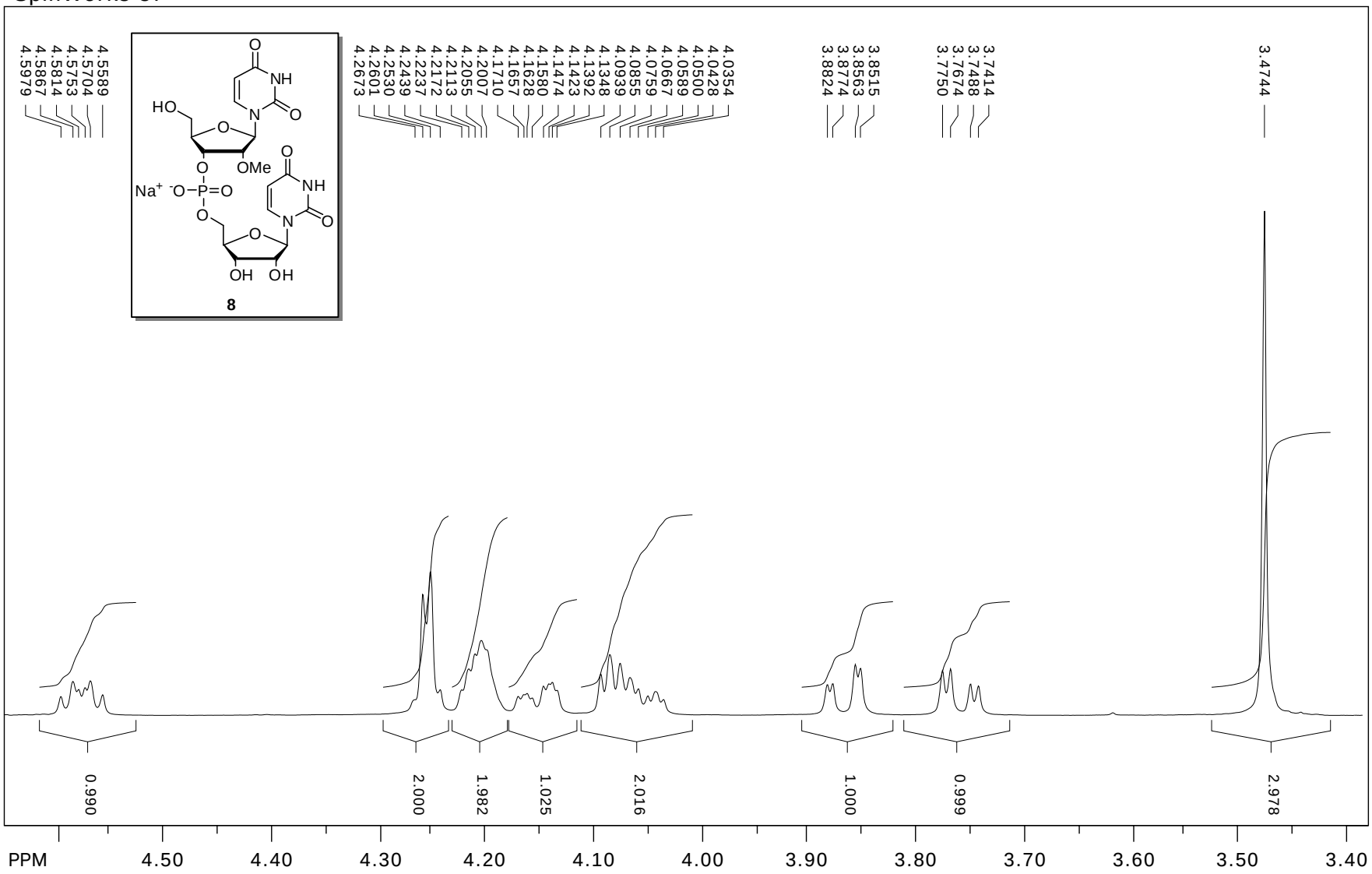
SpinWorks 3:



file: ...uments\NMR Data\TL159\TL159_1H\fid expt: <zg30>
transmitter freq.: 500.133089 MHz
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130000 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 53.137 ppm/cm: 0.10625

SpinWorks 3:



