Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Electronic Supplementary Information

Efficient synthesis of 2-nitroimidazole derivatives and the bioreductive clinical candidate Evofosfamide (TH-302).

Liam J. O'Connor,[a,b] Cindy Cazares-Körner,[a,b] Jaideep Saha,[a] Charles N. G. Evans,[a,b] Michael R. L. Stratford,[b] Ester M. Hammond[b] and Stuart J. Conway[a]*

[a]Department of Chemistry, Chemistry Research Laboratory, University of Oxford, Mansfield Road, Oxford, OX1 3TA, UK.

[b]Cancer Research UK/MRC Gray Institute for Radiation Oncology and Biology, Department of Oncology, University of Oxford, Old Road Campus Research Building, Oxford, OX3 7DQ, UK.

*To whom correspondence should be addressed: stuart.conway@chem.ox.ac.uk, telephone: +44 (0)1865 285 109, fax: +44 (0)1865 285 002.

Table of contents

General experimental procedures

^{1}H NMR spectra were recorded on Bruker AVC500, AVX500, DRX500 (500 MHz) or Bruker AV600 (600 MHz) using deuterochloroform (unless indicated otherwise) as a reference for the internal deuterium lock. The chemical shift data for each signal are given as δ_H in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ_H (TMS) = 0.00 ppm. The multiplicity of each signal is indicated by s (singlet); d (doublet); t (triplet); q (quartet); dd (doublet of doublets); or m (multiplet). The number of protons (n) for a given resonance signal is indicated by nH. Coupling constants (*J*) are quoted in Hz and are recorded to the nearest 0.1 Hz. Identical proton coupling constants (*J*) are averaged in each spectrum and reported to the nearest 0.1 Hz. The coupling constants are determined by analysis using Bruker TopSpin software. Spectra were assigned using COSY, HSQC and HMBC experiments as necessary.

^{13}C NMR spectra were recorded on Bruker DRX500, AVC500, AVX600 or AV600 (126 MHz) spectrometers in the stated solvents, with broadband proton decoupling and an internal deuterium lock. The chemical shift data for each signal are given as δ_C in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ_C (TMS) = 0.00 ppm. The shift values of resonances are quoted to 1 decimal place. The multiplicity of each signal is singlet unless indicated by: d (doublet). Where appropriate, coupling constants (*J*) are quoted to the nearest 0.1 Hz, and were determined using Bruker TopSpin software.

^{31}P NMR spectra were recorded on a Bruker AVB400 (162 MHz) or AVX500 (202 MHz) in the stated solvents as a reference for the internal deuterium lock with broadband proton decoupling. The chemical shift data for each signal are given as δ_P in units of parts per million (ppm). Signals are quoted as proton decoupled singlets.

Mass spectra were acquired on a VG platform spectrometer and an Agilent 6120 spectrometer (low resolution). Electro-spray ionisation spectra were obtained on Micromass LCT Premier and Bruker MicroTOF spectrometers, operating in positive or negative mode as

indicated, from solutions of MeOH or MeCN. *m/z* values are reported in Daltons and followed by their percentage abundance in parentheses.

Melting points were determined using a Leica Galen III hot stage microscope and are uncorrected.

Infrared spectra were obtained from neat samples, either as solids or liquids using a diamond ATR module. The spectra were recorded on a Bruker Tensor 27 spectrometer. Absorption maxima are recorded in wavenumbers (cm^{-1}), and reported as s (strong), m (medium), w (weak) or br (broad).

Analytical thin layer chromatography (TLC) was carried out on normal phase Merck silica gel 60 F_{254} aluminium-supported chromatography sheets. Visualisation was by absorption of UV light (λ_{max} 254 and 365 nm).

Flash column chromatography was performed manually using VWR Prolabo silica gel 60 (240-400 mesh) under a positive pressure of nitrogen.

Semi-preparative HPLC was carried out on a Waters system (2695 pump/autosampler, 2996 diode array detector and ZQ2000 mass spectrometer). The column was a Phenomenex Luna C18(2) 10 µm column, 250 x 10 mm, eluents water (A) and methanol (B), with a gradient from 50 – 70% B over 5 min, flow rate 5 ml/min.

In vacuo refers to removal of solvent on a Buchi® rotary evaporator under reduced pressure in a water bath at 40 °C.

Petroleum ether refers to the fraction in the boiling point range 40–60 °C.

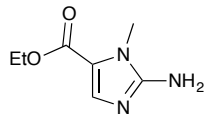
Chemicals were purchased from Sigma Aldrich UK and Alfa Aesar UK, and were used as supplied unless otherwise stated.

Compound purity for compounds was determined by elemental analysis or HPLC. Elemental analysis was obtained at the Elemental Analysis Service, London Metropolitan University, London. Elemental analysis was carried out in duplicate; average values are

reported in Supporting Information. For all tested compounds, experimentally determined hydrogen, carbon, and nitrogen composition was within 0.4% of the expected value, implying a purity of >95%.

HPLC was carried out on a Waters system (2695 pump/autosampler, 2996 diode array detector and ZQ2000 mass spectrometer). The column was a Hichrom RPB, 5 µm, 100 x 3.2 mm, and the eluents were 10 mM formic acid (A), and acetonitrile (B), with a gradient of 35 – 95% B in 5 min, flow rate 0.5 mL/min.

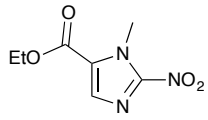
Ethyl 2-amino-1-methyl-1*H*-imidazole-5-carboxylate (4)



4

To a suspension of sarcosine ethyl ester hydrochloride (**5**, 2 g, 0.013 mol, 1 eq.) in ethyl formate (45 mL) and THF (45 mL), was added NaH (60% dispersion in mineral oil, 5 g, 0.13 mol, 10 eq.) slowly at ambient temperature, and allowed to stir for 3 h. After this time a yellow suspension had formed, and the mixture was concentrated and dried *in vacuo*. The resulting solid was triturated with hexane (2 × 75 mL), the hexane decanted, and the remaining solid dried *in vacuo*. EtOH (40 mL) and concentrated aqueous HCl (8 mL) were added to the solid, and the suspension heated under reflux for 2 h. The reaction mixture was filtered while hot, and the resulting colourless solid washed with boiling EtOH (2 × 30 mL). The filtrate was concentrated *in vacuo* to leave an aqueous solution, which was diluted with EtOH (70 mL) and distilled water (30 mL). The pH of the solution was adjusted to 3, using an aqueous 2 M solution of NaOH, and cyanamide (1.09 g, 0.026 mol, 2 eq.) was added. The resulting solution was heated under reflux for 1.5 h. After this time the solution was cooled to ambient temperature, and concentrated *in vacuo* to approximately $^1/_8$ of the original volume. The pH was then adjusted to 8-9 using solid K_2CO_3, resulting in the formation of a precipitate. The solid was removed by filtration, washed with aqueous K_2CO_3 solution (1 M, 1 × 20 mL), H_2O (2 × 10 mL), and dried *in vacuo*, to afford **4** as a pale yellow solid (1.10 g, 50%): (found C, 35.2; H, 2.8; N 24.4. $C_7H_{11}N_3O_2$ requires C, 35.1; H, 3.0; N, 24.6); R_f 0.42 (CH_2Cl_2 - MeOH, 9:1); mp 130-133 °C (from H_2O); ν_{max} (solid)/cm^{-1} 3126 (w), 1648 (s), 1246 (m), 1167 (s); δ_H ($CDCl_3$, 500 MHz) 7.44 (1H, s, C*H*), 4.41 (2H, s, N*H*$_2$), 4.27 (2H, q, *J* 7.1, C*H*$_2$CH$_3$), 3.68 (3H, s, C*H*$_3$), 1.34 (3H, t, *J* 7.1, CH$_2$C*H*$_3$); δ_C ($CDCl_3$, 126 MHz) 160.7, 152.1, 135.5, 119.1, 59.9, 30.6, 14.5; HRMS *m/z* (ESI$^+$) [found $(M+H)^+$ 170.0919, $C_7H_{11}N_3O_2$ requires M^+ 170.0924]; *m/z* (ES$^+$) 170.1 ($[M+H]^+$, 100 %). These data are in good agreement with the literature values.[1]

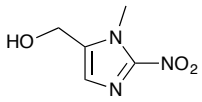
Ethyl 1-methyl-2-nitro-1*H*-imidazole-5-carboxylate (7)



7

To an aqueous solution of sodium nitrite (5 mL, 3.95 g, 57.24 mmol, 10 eq.), was added aminoimidazole **4** (0.97 g, 5.72 mmol) in acetic acid (10 mL) in a drop wise manner. The solution was stirred at room temperature for 4 h, after this time no more nitrogen gas was evolved. The organic mixture was extracted with CH_2Cl_2 (1 × 20 mL), washed with brine (1 × 20 mL) and a saturated aqueous solution of Na_2SO_3 (1 × 20 mL), dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo*, the residue adsorbed onto Celite®, and the product purified by silica gel column chromatography, eluting with CH_2Cl_2, to afford **7** (826 mg, 72 %) as a yellow solid: (found C, 42.3; H, 4.4; N, 21.0. $C_7H_9N_3O_4$ requires C, 42.2; H, 4.6; N, 21.1); R_f 0.85 (CH_2Cl_2 - MeOH, 9:1); mp 56-58 °C (from CH_2Cl_2) [lit.[2] mp 65-66 °C]; ν_{max} (solid)/cm^{-1} 2983, 1723, 1519, 1281, 1234; δ_H ($CDCl_3$, 500 MHz) 7.76 (1H, s, C*H*), 4.40 (2H, q, *J* 7.3, C*H*$_2$CH$_3$), 4.35 (3H, s, C*H*$_3$), 1.41 (3H, t, *J* 7.3, CH$_2$C*H*$_3$); δ_C ($CDCl_3$, 126 MHz) 159.1, 134.6, 126.3, 61.8, 35.4, 14.1; HRMS *m/z* (ESI^-) [found $(M+Na)^+$ 222.0480, $C_7H_9N_3NaO_4$ requires M^+ 222.0485]; *m/z* (ES^+) 222.1 ($[M+Na]^+$, 100 %). These data are in good agreement with the literature values.[2]

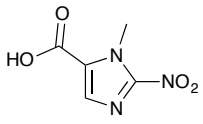
(1-Methyl-2-nitro-1*H*-imidazol-5-yl)methanol (8)



8

To a solution of nitroimidazole **7** (230 mg, 1.16 mmol, 1 eq.) in anhydrous THF (6 mL) and MeOH (0.5 mL) at 0 °C, was added sodium borohydride (131 mg, 3.47 mmol, 3 eq.) portion wise. The reaction mixture was stirred at 0 °C for 45 min, and then at ambient temperature for 1 h. The reaction mixture was cooled to 0 °C, quenched by addition of ice and the pH adjusted to 7 using 1 M aqueous HCl. The aqueous mixture was saturated with solid NaCl, and the organic components extracted with EtOAc (5 × 15 mL). The organic layers were combined, washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo*, the residue adsorbed onto Celite®, and the product purified by silica gel column chromatography, eluting with petroleum ether and EtOAc (gradient; 50-100% EtOAc) to afford **8** as pale yellow crystals (109 mg, 66%): (found C, 38.2; H, 4.5; N 26.6. $C_5H_7N_3O_3$ requires C, 38.2; H, 4.5; N, 26.7); R_f 0.5 (CH_2Cl_2 - MeOH, 9:1); mp 141-143 °C (from EtOAc) [lit.[2] mp 142-144 °C]; ν_{max} (solid)/cm^{-1} 3228 (br), 1490 (s), 1396 (s), 1038 (s); δ_H (DMSO-D_6, 400 MHz) 7.12 (1H, s, C*H*), 5.50 (1H, t, *J* 5.4, O*H*), 4.54 (2H, d, *J* 5.4, CH_2), 3.92 (3H, s, CH_3); δ_C (DMSO-D_6, 126 MHz) 146.7, 138.6, 126.5, 53.0, 34.1; HRMS *m/z* (ES+) [found $(M-H)^-$ 156.0412, $C_5H_6N_3O_3$ requires M^- 156.0414]; *m/z* (ESI^-) 156.04 ($[M-H]^-$, 100%). These data are in good agreement with the literature values.[2]

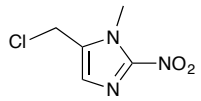
1-Methyl-2-nitro-1*H*-imidazole-5-carboxylic acid (9)



9

To nitroimidazole **7** (100 mg, 0.50 mmol, 1 eq.), was added a 0.75 M aqueous solution of NaOH (2 mL), and the resulting suspension was stirred for 2 h at room temperature, after which time a homogenous solution had formed. The reaction solution was acidified to pH 1 using conc. HCl, resulting in the formation of an off-white precipitate. The organic components were extracted with EtOAc (5 × 15 mL), the organic layers combined, dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo*, to afford **9** as an off-white solid (82 mg, 95 %): (found C, 35.2; H, 2.8; N 24.4. $C_5H_5N_3O_4$ requires C, 35.1; H, 3.0; N, 24.6); R_f 0.15 (AcOH-CH_2Cl_2-MeOH, 0.1:9:1); mp 155-157 °C (from $CHCl_3$) [lit.[2] mp 161-163 °C]; ν_{max} (solid) cm^{-1}: 2924 (br), 1712 (s), 1497 (s), 1362 (s), 1236 (s); δ_H (MeOD-D_4, 500 MHz) 7.73 (1H, s, C*H*), 4.33 (3H, s, CH_3); δ_C (MeOD-D_4, 126 MHz) 161.6, 149.0, 134.8, 128.5, 35.8; HRMS *m/z* (ESI^-) [found; $(M-H)^-$ 170.0207, $C_5H_4N_3O_4$ requires M^- 170.0207]; *m/z* (ESI^-) 170.02 ($[M-H]^-$, 68%). These data are in good agreement with the literature values.[2]

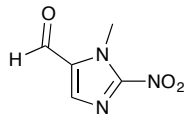
5-(Chloromethyl)-1-methyl-2-nitro-1*H*-imidazole (10)



