

Electronic Supplementary Information

For

Signal Transduction and Amplification through Enzyme-Triggered Ligand Release and Accelerated Catalysis

Sean Goggins ^a, Barrie J. Marsh ^b, Anneke Lubben ^a and Christopher G. Frost ^{a*}

^a Department of Chemistry, University of Bath, Bath, BA2 7AY, UK

^b Atlas, Derby Court, Epsom Square, White Horse Business Park, Trowbridge, Wiltshire,
BA14 0XG, UK

* Corresponding author. C.G.Frost@bath.ac.uk

Table of Contents

General information:	3
Materials:	3
Electrochemical Analysis:	3
Voltammogramatic Overlays	4
Real-time Mass Spectrometry Experiments.....	5
Mass Spectra acquired before ALP addition, 6.3–9.4 and 29.5–31.5 minutes after ALP addition	5
Individual traces acquired over time	6
Ligand Screen	7
LOD Calibration Curve.....	8
General Procedures	9
General procedure for the synthesis of mono- <i>N</i> -sulfonated ethylenediamines (L1–L10)	9
Procedure for the synthesis of both <i>N</i> - and <i>N'</i> -methyl- <i>N</i> -sulfonated ethylenediamines (L16–L17).....	13
General procedure for the synthesis of <i>N</i> -dimethyl mono- <i>N</i> -sulfonated ethylenediamines (L18–L19)	14
Procedure for the synthesis of <i>tert</i> -butyl (2-((2,4,6-trimethylphenyl)sulfonamido)ethyl)carbamate (L20)	15
Procedure for the synthesis of benzyl (2-((2,4,6-trimethylphenyl)sulfonamido)ethyl)carbamate (L21)	16
Procedure for the synthesis of 4-methoxybenzyl (2-((2,4,6-trimethylphenyl)sulfonamido)ethyl)carbamate (L22)	17
Procedure for the synthesis of diallyl (4-(hydroxymethyl)phenyl) phosphate (5).....	18
Procedure for the synthesis of 4-((bis(allyloxy)phosphoryl)oxy)benzyl (2-((2,4,6-trimethylphenyl)sulfonamido)ethyl)carbamate (6).....	19
Procedure for the synthesis of 4-(((2-((2,4,6-trimethylphenyl)sulfonamido)ethyl)carbamoyl)oxy)methyl)phenyl phosphate (PL1)	20
General procedures for the enzyme-triggered transfer hydrogenation of aldehydes to alcohols.....	21
General Procedure for the ³¹ P NMR Experiment	25
General Procedure for the Mass Spectrometry Experiment	25
References:.....	26
NMR Spectra:	27

General information:

Proton, carbon, fluorine and phosphorus nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 300 or 400 MHz spectrometer, or an Agilent Technologies 500 MHz spectrometer (^1H NMR at 300 or 400 MHz, ^{13}C NMR at 75.5 or 101 MHz, ^{31}P NMR at 121.5 MHz and ^{19}F NMR at 376.5 or 470.5 MHz). Chemical shifts for protons are reported downfield from tetramethylsilane and are referenced,¹ to residual protium in the solvent (^1H NMR: CHCl_3 at 7.26 ppm, C_6H_6 at 7.16 ppm, H_2O at 4.79 ppm, DMSO at 2.50 ppm). Chemical shifts for carbons are reported in parts per million downfield from tetramethylsilane and are referenced¹ to the carbon resonances of the solvent peak (^{13}C NMR: CDCl_3 at 77.0 ppm, C_6D_6 at 128.1 ppm, d_6 -DMSO at 39.5). Chemical shifts for fluorine resonances are reported in parts per million referenced to CFCl_3 . Chemical shifts for phosphorus are reported in parts per million referenced to 85% phosphoric acid. NMR data are represented as follows: chemical shift (integration, multiplicity [s = singlet, bs = broad singlet, d = double, dd = doublet of doublet, t = triplet, q = quartet, sept = septet, m = multiplet], coupling constants (Hz)). IR spectra were recorded on a Perkin-Elmer 1600 FT IR spectrophotometer, with absorbencies quoted as ν in cm^{-1} . High resolution mass spectrometry was performed on a Bruker Daltonik Electrospray Time-of-Flight mass spectrometry (ESI-UHR-TOF MS, negative ion mode). Melting points were obtained on a Bibby-Sterilin SMP10 melting point machine. Analytical thin layer chromatography (TLC) were performed using aluminium-backed plates coated with Alugram[®] SIL G/UV₂₅₄ purchased from Macherey-Nagel and visualised by UV light (254 nm) and/or KMnO_4 , 2,4-DNPH or ninhydrin staining. Silica gel column chromatography was carried out using 60 Å, 200–400 mesh particle size silica gel purchased from Sigma-Aldrich. Reverse phase (C18) silica gel column chromatography was carried out using VersaPak[®] 30g C18 cartridges (23 mm × 110 mm) preloaded with 20–45 μm spherical C18 bonded silica purchased from Sigma-Aldrich.

Materials:

All reactions were carried out under an atmosphere of nitrogen, in oven-dried glassware unless otherwise stated. Acetonitrile, dichloromethane, tetrahydrofuran and toluene were dried and degassed by passing through anhydrous alumina columns using an Innovative Technology Inc. PS-400-7 solvent purification system and stored under an atmosphere of argon prior to use. Dichloro(pentamethylcyclopentadienyl)iridium(III) dimer was purchased from Alfa Aesar and used as received. All other chemicals were purchased from Sigma-Aldrich. *N*-Chlorosuccinimide (NCS) and triethylamine (TEA) were purified by standard published methods prior to use.² All other chemicals were used as received. Alkaline phosphatase was purchased as a lyophilised solid from Sigma-Aldrich and stored in a $-20\text{ }^\circ\text{C}$ freezer. Prior to use, a stock solution of the enzyme was made up using 0.05 M pH 9.8 CO_3^{2-} buffer and stored at $4\text{ }^\circ\text{C}$ until immediate use.

Electrochemical Analysis:

Electrochemical analysis was performed on a Metrohm Autolab PGSTAT30 potentiostat using General Purpose Electrochemical System (GPES) software in differential pulse mode (modulation = 0.04 s, interval = 0.1 s, initial voltage = -200 mV , end voltage = 400 mV , step potential = 3 mV , modulation amplitude 49.95 mV).

Typically, each reaction was performed at a ferrocene concentration of 0.25 M and at the required interval, a 1 μL sample was taken from the reaction mixture and diluted into a 999 μL solution of 0.05 M pH 9.8 CO_3^{2-} buffer. After shaking, a 100 μL sample of this solution was diluted into 900 μL 0.05 M pH 9.8 CO_3^{2-} buffer to give a 25 μM ferrocene concentration. After shaking again, a 20 μL sample of this twice-diluted solution was applied to a screen printed electrochemical cell (GM Nameplate) consisting of carbon working and counter electrodes and a silver (pseudo Ag/AgCl) reference electrode. Post scan, the 'peak search' function was performed and the peak integrals that correspond to the appropriate ferrocene signal were input into the following equation to calculate reaction conversion:

$$\text{Conversion (\%)} = \left(\frac{f_2}{(f_2 + f_1)} \right) \times 100$$

Voltammogrammatic Overlays

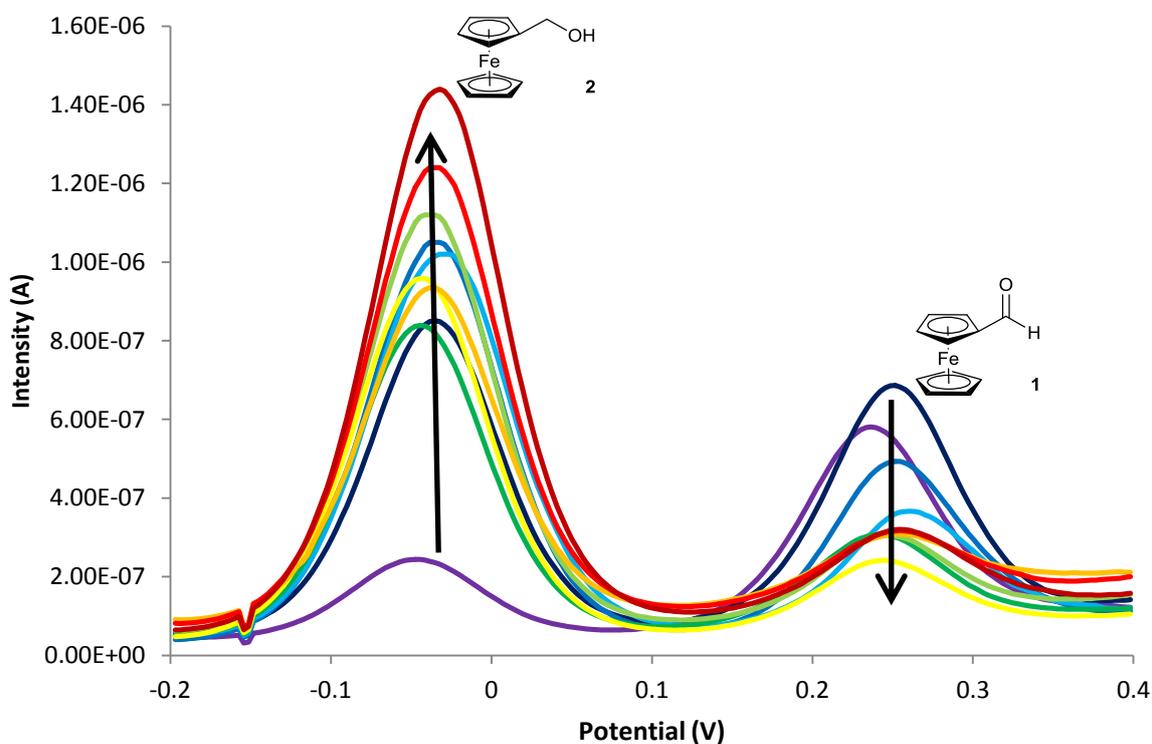


Fig S1 Typical voltammograms obtained and overlaid from a positive reaction (ALP + PL1) sampled every 3 minutes and analysed by differential pulse voltammetry. As seen, peak heights can vary tremendously due to errors accumulated during sampling and dilution, and also through the use of disposable screen-printed electrodes as electrode areas can vary from one cell to the next. This highlights the benefit of using ratiometric sensing to accurately obtain reaction conversions.

Real-time Mass Spectrometry Experiments

Mass Spectra acquired before ALP addition, 6.3–9.4 and 29.5–31.5 minutes after ALP addition

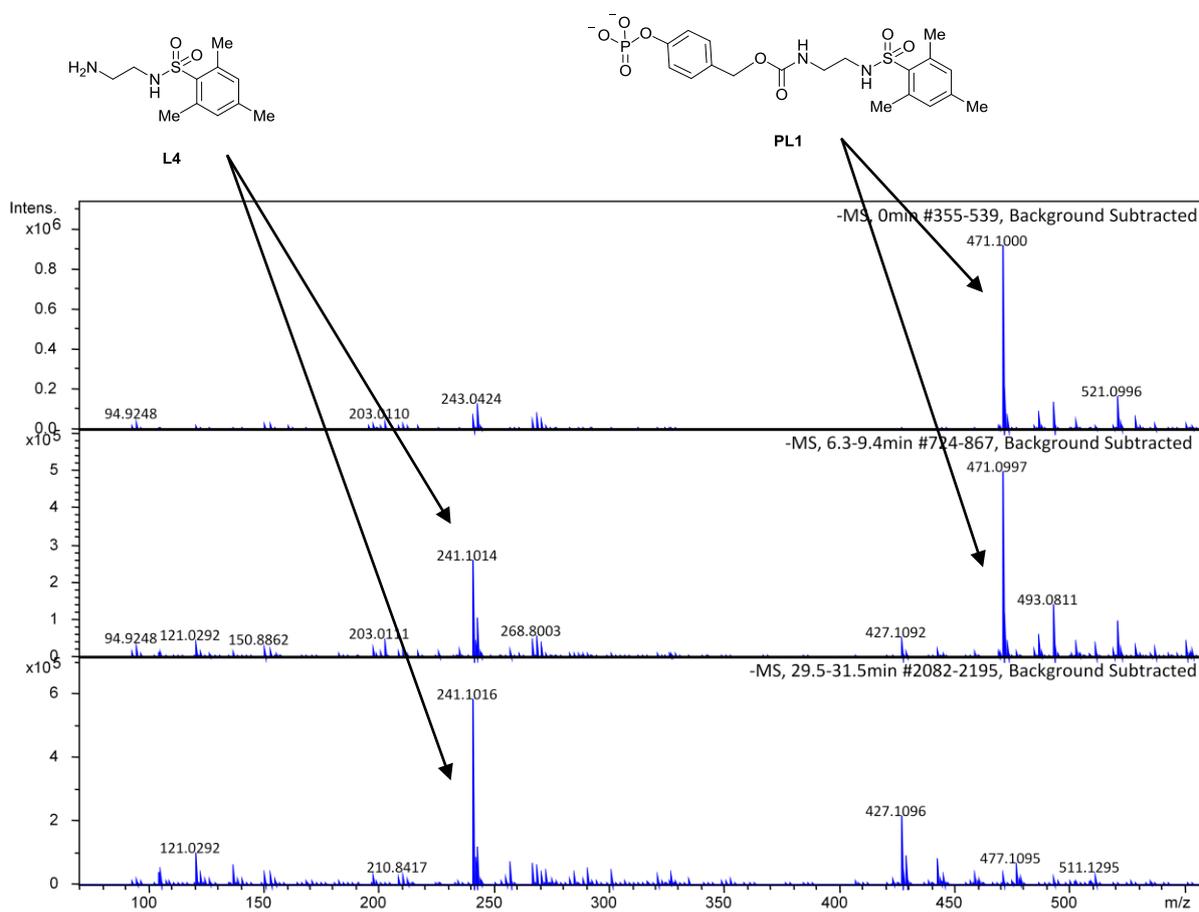


Fig. S2 Individual spectra acquired before and after enzyme additions

Individual traces acquired over time

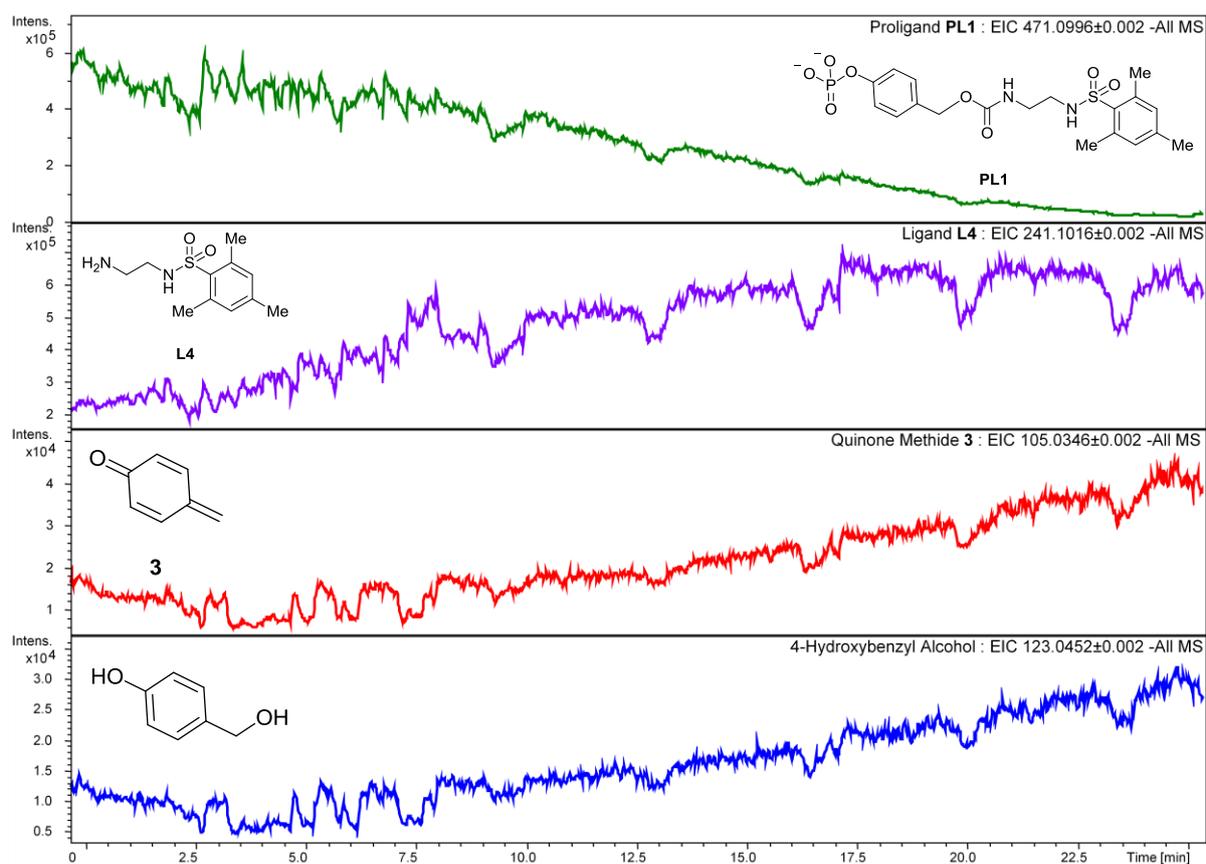
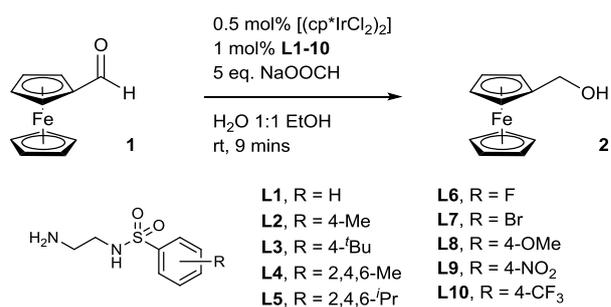


Fig. S3 Traces acquired over time

Table S1 Initial ligand screen

Entry	Ligand	Conversion ^a
1	None	1%
2	L1	22%
3	L2	40%
4	L3	39%
5	L4	43%
6	L5	3%
7	L6	39%
8	L7	18%
9	L8	18%
10	L9	25%
11	L10	32%

^a Determined by ratiometric electrochemical analysis

LOD Calibration Curve

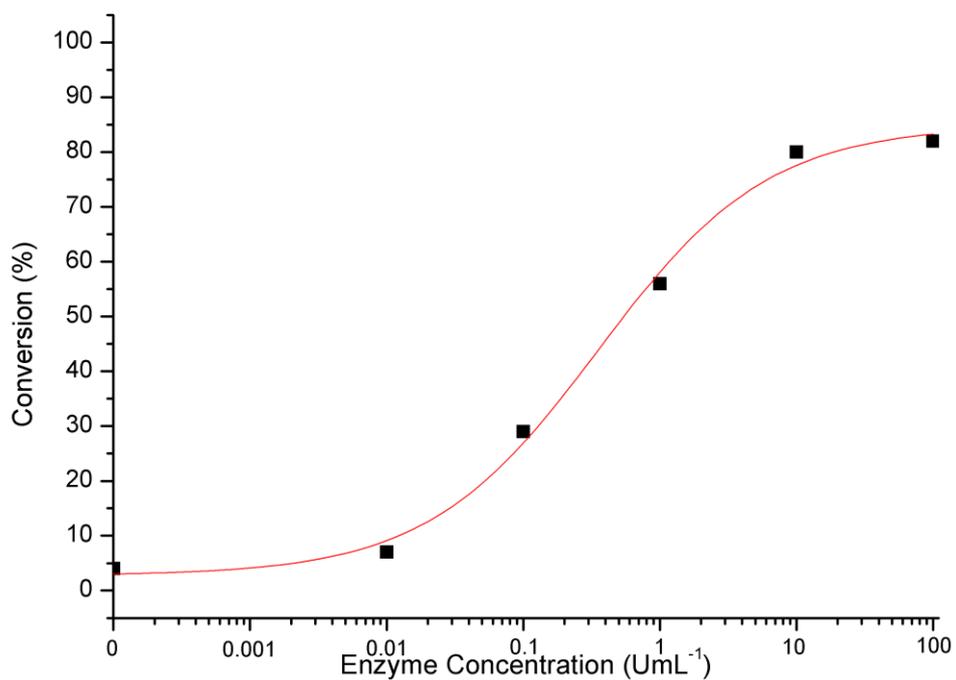


Fig. S4 Conversion of ferrocenecarboxaldehyde **1** to ferrocenemethanol **2** observed after 3 minutes at different enzyme concentration.

