Synthesis of Deuterium Labeled Dimethylallyl Diphosphate Derivatives

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Supporting Information

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General: All reactions were carried out in oven-dried glassware under argon unless otherwise noted. NMR spectra were collected at 23 °C. ^{1}H chemical shifts were referenced to internal $CDCl_3$ at 7.27 or D_2O at 4.80. ^{31}P NMR spectra were referenced to external phosphoric acid (5%) in D_2O at 0.00 ppm. Chemical shifts were reported in Hz.

[1,1-$^{2}H_2$]3-Methyl-2-buten-1-ol ([1,1-$^{2}H_2$]1-OH) and [1-^{2}H]3-Methyl-2-butenal ([1-^{2}H]3). **1,1-$^{2}H_2$]1-OH** and **[1-^{2}H]3** were synthesized by known procedures.[1] **[1,1-$^{2}H_2$]1-OH** was obtained as a colorless oil (86% yield, 99 % $^{2}H_2$); ^{1}H NMR ($CDCl_3$), δ 1.68 (d, *J* = 1.5 Hz, 3H), 1.74 (d, *J* = 1.5 Hz, 3H,), 5.39 (br s, 1H); ^{13}C NMR ($CDCl_3$), δ 17.9, 25.8, 58.6, 123.6, 136.2; Mass spectrum m/z (rel intensity) 88 (43), 73 (100), 55(10), 45 (10); HRMS (EI) calcd for $C_5H_8{}^{2}H_2O$ (M^+) 88.0855, found 88.0839. **[1-^{2}H]3** was obtained as a light yellow oil (78% yield, >99% ^{2}H); ^{1}H NMR ($CDCl_3$), δ 1.98 (d, *J* = 0.6 Hz, 3H), 2.17 (d, *J* = 0.9 Hz, 3H), 5.89 (t, *J* = 0.9 Hz, 1H); ^{13}C NMR ($CDCl_3$), δ 19.1, 27.5, 128.2, 161.0, 191.1. Mass spectrum m/z (rel intensity) 85 (100), 83 (38), 55 (26), 42 (12); HRMS (EI) calcd for $C_5H_7{}^{2}HO$ (M^+) 85.0637, found 85.0636.

(*R*)-[1-^{2}H] 3-Methyl-2-butenyl diphosphate ((*R*)-[1-^{2}H]1-OPP) and (*S*)-[1-^{2}H] 3-methyl-2-butenyl diphosphate ((*S*)-[1-^{2}H]1-OPP). Diphosphates **(*R*)-[1-^{2}H]1-OPP** and **(*S*)-[1-^{2}H]1-OPP** were prepared by direct phosphorylation of the corresponding alcohols.[2] To a solution of **(*R*)-[1-^{2}H]1-OH** (17.4 mg, 0.2 mmol) in 0.5 mL of trichloroacetonitrile was added a solution of bis-triethylammonium phosphate (0.5 mL) (TEAP, prepared by mixing 0.91 mL of solution of 25 mL of phosphoric acid in 94 mL acetonitrile and 1.5 mL of solution of 110 mL triethylamine in 100 mL acetonitrile) with stirring at room temperature. Two more additions of 0.5 mL of TEAP were carried out 5 min intervals. After an additional 5 min the mixture was concentrated and the residue was

chromatographed on silica gel (isopropanol-conc. NH_4OH-H_2O; 6:2:5:0.5) to give 17.7 mg (30 % yield; .99 % ^2H) of a white powder; ^1H NMR (D_2O) δ 1.67 (s, 3H), 1.71 (s, 3H), 4.38 (b t, J = 6.9 Hz, 1H), 5.39 (d, J = 7.2 Hz, 1H); ^{31}P NMR (D_2O) δ -7.5 (d, $J_{p,p}$ = 20.2 Hz, 1P), -10.3 (d, $J_{p,p}$ = 22.0 Hz, 1P); HRMS (FAB^-) $[M-1]^-$ calculated for $C_5H_{10}DP_2O_7$ 246.0042, found 246.0031.

Following the same procedure, **(*S*)-[1-^{2}H]1-OH** (17.4 mg, 0.2 mmol) gave 12.3 mg (21 %, >99 % ^2H) of a white powder; ^1H NMR (D_2O) δ 1.69 (s, 3H), 1.73 (s, 3H), 4.40 (b t, J = 7.2 Hz, 1H), 5.41 (d, J = 6.9 Hz, 1H); ^{31}P NMR (D_2O) δ -6.7 (d, $J_{p,p}$ = 22.0 Hz, 1P), -10.3 (d, $J_{p,p}$ = 22.0 Hz, 1P); HRMS (FAB^-) $[M-1]^-$ calculated for $C_5H_{10}{}^2HP_2O_7$ 246.0042, found 246.0046.

Preparation and analysis of Mosher esters [3] of (*R*)- and (*S*)- [1-^{2}H]3-Methyl-2-buten-1-ol ((*R*)- and (*S*)-[1-^{2}H]1-OH). To a stirred mixture of 4-(dimethylamino)pyridine (DMAP, 18 mg, 0.15 mmol) and 100 µl of triethylamine in 0.5 ml of CH_2Cl_2, added **(*R*)-** or **(*S*)-[1-^{2}H]1-OH** (13.5 mg, 0.15 mmol) via syringe. Immediately, (*R*)-(-)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (30 µl) was added. The clear solution became orange. After complertion of the reaction (~30 min), the solvent was removed under reduced pressure and the pure ester was obtained by flash chromatography on silicagel column (1.0 cm X 15 cm) by eluting with two column volume of CH_2Cl_2. 1HNMR analysis in $CDCl_3$ at 500 MHz focused on methylene proton. The chiral proton in diastereomeric esters from **(*R*)-** and **(*S*)-[1-^{2}H]1-OH** were cleanly resolved, giving doblets at 4.77 and 4.82 ppm, respectively. A comparison of peak intensities indicated that the enantiomeric ratios for **(*R*)- and (*S*)-[1-^{2}H]1-OH** were 96/4 and 94.5/5.5, respectively.

Ethyl [2,2-^{2}H]acetoacetate (**[2,2-^{2}H]4**). Ethyl acetoacetate (4.0 g, 4.1 ml) was stirred with D_2O (12 ml) for 24 h. The sample was extracted with ethyl ether and concentrated under reduced pressure. NMR analysis indicated 88% exchange of ^{1}H for ^{2}H at the activated methylene position.[4] The procedure was repeated to give a colorless oil (96% $^2H_2$). ^{1}H NMR ($CDCl_3$) δ 1.25 (t, *J* = 7.2 Hz, 3H), 2.24 (s, 3 H), 3.40 (br t, *J* = 2.1 Hz, 0.08 H), 4.16 (q, *J* = 7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14.2, 30.2, 49.8, 61.5, 167.3, 200.9.

Ethyl [2-^{2}H]3-(diethylphosphoryloxy)-2-butenoate ([2-^{2}H]5). To a stirred suspension of NaH (0.88 g, 60% suspension, 22 mmol, 1.1eq) in dry ether cooled in an ice bath was added a solution of **[2,2-^{2}H]4** (2.64 g, 20 mmol) of in ether. After 20 min at 0 °C, 1.1 eq (3.80 g, 22 mmol) of diethylchlorophosphate was introduced and stirring was continued for 2 h. The mixture was quenched with aqueous NH_4Cl and the aqueous phase was extracted with ether. The combined organic layers were washed with saturated $NaHCO_3$, dried over $MgSO_4$ and evaporated under reduced pressure. Flash chromatography on silica gel (CH_2Cl_2) gave 4.76 g (89 % yield, 93 % ^{2}H) of a colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.24 (t, *J* = 7.2 Hz, 3H,), 1.35 (td, J= 6.9 Hz & 1.2 Hz, 6H), 2.15 and 2.20 (2d, J = 1.5 Hz, 3H), 4.12 (q, *J* = 7.2 Hz, 2H), 4.25 (q, J= 6.9 Hz, 4H); ^{13}C NMR ($CDCl_3$) δ 14.4, 16.2, 16.2, 21.8, 59.9, 64.9, 65.0,105.5, 158.3, 163.8; P^{31} NMR ($CDCl_3$) δ –7.6; HRMS (CI) found [M^+ + H], calculated for $C_{10}H_{19}{}^2HO_6P$ requires 268.1059, found 268.1054.

[2-^{2}H]3-Methyl-2-butenol ([2-^{2}H]1-OH). To a solution of 516 mg (4.0 mmol) of **[2-^{2}H]5** in dry $CH_2Cl_2$was added diisobutylaluminium hydride (12 mL of 1M in CH_2Cl_2, 12 mmol)) at -78 °C. After 30 min, Na_2SO_4.10 H_2O/celite (1:1 w/w) was added and the

mixture was stirred overnight. The slurry was filtered, washed with CH_2Cl_2 and combined organic phase was concentrated. The residue was purified by flash chromatography on silica gel (CH_2Cl_2) to give 292 mg (84 % yield, 93 % ^2H) of a colorless oil; ^1H NMR ($CDCl_3$) δ 1.66 (s, 3H), 1.73 (s, 3H), 4.10 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 17.9, 25.8, 59.3, 123.5, 136.3; Mass spectrum m/z (rel intensity) 87 (32), 72 (100), 54 (15), 44 (21); HRMS (EI) calcd for $C_5H_9{}^2HO$ (M^+) 87.0793, found 87.0794.

[2-^{2}H]3-methyl-2-butenyl diphosphate ([2-^{2}H]1-OPP). To a solution of **[2-^{2}H]1-OH** (160 mg, 1.8 mmol) in pentane cooled to 4 °C was added PBr_3 (545.4 mg, 2.0 mmol) of in pentane. After 20 min methanol (173 µL) was added. The mixture was stirred for 5 min and the solvent was removed under reduced pressure at 4 °C to give 0.224 g (81%) of **[2-^{2}H]6**. The bromide obtained was immediately converted to **[2-^{2}H]1-OPP** by treatment with tris(tetra-n-butylammonium)hydrogen pyrophosphate (3.18 g, 3.53 mmol) in acetonitrile for 2 h. The mixture was concentrated under reduced pressure. The residue was dissolved in 10 mL of exchange buffer (2 mL of 25 mM NH_4HCO_3 with 2% (v/v) isopropanol/water) and passed through a column containing 108 meq of DOWEX AG 50W-X8 cation-exchange resin (NH_4^+ form).[5] The column was eluted with two column volumes of exchange buffer, and the eluent was lyopholized. The residue was dissolved in a minimal amount of water and chromatographed on cellulose with elution by 100 mM ammonium bicarbonate in 3.5:6.5 (v/v) water/1-propanol buffer. Fractions containing diphosphate were pooled, concentrated by rotary evaporator, and lyophilized to give 151 mg (34% yield, 93 % ^2H) of a white solid; ^1H NMR ($CDCl_3$) δ 1.65 (s, 3H), 1.69 (s, 3H), 4.37 (d. J = 6 Hz, 2H,); ^{31}P NMR (D_2O) δ -6.5 (d, $J_{p,p}$ = 21.0

Hz, 1P), -10.3 (d, $J_{p,p}$ = 21.0 Hz, 1P). HRMS (FAB$^-$) $[M-H]^-$ calculated for $C_5H_{10}{}^2HP_2O_7$ 246.0042, found 246.0037.

***(E)*- and (*Z*)-Ethyl 3-Phenylsulfanyl-2-butenoate ((*E*)- and (*Z*)-8).** NaOH (1.3 g, 0.03 mol) was added to a stirred solution of C_6H_5SH (3.3 g, 0.03 mol) in EtOH. The mixture was stirred for 30 min at room temp before a solution of ethyl but-2-ynoate (**7**) in EtOH was added. Stirring was continued for 4 h before an aqueous solution containing 2.1 g of acetic acid was added. The mixture was extracted with ether. The organic layers were combined, washed with 4% aqueous NaOH, water, and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was chromatographed over silica gel (10% ether/hexane) to give 4.1 g (70 %) of ***(E)*-8** as a viscous oil; ^{1}H NMR ($CDCl_3$) δ 1.20 (t, *J* = 7.2 Hz, 3H), 2.43 (s, 3H), 4.10 (q, *J* = 7.2 Hz, 2H), 5.26 (s, 1H), 7.41-7.51 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.4, 20.2, 60.0, 112.1, 129.2, 129.6, 131.0, 136.73, 158.4, 166.4; HRMS (CI) $[M+1]^+$ calculated for $C_{12}H_{15}SO_2$ 223.0793, found 223.0763.

The remaining material from the silica column was chromatographed on silica gel (ether) and re-chromatographed (10% ether/hexane) to give 1.5 g (25 %) of ***(Z)*-8** as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, *J* = 7.2 Hz, 3H), 1.81 (s, 3H), 4.22 (q, *J* = 7.2 Hz, 2H), 5.85 (s, 1H), 7.33-7.56 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.5, 25.2, 60.0, 112.1, 129.2, 129.6, 131.0, 136.3, 158.4, 166.4; HRMS (CI) $[M+1]^+$ calculated for $C_{12}H_{15}SO_2$, 223.0793, found 223.0769.

