Supporting information

Automated Solid Phase Synthesis of Teichoic Acids

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Experimental section

General

All chemicals (Acros, Fluka, Merck, Schleicher & Schuell, Sigma-Aldrich, Genscript) were used as received and reactions were carried out dry, under an argon atmosphere, at ambient temperature, unless stated otherwise. Column chromatography was performed on Screening Devices silica gel 60 (0.040-0.063 mm). TLC analysis was conducted on HPTLC aluminium sheets (Merck, silica gel 60, F245). Compounds were visualized by UV absorption (245 nm), by spraying with 20% H_2SO_4 in ethanol or with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ 25 g/l and $(NH_4)_4Ce(SO_4)_4\cdot 2\ H_2O$ 10 g/l, in 10% aqueous H_2SO_4 followed by charring at +/- 140 ^oC. Some unsaturated compounds were visualized by spraying with a solution of $KMnO_4$ (2%) and K_2CO_3 (1%) in water. Optical rotation measurements ($[\alpha]_D^{20}$) were performed on a Propol automated polarimeter (Sodium D-line, λ = 589 nm) with a concentration of 10 mg/ml (c = 1), unless stated otherwise. Infrared spectra were recorded on a Shimadzu FT-IR 8300. ^{31}P, ^1H, and ^{13}C NMR spectra were recorded with a Bruker AV 400 (161.7, 400 and 125 MHz respectively) or a Bruker DMX 600 (600 and 150 MHz respectively). NMR spectra were recorded in $CDCl_3$ with chemical shift (δ) relative to tetramethylsilane, unless stated otherwise. When D_2O was used, ^1H-NMR spectra were recorded with chemical shift relative (δ) to HDO (4.755 ppm), ^{31}P spectra were measured with chemical shift relative to 85 % H_3PO_4 (external standard) and ^{13}C-NMR spectra were recorded with chemical shift relative to TMS (external standard). High resolution mass spectra (HRMS) were recorded by direct injection (2 µl of a 2 µM solution in water/acetonitrile; 50/50; v/v and either 0.1 % formic acid or 10mM ammonium formate for the oligomers) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 275 ^oC) with resolution R = 60000 at *m/z* 400 (mass range *m/z* = 150-2000) and dioctylphthalate (*m/z* = 391.28428) as a lock mass. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). LC-MS analysis was performed

Procedure for automated solid-phase synthesis, purification and global deprotection of TA oligomers

Aminopropyl modified controlled pore glass support (CPG, Fluka) was loaded with (glucosyl)glycerol succinates **1b** or **2b** and the loading was determined (loading: 100 µmol/g CPG) using the method described by Pon.[1] The automated syntheses were performed on a synthesizer (ÄKTA™ oligopilot plus™, GE Healthcare) on a scale of 100-150 mg functionalized CPG (10-15 µmol glycerol derivative) and started off with acidolysis of the dimethoxytrityl ether using 3 % dichloroacetic acid in toluene (15 ml, 3 min). After flushing with acetonitrile (5ml, 1 min), the resulting alcohol was reacted with phosphoramidites **1a** or **2a** (0.1 M in ACN, 5 eq) and 5-benzylthiotetrazole (BTT, 0.3M in acetonitrile, 22.5 eq) for 5 min using a cycled flow. After flushing with acetonitrile (5ml, 1 min), oxidation of the intermediate phosphite was performed using I_2 (0.05 M in pyridine/H_2O 9/1, 2 ml, 1 min). A flushing step with acetonitrile (5ml, 1 min) was followed by a capping step (1 ml of a 1/1 mixture of capping solution A (20 v/v % *N*-methylimidazole in acetonitrile) and capping solution B (20 v/v % Ac_2O, 15 v/v % 2,6-lutidine in acetonitrile for 12s). After flushing with acetonitrile (5ml, 1 min), a detritylation step was performed using the before mentioned cocktail and the molecule was elongated using phosphoramidites **1a** or **2a** using the same set of reactions (coupling, oxidation, capping, detritylation). The average coupling efficiency was measured by quantitative UV-detection (400 nm) of the dimethoxytrityl cation during each detritylation step. When the desired length was obtained, spacer phosphoramidite **3** (0.1 M in ACN, 2 x 5 eq, 2 x 5 min) was coupled to the CPG-TA-oligomer using

BTT (0.3M in acetonitrile, 2 x 22.5 eq) and, subsequently treated with I_2 (0.05 M in pyridine/H_2O 9/1, 2 ml, 1 min), before it was released from the solid support using 25% NH_3OH (10 ml, 1 hr, the cyanoethyl protecting groups are concomitantly released at this stage). The solvents were then removed *in vacuo* before the crude oligomer was purified using method A or B.

Purification method A: RP-HPLC (Gilson preparative HPLC system; column: Alltima C18, particle size: 5 µm, dimensions: 10/250 mm; eluent: (10 mM NH_4OAc in H_2O)/acetonitrile, 9/1 → 1/9, detection: UV (215 and 254 nm), the fractions containing product were collected and the solvents were removed under reduced pressure. Repeated lyophilisation (twice) of the residue was followed by eluting the purified oligomer through a small column containing Dowex Na^+ cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in H_2O, flushed with H_2O and MeOH before use). Lyophilisation gave the partially protected oligomer of which the integrity and purity was confirmed by LC-MS, HRMS and NMR (^1H, ^{13}C, ^{31}P) analysis

Purification method B: Anion-exchange chromatography (device: ÄKTA Explorer™, GE Healthcare; column: Q-sepharose HR16/10, GE Healthcare; eluent: buffer A (50 mM NaOAc, 50 mM $NaClO_4$), buffer B (50mM NaOAc, 500mM $NaClO_4$), gradient 1/0 → 0/1) followed by desalination using size-exclusion chromatography (Sephadex G10 (hexamer **10**) or Sephadex G25 (all other oligomers), GE Healthcare, dimensions: 26/60 mm, eluent: 0.15 M NH_4HCO_3). The purified oligomer was lyophilized twice before it was eluted through a small column containing Dowex Na^+ cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in H_2O, flushed with H_2O and MeOH before use). Lyophilisation gave the partially protected oligomer of which the integrity and purity was confirmed by LC-MS, HRMS and NMR (^1H, ^{13}C, ^{31}P) analysis.

Deprotection: The oligomers (1-5 µmol) were dissolved in H_2O (3-6 ml) together with AcOH (3-6 drops) and treated for 3 days with Palladium black (20-40 mg)/H_2. Subsequently, the mixture was filtered and the solvents removed under reduced pressure before the residue was purified by size-exclusion chromatography (Sephadex HW40, Toyopearl, dimensions: 16/60 mm, eluent: 0.15 M Et_3NHOAc or 0.15 M NH_4OAc). After repeated lyophilisation, the purified product was eluted through a small column containing Dowex Na^+ cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in H_2O, flushed with H_2O and MeOH before use). Lyophilisation gave the partially protected oligomer of which the integrity and purity was confirmed by HRMS and NMR (^1H, ^{13}C, ^{31}P) analysis.

1-*O*-(Triethylammonium succinate)-2-*O*-benzyl-3-*O*-(4,4'-dimethoxytrityl)-*sn*-glycerol (1b)
To a solution of 2-*O*-benzyl-3-*O*-(4,4'-dimethoxytrityl)-*sn*-glycerol[5] (2.50 g, 5.16 mmol) and Et_3N (7.87 ml, 56.8 mmol) in DCM (35 ml) was added succinic anhydride (2.58 g, 25.8 mmol). The mixture was stirred for 10 minutes at 0 °C, followed by the addition of a catalytic amount of DMAP. After stirring 2 h at RT, the mixture was diluted with DCM (80 ml) and washed with 0.5 M HCl (50 ml), sat. aq. $NaHCO_3$ (40 ml) and brine (40 ml). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the residue by column chromatography (MeOH/DCM/Et_3N), afforded glycerol succinate **1b** (3.26 g, 4.75 mmol, 92%) as white foam. $[\alpha]_D^{20}$ (MeOH): +8.2; IR: 829, 1034, 1177, 1250, 1609, 1736, 2343, 2359; ^1H NMR (400 MHz, CD_3CN): δ = 1.11 (t, 9H, *J* = 7.3 Hz, 3 x CH_3 Et_3N), 2.35 - 2.45 (m, 4H, 2 x CH_2 succinyl), 2.85 (q, 6H, *J* = 7.3 Hz, 14.6 Hz, 3 x CH_2 Et_3N), 3.14 - 3.22 (m, 2H, 2 x CHH glycerol), 3.72 - 3.74 (m, 7H, 2 x OMe, CH glycerol), 4.15 - 4.23 (m, 2H, 2 x CHH glycerol), 4.57 (s, 2H, CH_2 Bn), 6.83 (d, 4H, *J* = 8.8 Hz, H_{arom}), 7.17 - 7.33 (m, 12H, H_{arom}), 7.44 (d, 2H, *J* = 7.6 Hz, H_{arom}); ^{13}C NMR (100 MHz, CD_3CN): δ = 9.2 (3 x CH_3 Et_3N), 30.9 (CH_2 succinyl), 31.7 (CH_2 succinyl), 45.9 (3 x CH_2 Et_3N), 55.8 (2 x OMe), 63.7 (CH_2 glycerol), 64.4 (CH_2 glycerol), 72.6 (CH_2 Bn), 77.2 (CH glycerol), 86.8 (C_q DMT), 113.9 (CH_{arom}), 127.6, 128.4, 128.6, 128.7, 128.8, 129.1, 130.8 (CH_{arom}), 136.9, 139.7, 146.1, 159.5 (C_q Bn, 4 x C_q DMTr), 173.8, 176.9 (2 x CO succinate); HRMS (free acid): $C_{35}H_{36}O_8$ + Na^+ requires 607.2302, found 607.2298.

1-*O*-(*tert*-Butyldiphenylsilyl)-2-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-3-*O*-allyl-*sn*-glycerol (6)
A solution of glucose donor **4** (2.70 g, 5.00 mmol), TTBP (2.86 g, 11.5 mmol) and Ph_2SO (1.11 g, 5.50 mmol) in freshly distilled DCM (100 ml), together with activated MS 3Å, was stirred under argon at RT for 30 min. The mixture was then cooled to -75 °C and stirred for another 15 min. After the addition of Tf_2O (0.93 ml, 5.5 mmol) the mixture was stirred for 45 min at -75 °C and, subsequently, glycerol acceptor **5** (2.22 g, 5.99 mmol) was added. After stirring for 2 hrs at -75 °C, the mixture was allowed to warm to room temperature overnight. The reaction was by the addition of moist Et_3N (3.4 ml, 25 mmol) and stirred for 30 minutes. After washing with sat. aq. $NaHCO_3$ (30 ml) and brine (30

ml), the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The resulting oil was dissolved in pyridine (50 ml) and Ac_2O (10 ml) and stirred for 2 hrs. The solvents were removed *in vacuo* before the residue was redissolved in Et_2O (125 ml) and washed with H_2O (2 x 40 ml) and brine (40 ml). The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure. Column chromatography (EtOAc/PE) of the residue gave α-glucoside **6** (3.10 g, 3.87 mmol, 78 %), as a colourless oil with a minor amount (< 7 %, based on ^{1}H-NMR analysis) of the β-product. $[\alpha]_D^{20}$ ($CHCl_3$): +4.2; IR: 737, 1088, 1369, 1454, 1751, 2855, 2924; ^{1}H NMR (400 MHz, α-anomer): δ = 1.05 (s, 9H, 3 x CH_3 TBDPS), 3.53 - 3.69 (m, 4H, CH glycerol, C<u>H</u>H glycerol, H-2, H-6), 3.71 - 3.81 (m, 3H, 3 x C<u>H</u>H glycerol), 3.86 - 3.92 (m, 1H, H-5), 3.99 - 4.04 (m, 5H, CH_2 allyl, H-3, H-4, H-6), 4.74 (d, 1H, *J* = 12.0 Hz, C<u>H</u>H Bn), 4.78 (d, 1H, *J* = 11.8 Hz, C<u>H</u>H Bn), 4.80 (d, 1H, *J* = 11.2 Hz, C<u>H</u>H Bn), 4.87 (d, 1H, *J* = 11.3 Hz, C<u>H</u>H Bn), 5.16 (dd, 1H, *J* = 1.3 Hz, 10.4 Hz, C<u>H</u>H allyl), 5.26 (dd, 1H, *J* = 1.6 Hz, 17.2 Hz, C<u>H</u>H allyl), 5.26 (d, 1H, *J* = 3.8 Hz, H-1), 5.50 (s, 1H, CH benzylidene), 5.88 (ddd, 1H, *J* = 5.2 Hz, 10.4 Hz, 22.4 Hz, CH allyl), 7.24 - 7.46 (m, 21H, H_{arom}), 7.65 - 7.68 (m, 4H, H_{arom}); ^{13}C NMR (100 MHz, α-anomer): δ = 19.2 (C_q *t*-butyl, 26.8 (3 x CH_3 TBDPS), 62.4 (C-5), 63.9 (CH_2 glycerol), 68.8 (C-6), 70.8 (CH_2 glycerol), 72.3 (CH_2 allyl), 72.5 (CH_2 Bn), 75.2 (CH_2 Bn), 76.7, 78.2 (C-3, C-4), 79.0 (CH glycerol), 82.1 (C-2), 97.2 (C-1), 101.2 (CH benzylidene), 116.9 (CH_2 allyl), 126.0 - 129.7 (CH_{arom}), 133.1, 133.2 (2 x C_q phenyl), 134.6 (CH allyl), 135.5 (CH_{arom}), 137.5, 138.3, 138.8 (2 x C_q Bn, C_q benzylidene); HRMS: $C_{49}H_{56}O_8Si + Na^+$ requires 823.3637, found 823.3631.

1-*O*-(*tert*-Butyldiphenylsilyl)-2-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-*sn*-glycerol (7)

A solution of glycoside **6** (0.400 g, 0.499 mmol) in freshly distilled THF (3.0 ml) was stirred under argon for 30 min. After the addition of $Ir(COD)(Ph_2MeP)_2PF_6$ (0.042 g, 10 mol %) the solution turned red and the mixture was purged with H_2 (g) until the solution turned colourless again (~30s). After stirring under argon for 2 hrs, the mixture was diluted with THF (7.0 ml) and sat. aq. $NaHCO_3$ (25 ml). Upon addition of I_2 (0.190 g, 0.75 mmol), the mixture was allowed to stir for 1.5 hrs at room temperature. The mixture was then diluted with EtOAc (100 ml) and washed with, respectively, sat. aq. NaS_2O_3 (2 x 20 ml) and brine (20 ml). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography (EtOAc/toluene/PE) afforded **7** (281 mg, 0.369 mmol, 74 %) as a colourless oil. $[\alpha]_D^{20}$ ($CHCl_3$): +3.2; IR: 737, 995, 1030, 1076, 1369, 1736, 2858, 2932; ^{1}H NMR (400 MHz): δ = 1.05 (s, 9H, 3 x CH_3 TBDPS), 3.17 (bs, 1H, OH), 3.53 - 3.68 (m, 5H, 2 x C<u>H</u>H glycerol, H-2, H-4, H-6), 3.72 - 3.90 (m, 4H, 2 x C<u>H</u>H glycerol, CH glycerol, H-5), 3.99 (dd, 1H, *J* = 4.9 Hz, 10.1 Hz, H-6), 4.04 (at, 1H, *J* = 9.3 Hz, H-3), 4.70 (d, 1H, *J* = 11.6 Hz, C<u>H</u>H Bn), 4.78 (d, 1H, *J* = 11.2 Hz, C<u>H</u>H Bn), 4.86 (d, 1H, *J* = 3.9 Hz, H-1), 4.88 (d, 1H, *J* = 11.7 Hz, C<u>H</u>H Bn), 4.93 (d, 1H, *J* = 11.2 Hz, C<u>H</u>H Bn), 5.50 (s, 1H, CH benzylidene), 7.25 - 7.46 (m, 21H, H_{arom}), 7.63 - 7.66 (m, 4H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.1 (C_q *t*-butyl, 26.8 (3 x CH_3 TBDPS), 62.7 (C-5), 62.9 (CH_2 glycerol), 63.9 (CH_2 glycerol), 68.8 (C-6), 74.5 (CH_2 Bn), 75.2 (CH_2 Bn), 78.9 (C-2, C-3), 81.8 (CH glycerol), 82.3 (C-4), 99.6 (C-1), 101.2 (CH benzylidene), 126.0 - 129.8 (CH_{arom}), 133.0, 133.1 (2 x C_q phenyl), 135.5 (CH_{arom}), 137.3, 137.5, 138.5 (2 x C_q Bn, C_q benzylidene); HRMS: $C_{46}H_{52}O_8Si + Na^+$ requires 783.3324, found 783.3325.

1-*O*-(*tert*-Butyldiphenylsilyl)-2-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-3-*O*-(4,4'-dimethoxytrityl)-*sn*-glycerol (8)

To a solution of alcohol **7** (4.68 g, 6.14 mmol) in DCM (50 ml) were added, respectively, DIPEA (1.61 ml, 9.22 mmol) and DMTr-Cl (2.50 g, 7.37 mmol). The mixture was allowed to stir overnight after which it was quenched by the addition of MeOH (5.0 ml). After stirring 15 min the mixture was washed with H_2O (20 ml) and brine (20 ml) and the organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. Purification of the residue by column chromatography (EtOAc/PE/Et_3N) yielded **8** (6.51 g, 6.12 mmol, 100 %) as white foam. $[\alpha]_D^{20}$ (MeOH): +17.2; IR: 1034, 1088, 1250, 1508, 2343, 2361; ^{1}H NMR (400 MHz): δ = 0.97 (s, 9H, 3 x CH_3 TBDPS), 3.30 (dd, 1H, *J* = 6.2 Hz, 9.8 Hz, C<u>H</u>H glycerol), 3.46 (dd, 1H, *J* = 4.4 Hz, 10.0 Hz), 3.52 - 3.64 (m, 3H, H-2, H-4, H-6), 3.72 - 3.80 (m, 8H, 2 x C<u>H</u>H glycerol, 2 x OMe), 3.90 - 3.97 (m, 1H, H-5), 4.00 - 4.07 (m, 3H, CH glycerol, H-3, H-6), 4.59 (d, 1H, *J* = 12.4 Hz, C<u>H</u>H Bn), 4.64 (d, 1H, *J* = 12.0 Hz, C<u>H</u>H Bn), 4.79 (d, 1H, *J* = 11.6 Hz, C<u>H</u>H Bn), 4.87 (d, 1H, *J* = 11.2 Hz, C<u>H</u>H Bn), 5.21 (d, 1H, *J* = 3.6 Hz, H-1), 5.50 (s, 1H, CH benzylidene), 6.74 - 6.76 (m, 4H, H_{arom}), 7.12 - 7.46 (m, 30H, H_{arom}), 7.58 - 7.62 (m, 4H, H_{arom}); ^{13}C NMR (100 MHz): δ = 19.1 (C_q *t*-butyl, 26.8 (3 x CH_3 TBDPS), 55.1 (2 x OMe), 62.6 (C-5), 64.1 (2 x CH_2 glycerol), 68.9 (C-6), 72.5 (CH_2 Bn), 75.2 (CH_2 Bn), 77.5, 78.3 (CH glycerol, C-3), 78.9 (C-2), 82.2 (C-4), 86.5 (C_q DMTr), 97.2 (C-1), 101.3 (CH benzylidene), 113.1 (CH_{arom}), 126.1 - 130.1 (CH_{arom}), 133.2, 133.3 (2 x C_q

phenyl), 135.5 (CH_{arom}), 136.0, 136.0, 137.6, 138.1, 138.8 (2 x C_q Bn, C_q benzylidene, 5 x C_q DMTr); HRMS: $C_{67}H_{70}O_{10}Si + H^+$ requires 1063.4811, found 1063.4804.

2-*O*-(2,3-di-*O*-Benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-3-*O*-(4,4'-dimethoxytrityl)-*sn*-glycerol (9)

Compound **8** (6.33 g, 5.95 mmol) was dissolved in THF (60 ml) and after addition of TBAF (1M in THF, 10.7 ml) stirred overnight. After evaporation of the solvents under reduced pressure the resulting oil was purified by column chromatography (EtOAc/PE/Et_3N), giving alcohol **9** (4.37 g, 5.30 mmol, 89 %) as a white foam. $[\alpha]_D^{20}$ (MeOH): +29.2; IR: 1032, 1076, 1250, 1508, 1609, 2343, 2361; ^{1}H NMR (400 MHz): δ = 2.40 (bs, 1H, OH), 3.28 (dd, 1H, *J* = 6.3 Hz, 9.8 Hz, CH_2 glycerol), 3.34 (dd, 1H, *J* = 5.4 Hz, 9.9 Hz, CH_2 glycerol), 3.54 (dd, 1H, *J* = 3.8 Hz, 9.4 Hz, H-2), 3.61 (at, 1H, *J* = 9.5 Hz, H-4), 3.66 - 3.77 (m, 9H, CH_2 glycerol, H-6, 2 x OMe), 3.87 (ddd, 1H, *J* = 3.5 Hz, 6.1 Hz, 11.8 Hz, CH glycerol), 3.98 (dd, 1H, *J* = 4.8 Hz, 10.0 Hz, H-5), 4.02 (at, 1H, *J* = 9.3 Hz, H-3), 4.25 (dd, 1H, *J* = 4.9 Hz, 10.2 Hz, H-6), 4.59 (d, 1H, *J* = 12.1 Hz, CH_2 Bn), 4.68 (d, 1H, *J* = 12.1 Hz, CH_2 Bn), 4.80 (d, 1H, *J* = 11.3 Hz, CH_2 Bn), 4.90 (d, 1H, *J* = 11.3 Hz, CH_2 Bn), 4.97 (d, 1H, *J* = 3.9 Hz, H-1), 5.54 (s, 1H, CH benzylidene), 6.80 (4, 4H, *J* = 8.9 Hz, H_{arom}), 7.16 - 7.36 (m, 20H, H_{arom}), 7.43 - 7.49 (m, 4H, H_{arom}); ^{13}C NMR (100 MHz): δ = 55.1 (2 x OMe), 62.9 (C-5), 63.3 (CH_2 glycerol), 63.9 (CH_2 glycerol), 68.9 (C-6), 73.2 (CH_2 Bn), 75.3 (CH_2 Bn), 78.4 (C-3), 78.8 (C-2), 78.8 (CH glycerol), 82.0 (C-4), 86.6 (C_q DMT), 97.4 (C-1), 101.2 (CH benzylidene), 113.1 (CH_{arom}), 126.0 - 130.0 (CH_{arom}), 133.0 (2 x C_q phenyl), 135.8, 137.3, 137.9, 138.7, 144.6, 158.5 (2 x C_q Bn, C_q benzylidene, 5 x C_q DMTr); HRMS: $C_{51}H_{52}O_{10} + Na^+$ requires 847.3453, found 847.3455.

1-*O*-([*N*,*N*-diisopropylamino]-2-cyanoethoxy-phosphite)-2-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-3-*O*-(4,4'-dimethoxytrityl)-*sn*-glycerol (2a)

To a cooled (0 °C) solution of **9** (1.24 g, 1.50 mmol) and DIPEA (0.42 ml, 2.4 mmol) in freshly distilled DCM (30 ml) was added 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite (0.462 g, 1.95 mmol). After stirring overnight, the reaction was quenched by the addition of H_2O (2.0 ml) and washed with, respectively, H_2O (10 ml) and brine (10 ml). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. Purification of the residue by column chromatography (EtOAc/PE/Et_3N) gave phosphoramidite **2a** (1.50 g, 1.46 mmol, 98 %) as white foam. ^{31}P NMR (161.7 MHz, CD_3CN): δ = 149.0, 149.3 (diastereoisomers); ^{1}H NMR (400 MHz, CD_3CN): δ = 1.07 - 1.15 (m, 12H, 4 x CH_3 isopropylamino), 2.46 - 2.50 (m, 2H, CH_2 cyanoethoxy), 3.11 - 3.29 (m, 2H, 2 x CH isopropylamino), 3.51 - 4.27 (m, 19H, CH_2 cyanoethoxy, 2 x OMe, 2 x CH_2 glycerol, CH glycerol, H-2, H-3, H-4, H-5, H-6, H-6), 4.57 - 4.62 (m, 2H, CH_2 Bn), 4.79 (m, 2H, C_2 Bn), 5.15 (m, 1H, H-1), 5.60 (s, 1H, CH benzylidene), 6.80 (d, 4H, *J* = 8.1 Hz, H_{arom}), 7.11 - 7.47 (m, 24H, H_{arom}); HRMS: $C_{60}H_{69}N_2O_{11}P + H^+$ requires 1025.4712, found 1025.4720.

1-*O*-(Triethylammoniumsuccinate)-2-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-3-*O*-(4,4'-dimethoxytrityl)-*sn*-glycerol (2b)

To a solution of **9** (3.21 g, 3.89 mmol) and Et_3N (5.93 ml, 42.8 mmol) in DCM (40 ml) was added succinic anhydride (1.95 g, 19.45 mmol). A catalytic amount of DMAP was added and the reaction was stirred for 100 min.. The mixture was diluted with DCM (50 ml) and washed with H_2O (2 x 50 ml) after which the organic layer was concentrated under reduced pressure. The triethylammonium salt of succinyl ester **2b** (3.93 g, 3.83 mmol, 99 %) was obtained as white foam. $[\alpha]_D^{20}$ (MeOH): +31.6 °; IR: 829, 1034, 1250, 1508, 1738, 2343, 2361; ^{1}H NMR (400 MHz, CD_3CN): δ = 1.11 (t, 9H, *J* = 7.3 Hz, 3 x CH_3 Et_3NH), 2.36 - 2.39 (m, 2H, CH_2 succinyl), 2.47 - 2.50 (m, 2H, CH_2 succinyl), 2.82 (q, 6H, *J* = 7.3 Hz, 14.6 Hz, 3 x CH_2 Et_3N), 3.21 (m, 2H, CH_2 glycerol), 3.56 (dd, 1H, *J* = 3.7 Hz, 9.3 Hz, H-2), 3.64 (at, 1H, *J* = 9.5 Hz, H-4), 3.69 - 3.75 (m, 7H, 2 x OMe, H-6), 3.87 (at, 1H, *J* = 9.3 Hz, H-3), 3.94 - 4.00 (m, 2H, CH glycerol, H-5), 4.15 (dd, 1H, *J* = 6.4 Hz, 11.6 Hz, CH_2 glycerol), 4.23 - 4.26 (m, 2H, CH_2 glycerol, H-6), 4.58 (s, 2H, CH_2 Bn), 4.79 (s, 2H, CH_2 Bn), 5.10 (d, 1H, *J* = 3.7 Hz, H-1), 5.61 (s, 1H, CH benzylidene), 6.84 (d, 4H, *J* = 8.9 Hz, H_{arom}), 7.14 - 7.50 (m, 24H, H_{arom}); ^{13}C NMR (100 MHz, CD_3CN): δ = 9.5 (3 x CH_3 Et_3N), 30.9 (CH_2 succinyl), 31.7 (CH_2 succinyl), 45.9 (3 x CH_2 Et_3N), 55.9 (2 x OMe), 63.7 (CH_2 glycerol), 63.7 (C-5), 65.0 (CH_2 glycerol), 69.5 (C-6), 73.4 (CH_2 Bn), 75.3 (CH_2 Bn), 75.7 (CH glycerol), 79.0 (C-3), 80.3 (C-2), 82.6 (C-4), 87.3 (C_q DMT), 114.1 (CH_{arom}), 127.2 - 131.0 (CH_{arom}), 136.7, 136.8, 138.9, 139.4, 140.1, 146.0, 159.6 (2 x C_q Bn, 4 x C_q DMT, C_q benzylidene), 174.0, 176.8 (2 x CO succinate); HRMS (as free acid): $C_{55}H_{56}O_{13} + Na^+$ requires 947.3613, found 947.3627.

Hexaglycerolphosphate (16)

Synthesis on 10 µmol scale (100 mg glycerol-CPG). Average coupling efficiency: 98.3 % (5 couplings). Purification method A gave the semiprotected hexamer (**10**) as white amorphous solid (3.4 mg, 1.8 µmol, 18 %). Method B (synthesis on 15 µmol scale) gave **10** as white amorphous solid (2.7 mg, 1.5 µmol, 10 %). LC-MS (gradient: 10 mM NH_4OAc in H_2O/acetonitrile 1/0 → 1/9 in 13.5 min): r.t. 5.85 min, $C_{74}H_{99}NO_{33}P_6 + H^+$ requires 1716.5 found 1716.6.; ^{31}P-NMR (162 MHz, D_2O): δ = 1.0 (4P), 1.1 (1P), 1.2 (1P); ^{1}H-NMR (600 MHz, D_2O): δ = 1.12 (m, 4H, 2 x CH_2 hexylspacer), 1.26 (m, 2H, CH_2 hexylspacer), 1.42 (m, 2H, CH_2 hexylspacer), 2.92 (t, 2H, *J* = 6.9 Hz, CH_2-N hexylspacer), 3.50 - 3.61 (m, 3H, CH glycerol, CH_2 glycerol), 3.68 - 3.93 (m, 29H, CH_2-O hexylspacer, 11 x CH_2 glycerol, 5 x CH glycerol), 4.41 - 4.54 (m, 12H, 6 x CH_2 Bn), 4.92 (s, 2H, CH_2 benzylcarbamate), 7.16 - 7.32 (m, 35H, H_{arom}); ^{13}C NMR (150 MHz, D_2O): δ = 25.6, 26.5, 29.7, 30.7 (4 x CH_2 hexylspacer), 41.3 (CH_2-N hexylspacer), 61.4 (CH_2 glycerol), 65.1, 65.4, 65.5 - 65.7, 67.2, 67.6 (CH_2-O hexylspacer, 11 x CH_2 glycerol, CH_2 benzylcarbamate), 72.7, 73.0 (6 x CH_2 Bn), 78.0 - 78.1 (5 x CH glycerol), 79.2 (CH glycerol), 128.6 - 129.7 (CH_{arom}), 137.5, 138.4 - 138.5 (6 x C_q Bn, C_q benzylcarbamate), 159.2 (C_q benzylcarbamate); HRMS: $[C_{74}H_{99}NO_{33}P_6 + 2H]^{2+}$ requires 858.7335, found 858.7340; Deprotection: The semiprotected hexamer (**10**, 4.7 mg, 2.5 µmol) was deprotected using the standard procedure yielding hexamer **16** (1.9 mg, 1.6 µmol, 65 %) as an amorphous off-white solid. ^{31}P-NMR (162 MHz, D_2O): δ = 1.2 (1P), 1.2 (3P), 1.3 (2P); ^{1}H-NMR (600 MHz): δ = 1.36 - 1.38 (m, 4H, 2 x CH_2 hexylspacer), 1.58 - 1.64 (m, 4H, 2 x CH_2 hexylspacer), 2.94 (at, 2H, *J* = 7.5 Hz, CH_2-N hexylspacer), 3.54 (dd, 1H, *J* = 6.1 Hz, 11.8 Hz, C<u>H</u>H glycerol), 3.62 (dd, 1H, *J* = 4.2 Hz, 11.8 Hz, C<u>H</u>H glycerol), 3.78 - 3.91 (m, 25H, CH_2-O hexylspacer, 11 x CH_2 glycerol, CH glycerol), 3.96 - 4.01 (m, 5H, 5 x CH glycerol); ^{13}C NMR (150 MHz, D_2O): δ = 25.4, 26.1, 27.6, 30.3 (4 x CH_2 hexylspacer), 40.4 (CH_2-N hexylspacer), 63.0 (CH_2 glycerol), 66.9 - 67.3 (CH_2-O hexylspacer, 11 x CH_2 glycerol), 70.4 - 70.6, 71.7 (6 x CH glycerol); HRMS: $C_{24}H_{57}NO_{31}P_6 + H^+$ requires 1042.1413, found 1042.1425.