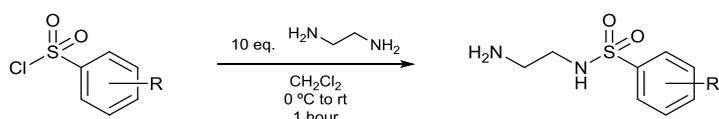
Curve fit equation $y = A2 + (A1 - A2) / (1 + (x / x^0)^p)$

$$R^2 = 0.99105$$

When enzyme concentrations are in units of UL^{-1} , $A2 = 84.9$, $A1 = 2.8$, $x^0 = 352.1$ and $p = 0.7$. The mean conversion of 3 background runs after 3 minutes is 3.67%. $LOD = \text{mean} + 3 * \text{StDev} = 8\%$ conversion. Entering this into the curve fit equation gives an enzyme concentration LOD of $7 UL^{-1}$. Using 6500 Umg^{-1} ALP, this approximates to a 7 pM concentration ALP.

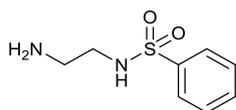
General Procedures

General procedure for the synthesis of mono-*N*-sulfonated ethylenediamines (L1–L10)



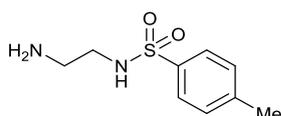
A solution of the sulfonyl chloride (10 mmol) in anhydrous dichloromethane (25 mL) was added, *via* a dropping funnel, to a stirring solution of ethylenediamine (100 mmol, 6.7 mL) in anhydrous dichloromethane (25 mL) at 0 °C. After complete addition, the reaction mixture was allowed to warm to room temperature and stirred for 1 hour. The reaction was then quenched with water (50 mL) and the organics separated. The aqueous layer was then extracted with further dichloromethane (2 × 25 mL). The combined organics were then washed with water (50 mL) followed by brine (50 mL), then dried over MgSO₄ and concentrated. The crude residue was then purified by silica gel column chromatography (dichloromethane 9:1 methanol + 1% triethylamine).

N-(2-aminoethyl)benzenesulfonamide (L1)



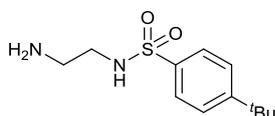
Benzenesulfonyl chloride (1.28 mL, 10 mmol) was reacted according to the general procedure to give the title compound as an off-white waxy solid (0.49 g, 25%). ¹H NMR (300 MHz, *d*₆-DMSO); δ 7.79 (2H, m), 7.59 (3H, m), 3.90 (2H, br s), 2.73 (2H, t, *J* = 6.4 Hz), 2.52 (2H, m). ¹³C NMR (75.5 MHz, *d*₆-DMSO); δ 140.6, 132.3, 129.2, 126.4, 46.0, 41.3. NMR data in accordance with literature precedent.³

N-(2-aminoethyl)benzenesulfonamide (L2)



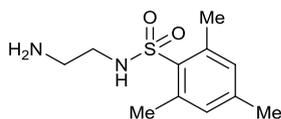
4-methylbenzenesulfonyl chloride (1.77 g, 10 mmol) was reacted according to the general procedure to give the title compound as a white solid (1.06 g, 49%). ¹H NMR (300 MHz, *d*₆-DMSO); δ 7.66 (2H, d, *J* = 8.2 Hz), 7.37 (2H, d, *J* = 8.2 Hz), 3.37 (2H, br s), 2.68 (2H, t, *J* = 6.5 Hz), 2.49 (2H, t, *J* = 6.5 Hz), 2.36 (3H, s). ¹³C NMR (75.5 MHz, *d*₆-DMSO); δ 142.8, 138.1, 130.0, 126.9, 46.6, 41.7, 21.3. NMR data in accordance with literature precedent.³

N-(2-aminoethyl)-4-(*tert*-butyl)benzenesulfonamide (L3)



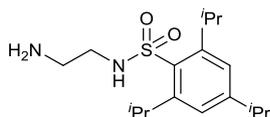
4-(*tert*-butyl)benzenesulfonyl chloride (2.33 g, 10 mmol) was reacted according to the general procedure to give the title compound as a white solid (1.87 g, 77%). $^1\text{H NMR}$ (300 MHz, d_6 -DMSO); δ 7.70 (2H, d, $J = 8.7$ Hz), 7.59 (2H, d, $J = 8.7$ Hz), 3.36 (2H, br s), 2.69 (2H, t, $J = 6.4$ Hz), 2.50 (2H, m), 1.29 (9H, s). $^{13}\text{C NMR}$ (75.5 MHz, d_6 -DMSO); δ 155.2, 137.7, 126.3, 126.0, 46.2, 41.4, 34.8, 30.8. NMR data in accordance with literature precedent.³

***N*-(2-aminoethyl)-2,4,6-trimethylbenzenesulfonamide (L4)**



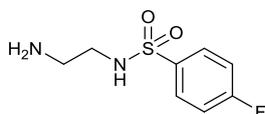
2,4,6-trimethylbenzenesulfonyl chloride (2.19 g, 10 mmol) was reacted according to the general procedure to give the title compound as a white solid (1.87 g, 77%). $^1\text{H NMR}$ (300 MHz, d_6 -DMSO); δ 7.01 (2H, s), 3.31 (2H, br s), 2.68 (2H, t, $J = 6.5$ Hz), 2.54 (6H, s), 2.47 (2H, t, $J = 6.5$ Hz), 2.24 (3H, s). $^{13}\text{C NMR}$ (75.5 MHz, d_6 -DMSO); δ 141.6, 138.6, 134.9, 132.0, 45.7, 41.6, 22.9, 20.7. NMR data in accordance with literature precedent.³

***N*-(2-aminoethyl)-2,4,6-triisopropylbenzenesulfonamide (L5)**



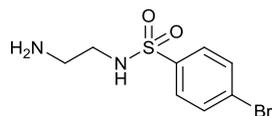
2,4,6-triisopropylbenzenesulfonyl chloride (3.03 g, 10 mmol) was reacted according to the general procedure to give the title compound as a white solid (2.52 g, 77%). $^1\text{H NMR}$ (300 MHz, d_6 -DMSO); δ 7.21 (2H, s), 4.13 (2H, sept, $J = 6.7$ Hz), 3.39 (2H, br s), 2.89 (1H, sept, $J = 6.9$ Hz), 2.78 (2H, t, $J = 6.4$ Hz), 2.53 (2H, t, $J = 6.4$ Hz), 2.49 (1H, t, $J = 1.8$ Hz), 1.20-1.17 (18H, m). $^{13}\text{C NMR}$ (75.5 MHz, d_6 -DMSO); δ 152.2, 149.9, 133.6, 123.8, 45.7, 41.7, 33.7, 29.1, 25.1, 23.8. NMR data in accordance with literature precedent.³

***N*-(2-aminoethyl)-4-fluorobenzenesulfonamide (L6)**



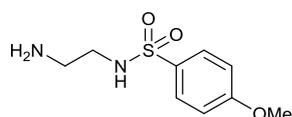
4-fluorobenzenesulfonyl chloride (2.45 g, 10 mmol) was reacted according to the general procedure to give the title compound as a white solid (0.46 g, 21%). $^1\text{H NMR}$ (400 MHz, d_6 -DMSO); δ 7.86 (2H, m), 7.43 (2H, m), 3.50 (2H, br s), 2.73 (2H, t, $J = 6.4$ Hz), 2.52 (2H, m). $^{13}\text{C NMR}$ (101 MHz, d_6 -DMSO); δ 164.1 (d, $^1J_{\text{C-F}} = 251$ Hz), 137.0 (d, $^4J_{\text{C-F}} = 3$ Hz), 129.5 (d, $^3J_{\text{C-F}} = 9$ Hz), 116.3 (d, $^2J_{\text{C-F}} = 23$ Hz), 46.2, 41.3. $^{19}\text{F NMR}$ (376.5 MHz, d_6 -DMSO); δ -107.2. **IR** (film, cm^{-1}); ν 3349, 3298, 3068, 2951, 2897, 2854, 2591, 2161, 2029, 1658, 1590, 1512, 1490, 1404, 1315, 1286, 1234, 1155, 1143, 1088, 1063, 1013, 967, 832, 819, 797, 707, 661. **HRMS** (ESI); calc'd for $\text{C}_8\text{H}_{12}\text{FN}_2\text{O}_2\text{S}$ $[\text{M}+\text{H}]^+$: m/z 219.0598, found 219.0547.

***N*-(2-aminoethyl)-4-bromobenzenesulfonamide (L7)**



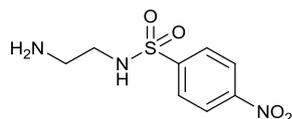
4-bromobenzenesulfonyl chloride (2.56 g, 10 mmol) was reacted according to the general procedure to give the title compound as a white solid (1.84 g, 66%). **¹H NMR** (300 MHz, *d*₆-DMSO); δ 7.81 (2H, d, *J* = 8.7 Hz), 7.72 (2H, d, *J* = 8.7 Hz), 3.48 (2H, br s), 2.73 (2H, t, *J* = 6.5 Hz), 2.50 (2H, m). **¹³C NMR** (75.5 MHz, *d*₆-DMSO); δ 139.9, 132.3, 128.5, 126.1, 46.2, 41.3. **IR** (film, cm⁻¹); ν 3401, 3350, 3299, 3056, 2951, 2861, 2582, 2162, 2026, 1591, 1574, 1491, 1469, 1388, 1316, 1294, 1235, 1145, 1089, 1065, 1008, 968, 926, 817, 779, 736, 702, 662. **HRMS** (ESI); calc'd for C₈H₁₂BrN₂O₂S [M+H]⁺ : *m/z* 278.9797, found 278.9715.

***N*-(2-aminoethyl)-4-methoxybenzenesulfonamide (L8)**



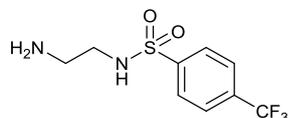
4-methoxybenzenesulfonyl chloride (2.07 g, 10 mmol) was reacted according to the general procedure to give the title compound as a white crystalline solid (0.84 g, 37%). **¹H NMR** (300 MHz, *d*₆-DMSO); δ 7.71 (2H, d, *J* = 8.9 Hz), 7.09 (2H, d, *J* = 8.9 Hz), 3.82 (3H, s), 3.36 (2H, br s), 2.67 (2H, t, *J* = 6.5 Hz), 2.49 (2H, t, *J* = 6.5 Hz). **¹³C NMR** (75.5 MHz, *d*₆-DMSO); δ 162.4, 132.6, 129.0, 114.6, 56.0, 46.5, 41.7. **IR** (film, cm⁻¹); ν 3367, 3307, 3056, 2944, 2839, 2603, 2161, 2039, 1595, 1577, 1495, 1459, 1442, 1412, 1319, 1296, 1254, 1180, 1143, 1092, 1025, 927, 832, 806, 763, 661, 627. **HRMS** (ESI); calc'd for C₉H₁₄N₂O₃S [M+H]⁺ : *m/z* 231.0803, found 231.0785.

***N*-(2-aminoethyl)-4-nitrobenzenesulfonamide (L9)**



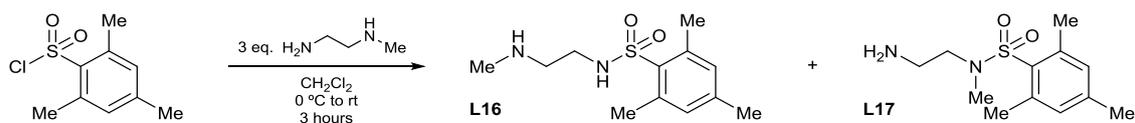
4-nitrobenzenesulfonyl chloride (2.22 g, 10 mmol) was reacted according to the general procedure to give the title compound as a pale-yellow crystalline solid (0.71 g, 29%). **¹H NMR** (300 MHz, *d*₆-DMSO); δ 8.40 (2H, d, *J* = 8.9 Hz), 8.03 (2H, d, *J* = 8.9 Hz), 3.67 (2H, br s), 2.78 (2H, t, *J* = 6.5 Hz), 2.51 (2H, t, *J* = 6.5 Hz). **¹³C NMR** (75.5 MHz, *d*₆-DMSO); δ 149.9, 146.7, 128.4, 124.9, 46.4, 41.7. **IR** (film, cm⁻¹); ν 3362, 3274, 3111, 2931, 2868, 2162, 2032, 1602, 1528, 1469, 1441, 1401, 1349, 1297, 1154, 1111, 1090, 1055, 1009, 959, 854, 830, 776, 737, 698, 681, 640, 606. **HRMS** (ESI); calc'd for C₈H₁₂N₃O₄S [M+H]⁺ : *m/z* 246.0543, found 246.0482.

***N*-(2-aminoethyl)-4-(trifluoromethyl)benzenesulfonamide (L10)**



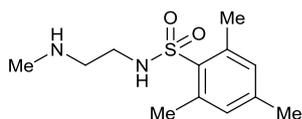
4-(trifluoromethyl)benzenesulfonyl chloride (2.45 g, 10 mmol) was reacted according to the general procedure to give the title compound as a white crystalline solid (1.58 g, 59%). **¹H NMR** (400 MHz, *d*₆-DMSO); δ 8.03-8.00 (4H, m), 3.59 (2H, br s), 2.77 (2H, t, *J* = 6.0 Hz), 2.53 (2H, t, *J* = 6.0 Hz). **¹³C NMR** (101 MHz, *d*₆-DMSO); δ 145.1, 132.6 (q, ²*J*_{C-F} = 32 Hz), 127.9, 126.9 (q, ³*J*_{C-F} = 4 Hz), 124.0 (q, ¹*J*_{C-F} = 273 Hz), 46.6, 41.8. **¹⁹F NMR** (376.5 MHz, *d*₆-DMSO); δ -61.6. NMR data in accordance with literature precedent.³

Procedure for the synthesis of both *N*- and *N'*-methyl-*N*-sulfonated ethylenediamines (L16–L17)



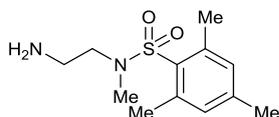
A solution of mesitylsulfonyl chloride (3.82 mmol, 836 mg) in anhydrous dichloromethane (10 mL) was added, *via* a dropping funnel, to a stirring solution of *N*-methylethylenediamine (11.47 mmol, 1.0 mL) in anhydrous dichloromethane (1 mL) at 0 °C. After complete addition, the reaction mixture was allowed to warm to room temperature and stirred for 3 hours. The reaction was then quenched with water (10 mL) and the organics separated. The aqueous layer was then extracted with further dichloromethane (2 × 10 mL). The combined organics were then washed with water (30 mL) followed by brine (30 mL), then dried over MgSO₄ and concentrated. The crude residue contained a mixture of both compounds which were purified by silica gel column chromatography (chloroform 9:1 methanol + 1% triethylamine) to give **L16** as a waxy pale-yellow solid (0.18 g, 7%) and **L17** as a waxy dark-yellow solid (0.55 g, 22%).

2,4,6-trimethyl-*N*-(2-(methylamino)ethyl)benzenesulfonamide (L16)



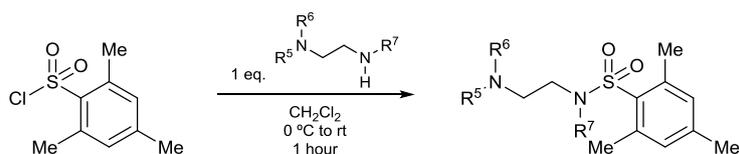
¹H NMR (300 MHz, CDCl₃); δ 6.92 (2H, s), 3.95 (2H, br s), 2.94-2.91 (2H, m), 2.69-2.65 (2H, m), 2.61 (6H, s), 2.31 (3H, s), 2.26 (2H, s). **¹³C NMR** (75.5 MHz, CDCl₃); δ 142.1, 139.1, 133.5, 131.9, 49.8, 41.1, 35.4, 22.9, 20.9. **IR** (film, cm⁻¹); ν 3318, 2975, 2935, 2846, 2162, 1977, 1664, 1603, 1563, 1454, 1404, 1382, 1310, 1262, 1186, 1146, 1118, 1098, 1056, 1035, 966, 933, 845, 796, 757, 718. **HRMS** (ESI); calc'd for C₁₂H₂₁N₂O₂S [M+H]⁺ : *m/z* 257.1324, found 257.1357.

N-(2-aminomethyl)-*N*,2,4,6-tetramethylbenzenesulfonamide (L17)



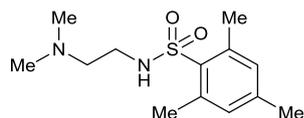
¹H NMR (300 MHz, CDCl₃); δ 6.84 (2H, s), 3.11 (2H, t, *J* = 6.2 Hz), 2.74 (2H, t, *J* = 6.2 Hz), 2.62 (3H, s), 2.50 (6H, s), 2.19 (3H, s), 1.27 (2H, br s). **¹³C NMR** (75.5 MHz, CDCl₃); δ 142.3, 140.0, 132.1, 131.8, 51.9, 39.3, 32.9, 22.6, 20.7. **IR** (film, cm⁻¹); ν 3357, 2975, 2935, 2161, 2024, 1603, 1563, 1457, 1401, 1380, 1309, 1256, 1208, 1143, 1104, 1076, 1053, 1039, 999, 950, 852, 749, 713, 644. **HRMS** (ESI); calc'd for C₁₂H₂₁N₂O₂S [M+H]⁺ : *m/z* 257.1324, found 257.1363.

General procedure for the synthesis of *N*-dimethyl mono-*N*-sulfonated ethylenediamines (L18–L19)



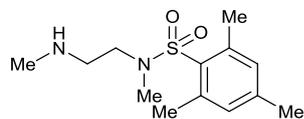
A solution of mesitylsulfonyl chloride (10 mmol, 2.19 g) in anhydrous dichloromethane (25 mL) was added, *via* a dropping funnel, to a stirring solution of the *N*-dimethylethylenediamine (10 mmol) in anhydrous dichloromethane (25 mL) at 0 °C. After complete addition, the reaction mixture was allowed to warm to room temperature and stirred for 1 hour. The reaction was then quenched with water (50 mL) and the organics separated. The aqueous layer was then extracted with further dichloromethane (2 × 25 mL). The combined organics were then washed with water (50 mL) followed by brine (50 mL), then dried over MgSO₄ and concentrated. The crude residue was then purified by silica gel column chromatography (dichloromethane 9:1 methanol + 1% triethylamine).

N-(2-(dimethylamino)ethyl)-2,4,6-trimethylbenzenesulfonamide (L18)



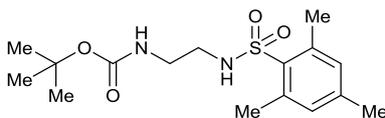
N,N-dimethylethylenediamine (10 mmol, 1.1 mL) was reacted according to the general procedure to give the title compound as an amorphous crystalline solid (2.20g, 81%). ¹H NMR (300 MHz, CDCl₃); δ 6.95 (2H, s), 5.45 (1H, br s), 2.95-2.91 (2H, m), 2.64 (6H, s), 2.39-2.35 (2H, m), 2.29 (3H, s), 2.15 (6H, s). ¹³C NMR (75.5 MHz, CDCl₃); δ 142.1, 139.2, 133.5, 131.9, 57.0, 44.7, 39.4, 22.8, 20.9. IR (film, cm⁻¹); ν 3303, 2943, 2862, 2824, 2775, 2160, 2026, 1604, 1564, 1457, 1384, 1319, 1232, 1186, 1152, 1091, 1152, 1091, 1039, 958, 851, 759, 653. HRMS (ESI); calc'd for C₁₃H₂₃N₂O₂S [M+H]⁺ : *m/z* 271.1480, found 271.1504.