10

To a solution of nitroimidazole **8** (200 mg, 1.27 mmol, 1 eq.) in dry CH_2Cl_2 (8 mL) and distilled pyridine (0.30 ml, 3.63 mmol, 2.9 eq.), was added $SOCl_2$ (0.28 mL, 3.90 mmol, 3 eq.) in dry CH_2Cl_2 (2 mL) drop wise at 0 °C. The reaction solution was stirred at 0 °C for 1 h, followed by 1 h at room temperature. The excess $SOCl_2$ was quenched by addition of ice, and saturated aqueous $NaHCO_3$ solution (5 mL) was added. The organic mixture was extracted with CH_2Cl_2 (2 × 15 mL), the organic layers combined, washed with brine (30 mL), dried over $MgSO_4$, and filtered. The filtrate was concentrated *in vacuo*, the residue adsorbed onto Celite®, and the product purified by silica gel column chromatography, eluting with petroleum ether and EtOAc (gradient; 50-100% EtOAc) to afford **10** as pale yellow crystals (147 mg, 66%): (found C, 34.3; H, 3.5; N 23.8. $C_5H_6ClN_3O_2$ requires C, 34.2; H, 3.4; N, 23.9); R_f 0.4 (petroleum ether - EtOAc, 1:1); mp 87-90 °C (from EtOAc) [lit.[3] mp 94-96 °C]; ν_{max} (solid) cm^{-1}: 1483 (s), 1360 (s); δ_H ($CDCl_3$, 500 MHz) 7.17 (1H, s, C*H*), 4.63 (2H, s, CH_2), 4.06 (3H, s, CH_3); δ_C ($CDCl_3$, 126 MHz) 146.3, 132.9, 128.5, 34.2, 33.9; HRMS *m/z* (ESI^+) [found $(M+Na)^+$ 198.0041, $C_5H_6ClN_3NaO_2$ requires M^+ 198.0041]; *m/z* (ESI^+) 198.1 ($[M+Na]^+$, 100%). These data are in good agreement with the literature values.[3]

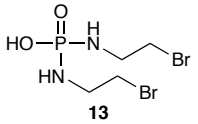
1-Methyl-2-nitro-1*H*-imidazole-5-carbaldehyde (11)



11

To a solution of nitroimidazole **8** (50 mg, 0.32 mmol, 1 eq.) in acetone (0.3 mL) and CH_2Cl_2 (2.7 mL), was added manganese(IV) dioxide (157 mg, 1.8 mmol, 6 eq.), and the reaction solution was stirred at ambient temperature for 3 d, under an argon atmosphere. The reaction suspension was filtered through Celite®, and washed with CH_2Cl_2 (10 mL). The elutant was adsorbed onto Celite®, and the product purified using silica gel column chromatography eluting with acetone and petroleum ether (1:4), to afford the carbaldehyde **11** as a colourless solid (25 mg, 52%): R_f 0.72 (acetone - petroleum ether, 1:1); mp 113-115 °C (from acetone) [lit.[2] mp 114-115 °C]; ν_{max} (solid)/cm^{-1} 1681 (s), 1490 (s), 1327 (s); δ_H ($CDCl_3$, 500 MHz) 9.93 (1H, s, C*H*O), 7.82 (1H, s, C*H*), 4.36 (3H, s, CH_3); δ_C ($CDCl_3$, 126 MHz) 180.7, 148.6, 139.8, 132.7, 35.9; HRMS *m/z* (ESI^+) [found $(M+MeOH+Na)^+$ 210.0484, $C_6H_9N_3NaO_4$ requires M^+ 210.0485]; *m/z* (ES^+) 210.1 ($[M+MeOH+Na]^+$, 100 %). These data are in good agreement with the literature values.[2]

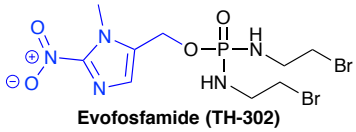
***N,N'*-bis(2-Bromoethyl)phosphorodiamidic acid (Br-IPM, 13)**



13

Following the procedure of Duan *et al.*[4]. To a vigorously stirring suspension of 2-bromoethylamine hydrobromide (12.6 g, 62 mmol, 1 eq.) in CH_2Cl_2 (110 mL), was added $POCl_3$ (2.84 mL, 31 mmol, 0.5 eq.) drop wise, while maintaining the temperature between −10 °C and −15 °C. A solution of triethylamine (17.5 mL, 124 mmol, 2 eq.) in CH_2Cl_2 (30 mL) was then added, drop wise, over a period of 30 min, while maintaining the temperature between −10 °C and −15 °C. The reaction mixture was then filtered, and the filtrate was concentrated *in vacuo* to a volume of approximately 30 mL. The residue was filtered, the combined solids were washed with cold CH_2Cl_2, and the filtrate concentrated and dried *in vacuo*. The residue was dissolved in THF (7 mL), H_2O added (10 mL), and the mixture stirred at room temperature for 2 h. The THF was removed under a flow of N_2 gas, and the remaining aqueous solution cooled to 4 °C overnight. The resulting solid was filtered, and washed with cold H_2O (5 mL) and diethyl ether (5 mL) to afford **13** as a colourless solid that was contaminated with its triethylamine salt: mp 109-112 °C (from H_2O) [lit. mp 134-135 °C]; ν_{max} (solid) cm^{-1}: 3305 (w), 1249 (s), 1126 (s); δ_H (DMSO-D_6, 600 MHz) 3.43 (t, 4H, *J* 7.2, CH_2Br), 3.08 (dt, 4.37H, *J* 12.2, 7.2, NCH_2/CH_3CH_2N), 1.19 (0.7H, t, *J* 7.3, C$H_3$$CH_2$N); δ_C (DMSO-D_6, 126 MHz) 45.7, 43.0, 34.0 (d, *J* 4.4, *C*H_2Br), 8.6; δ_P (DMSO-D_6, 162 MHz) 11.9. The ^{1}H NMR data are in good agreement with the literature values.[5]

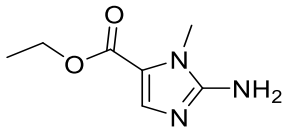
(1-Methyl-2-nitro-1*H*-imidazol-5-yl)-*N*,*N*–bis(2-bromoethyl) phosphordiamidate (TH-302)



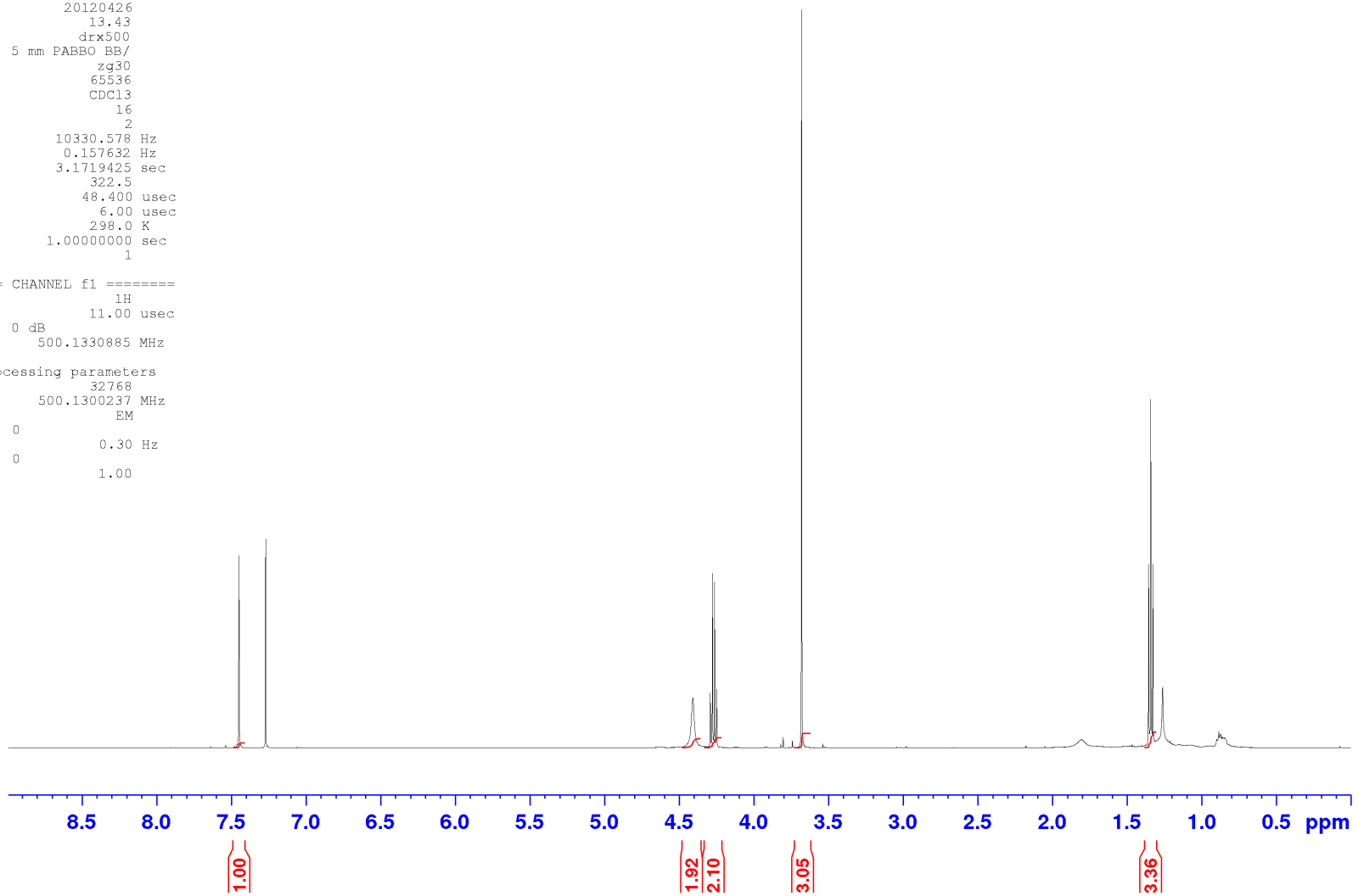
Evofosfamide (TH-302)

To a suspension of Br-IPM (78 mg, 0.25 mmol, 1 eq.), was added nitroimidazole alcohol **8** (80 mg, 0.50 mmol, 2 eq.), and triphenylphosphine (133 mg, 0.50 mmol, 2 eq.) in THF (7 mL), and DIAD (100 µL, 101 mg, 0.50 mmol, 2 eq.) at 0 °C. The reaction mixture was stirred at room temperature for 3 h, the solvent removed under a flow of N_2 gas, and the resulting residue dried *in vacuo*. The residue was then purified by semi-preparative HPLC on a Phenomenex Luna (C18(2), 10 µm, 250 × 10 mm) column, eluting with H_2O and methanol (50 – 70% methanol over 10 min, then 1 min wash with methanol, 5 mL/min flow rate) to afford **TH-302** as a yellow gum: ν_{max} (solid) cm^{-1}: 3212 (br), 1489 (m), 1350 (m), 1105 (m), 1004 (s); δ_H (DMSO-D_6, 400 MHz) 7.25 (1H, s, C*H*), 5.10–4.90 (2H, m, N*H*CH_2CH_2Br), 4.98 (2H, d, *J* 7.8, CH_2O), 3.94 (3H, s, CH_3), 3.42 (4H, t, *J* 7.0, NHCH_2CH_2Br), 3.11 (4H, dt, *J* 9.8, 7.2, NHCH_2CH_2Br); δ_C (DMSO-D_6, 126 MHz) 146.1, 134.2 (d, *J* 7.5, OCH_2CN), 128.2, 55.6 (d, *J* 4.6, CH_2O), 42.7, 34.2 (d, *J* 26.4, CH_2Br), 34.1; δ_P (DMSO-D_6, 202 MHz) 15.4; HRMS *m/z* (ESI^-) [found; $(M-H)^-$ 447.9216, $C_9H_{16}{}^{79}Br^{81}BrN_5O_4P$ requires $(M-H)^-$ 447.9213]; *m/z* (ESI^+) 448.0 ($[M-H]^-$, 60%, $[C_9H_{15}{}^{79}Br^{81}BrN_5O_4P]^-$), 493.9 ($[M+formate]^-$, 100%, $[C_{10}H_{17}{}^{79}Br^{81}BrN_5O_6P]^-$). These data are in good agreement with the literature values.[4]

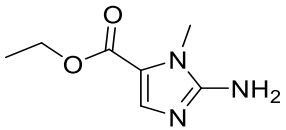
Ethyl 2-amino-1-methyl-1*H*-imidazole-5-carboxylate (**4**)