14-mer (17)

Synthesis on 10 µmol scale (100 mg glycerol-CPG). Average coupling efficiency: 98.0 % (9 couplings). Purification method A gave the semiprotected decamer (**11**) as white amorphous solid (8.5 mg, 2.9 µmol, 29 %). Method B (synthesis on 15 µmol scale) gave the semiprotected decamer (**11**) as white amorphous solid (7.1 mg, 2.4 µmol, 16 %). LC-MS (gradient: 10 mM NH_4OAc in H_2O/acetonitrile 1/0 → 1/9 in 13.5 min): r.t. 6.15 min, $[C_{114}H_{151}NO_{53}P_{10} + 2H]^{2+}$ requires 1346.8 found 1346.8.; ^{31}P-NMR (162 MHz, D_2O): δ = 1.0 (8P), 1.1 (1P), 1.2 (1P); ^{1}H-NMR (600 MHz, D_2O): δ = 1.03 (m, 2H, CH_2 hexylspacer), 1.08 (m, 2H, CH_2 hexylspacer), 1.20 (m, 2H, CH_2 hexylspacer), 1.38 (m, 2H, CH_2 hexylspacer), 2.86 (m, 2H, CH_2-N hexylspacer), 3.49 - 3.59 (m, 3H, CH glycerol, CH_2 glycerol), 3.64 - 3.92 (m, 49H, CH_2-O hexylspacer, 19 x CH_2 glycerol, 9 x CH glycerol), 4.33 - 4.47 (m, 20H, 10 x CH_2 Bn), 4.82 (bs, 2H, CH_2 benzylcarbamate), 7.00 - 7.22 (m, 55H, H_{arom}); ^{13}C NMR (150 MHz, D_2O): δ = 25.7, 26.6, 29.8, 30.7 (4 x CH_2 hexylspacer), 41.2 (CH_2-N hexylspacer), 61.3 (CH_2 glycerol), 65.1, 65.5 - 65.6, 67.1, 67.5 (CH_2-O hexylspacer, 19 x CH_2 glycerol, CH_2 benzylcarbamate), 72.6, 72.8 - 72.9 (10 x CH_2 Bn), 78.0 - 78.1 (9 x CH glycerol), 79.2 (CH glycerol), 128.6 - 129.6 (CH_{arom}), 138.4 - 138.5 (10 x C_q Bn, C_q benzylcarbamate) 158.9 (C_q benzylcarbamate); HRMS: $[C_{114}H_{151}NO_{53}P_{10} + 2Na]^{2+}$ requires 1369.3173, found 1369.3181; Deprotection: The partially protected decamer (**11**, 7.10 mg, 2.44 µmol) was deprotected using the standard procedure yielding decameric GTA **17** (3.6 mg, 1.9 µmol, 78 %) as an amorphous off-white solid. ^{31}P-NMR (162 MHz, D_2O): δ = 1.2 (1P), 1.3 (7P), 1.3 (2P); ^{1}H-NMR (600 MHz, D_2O): δ = 1.36 - 1.39 (m, 4H, 2 x CH_2 hexylspacer), 1.58 - 1.65 (m, 4H, 2 x CH_2 hexylspacer), 2.95 (at, 2H, *J* = 7.5 Hz, CH_2-N hexylspacer), 3.55 (dd, 1H, *J* = 6.1 Hz, 11.8 Hz, C<u>H</u>H glycerol), 3.62 (dd, 1H, *J* = 4.2 Hz, 11.8 Hz, C<u>H</u>H glycerol), 3.72 (m, 1H, C<u>H</u>H glycerol), 3.80 - 4.02 (m, 49H, CH_2-O hexylspacer, 18 x CH_2 glycerol, 1 x C<u>H</u>H glycerol, 10 x CH glycerol); ^{13}C NMR (150 MHz, D_2O): δ = 25.4, 26.0, 27.5, 30.3 (4 x CH_2 hexylspacer), 40.3 (CH_2-N hexylspacer), 63.0 (CH_2 glycerol), 66.9 - 67.3 (CH_2-O hexylspacer, 19 x CH_2 glycerol), 70.4 - 70.5, 71.3, 71.6, 71.7 (10 x CH glycerol); HRMS: $C_{36}H_{85}NO_{51}P_{10} + H^+$ requires 1658.1537, found 1658.1553.

14-mer (18)

Synthesis on 10 µmol scale (100 mg glycerol-CPG). Average coupling efficiency: 98.1 % (13 couplings). Purification method A gave the semiprotected 14-mer (**12**) as white amorphous solid (3.0 mg, 0.75 µmol, 8 %). Method B gave the semiprotected 14-mer (**12**) as white amorphous solid (8.2 mg, 2.1 µmol, 21 %). LC-MS (gradient: 10 mM NH_4OAc in H_2O/acetonitrile 1/0 → 1/9 in 13.5 min): r.t. 5.84 min, $[C_{154}H_{203}NO_{73}P_{14} + 3H]^{3+}$ requires 1224.0 found 1224.4.; ^{31}P-NMR (162 MHz, D_2O): δ = 0.7 - 1.1 (14P); ^{1}H-NMR (600 MHz, D_2O): δ = 0.99 (m, 2H, CH_2 hexylspacer), 1.05 (m, 2H, CH_2

hexylspacer), 1.15 (m, 2H, CH_2 hexylspacer), 1.35 (m, 2H, CH_2 hexylspacer), 2.83 (m, 2H, CH_2-N hexylspacer), 3.47 - 3.58 (m, 3H, CH glycerol, CH_2 glycerol), 3.60 - 3.92 (m, 69H, CH_2-O hexylspacer, 27 x CH_2 glycerol, 13 x CH glycerol), 4.29 - 4.44 (m, 28H, 14 x CH_2 Bn), 4.72 (bs, 2H, CH_2 benzylcarbamate), 6.91 - 7.15 (m, 75H, H_{arom}); ^{13}C NMR (150 MHz, D_2O): δ = 24.7, 25.6, 28.9, 29.8 (4 x CH_2 hexylspacer), 40.3 (CH_2-N hexylspacer), 60.2 (CH_2 glycerol), 64.1, 64.4 - 64.6, 66.1, 66.4 (CH_2-O hexylspacer, 27 x CH_2 glycerol, CH_2 benzylcarbamate), 71.6, 71.8 (14 x CH_2 Bn), 77.0 - 77.1 (13 x CH glycerol), 78.2 (CH glycerol), 127.6 - 128.6 (CH_{arom}), 137.5 - 137.6 (14 x C_q Bn, C_q benzylcarbamate); HRMS: $[C_{154}H_{203}NO_{73}P_{14} + 2Na]^{2+}$ requires 1857.4174 found 1857.4167; Deprotection: The semiprotected 14-mer (**12**, 5.5 mg, 1.4 µmol) was deprotected using the standard procedure yielding 14-mer glycerol TA **18** (3.0 mg, 1.2 µmol, 84 %) as an amorphous off-white solid. ^{31}P-NMR (162 MHz, D_2O): δ = 1.1 - 1.3 (14P); ^{1}H-NMR (600 MHz, D_2O): δ =1.37 - 1.41 (m, 4H, 2 x CH_2 hexylspacer), 1.60 - 1.67 (m, 4H, 2 x CH_2 hexylspacer), 2.97 (at, 2H, *J* = 7.5 Hz, CH_2-N hexylspacer), 3.57 (dd, 1H, *J* = 6.1 Hz, 11.8 Hz, C<u>H</u>H glycerol), 3.64 (dd, 1H, *J* = 4.1 Hz, 11.8 Hz, C<u>H</u>H glycerol), 3.71 - 3.76 (m, 1H, C<u>H</u>H glycerol), 3.78 - 4.04 (m, 69H, CH_2-O hexylspacer, 26 x CH_2 glycerol, C<u>H</u>H glycerol, 14 x CH glycerol); ^{13}C NMR (150 MHz, D_2O): δ = 25.4, 26.1, 27.6, 30.4 (4 x CH_2 hexylspacer), 40.4 (CH_2-N hexylspacer), 63.1 (CH_2 glycerol), 65.8 (CH_2 glycerol), 67.3 - 67.7 (CH_2-O hexylspacer, 26 x CH_2 glycerol), 70.5 - 70.6, 71.3, 71.7 (14 x CH glycerol); HRMS: $C_{48}H_{112}NO_{71}P_{14} + H^+$ requires 2273.1584, found 2273.1562.

20-mer (19)

Synthesis on 15 µmol scale (150 mg glycerol-CPG). Average coupling efficiency: 98.5 % (19 couplings). Purification method B gave the semiprotected 20-mer (**13**) as white amorphous solid (20.2 mg, 3.62 µmol, 24 %). LC-MS (gradient: 10 mM NH_4OAc in H_2O/acetonitrile 9/1 → 1/9 in 13.5 min): r.t. 5.23 min, $[C_{214}H_{281}NO_{103}P_{20} + 4H]^{4+}$ requires 1284.6 found 1284.8.; ^{31}P-NMR (162 MHz, D_2O): δ = 0.8 (1P), 1.0 (18P), 1.1 (1P); ^{1}H-NMR (600 MHz, D_2O): δ = 0.96 (m, 2H, CH_2 hexylspacer), 1.03 (m, 2H, CH_2 hexylspacer), 1.13 (m, 2H, CH_2 hexylspacer), 1.33 (m, 2H, CH_2 hexylspacer), 2.82 (m, 2H, CH_2-N hexylspacer), 3.43 - 3.55 (m, 3H, CH glycerol, CH_2 glycerol), 3.57 - 4.08 (m, 99H, CH_2-O hexylspacer, 39 x CH_2 glycerol, 19 x CH glycerol), 4.17 - 4.44 (m, 40H, 20 x CH_2 Bn), 4.69 (bs, 2H, CH_2 benzylcarbamate), 6.85 - 7.13 (m, 105H, H_{arom}), 7.68 (s, 1H, NH); ^{13}C NMR (150 MHz, D_2O): δ = 25.7, 26.6, 30.0, 30.7 (4 x CH_2 hexylspacer), 41.3 (CH_2-N hexylspacer), 61.1 (CH_2 glycerol), 64.9 - 65.6, 67.1, 67.2, 67.6 (CH_2-O hexylspacer, 39 x CH_2 glycerol, CH_2 benzylcarbamate), 72.5 - 72.8 (20 x CH_2 Bn), 77.7 - 78.1 (19 x CH glycerol), 79.1 (CH glycerol), 128.6 - 129.6 (CH_{arom}), 137.5, 138.4 - 138.7 (20 x C_q Bn, C_q benzylcarbamate), 158.4 (C_q benzylcarbamate); HRMS: $[C_{214}H_{296}N_6O_{103}P_{20} + 3H]^{3+}$ (mass + 5 x NH_3) requires 1740.7715, found 1740.7691; Deprotection: The partially protected 20-mer (**13**, 6.2 mg, 1.1 µmol) was deprotected using the standard procedure yielding 20-mer glycerol TA **19** (3.8 mg, 1.1 µmol, 95 %) as an amorphous off-white solid. ^{31}P-NMR (162 MHz, D_2O): δ = 1.2 - 1.4 (20P); ^{1}H-NMR (600 MHz, D_2O): δ = 1.36 - 1.40 (m, 4H, 2 x CH_2 hexylspacer), 1.59 - 1.71 (m, 4H, 2 x CH_2 hexylspacer), 2.95 (at, 2H, *J* = 7.5 Hz, CH_2-N hexylspacer), 3.56 (dd, 1H, *J* = 6.2 Hz, 11.8 Hz, C<u>H</u>H glycerol), 3.63 (dd, 1H, *J* = 4.3 Hz, 11.9 Hz, C<u>H</u>H glycerol), 3.72 (m, 1H, C<u>H</u>H glycerol), 3.81 - 4.04 (m, 99H, CH_2-O hexylspacer, 38 x CH_2 glycerol, C<u>H</u>H glycerol, 20 x CH glycerol); ^{13}C NMR (150 MHz, D_2O): δ = 25.4, 26.0, 27.6, 30.4 (4 x CH_2 hexylspacer), 40.4 (CH_2-N hexylspacer), 63.0 (CH_2 glycerol), 65.7 (CH_2 glycerol), 66.9 - 67.5 (CH_2-O hexylspacer, 37 x CH_2 glycerol), 67.7 (CH_2 glycerol), 70.4 - 70.7, 71.3, 71.7 (20 x CH glycerol); HRMS: $[C_{66}H_{155}NO_{101}P_{20} + 2H]^{2+}$ requires 1599.5961, found 1599.5971.

glucosylated hexamer (20)

Synthesis on 15 µmol scale (150 mg glycerol-CPG). Average coupling efficiency: 98.2 % (5 couplings). Purification method B gave the semiprotected hexamer (**14**) as white amorphous solid (10.5 mg, 4.86 µmol, 32 %). LC-MS (gradient: 10 mM NH_4OAc in H_2O/acetonitrile 9/1 → 1/1 in 25 min): r.t. 13.80 min, $[C_{94}H_{119}NO_{38}P_6 + 2H]^{2+}$ requires 1029.3 found 1029.2.; ^{31}P-NMR (162 MHz, D_2O): δ = 1.0 (1P), 1.1 (3P), 1.1 (1P), 1.2 (1P); ^{1}H-NMR (600 MHz, D_2O): δ = 0.85 - 1.17 (m, 6H, 3 x CH_2 hexylspacer), 1.37 (m, 2H, CH_2 hexylspacer), 2.84 (m, 2H, CH_2-N hexylspacer), 3.34 - 4.11 (m, 38H, CH_2-O hexylspacer, 12 x CH_2 glycerol, 6 x CH glycerol, H-2, H-3, H-4, H-5, 2 x H-6), 4.29 - 4.71 (m, 16H, 7 x CH_2 Bn, CH_2 benzylcarbamate), 5.22 (bs, 1H, H-1), 5.28 (bs, 1H, CH benzylidene), 6.86 - 7.43 (m, 45H, H_{arom}); HRMS: $[C_{94}H_{119}NO_{38}P_6 + NH_4 + H]^{2+}$ requires 1037.3123, found 1037.3120; Deprotection: The partially protected hexamer (**14**, 10.5 mg, 4.86 µmol) was deprotected using the standard procedure yielding hexamer monoglucosylglycerol TA **20** (4.4 mg, 3.3 µmol, 68 %) as an amorphous off-white solid. ^{31}P-NMR (162 MHz, D_2O): δ = 0.9 (1P), 1.2 (3P), 1.3 (1P), 1.3 (1P); ^{1}H-NMR (600 MHz, D_2O): δ =1.36 - 1.40 (m, 4H, 2 x CH_2 hexylspacer), 1.58 - 1.65 (m, 4H, 2 x CH_2

hexylspacer), 2.94 (at, 2H, *J* = 7.5 Hz, CH_2-N hexylspacer), 3.34 (at, 1H, *J* = 9.6 Hz, H-4), 3.46 (dd, 1H, *J* = 3.7 Hz, 9.9 Hz, H-2), 3.55 (dd, 1H, *J* = 6.1 Hz, 11.8 Hz, CHH glycerol), 3.62 (dd, 1H, *J* = 4.2 Hz, 11.8 Hz, CHH glycerol), 3.69 - 3.72 (m, 3H, H-3, H-5, H-6), 3.79 - 4.00 (m, 30H, CH_2-O hexylspacer, 11 x CH_2 glycerol, 5 x CH glycerol, H-6), 4.05 (m, 1H, CH glycerol), 5.11 (d, 1H, *J* = 3.7 Hz, H-1); ^{13}C NMR (150 MHz, D_2O): δ = 25.4, 26.1, 27.6, 30.4 (4 x CH_2 hexylspacer), 40.4 (CH_2-N hexylspacer), 61.5 (C-6), 63.0 (CH_2 glycerol), 65.2 (CH_2 glycerol), 66.1 (CH_2 glycerol) 67.0 - 67.4 (CH_2-O hexylspacer, 9 x CH_2 glycerol), 70.4 - 70.6 (4 x CH glycerol, C-4), 71.7 (CH glycerol), 72.5 (C-2), 72.8 (C-5), 73.9 (C-3), 76.3 (CH glycerol), 98.7 (C-1); HRMS: $C_{30}H_{67}NO_{36}P_6$ + H^+ requires 1204.1941, found 1204.1957.

glucosylated hexamer (21)

Synthesis on 15 µmol scale (150 mg glucosylglycerol-CPG). Average coupling efficiency: 96.9 % (5 couplings). Purification method B gave the semiprotected hexamer (**15**) as white amorphous solid (11.6 mg, 5.37 µmol, 36 %). LC-MS (gradient: 10 mM NH_4OAc in H_2O/acetonitrile 9/1 → 1/1 in 25 min): r.t. 13.55 min, $C_{94}H_{119}NO_{38}P_6$ + H^+ requires 2057.6 found 2058.0.; ^{31}P-NMR (162 MHz, D_2O): δ = 1.0 - 1.3 (6P); ^{1}H-NMR (600 MHz, D_2O): δ = 0.90 - 1.15 (m, 6H, 3 x CH_2 hexylspacer), 1.33 (m, 2H, CH_2 hexylspacer), 2.80 (m, 2H, CH_2-N hexylspacer), 3.22 - 4.18 (m, 38H, CH_2-O hexylspacer, 12 x CH_2 glycerol, 6 x CH glycerol, H-2, H-3, H-4, H-5, 2 x H-6), 4.30 - 4.69 (m, 16H, 7 x CH_2 Bn, CH_2 benzylcarbamate), 5.12 - 5.20 (m, 2H, H-1, CH benzylidene), 6.86 - 7.26 (m, 45H, H_{arom}); HRMS: $[C_{94}H_{119}NO_{38}P_6 + 2H]^{2+}$ requires 1028.7991, found 1028.7996; Deprotection: The partially protected hexamer (**15**, 2.2 mg, 0.99 µmol) was deprotected using the standard procedure yielding hexamer monoglucosyl TA **21** (1.1 mg, 0.85 µmol, 86 %) as an amorphous off-white solid. ^{31}P-NMR (162 MHz, D_2O): δ = 1.2 (1P), 1.2 (3P), 1.3 (1P), 1.3 (1P); ^{1}H-NMR (600 MHz, D_2O): δ = 1.36 - 1.40 (m, 4H, 2 x CH_2 hexylspacer), 1.59 - 1.65 (m, 4H, 2 x CH_2 hexylspacer), 2.94 (at, 2H, *J* = 7.5 Hz, CH_2-N hexylspacer), 3.36 (at, 1H, *J* = 9.7 Hz, H-4), 3.48 (dd, 1H, *J* = 3.8 Hz, 9.9 Hz, H-2), 3.68 - 3.73 (m, 4H, H-3, 2 x H-6, CHH glycerol), 3.77 - 4.02 (m, 32H, CH_2-O hexylspacer, 11 x CH_2 glycerol, CHH glycerol, 6 x CH glycerol, H-5), 5.12 (d, 1H, *J* = 3.7 Hz, H-1); ^{13}C NMR (150 MHz, D_2O): δ = 25.4, 26.0, 27.6, 30.3 (4 x CH_2 hexylspacer), 40.4 (CH_2-N hexylspacer), 61.5 (CH_2 glycerol), 62.2 (C-6), 65.2 (CH_2 glycerol), 66.9 - 67.2 (CH_2-O hexylspacer, 11 x CH_2 glycerol), 70.4 - 70.6 (5 x CH glycerol, C-4), 72.4 (C-2), 72.9 (C-5), 73.8 (C-3), 77.8 (CH glycerol), 98.8 (C-1); HRMS: $C_{30}H_{67}NO_{36}P_6$ + H^+ requires 1204.1941, found 1204.1956.

Opsonophagocytic killing assay

White blood cells (WBC) were prepared from fresh human blood collected from healthy adult volunteers. 25 ml were mixed with an equal volume of dextran-heparin buffer and incubated at 37°C for 1 h. The upper layer containing the leukocytes was collected, the cells were pelleted by centrifugation, and hypotonic lysis of the remaining erythrocytes was accomplished by resuspension of the cell pellet in 1% NH_4Cl and incubation for 10 min at room temperature. WBC were then washed and resuspended with RPMI with 15% FBS (RPMI-FBS). With trypan blue staining to differentiate dead from live leukocytes, the final WBC count was adjusted to 5 x 10^6 WBC per mL.

The complement source (1 mL of baby rabbit serum diluted 1:15 in RPMI-FBS) was adsorbed with target bacteria at 4°C for 30 min with continual mixing. After adsorption, the complement solution was centrifuged and filter sterilized.

The test sera were diluted with RPMI-FBS.

The bacterial strains to be evaluated were grown in TSB and a 1:100 dilution in RPMI-FBS was made for use in the killing assay.The actual phagocytic killing assay was performed by mixing 100 µL (each) of the WBC suspension, target bacteria, dilutions of test sera, and the complement source. The reaction mixture was incubated on a rotor rack at 37°C for 90 min; samples were taken at time zero and after 90 min. A 10-fold dilution was made in TSB, and samples were plated onto tryptic soy agar plates. Tubes lacking any serum were used as controls, as were tubes containing serum and complement but lacking WBC to control for potential aggregation of bacteria by the antibody, which would reduce the apparent CFU counts at the end of the assay. The percentage of killing was calculated by determining the ratio of the number of CFU surviving in the tubes with bacteria, leukocytes, complement, and sera to the number of CFU surviving in tubes lacking sera but containing bacteria, complement, and leukocytes.

For inhibition studies, serum was diluted and incubated for 60 min at 4°C with an equal volume of a solution containing 100 µg/mL of synthetic TA oligomers **16-21**. Subsequently, the antiserum was used in the opsonophagocytic assay as described above. Inhibition of >40% was considered biologically significant, as this represented twice the upper limit of nonspecific inhibition seen in control tubes containing irrelevant polysaccharide antigens as inhibitors.

In table 1 the results from the opsonophagocytic inhibition assay (given is percentual killing of the bacteria), using synthetic TAs **16-21** (at a concentration of 100 µg/ml) are depicted. Native LTA is used as a positive control at the same concentration.[2]

Table 1. Killing (%) in an opsonophagocytic inhibition assay using synthetic TA **16-21** (100 ug/ml).

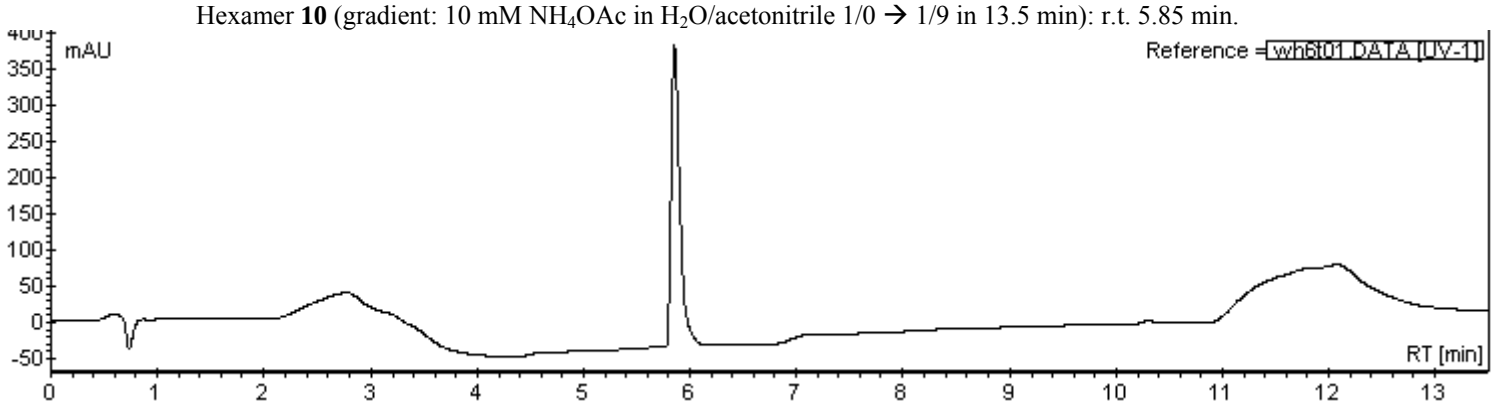
TA	**16**	**17**	**18**	**19**	**20**	**21**	**native LTA**	**α-LTA 12030 (1:200)**
Experiments 1-4	35	50	45	25	20	8	4	79
	37	58	46	29	9	4	7	78
	30	65	65	18	18	18	7	88
	7	42	30	30	7	7	-5	100
Mean	**27**	**54**	**47**	**26**	**13**	**9**	**3**	**86**

Reference

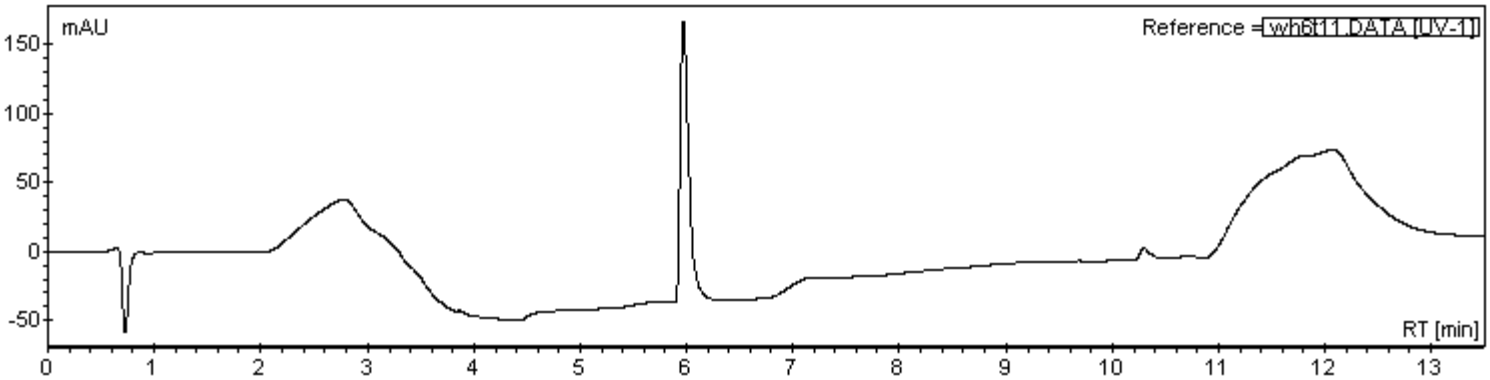
1) Pon, R.T. *Methods in Molecular Biology*; Agrawal, S., Ed. ; Humana Press, Totowa, New Jersey, 1993, Vol. 20, pp 465-496.
2) C. Theilacker, Z. Kaczynski, A. Kropec, F. Fabretti, T. Sange, O. Holst, J. Huebner, *Inf. Immun.*, 2006, **74**, 5703.

LC traces compounds 10-15 (UV 215nm)

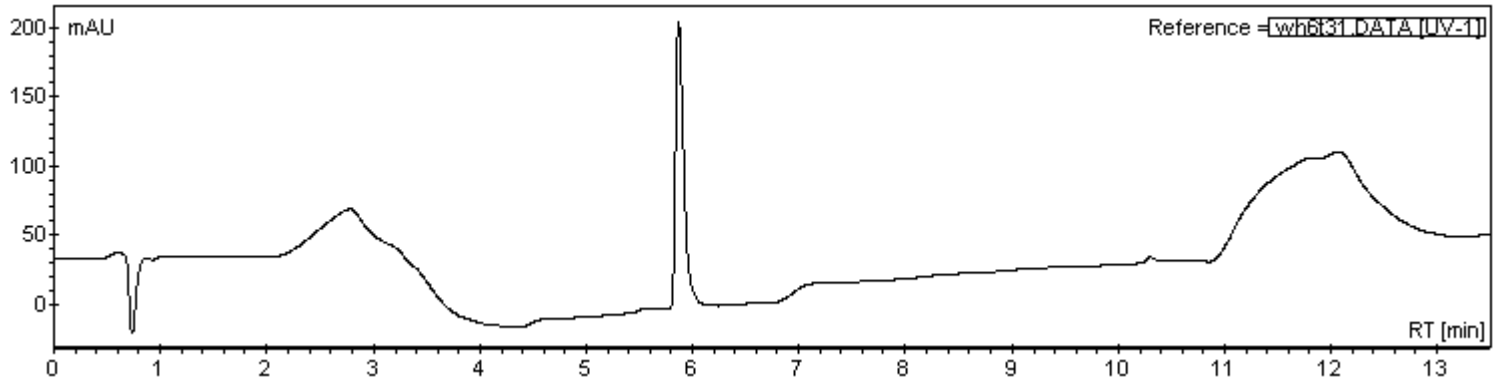
Hexamer **10** (gradient: 10 mM NH_4OAc in H_2O/acetonitrile 1/0 → 1/9 in 13.5 min): r.t. 5.85 min.