N,2,4,6-tetramethyl-*N*-(2-(methylamino)ethyl)benzenesulfonamide (L19)



N,N'-dimethylethylenediamine (10 mmol, 1.1 mL) was reacted according to the general procedure to give the title compound as a yellow oil (0.20 g, 7%). ¹H NMR (300 MHz, CDCl₃); δ 6.90 (2H, s), 3.22 (2H, t, *J* = 6.4 Hz), 2.74-2.69 (5H, m), 2.56 (6H, s), 2.32 (3H, s), 2.25 (3H, s), 1.56 (1H, br s). ¹³C NMR (75.5 MHz, CDCl₃); δ 142.5, 140.3, 132.1, 132.0, 48.9, 48.2, 36.0, 33.3, 22.8, 20.9. IR (film, cm⁻¹); ν 2937, 2161, 2031, 1603, 1564, 1454, 1403, 1382, 1312, 1187, 1148, 1054, 1035, 956, 889, 853, 743, 727, 702, 645. HRMS (ESI); calc'd for C₁₃H₂₃N₂O₂S [M+H]⁺ : *m/z* 271.1480, found 271.1517.

Procedure for the synthesis of *tert*-butyl (2-((2,4,6-trimethylphenyl)sulfonamido)ethyl)carbamate (L20)



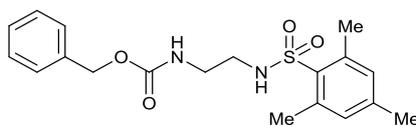
Di-*tert*-butyl dicarbonate (2 mmol, 436 mg) dissolved in chloroform (10 mL) was added dropwise to a stirring solution of ethylenediamine (10 mmol, 0.67 mL) in chloroform (50 mL) at 0 °C. The reaction mixture was left to warm to room temperature and left to stir overnight. The reaction was then quenched with NaHCO₃ (sat.) and the organics separated. The organic layer was then washed with brine before being dried over Na₂SO₄ and concentrated. The residue was then taken up in anhydrous dichloromethane (5 mL) and cooled to 0 °C. A solution of 2,4,6-trimethylbenzenesulfonyl chloride **L4** (2 mmol, 437 mg) in anhydrous dichloromethane (45 mL) was then added dropwise. After complete addition, the reaction mixture was allowed to warm to room temperature and stirred for 3 hours. The reaction was then quenched with water (25 mL) and the organics separated. The aqueous layer was then extracted further with dichloromethane (2 × 25 mL) before the combined organics were dried over Na₂SO₄ and concentrated. The residue was then purified by silica gel column chromatography (hexane 8:2 ethyl acetate) to give the title compound as a colourless oil which solidifies to a white solid upon standing (137 mg, 20%).

¹H NMR (300 MHz, CDCl₃); δ 6.92 (2H, s), 5.57 (1H, br s), 5.04 (1H, br s), 3.20-3.19 (2H, m), 3.00-2.94 (2H, m), 2.60 (6H, s), 2.27 (3H, s), 1.39 (9H, s).

¹³C NMR (75.5 MHz, CDCl₃); δ 156.5, 142.2, 139.1, 133.5, 132.0, 79.7, 43.0, 40.3, 28.3, 22.9, 20.9.

NMR data in accordance with literature precedent.⁴

Procedure for the synthesis of benzyl (2-((2,4,6-trimethylphenyl)sulfonamido)ethyl)carbamate (L21)



To a stirring solution of 1,1'-carbonyldiimidazole (5 mmol, 0.81 g) in anhydrous acetonitrile (20 mL) was added a solution of benzyl alcohol (5 mmol, 0.52 mL) in anhydrous acetonitrile (20 mL) dropwise at room temperature. The reaction mixture was left to stir for 2 hours before the portionwise addition of *N*-(2-aminoethyl)-2,4,6-trimethylbenzenesulfonamide **L4** (5 mmol, 1.21 g). The reaction mixture was left to stir overnight before being concentrated. The residue was then taken up in ethyl acetate (20 mL) and washed with water (3 × 20 mL), then brine (20 mL), dried over Na₂SO₄ and concentrated. The residue was purified by silica gel column chromatography (hexane 7:3 ethyl acetate) to give the title compound a white solid (0.95 g, 50%).

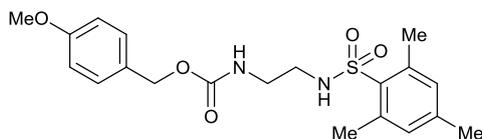
¹H NMR (300 MHz, CDCl₃); δ 7.34-7.29 (5H, m), 6.92 (2H, s), 5.47 (1H, br s), 5.34 (1H, br s), 3.30-3.24 (2H, m), 3.01-2.99 (2H, m), 2.60 (6H, s), 2.28 (3H, s).

¹³C NMR (75.5 MHz, CDCl₃); δ 157.0, 142.4, 139.1, 136.4, 133.4, 132.1, 128.6, 128.2, 128.1, 66.9, 42.7, 40.8, 23.0, 21.0.

IR (film, cm⁻¹); ν 3435, 3200, 2956, 2164, 1978, 1680, 1604, 1506, 1456, 1439, 1402, 1385, 1311, 1261, 1217, 1149, 1118, 1094, 1046, 990, 966, 916, 861, 833, 780, 755, 706, 653.

HRMS (ESI); calc'd for C₁₉H₂₄N₂O₄SNa [M+Na]⁺ : *m/z* 399.1354, found 399.1322.

**Procedure for the synthesis of 4-methoxybenzyl (2-((2,4,6-trimethylphenyl)sulfonamido)ethyl)carbamate
(L22)**



To a stirring solution of 1,1'-carbonyldiimidazole (1.3 mmol, 211 mg) in anhydrous acetonitrile (7.5 mL) was added a solution of 4-methoxybenzyl alcohol (5 mmol, 0.52 mL) in anhydrous acetonitrile (20 mL) dropwise at room temperature and the reaction mixture was left to stir overnight. The reaction was then concentrated, quenched with water (10 mL) and extracted with chloroform (3 × 10 mL). The combined organics were then washed with water (15 mL), then brine (15 mL) before being dried over Na₂SO₄ and concentrated. The residue was then taken up in anhydrous tetrahydropyran (15 mL) and *N*-(2-aminoethyl)-2,4,6-trimethylbenzenesulfonamide **L4** (1 mmol, 242 mg) was added. The reaction mixture was left to stir overnight before being concentrated. The residue was taken up in ethyl acetate (15 mL), washed with water (15 mL), then brine (15 mL), dried over Na₂SO₄ and concentrated. The residue was purified by silica gel column chromatography (hexane 7:3 ethyl acetate) to give the title compound a white crystalline solid (283 mg, 72%).

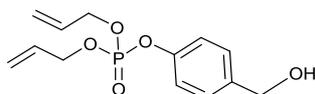
¹H NMR (300 MHz, CDCl₃); δ 7.27 (2H, d, *J* = 8.6 Hz), 6.93 (2H, d, *J* = 8.6 Hz), 5.21 (1H, br t, *J* = 5.4 Hz), 5.10 (1H, br t, *J* = 5.7 Hz), 5.00 (2H, s), 3.80 (3H, s), 3.29-3.24 (2H, m), 3.04-2.98 (2H, m), 2.60 (6H, s), 2.29 (3H, s).

¹³C NMR (75.5 MHz, CDCl₃); δ 159.7, 157.1, 142.4, 139.2, 133.5, 132.2, 130.1, 128.4, 114.0, 66.9, 55.4, 42.9, 40.9, 23.0, 21.1.

IR (film, cm⁻¹); ν 3328, 2939, 2161, 2032, 1682, 1616, 1532, 1445, 1397, 1329, 1303, 1268, 1242, 1157, 1077, 1027, 976, 911, 849, 826, 806, 779, 652.

HRMS (ESI); calc'd for C₂₀H₂₆N₂O₅SNa [M+Na]⁺ : *m/z* 429.1460, found 429.1455.

Procedure for the synthesis of diallyl (4-(hydroxymethyl)phenyl) phosphate (5)



Phosphorus trichloride (8.7 mL, 100 mmol, 2 eq.), allyl alcohol (13.6 mL, 200 mmol, 4 eq.), anhydrous TEA (31 mL, 220 mmol, 4.4 eq.), *N*-chlorosuccinimide (11.7 g, 87.5 mmol, 1.75 eq.), 4-hydroxybenzaldehyde (6.1 g, 50 mmol, 1 eq.), anhydrous TEA (10.5 mL, 75 mmol, 1.5 eq.) and sodium borohydride (3.8 g, 100 mmol, 2 eq.) were reacted together in a telescoped manner according to a previously reported literature procedure.⁵ The crude colourless oil was then purified by silica gel column chromatography (ethyl acetate 1:1 hexane ($R_f = 0.20$, UV_{254} nm & $KMnO_4$)) gave the title compound as a colourless liquid (4.0 g, 28%).

¹H NMR (300 MHz, $CDCl_3$); δ 7.22 (2H, d, $J = 8.6$ Hz), 7.08 (2H, d, $J = 8.6$ Hz), 5.89-5.80 (2H, m), 5.29 (2H, ddd, $J = 17.1, 2.6, 1.5$ Hz), 5.18 (2H, ddd, $J = 10.4, 2.6, 1.1$ Hz), 4.56-4.51 (6H, m), 2.90 (1H, br s).

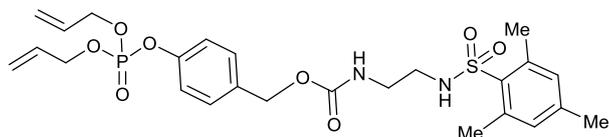
¹³C NMR (75.5 MHz, $CDCl_3$); δ 149.7 (d, $J_{C-P} = 7$ Hz), 138.3 (d, $J_{C-P} = 1$ Hz), 132.0 (d, $J_{C-P} = 7$ Hz), 128.2, 119.9 (d, $J_{C-P} = 5$ Hz), 118.8, 68.9 (d, $J_{C-P} = 6$ Hz), 64.2.

³¹P NMR (121.5 MHz, $CDCl_3$); $\delta - 5.52$.

IR (film, cm^{-1}); ν 3419, 2881, 1651, 1608, 1506, 1459, 1425, 1365, 1267, 1210, 1164, 1097, 1013, 988, 931, 874, 824, 733, 693, 638.

HRMS (ESI); calc'd for $C_{13}H_{17}O_3P$ $[M+Na]^+$: m/z 307.0706, found 307.0760.

Procedure for the synthesis of 4-((bis(allyloxy)phosphoryl)oxy)benzyl 2-((2,4,6-trimethylphenyl)sulfonamido)ethyl)carbamate (6)



To a stirring solution of 1,1'-carbonyldiimidazole (8.4 mmol, 1.36 g) in anhydrous acetonitrile (25 mL), was added a solution of diallyl (4-(hydroxymethyl)phenyl) phosphate **5** (6.5 mmol, 1.86 g) in anhydrous acetonitrile (25 mL) slowly *via* a dropping funnel. After complete addition, the reaction mixture was allowed to stir overnight before concentrated under reduced pressure. The residue was taken up in chloroform (50 mL) and washed with water (50 mL). The organics were separated and the aqueous layer extracted with chloroform (2 × 50 mL). The combined organics were washed with water (3 × 50 mL), dried over MgSO₄ and concentrated. The residue was taken up in anhydrous acetonitrile (50 mL) and *N*-(2-aminoethyl)-2,4,6-trimethylbenzenesulfonamide **L4** (8.4 mmol, 2.71 g) was added. The reaction mixture was then allowed to stir at room temperature for 4 hours before being concentrated under reduced pressure. The residue was taken up in chloroform (50 mL) and washed with water (50 mL). The organics were separated and the aqueous layer extracted with chloroform (2 × 50 mL). The combined organics were then washed with water (2 × 50 mL) and brine (50 mL) before dried over MgSO₄ and concentrated under reduced pressure to obtain the crude product. The product was then purified by silica gel column chromatography (hexane 1:1 ethyl acetate) to give the title compound as a colourless oil (2.71 g, 76%).

¹H NMR (300 MHz, CDCl₃); δ 7.03 (2H, d, *J* = 8.3 Hz), 6.91 (2H, d, *J* = 8.3 Hz), 6.66 (2H, s), 5.64 (4H, m), 5.12 (2H, dq, *J* = 17.2, 1.3 Hz), 5.01 (2H, ap dd, *J* = 10.4, 1.3 Hz), 4.74 (2H, s), 4.38 (4H, ddd, *J* = 8.4, 5.7, 1.3 Hz), 2.97 (2H, ap dd, *J* = 10.9, 5.5 Hz), 2.71 (2H, ap dd, *J* = 10.9, 5.5 Hz), 2.33 (6H, s), 2.02 (3H, s).

¹³C NMR (75.5 MHz, CDCl₃); δ 156.8, 150.2 (d, *J*_{C-P} = 7 Hz), 142.1, 139.0, 133.6, 132.0, 132.0, 131.9, 129.6, 121.1 (d, *J*_{C-P} = 5 Hz), 118.8, 69.0 (d, *J*_{C-P} = 5 Hz), 65.9, 42.5, 40.7, 22.9, 20.9.

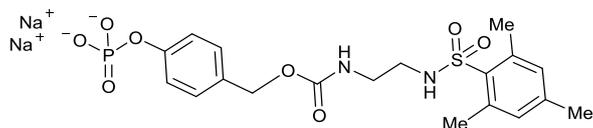
³¹P NMR (121.5 MHz, CDCl₃); δ -5.65.

IR (film, cm⁻¹); ν 3299, 2942, 1707, 1605, 1508, 1456, 1425, 1382, 1324, 1255, 1218, 1153, 1095, 1015, 989, 936, 853, 828, 776, 734, 654.

HRMS (ESI); calc'd for C₂₅H₃₃N₂O₈PS [M+H]⁺ : *m/z* 553.1773, found 553.1765.

Procedure for the synthesis of

4-(((2-((2,4,6-trimethylphenyl)sulfonamido)ethyl)carbamoyloxy)methyl)phenyl phosphate (PL1)



To a stirring solution of 4-((bis(allyloxy)phosphoryl)oxy)benzyl (2-((2,4,6-trimethylphenyl)sulfonamido)ethyl) carbamate **7** (276 mg, 0.5 mmol) in anhydrous tetrahydrofuran (5 mL), was sequentially added polymer-bound tetrakis(triphenylphosphine)palladium (14 mg, 0.01 mmol), formic acid (0.3 mL, 7.5 mmol) and triethylamine (0.7 mL, 5 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature and left to stir overnight. The reaction mixture was then filtered through filter paper and washed through with tetrahydrofuran (15 mL). The filtrate was concentrated under reduced pressure with the excess formic acid being removed *via* its hexane azeotrope. The residue was then cooled to 0 °C and 1M NaOH (6 mL, 6 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 1 hour. The reaction mixture was then concentrated under reduced pressure with the excess triethylamine removed *via* its toluene azeotrope to obtain the crude product. The product was then purified by reverse phase (C18) silica gel column chromatography (water) to give the title compound as a white powder (257 mg, *quant.*).

¹H NMR (300 MHz, D₂O/NaOD); δ 7.23 (2H, d, *J* = 8.2 Hz), 7.12 (2H, d, *J* = 8.2 Hz), 6.92 (2H, s), 4.79 (2H, s), 2.93 (2H, m), 2.78 (2H, s), 2.49 (6H, s), 2.16 (3H, s).

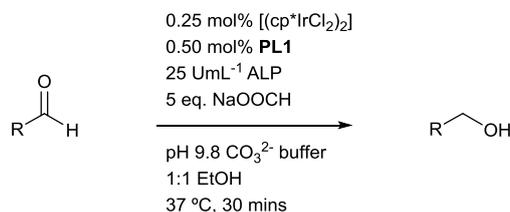
³¹P NMR (121.5 MHz, D₂O/NaOD); δ 1.05.

IR (solid, cm⁻¹); ν 3299, 1707, 1605, 1508, 1456, 1425, 1382, 1324, 1255, 1218, 1153, 1095, 1015, 989, 936, 853, 828, 776, 734, 654.

HRMS (ESI); calc'd for C₁₉H₂₄N₂O₈PS [M-H]⁻ : *m/z* 471.0991, found 471.1025.

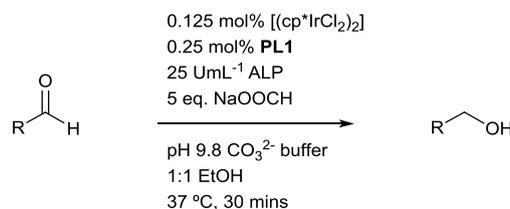
General procedures for the enzyme-triggered transfer hydrogenation of aldehydes to alcohols

Procedure A:



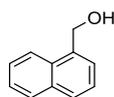
To a medium screw-top vial equipped with a magnetic flea was added dichloro(pentamethylcyclopentadienyl)iridium(III) dimer (1 mg, 0.00125 mmol), 4-(((2-((2,4,6-trimethylphenyl)sulfonamido)ethyl)carbamoyloxy)methyl)phenyl phosphate **PL1** (1.3 mg, 0.0025 mmol) and sodium formate (170 mg, 2.5 mmol) and dissolved in pH 9.8 0.05M sodium carbonate buffer (1 mL). Alkaline phosphatase (2.5 mg, 10 U mg⁻¹) was then added. Immediately thereafter, a solution of the aldehyde (0.5 mmol) in ethanol (1 mL) was added. The vial was then secured into a DrySyn vial holder upon a stirrer hotplate and stirred at 1000 rpm for 30 minutes at 37 °C. The reaction was then poured into a 100 mL conical flask containing water (20 mL) and ethyl acetate (20 mL). The organics were extracted and the aqueous layer was extracted further with ethyl acetate (2 × 20 mL). The combined organics were dried over MgSO₄ and concentrated under reduced pressure. The crude residue was then analysed by ¹H NMR to obtain conversions. To confirm the diagnostic peaks used to calculate conversions corresponded with those of the product; analytically pure samples of the product were obtained through purification of the crude material by flash silica gel column chromatography (hexane 9:1 ethyl acetate).

Procedure B:



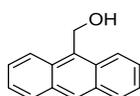
To a medium screw-top vial equipped with a magnetic flea was added dichloro(pentamethylcyclopentadienyl)iridium(III) dimer (1 mg, 0.00125 mmol), 4-(((2-((2,4,6-trimethylphenyl)sulfonamido)ethyl)carbamoyloxy)methyl)phenyl phosphate **PL1** (1.3 mg, 0.0025 mmol) and sodium formate (340 mg, 5 mmol) and dissolved in pH 9.8 0.05M sodium carbonate buffer (2 mL). Alkaline phosphatase (2.5 mg, 10 U mg⁻¹) was then added. Immediately thereafter, a solution of the aldehyde (1 mmol) in ethanol (2 mL) was added. The vial was then secured into a DrySyn vial holder upon a stirrer hotplate and stirred at 1000 rpm for 30 minutes at 37 °C. The reaction was then poured into a 100 mL conical flask containing water (20 mL) and ethyl acetate (20 mL). The organics were extracted and the aqueous layer was extracted further with ethyl acetate (2 × 20 mL). The combined organics were dried over MgSO₄ and concentrated under reduced pressure. The crude residue was then analysed by ¹H NMR to obtain conversions. To confirm the diagnostic peaks used to calculate conversions corresponded with those of the product; analytically pure samples of the product were obtained through purification of the crude material by flash silica gel column chromatography (hexane 9:1 ethyl acetate).