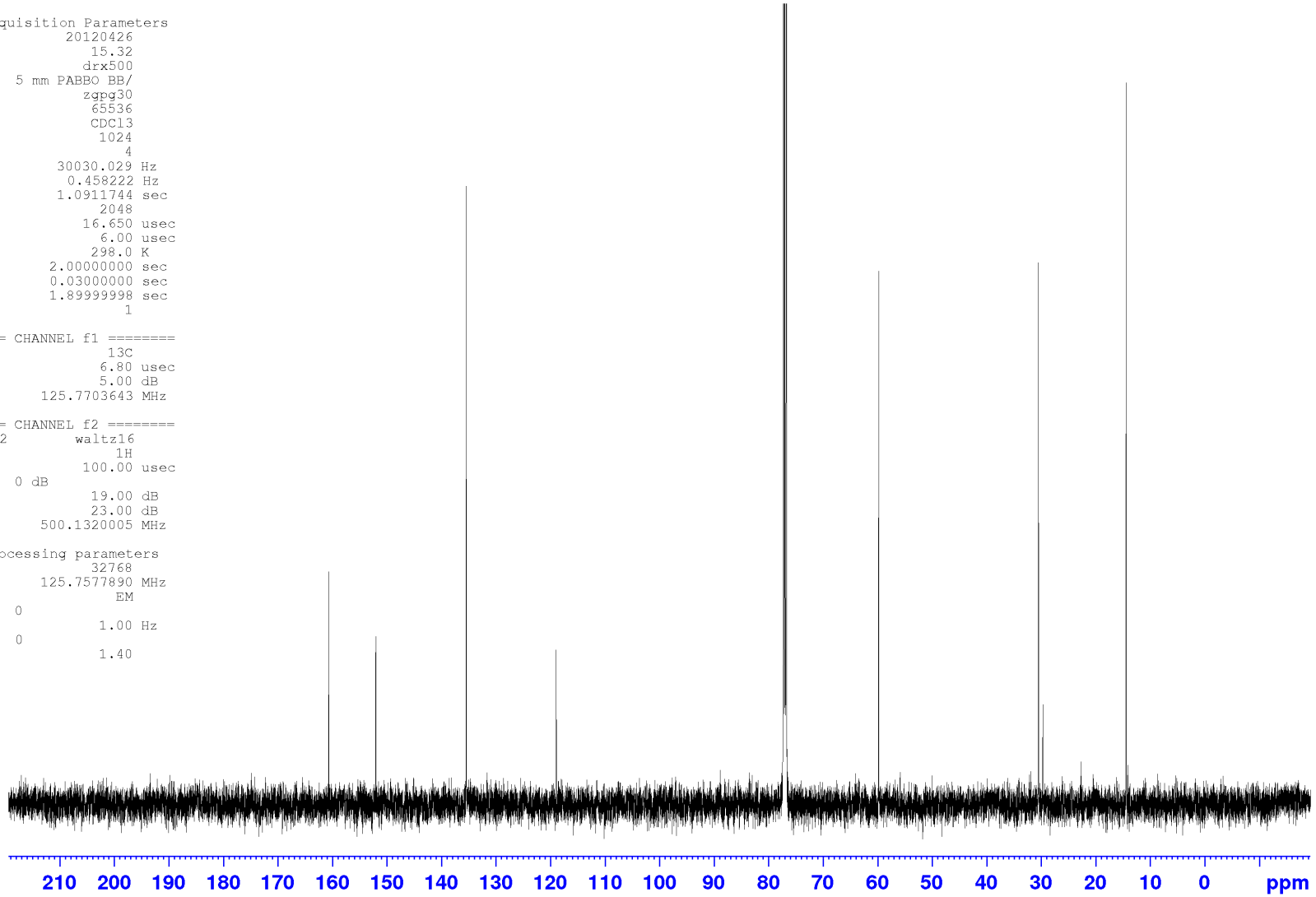

```
Current Data Parameters
NAME      ethyl 2-amino-1-methyl-1H-imidazole-5-carboxylate
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20120426
Time              13.43
INSTRUM          drx500
PROBHD   5 mm PABBO BB/
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                322.5
DW               48.400 usec
DE                6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                  1H
P1                11.00 usec
PL1       0 dB
SFO1         500.1330885 MHz

F2 - Processing parameters
SI                32768
SF           500.1300237 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```



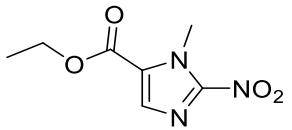
Ethyl 2-amino-1-methyl-1*H*-imidazole-5-carboxylate (**4**)



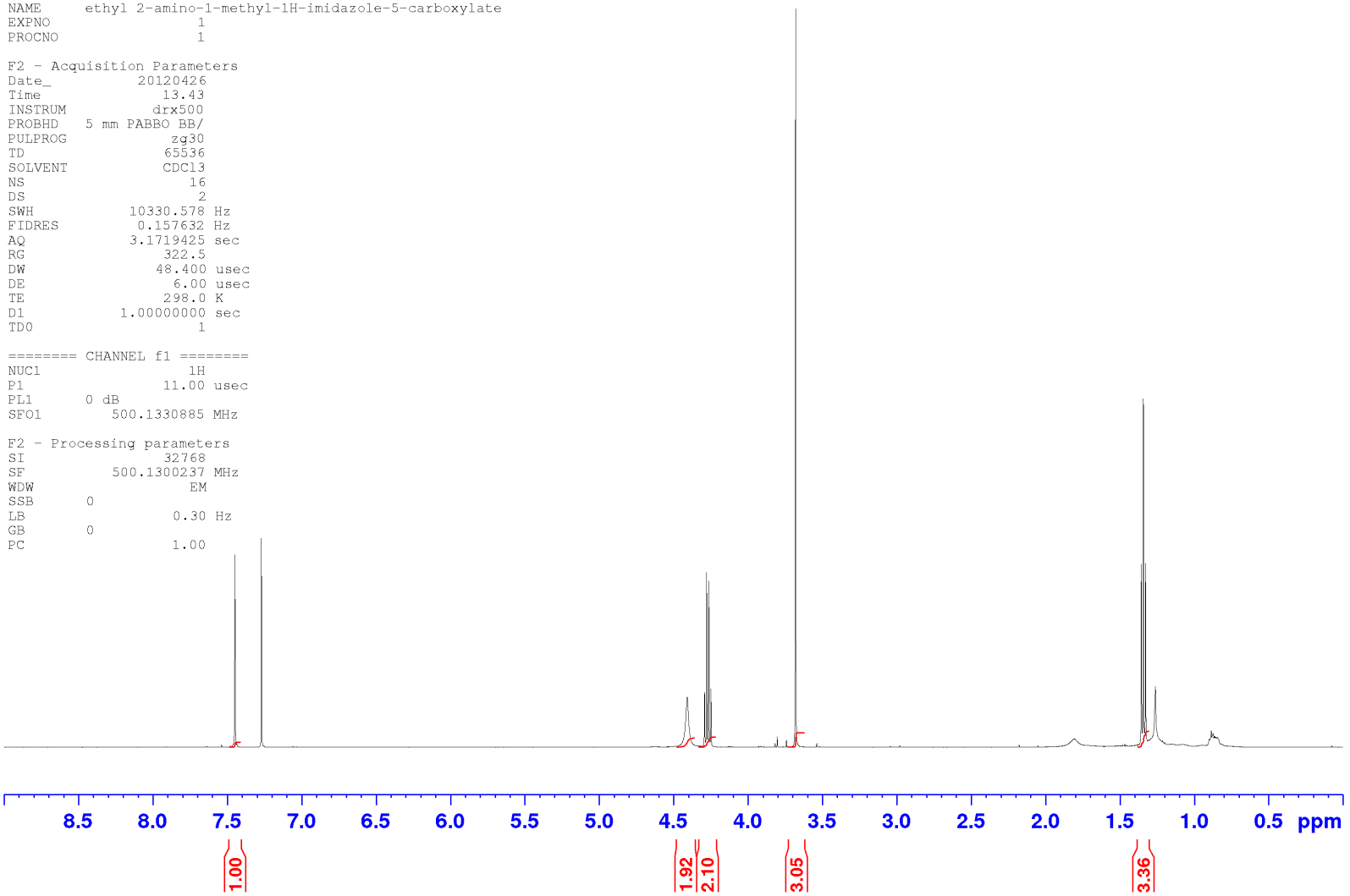

```
Current Data Parameters
NAME     ethyl 2-amino-1-methyl-1H-imidazole-5-carboxylate
EXPNO                 5
PROCNO                1

F2 - Acquisition Parameters
Date_          20120426
Time              15.32
INSTRUM          drx500
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    4
SWH           30030.029 Hz
FIDRES         0.458222 Hz
AQ            1.0911744 sec
RG                 2048
DW               16.650 usec
DE                6.00 usec
TE               298.0 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                  6.80 usec
PL1                 5.00 dB
SFO1        125.7703643 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             100.00 usec
PL2      0 dB
PL12                19.00 dB
PL13                23.00 dB
SFO2        500.1320005 MHz

F2 - Processing parameters
SI                 32768
SF          125.7577890 MHz
WDW                   EM
SSB      0
LB                  1.00 Hz
GB       0
PC                  1.40
```



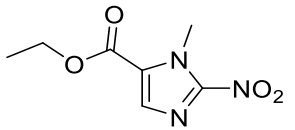
Ethyl 2-nitro-1-methyl-1*H*-imidazole-5-carboxylate (**7**)



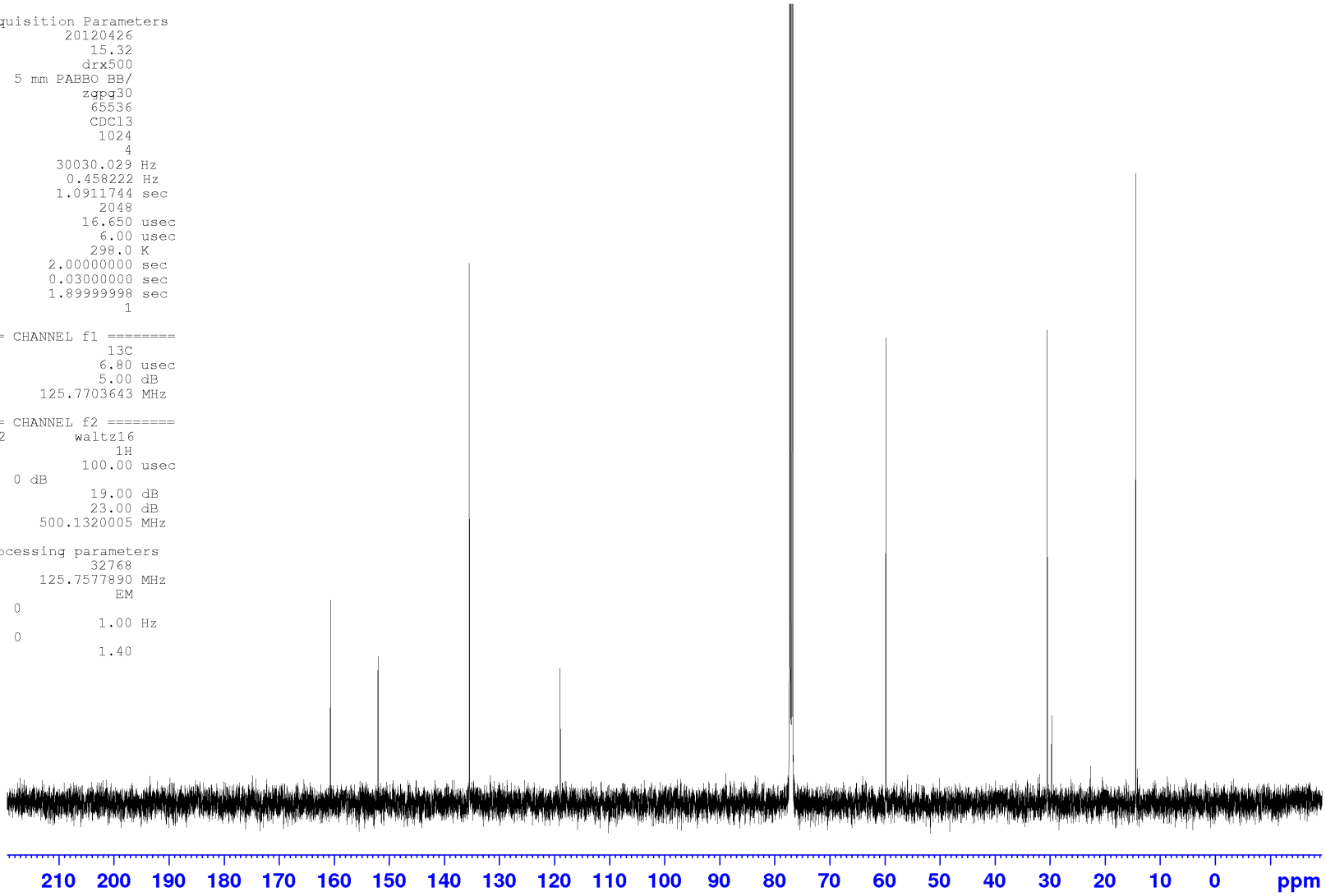

```
Current Data Parameters
NAME      ethyl 2-amino-1-methyl-1H-imidazole-5-carboxylate
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20120426
Time              13.43
INSTRUM          drx500
PROBHD   5 mm PABBO BB/
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                322.5
DW               48.400 usec
DE                6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                  1H
P1                11.00 usec
PL1       0 dB
SFO1         500.1330885 MHz

F2 - Processing parameters
SI                32768
SF          500.1300237 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```



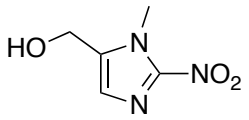
Ethyl 2-nitro-1-methyl-1*H*-imidazole-5-carboxylate (**7**)



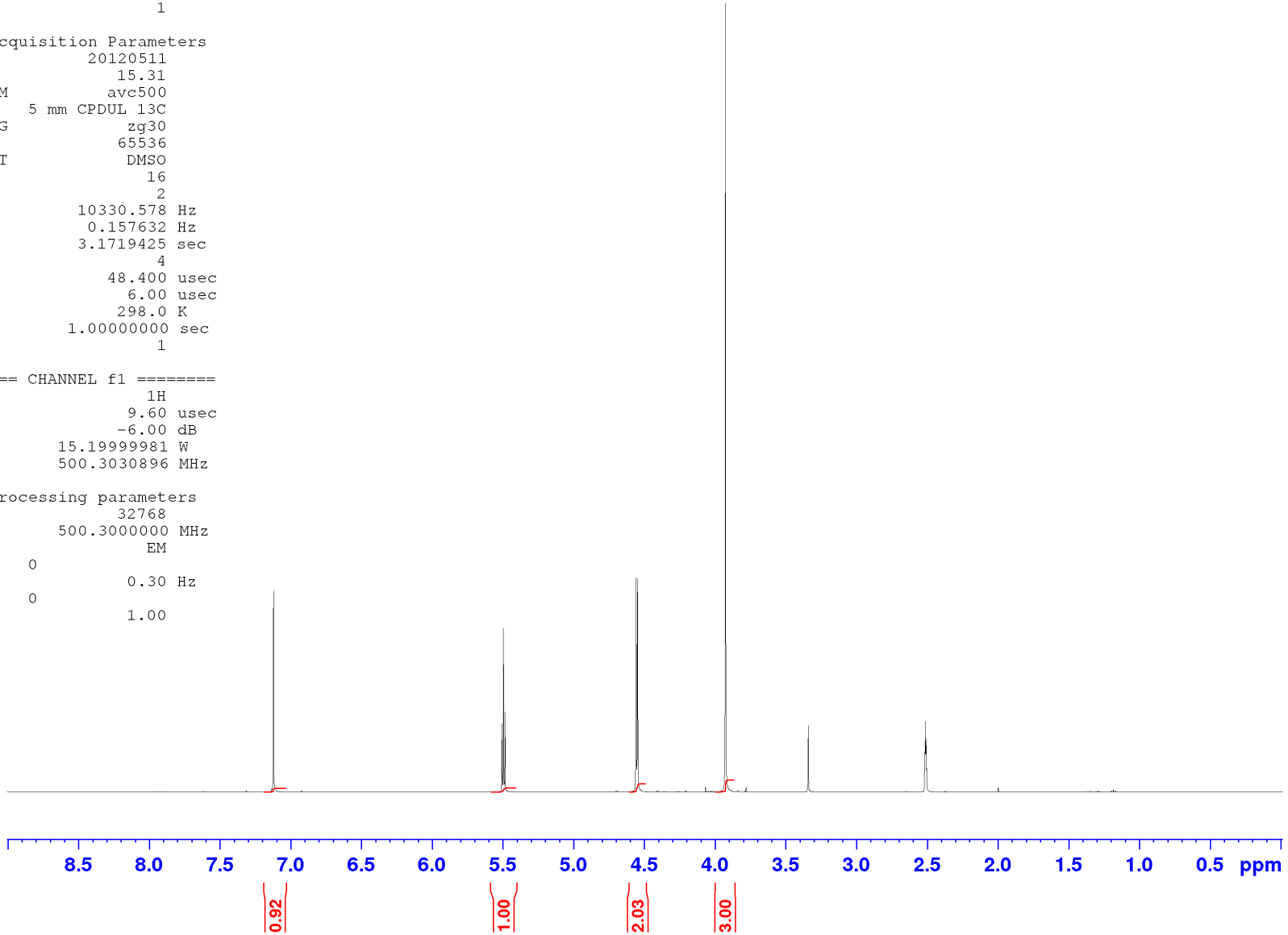

```
Current Data Parameters
NAME     ethyl 2-amino-1-methyl-1H-imidazole-5-carboxylate
EXPNO                 5
PROCNO                1

F2 - Acquisition Parameters
Date_          20120426
Time              15.32
INSTRUM          drx500
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    4
SWH           30030.029 Hz
FIDRES         0.458222 Hz
AQ            1.0911744 sec
RG                 2048
DW                16.650 usec
DE                 6.00 usec
TE                298.0 K
D1           2.00000000 sec
d11          0.03000000 sec
DELTA        1.89999998 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                  6.80 usec
PL1                 5.00 dB
SFO1        125.7703643 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             100.00 usec
PL2      0 dB
PL12                19.00 dB
PL13                23.00 dB
SFO2        500.1320005 MHz

F2 - Processing parameters
SI                  32768
SF          125.7577890 MHz
WDW                    EM
SSB      0
LB                  1.00 Hz
GB       0
PC                  1.40
```