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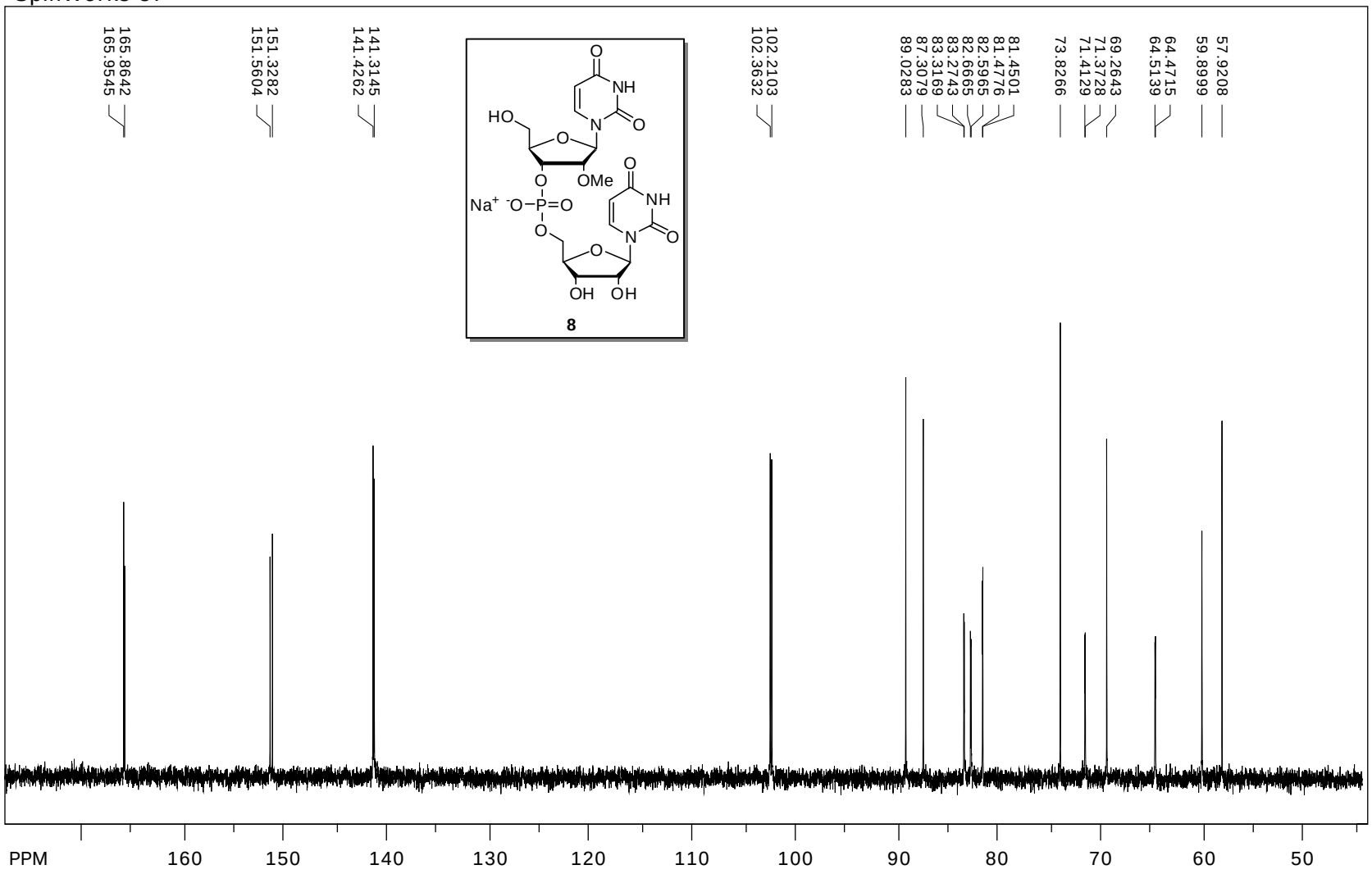
time domain size: 65536 points
width: 10330.58 Hz = 20.6557 ppm = 0.157632 Hz/pt
number of scans: 16

freq. of 0 ppm: 500.130000 MHz
processed size: 32768 complex points
LB: 0.300 GF: 0.0000
Hz/cm: 25.373 ppm/cm: 0.05073