Naphthalen-1-ylmethanol (7)



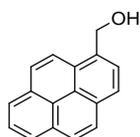
1-Naphthaldehyde (68 μ L, 0.5 mmol) was reacted according to general procedure **A** to give the title compound as a colourless oil (>99% conversion by ¹H NMR). ¹H NMR (300 MHz, CDCl₃); δ 8.13-8.10 (1H, m), 7.91-7.88 (1H, m), 7.84-7.81 (1H, m), 7.59-7.42 (4H, m), 5.13 (2H, s), 1.95 (1H, br s). ¹³C NMR (75.5 MHz, CDCl₃); δ 136.3, 133.9, 131.3, 128.8, 128.7, 126.5, 126.0, 125.5, 125.4, 123.8, 63.8. NMR data in accordance with literature precedent.⁶

Anthracen-9-ylmethanol (8)



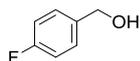
Anthracene-9-carboxaldehyde (103 mg, 0.5 mmol) was reacted according to general procedure **A** to give the title compound as a pale yellow crystalline solid (77% conversion by ¹H NMR). ¹H NMR (300 MHz, CDCl₃); δ 8.44 (1H, s), 8.37 (2H, d, J = 8.7 Hz), 8.02 (2H, d, J = 8.2 Hz), 7.58-7.46 (4H, m), 5.61 (2H, s), 1.87 (1H, br s). ¹³C NMR (75.5 MHz, CDCl₃); δ 131.6, 131.1, 130.2, 129.3, 128.5, 126.6, 125.2, 124.0, 57.5. NMR data in accordance with literature precedent.⁷

Pyren-1-ylmethanol (9)



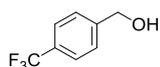
Pyrene-1-carboxaldehyde (115 mg, 0.5 mmol) was reacted according to general procedure **A** to give the title compound as a pale yellow crystalline solid (95% conversion by ¹H NMR). ¹H NMR (300 MHz, CDCl₃); δ 8.26 (1H, d, J = 9.2 Hz), 8.17 (2H, d, J = 7.6 Hz), 8.09-7.94 (6H, m), 5.31 (2H, s), 2.02 (1H, br s). ¹³C NMR (75.5 MHz, CDCl₃); δ 133.8, 131.29, 131.26, 130.8, 128.8, 127.9, 127.49, 127.45, 126.04, 126.01, 125.35, 125.33, 124.9, 124.8, 123.0, 63.8. NMR data in accordance with literature precedent.⁸

4-Fluorobenzyl alcohol (10)



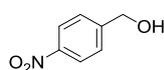
4-Fluorobenzaldehyde (94 μ L, 1 mmol) was reacted according to general procedure **B** to give the title compound as a colourless oil (>99% conversion by ¹H NMR). ¹H NMR (300 MHz, CDCl₃); δ 7.35-7.29 (2H, m), 7.08-7.00 (2H, m), 4.64 (2H, s), 1.97 (1H, br s). ¹³C NMR (75.5 MHz, CDCl₃); δ 162.4 (d, ¹ J_{C-F} = 246 Hz), 136.7 (d, ⁴ J_{C-F} = 3 Hz), 128.9 (d, ³ J_{C-F} = 8 Hz), 115.5 (d, ² J_{C-F} = 21 Hz), 64.7. ¹⁹F NMR (470.5 MHz, CDCl₃); δ -114.9.⁹

4-(Trifluoromethyl)benzyl alcohol (11)



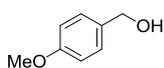
4-(Trifluoromethyl)benzaldehyde (136 μ L, 1 mmol) was reacted according to general procedure **B** to give the title compound as a colourless oil (>99% conversion by $^1\text{H NMR}$). $^1\text{H NMR}$ (300 MHz, CDCl_3); δ 7.57 (2H, d, $J = 8.1$ Hz), 7.39 (2H, d, $J = 8.1$ Hz), 4.65 (2H, s), 3.24 (1H, br s). $^{13}\text{C NMR}$ (75.5 MHz, CDCl_3); δ 144.8 (q, $^4J_{\text{C-F}} = 1$ Hz), 129.8 (q, $^2J_{\text{C-F}} = 32$ Hz), 126.9, 125.5 (q, $^3J_{\text{C-F}} = 32$ Hz), 124.3 (q, $^1J_{\text{C-F}} = 272$ Hz), 64.3. $^{19}\text{F NMR}$ (470.5 MHz, CDCl_3); δ -62.5. NMR data in accordance with literature precedent.¹⁰

4-Nitrobenzyl alcohol (12)



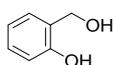
4-Nitrobenzaldehyde (76 mg, 0.5 mmol) was reacted according to general procedure **B** to give the title compound as a white solid (>99% conversion by $^1\text{H NMR}$). $^1\text{H NMR}$ (300 MHz, CDCl_3); δ 8.18 (2H, d, $J = 8.2$ Hz), 7.51 (2H, d, $J = 8.2$ Hz), 4.82 (2H, s), 2.29 (1H, br s). $^{13}\text{C NMR}$ (75.5 MHz, CDCl_3); δ 148.4, 147.3, 127.1, 123.8, 64.0. NMR data in accordance with literature precedent.¹¹

4-Methoxybenzyl alcohol (13)



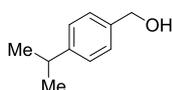
p-Anisaldehyde (61 μ L, 0.5 mmol) was reacted according to general procedure **A** to give the title compound as a colourless oil (>99% conversion by $^1\text{H NMR}$). $^1\text{H NMR}$ (300 MHz, CDCl_3); δ 7.20 (2H, d, $J = 8.7$ Hz), 6.81 (2H, d, $J = 8.7$ Hz), 4.51 (2H, s), 3.72 (3H, s), 1.89 (1H, br s). $^{13}\text{C NMR}$ (75.5 MHz, CDCl_3); δ 159.3, 133.2, 128.8, 114.0, 65.1, 55.4. NMR data in accordance with literature precedent.¹²

2-Hydroxybenzyl alcohol (14)



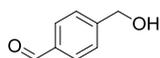
Salicylaldehyde (53 μ L, 0.5 mmol) was reacted according to general procedure **A** to give the title compound as a white crystalline solid (>99% conversion by $^1\text{H NMR}$). $^1\text{H NMR}$ (300 MHz, CDCl_3); δ 7.34 (1H, br s), 7.24-7.18 (1H, m), 7.04 (1H, dd, $J = 7.4, 1.3$ Hz), 4.85 (2H, s), 2.46 (1H, br s). $^{13}\text{C NMR}$ (75.5 MHz, CDCl_3); δ 156.1, 129.7, 128.0, 124.8, 120.3, 116.6, 64.7. NMR data in accordance with literature precedent.¹³

4-Isopropylbenzyl alcohol (15)



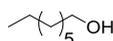
Cuminaldehyde (76 μL , 0.5 mmol) was reacted according to general procedure **A** to give the title compound as a colourless oil (>99% conversion by ^1H NMR). ^1H NMR (300 MHz, CDCl_3); δ 7.31 (2H, d, $J = 8.2$ Hz), 7.24 (2H, d, $J = 8.2$ Hz), 4.66 (2H, s), 2.92 (1H, hept, $J = 6.9$ Hz), 1.71 (1H, br s), 1.26 (6H, d, $J = 6.9$ Hz). ^{13}C NMR (75.5 MHz, CDCl_3); δ 148.5, 138.3, 127.2, 126.7, 65.3, 33.9, 24.1. NMR data in accordance with literature precedent.¹⁴

4-(Hydroxymethyl)benzaldehyde (**16**)



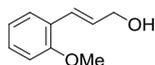
Terephthalaldehyde (67 mg, 0.5 mmol) was reacted according to general procedure **A** to give the title compound as a white solid (>99% conversion by ^1H NMR). ^1H NMR (300 MHz, CDCl_3); δ 9.98 (1H, s), 7.85 (2H, d, $J = 8.1$ Hz), 7.51 (2H, d, $J = 8.1$ Hz), 4.79 (2H, s), 2.28 (1H, br s). ^{13}C NMR (75.5 MHz, CDCl_3); δ 192.2, 148.0, 135.8, 130.1, 127.1, 64.6. NMR data in accordance with literature precedent.¹⁵

1-Octanol (**17**)



Octanal (79 μL , 0.5 mmol) was reacted according to general procedure **A** to give the title compound as a colourless oil (>99% conversion by ^1H NMR). ^1H NMR (300 MHz, CDCl_3); δ 3.64 (2H, t, $J = 6.6$ Hz), 1.61-1.52 (2H, m), 1.39-1.28 (11H, m), 0.88 (3H, t, $J = 6.8$ Hz). ^{13}C NMR (75.5 MHz, CDCl_3); δ 63.2, 33.0, 29.5, 29.4, 25.9, 22.8, 14.2. NMR data in accordance with literature precedent.⁹

2-Methoxycinnamyl alcohol (**18**)



2-Methoxycinnamaldehyde (81 mg, 0.5 mmol) was reacted according to general procedure **A** to give the title compound as a colourless oil (75% conversion by ^1H NMR). ^1H NMR (300 MHz, CDCl_3); δ 7.44 (1H, dd, $J = 7.6, 1.7$ Hz), 7.27-7.21 (1H, m), 6.96-6.86 (3H, m), 6.38 (1H, dt, $J = 16.0, 5.9$ Hz), 4.32 (2H, d, $J = 5.0$ Hz), 3.84 (3H, s), 1.81 (1H, br s). ^{13}C NMR (75.5 MHz, CDCl_3); δ 156.8, 129.4, 128.9, 127.1, 126.2, 125.8, 120.8, 110.9, 64.3, 55.5. NMR data in accordance with literature precedent.¹⁶

General Procedure for the ^{31}P NMR Experiment

13 mg of **PL1** was dissolved in 400 μL of pH 9.8 CO_3^{2-} buffer and to this solution was added 100 μL of 2.5 mg of ALP in 1 mL of pH 9.8 CO_3^{2-} buffer to give an overall reaction concentration of 50 mM **PL1** and 5 U mL^{-1} ALP. The mixture was then spiked with a small amount of D_2O for NMR locking and analysed. At regular intervals, the NMR tube was inverted and return to its upright position to induce reagent mixing before being re-analysed. This was repeated until complete consumption of **PL1** was observed.

General Procedure for the Mass Spectrometry Experiment

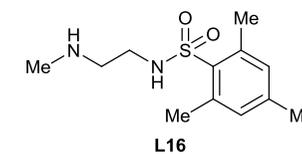
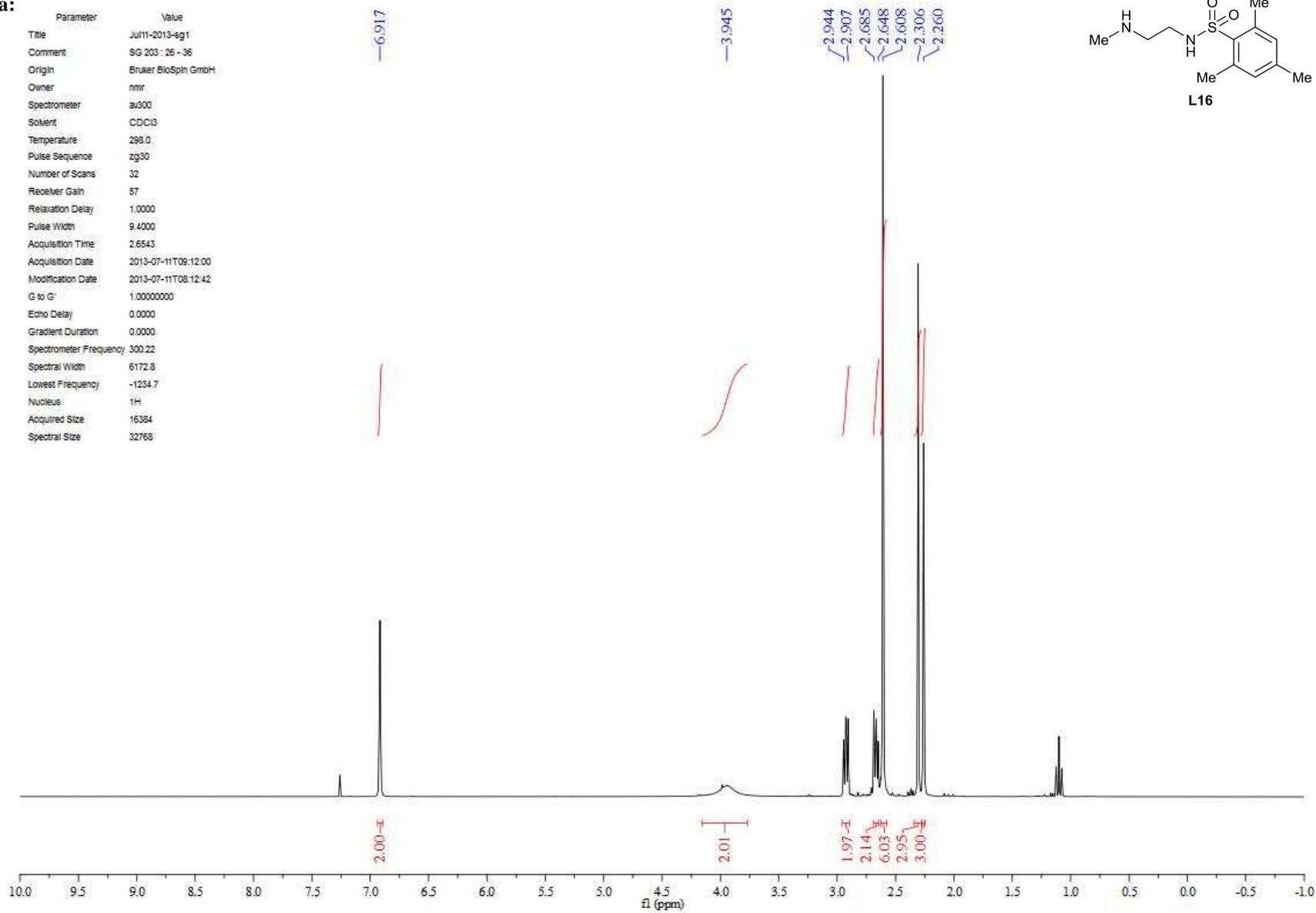
1.3 mg of **PL1** was dissolve in 900 μL of water and to this solution was added 100 μL of a solution containing 2.5 mg of 10 U mg^{-1} ALP in 1 mL of μL of pH 9.8 CO_3^{2-} buffer. The reaction mixture was then taken up in a micro-syringe and injected at 3 $\mu\text{L min}^{-1}$ directly into an Electrospray Time-of-Flight Mass Spectrometer (ESI-UHR-TOF MS, negative ion mode). Injection was stopped after complete consumption of **PL1** was observed.

References:

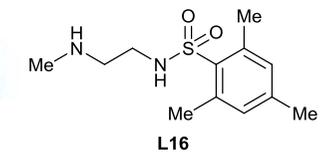
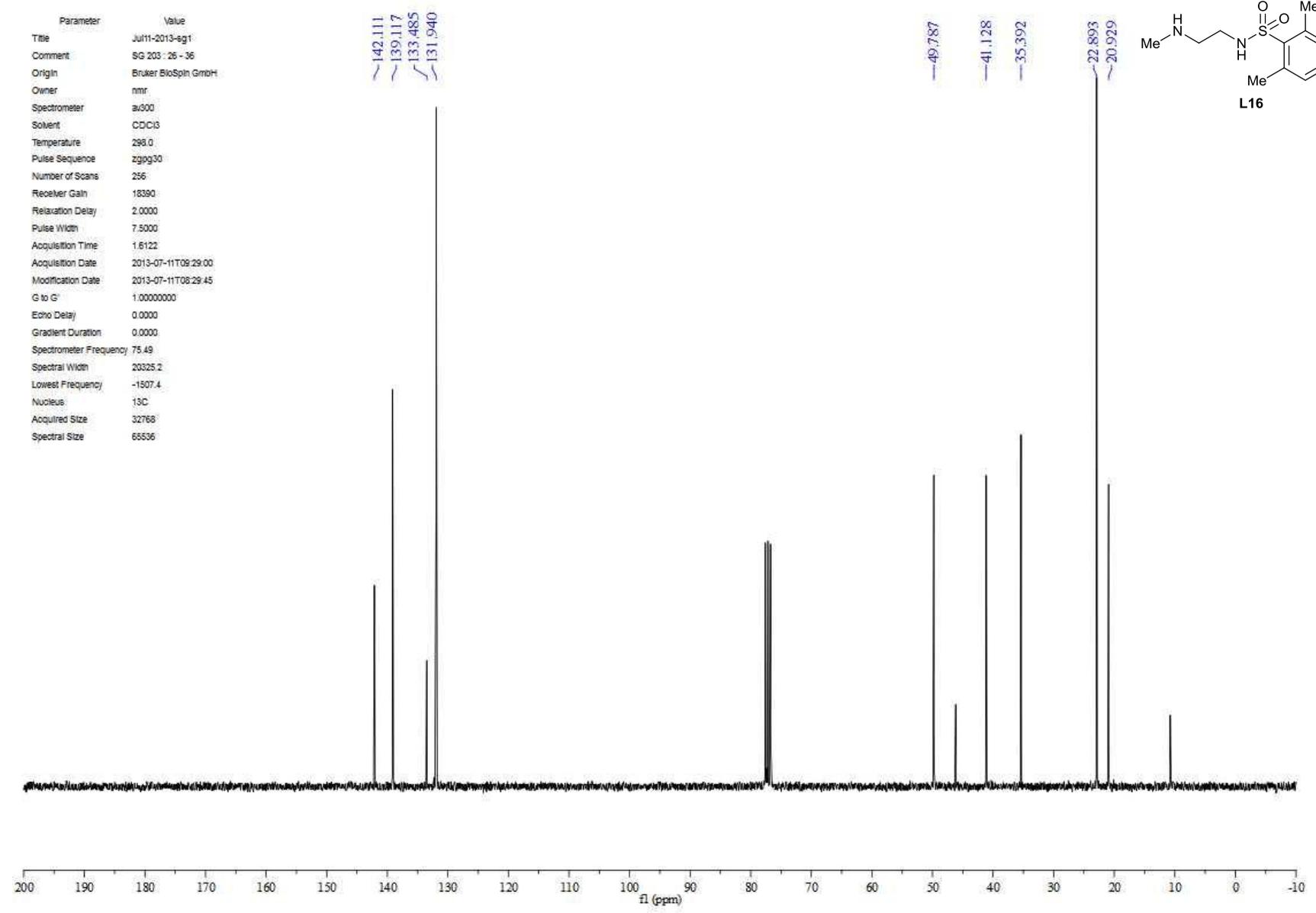
1. H.E. Gottlieb, V. Kotlyar and A. Nudelman, *J. Org. Chem.*, 1997, **62**, 7512.
2. *Purification of Laboratory Chemicals* 3rd ed., C. L. L. Chai and W. L. F. Amarego, Pergamon Press, Oxford, 1988.
3. J. Tan, W. Tang, Y. Sun, Z. Jiang, F. Chen, L. Xu, Q. Fan and J. Xiao, *Tetrahedron*, **67**, 6206.
4. D. G. Batt, J. J. Petraitis, G. C. Houghton, D. P. Modi, G. A. Cain, M. H. Corjay, S. A. Mousa, P. J. Bouchard, M. S. Forsythe, P. B. Harlow, F. A. Barbera, S. M. Spitz, R. R. Wexler and P. K. Jadhav, *J. Med. Chem.*, 2000, **43**, 41.
5. S. Goggins, C. Naz, B. J. Marsh and C. G. Frost, *Chem. Commun.*, 2015, **51**, 561.
6. U. Ragnarsson, L. Grehn, L. S. Monteiro and H. L. S. Maia, *Synlett*, 2013, **15**, 2386.
7. N. Mase, T. Ando, F. Shibagaki, A. Sugita, T. Narumi, M. Toda, N. Watanabe and F. Tanaka, *Tetrahedron Lett.*, 2014, **55**, 1946.
8. S. Chen, L. Wang, N. E. Fahmi, S. J. Benkovic and S. M. Hecht, *J. Am. Chem. Soc.*, 2012, **134**, 18883.
9. N. S. Shaikh, K. Junge and M. Beller, *Org. Lett.*, 2007, **9**, 5429.
10. A. P. Dieskau, J.-M. Begouin and B. Plietker, *Eur. J. Org. Chem.*, 2011, **2011**, 5291.
11. N. Murai, M. Yonaga and K. Tanaka, *Org. Lett.*, 2012, **14**, 1278.
12. R. Cano, M. Yus and D. J. Ramón, *Tetrahedron*, 2011, **67**, 8079.
13. Y. Zhou, G. Gao, H. Li and J. Qu, *Tetrahedron Lett.*, 2008, **49**, 3260.
14. B. Basu, B. Mandal, S. Das, P. Das and A. K. Nanda, *Beilstein J. Org. Chem.*, 2008, **4**, 53.
15. E. Kawabata, K. Kikuchi, Y. Urano, H. Kojima, A. Odani and T. Nagano, *J. Am. Chem. Soc.*, 2005, **127**, 818.
16. C. Morrill and R. H. Grubbs, *J. Am. Chem. Soc.*, 2005, **127**, 2842.