***(E)*- and *(Z)*-[4-^{2}H$_3$]3-Methyl-2-butenol (*(E)*- and *(Z)*-[4,4,4-^{2}H$_3$]1-OH).** To a stirred suspension of LAH (0.15 g, 3.8 mmol) in dry ether at 0 °C was added a solution of ***(E)*-[4,4,4-^{2}H$_3$]4** (0.63 g, 4.8 mmol) of in ether. After 150 min, water (0.9 mL) and 10%

aq NaOH (0.15 mL) were added and stirring was continued for 40 min at room temp. The mixture was then passed through a 1:1 (v/v) mixture of celite/$MgSO_4$, which was washed with ether. The ether fractions were dried over $MgSO_4$ and concentrated at reduced pressure. The residue was chromatographed over silica gel (1:1 hexane/ether) to give 0.35 g (82% yield; >99 % ^2H) of ***(E)*-[4,4,4-$^2H_3$]1-OH** [6] as a colorless oil; ^1H NMR ($CDCl_3$) δ 1.68 (bs, 3H), 4.13 (d, *J* = 7.2 Hz, 2H), 5.41 (t, *J* = 7.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 18.0, 59.6, 123.8, 136.6; HRMS (CI) $[M+1]^+$ calculated for $C_5H_7{}^2H_3O$, 90.0995, found 90.0995.

Following the same procedure, ***(Z)*-[4,4,4-$^2H_3$]4** (0.63 g, 4.8 mmol) gave 0.32 g (75% yield; >99 % ^2H) of ***(Z)*-[4,4,4-$^2H_3$]1-OH** [7] as an oil; ^1H NMR ($CDCl_3$) δ 1.74 (bs, 3H), 4.12 (d, *J* = 7.2 Hz, 2H), 5.41 (t, J = 7.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 25.9, 59.6, 123.8, 136.5; HRMS (CI) [M+1]+ calculated for $C_5H_7{}^2H_3O$, 90.0995; found 90.0996.

***(E)*- and *(Z)*- [4-$^2H_3$]3-Methyl-2-butenyl diphosphate (*(E)*- and (*Z)*-[4,4,4-$^2H_3$]1-OPP).** To a solution of N-chlorosuccinimide (0.77 g, 5.8 mmol) in CH_2Cl_2 at –30 °C was added dimethyl sulfide (0.50 mL, 0.42 g, 6.7 mmol). The mixture was allowed to warm to 0 °C for 5 min before the temperature was lowered to –40 °C. A solution of ***(E)*-[4,4,4-$^2H_3$]1-OH** (0.40 g, 4.5 mmol) in CH_2Cl_2 was added slowly. The stirred mixture was allowed to warm up to 0 °C for 1 h and then warmed to room temp for 14 h. The mixture was diluted with brine and extracted twice ether. The organic layer was washed with brine, and the aqueous layer was back-extracted with ether. The combined organic layers were dried over $MgSO_4$ and concentrated at reduced pressure to give 0.375 g (78 %, >99 % ^2H) of ***(E)*-[4,4,4-$^2H_3$]7** as a colorless oil; ^1H NMR ($CDCl_3$) δ 1.69 (s, 3H), 4.1 (d, *J* = 8.0 Hz, 2H), 5.4 (t, *J* = 8.1 Hz, 1H).

The identical procedure was used to convert ***(Z)*-[4,4,4-^{2}H$_3$]1-OH** (0.40 g, 4.5 mmol) to 0.391 g (82 %, >99 % ^{2}H) of ***(Z)*-[4,4,4-^{2}H$_3$]7** as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.73 (s, 3H), 4.1 (d, *J* = 7.1 Hz, 2H), 5.4 (t, *J* = 7.9 Hz, 1H).

To a solution of *tris*-tetra-*n*-butylammonium hydrogen diphosphate trihydrate (4.0 g, 4.2 mmol) in acetonitrile at 0 °C was added ***(E)*-[4,4,4-^{2}H$_3$]7** (200 mg, 1.9 mmol) in acetonitrile over a 10 min period. After 1 h, the ice bath was removed, and the reaction was allowed to stir at room temperature for 4 h before solvent was removed by rotary evaporation. The residue was dissolved in 10 mL of exchange buffer (2 mL of 25 mM NH_4HCO_3 containing 2% (v/v) isopropanol/water), and the solution was passed through a 2x50 cm column of DOWEX AG 50W-X8 cation-exchange resin (NH_4^+ form). The product was eluted with two column volumes of exchange buffer, and the eluent was lyophilized. The residue was dissolved in a minimal volume of water and chromatographed on cellulose (100 mM NH_4HCO_3 in 3.5:6.5 (v/v) water/1-propanol buffer). Fractions containing product were pooled, concentrated by at reduced pressure, and lyophilized to give 334 mg (60 %, >99 % ^{2}H) of **(*E*)-[4,4,4-^{2}H$_3$]1-OPP** [6] as a white powder; ^{1}H NMR (D_2O) δ 1.64 (s, 3H), 4.37 (t, *J* = 6.4 Hz, 2H), 5.37 (t, *J* = 7.2, 1H); ^{31}P NMR (D_2O) δ –6.7 (d, *J* = 20.2 Hz, 1P), -10.2 (d, *J* = 20.8 Hz, 1P); HRMS (FAB^-) $[M-1]^-$ calculated for $C_5H_8{}^2H_3P_2O_7$ 248.0168, found 248.0160.

The identical procedure was used to convert ***(Z)*-[4,4,4-^{2}H$_3$]7** (200 mg, 1.9 mmol) to 350 mg (63 %, >99 % $^2H_3$) of ***(Z)*-[4,4,4-^{2}H$_3$]1-OPP** [7] as a white powder; ^{1}H NMR (D_2O) δ 1.70 (s, 3H), 4.37 (q, *J* = 6.3 Hz, 2H), 5.40 (t, *J* = 5.4 Hz, 1H); ^{31}P NMR (D_2O) δ –6.4 (d, *J* = 21.9 Hz, 1P), -10.2 (d, *J* = 21.4 Hz, 1P); HRMS (FAB^-) $[M-1]^-$ calculated for $C_5H_8{}^2H_3P_2O_7$ 248.0168, found 248.0177.

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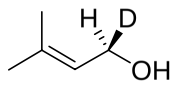

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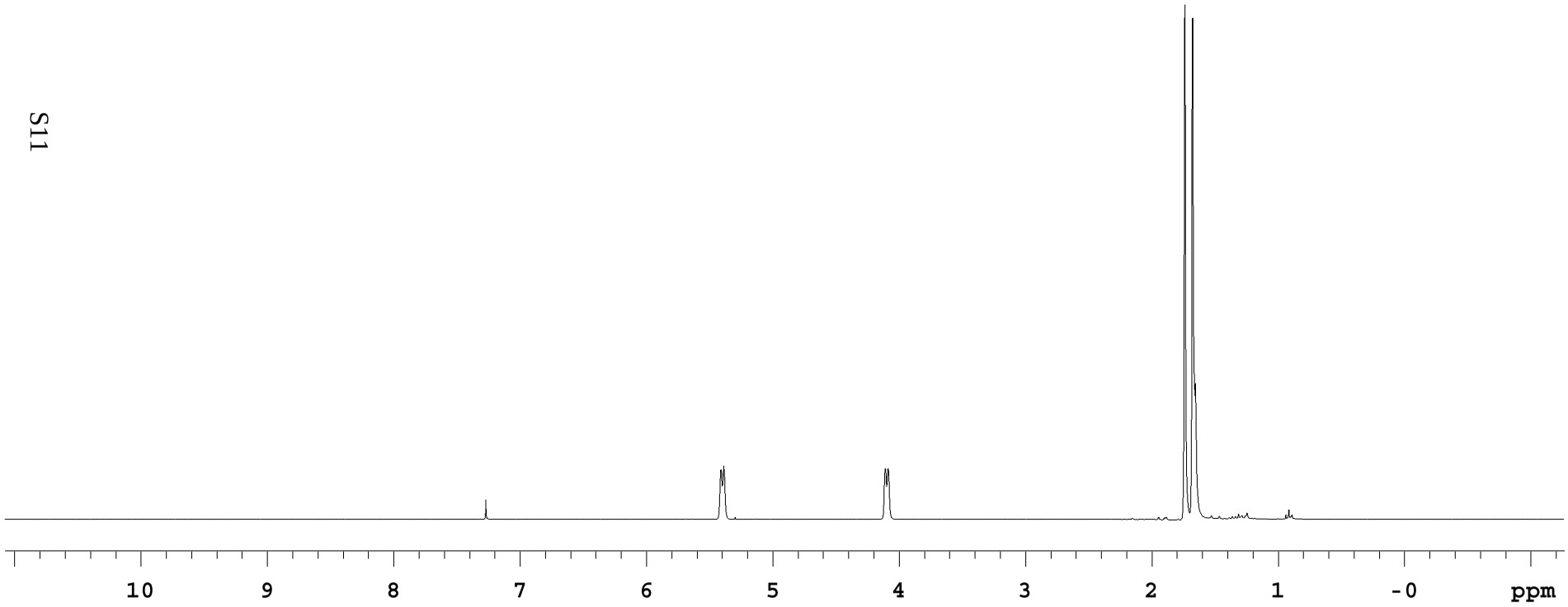

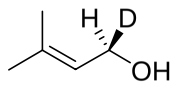

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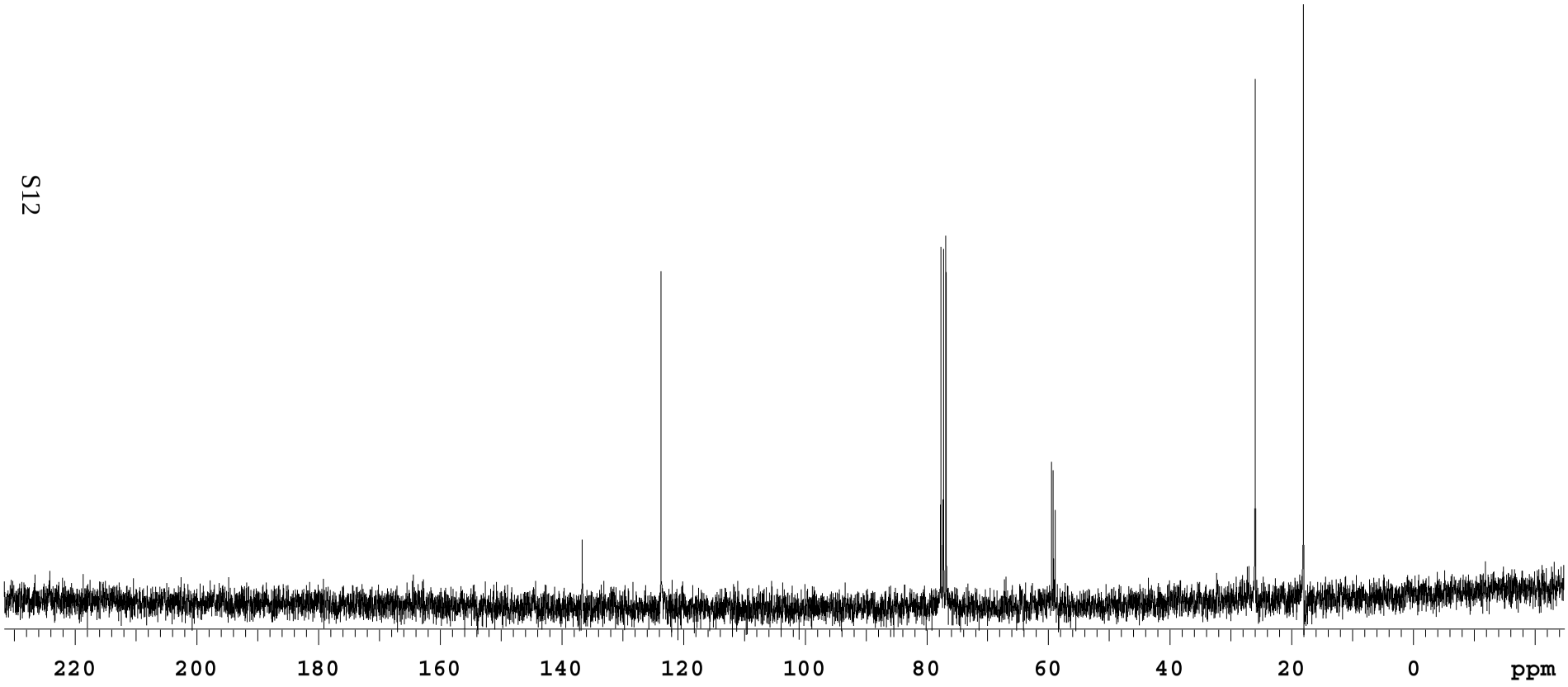