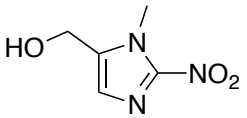
(1-Methyl-2-nitro-1*H*-imidazol-5-yl)methanol (**8**)



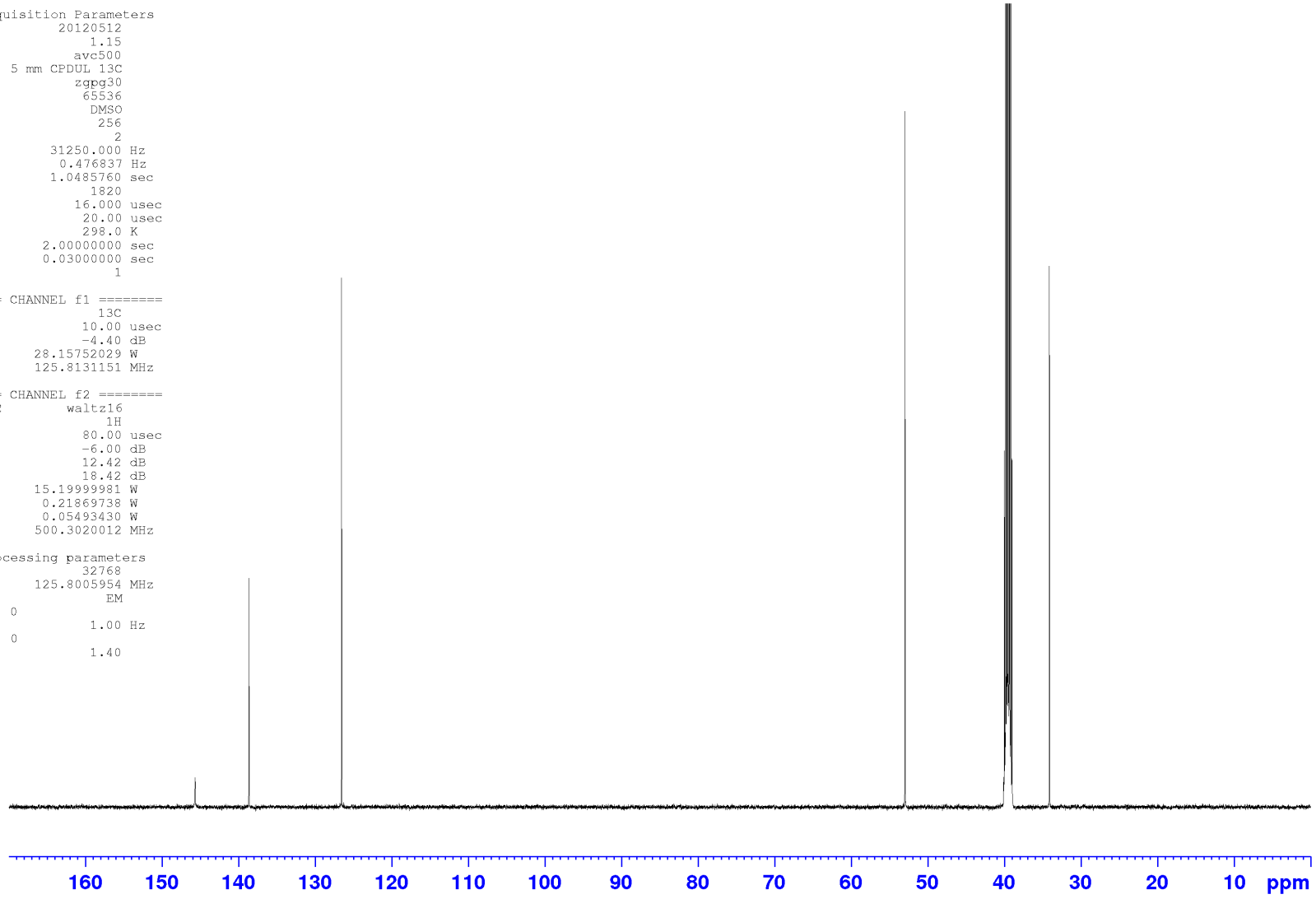

```
Current Data Parameters
NAME      (1-methyl-2-nitro-1H-imidazol-5-yl)methanol
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20120511
Time               15.31
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                    16
DS                     2
SWH            10330.578 Hz
FIDRES          0.157632 Hz
AQ             3.1719425 sec
RG                     4
DW                48.400 usec
DE                 6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                  9.60 usec
PL1                -6.00 dB
PL1W        15.19999981 W
SFO1        500.3030896 MHz

F2 - Processing parameters
SI                 32768
SF          500.3000000 MHz
WDW                   EM
SSB      0
LB                 0.30 Hz
GB       0
PC                 1.00
```



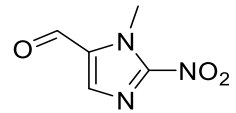
(1-Methyl-2-nitro-1*H*-imidazol-5-yl)methanol (**8**)



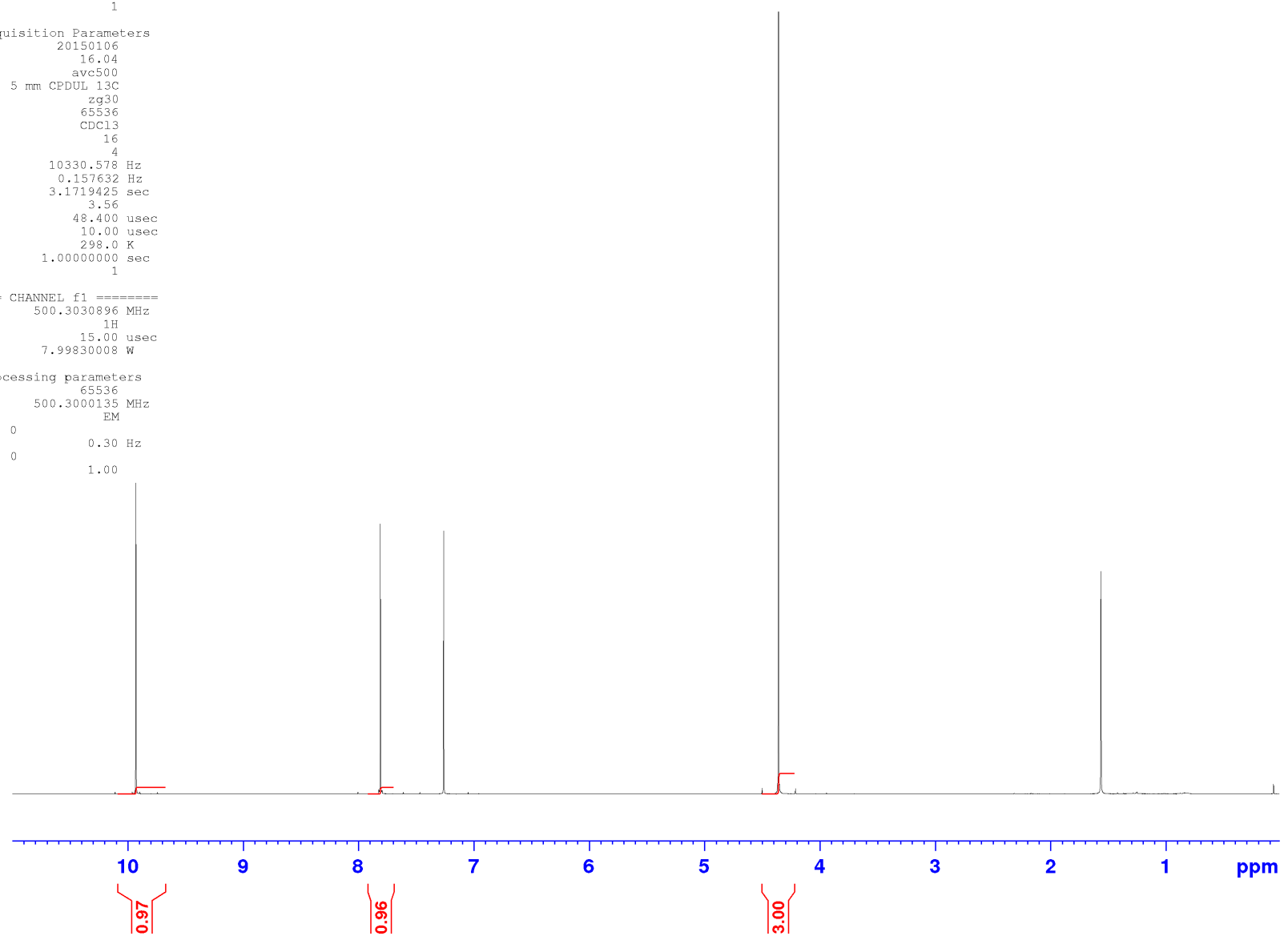

```
Current Data Parameters
NAME      (1-methyl-2-nitro-1H-imidazol-5-yl)methanol
EXPNO                  4
PROCNO                 1

F2 - Acquisition Parameters
Date_           20120512
Time                1.15
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG           zgpg30
TD                 65536
SOLVENT             DMSO
NS                   256
DS                     2
SWH            31250.000 Hz
FIDRES          0.476837 Hz
AQ             1.0485760 sec
RG                  1820
DW                16.000 usec
DE                20.00 usec
TE                298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                 13C
P1                 10.00 usec
PL1                -4.40 dB
PL1W         28.15752029 W
SFO1         125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2          waltz16
NUC2                 1H
PCPD2              80.00 usec
PL2                -6.00 dB
PL12               12.42 dB
PL13               18.42 dB
PL2W         15.19999981 W
PL12W         0.21869738 W
PL13W         0.05493430 W
SFO2         500.3020012 MHz

F2 - Processing parameters
SI                 32768
SF           125.8005954 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```



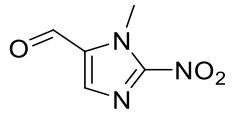
1-Methyl-2-nitro-1*H*-imidazole-5-carbaldehyde (**11**)



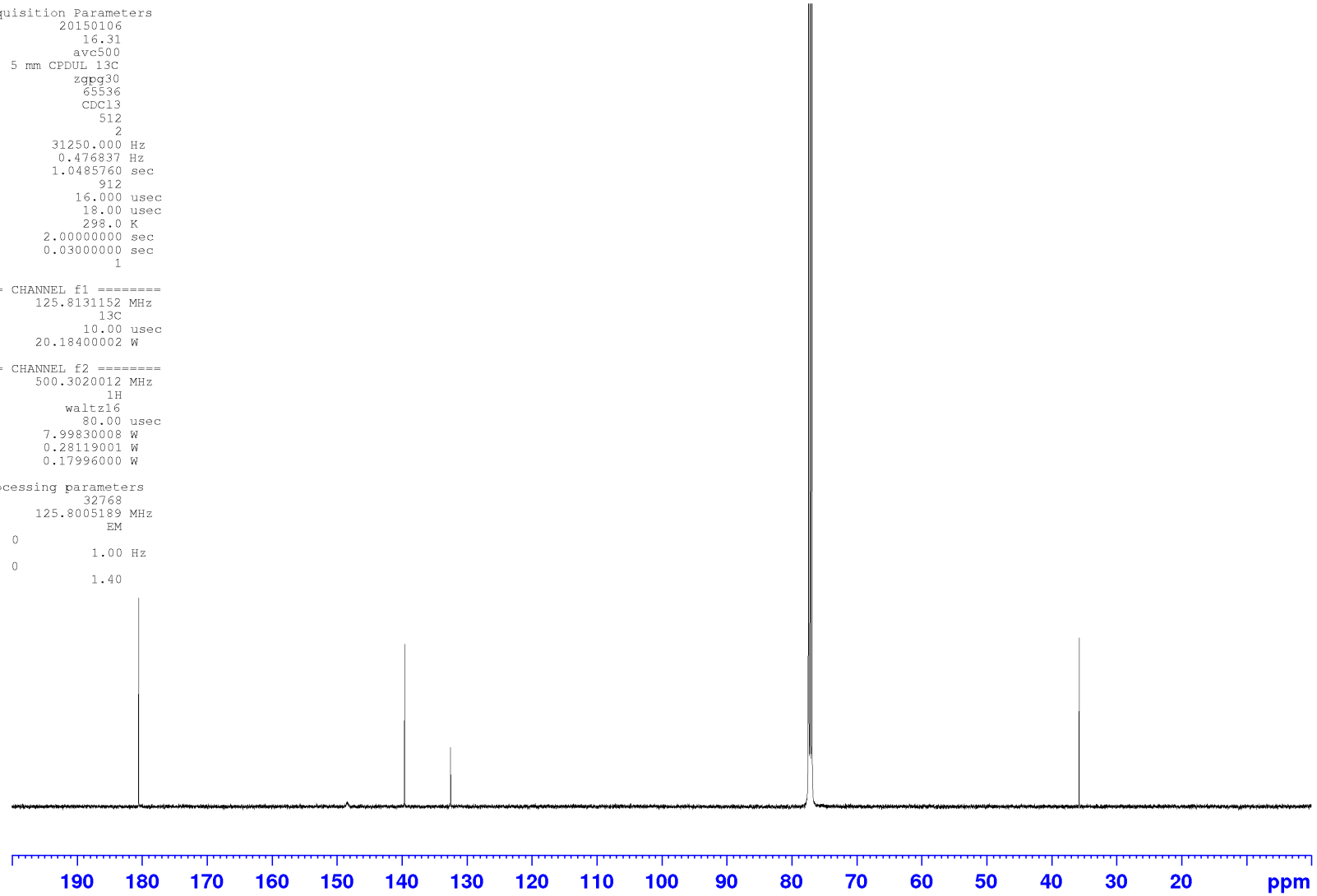

```
Current Data Parameters
NAME      1-methyl-2-nitro-1H-imidazole-5-carbaldehyde
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20150106
Time              16.04
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     4
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                  3.56
DW                48.400 usec
DE                10.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         500.3030896 MHz
NUC1                   1H
P1                 15.00 usec
PLW1          7.99830008 W

F2 - Processing parameters
SI                 65536
SF           500.3000135 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```



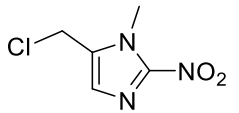
1-Methyl-2-nitro-1*H*-imidazole-5-carbaldehyde (**11**)