NMR Spectra:

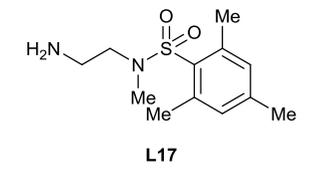
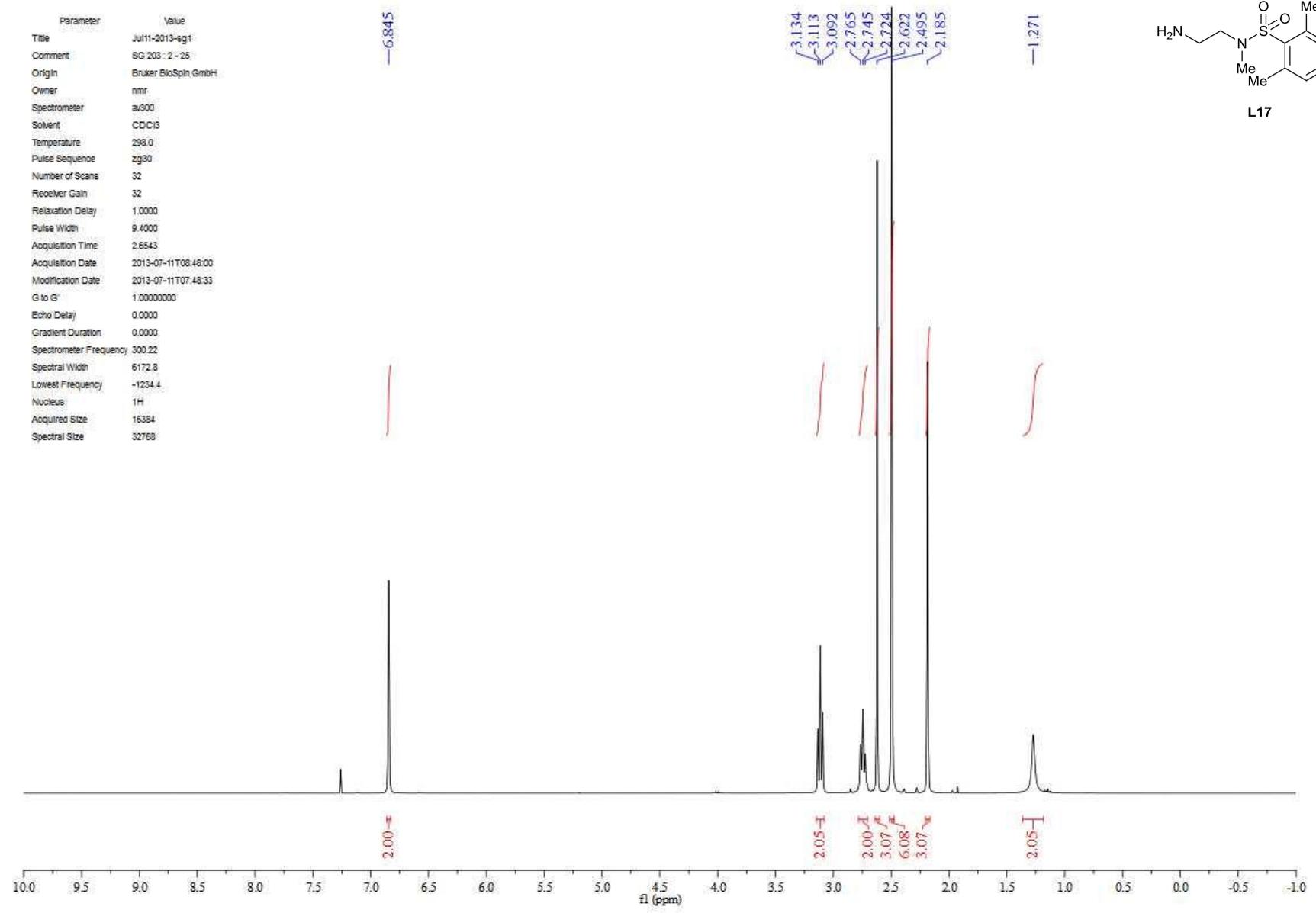
Parameter	Value
Title	Jul11-2013-eg1
Comment	SG 203 - 26 - 36
Origin	Bruker BioSpin GmbH
Owner	nmr
Spectrometer	av300
Solvent	CDCl3
Temperature	298.0
Pulse Sequence	zg30
Number of Scans	32
Receiver Gain	57
Relaxation Delay	1.0000
Pulse Width	9.4000
Acquisition Time	2.6543
Acquisition Date	2013-07-11T09:12:00
Modification Date	2013-07-11T08:12:42
G to G'	1.00000000
Echo Delay	0.0000
Gradient Duration	0.0000
Spectrometer Frequency	300.22
Spectral Width	6172.8
Lowest Frequency	-1234.7
Nucleus	1H
Acquired Size	16384
Spectral Size	32768



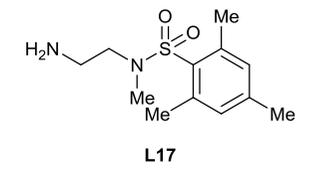
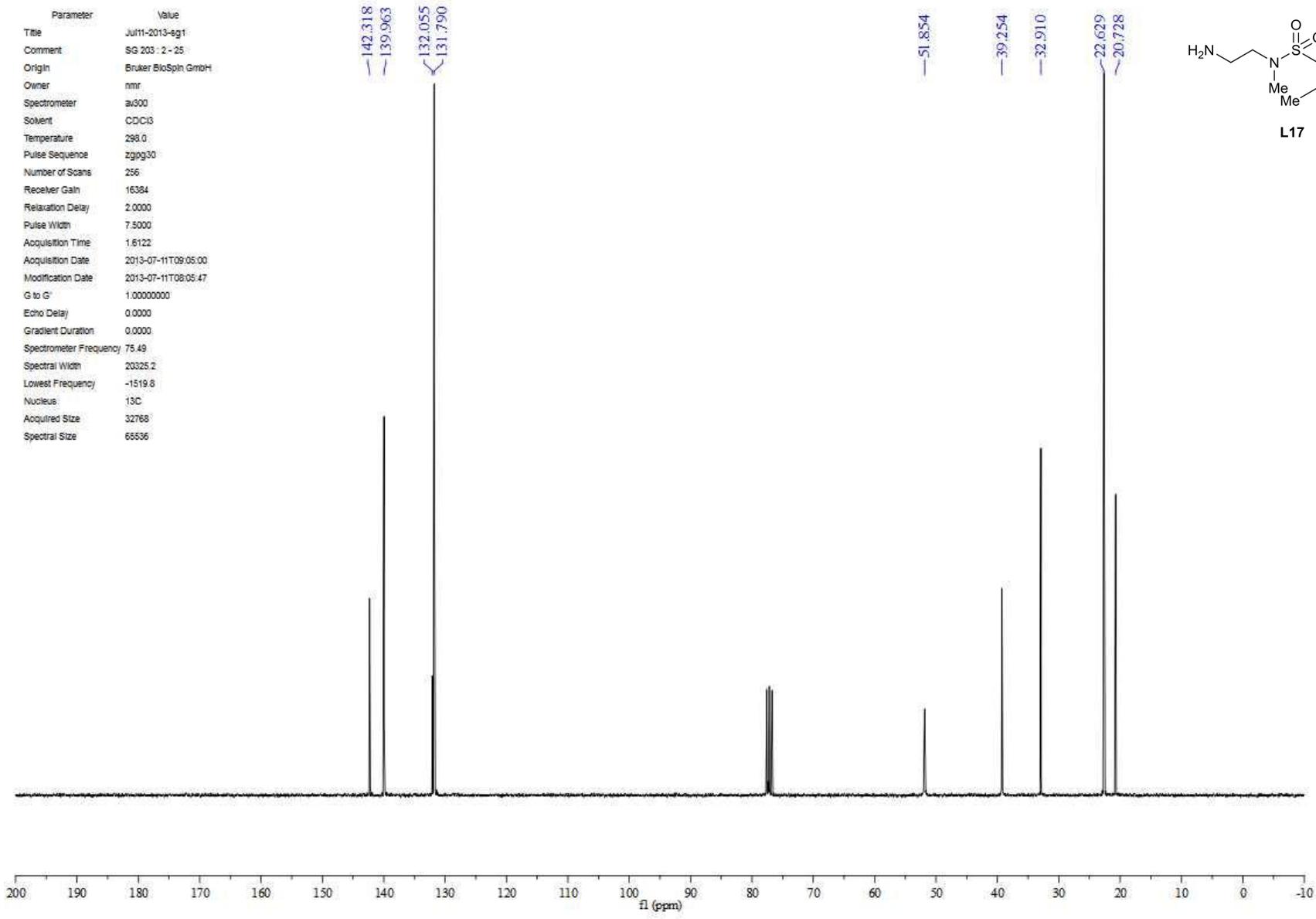
Parameter	Value
Title	Jul11-2013-eg1
Comment	SG 203 : 26 - 36
Origin	Bruker BioSpin GmbH
Owner	nmr
Spectrometer	av500
Solvent	CDCl3
Temperature	298.0
Pulse Sequence	zgpg30
Number of Scans	256
Receiver Gain	18390
Relaxation Delay	2.0000
Pulse Width	7.5000
Acquisition Time	1.6122
Acquisition Date	2013-07-11T09:29:00
Modification Date	2013-07-11T08:29:45
G to G'	1.0000000
Echo Delay	0.0000
Gradient Duration	0.0000
Spectrometer Frequency	75.49
Spectral Width	20325.2
Lowest Frequency	-1507.4
Nucleus	¹³ C
Acquired Size	32768
Spectral Size	65536



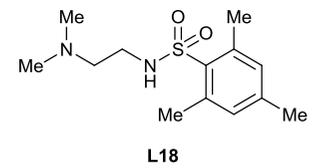
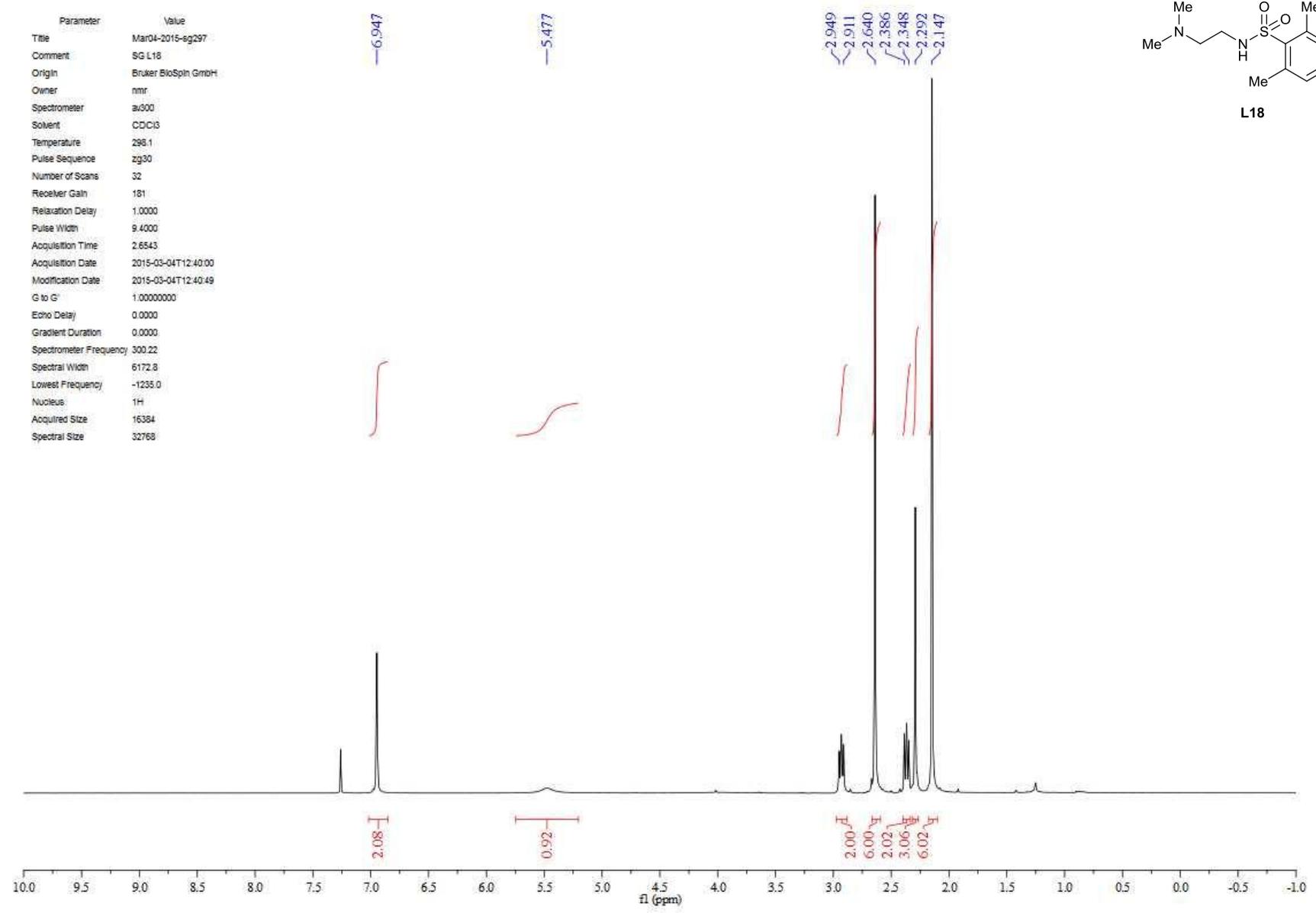
Parameter	Value
Title	Jul11-2013-eg1
Comment	SG 203 : 2 - 25
Origin	Bruker BioSpin GmbH
Owner	nmr
Spectrometer	av500
Solvent	CDCl3
Temperature	298.0
Pulse Sequence	zg30
Number of Scans	32
Receiver Gain	32
Relaxation Delay	1.0000
Pulse Width	9.4000
Acquisition Time	2.6543
Acquisition Date	2013-07-11T08:48:00
Modification Date	2013-07-11T07:48:33
G to G'	1.0000000
Echo Delay	0.0000
Gradient Duration	0.0000
Spectrometer Frequency	300.22
Spectral Width	6172.8
Lowest Frequency	-1234.4
Nucleus	1H
Acquired Size	16384
Spectral Size	32768



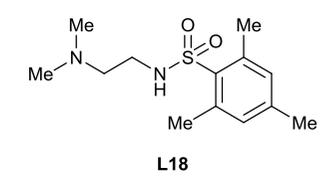
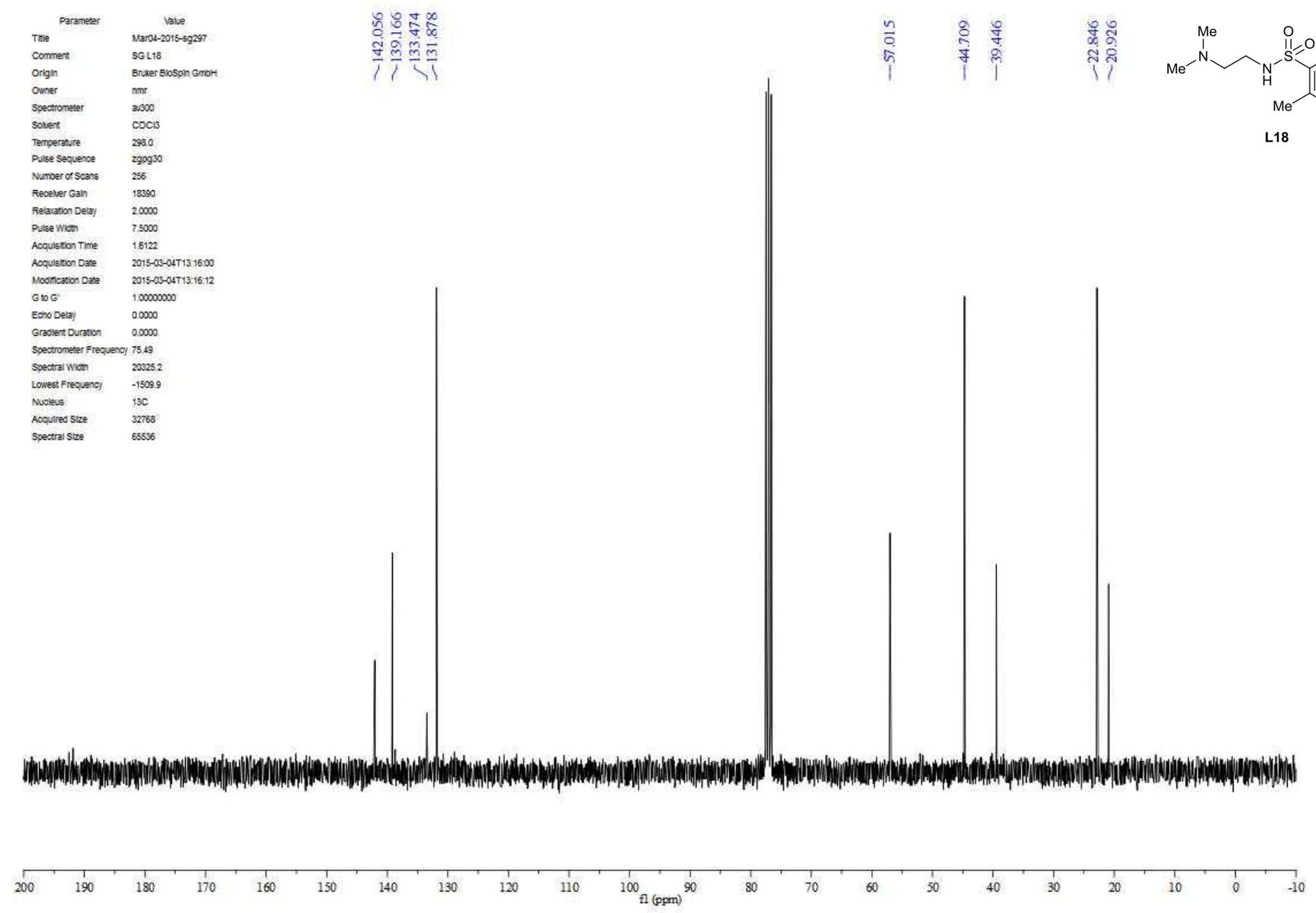
Parameter	Value
Title	Jul11-2013-eg1
Comment	SG 203 : 2 - 25
Origin	Bruker BioSpin GmbH
Owner	nmr
Spectrometer	av500
Solvent	CDCl3
Temperature	298.0
Pulse Sequence	zgpg30
Number of Scans	256
Receiver Gain	16384
Relaxation Delay	2.0000
Pulse Width	7.5000
Acquisition Time	1.6122
Acquisition Date	2013-07-11T09:05:00
Modification Date	2013-07-11T08:05:47
G to G'	1.00000000
Echo Delay	0.0000
Gradient Duration	0.0000
Spectrometer Frequency	75.49
Spectral Width	20325.2
Lowest Frequency	-1519.8
Nucleus	¹³ C
Acquired Size	32768
Spectral Size	65536