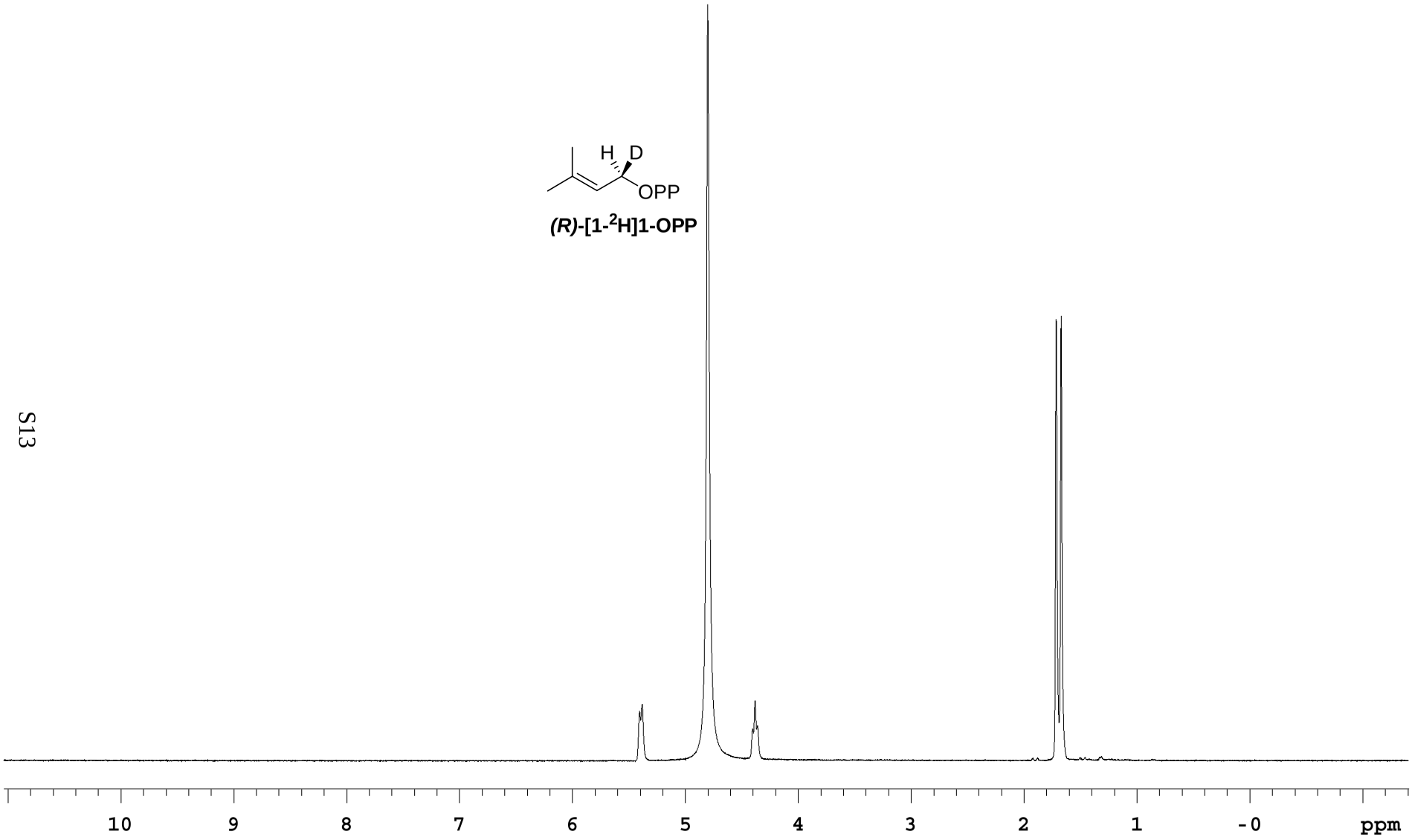
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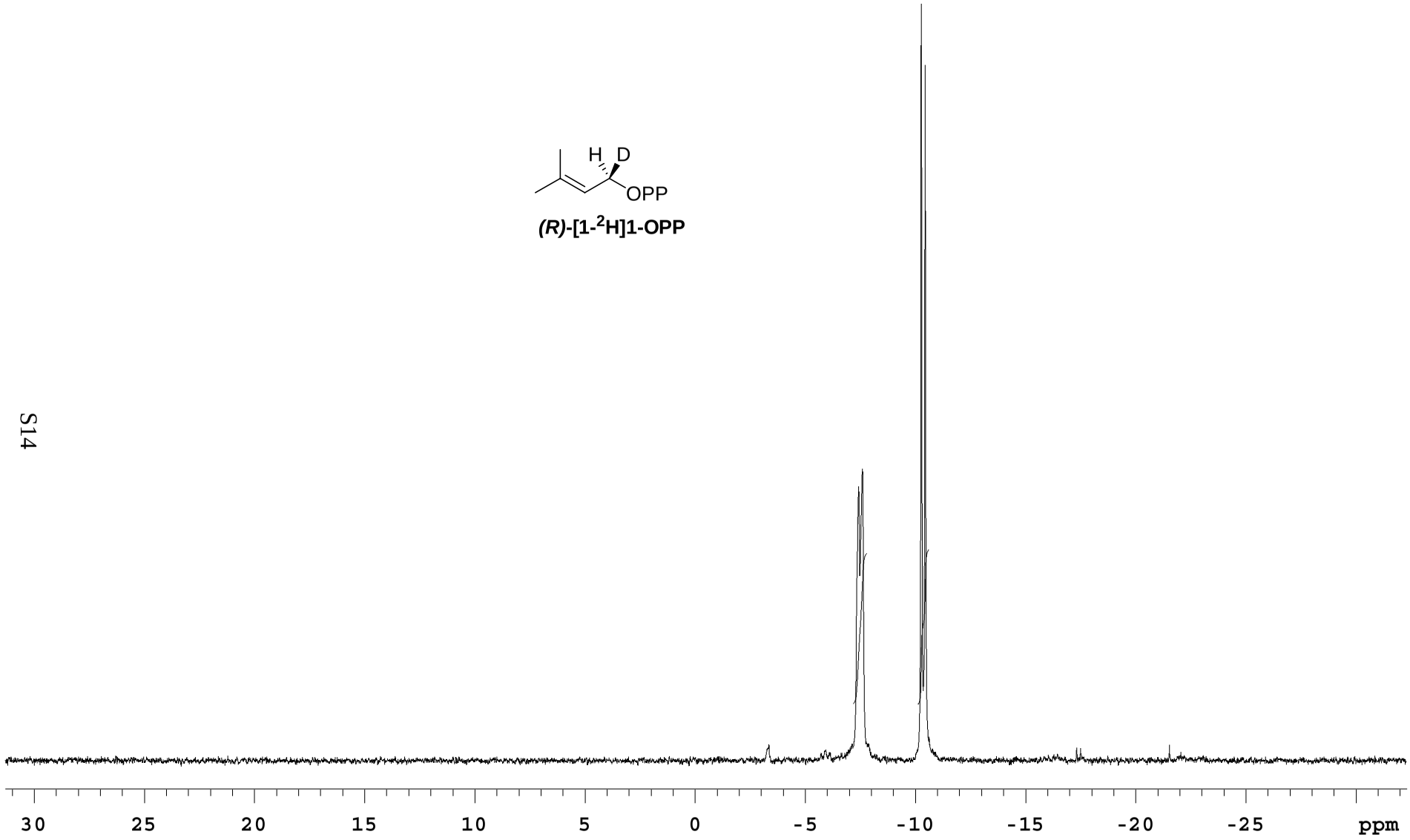

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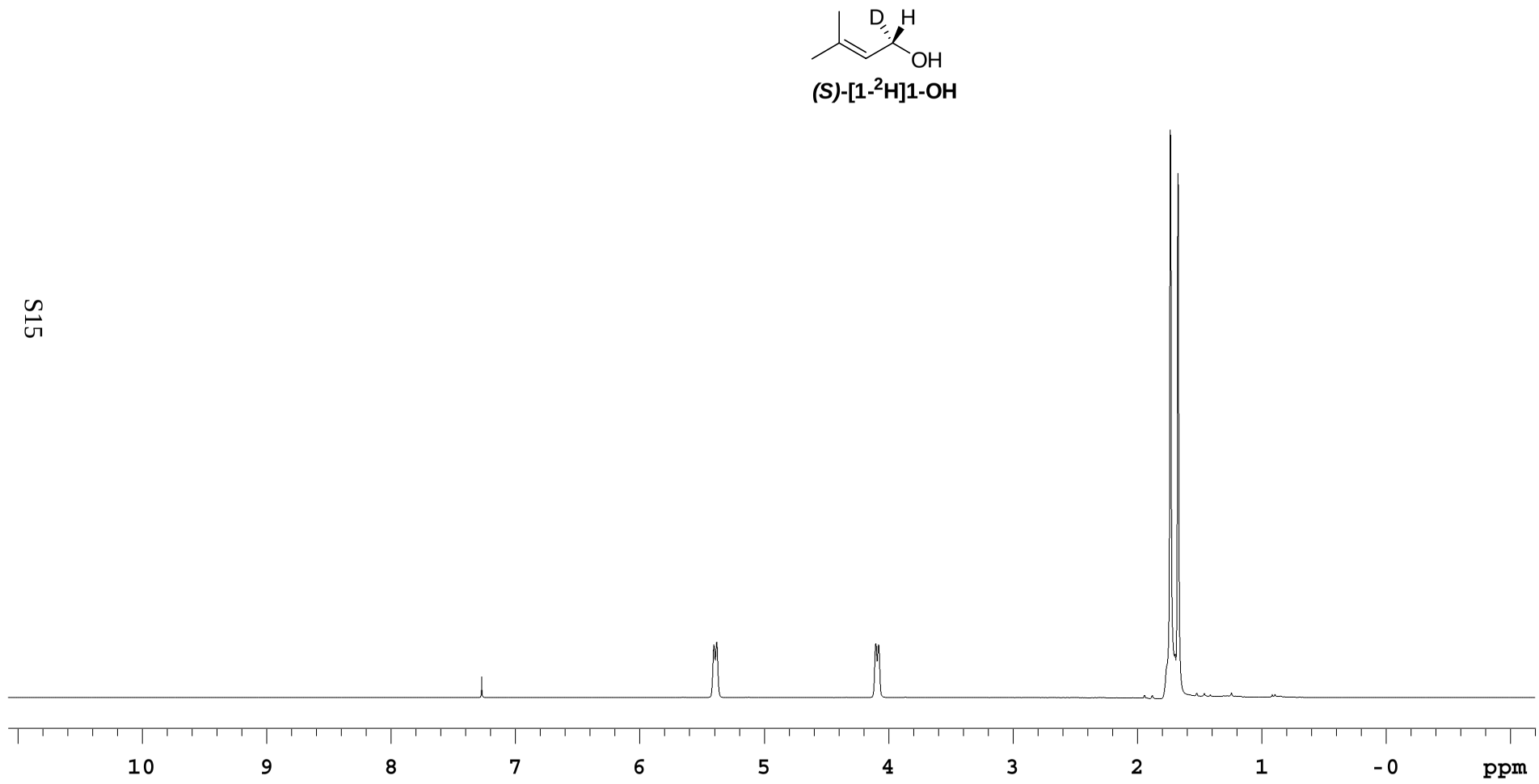
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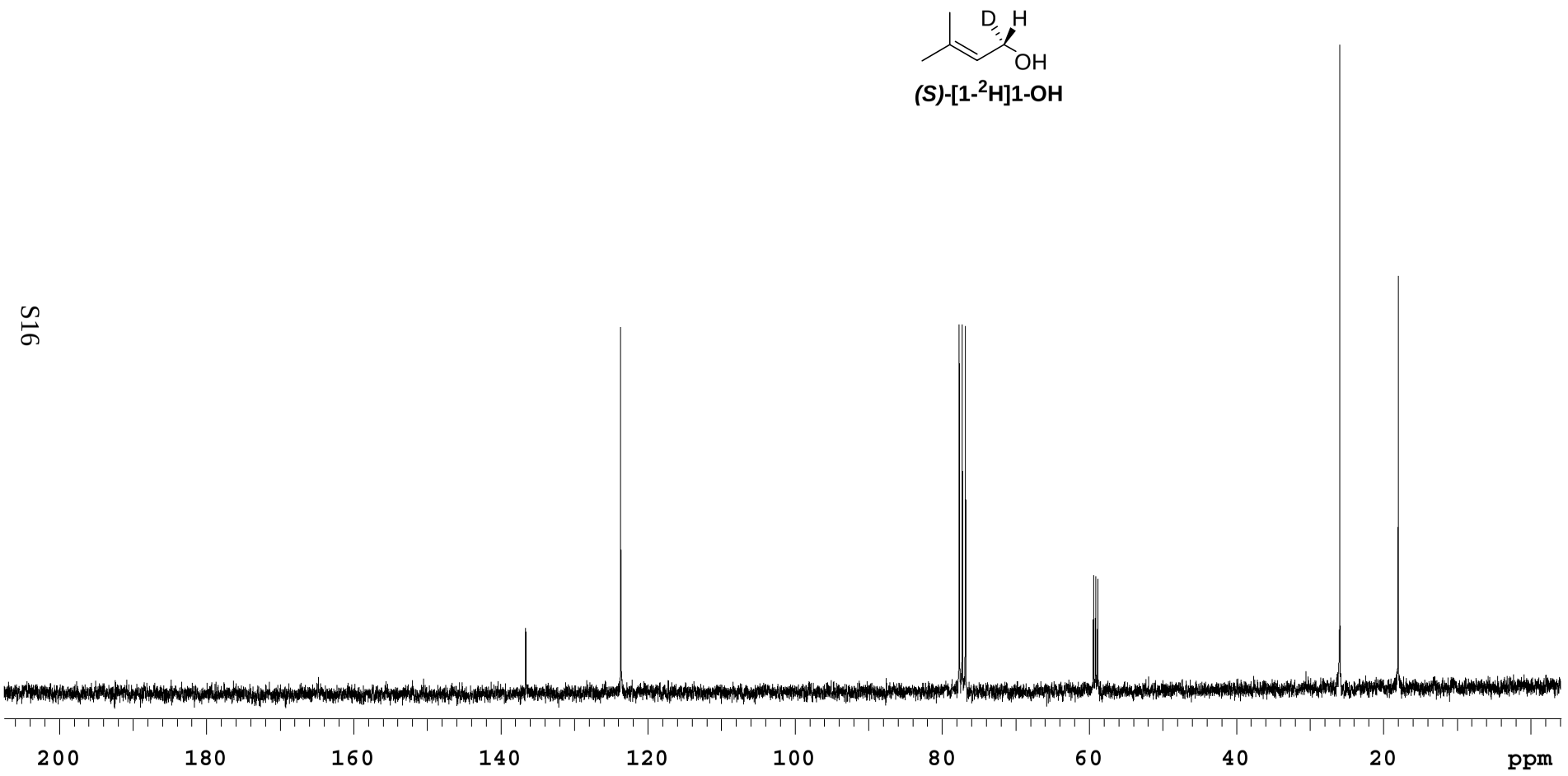
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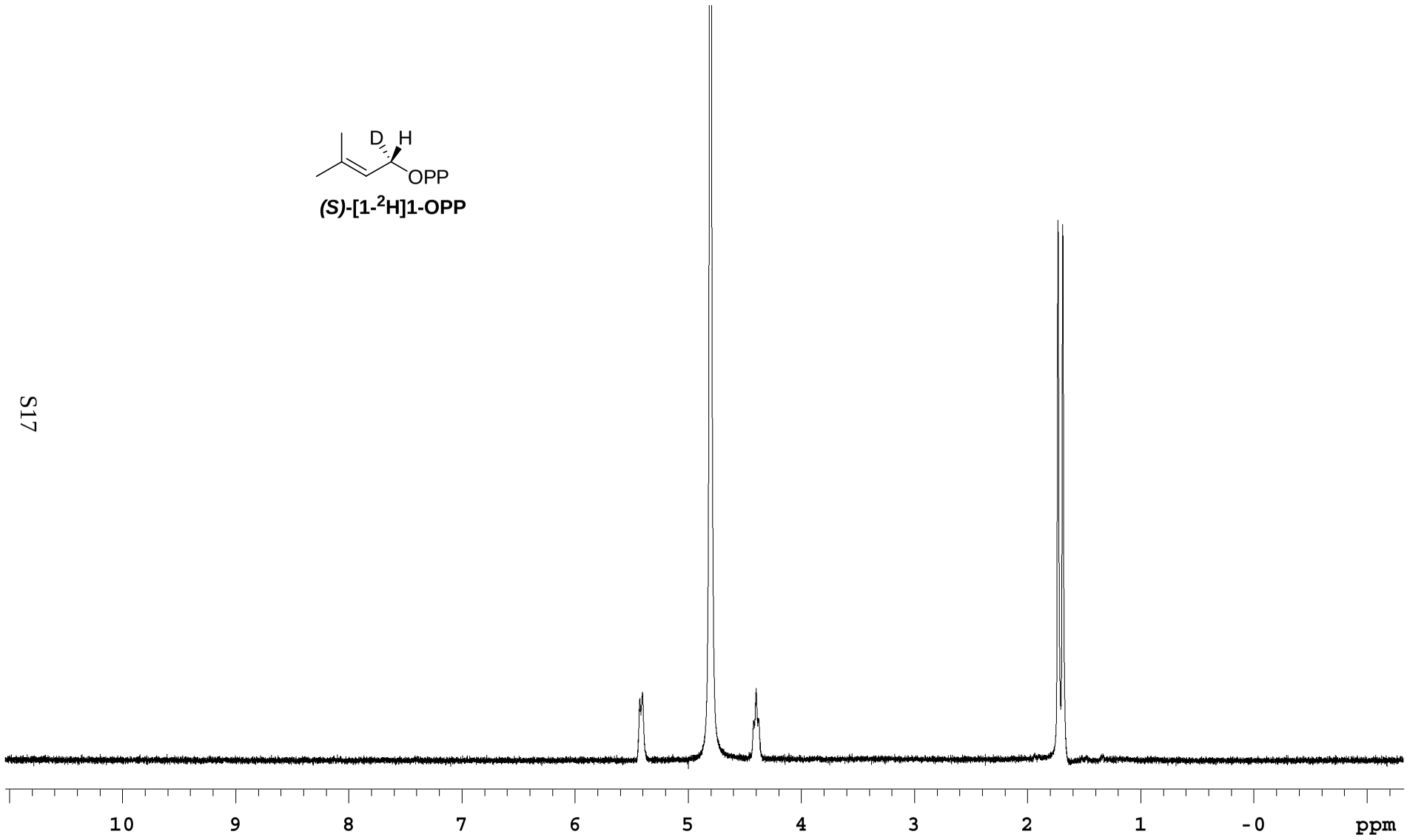