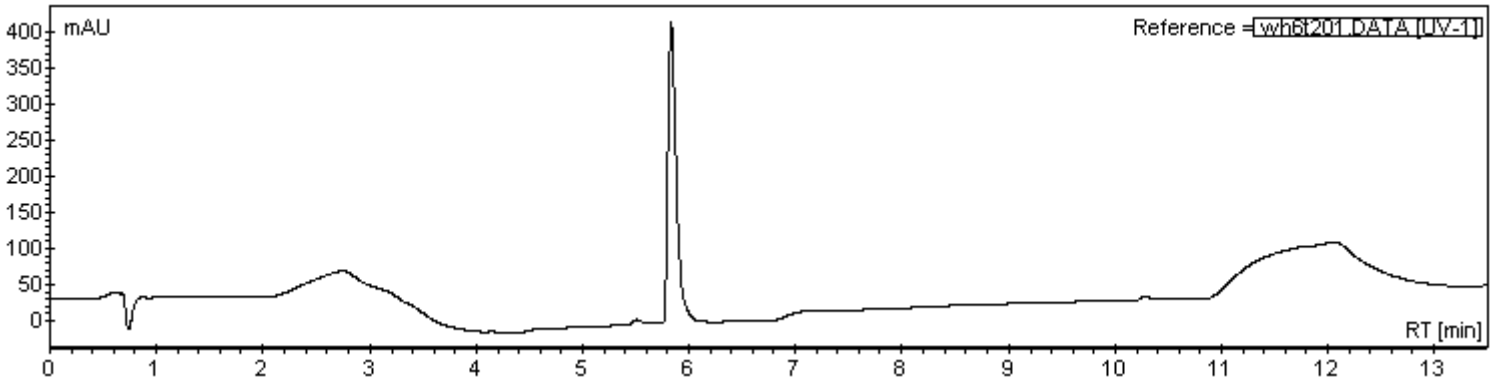
Hexamer **10** after 1 hr with 25 % NH_3OH at RT



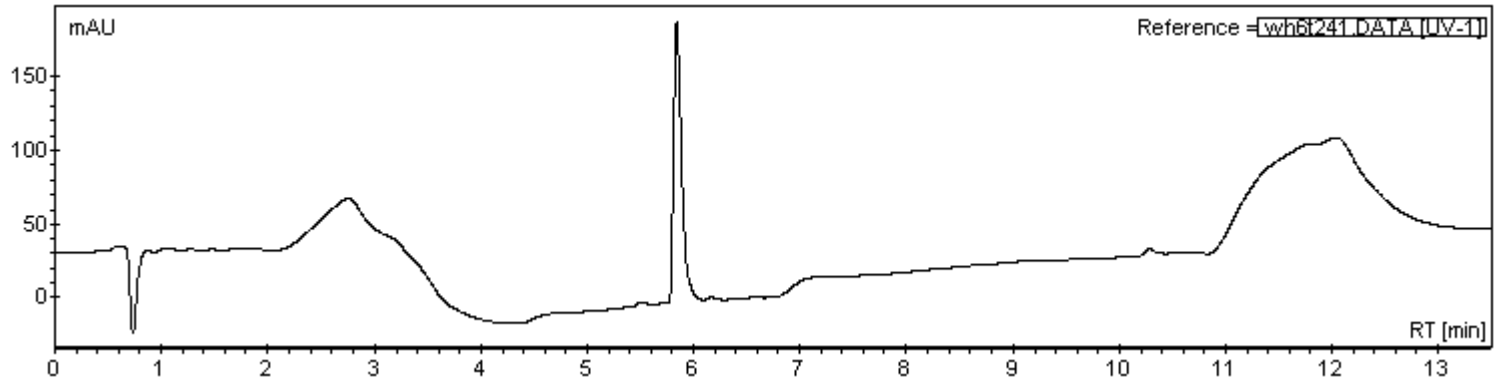
Hexamer **10** after 3 hr with 25 % NH_3OH at RT



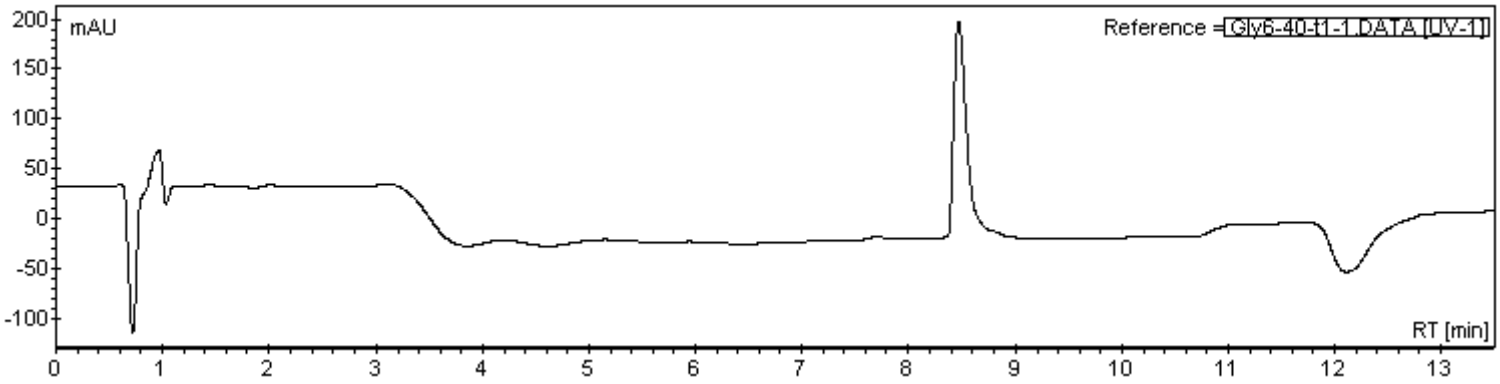
Hexamer **10** after 20 hr with 25 % NH_3OH at RT



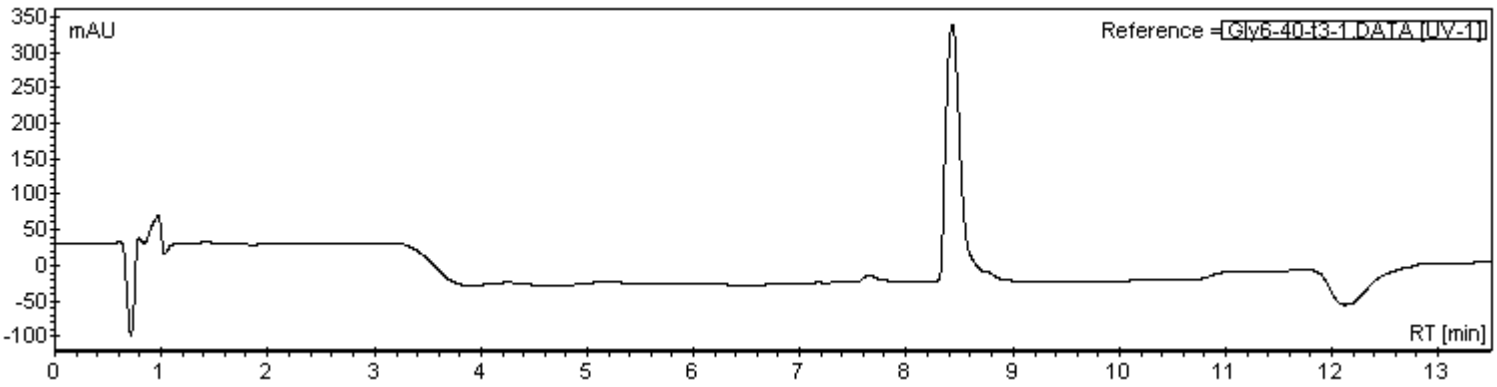
Hexamer **10** after 24 hr with 25 % NH_3OH at RT



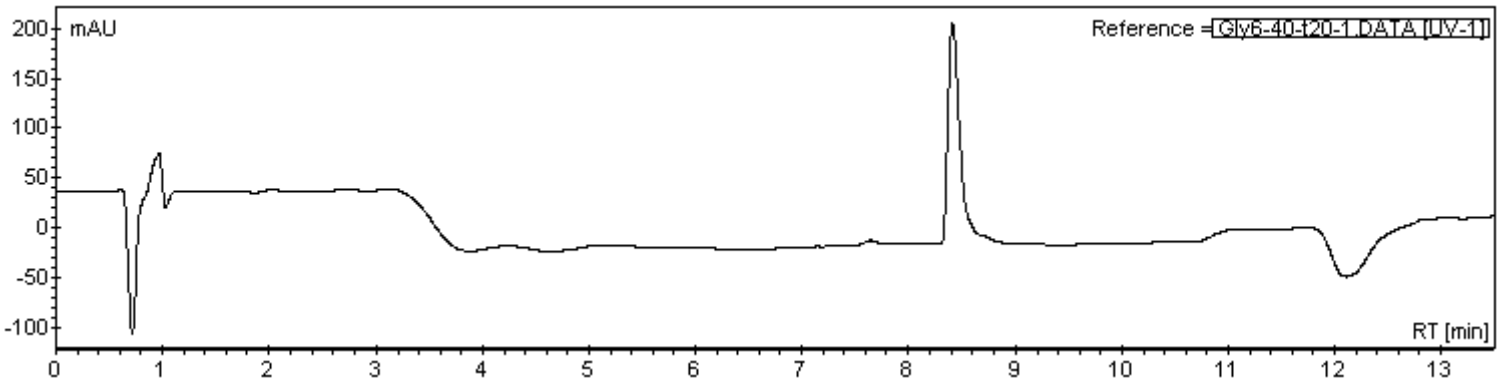
Hexamer **10** after 1 hr with 25 % NH_3OH at 40 ^oC (gradient: 10 mM NH_4OAc in H_2O/acetonitrile 19/1 → 1/1 in 13.5 min): r.t. 8.43 min.



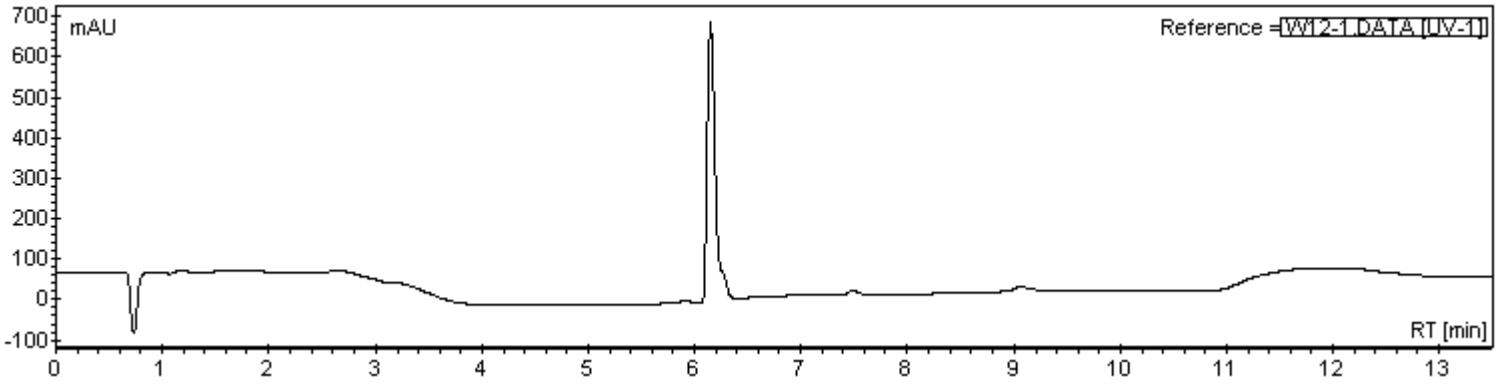
Hexamer **10** after 3 hr with 25 % NH_3OH at 40 ^oC



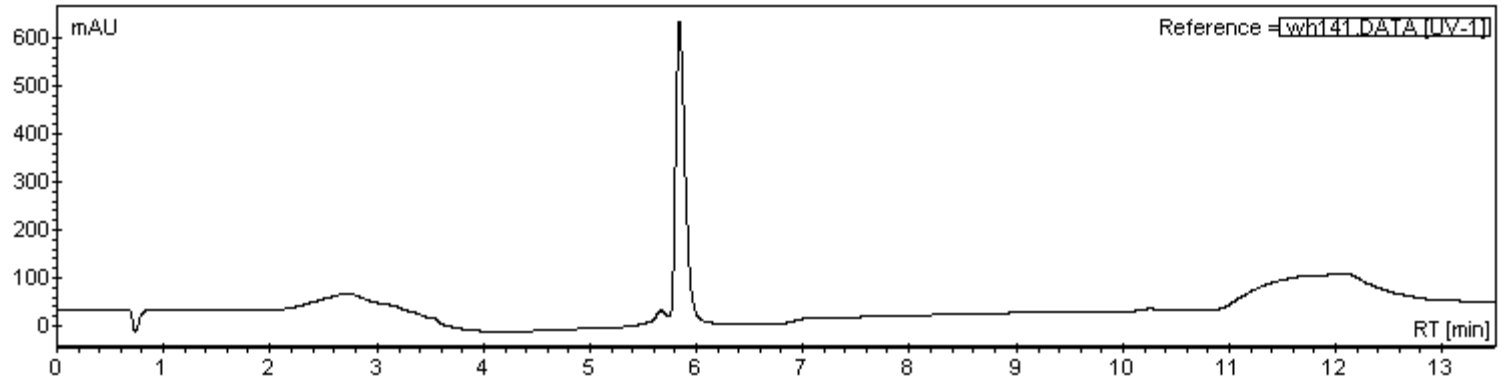
Hexamer **10** after 20 hr with 25 % NH_3OH at 40 ^oC



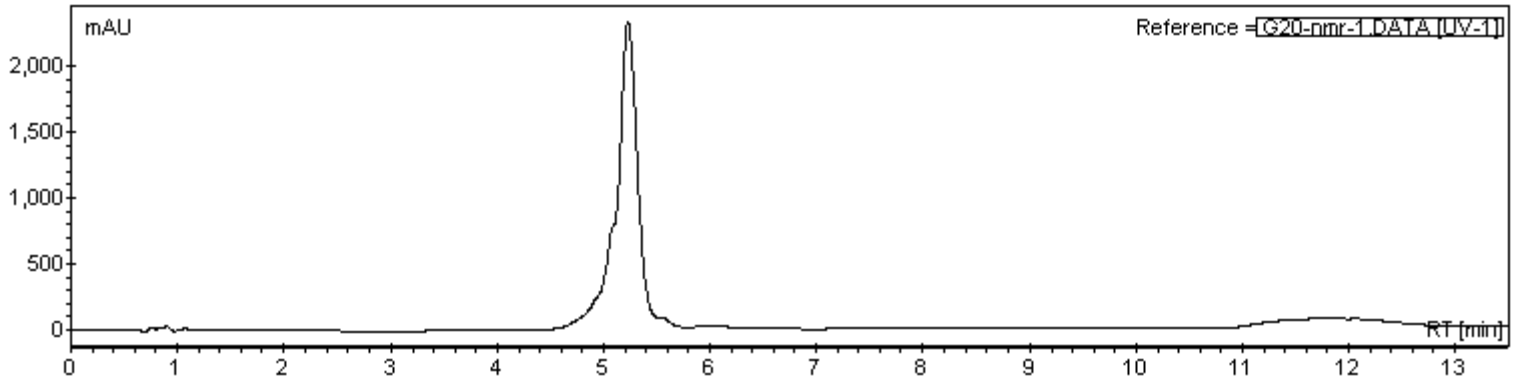
10-mer **11** (gradient: 10 mM NH_4OAc in H_2O/acetonitrile 1/0 → 1/9 in 13.5 min): r.t. 6.15 min.



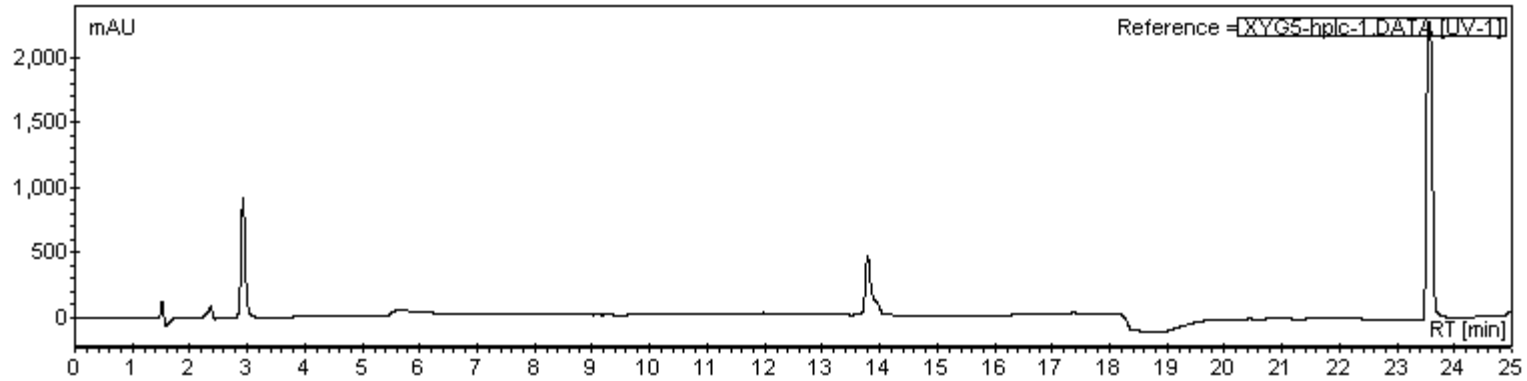
14-mer **12** (gradient: 10 mM NH_4OAc in H_2O/acetonitrile 1/0 → 1/9 in 13.5 min): r.t. 5.84 min



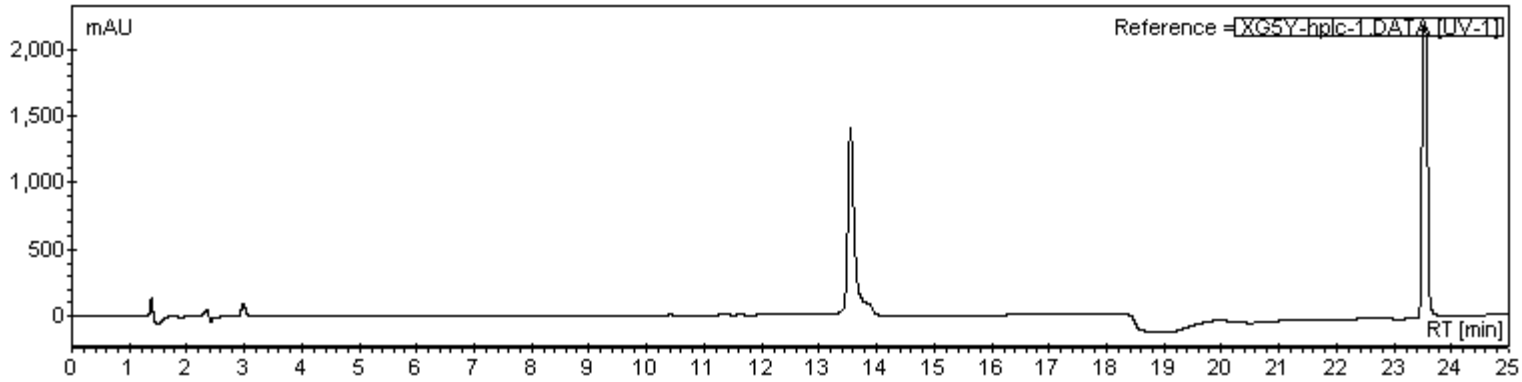
20-mer **13** (gradient: 10 mM NH_4OAc in H_2O/acetonitrile 9/1 → 1/9 in 13.5 min): r.t. 5.23 min.



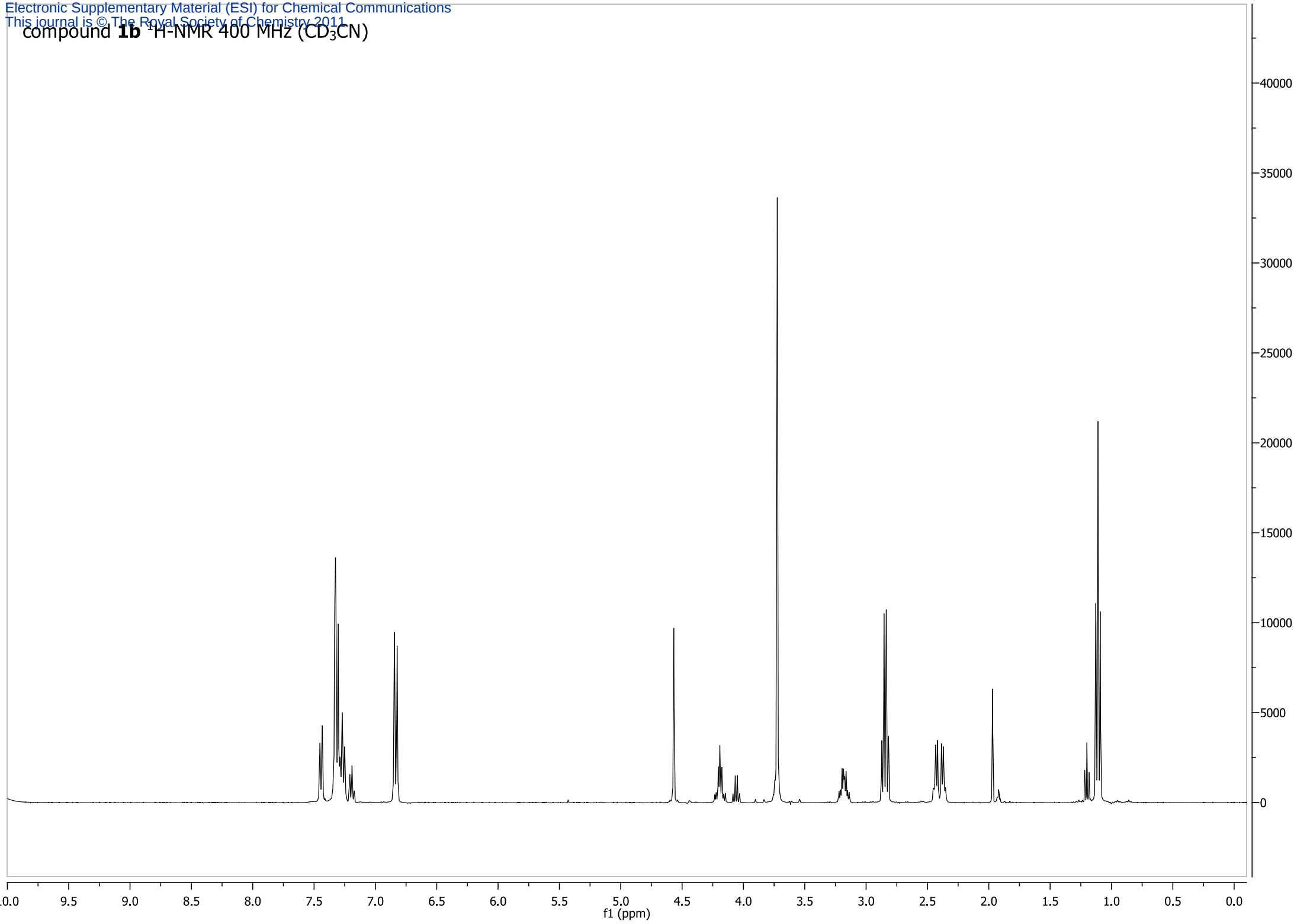
Glucosylated hexamer **14** (gradient: 10 mM NH_4OAc in H_2O/acetonitrile 9/1 → 1/1 in 25 min): r.t. 13.80 min.



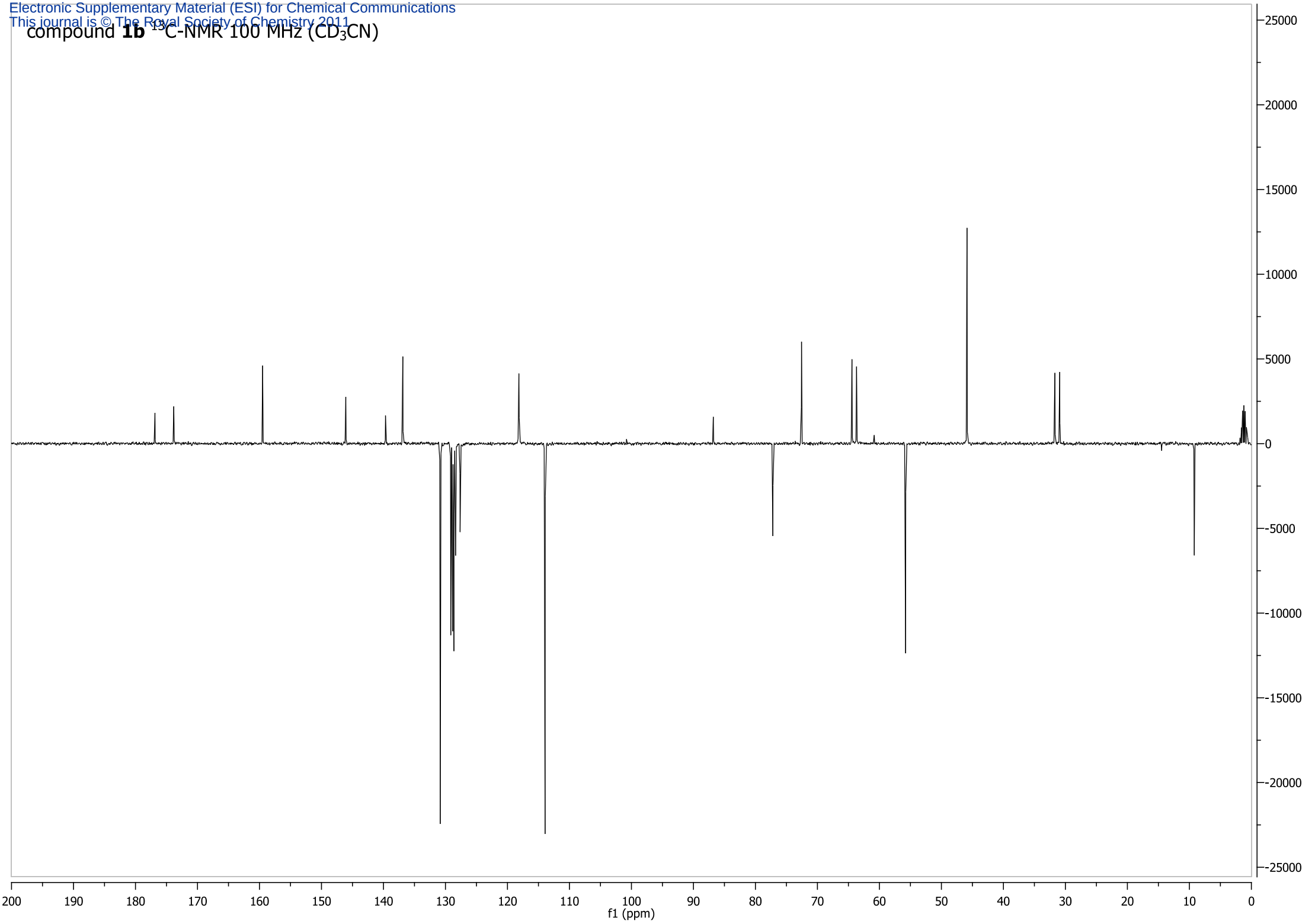
Glucosylated hexamer **15** (gradient: 10 mM NH_4OAc in H_2O/acetonitrile 9/1 → 1/1 in 25 min): r.t. 13.55 min.



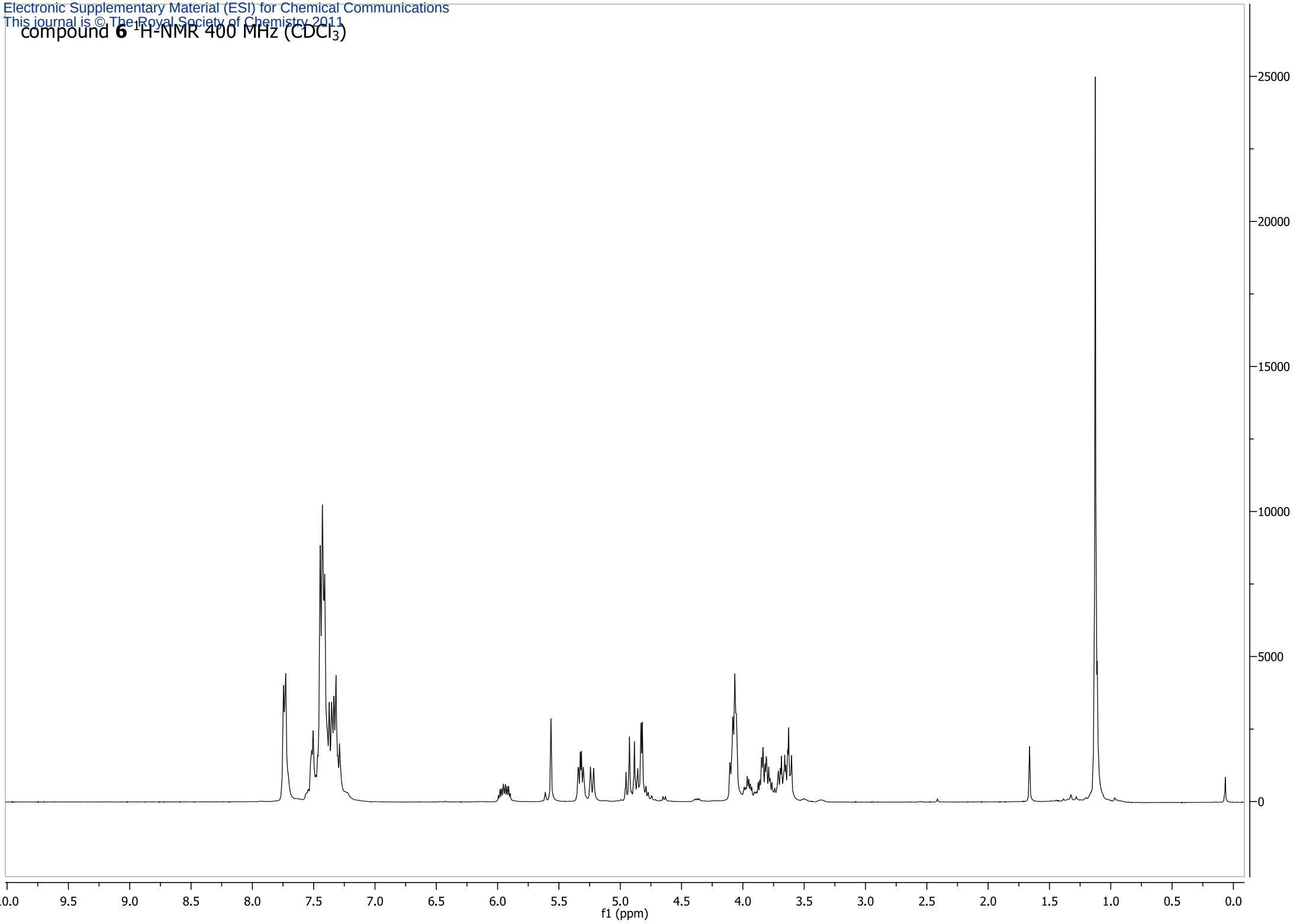
compound **1b** ^{1}H-NMR 400 MHz (CD_3CN)

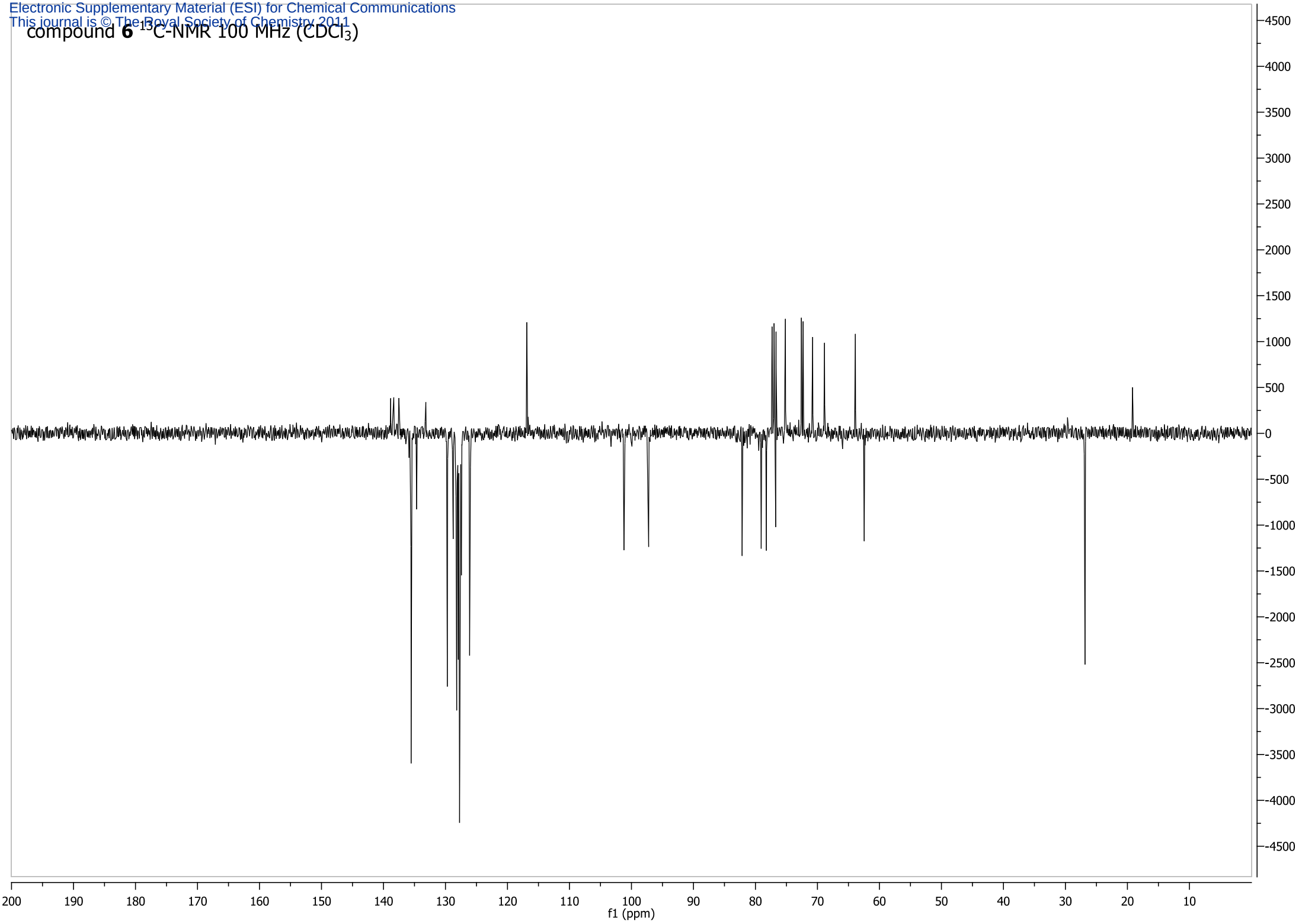


compound **1b** ^{13}C-NMR 100 MHz (CD_3CN)