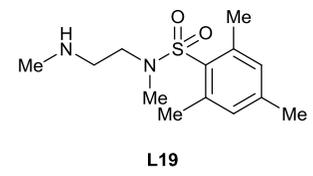
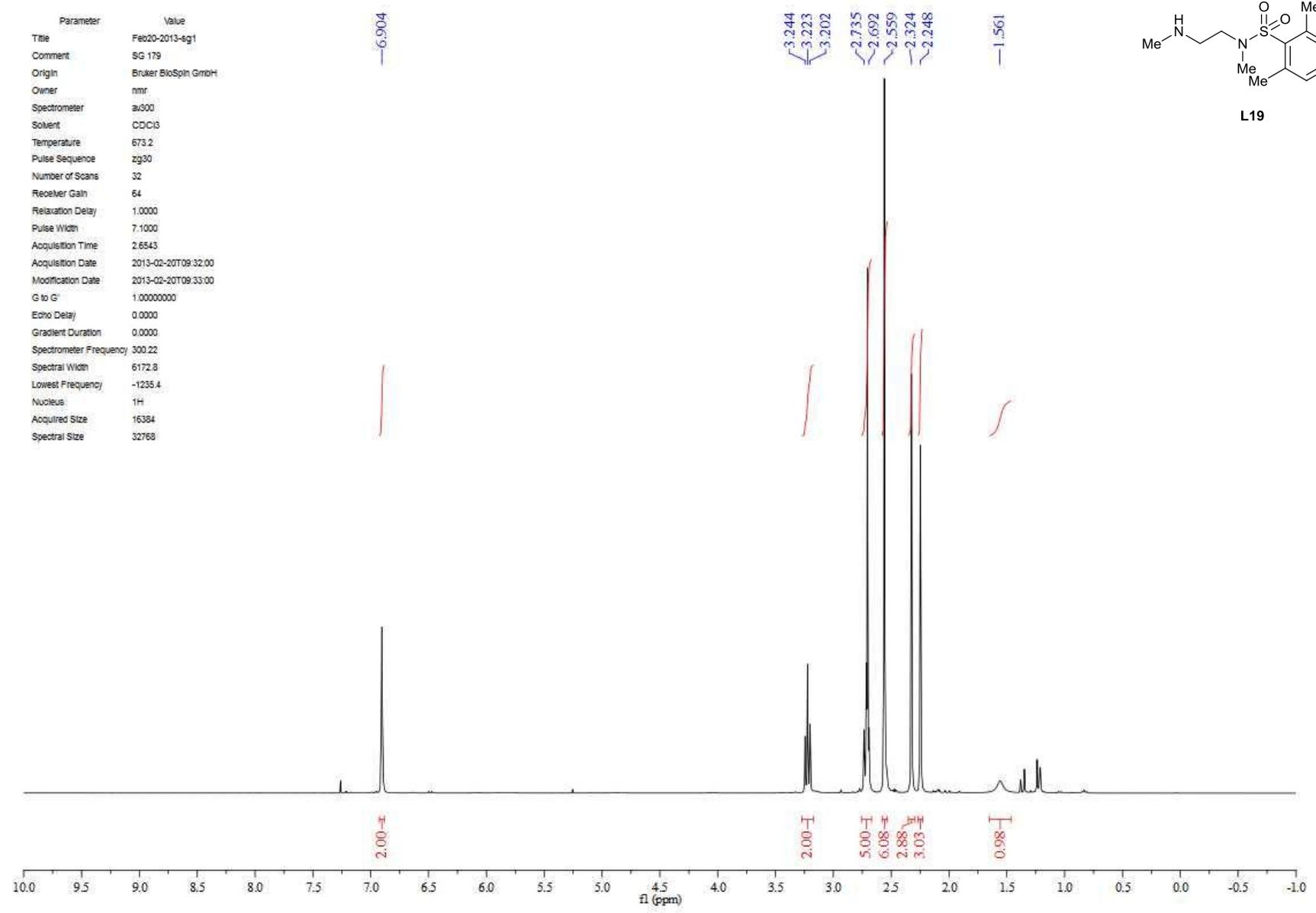
Parameter	Value
Title	Mar04-2015-eg297
Comment	SG L18
Origin	Bruker BioSpin GmbH
Owner	nmr
Spectrometer	av500
Solvent	CDCl3
Temperature	298.1
Pulse Sequence	zg30
Number of Scans	32
Receiver Gain	181
Relaxation Delay	1.0000
Pulse Width	9.4000
Acquisition Time	2.6543
Acquisition Date	2015-03-04T12:40:00
Modification Date	2015-03-04T12:40:49
G to G'	1.00000000
Echo Delay	0.0000
Gradient Duration	0.0000
Spectrometer Frequency	300.22
Spectral Width	6172.8
Lowest Frequency	-1235.0
Nucleus	¹ H
Acquired Size	16384
Spectral Size	32768



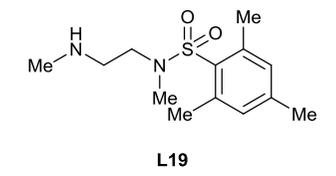
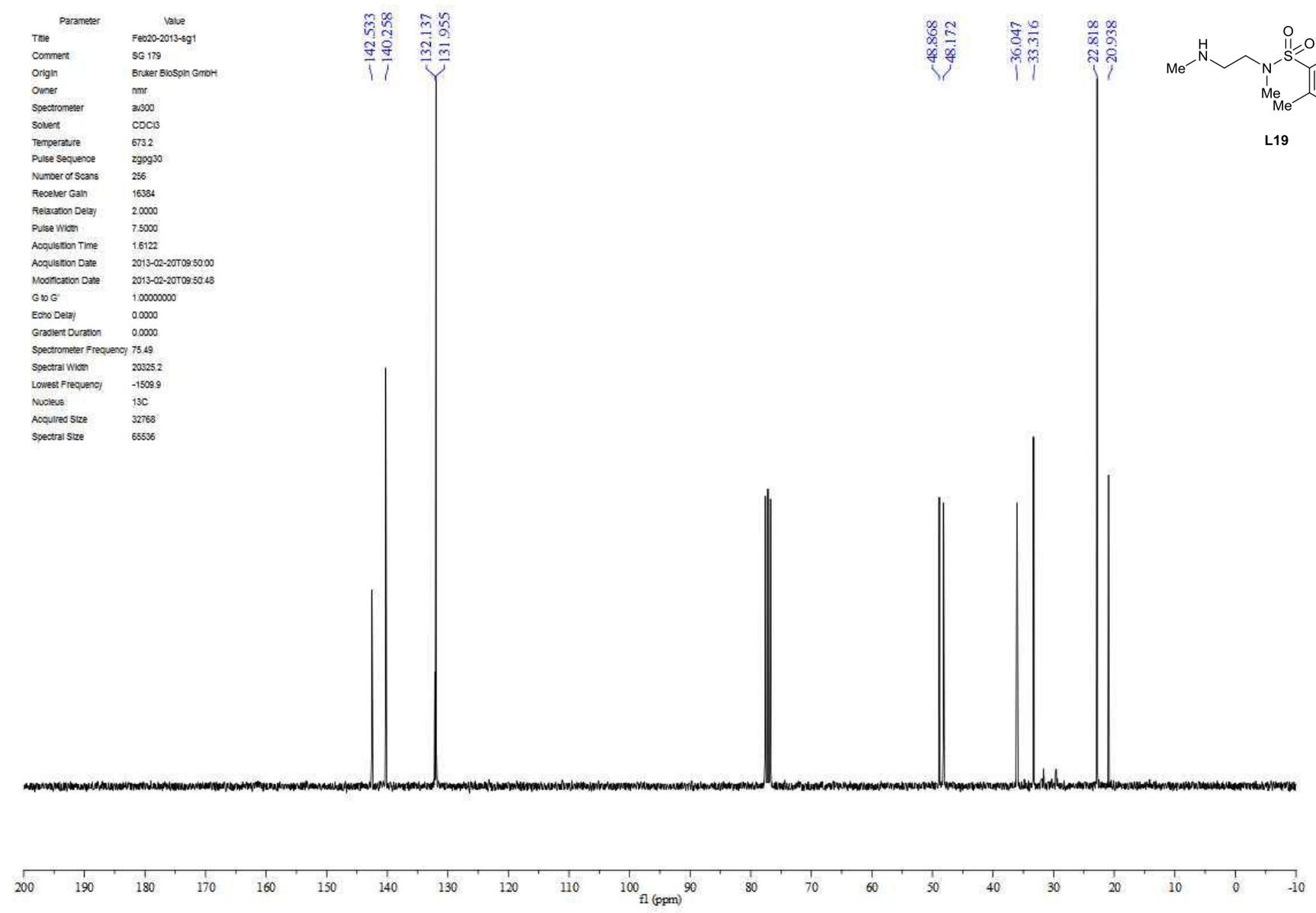
Parameter	Value
Title	Mar04-2015-eg297
Comment	SG L18
Origin	Bruker BioSpin GmbH
Owner	nmr
Spectrometer	av500
Solvent	CDCl3
Temperature	298.0
Pulse Sequence	zgpg30
Number of Scans	256
Receiver Gain	18390
Relaxation Delay	2.0000
Pulse Width	7.5000
Acquisition Time	1.6122
Acquisition Date	2015-03-04T13:16:00
Modification Date	2015-03-04T13:16:12
G to G'	1.0000000
Echo Delay	0.0000
Gradient Duration	0.0000
Spectrometer Frequency	75.49
Spectral Width	20325.2
Lowest Frequency	-1509.9
Nucleus	¹³ C
Acquired Size	32768
Spectral Size	65536



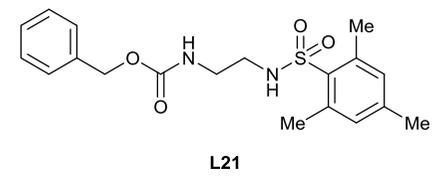
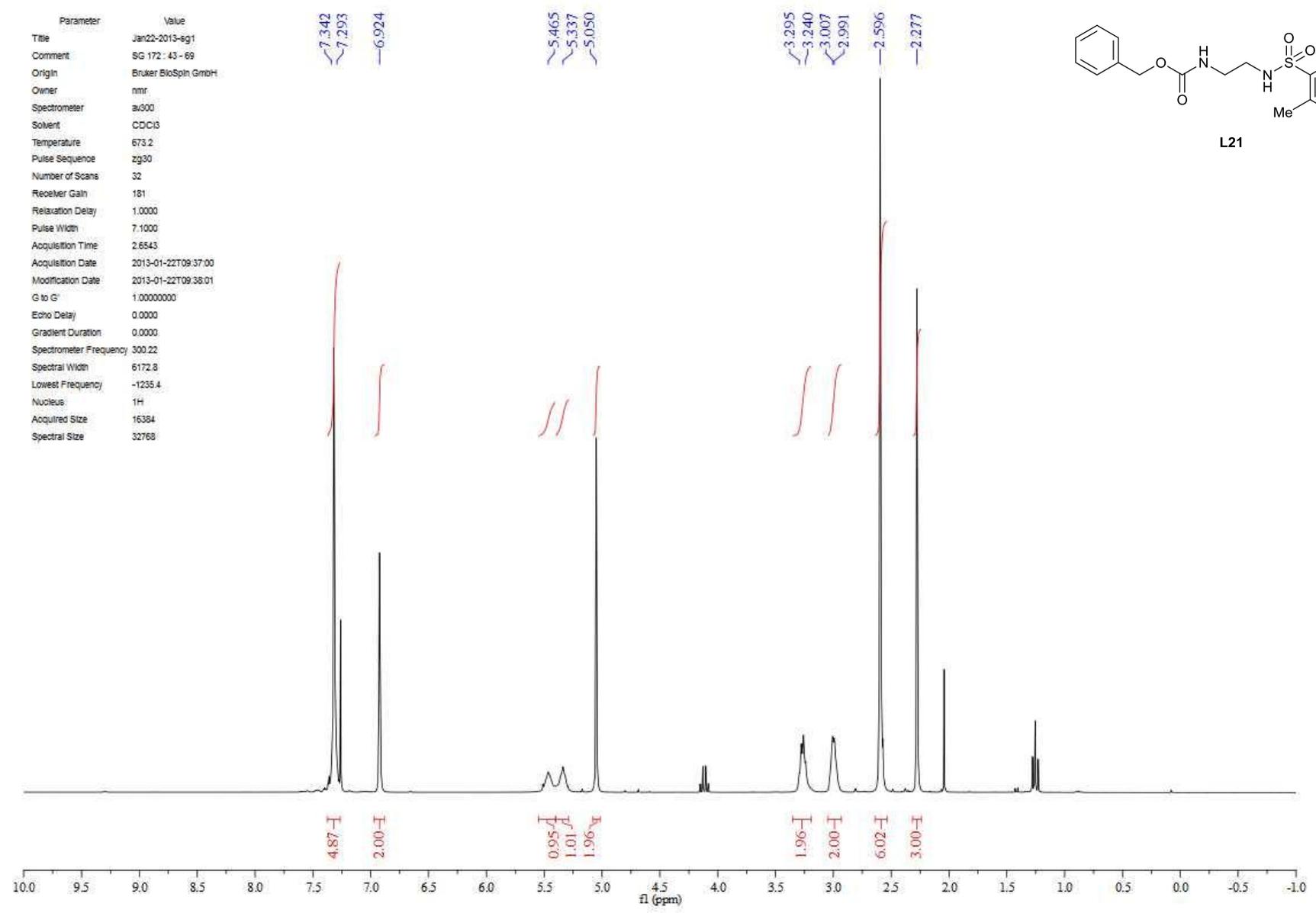
Parameter	Value
Title	Fel20-2013-eg1
Comment	SG 179
Origin	Bruker BioSpin GmbH
Owner	nmr
Spectrometer	av500
Solvent	CDCl3
Temperature	673.2
Pulse Sequence	zg30
Number of Scans	32
Receiver Gain	64
Relaxation Delay	1.0000
Pulse Width	7.1000
Acquisition Time	2.6543
Acquisition Date	2013-02-20T09:32:00
Modification Date	2013-02-20T09:33:00
G to G'	1.00000000
Echo Delay	0.0000
Gradient Duration	0.0000
Spectrometer Frequency	300.22
Spectral Width	6172.8
Lowest Frequency	-1235.4
Nucleus	1H
Acquired Size	16384
Spectral Size	32768



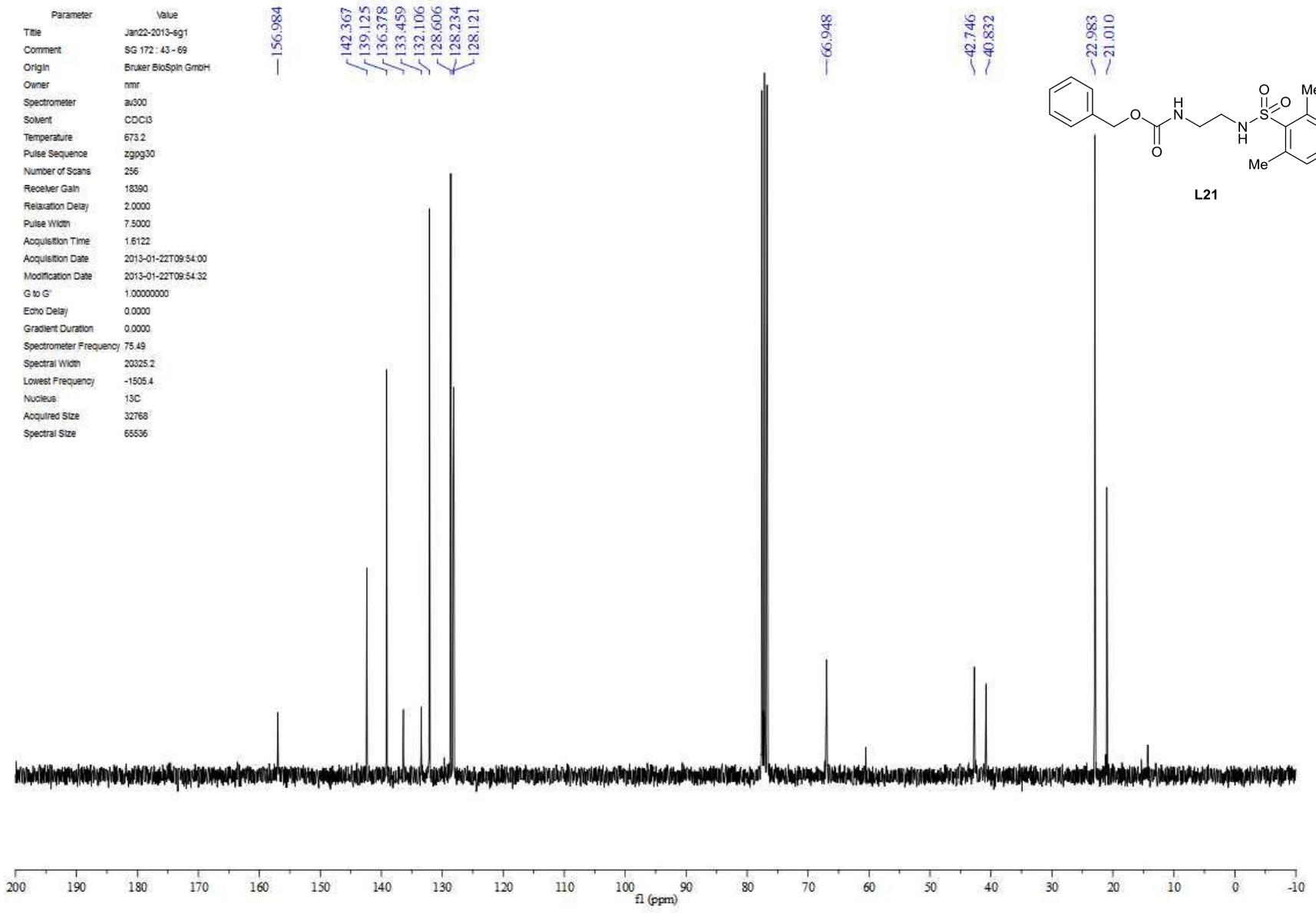
Parameter	Value
Title	Fel20-2013-eg1
Comment	SG 179
Origin	Bruker BioSpin GmbH
Owner	nmr
Spectrometer	av500
Solvent	CDCl3
Temperature	673.2
Pulse Sequence	zgpg30
Number of Scans	256
Receiver Gain	16384
Relaxation Delay	2.0000
Pulse Width	7.5000
Acquisition Time	1.6122
Acquisition Date	2013-02-20T09:50:00
Modification Date	2013-02-20T09:50:48
G to G'	1.00000000
Echo Delay	0.0000
Gradient Duration	0.0000
Spectrometer Frequency	75.49
Spectral Width	20325.2
Lowest Frequency	-1509.9
Nucleus	¹³ C
Acquired Size	32768
Spectral Size	65536