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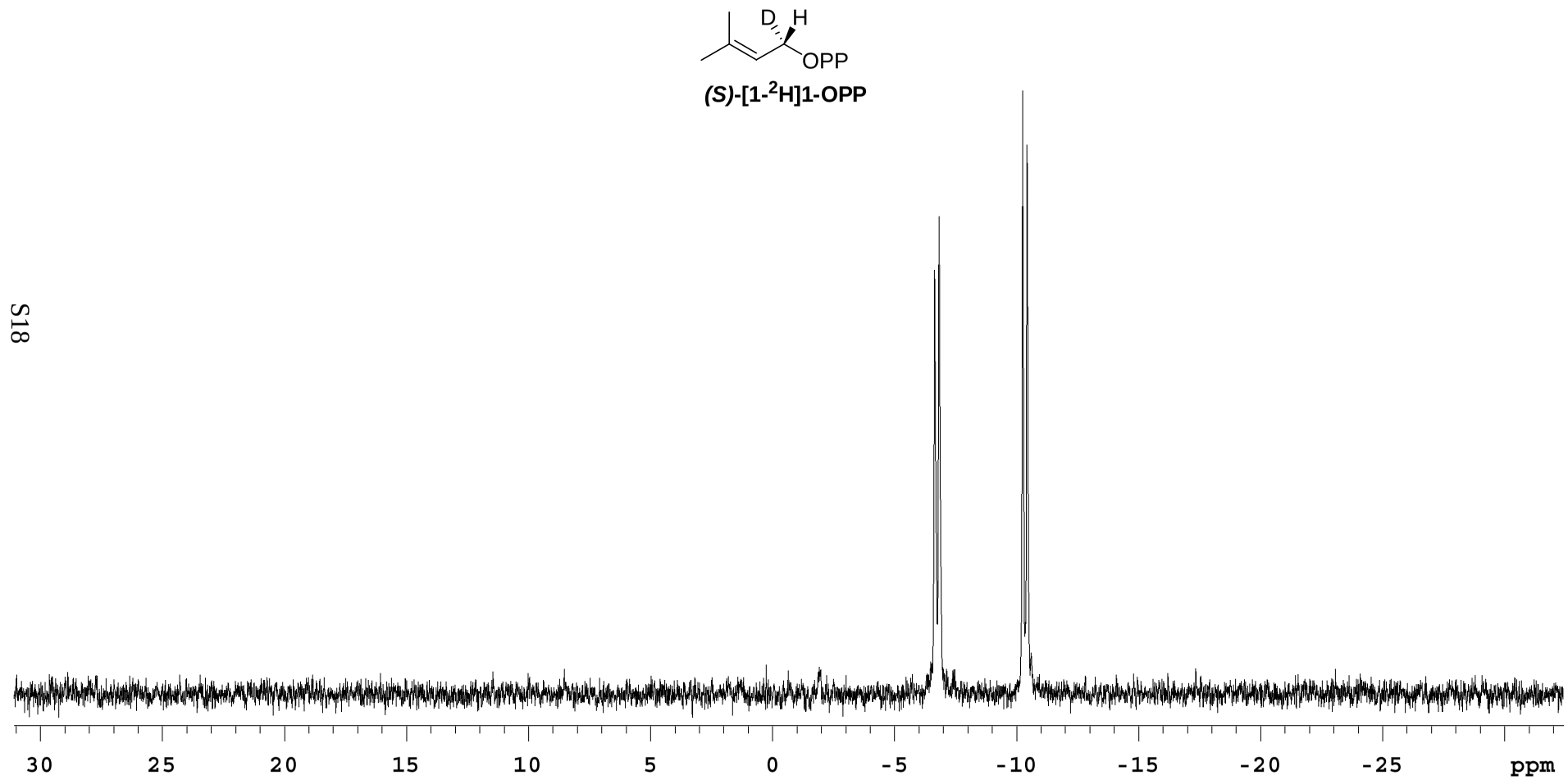
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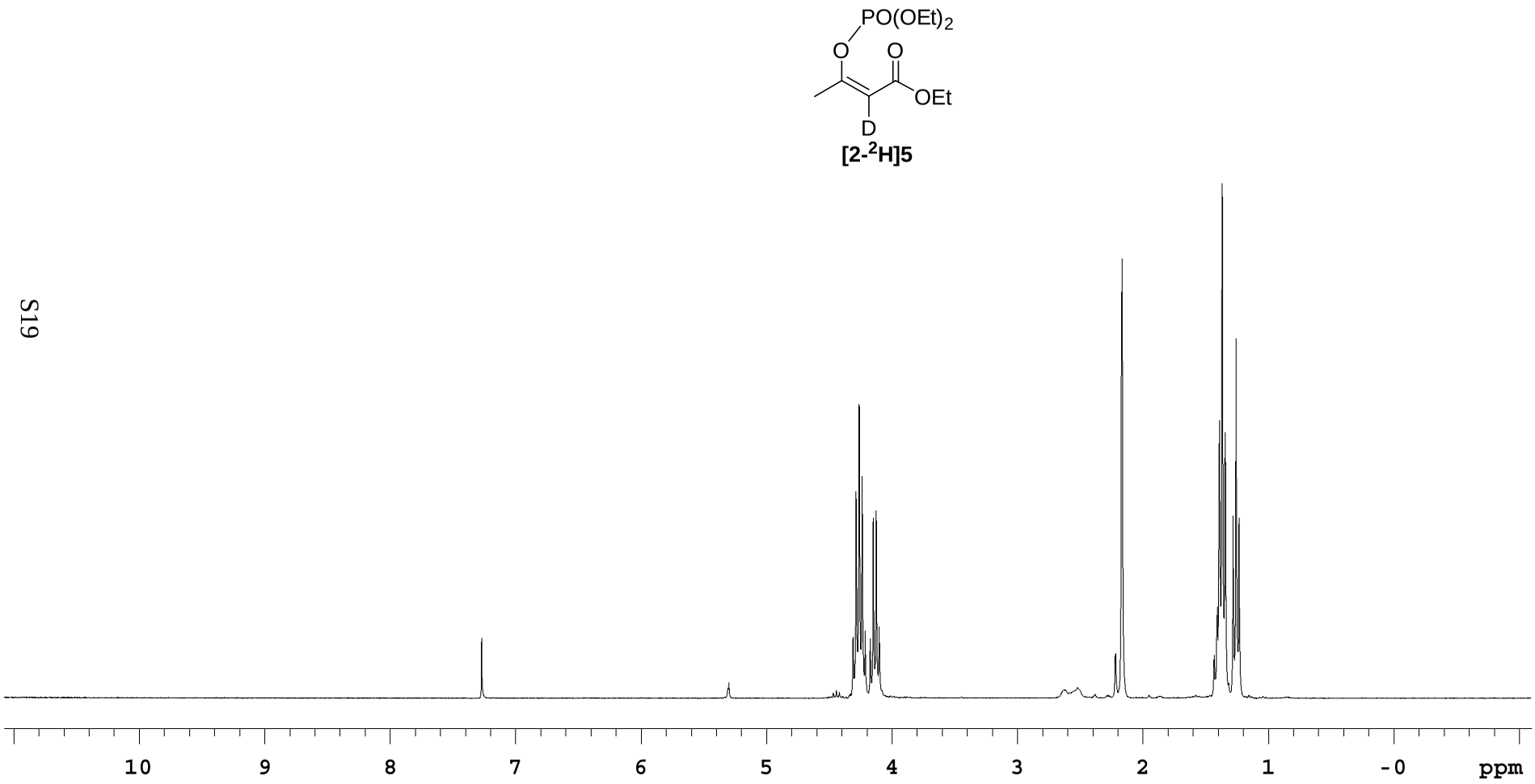