SpinWorks 3:



file: ...ments\NMR Data\TL159\TL159_13C\fid expt: <zgpg30>
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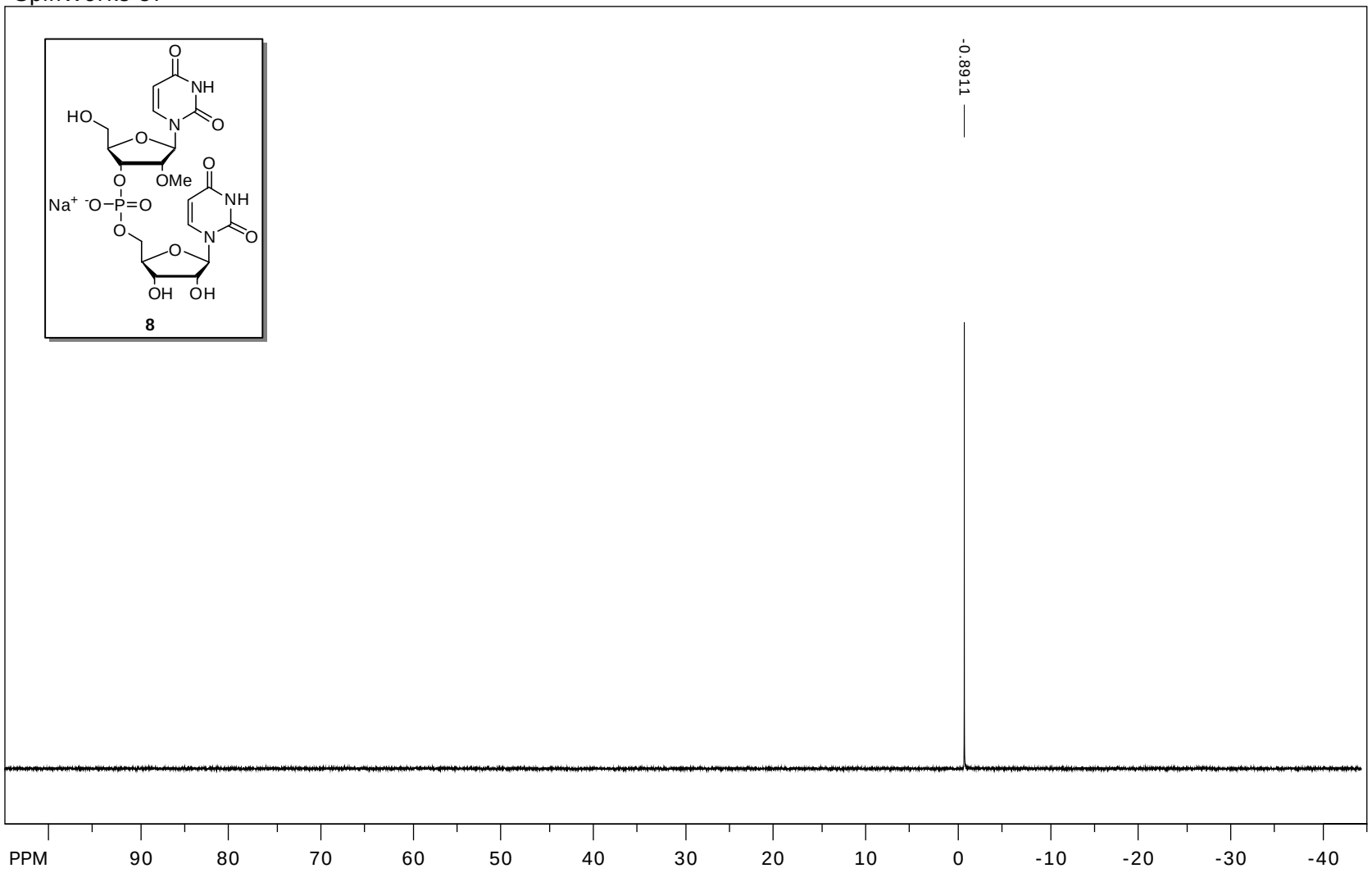
time domain size: 65536 points
width: 30030.03 Hz = 238.7687 ppm = 0.458222 Hz/pt
number of scans: 685

freq. of 0 ppm: 125.757789 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 671.659 ppm/cm: 5.34036

SpinWorks 3:



8

file: ...ments\NMR Data\TL159\TL159_31P\fid expt: <zgpg30>
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time domain size: 65536 points
width: 80645.16 Hz = 398.3536 ppm = 1.230548 Hz/pt
number of scans: 19

freq. of 0 ppm: 202.456310 MHz
processed size: 32768 complex points
LB: 1.000 GF: 0.0000
Hz/cm: 1213.020 ppm/cm: 5.99181