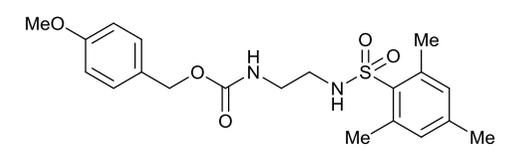
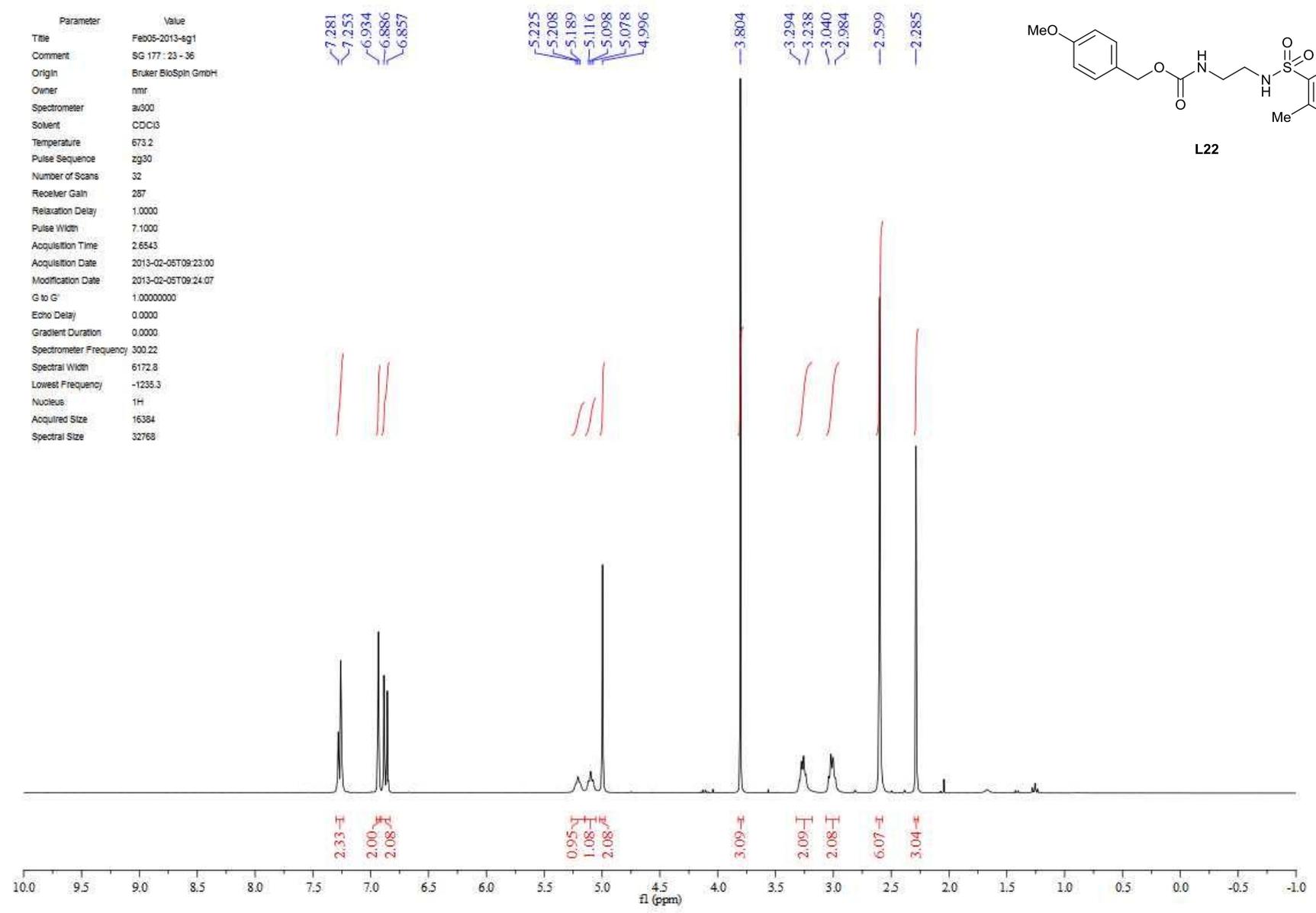
Parameter	Value
Title	Jan22-2013-eg1
Comment	SG 172: 43 - 69
Origin	Bruker BioSpin GmbH
Owner	nmr
Spectrometer	av500
Solvent	CDCl3
Temperature	673.2
Pulse Sequence	zg30
Number of Scans	32
Receiver Gain	181
Relaxation Delay	1.0000
Pulse Width	7.1000
Acquisition Time	2.6543
Acquisition Date	2013-01-22T09:37:00
Modification Date	2013-01-22T09:38:01
G to G ⁺	1.00000000
Echo Delay	0.0000
Gradient Duration	0.0000
Spectrometer Frequency	300.22
Spectral Width	6172.8
Lowest Frequency	-1235.4
Nucleus	¹ H
Acquired Size	16384
Spectral Size	32768



Parameter	Value
Title	Jan22-2013-eg1
Comment	SG 172 : 43 - 69
Origin	Bruker BioSpin GmbH
Owner	nmr
Spectrometer	av500
Solvent	CDCl3
Temperature	673.2
Pulse Sequence	zgpg30
Number of Scans	256
Receiver Gain	18390
Relaxation Delay	2.0000
Pulse Width	7.5000
Acquisition Time	1.6122
Acquisition Date	2013-01-22T09:54:00
Modification Date	2013-01-22T09:54:32
G to G'	1.00000000
Echo Delay	0.0000
Gradient Duration	0.0000
Spectrometer Frequency	75.49
Spectral Width	20325.2
Lowest Frequency	-1505.4
Nucleus	¹³ C
Acquired Size	32768
Spectral Size	65536

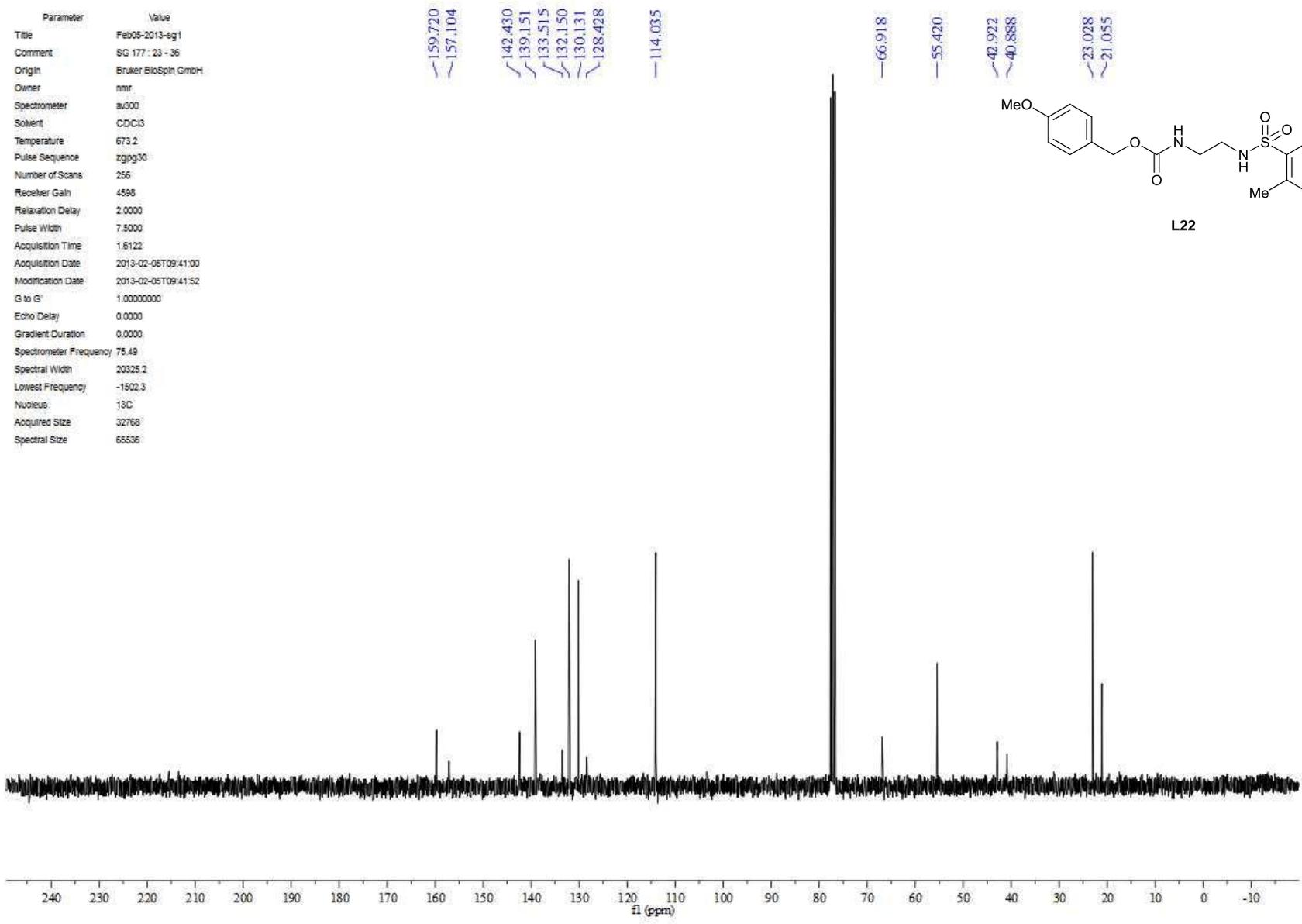


Parameter	Value
Title	Feb05-2013-eg1
Comment	SG 177 : 23 - 36
Origin	Bruker BioSpin GmbH
Owner	nmr
Spectrometer	av500
Solvent	CDCl3
Temperature	673.2
Pulse Sequence	zg30
Number of Scans	32
Receiver Gain	287
Relaxation Delay	1.0000
Pulse Width	7.1000
Acquisition Time	2.6543
Acquisition Date	2013-02-05T09:23:00
Modification Date	2013-02-05T09:24:07
G to G'	1.00000000
Echo Delay	0.0000
Gradient Duration	0.0000
Spectrometer Frequency	300.22
Spectral Width	6172.8
Lowest Frequency	-1235.3
Nucleus	1H
Acquired Size	16384
Spectral Size	32768

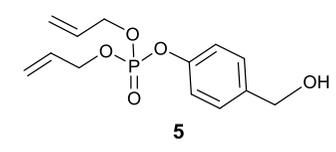
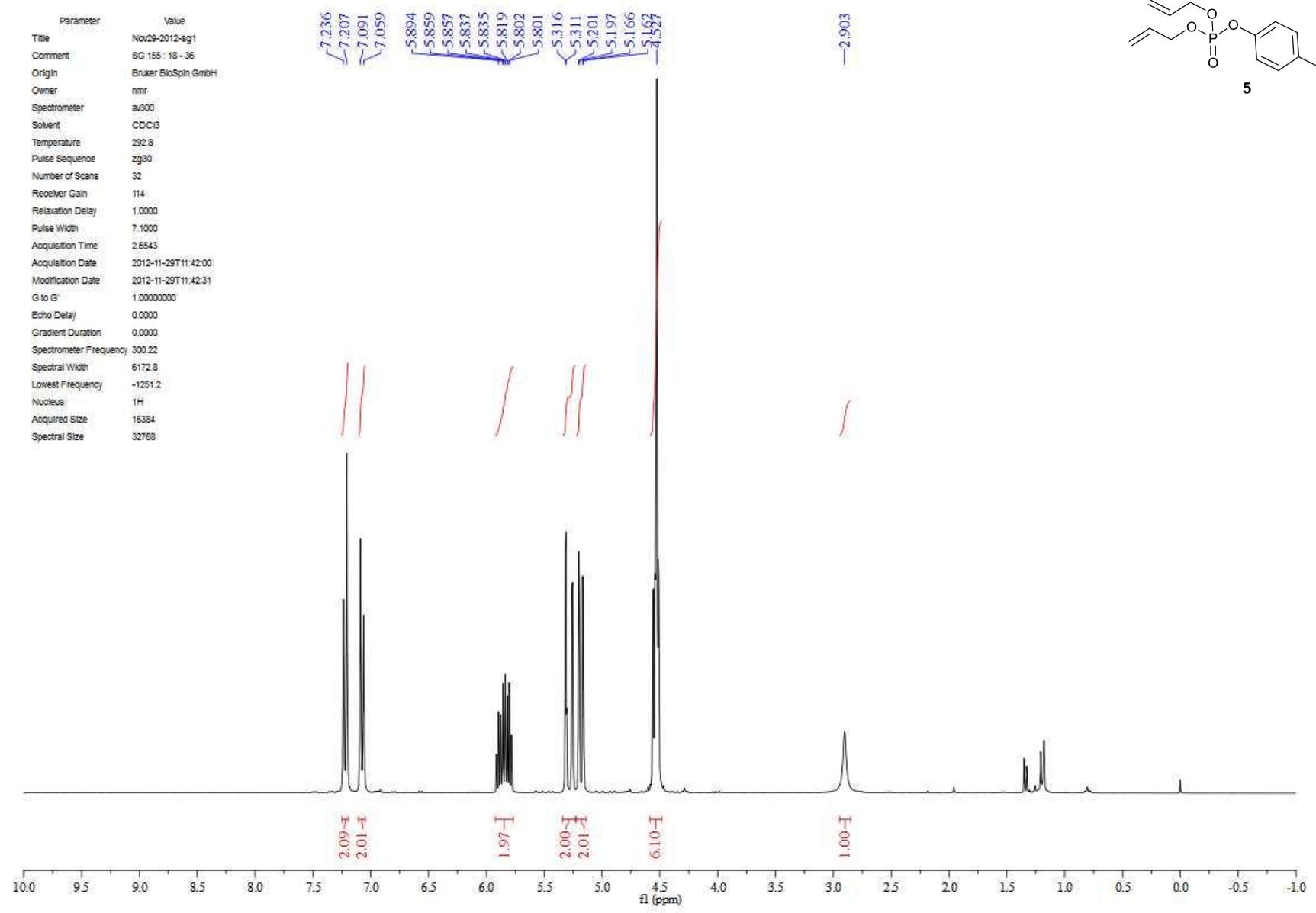


L22

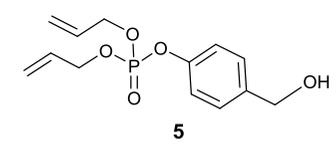
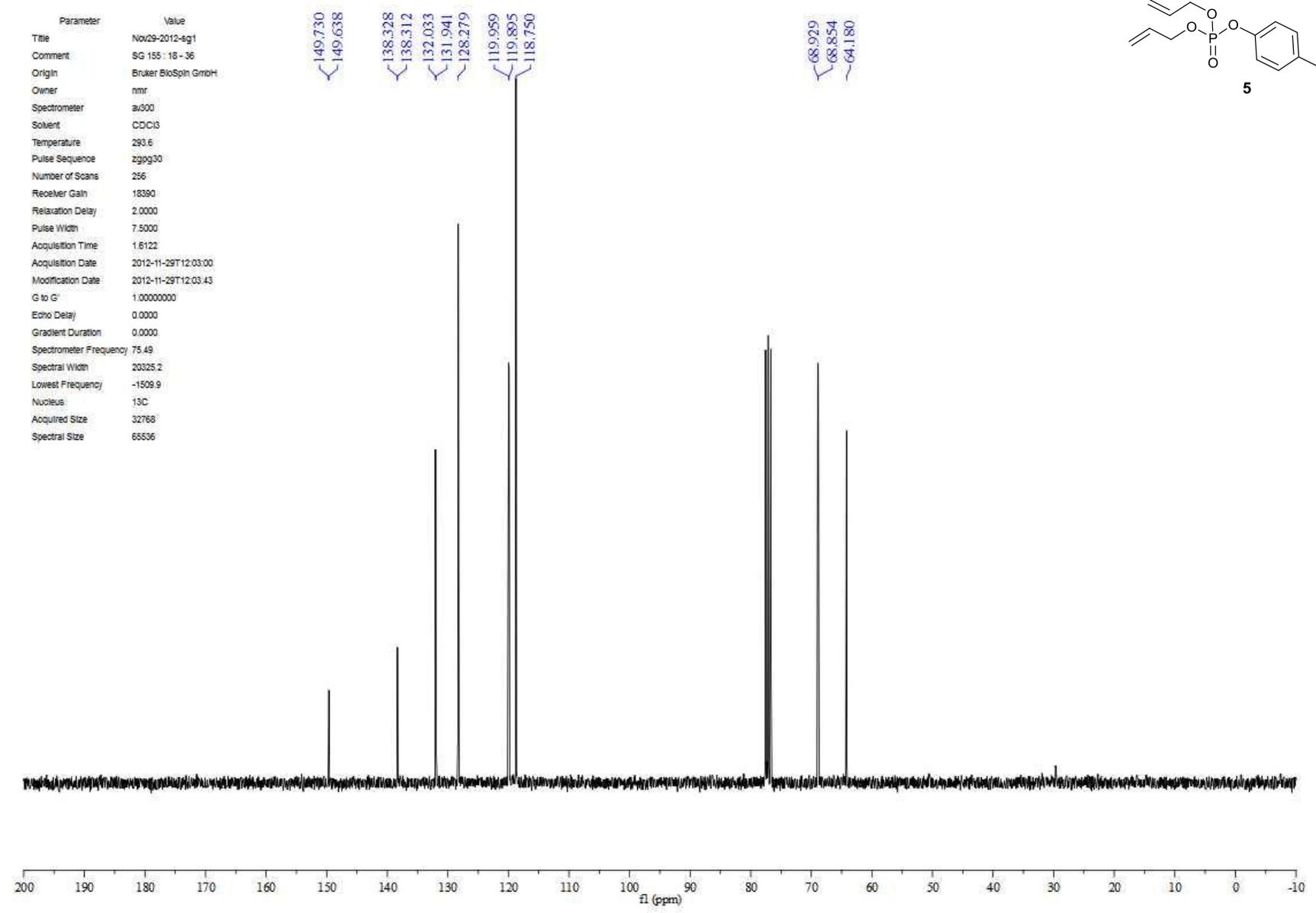
Parameter	Value
Title	Feb05-2013-eg1
Comment	SG 177 : 23 - 36
Origin	Bruker BioSpin GmbH
Owner	nmr
Spectrometer	av500
Solvent	CDCl3
Temperature	673.2
Pulse Sequence	zgpg30
Number of Scans	256
Receiver Gain	4598
Relaxation Delay	2.0000
Pulse Width	7.5000
Acquisition Time	1.6122
Acquisition Date	2013-02-05T09:41:00
Modification Date	2013-02-05T09:41:52
G to G'	1.0000000
Echo Delay	0.0000
Gradient Duration	0.0000
Spectrometer Frequency	75.49
Spectral Width	20325.2
Lowest Frequency	-1502.3
Nucleus	¹³ C
Acquired Size	32768
Spectral Size	65536



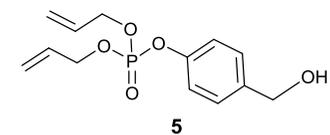
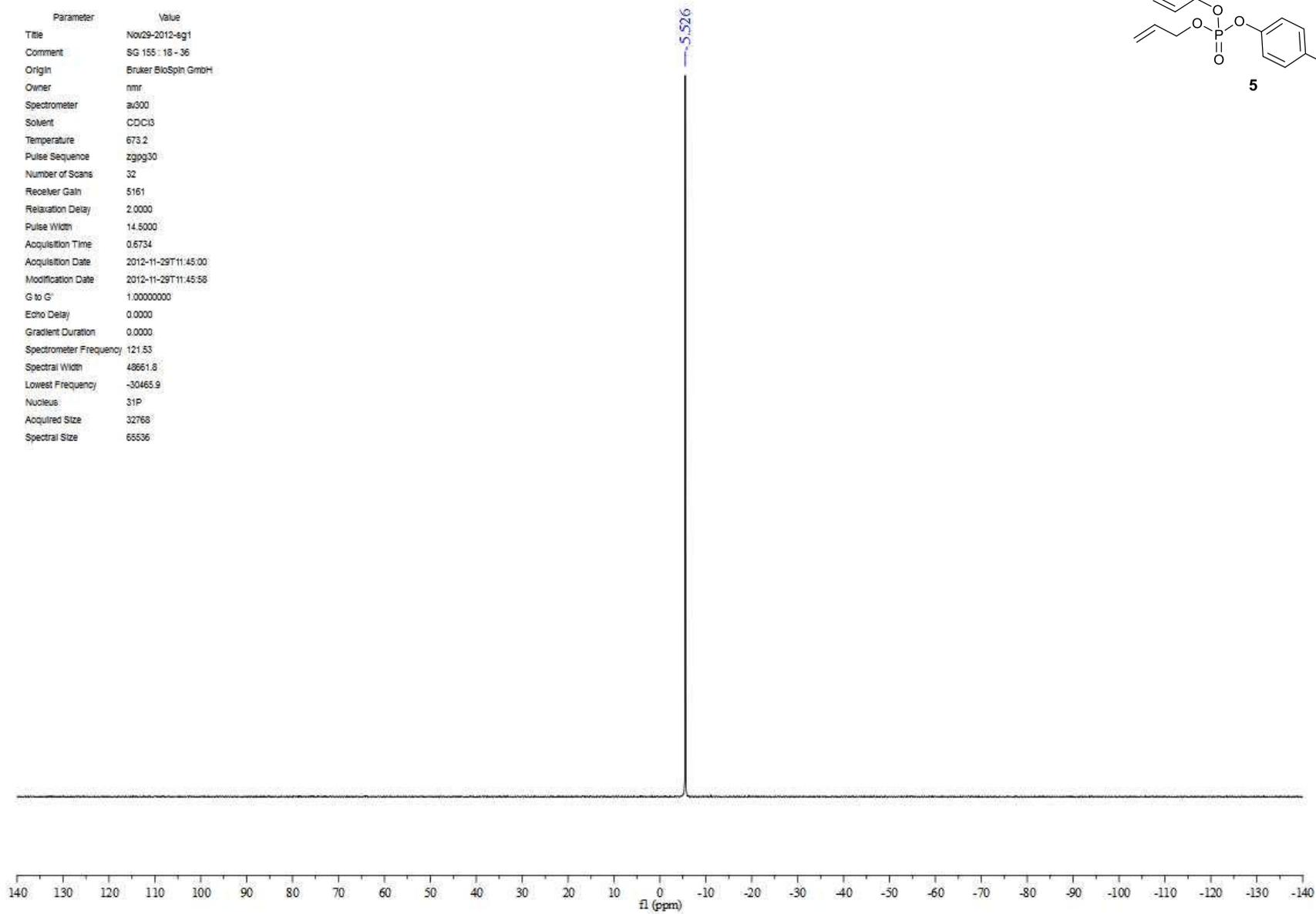
Parameter	Value
Title	Nov29-2012-eg1
Comment	SG 155 - 18 - 36
Origin	Bruker BioSpin GmbH
Owner	nmr
Spectrometer	av500
Solvent	CDCl3
Temperature	292.8
Pulse Sequence	zg30
Number of Scans	32
Receiver Gain	114
Relaxation Delay	1.0000
Pulse Width	7.1000
Acquisition Time	2.6543
Acquisition Date	2012-11-29T11:42:00
Modification Date	2012-11-29T11:42:31
G to G'	1.00000000
Echo Delay	0.0000
Gradient Duration	0.0000
Spectrometer Frequency	300.22
Spectral Width	6172.8
Lowest Frequency	-1251.2
Nucleus	1H
Acquired Size	16384
Spectral Size	32768