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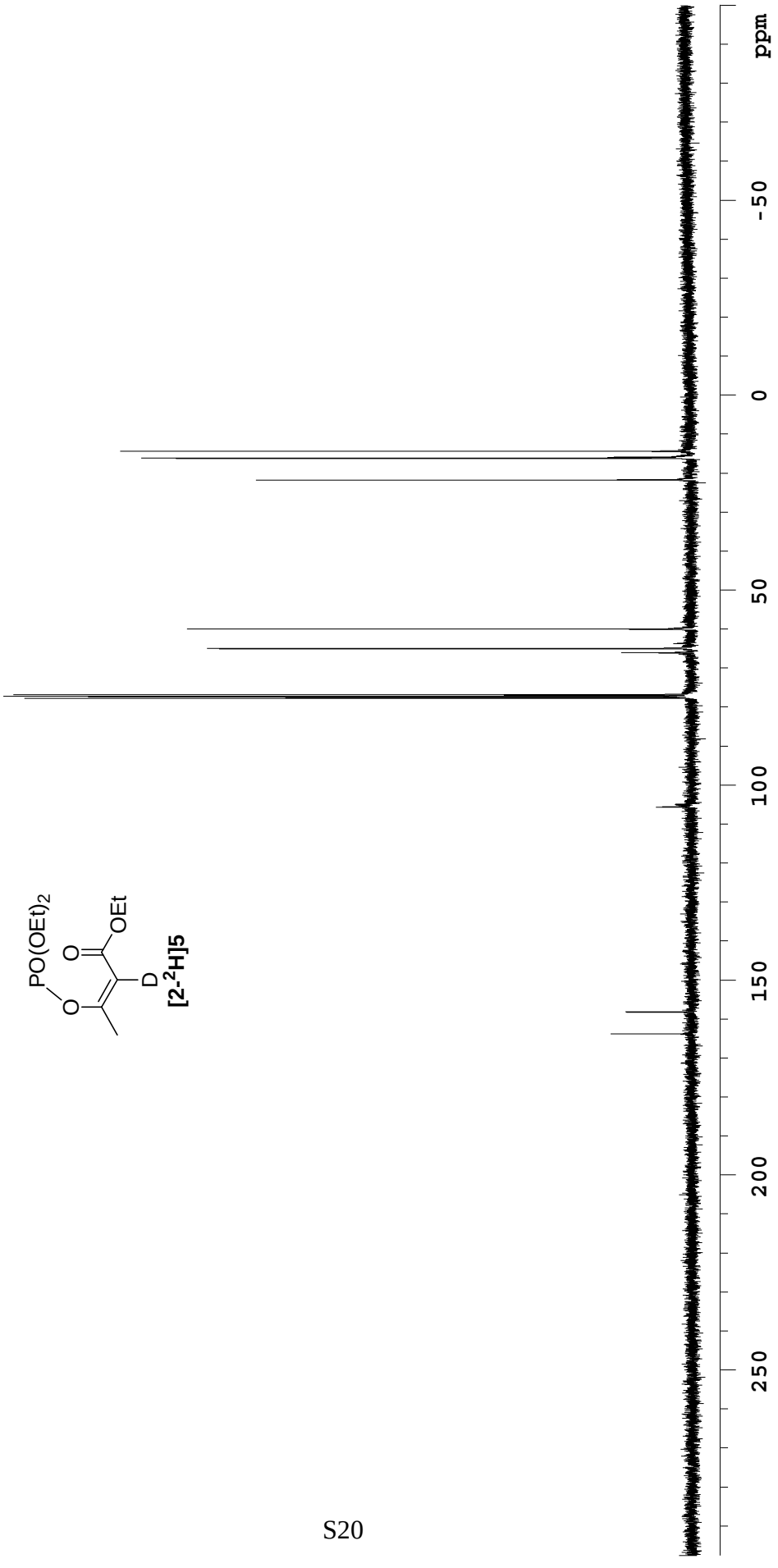
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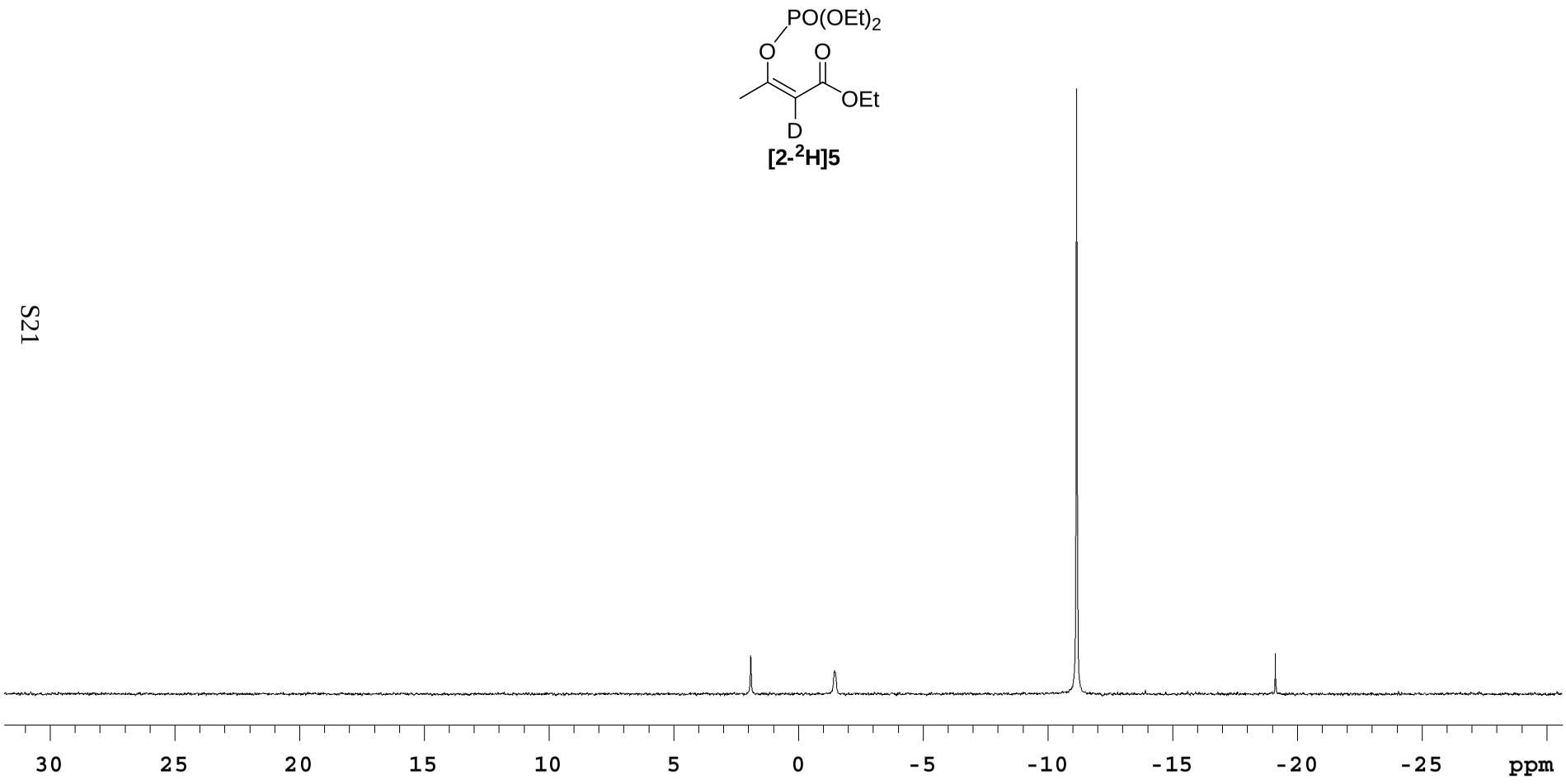
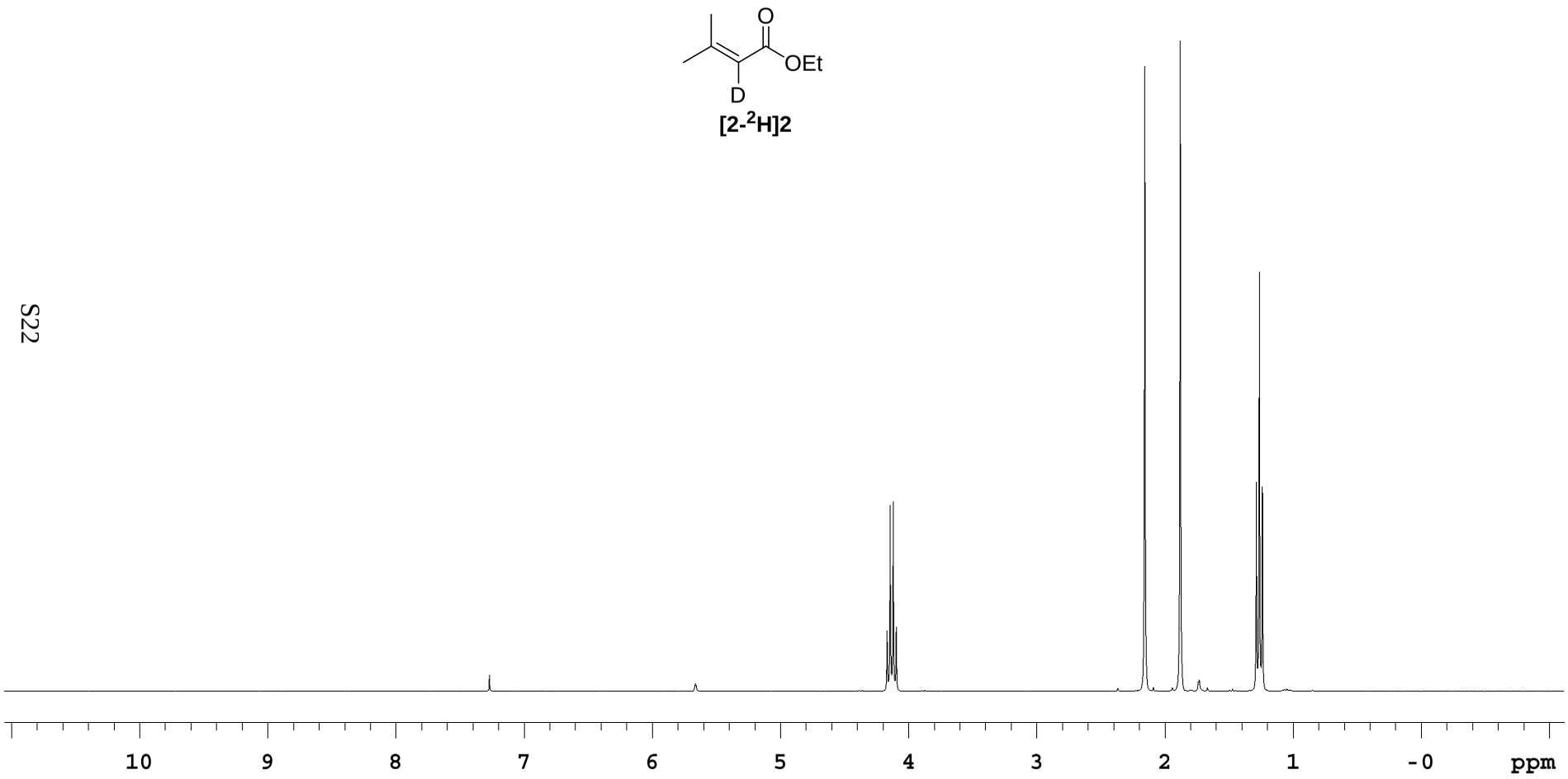
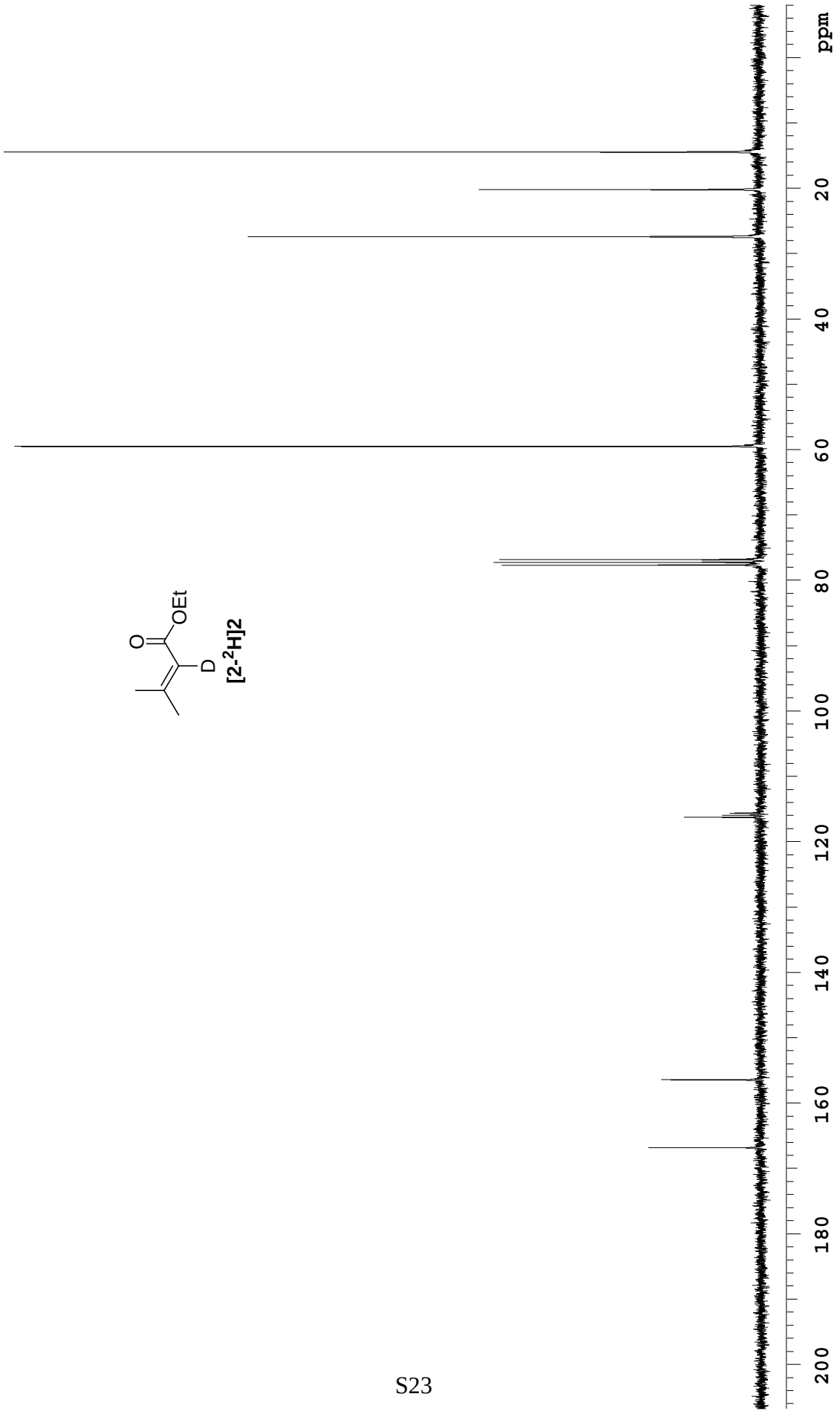
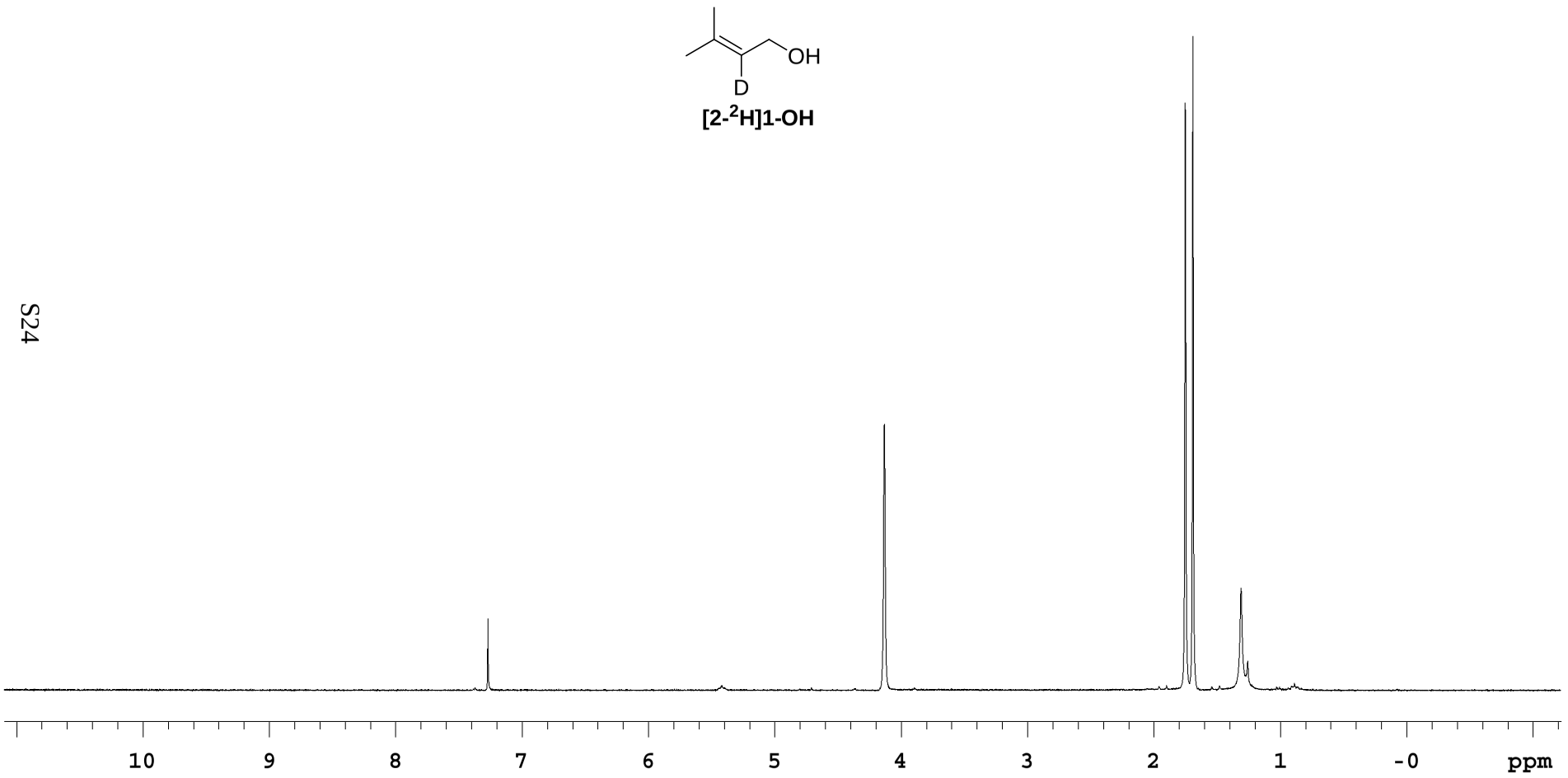
0
-50