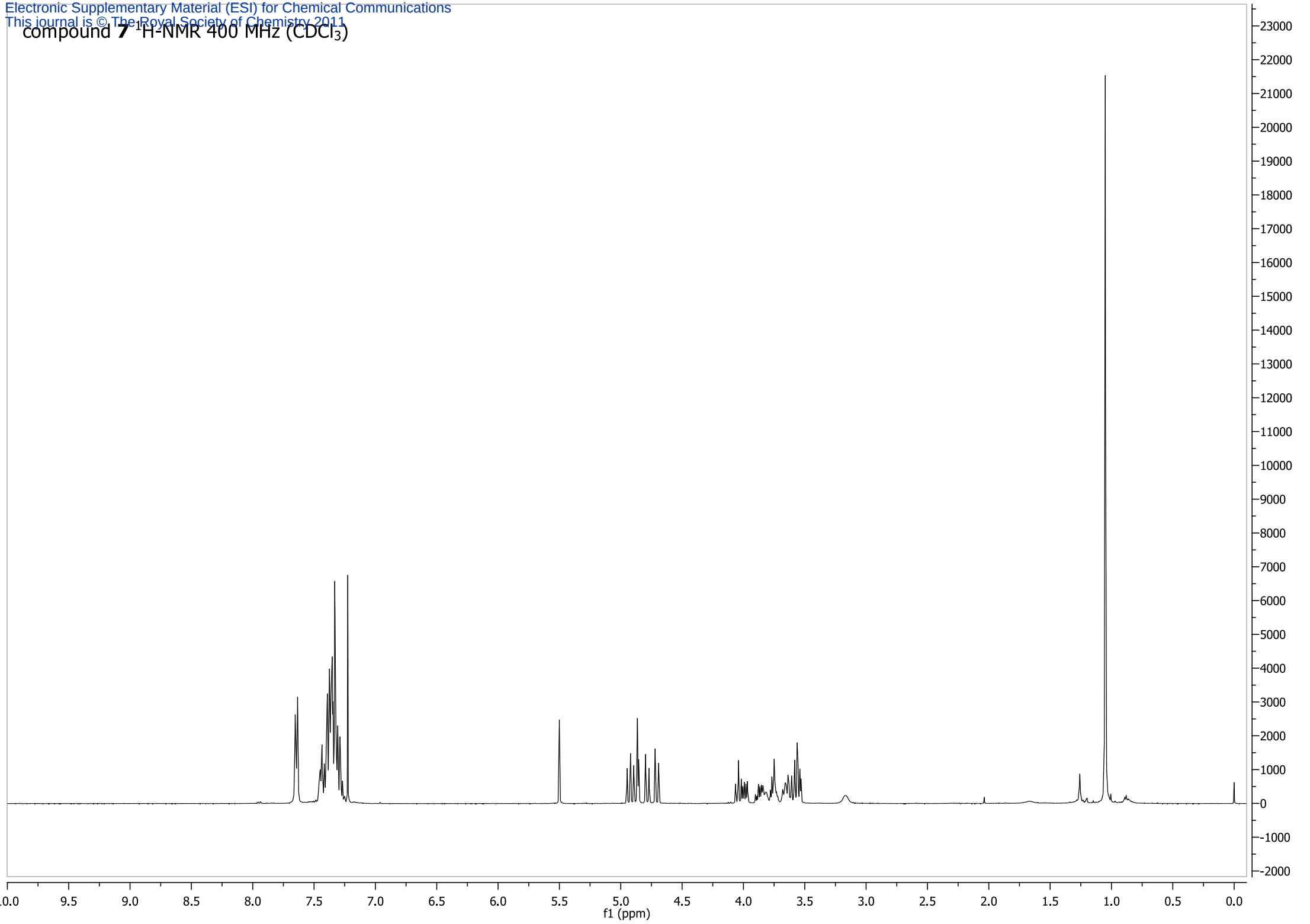


compound **6** ^{1}H-NMR 400 MHz ($CDCl_3$)

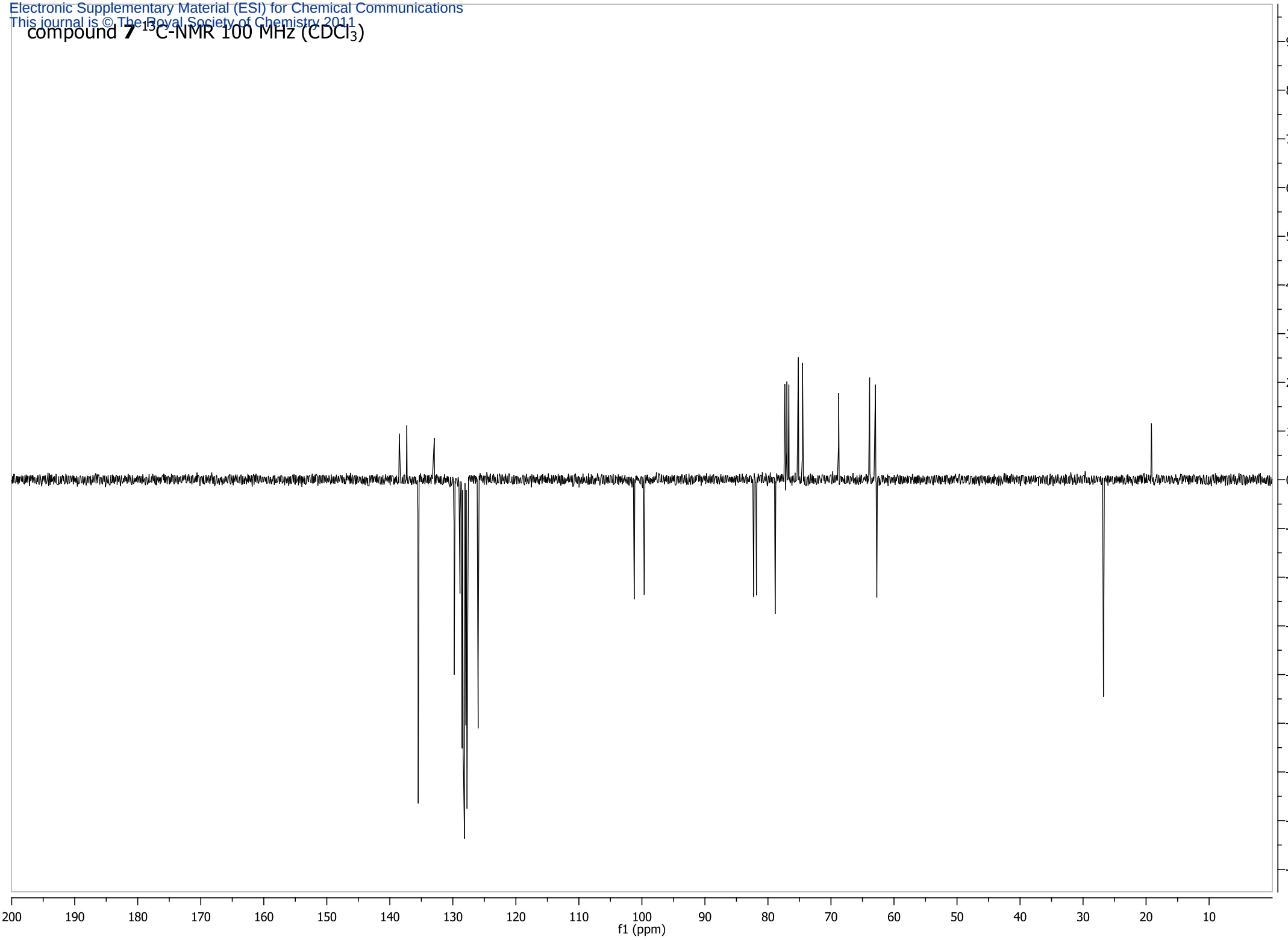


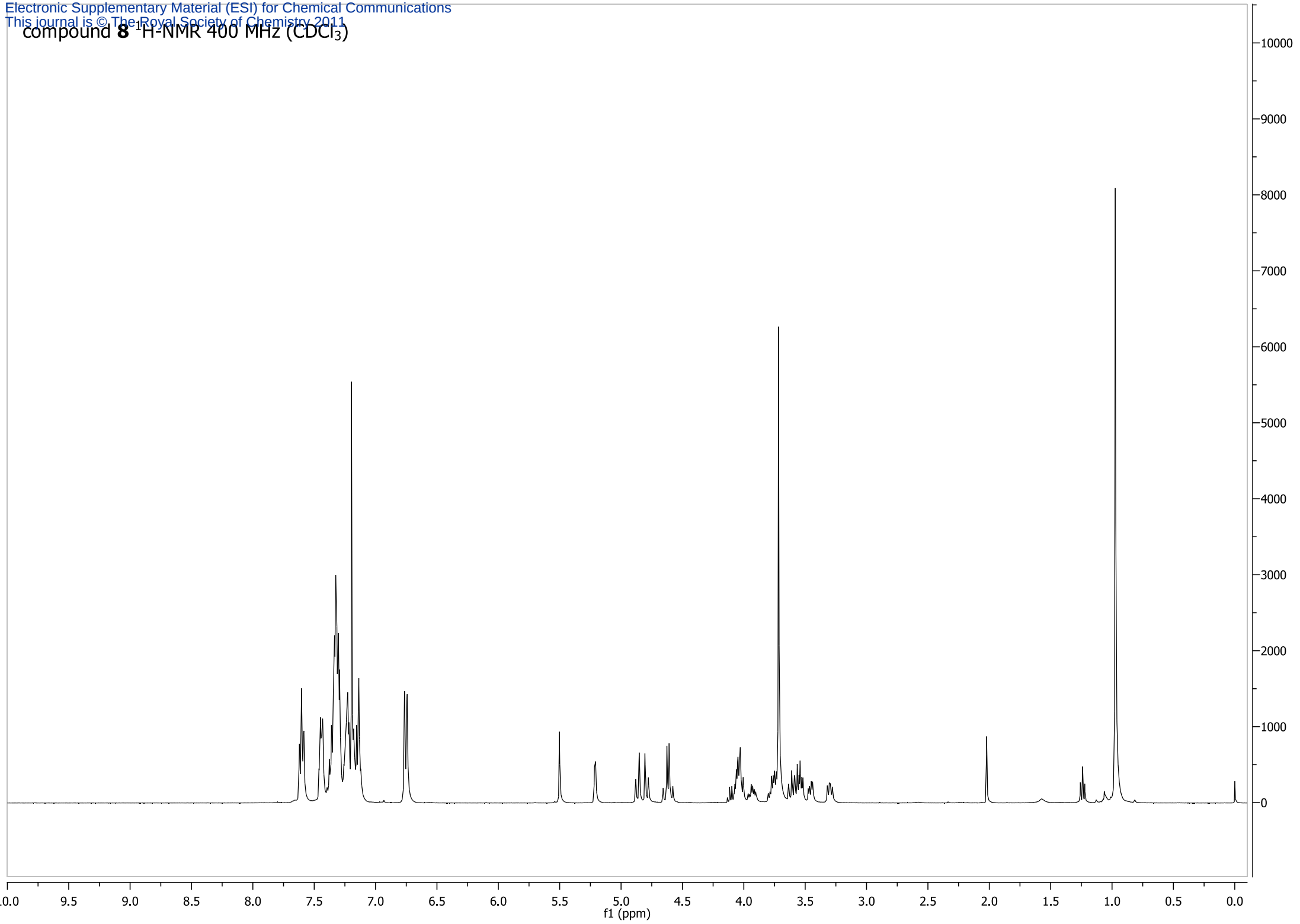


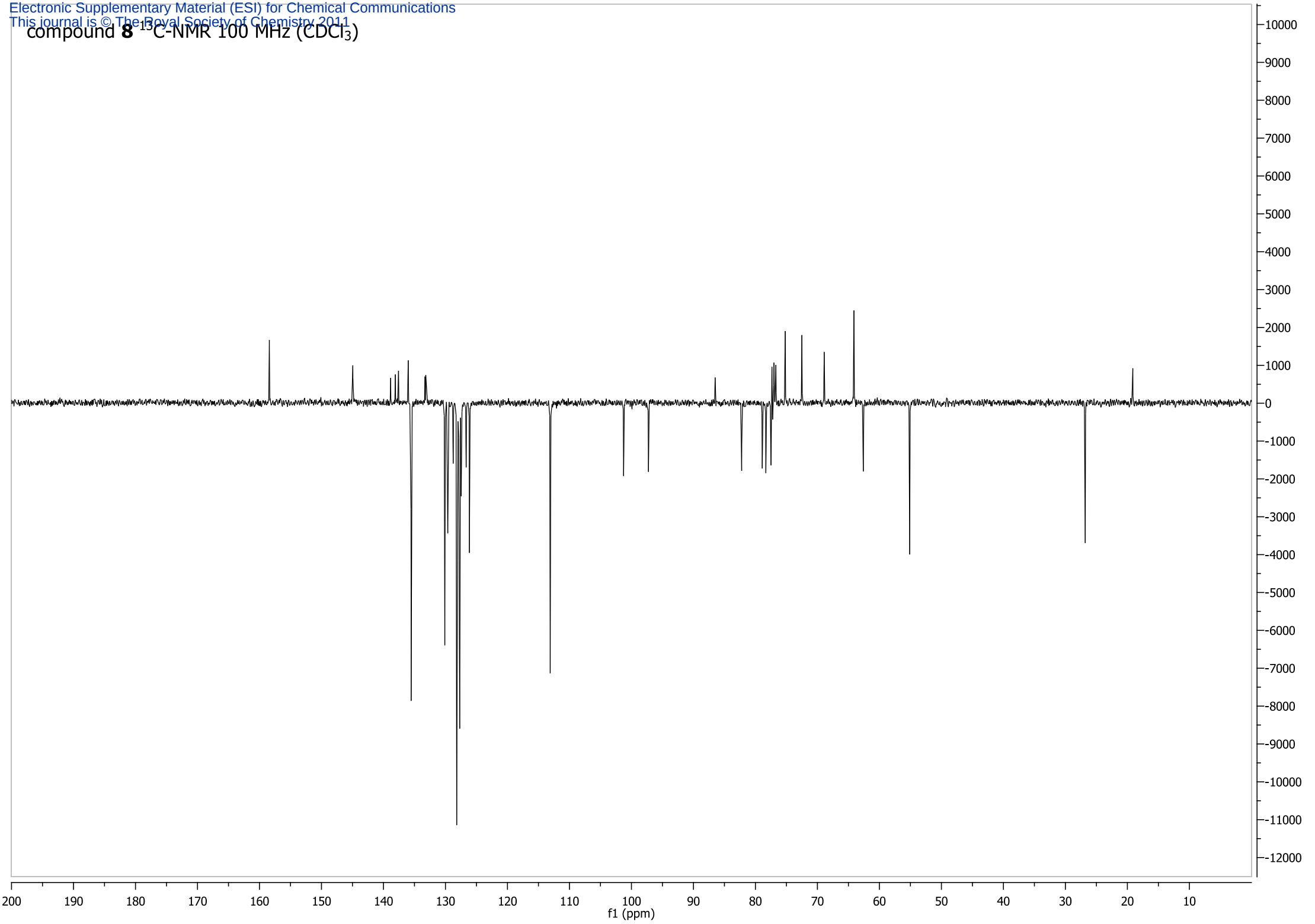
compound **7** ^{1}H-NMR 400 MHz ($CDCl_3$)



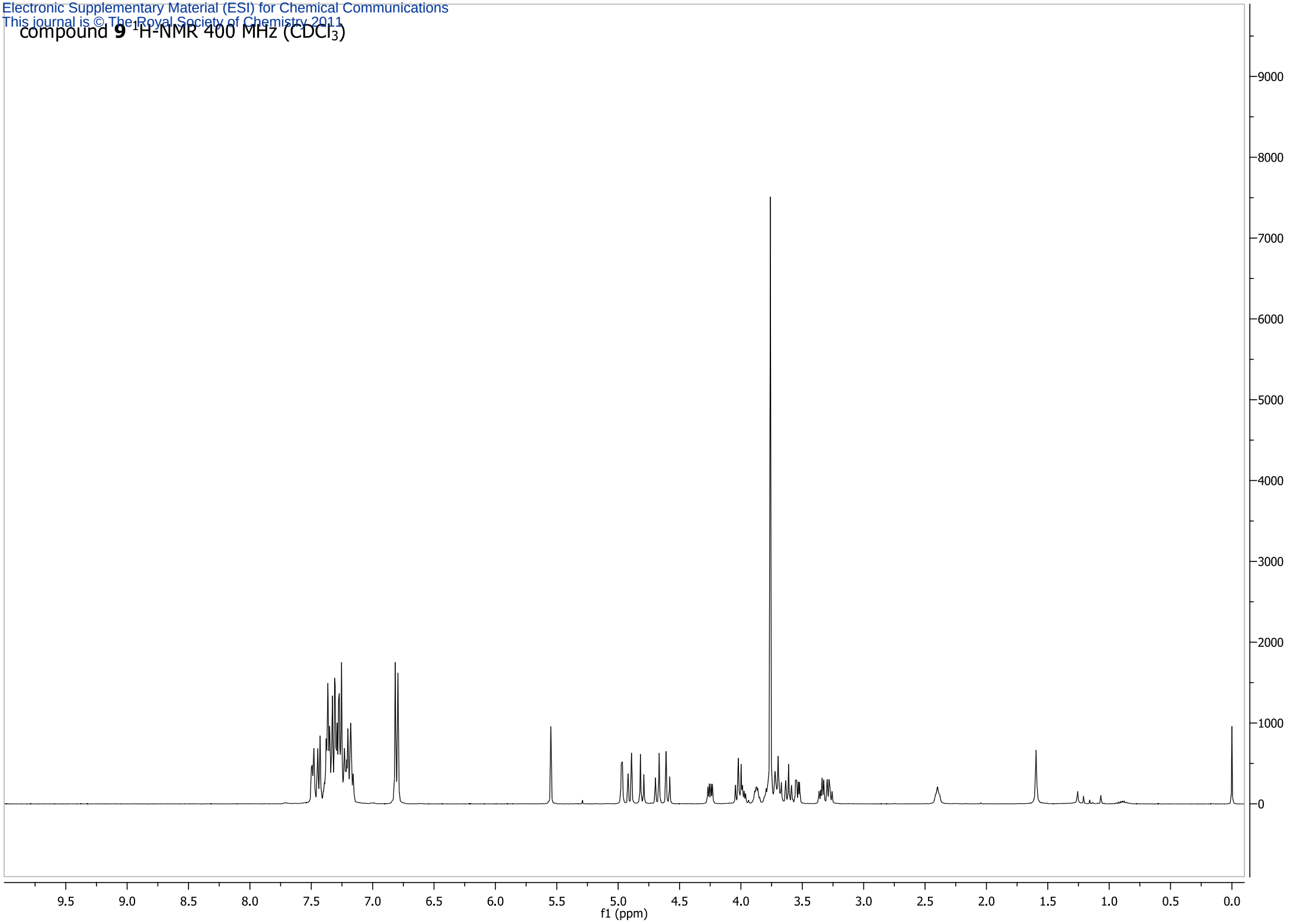
compound **7** ^{13}C-NMR 100 MHz ($CDCl_3$)

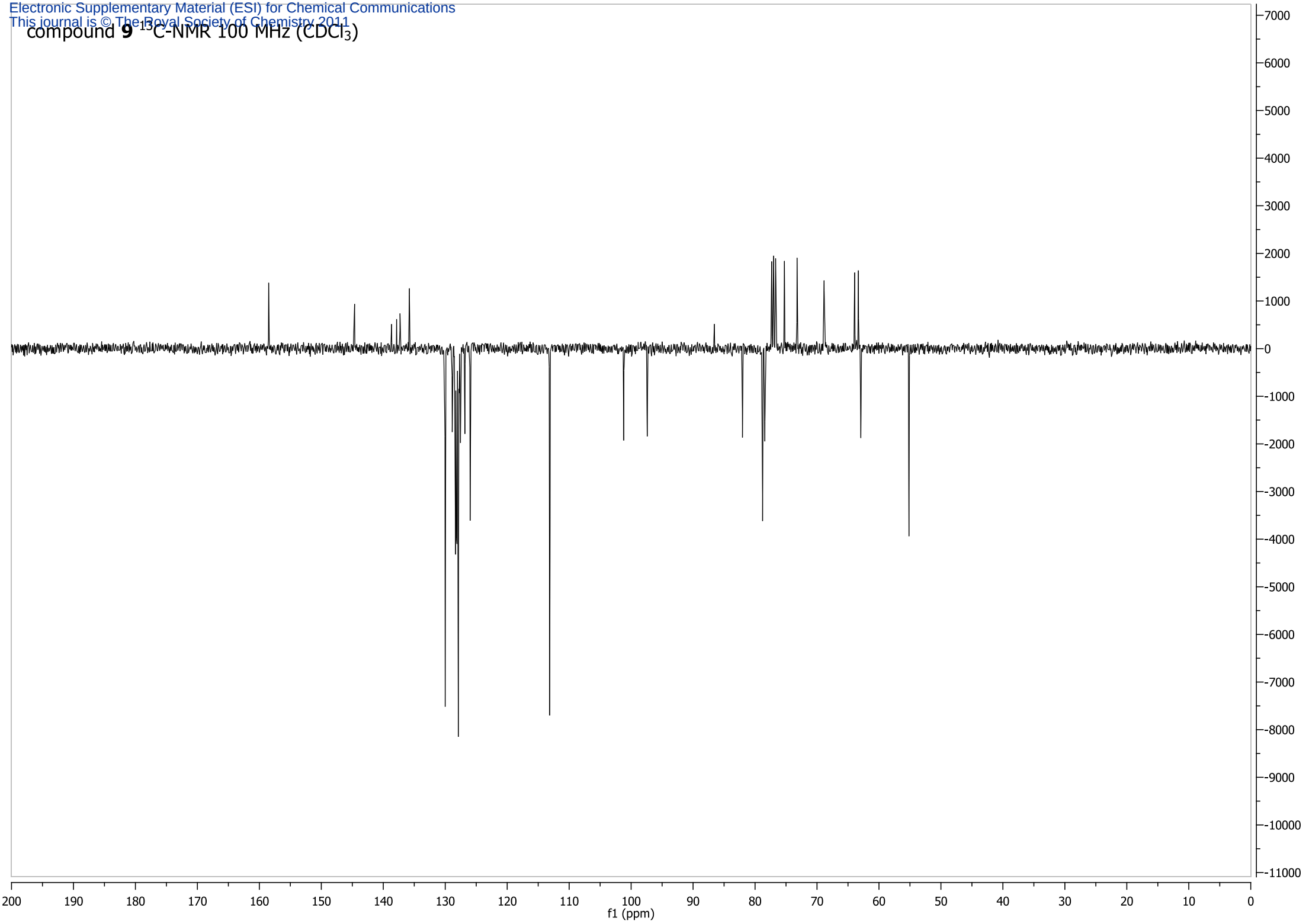




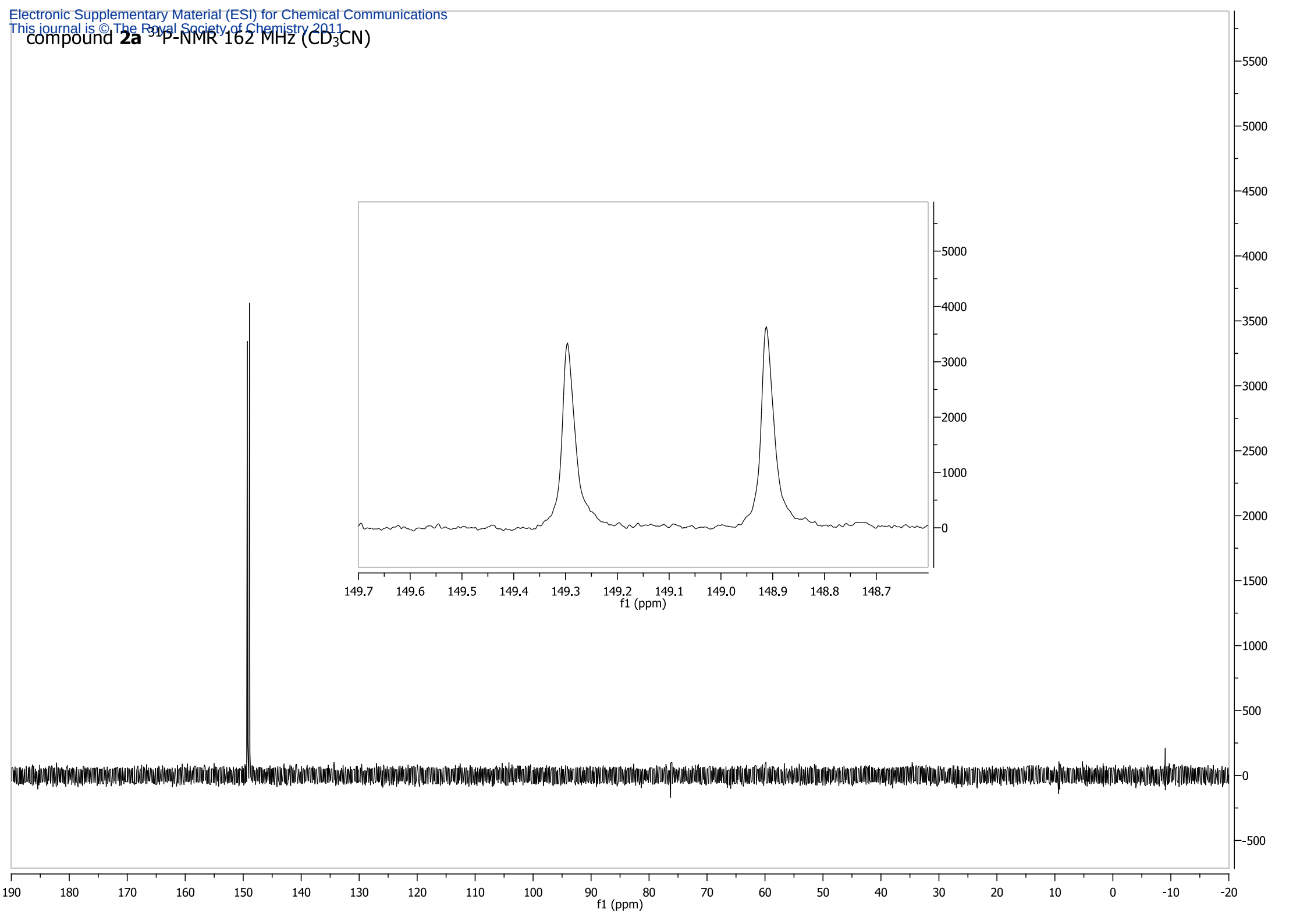


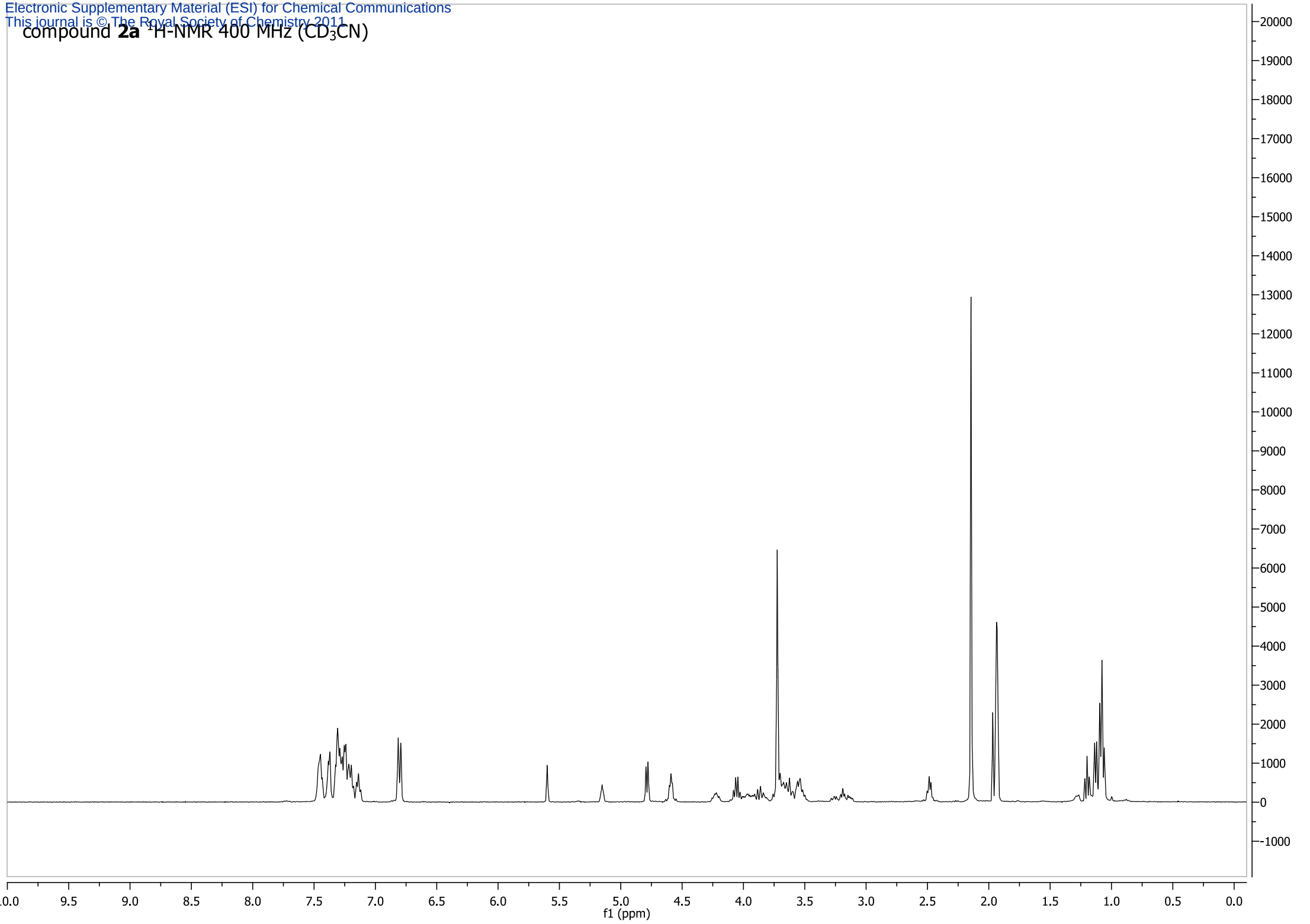
compound **9** ^{1}H-NMR 400 MHz ($CDCl_3$)