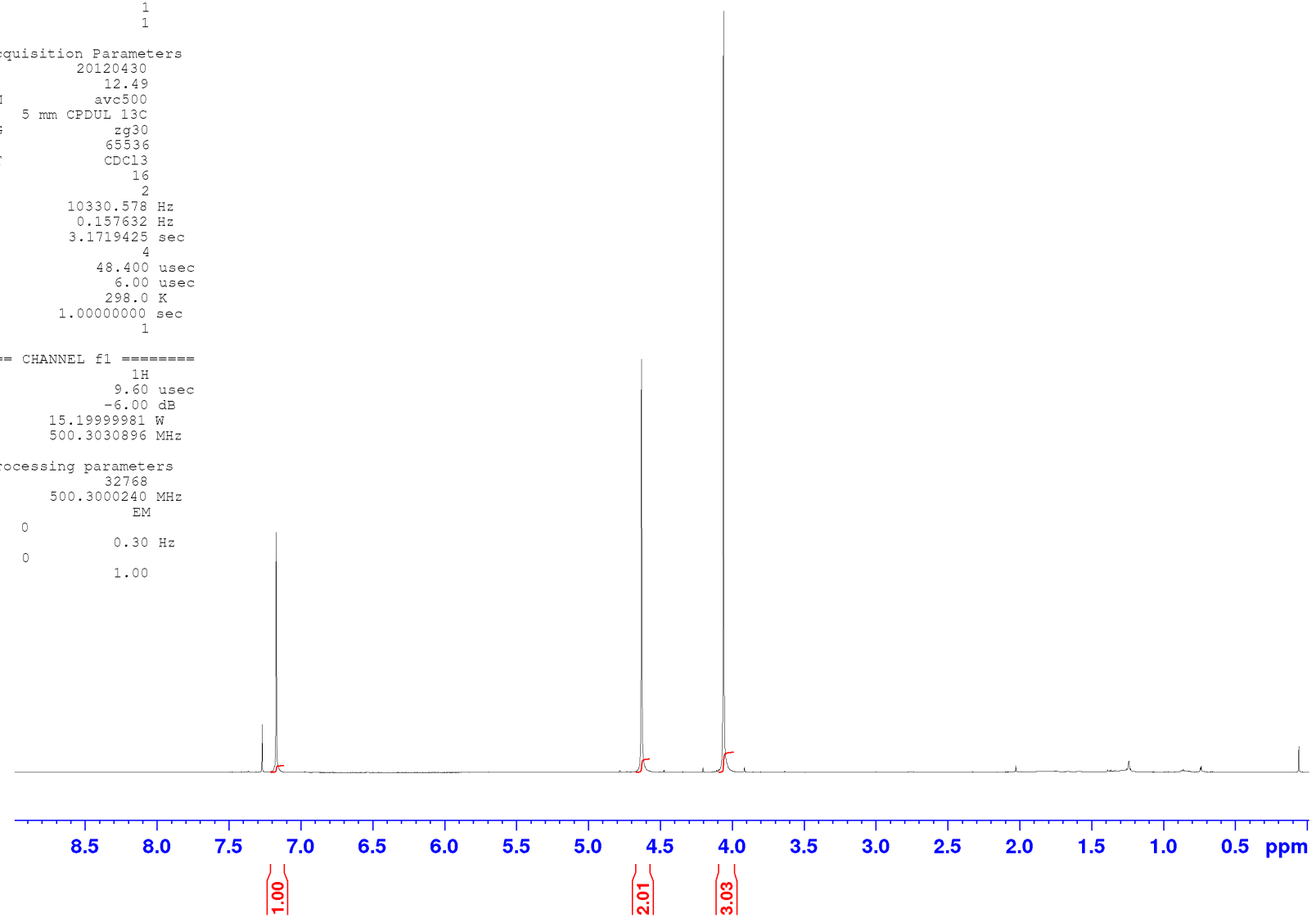

```
Current Data Parameters
NAME      1-methyl-2-nitro-1H-imidazole-5-carbaldehyde
EXPNO                   2
PROCNO                  1

F2 - Acquisition Parameters
Date_            20150106
Time                16.31
INSTRUM           avc500
PROBHD    5 mm CPDUL 13C
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                   512
DS                     2
SWH            31250.000 Hz
FIDRES          0.476837 Hz
AQ             1.0485760 sec
RG                   912
DW                16.000 usec
DE                18.00 usec
TE                298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         125.8131152 MHz
NUC1                  13C
P1                 10.00 usec
PLW1         20.18400002 W

======== CHANNEL f2 ========
SFO2         500.3020012 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2              80.00 usec
PLW2          7.99830008 W
PLW12         0.28119001 W
PLW13         0.17996000 W

F2 - Processing parameters
SI                 32768
SF           125.8005189 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```



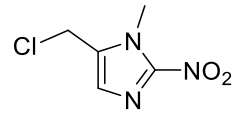
5-(Chloromethyl)-1-methyl-2-nitro-1*H*-imidazole (**10**)



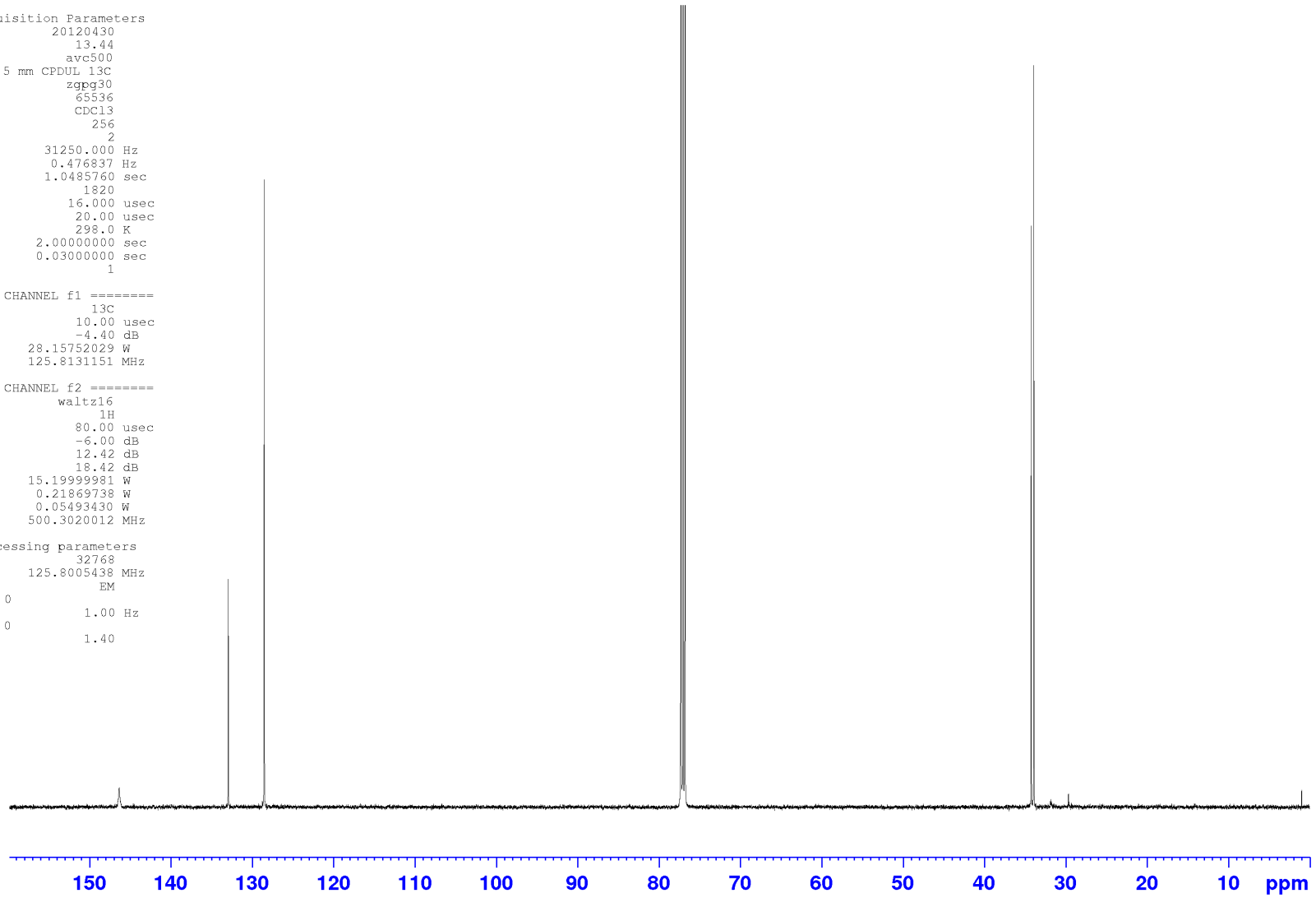

```
Current Data Parameters
NAME      5-(chloromethyl)-1-methyl-2-nitro-1H-imidazole
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20120430
Time               12.49
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH            10330.578 Hz
FIDRES          0.157632 Hz
AQ             3.1719425 sec
RG                     4
DW                48.400 usec
DE                 6.00 usec
TE                298.0 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                  9.60 usec
PL1                -6.00 dB
PL1W         15.19999981 W
SFO1         500.3030896 MHz

F2 - Processing parameters
SI                 32768
SF           500.3000240 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```



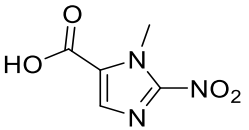
5-(Chloromethyl)-1-methyl-2-nitro-1*H*-imidazole (**10**)



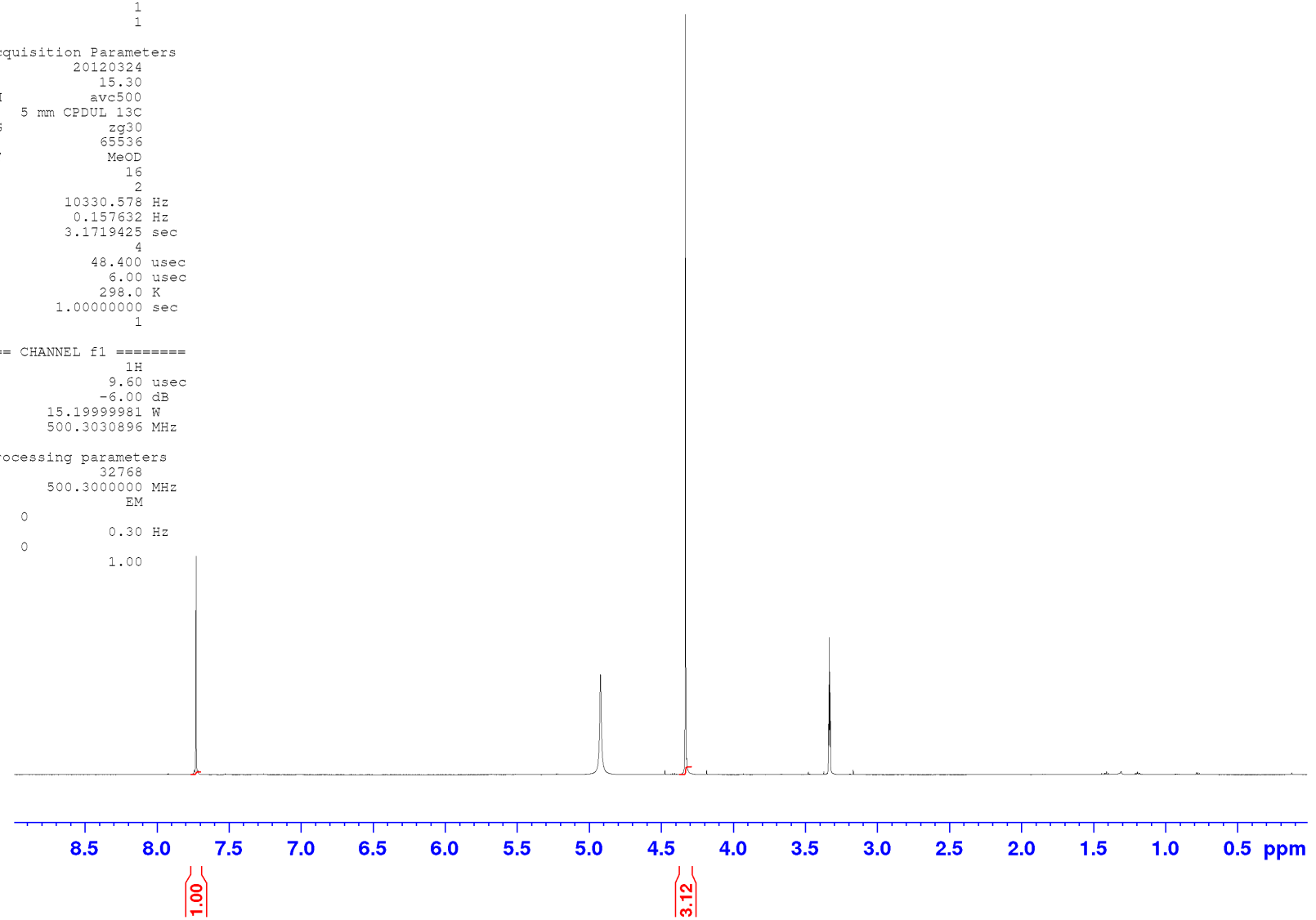

```
Current Data Parameters
NAME      5-(chloromethyl)-1-methyl-2-nitro-1H-imidazole
EXPNO                  4
PROCNO                 1

F2 - Acquisition Parameters
Date_           20120430
Time              13.44
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT           CDC13
NS                  256
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                 1820
DW               16.000 usec
DE               20.00 usec
TE               298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                13C
P1                10.00 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                1H
PCPD2             80.00 usec
PL2               -6.00 dB
PL12              12.42 dB
PL13              18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2        500.3020012 MHz

F2 - Processing parameters
SI                32768
SF          125.8005438 MHz
WDW                 EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```



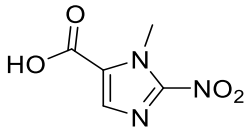
1-Methyl-2-nitro-1*H*-imidazole-5-carboxylic acid (**9**)



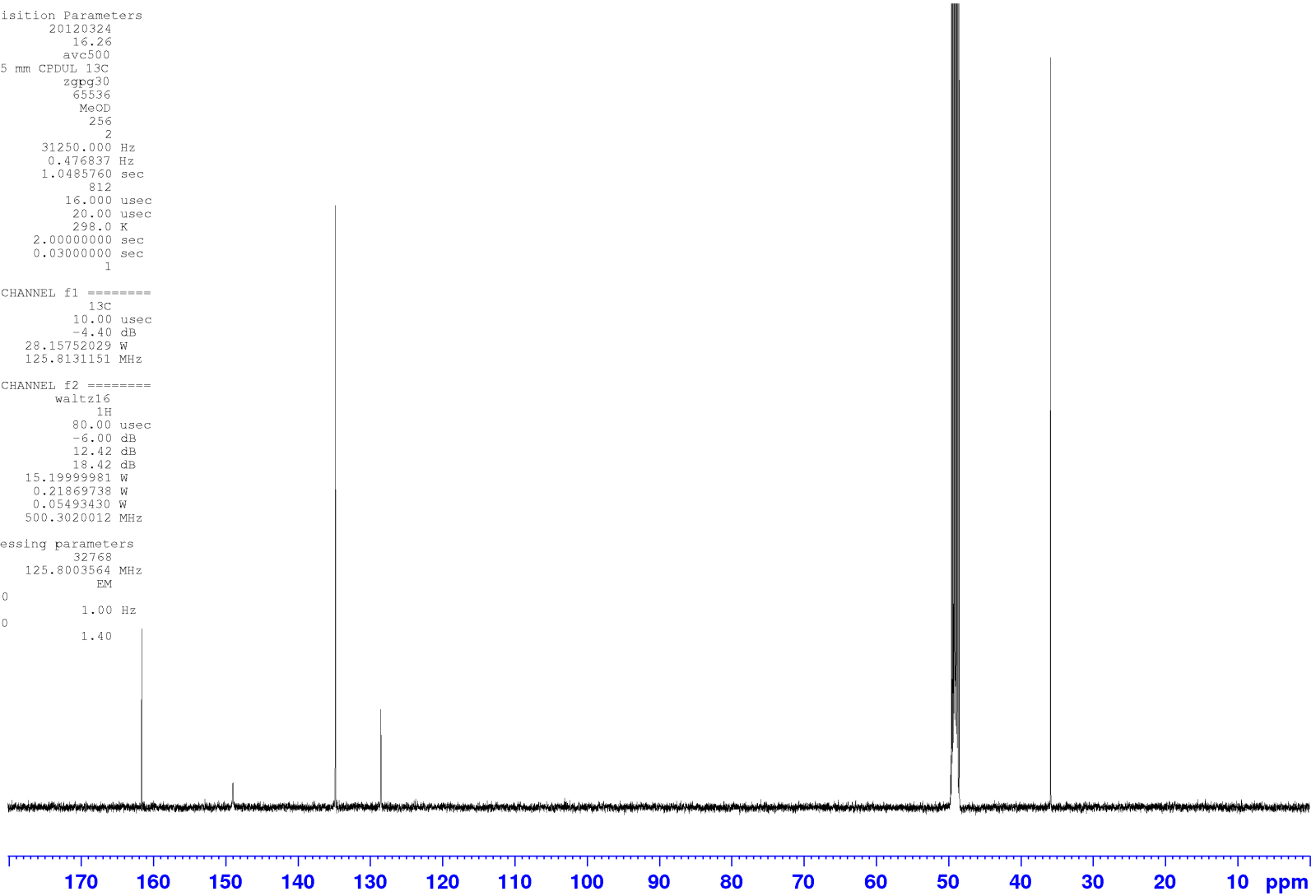