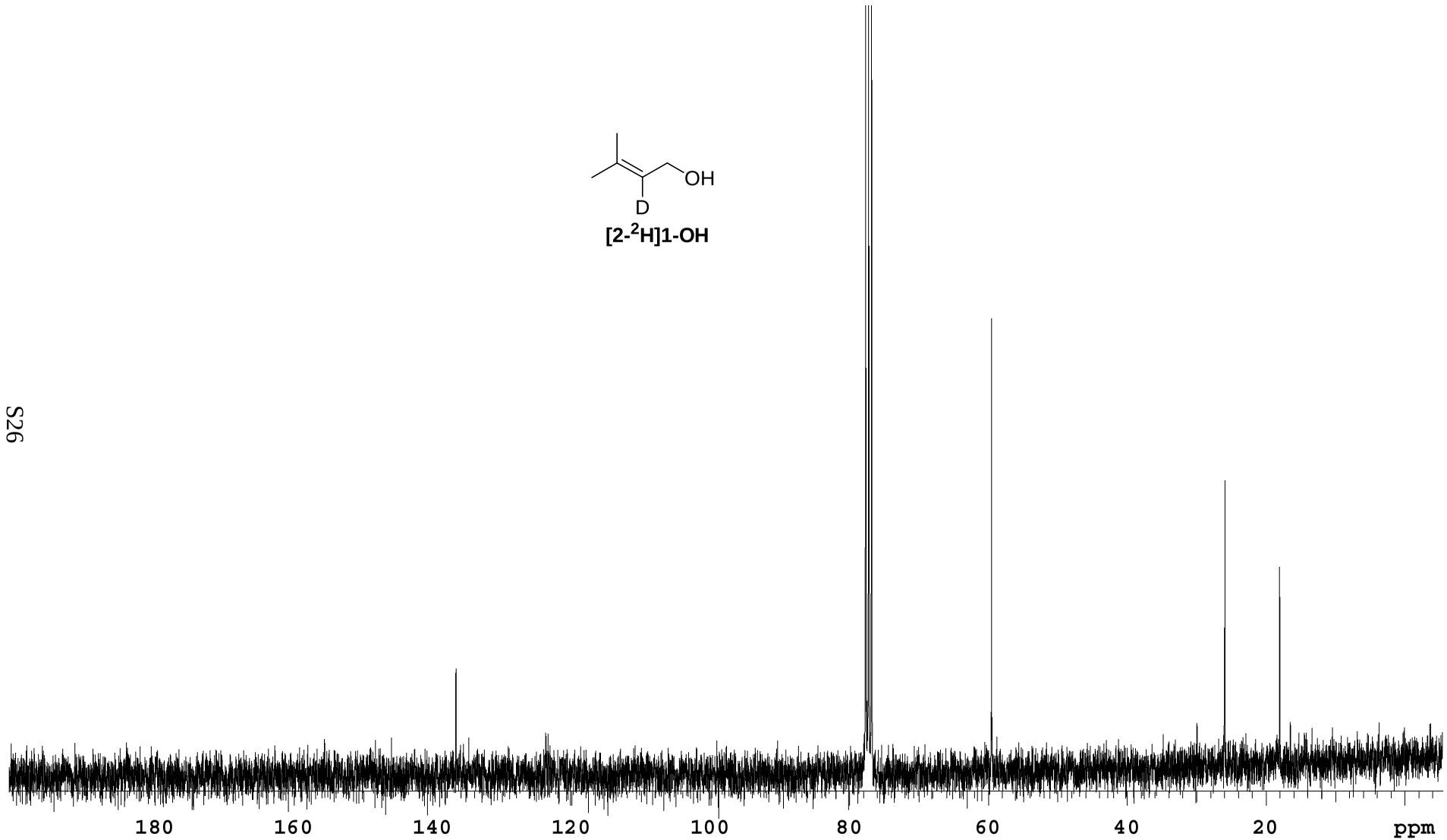

PO(OEt)2
O
O
OEt
D
[2-2H]5
30
25
20
15
10
5
0
-5
-10
-15
-20
-25
ppm

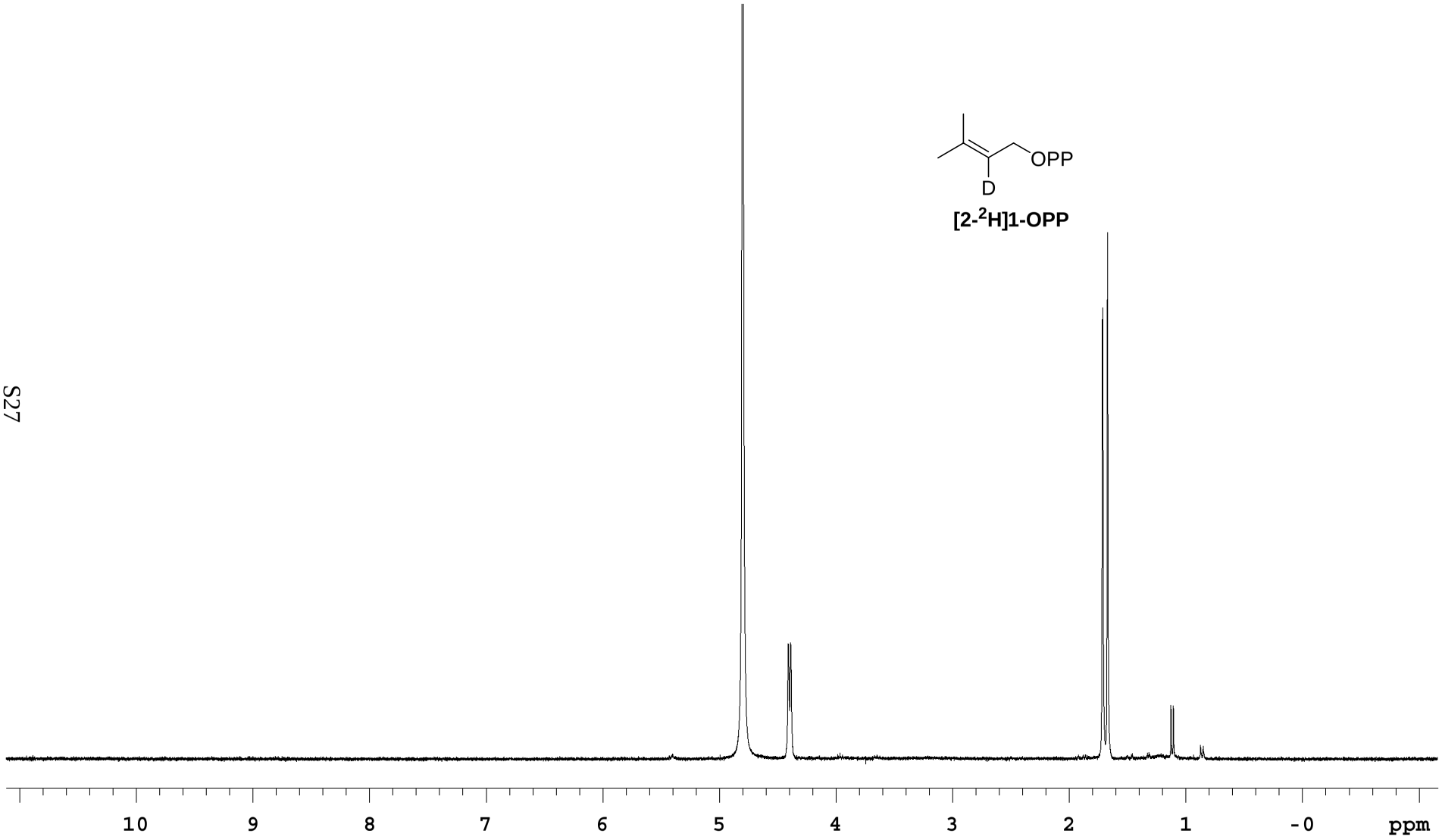

O
OEt
D
[2-2H]2
10
9
8
7
6
5
4
3
2
1
-0
ppm

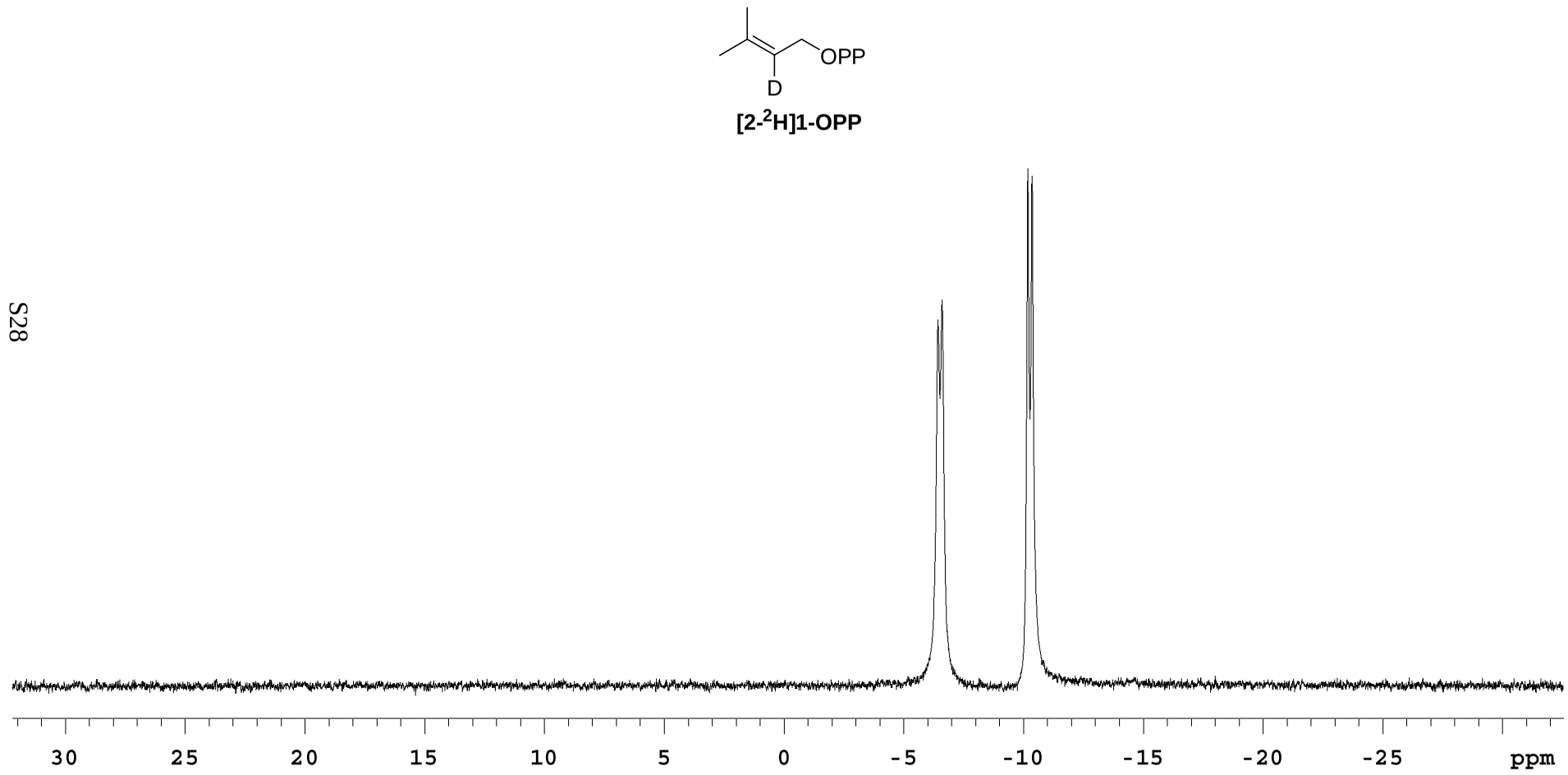

O
OEt
D
[2-2H]2
ppm
20
40
60
80
100
120
140
160
180
200



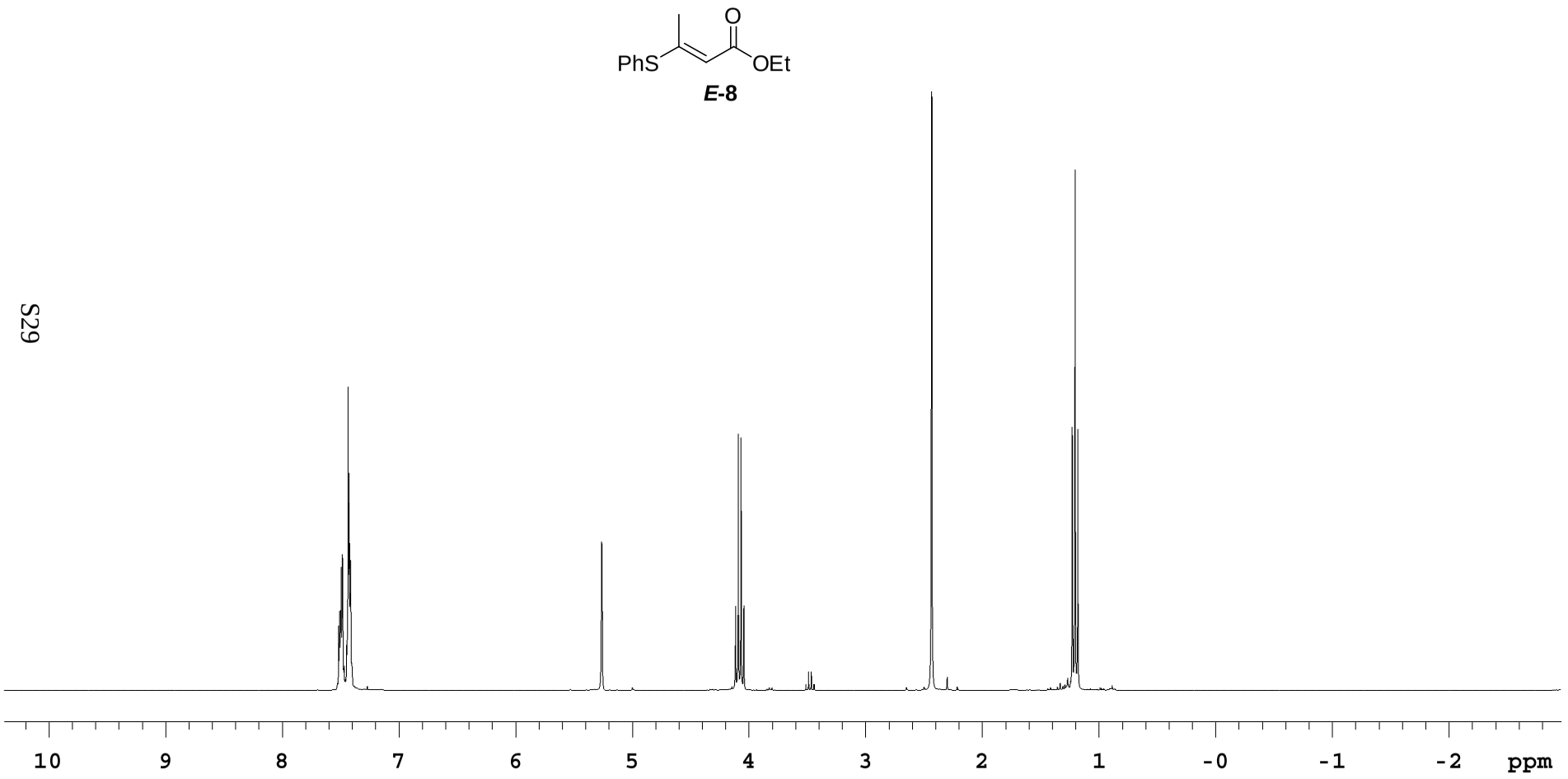