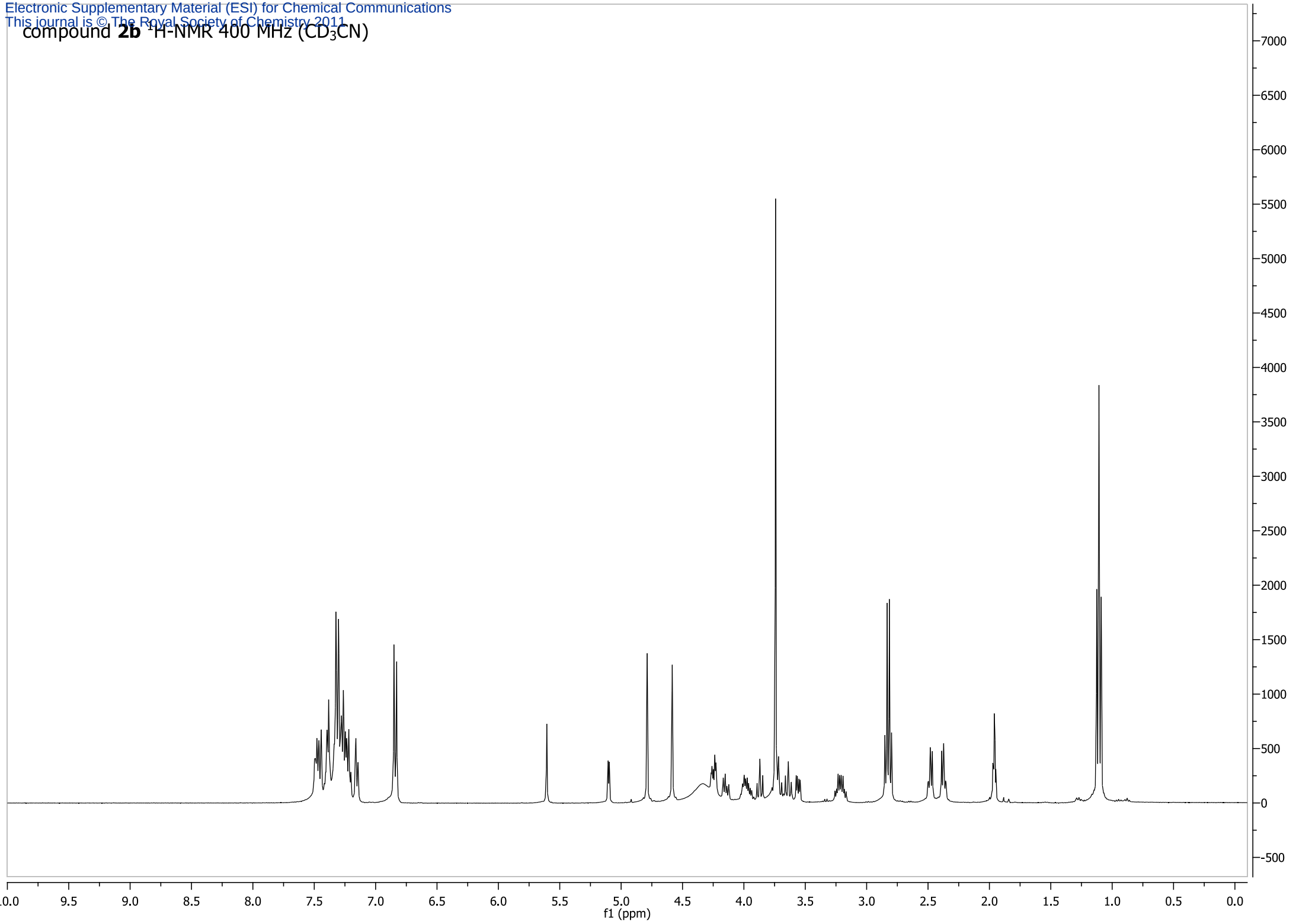


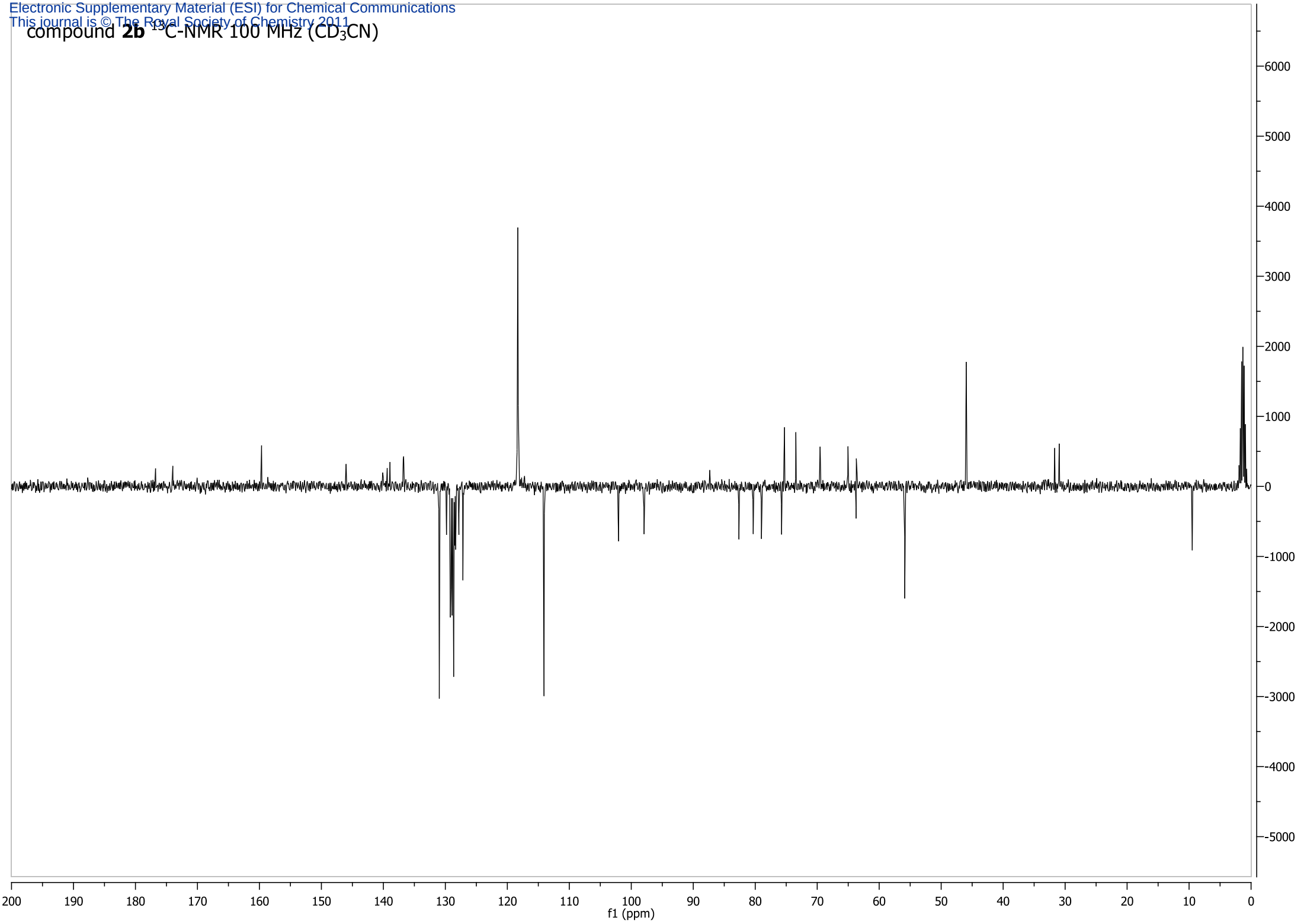
compound **2a** ^{31}P-NMR 162 MHz (CD_3CN)



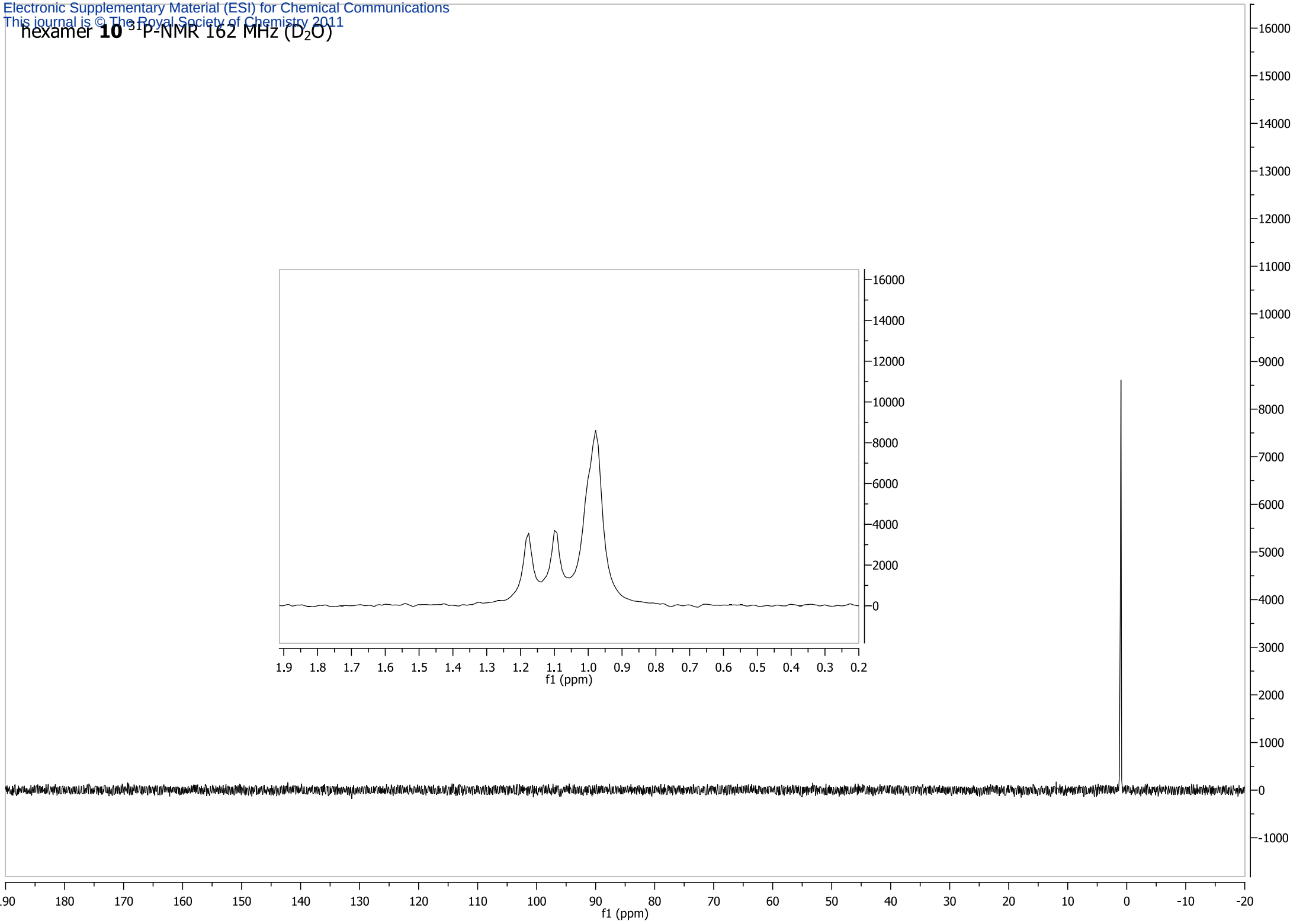


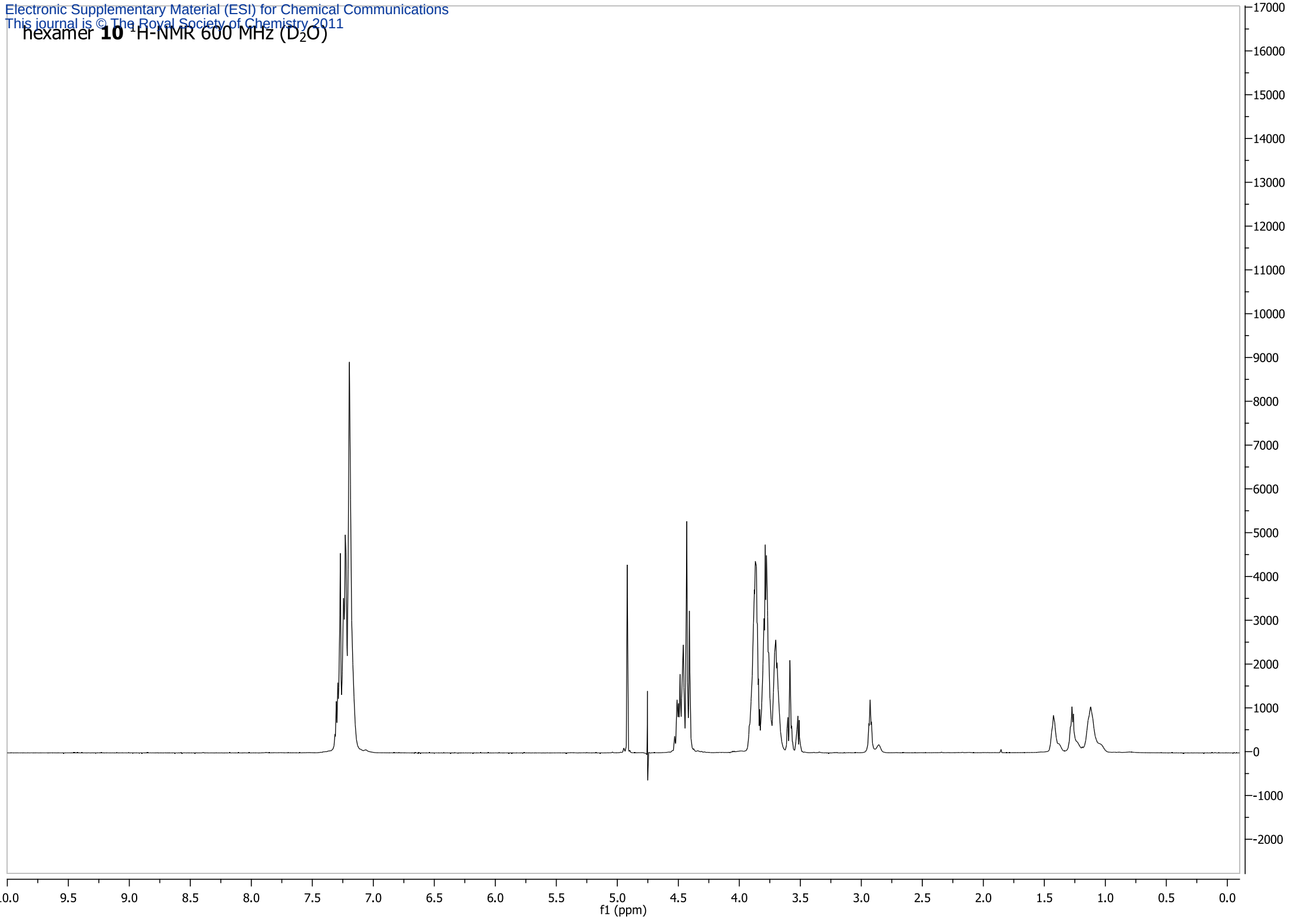
compound **2b** ^{1}H-NMR 400 MHz (CD_3CN)

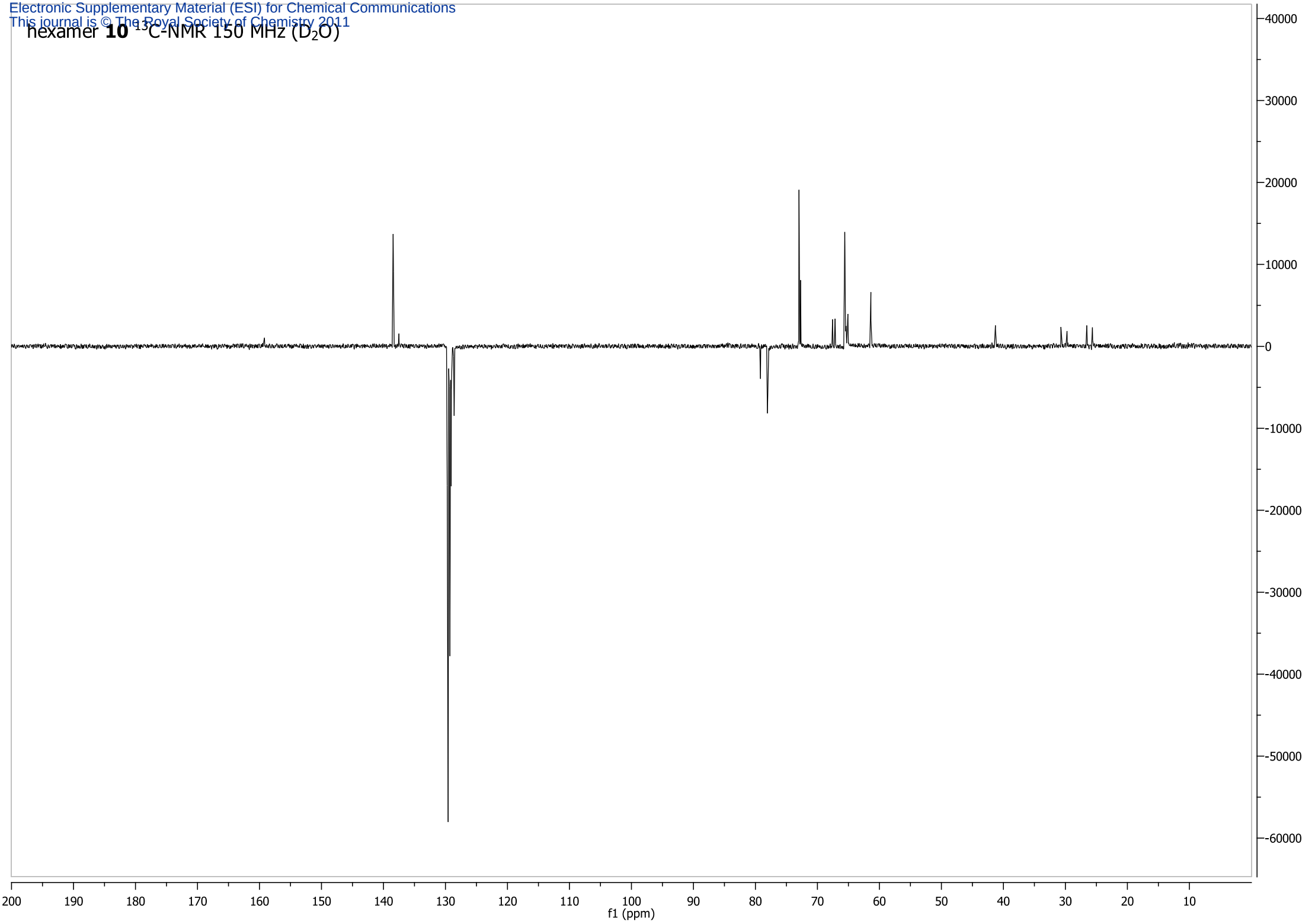


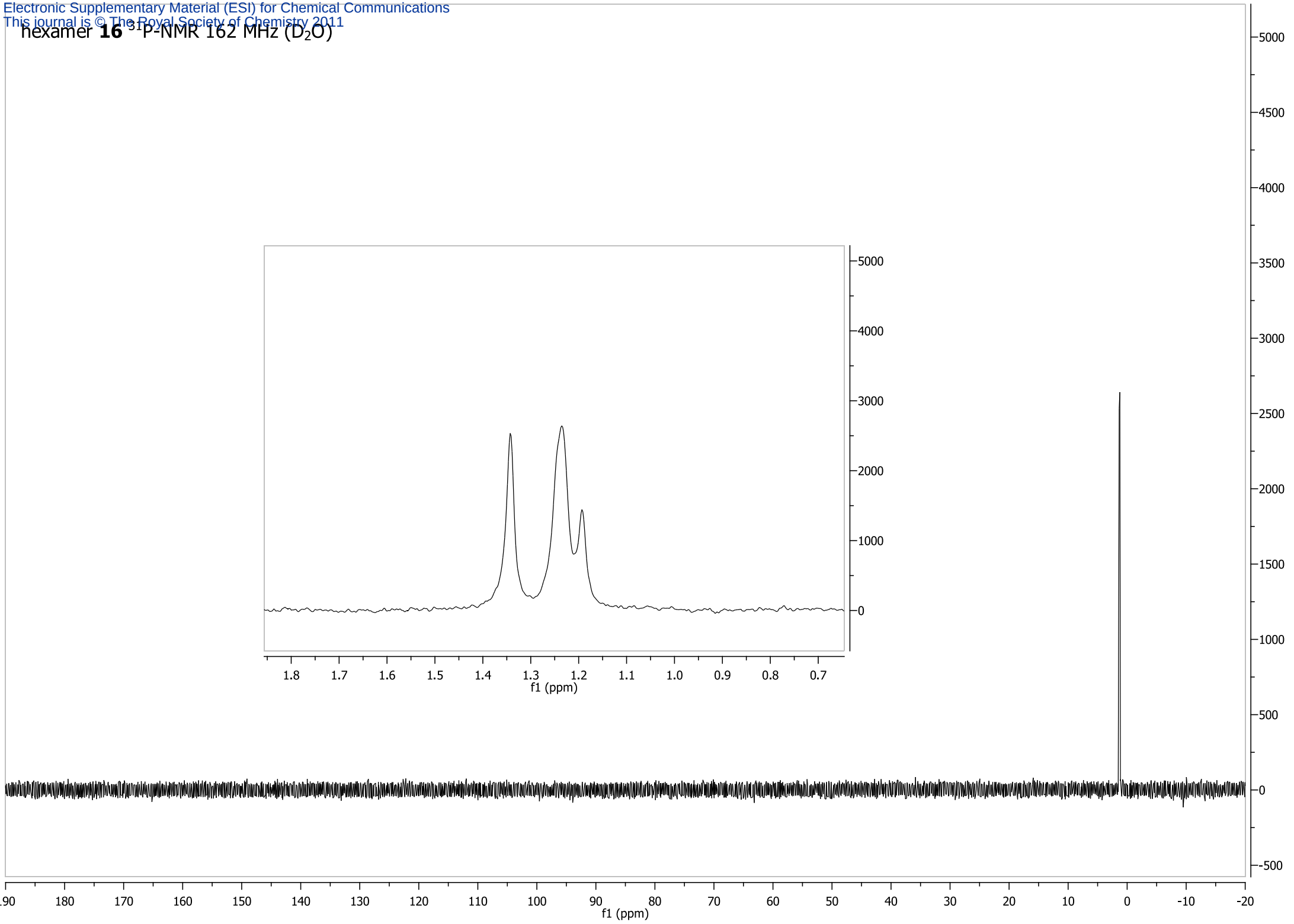


hexamer **10** ^{31}P-NMR 162 MHz (D_2O)

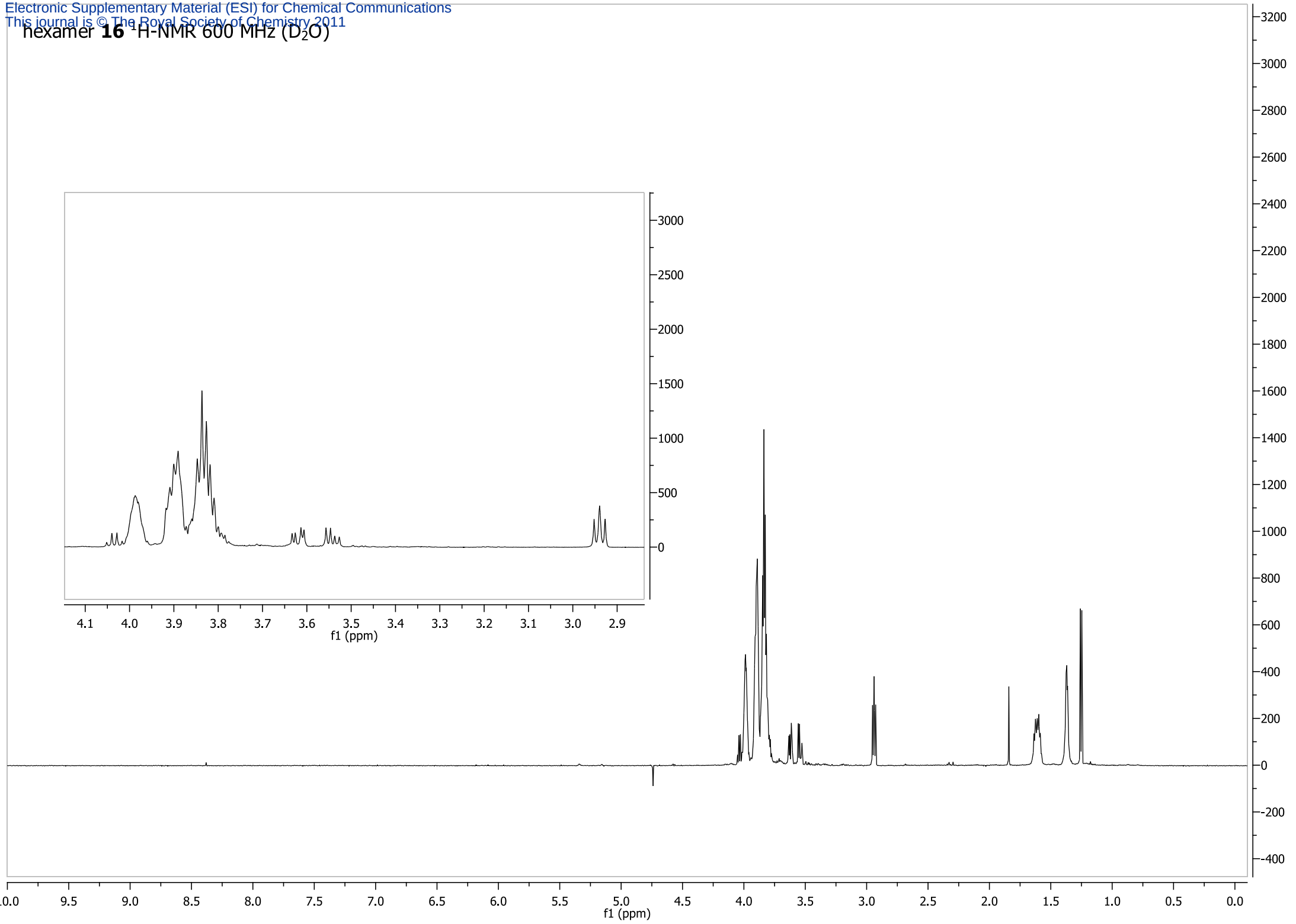


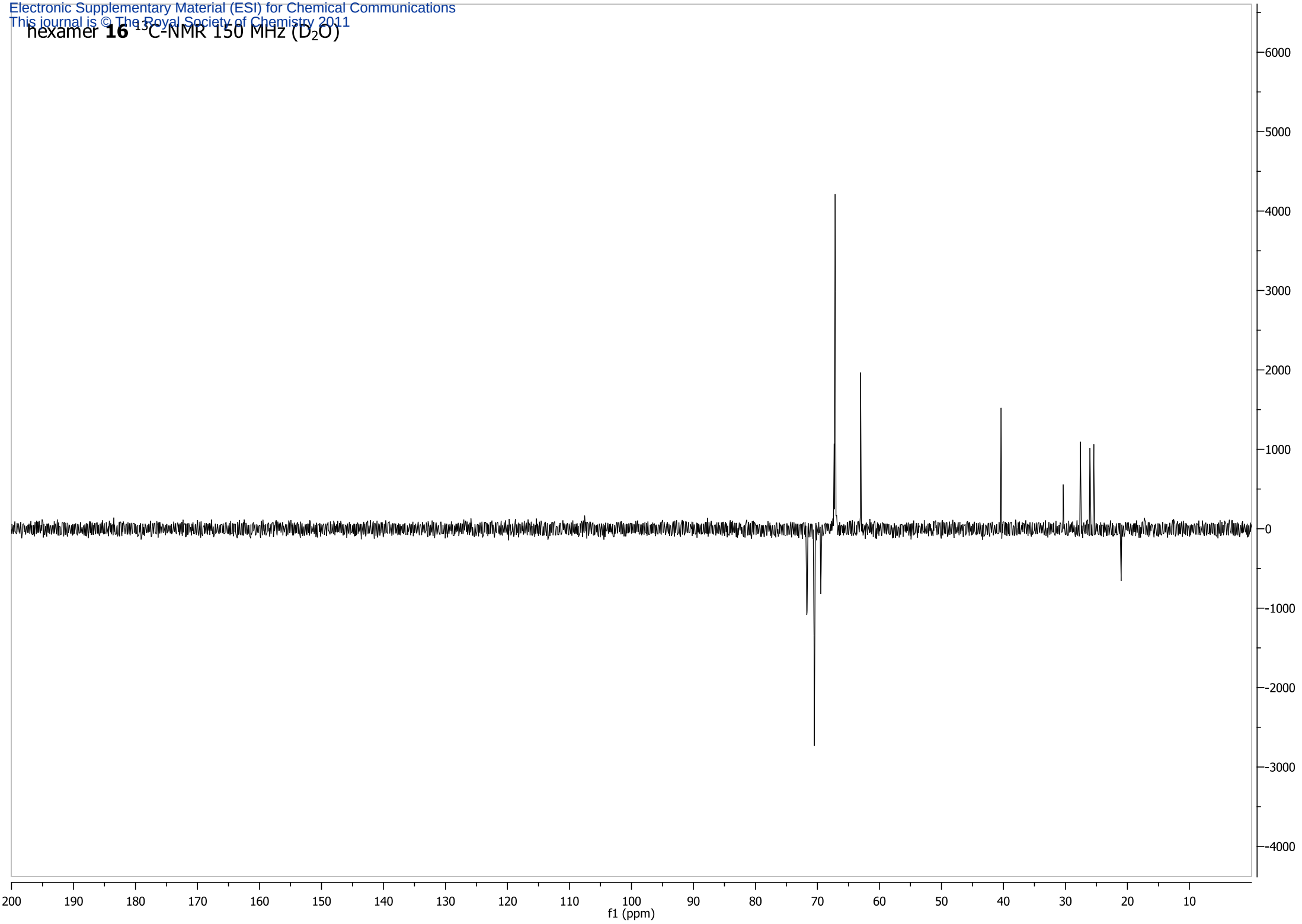




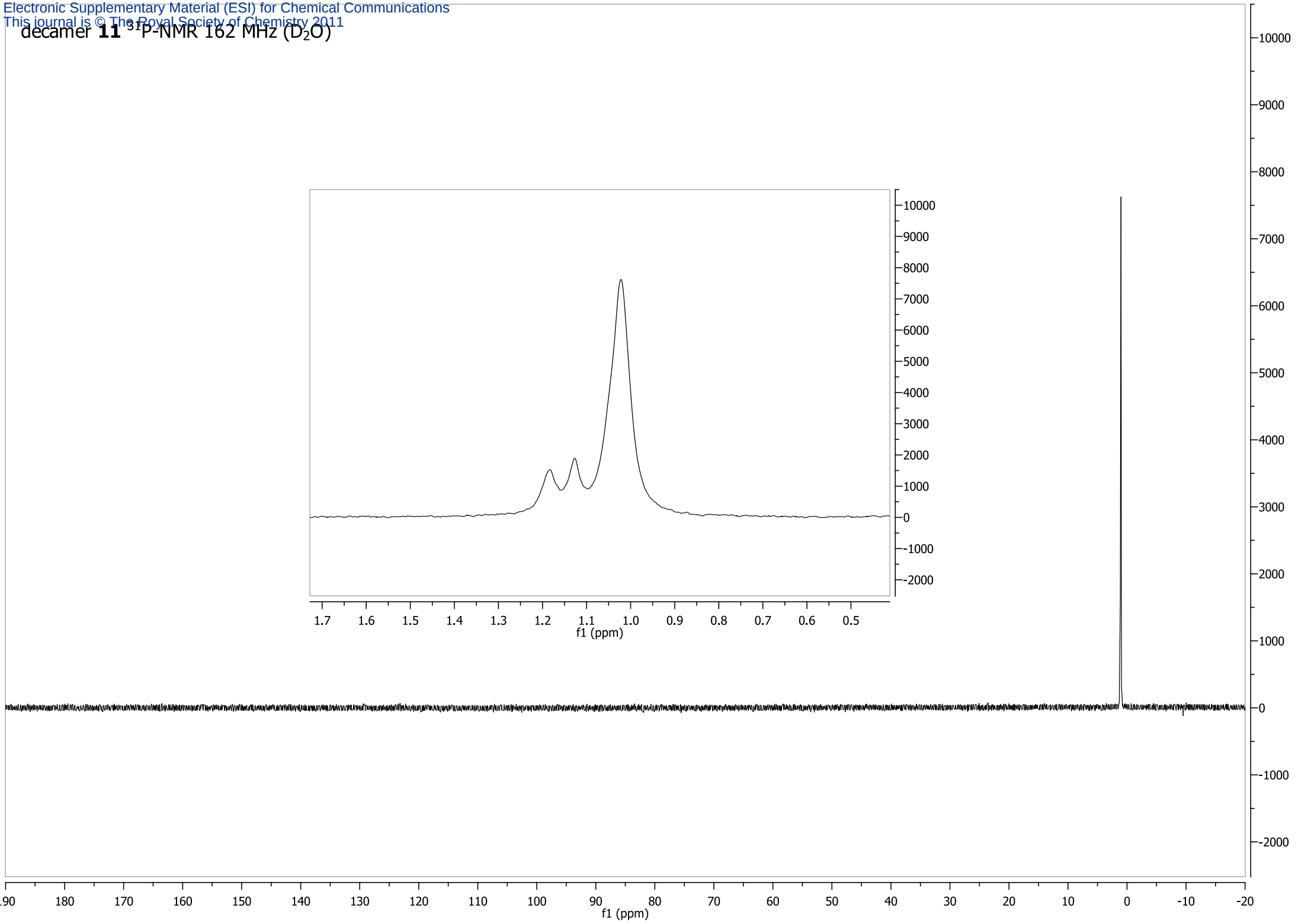


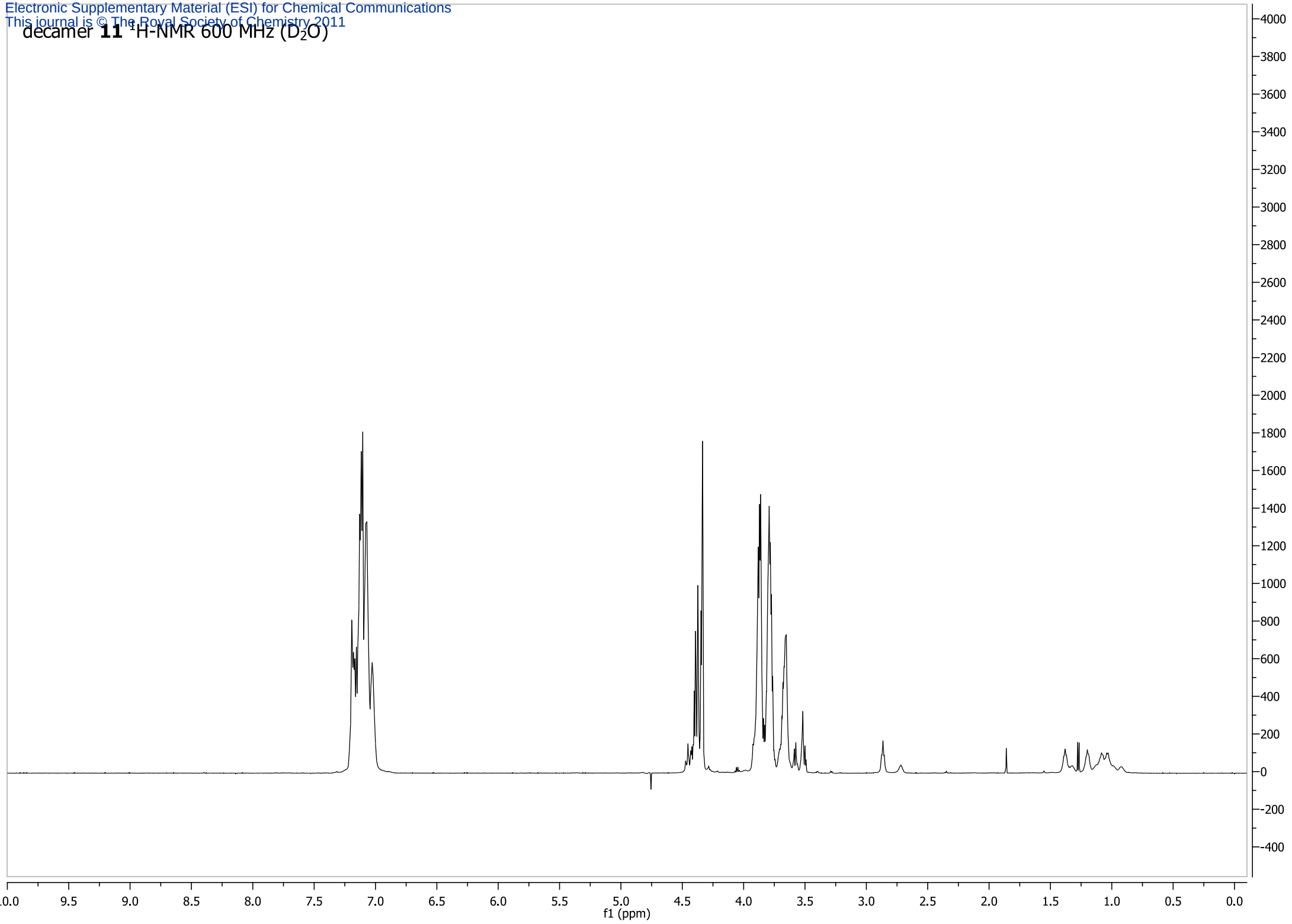
hexamer **16** ^{1}H-NMR 600 MHz (D_2O)