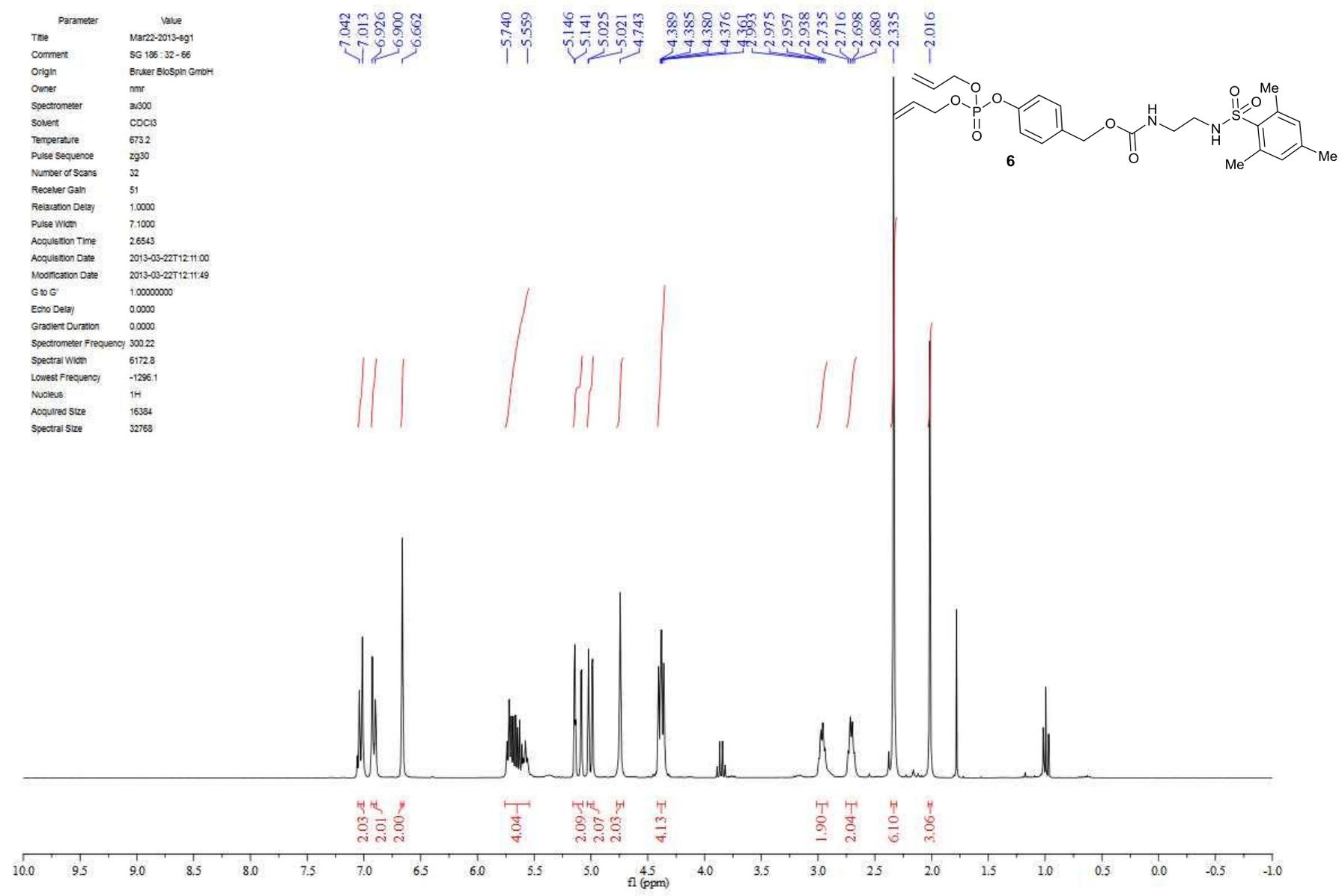
Parameter	Value
Title	Nov29-2012-eg1
Comment	SG 155 - 18 - 36
Origin	Bruker BioSpin GmbH
Owner	nmr
Spectrometer	av500
Solvent	CDCl3
Temperature	293.6
Pulse Sequence	zgpg30
Number of Scans	256
Receiver Gain	18390
Relaxation Delay	2.0000
Pulse Width	7.5000
Acquisition Time	1.6122
Acquisition Date	2012-11-29T12:03:00
Modification Date	2012-11-29T12:03:43
G to G'	1.00000000
Echo Delay	0.0000
Gradient Duration	0.0000
Spectrometer Frequency	75.49
Spectral Width	20325.2
Lowest Frequency	-1509.9
Nucleus	¹³ C
Acquired Size	32768
Spectral Size	65536



Parameter	Value
Title	Nov29-2012-eg1
Comment	SG 155 - 18 - 36
Origin	Bruker BioSpin GmbH
Owner	nmr
Spectrometer	av500
Solvent	CDCl3
Temperature	673.2
Pulse Sequence	zgpg30
Number of Scans	32
Receiver Gain	5161
Relaxation Delay	2.0000
Pulse Width	14.5000
Acquisition Time	0.6734
Acquisition Date	2012-11-29T11:45:00
Modification Date	2012-11-29T11:45:58
G to G'	1.00000000
Echo Delay	0.0000
Gradient Duration	0.0000
Spectrometer Frequency	121.53
Spectral Width	48661.8
Lowest Frequency	-30465.9
Nucleus	31P
Acquired Size	32768
Spectral Size	65536



Parameter	Value
Title	Mar22-2013-eg1
Comment	SG 186 : 32 - 66
Origin	Bruker BioSpin GmbH
Owner	nmr
Spectrometer	av500
Solvent	CDCl3
Temperature	673.2
Pulse Sequence	zg30
Number of Scans	32
Receiver Gain	51
Relaxation Delay	1.0000
Pulse Width	7.1000
Acquisition Time	2.6543
Acquisition Date	2013-03-22T12:11:00
Modification Date	2013-03-22T12:11:49
G to G'	1.0000000
Echo Delay	0.0000
Gradient Duration	0.0000
Spectrometer Frequency	300.22
Spectral Width	6172.8
Lowest Frequency	-1296.1
Nucleus	1H
Acquired Size	16384
Spectral Size	32768



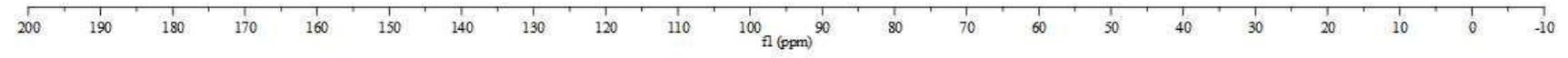
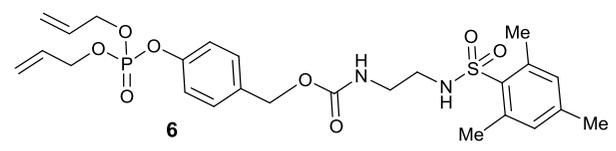
Parameter	Value
Title	Mar22-2013-eg1
Comment	SG 186 - 32 - 66
Origin	Bruker BioSpin GmbH
Owner	nmr
Spectrometer	av500
Solvent	CDCl3
Temperature	673.2
Pulse Sequence	zgpg30
Number of Scans	256
Receiver Gain	11685
Relaxation Delay	2.0000
Pulse Width	7.5000
Acquisition Time	1.6122
Acquisition Date	2013-03-22T12:33:00
Modification Date	2013-03-22T12:33:31
G to G'	1.00000000
Echo Delay	0.0000
Gradient Duration	0.0000
Spectrometer Frequency	75.49
Spectral Width	20325.2
Lowest Frequency	-1509.9
Nucleus	13C
Acquired Size	32768
Spectral Size	65536

156.765
 150.263
 150.171
 142.093
 139.001
 133.565
 131.975
 131.950
 131.885
 129.555
 120.085
 120.021
 118.801

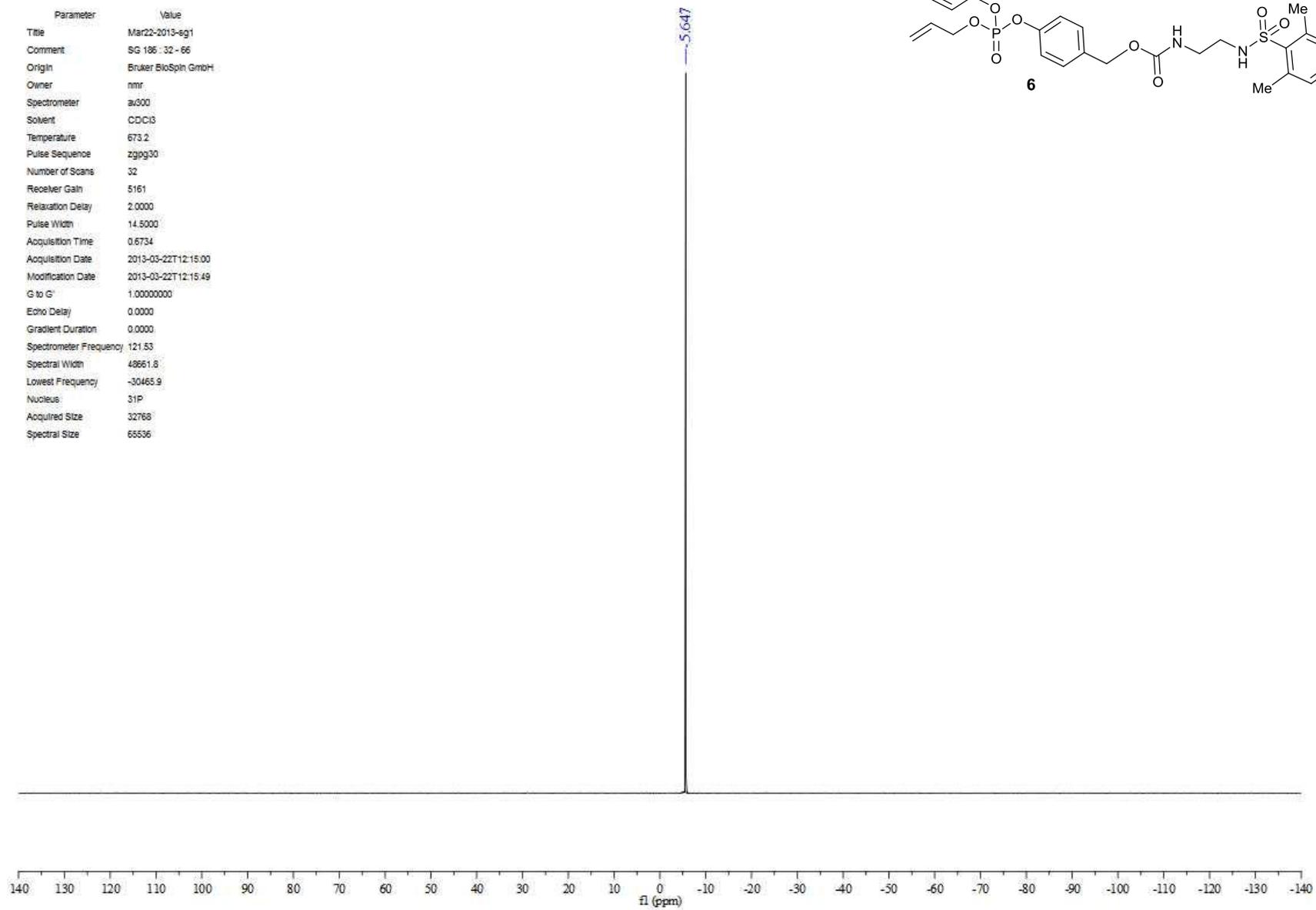
68.994
 68.918
 65.857

42.533
 40.701

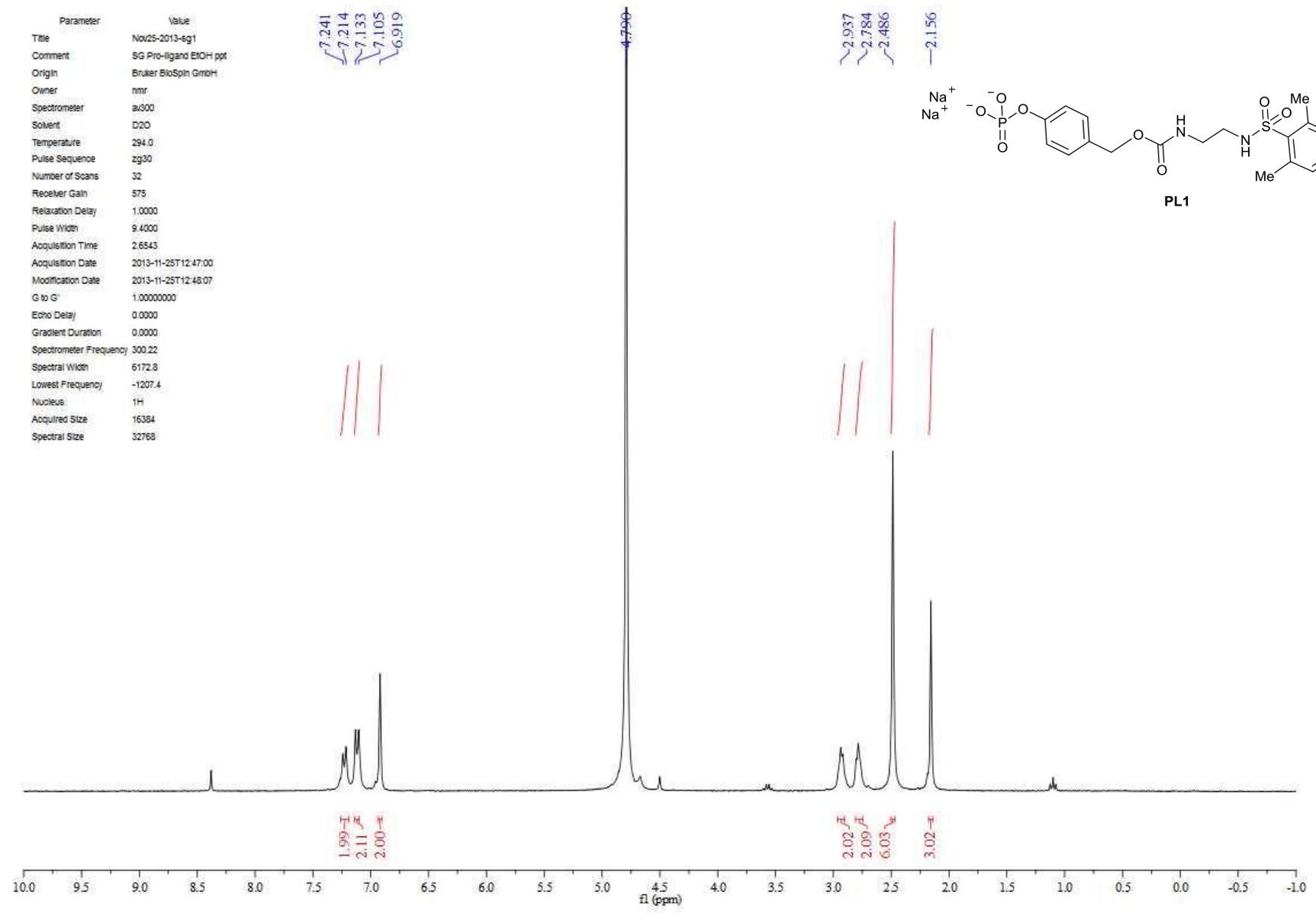
22.864
 20.889



Parameter	Value
Title	Mar22-2013-eg1
Comment	SG 186 : 32 - 66
Origin	Bruker BioSpin GmbH
Owner	nmr
Spectrometer	av500
Solvent	CDCl3
Temperature	673.2
Pulse Sequence	zgpg30
Number of Scans	32
Receiver Gain	5161
Relaxation Delay	2.0000
Pulse Width	14.5000
Acquisition Time	0.6734
Acquisition Date	2013-03-22T12:15:00
Modification Date	2013-03-22T12:15:49
G to G'	1.00000000
Echo Delay	0.0000
Gradient Duration	0.0000
Spectrometer Frequency	121.53
Spectral Width	48661.8
Lowest Frequency	-30465.9
Nucleus	31P
Acquired Size	32768
Spectral Size	65536



Parameter	Value
Title	Nov25-2013-eg1
Comment	SG Pro-ligand EtOH ppt
Origin	Bruker BioSpin GmbH
Owner	nmr
Spectrometer	av500
Solvent	D2O
Temperature	294.0
Pulse Sequence	zg30
Number of Scans	32
Receiver Gain	575
Relaxation Delay	1.0000
Pulse Width	9.4000
Acquisition Time	2.6543
Acquisition Date	2013-11-25T12:47:00
Modification Date	2013-11-25T12:48:07
G to G ⁺	1.0000000
Echo Delay	0.0000
Gradient Duration	0.0000
Spectrometer Frequency	300.22
Spectral Width	6172.8
Lowest Frequency	-1207.4
Nucleus	¹ H
Acquired Size	16384
Spectral Size	32768



Parameter	Value
Title	Nov25-2013-eg1
Comment	SG Pro-ligand EtOH ppt
Origin	Bruker BioSpin GmbH
Owner	nmr
Spectrometer	av500
Solvent	D2O
Temperature	294.2
Pulse Sequence	zgpg30
Number of Scans	32
Receiver Gain	18390
Relaxation Delay	2.0000
Pulse Width	14.5000
Acquisition Time	0.6734
Acquisition Date	2013-11-25T12:51:00
Modification Date	2013-11-25T12:51:48
G to G'	1.00000000
Echo Delay	0.0000
Gradient Duration	0.0000
Spectrometer Frequency	121.53
Spectral Width	48661.8
Lowest Frequency	-30447.6
Nucleus	31P
Acquired Size	32768
Spectral Size	65536