```
Current Data Parameters
NAME     1-methyl-2-nitro-1H-imidazole-5-carboxylic acid
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_          20120324
Time              15.30
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG             zg30
TD                 65536
SOLVENT             MeOD
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                     4
DW                48.400 usec
DE                 6.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                  9.60 usec
PL1                -6.00 dB
PL1W        15.19999981 W
SFO1        500.3030896 MHz

F2 - Processing parameters
SI                 32768
SF          500.3000000 MHz
WDW                   EM
SSB      0
LB                 0.30 Hz
GB       0
PC                 1.00
```



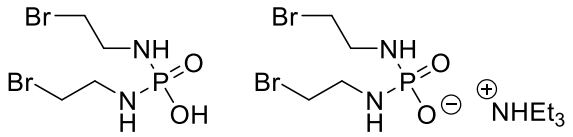
1-Methyl-2-nitro-1*H*-imidazole-5-carboxylic acid (**9**)



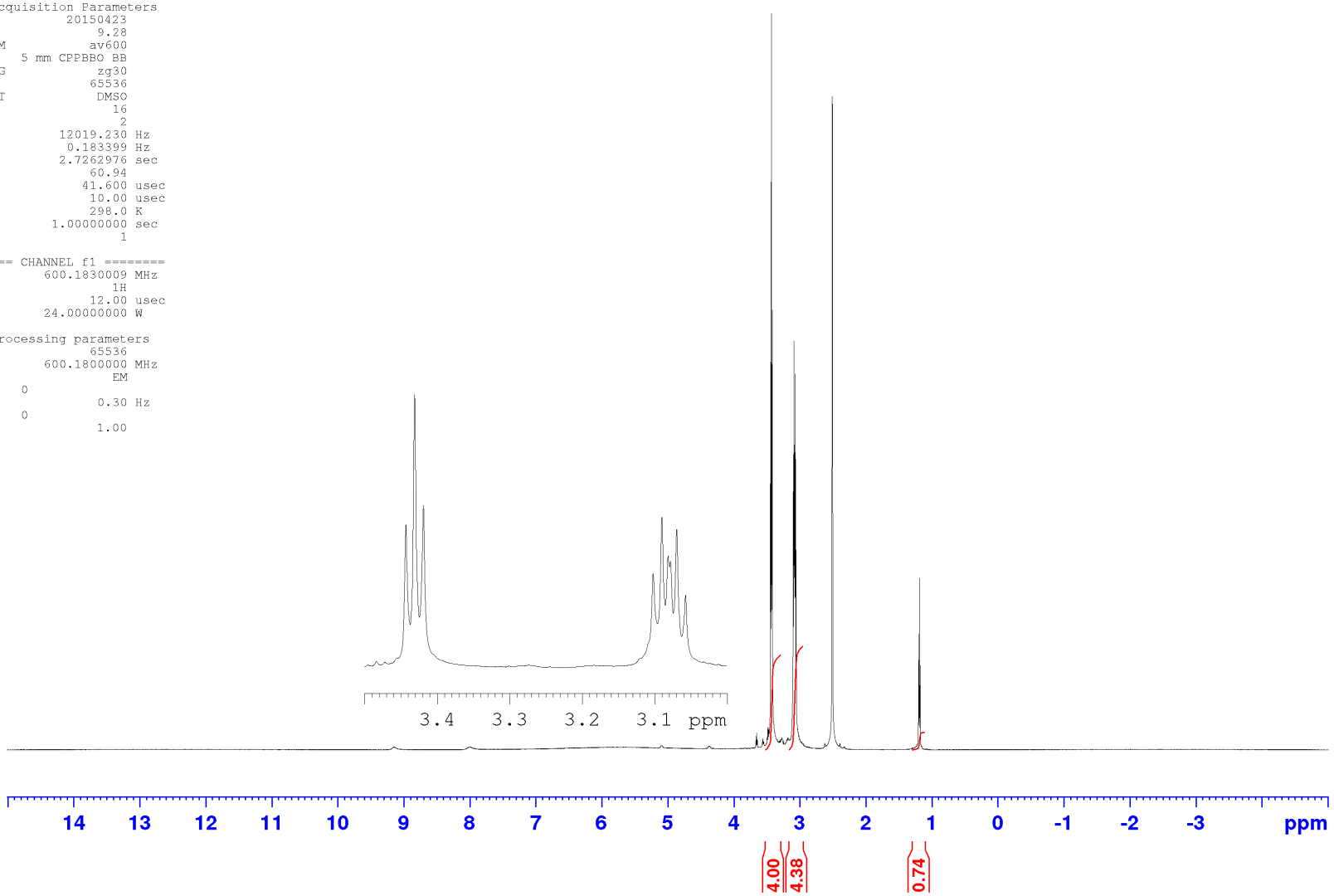

```
Current Data Parameters
NAME     1-methyl-2-nitro-1H-imidazole-5-carboxylic acid
EXPNO                 4
PROCNO                1

F2 - Acquisition Parameters
Date_          20120324
Time              16.26
INSTRUM          avc500
PROBHD   5 mm CPDUL 13C
PULPROG          zgpg30
TD                65536
SOLVENT            MeOD
NS                  256
DS                    2
SWH           31250.000 Hz
FIDRES         0.476837 Hz
AQ            1.0485760 sec
RG                  812
DW               16.000 usec
DE               20.00 usec
TE               298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                10.00 usec
PL1               -4.40 dB
PL1W        28.15752029 W
SFO1        125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                 1H
PCPD2             80.00 usec
PL2               -6.00 dB
PL12              12.42 dB
PL13              18.42 dB
PL2W        15.19999981 W
PL12W        0.21869738 W
PL13W        0.05493430 W
SFO2        500.3020012 MHz

F2 - Processing parameters
SI                32768
SF          125.8003564 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```



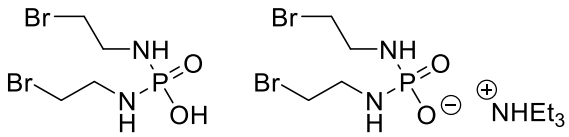
N,N'-bis(2-Bromoethyl)phosphorodiamidic acid (Br-IPM, **13**)



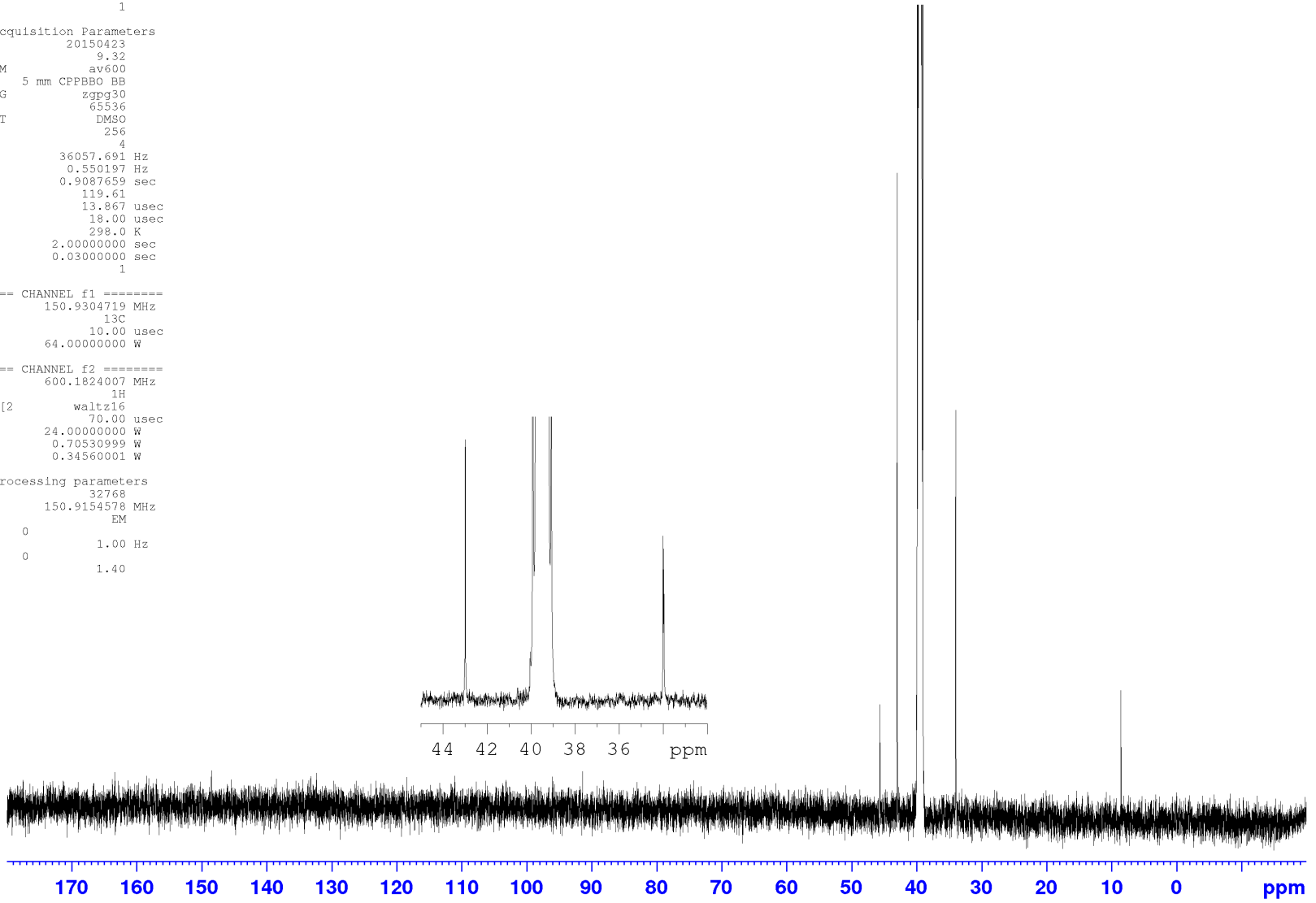

```
Current Data Parameters
NAME      LOC297 Service lo10192304
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20150423
Time               9.28
INSTRUM           av600
PROBHD   5 mm CPPBBO BB
PULPROG             zg30
TD                65536
SOLVENT            DMSO
NS                   16
DS                    2
SWH           12019.230 Hz
FIDRES         0.183399 Hz
AQ            2.7262976 sec
RG                60.94
DW               41.600 usec
DE               10.00 usec
TE                298.0 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1        600.1830009 MHz
NUC1                1H
P1                12.00 usec
PLW1        24.00000000 W

F2 - Processing parameters
SI                65536
SF          600.1800000 MHz
WDW                 EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```



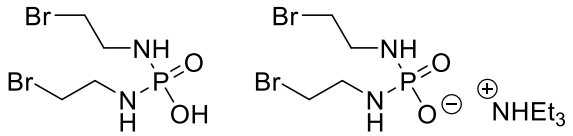
N,N′-bis(2-Bromoethyl)phosphorodiamidic acid (Br-IPM, **13**)



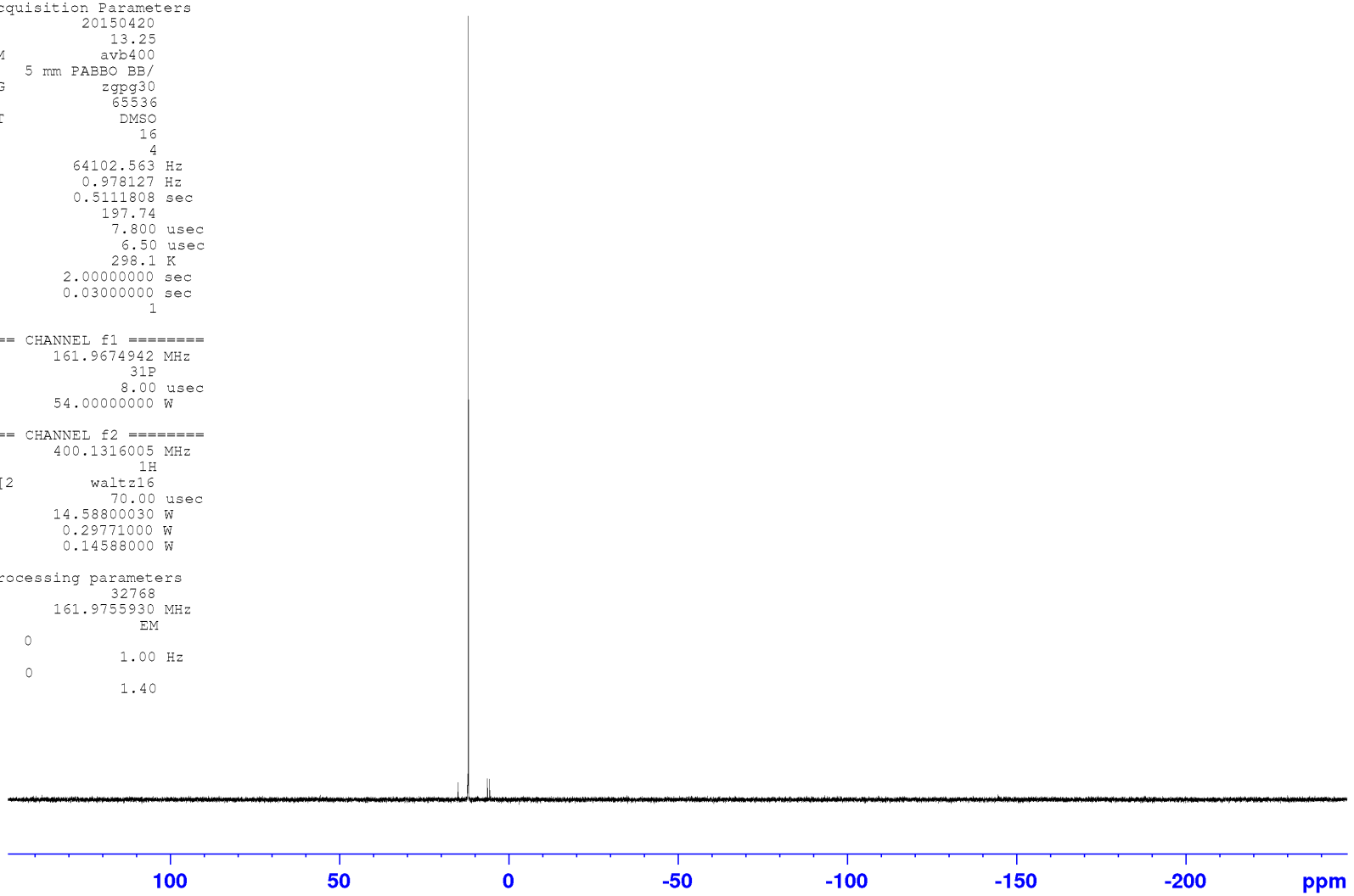