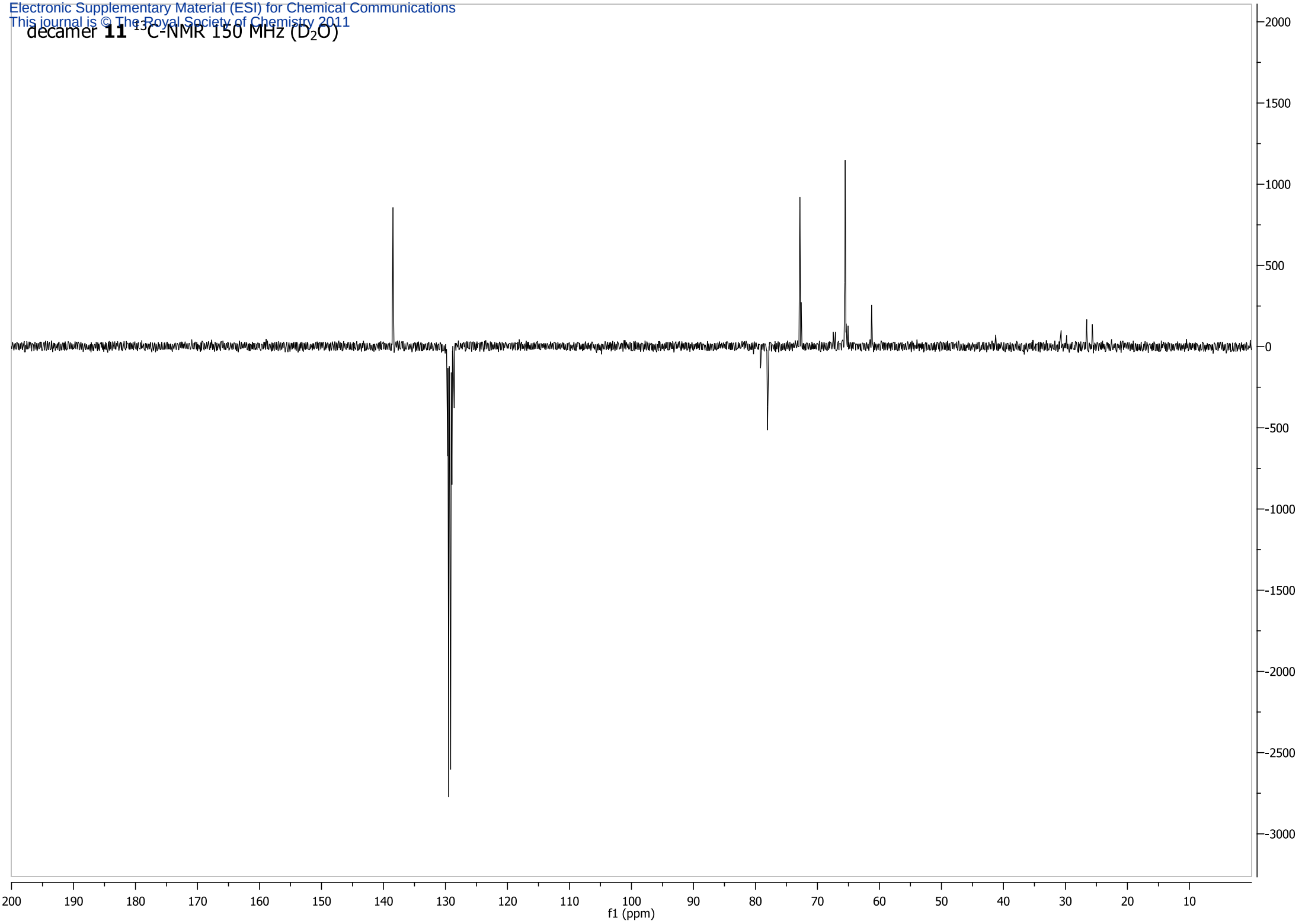




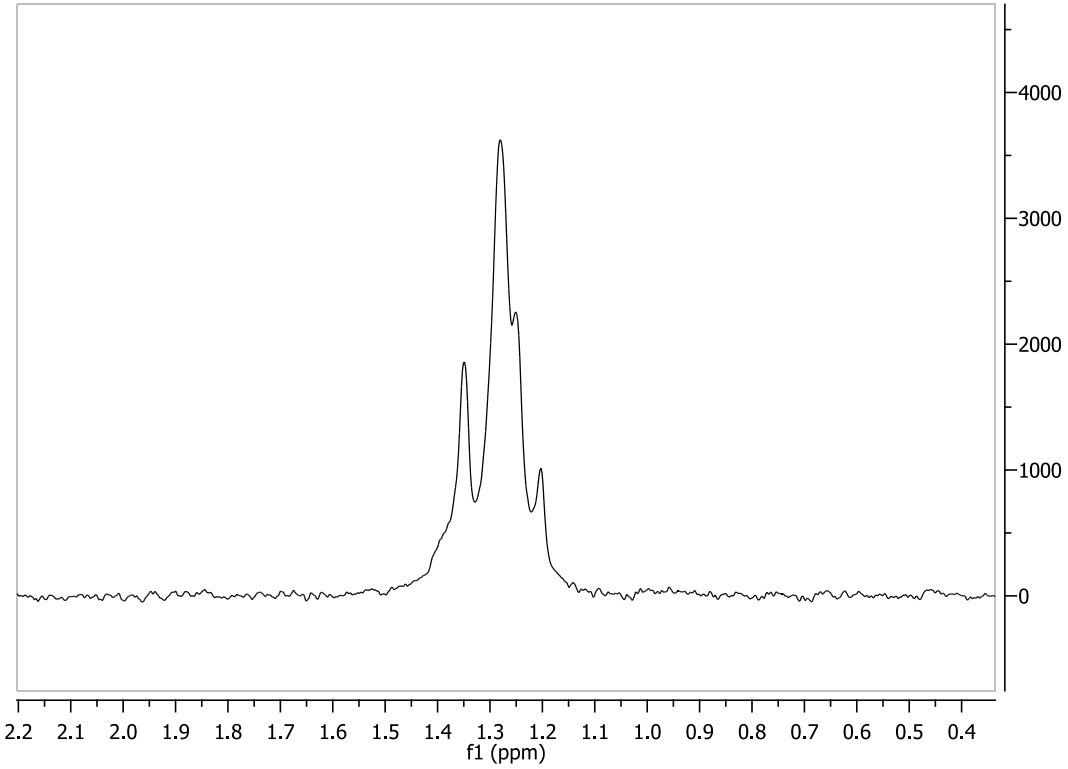
decamer **11** ^{31}P-NMR 162 MHz (D_2O)



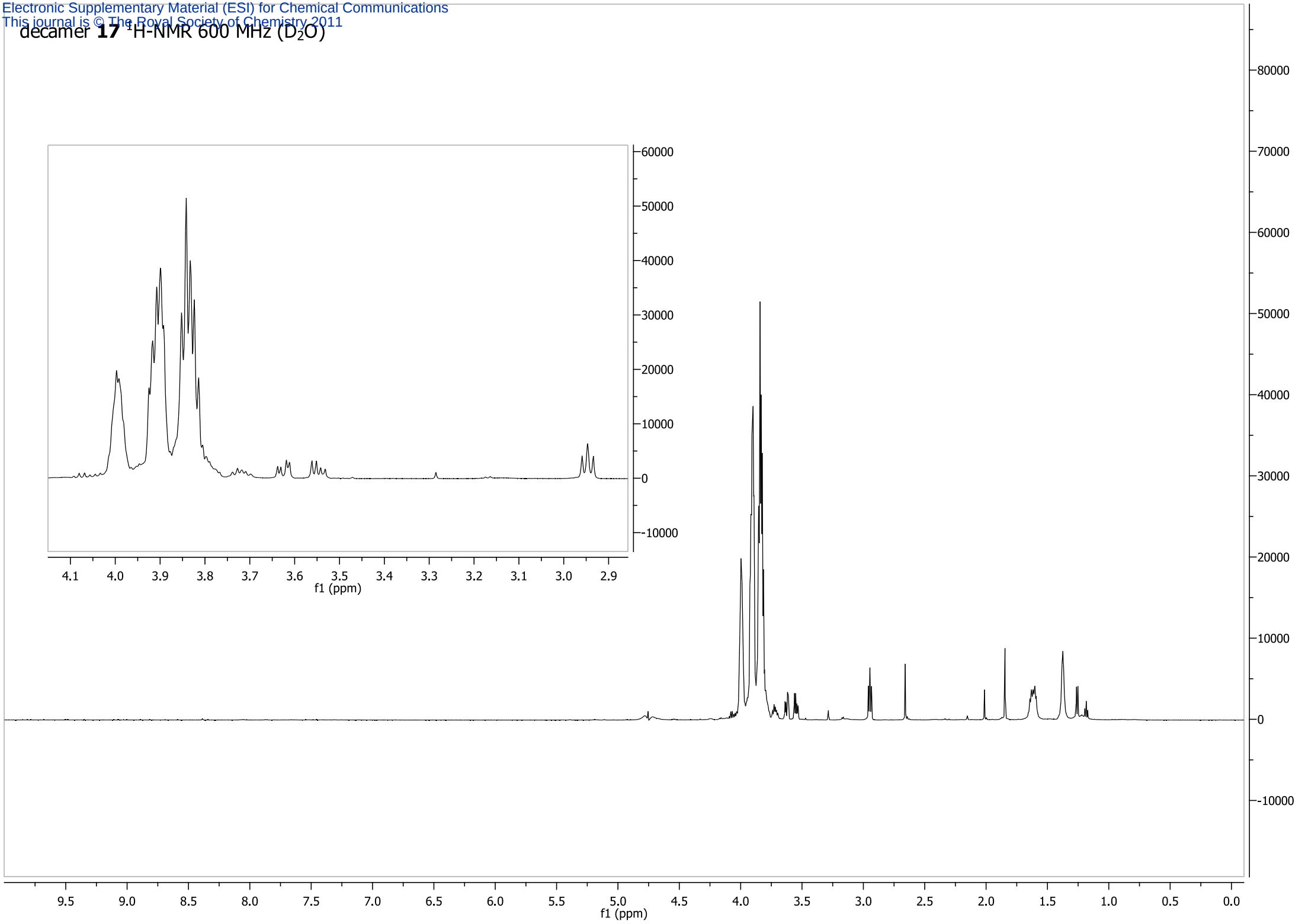


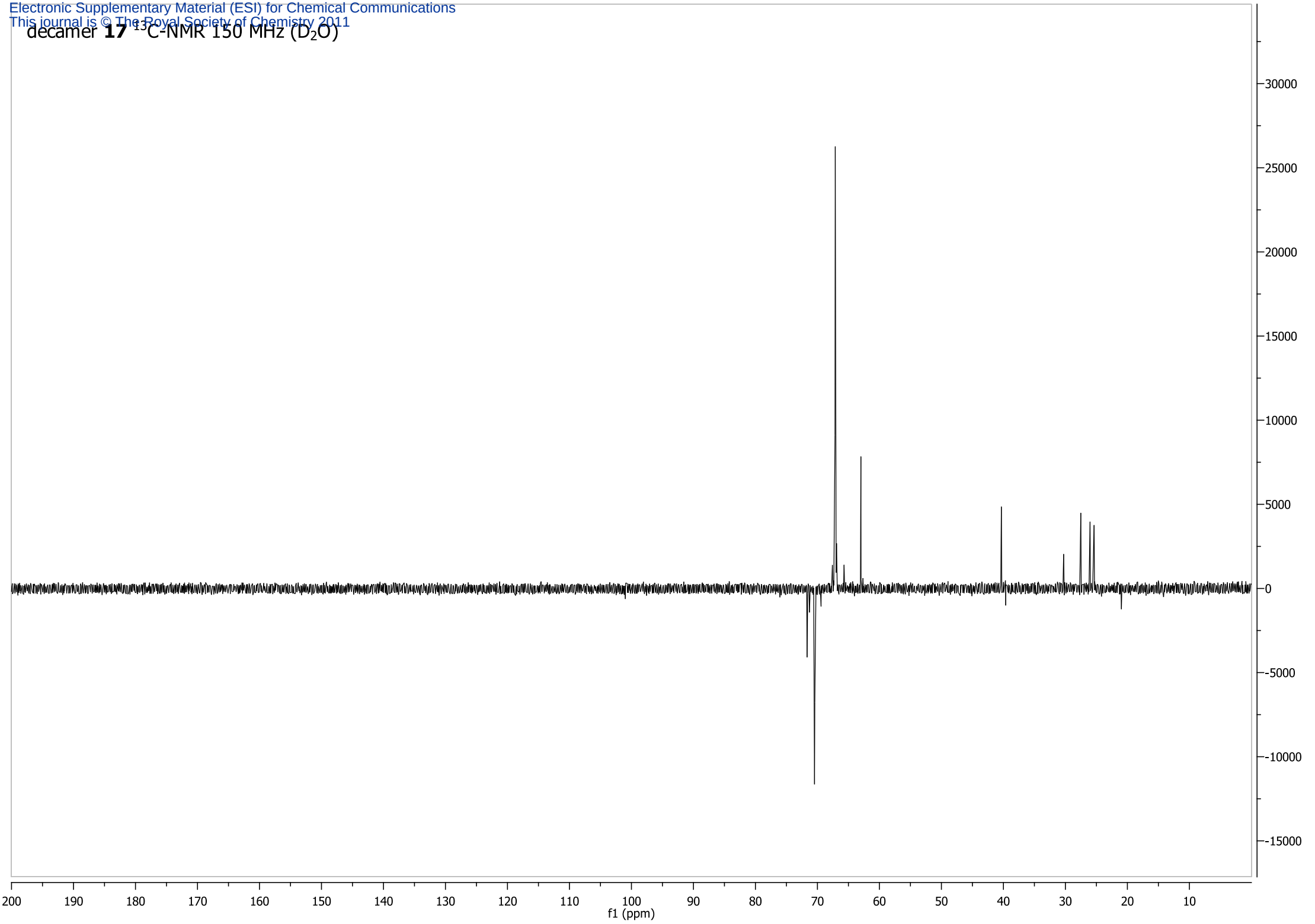


decamer **17** ^{31}P-NMR 162 MHz (D_2O)

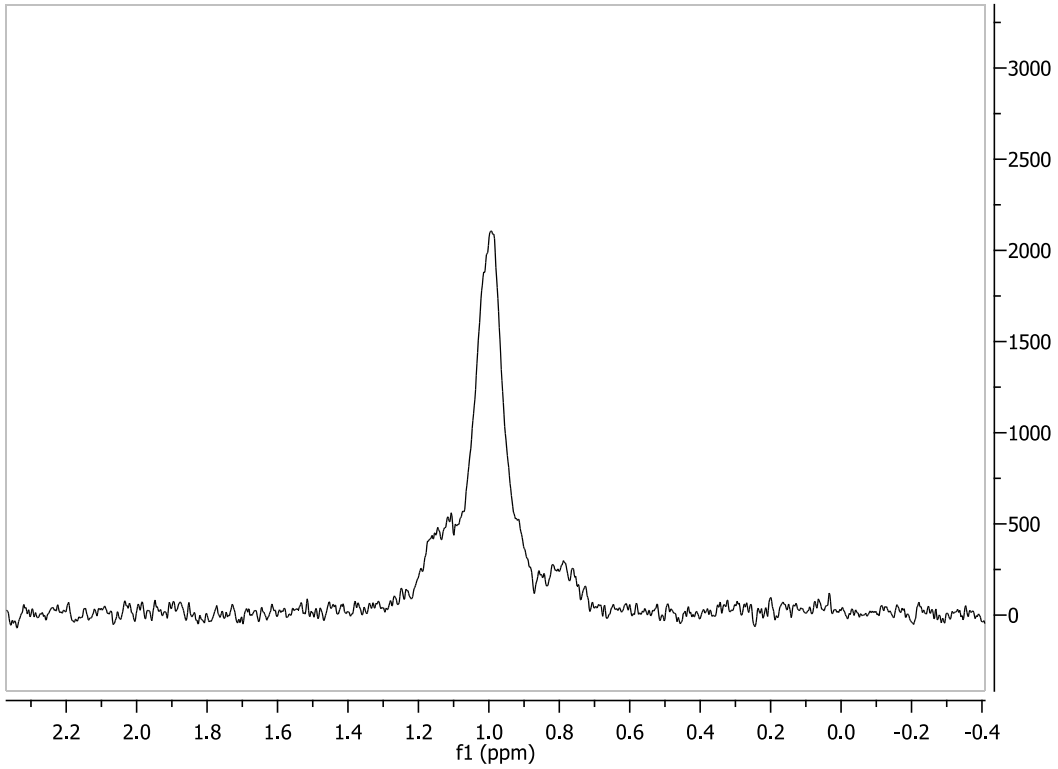


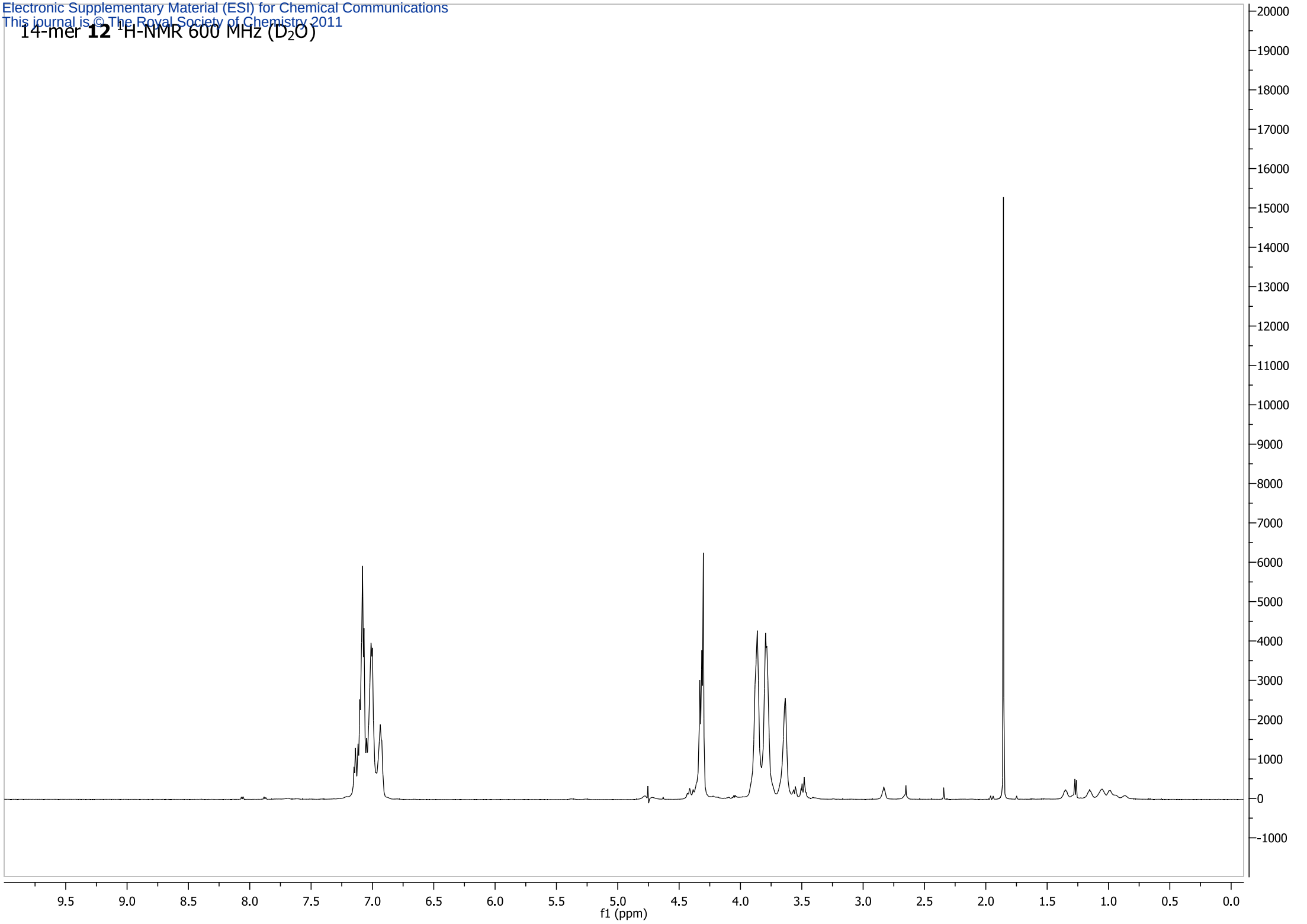
decamer **17** ^{1}H-NMR 600 MHz (D_2O)

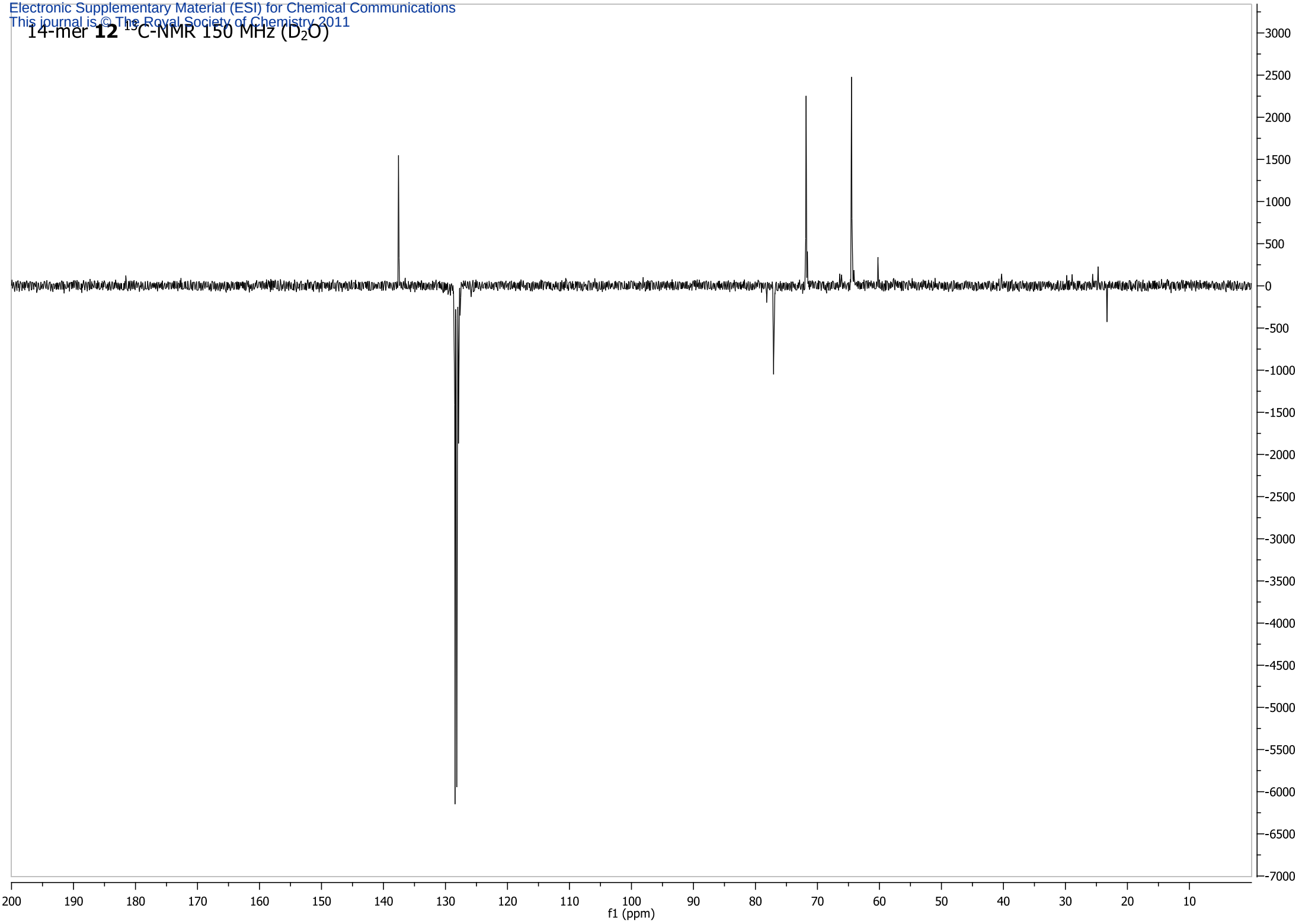




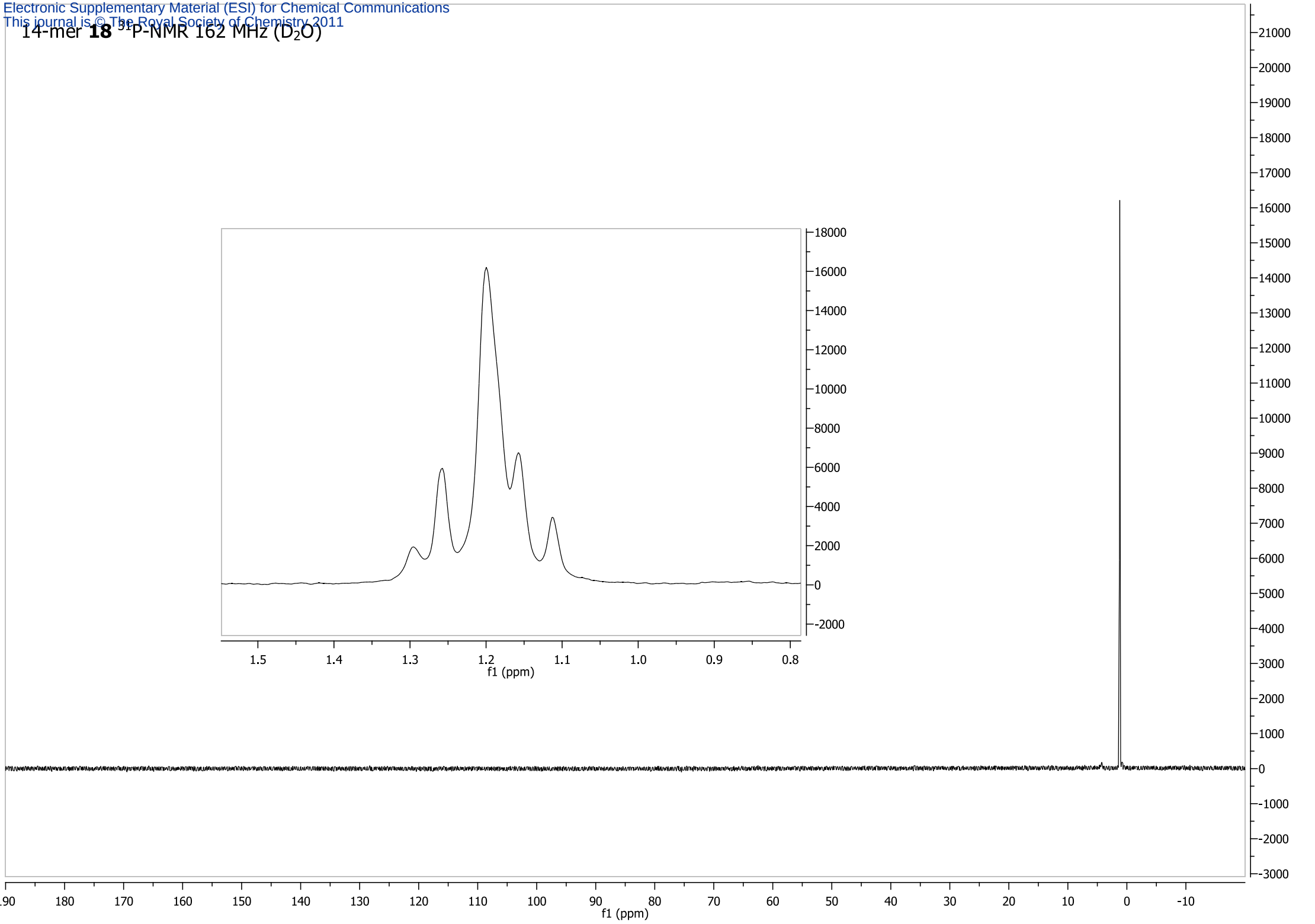
14-mer **12** ^{31}P-NMR 162 MHz (D_2O)