[2-^{2}H]1-OH

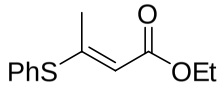

OH
D
[2-^{2}H]1-OH
180
160
140
120
100
80
60
40
20
ppm


OPP
D
[2-^{2}H]1-OPP
10
9
8
7
6
5
4
3
2
1
-0
ppm

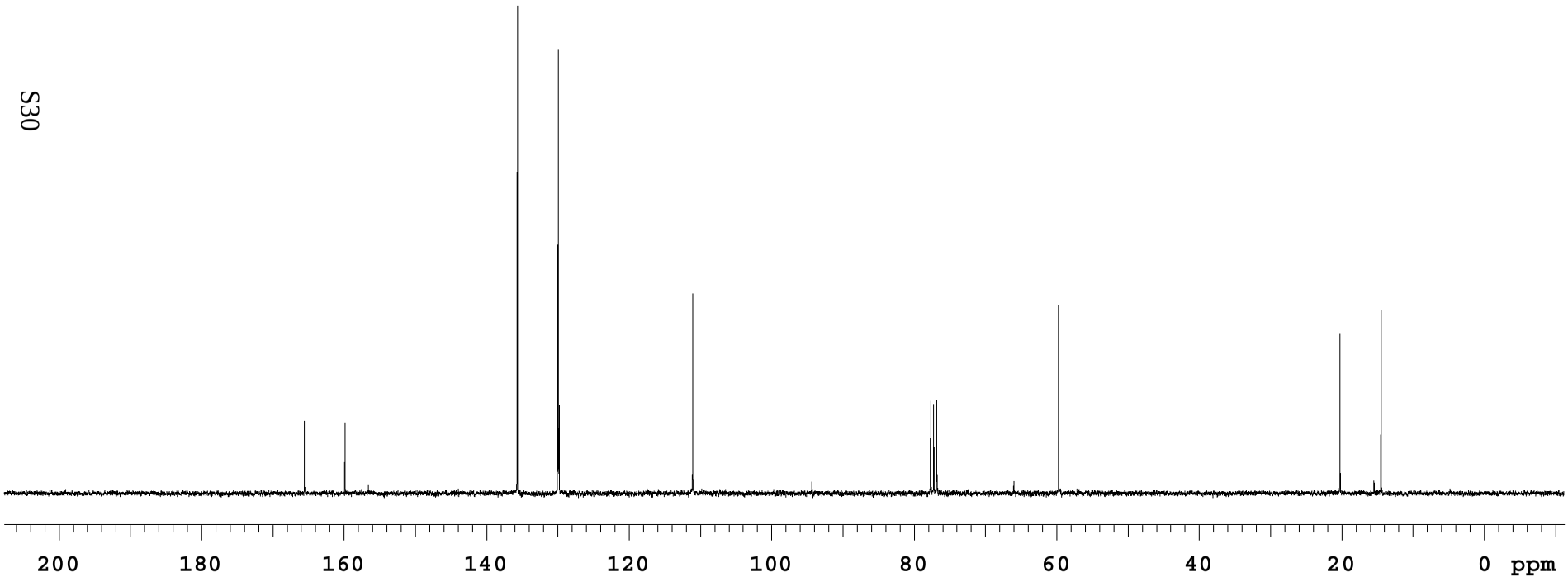


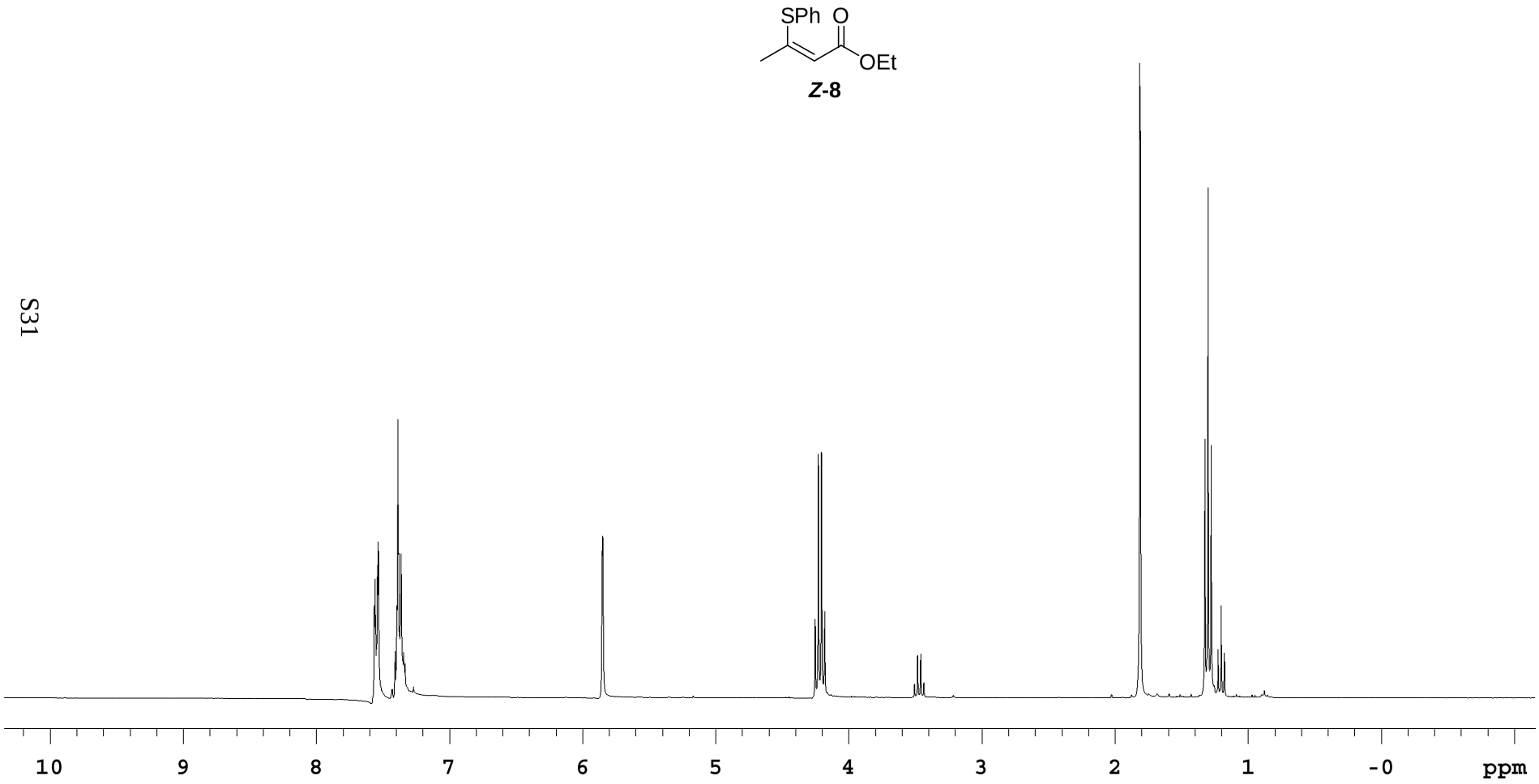
[2-^{2}H]1-OPP

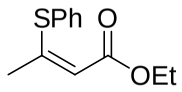

O
PhS
OEt
E-8
10
9
8
7
6
5
4
3
2
1
-0
-1