```
Current Data Parameters
NAME      LOC297 Service lo10192304
EXPNO                 2
PROCNO                1

F2 - Acquisition Parameters
Date_          20150423
Time               9.32
INSTRUM           av600
PROBHD   5 mm CPPBBO BB
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                   256
DS                     4
SWH           36057.691 Hz
FIDRES         0.550197 Hz
AQ            0.9087659 sec
RG               119.61
DW               13.867 usec
DE                18.00 usec
TE                298.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1        150.9304719 MHz
NUC1               13C
P1                10.00 usec
PLW1        64.00000000 W

======== CHANNEL f2 ========
SFO2        600.1824007 MHz
NUC2               1H
CPDPRG[2         waltz16
PCPD2             70.00 usec
PLW2        24.00000000 W
PLW12        0.70530999 W
PLW13        0.34560001 W

F2 - Processing parameters
SI                32768
SF          150.9154578 MHz
WDW                EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```



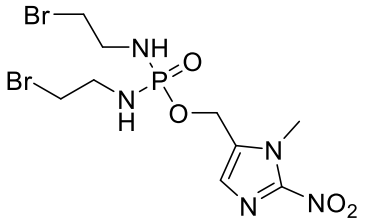
N,N'-bis(2-Bromoethyl)phosphorodiamidic acid (Br-IPM, **13**)



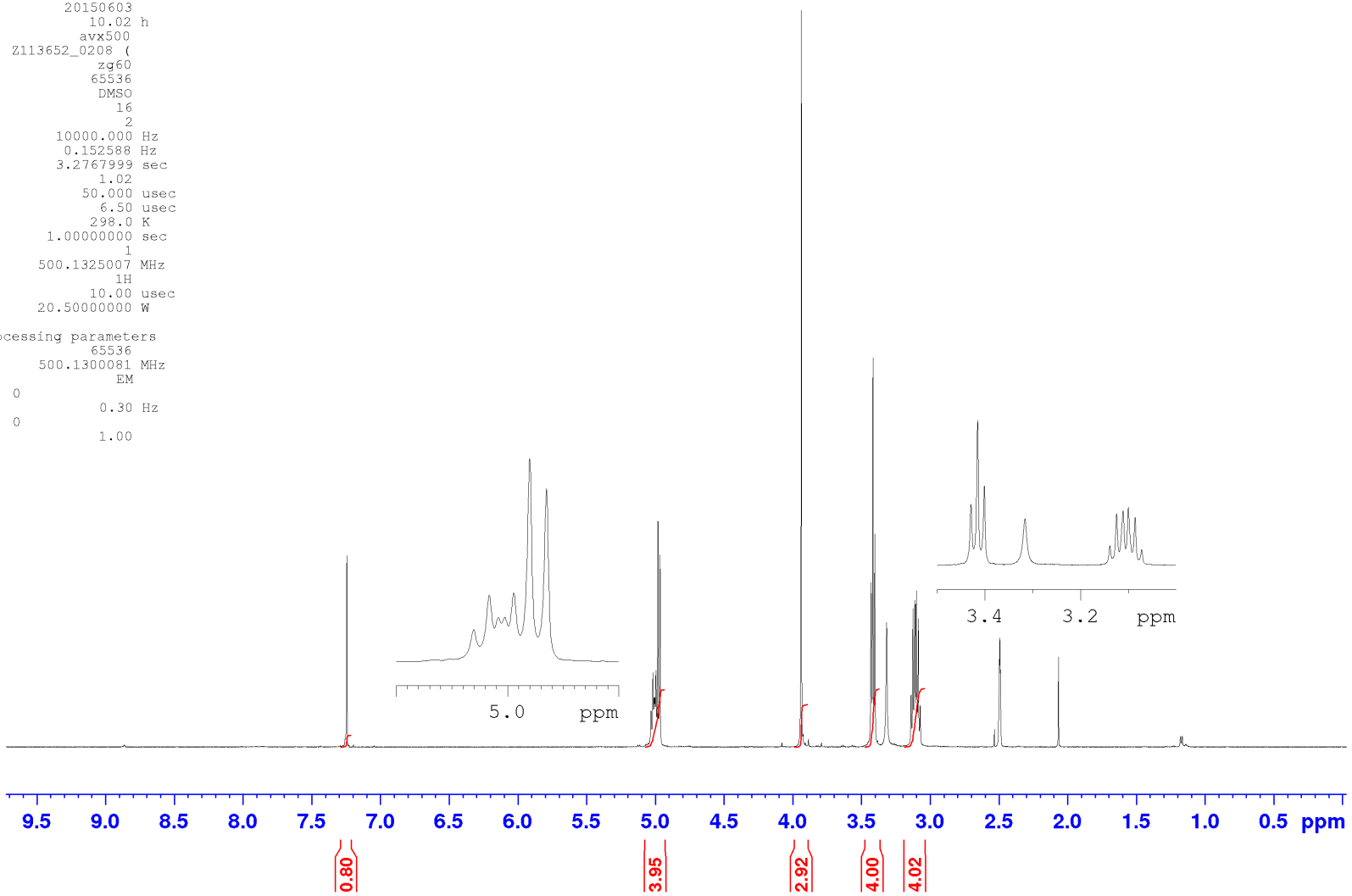

```
Current Data Parameters
NAME       LOC297 AVB400
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_           20150420
Time              13.25
INSTRUM          avb400
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT             DMSO
NS                    16
DS                     4
SWH            64102.563 Hz
FIDRES          0.978127 Hz
AQ             0.5111808 sec
RG                197.74
DW                 7.800 usec
DE                 6.50 usec
TE                298.1 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1         161.9674942 MHz
NUC1                31P
P1                 8.00 usec
PLW1         54.00000000 W

======== CHANNEL f2 ========
SFO2         400.1316005 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             70.00 usec
PLW2         14.58800030 W
PLW12         0.29771000 W
PLW13         0.14588000 W

F2 - Processing parameters
SI                 32768
SF           161.9755930 MHz
WDW                  EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```



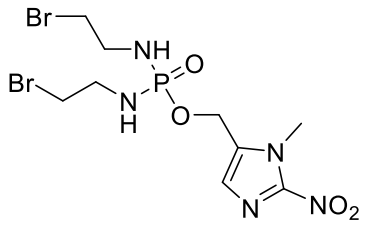
(1-Methyl-2-nitro-1*H*-imidazol-5-yl) *N*, *N*–bis(2-bromoethyl) phosphordiamidate (**TH-302**)



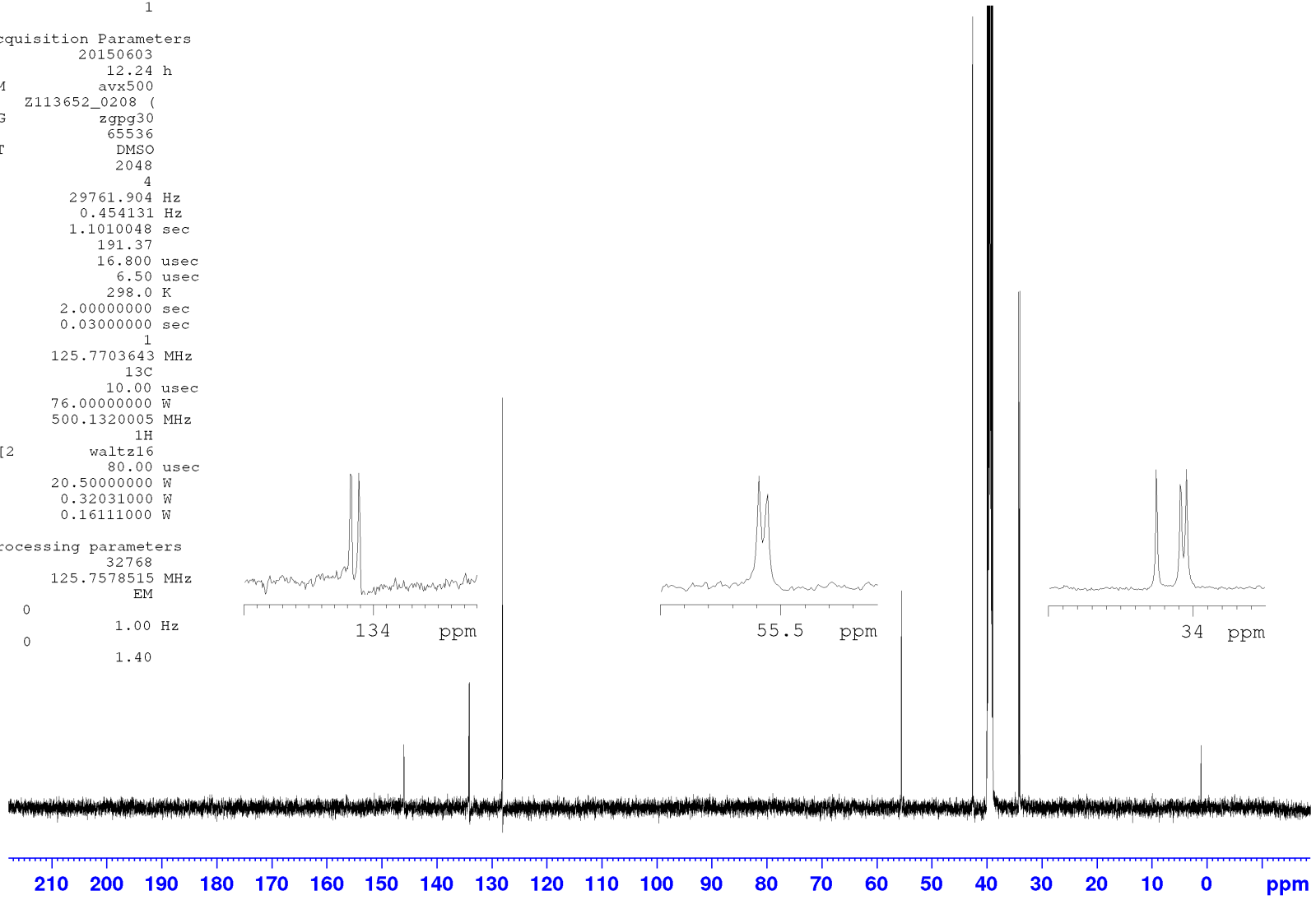

```
Current Data Parameters
NAME      LOC300 Service lo14870306
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20150603
Time              10.02 h
INSTRUM          avx500
PROBHD   Z113652_0208 (
PULPROG             zg60
TD                 65536
SOLVENT             DMSO
NS                    16
DS                     2
SWH            10000.000 Hz
FIDRES          0.152588 Hz
AQ             3.2767999 sec
RG                  1.02
DW                50.000 usec
DE                 6.50 usec
TE                298.0 K
D1           1.00000000 sec
TD0                    1
SFO1         500.1325007 MHz
NUC1                  1H
P1                 10.00 usec
PLW1         20.50000000 W

F2 - Processing parameters
SI                 65536
SF           500.1300081 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```



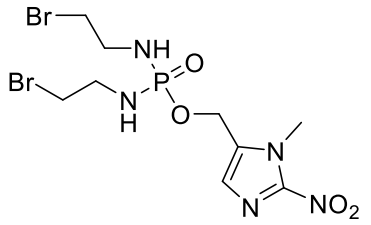
(1-Methyl-2-nitro-1*H*-imidazol-5-yl) *N*, *N*–bis(2-bromoethyl) phosphordiamidate (**TH-302**)