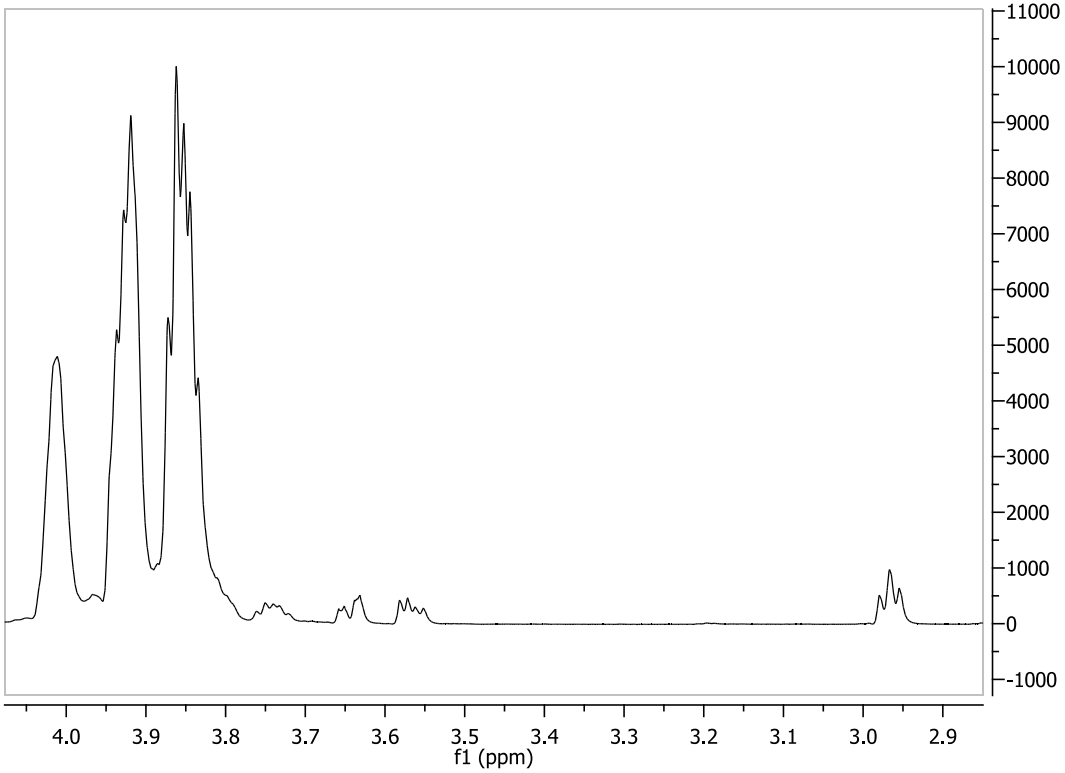


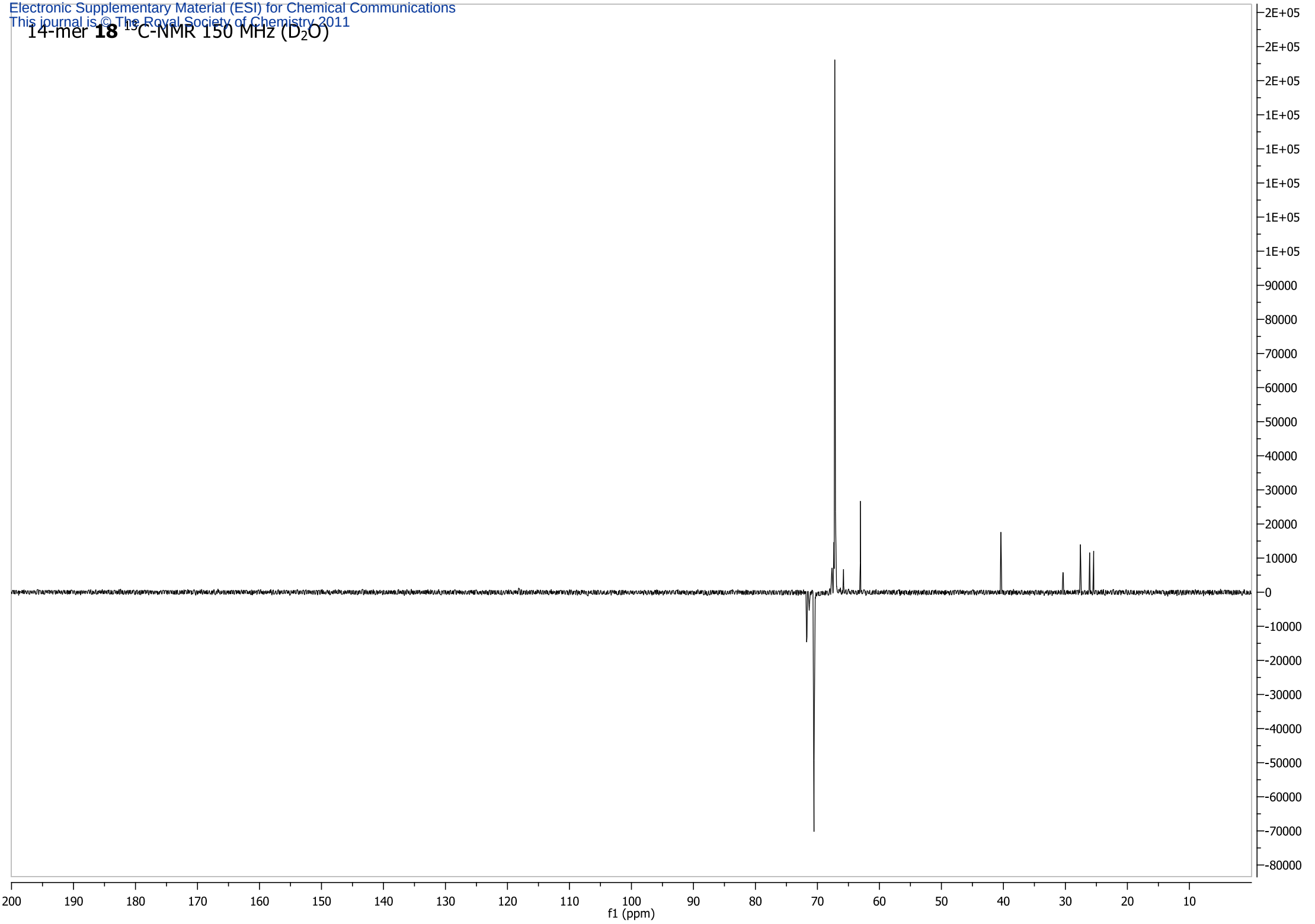


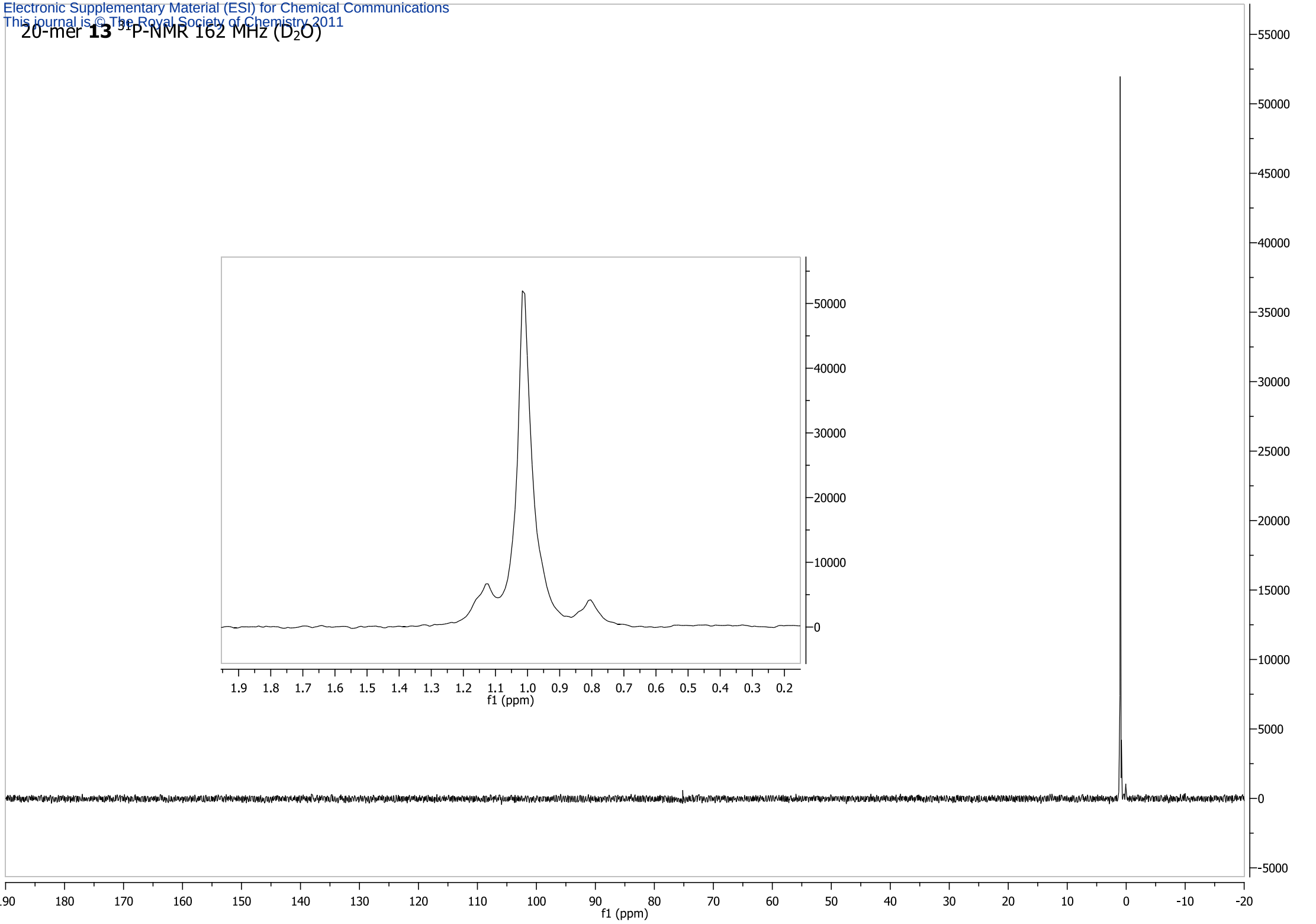
14-mer **18** ^{31}P-NMR 162 MHz (D_2O)

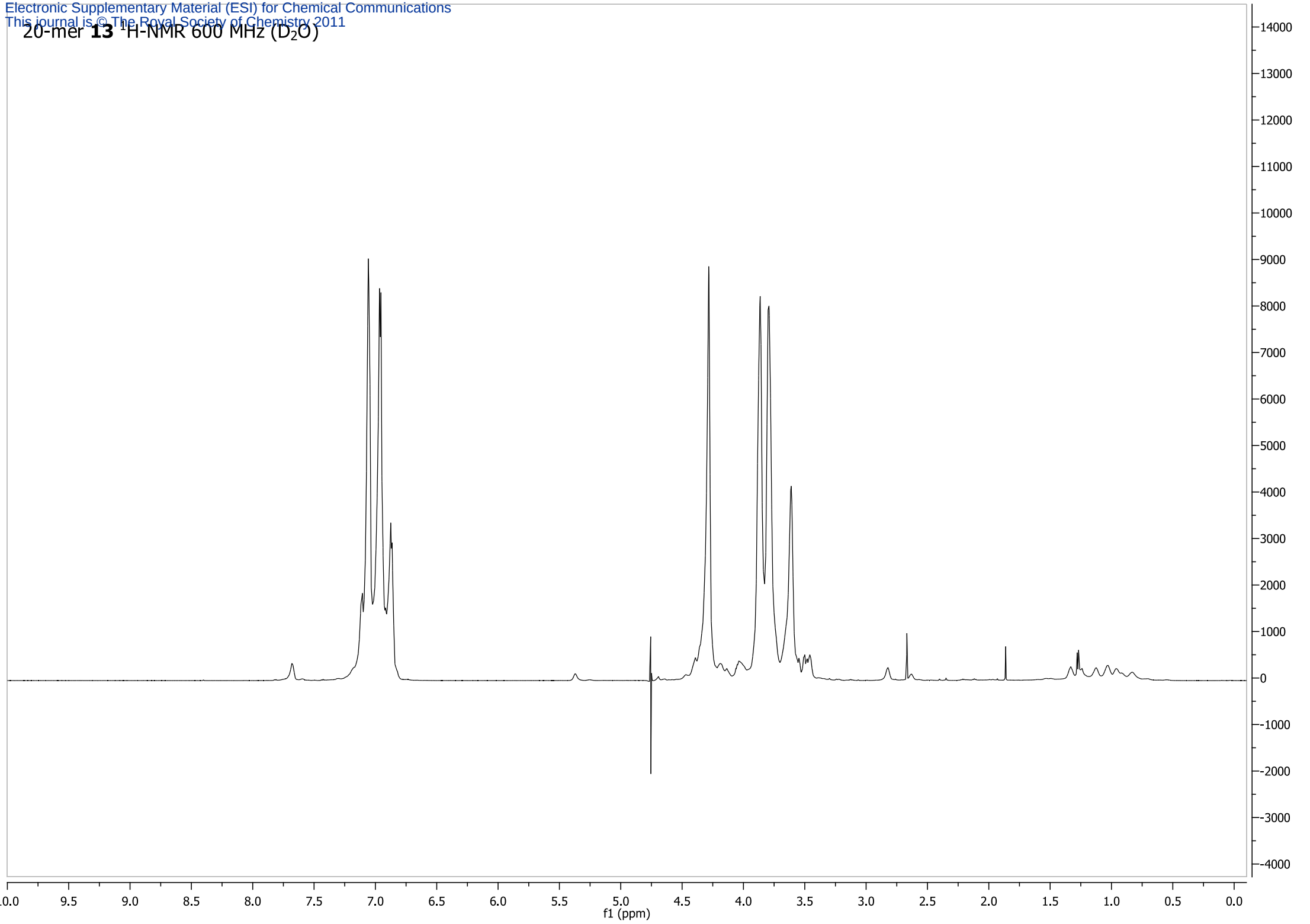


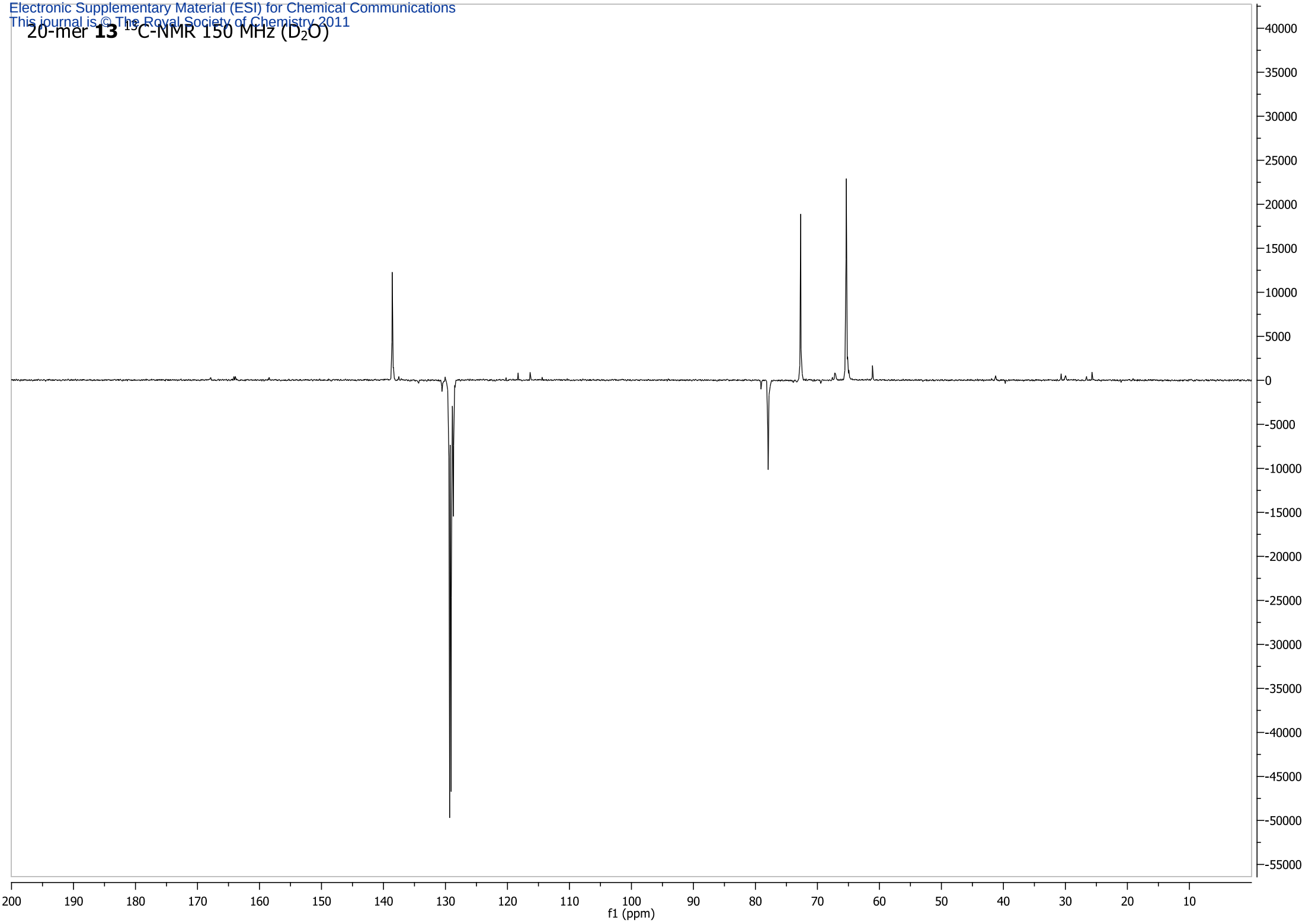
14-mer **18** ^{1}H-NMR 600 MHz (D_2O)



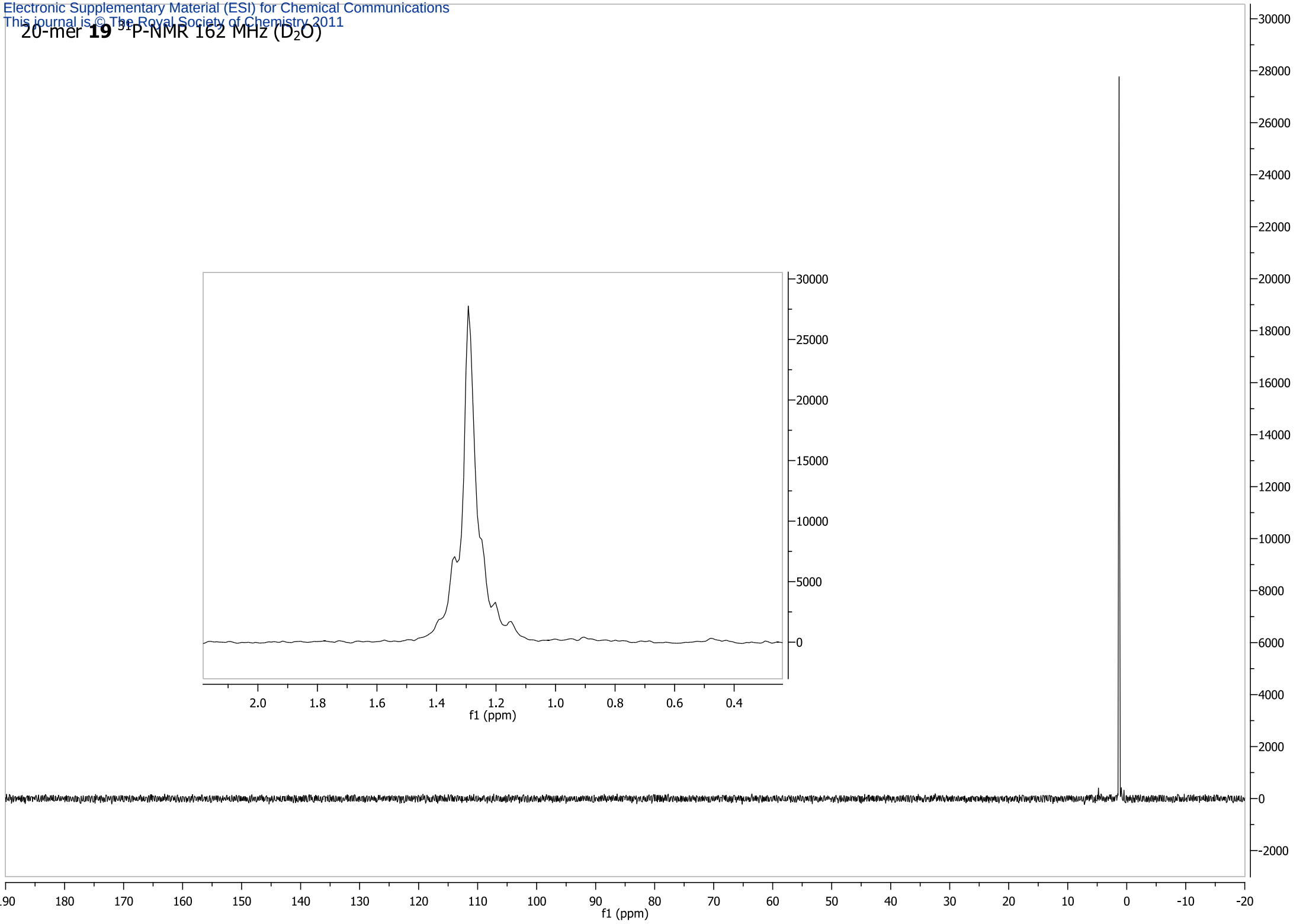




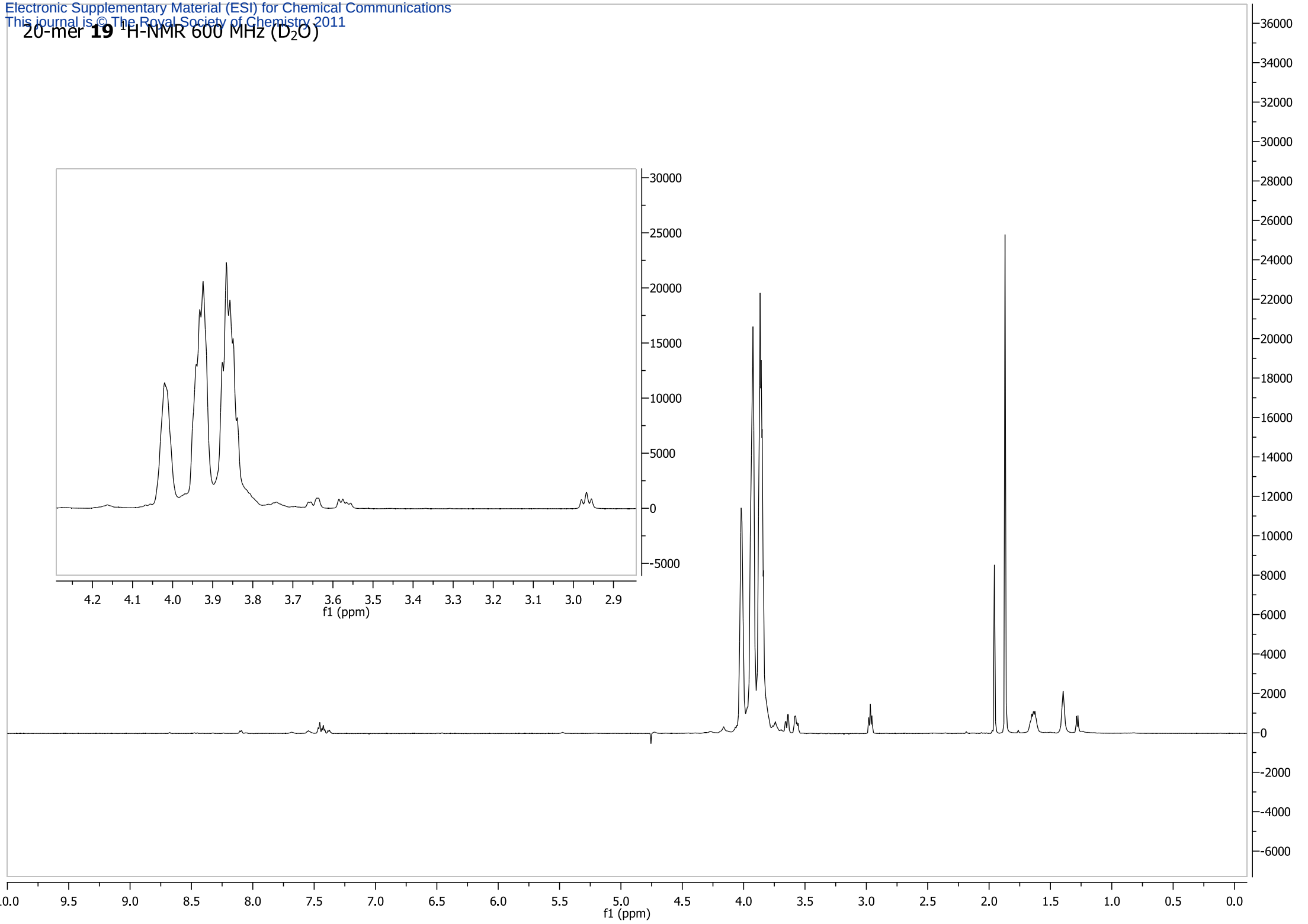


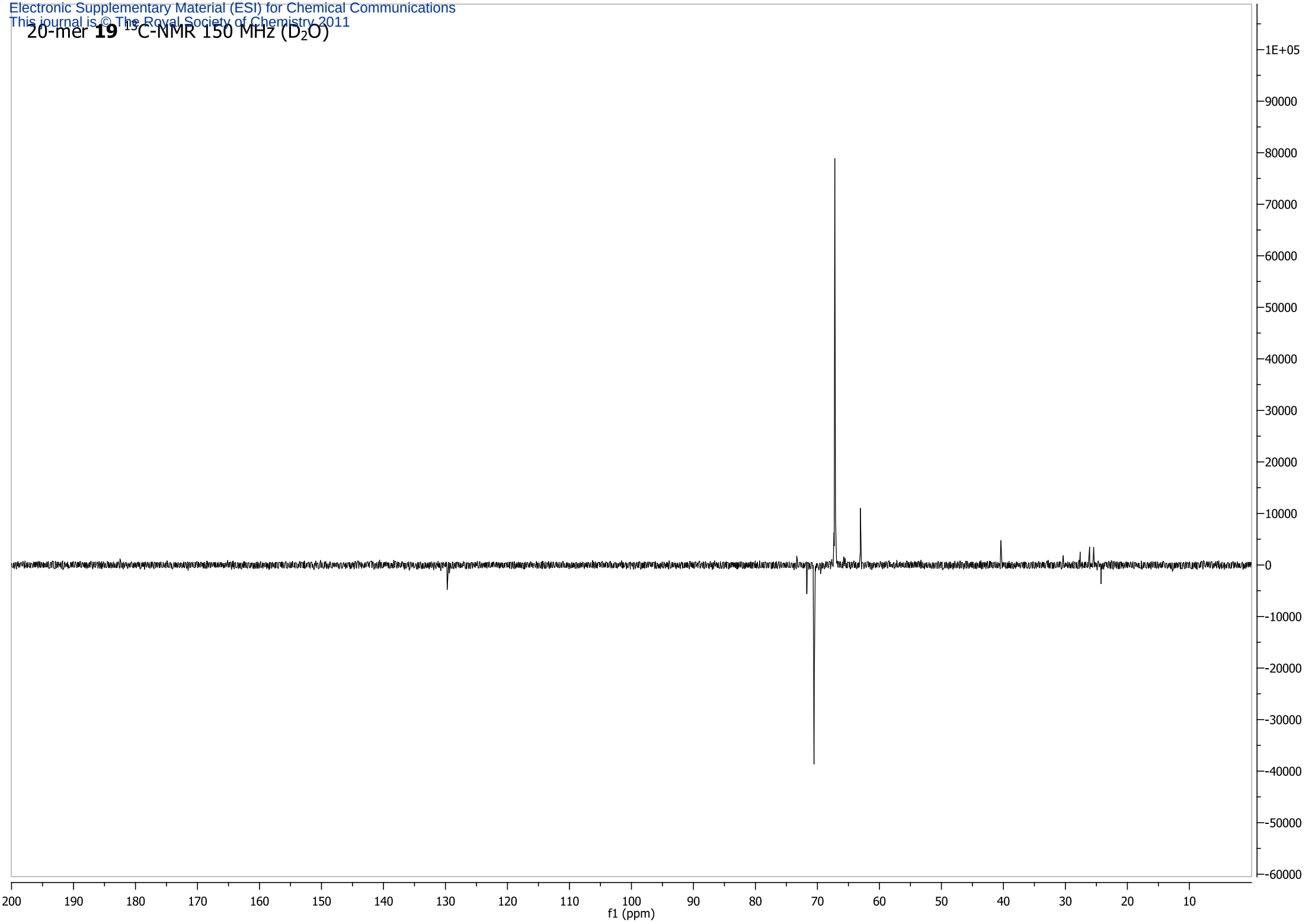


20-mer **19** ^{31}P-NMR 162 MHz (D_2O)

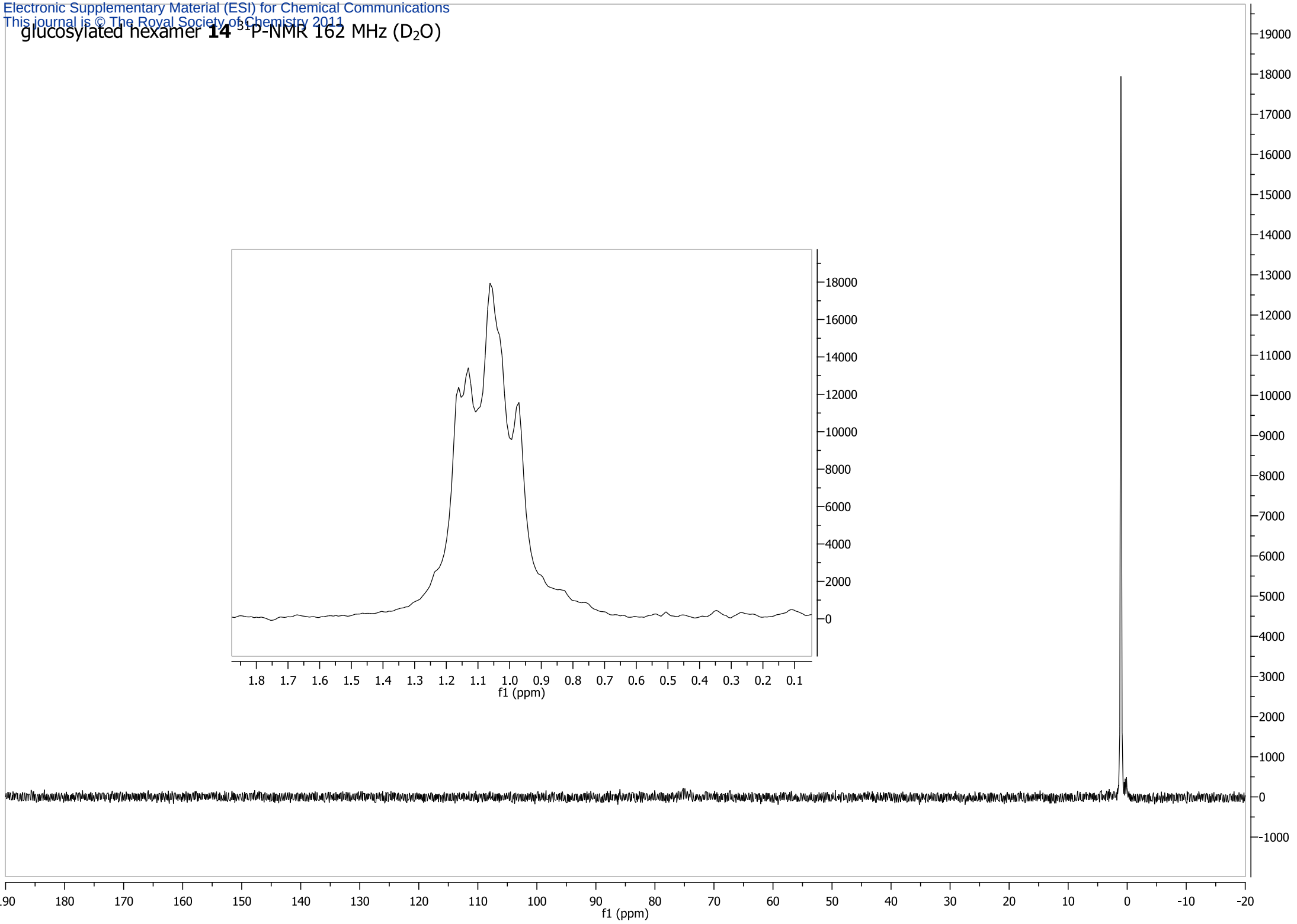


20-mer **19** ^{1}H-NMR 600 MHz (D_2O)