-2
ppm



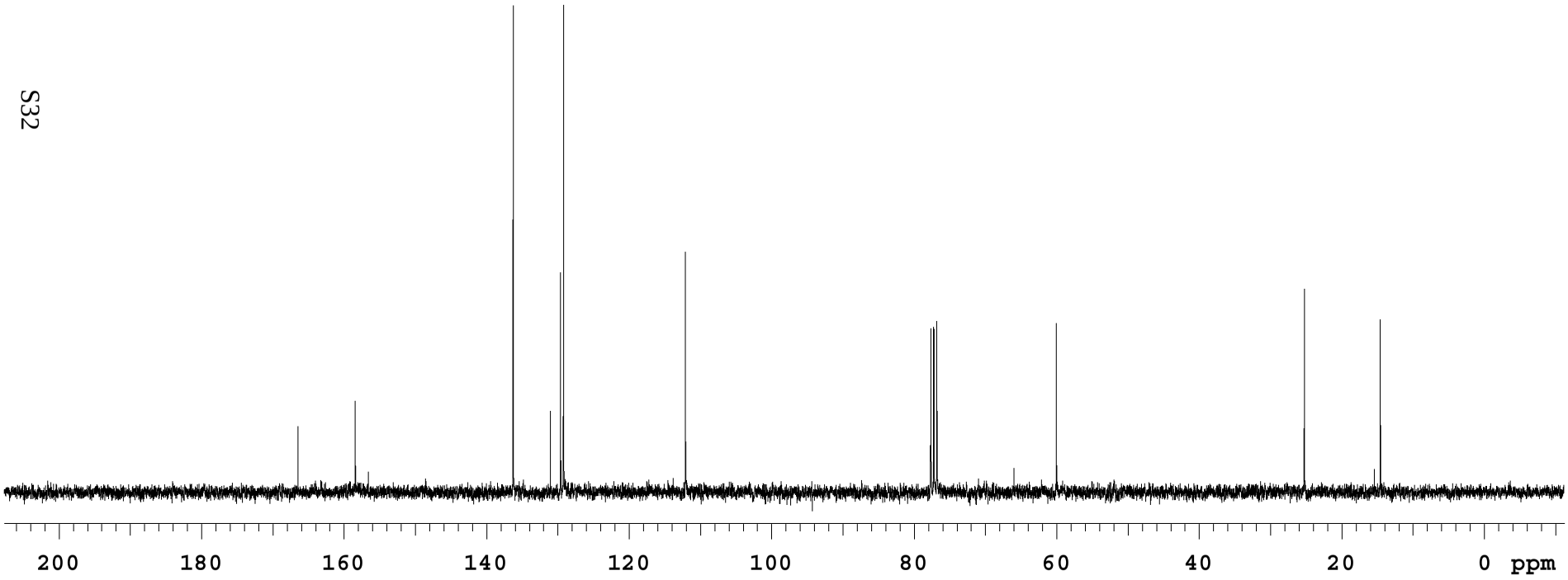
***E*-8**

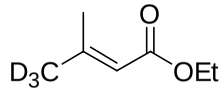



SPh
O
OEt
Z-8
10
9
8
7
6
5
4
3
2
1
-0
ppm

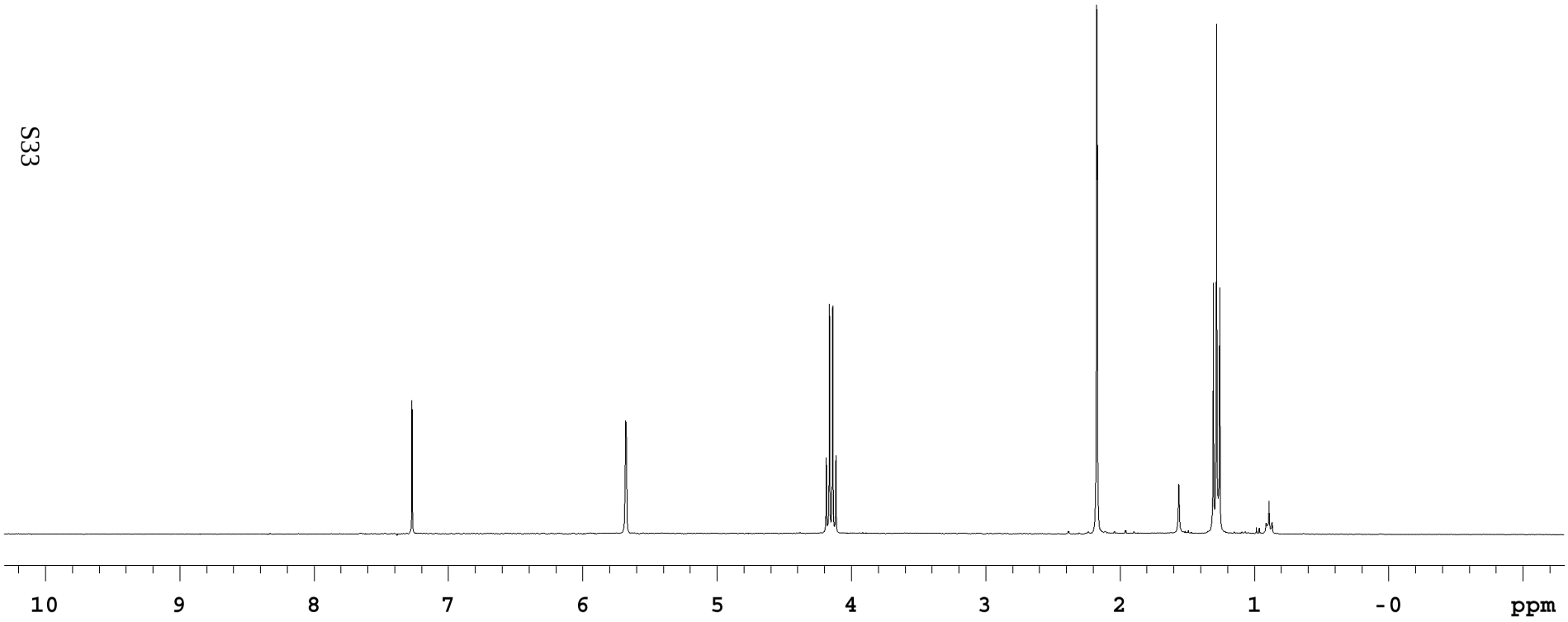


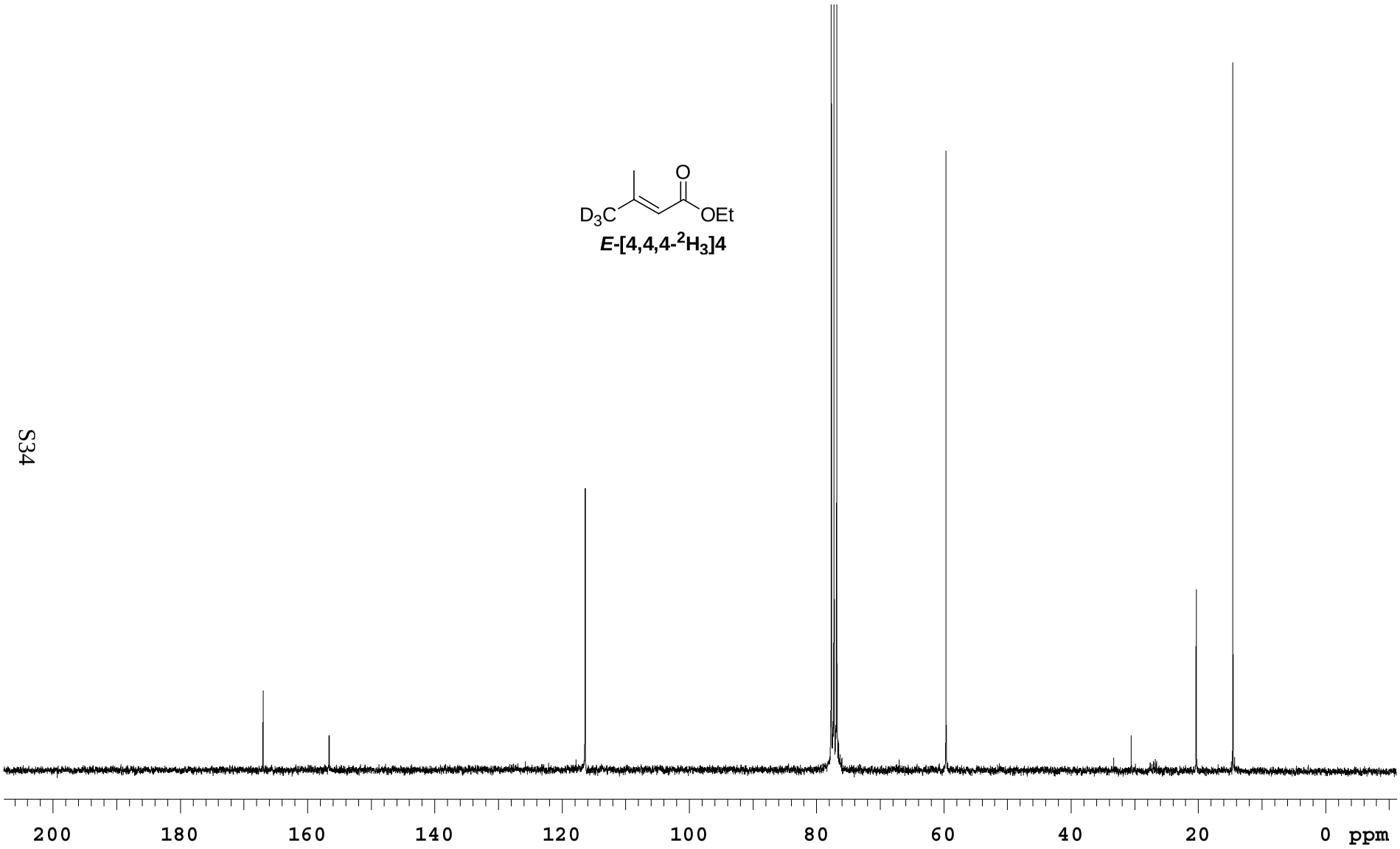
***Z*-8**





***E*-[4,4,4-$^{2}H_3$]4**




O
D3C
OEt
E-[4,4,4-2H3]4
200
180
160
140
120
100
80
60
40
20
0
ppm