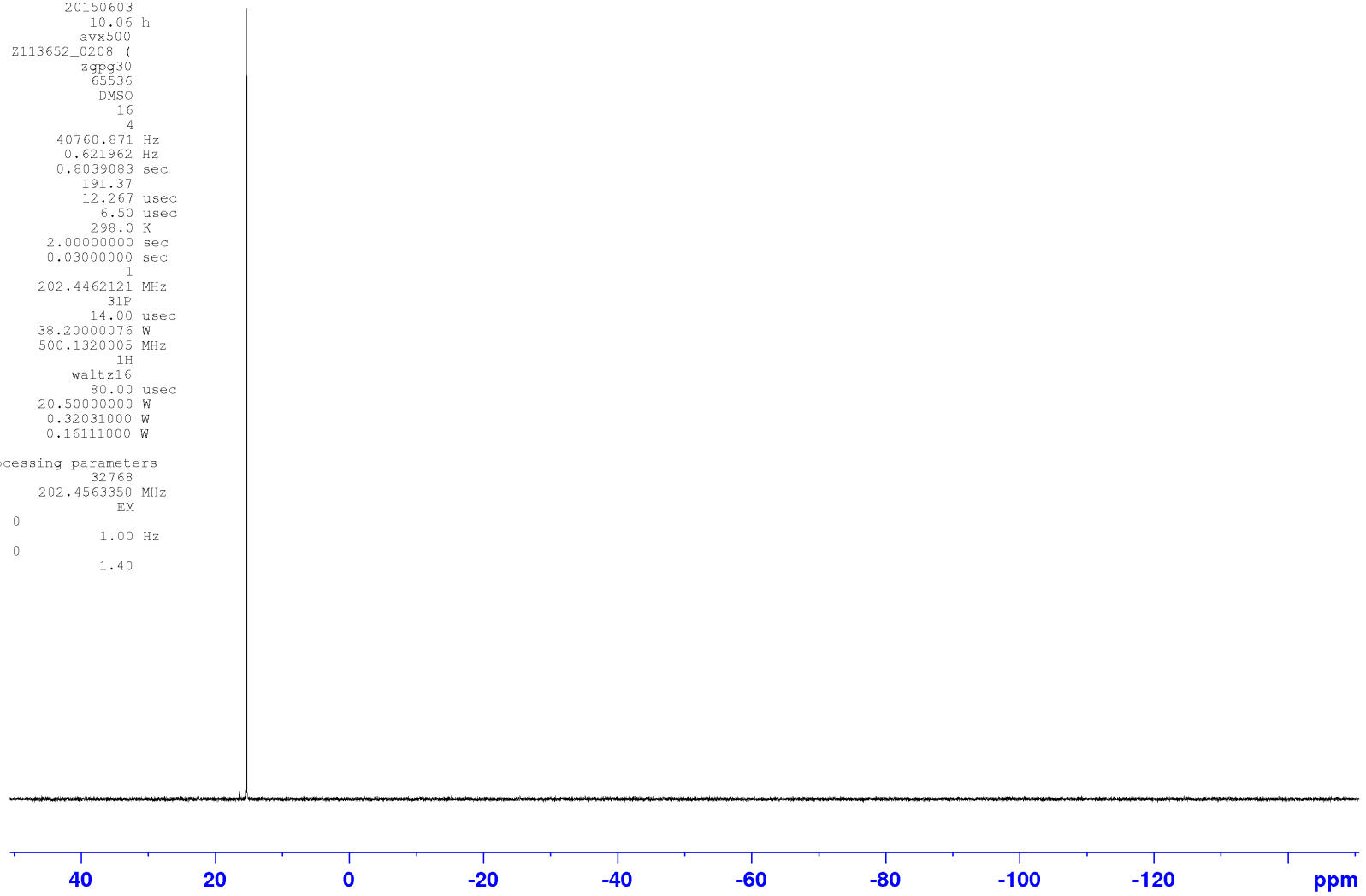

```
Current Data Parameters
NAME      LOC300 Service lo14870306
EXPNO                 8
PROCNO                1

F2 - Acquisition Parameters
Date_           20150603
Time              12.24 h
INSTRUM           avx500
PROBHD   Z113652_0208 (
PULPROG          zgpg30
TD                65536
SOLVENT             DMSO
NS                  2048
DS                     4
SWH            29761.904 Hz
FIDRES          0.454131 Hz
AQ             1.1010048 sec
RG                191.37
DW                16.800 usec
DE                 6.50 usec
TE                298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1
SFO1         125.7703643 MHz
NUC1                13C
P1                10.00 usec
PLW1         76.00000000 W
SFO2         500.1320005 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2         20.50000000 W
PLW12         0.32031000 W
PLW13         0.16111000 W

F2 - Processing parameters
SI                32768
SF           125.7578515 MHz
WDW                  EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```



(1-Methyl-2-nitro-1*H*-imidazol-5-yl) *N*, *N*–bis(2-bromoethyl) phosphordiamidate (**TH-302**)

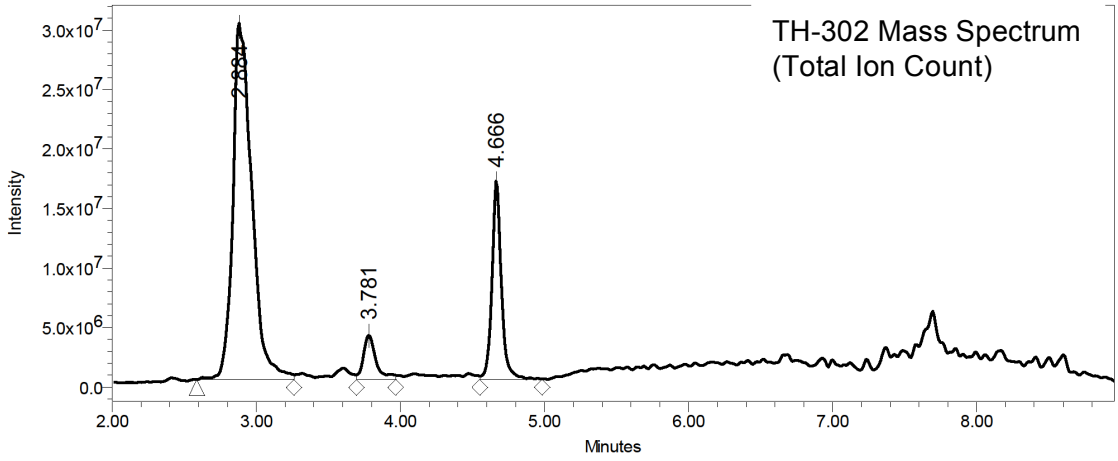



```
Current Data Parameters
NAME      LOC300 Service lol4870306
EXPNO                  3
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150603
Time              10.06 h
INSTRUM           avx500
PROBHD   Z113652_0208 (
PULPROG           zgpg30
TD                65536
SOLVENT             DMSO
NS                    16
DS                     4
SWH            40760.871 Hz
FIDRES          0.621962 Hz
AQ             0.8039083 sec
RG                191.37
DW                12.267 usec
DE                 6.50 usec
TE                298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1
SFO1         202.4462121 MHz
NUC1                31P
P1                14.00 usec
PLW1         38.20000076 W
SFO2         500.1320005 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2         20.50000000 W
PLW12         0.32031000 W
PLW13         0.16111000 W

F2 - Processing parameters
SI                 32768
SF           202.4563350 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```

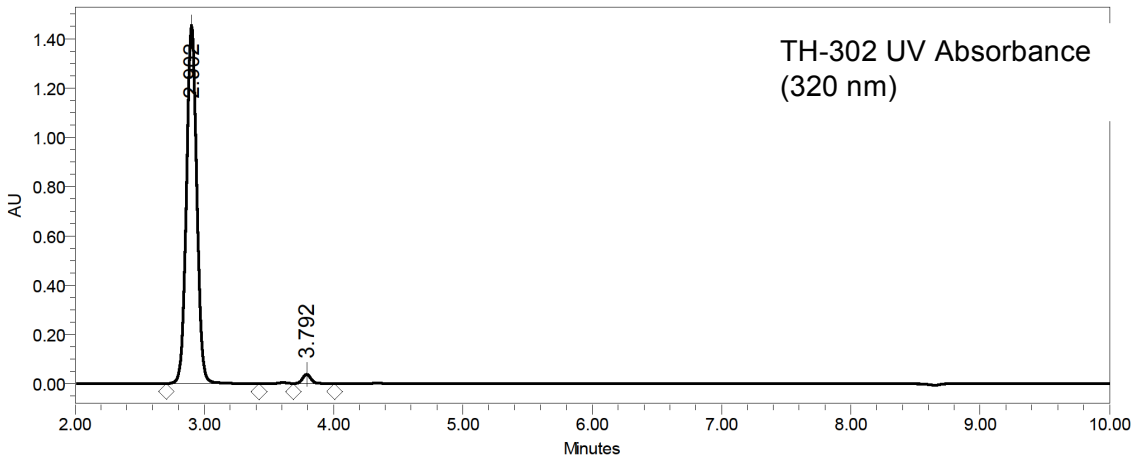


Sample Name:	loc300 bulk prep	Acq. Method Set:	MS_35_95 Liam
Vial:	7	Processing Method:	PM_TIC, PM_PDA
Injection Volume:	0.50 ul	Channel Name:	320NM, MS TIC
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	ZQ F1 Scan MS TIC, Smoothed by
Date Acquired:	14/05/2015 12:21:52 BST		

Auto-Scaled Chromatogram

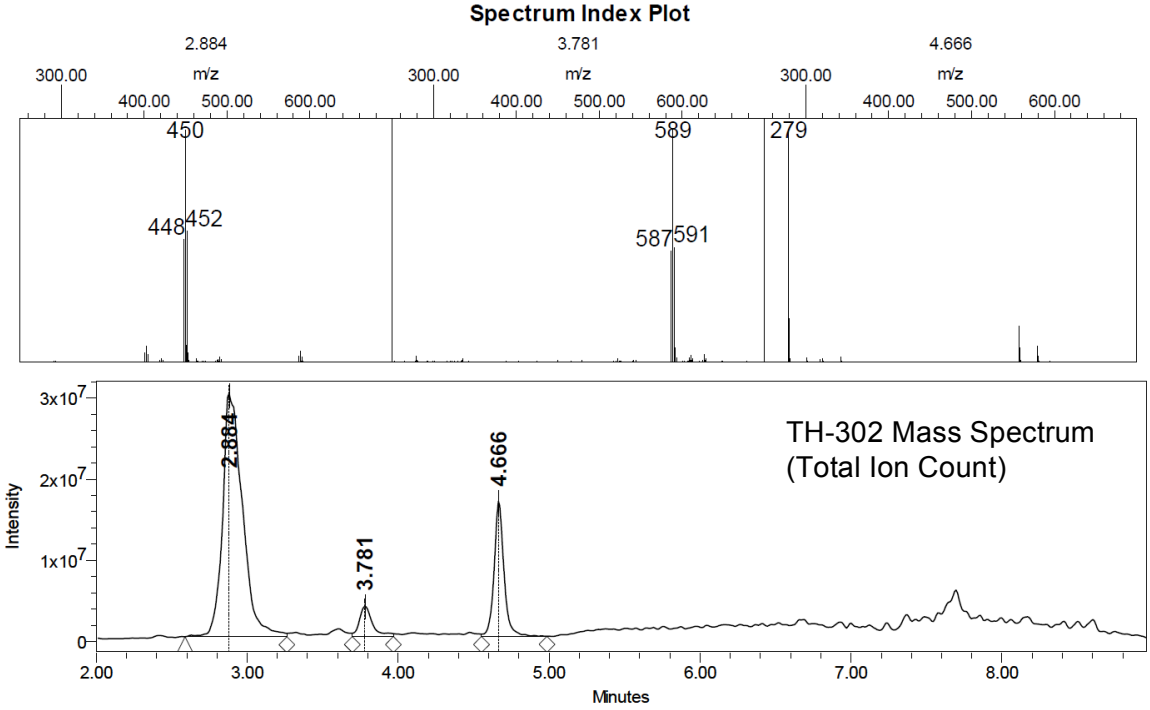


Auto-Scaled Chromatogram

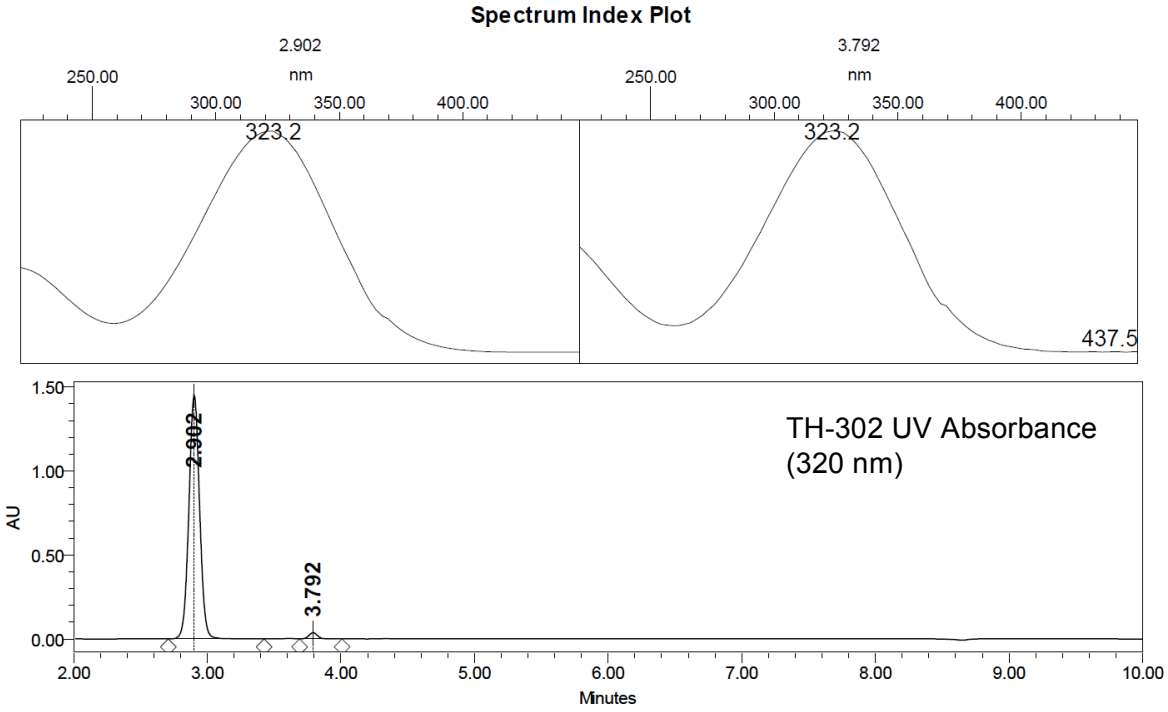


Peak Results

	Name	RT	Area	% Area	Channel Name
1		2.902	8097904	97.86	320NM
2		3.792	177107	2.14	320NM
3		2.884	275854110	73.71	MS TIC
4		3.781	22717370	6.07	MS TIC
5		4.666	75667782	20.22	MS TIC



SampleName: loc300 bulk prep; Vial: 7; Injection: 1; Date Acquired: 14/05/2015 12:21:52 BST



SampleName: loc300 bulk prep; Vial: 7; Injection: 1; Date Acquired: 14/05/2015 12:21:52 BST

References

1 B. Cavalleri, R. Ballotta and G. Lancini, *J. Heterocycl. Chem.*, 1972, **9**, 979–984.

2 B. Cavalleri, R. Ballotta, V. Arioli and G. Lancini, *J. Med. Chem.*, 1973, **16**, 557–560.

3 I. Parveen, D. P. Naughton, W. Whish and M. D. Threadgill, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 2031–2036.

4 J.-X. Duan, H. Jiao, J. Kaizerman, T. Stanton, J. W. Evans, L. Lan, G. Lorente, M. Banica, D. Jung, J. Wang, H. Ma, X. Li, Z. Yang, R. M. Hoffman, W. S. Ammons, C. P. Hart and M. Matteucci, *J. Med. Chem.*, 2008, **51**, 2412–2420.

5 R. F. Struck, S. M. Schmid and W. R. Waud, *Cancer Chemotherapy and Pharmacology*, 1994, **34**, 191–196.