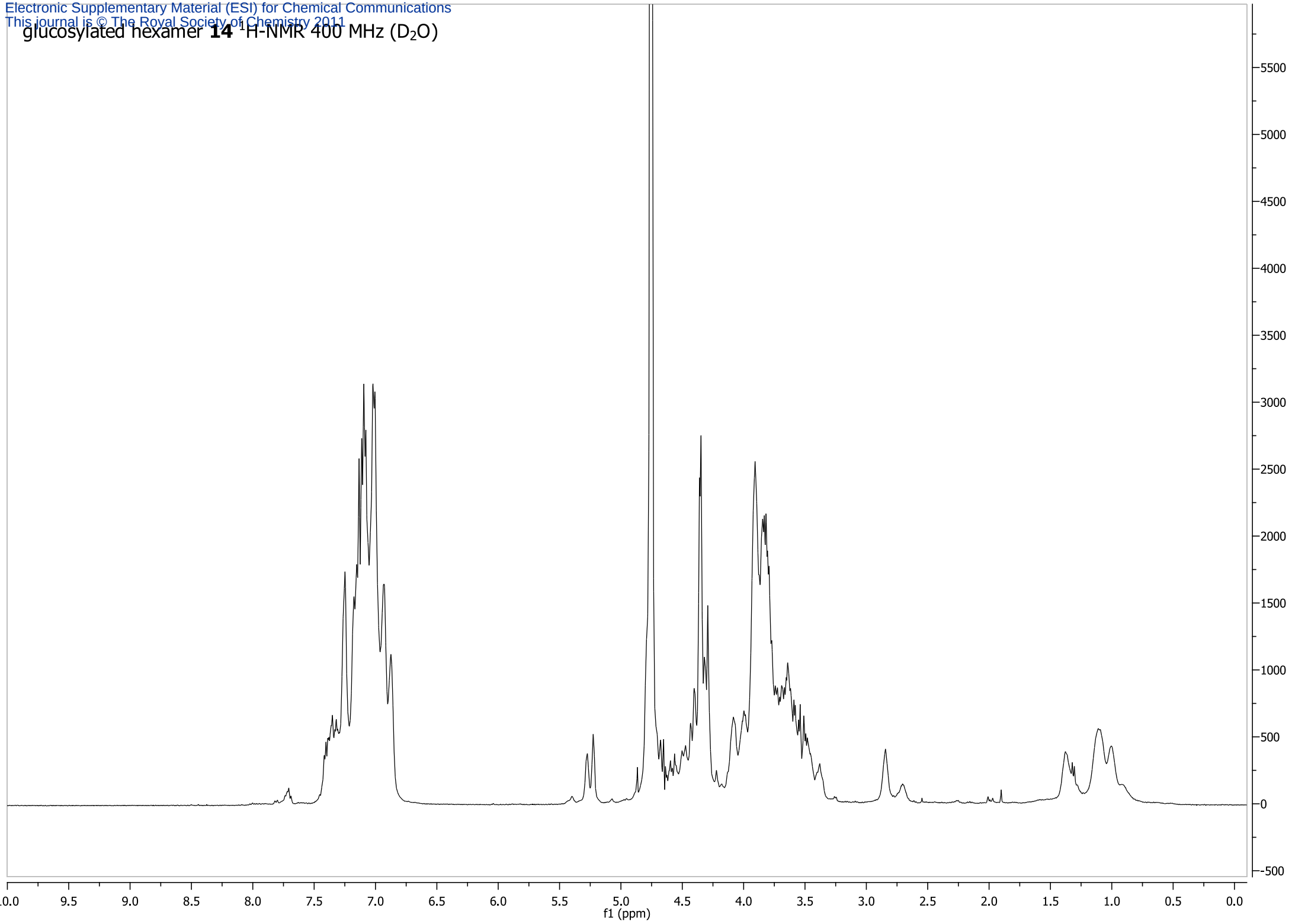




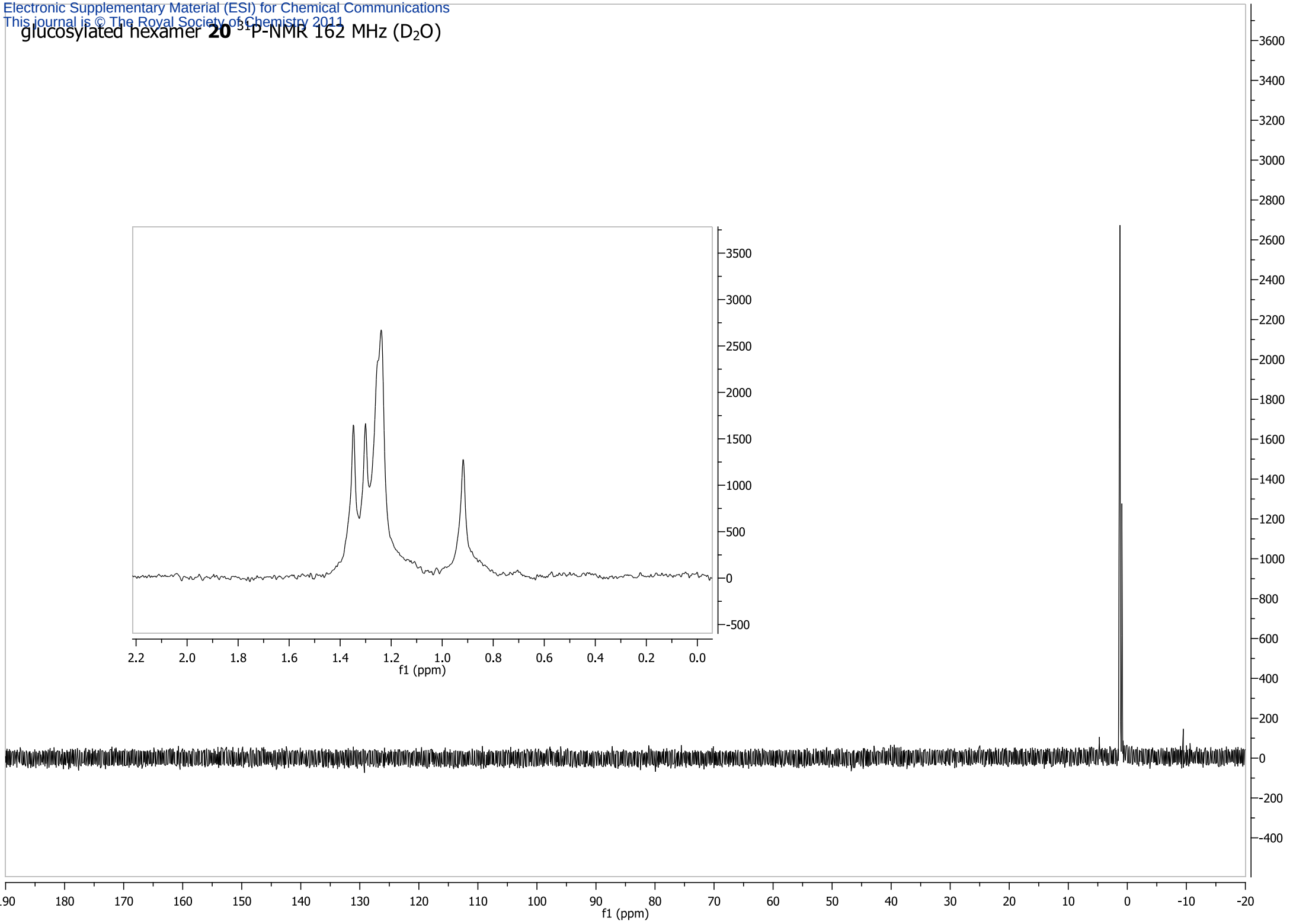
glucosylated hexamer **14** ^{31}P-NMR 162 MHz (D_2O)



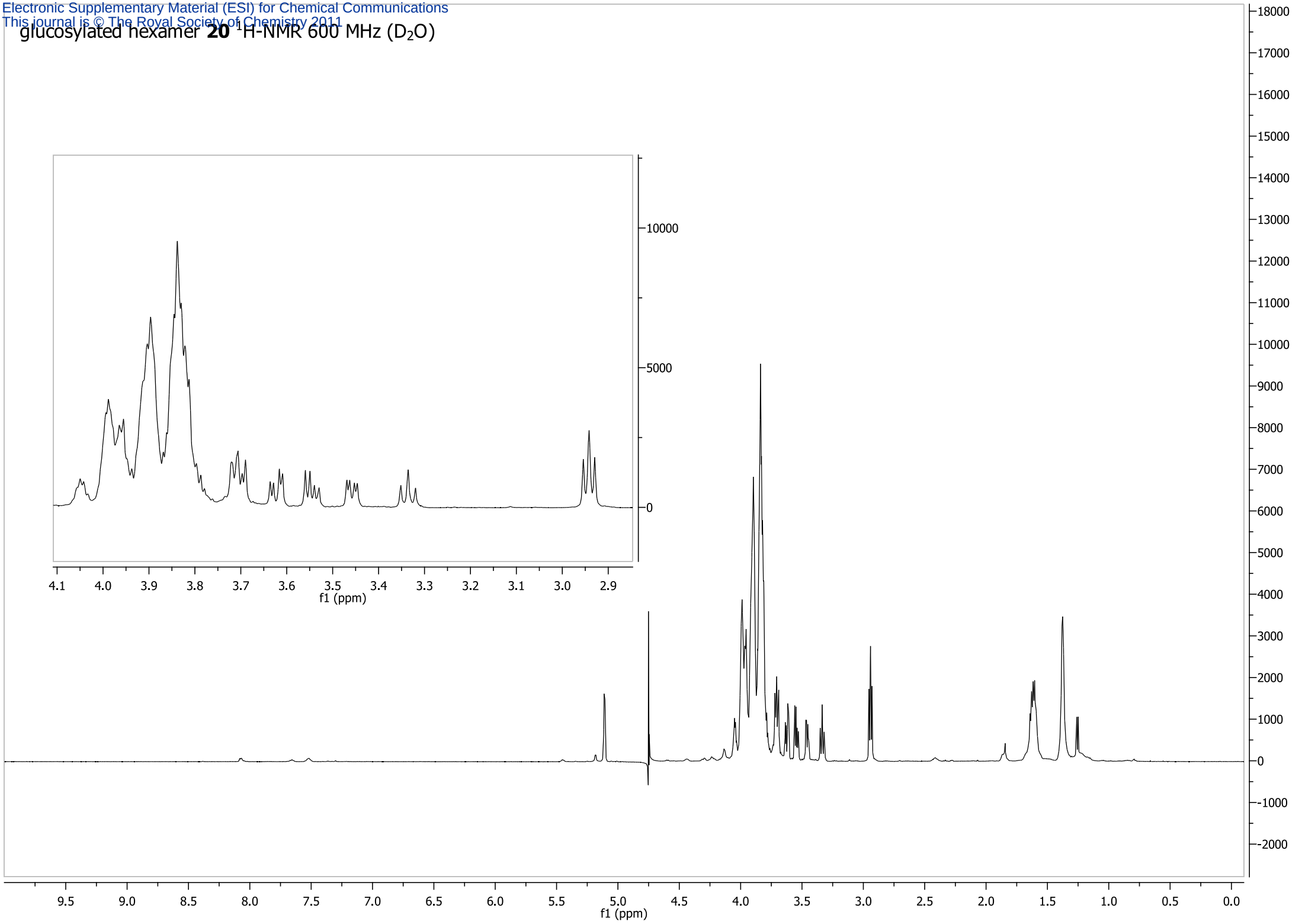
glucosylated hexamer **14** ^{1}H-NMR 400 MHz (D_2O)



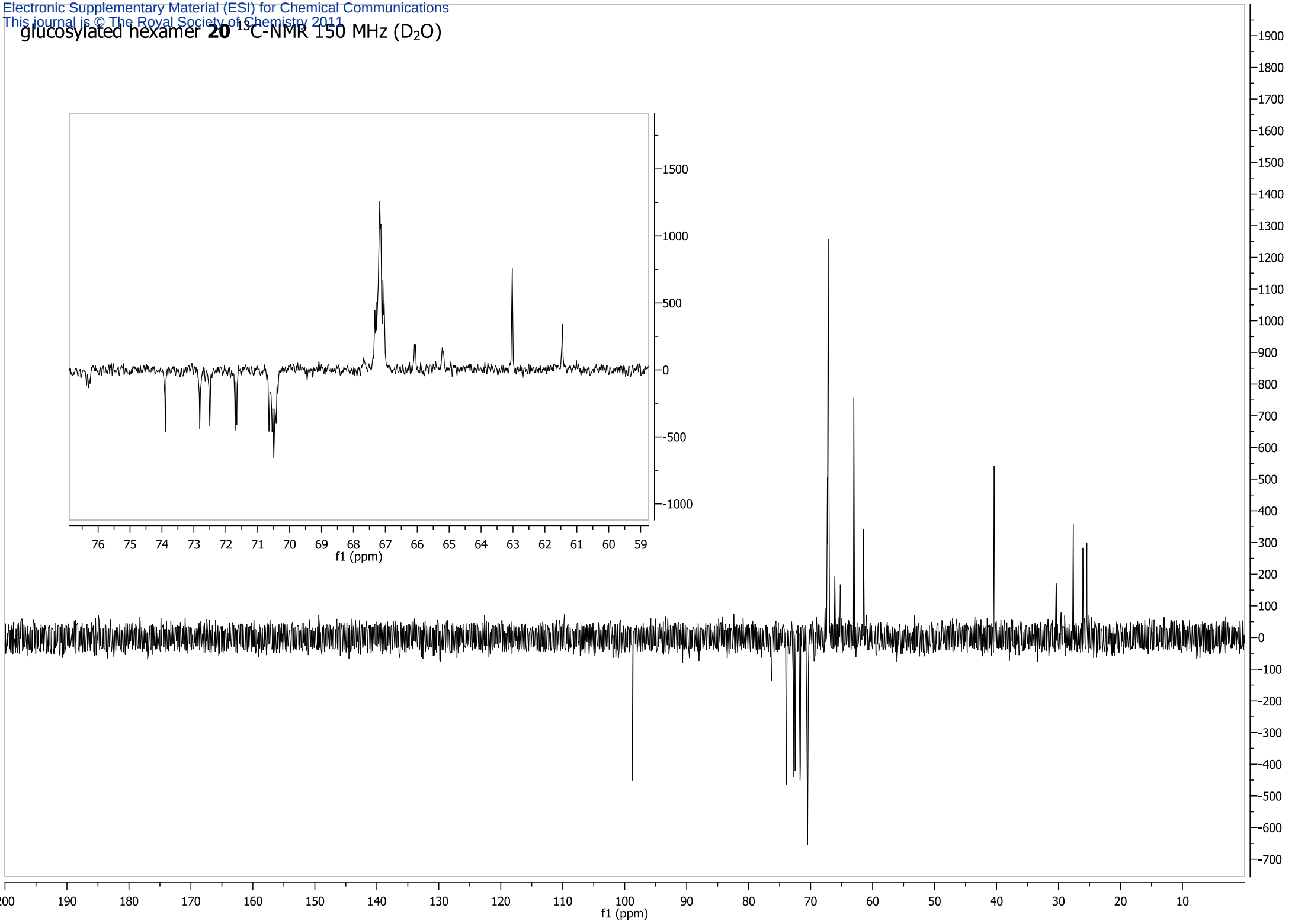
glucosylated hexamer **20** ^{31}P-NMR 162 MHz (D_2O)



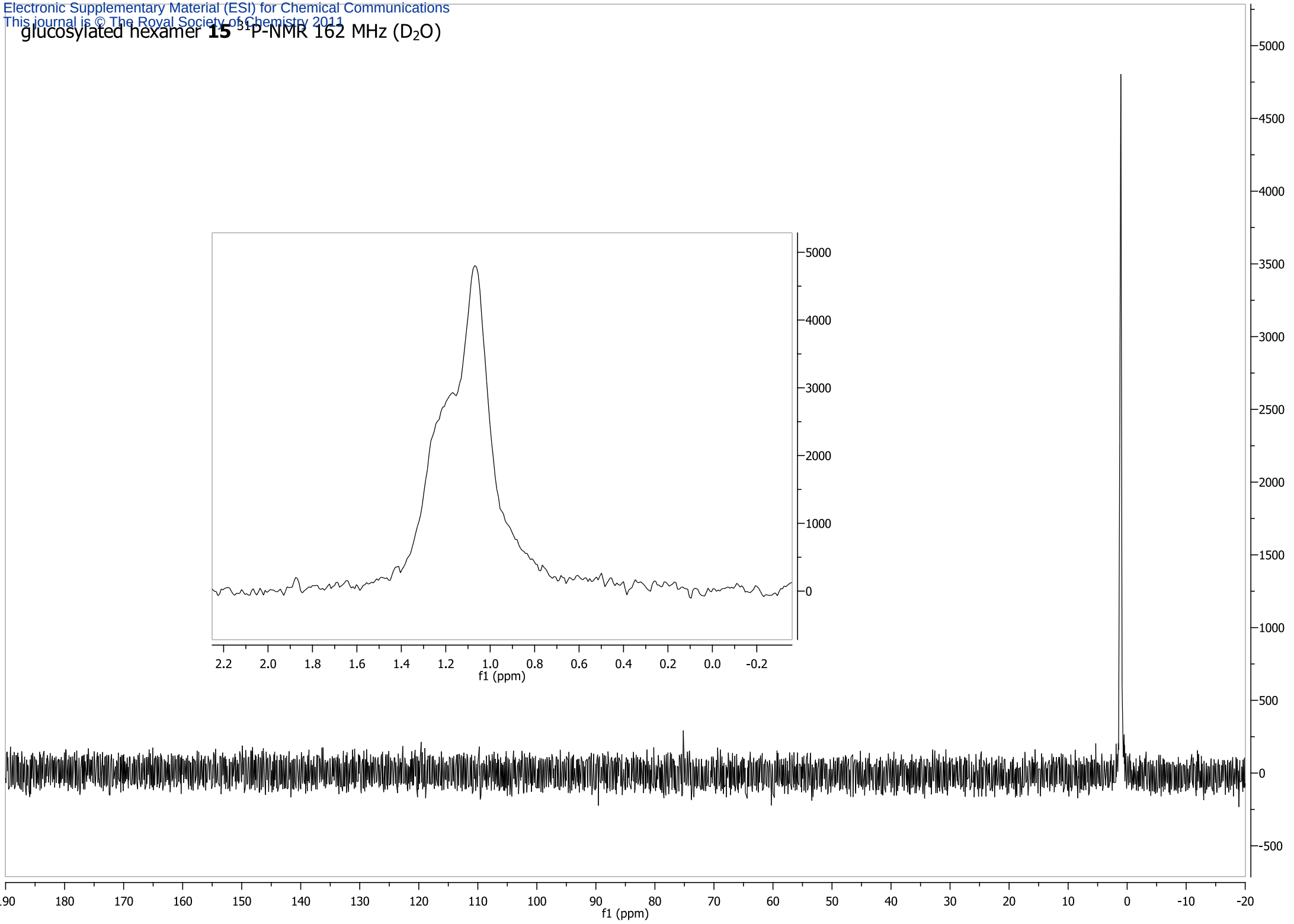
glucosylated hexamer **20** ^{1}H-NMR 600 MHz (D_2O)

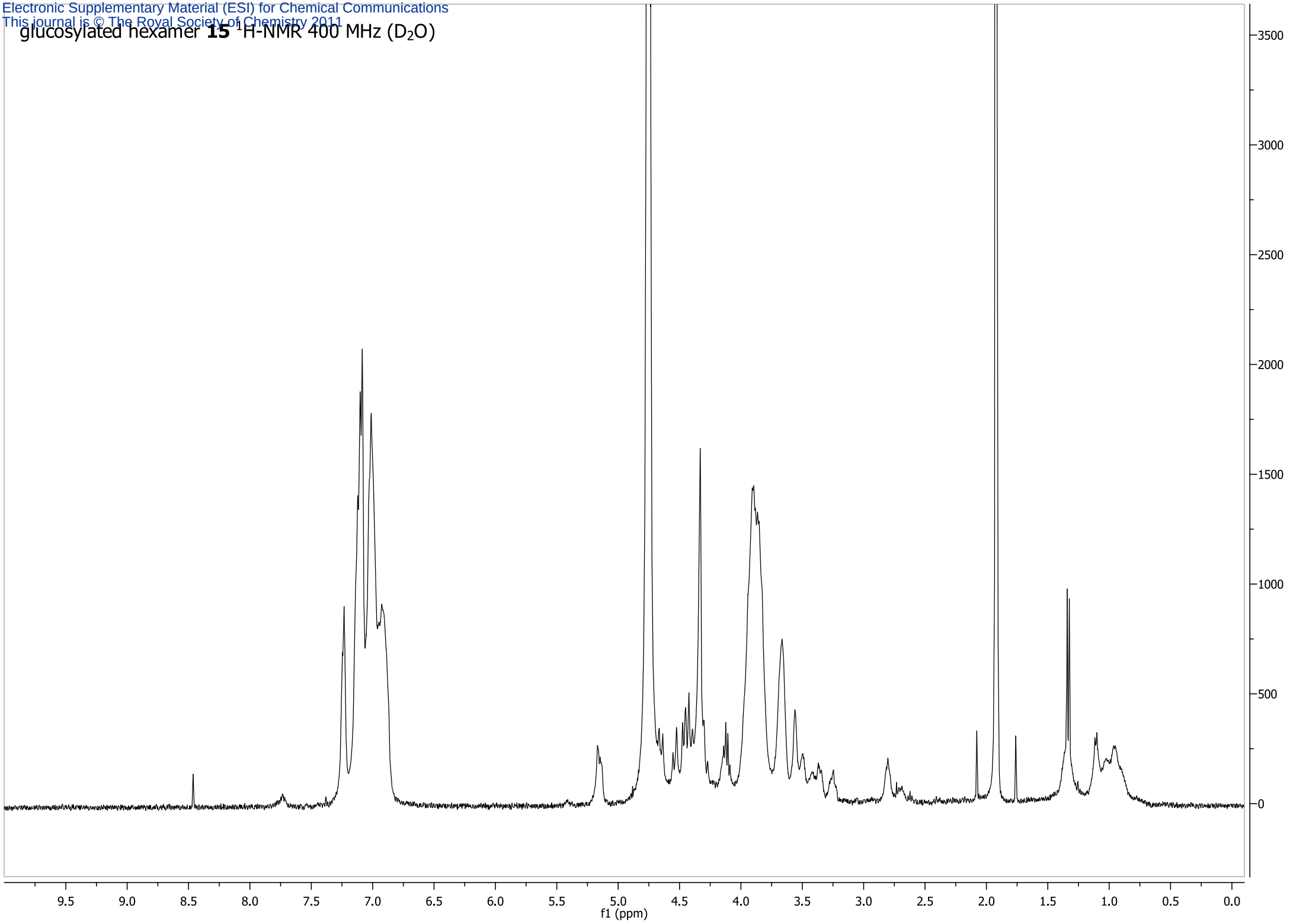


glucosylated hexamer **20** ^{13}C-NMR 150 MHz (D_2O)

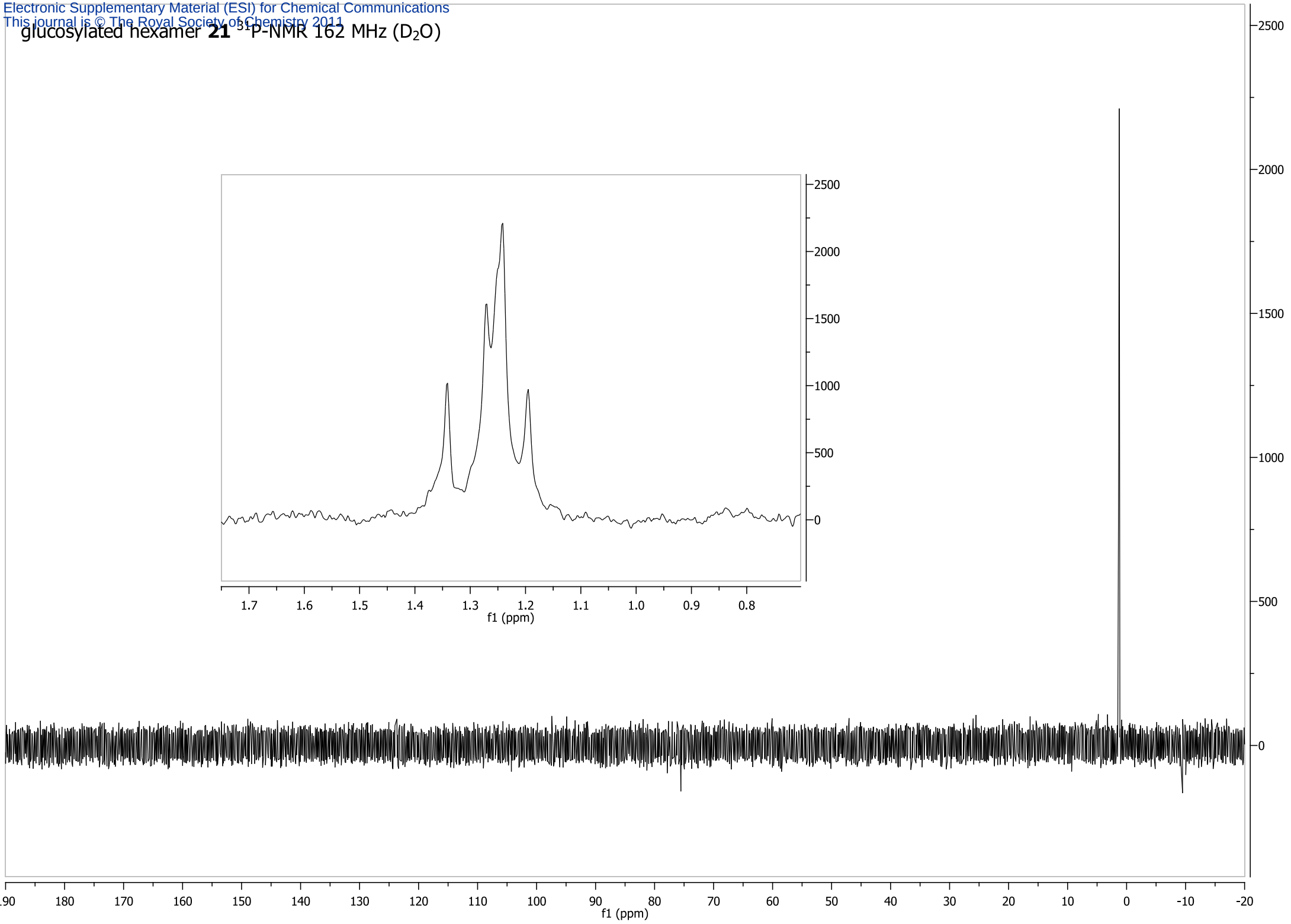


glucosylated hexamer **15** ^{31}P-NMR 162 MHz (D_2O)

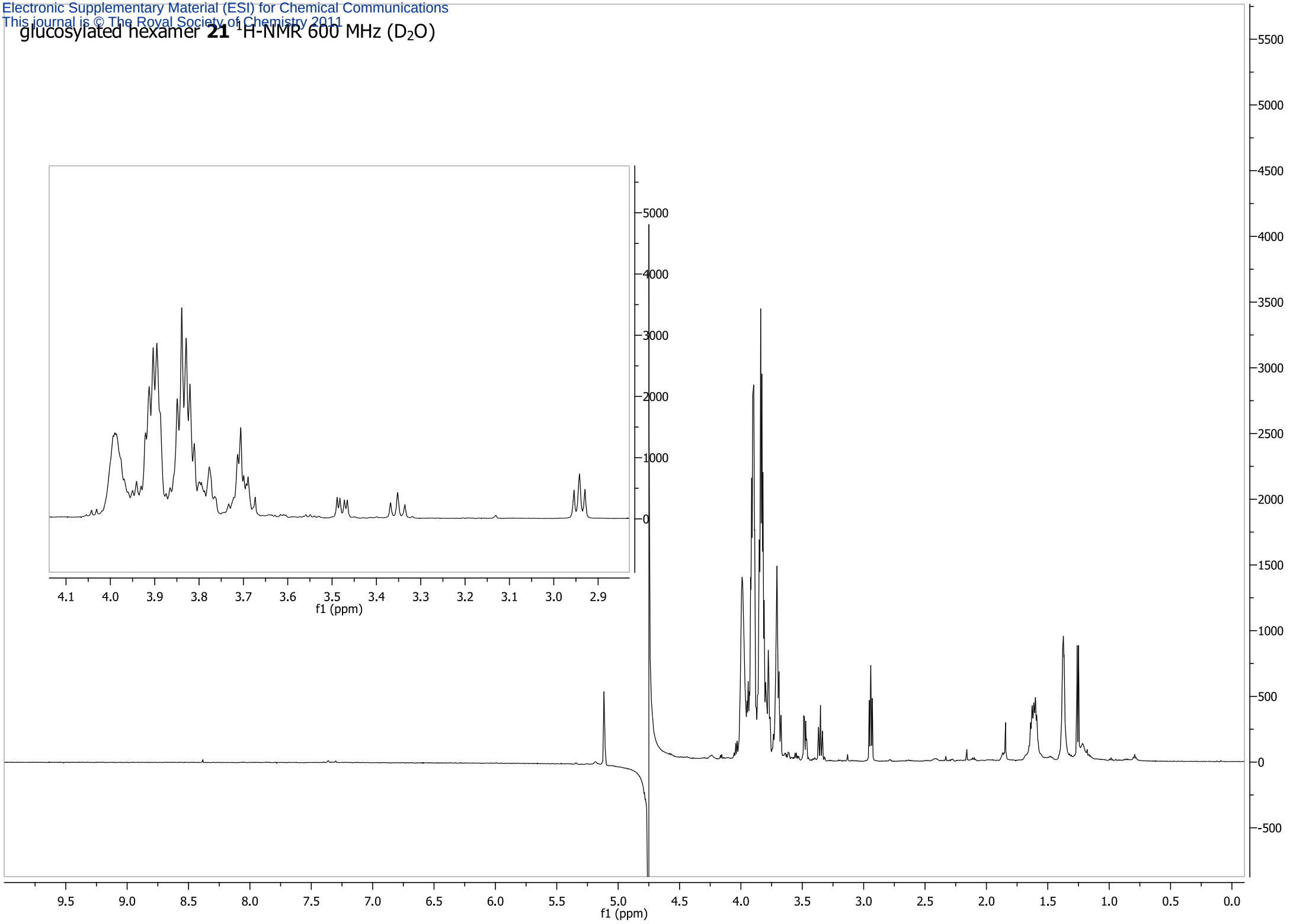




glucosylated hexamer **21** ^{31}P-NMR 162 MHz (D_2O)



glucosylated hexamer **21** ^{1}H-NMR 600 MHz (D_2O)



glucosylated hexamer **21** ^{13}C-NMR 150 MHz (D_2O)