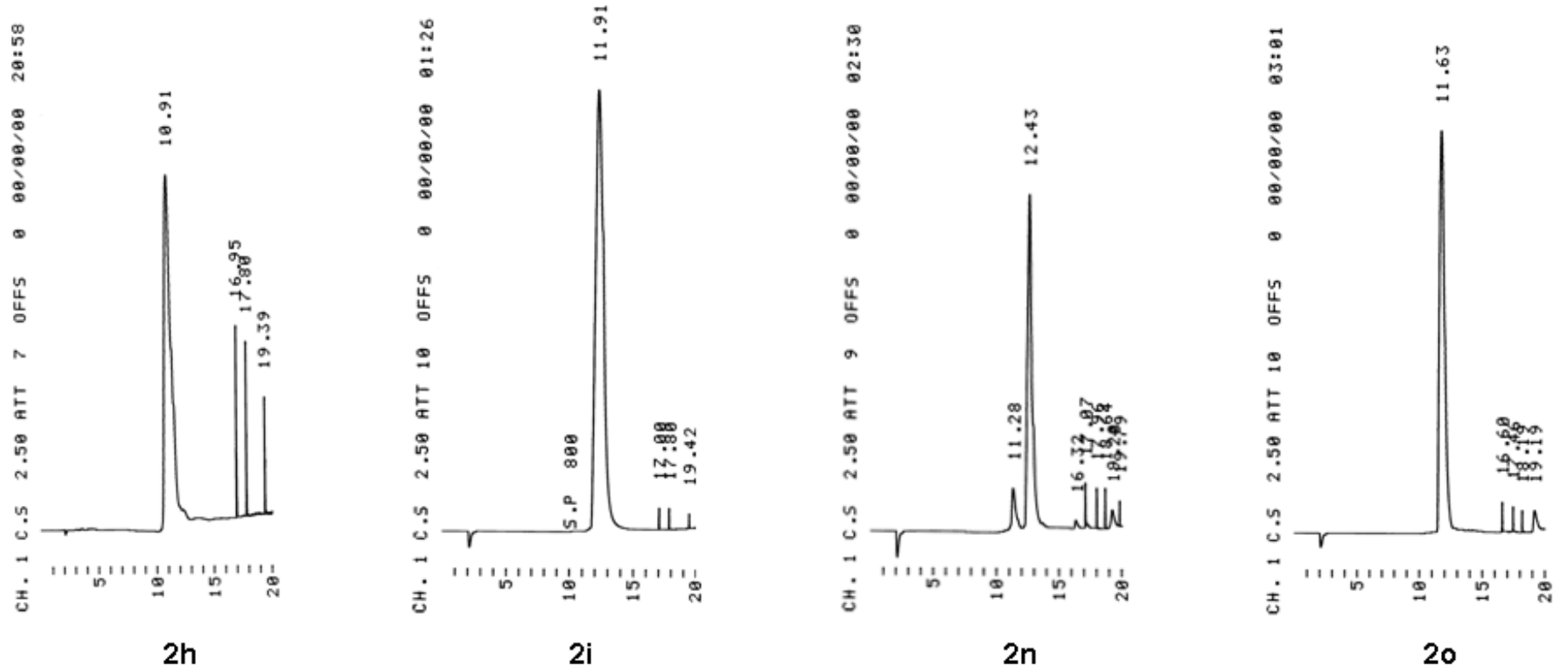

Figure S2. HPLC chromatograms of compounds **2h**, **2i**, **2n** and **2o** [Thermo Hypersil HyPurity C18 column (150 × 4.6 mm, 5 μm); flow rate = 1 mL min^{-1}; 0.1% aq. TFA and a linear gradient of 0→30% of 0.1% TFA in MeCN during 15 min, after which 30% of 0.1% TFA in MeCN for 5 min, λ = 220 nm]. The sharp peaks at the end of each chromatogram are artifacts that appear at random retention times even when no sample is injected. The most probable explanation for these peaks is air from the MeCN line.

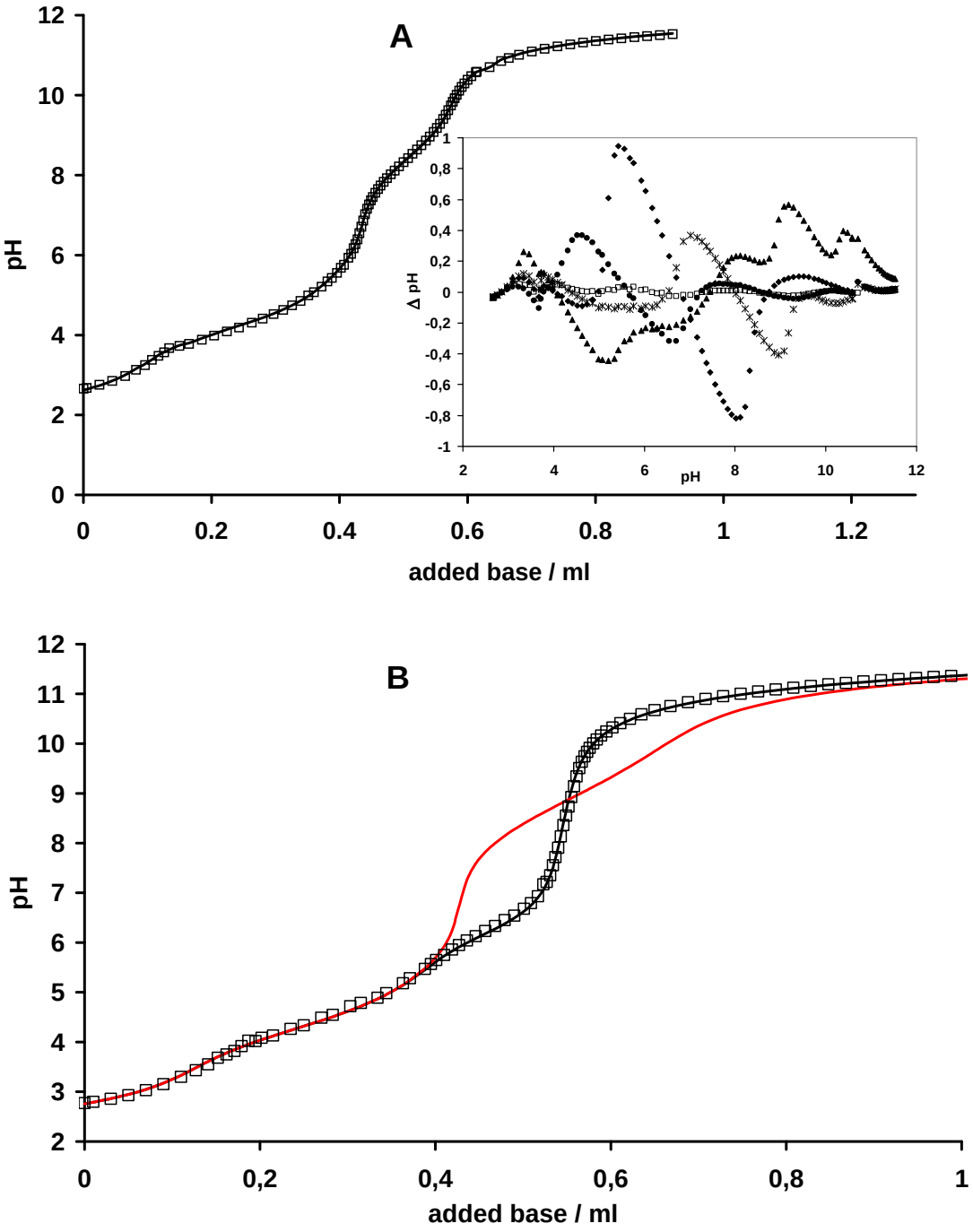

Figure S3. Examples illustrating the excellence of fit of the potentiometric data to the proposed model, as well as the fact that simpler models fail to accurately describe the protonation equilibria prevalent in the system. (A) An example of experimental titration data (□) and the fit (solid line) of the binary system using the model described in the text ($[Zn^{II}]_{TOT} = 2.91\times10^{-3}$ M, $[L]_{TOT} = 1.57\times10^{-3}$ M, $V_0 = 4.55$ cm^3). Insert shows the ΔpH = pH(observed) – pH(calculated) differences of the best fit of the accepted model (□) and when the species Zn_2HL (●), Zn_2L (♦), $Zn_2H_{-1}L$ (∗) or $Zn_2H_{-2}L$ (▲) is excluded from the model. (B) An example of experimental titration data (□) and the fit (solid black line) of the ternary system using the model described in the text ($[Zn^{II}]_{TOT} = 2.30\times10^{-3}$ M, $[L]_{TOT} = 1.29\times10^{-3}$ M, $[mUpU]_{TOT} = 1.22\times10^{-3}$ M, $V_0 = 5$ cm^3). Red line shows the best fit when the ternary $Zn_2L(mUpU)$ species is omitted from the model.