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Discovery of diarylphosphoryl and diaminophosphoryl substituted 1,3,4-thiadiazoles for combination therapy of breast cancer

Denis Yu. Uvarov, Alexander M. Scherbakov, Diana I. Salnikova, Tatiana A. Bogush, Igor V. Zavarzin and Yulia A. Volkova

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Experimental Section

Chemistry

General Information

NMR spectra were acquired at room temperature; the chemical shifts δ were measured in ppm relative to the solvent (^{1}H: $CDCl_3$, δ = 7.27 ppm, DMSO-d_6, δ = 2.50 ppm; ^{13}C: $CDCl_3$, δ = 77.00 ppm, DMSO-d_6, δ = 39.50 ppm). Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, double doublet;. The coupling constants (J) are in Hertz. High-resolution and accurate mass spectra were obtained on BrukermicrOTOF-QTM ESI-TOF (Electrospray Ionization/Time of Flight) and Thermo Fisher Scientific* LTQ Orbitrap mass spectrometers. Mass spectra were recorded on a Finnigan Mat INCOS 50 Quadrupole Mass Spectrometer. Melting points (m.p.) are uncorrected and were measured on a Boetius capillary melting point apparatus. Analytical thin layer chromatography (TLC) was carried out on silica gel plates (silica gel 60 F254 aluminum supported plates); the visualization was accomplished with an UV lamp (365 nm). Column chromatography was performed on silica gel 60 (230-400 mesh, Merck). Aldehydes and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone were commercially available and were used without additional purification. Parent phosphorylthioformic acid hydrazides were prepared according to published procedures.[S1,S2] All reactions were carried out using freshly distilled and dry solvents.

Procedures

General procedures for the synthesis of 1,3,4-thiadiazoles 3.

Procedure A. Aldehyde **1** (0.17 mmol, 1.1 equiv) was added to a boiling solution of thiohydrazide **2a,b** (0.15 mmol, 1.0 equiv) in MeOH (7 mL). Resulting mixture was refluxed for 5 min and cooled to r.t. Next DDQ (37 mg, 0.17 mmol, 1.1 equiv) was added a solution and mixture was stirred for 30 min at r.t. Solvent was removed under reduced pressure and product was isolated by column chromatography (eluent: CH_2Cl_2 – MeOH, 1 : 0 → 150 : 1).

Procedure B. Aldehyde **1** (0.78 mmol, 5.2 equiv) was added to a boiling solution of complex **2c**·$2NH_2NH_2$·H_2O (58 mg, 0.15 mmol, 1.0 equiv) in MeOH (7 mL). Resulting mixture was refluxed for 5 min and cooled to r.t. (in case of azine formation, it was removed by filtration). Next DDQ (37 mg, 0.17 mmol, 1.1 equiv) was added a solution and mixture was stirred for 30 min at r.t. Solvent was removed under reduced pressure and product was isolated by column chromatography (eluent: $CHCl_3$ – MeOH, 1 : 0 → 100 : 1).

Diphenyl(5-phenyl-1,3,4-thiadiazol-2-yl)phosphine oxide (3a). The general procedure **A** was followed using 18 mg of benzaldehyde. Product was obtained as a pale yellow solid (42 mg, 71% yield), m.p. 171 - 174 °C, R_f 0.46 ($CHCl_3$ – MeOH, 50 : 1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.07 – 7.94 (m, 6H, 6 × CH), 7.66 – 7.46 (m, 9H, 9 × CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.6 (d, $J_{C\text{-}P}$ = 2.9 Hz, C-P), 166.5 (d, $J_{C\text{-}P}$ = 114.0 Hz, C-P), 133.0 (d, $J_{C\text{-}P}$ = 2.9 Hz, C-P), 132.0 (CH), 132.0 (2 × CH), 131.8 (2 × CH), 130.7 (d, $J_{C\text{-}P}$ = 111.1 Hz, C-P), 129.3 (C), 129.3 (d, $J_{C\text{-}P}$ = 37.1 Hz, C-P), 128.7 (d, $J_{C\text{-}P}$ = 23.2 Hz, C-P).^{31}P NMR (122 MHz, $CDCl_3$): δ = 18.96. HRMS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_{20}H_{16}N_2OPS^+$: 363.0715; found: 363.0712.

Diphenyl(5-(p-tolyl)-1,3,4-thiadiazol-2-yl)phosphine oxide (3b). The general procedure **A** was followed using 20 mg of *p*-tolualdehyde. Product was obtained as a pale yellow solid (39 mg, 68% yield), m.p. 174 - 178 °C, R_f 0.46 ($CHCl_3$ – MeOH, 50 : 1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.05 – 7.94 (m, 4H, 4 × CH), 7.91 (d, $J_{H\text{-}H}$ = 8.1 Hz, 2 × CH), 7.64 – 7.48 (m, 6H, 6 × CH), 7.30 (d, $J_{H\text{-}H}$ = 8.1 Hz, 2H, 2 × CH), 2.43 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 142.6 (C), 132.9 (d, $J_{C\text{-}P}$ = 3.0 Hz, C-P), 131.8 (d, $J_{C\text{-}P}$ = 10.5 Hz, C-P), 130.7 (d, $J_{C\text{-}P}$ = 111.1 Hz, C-P), 130.1 (2 × CH), 128.7 (2 × CH), 128.6 (d, $J_{C\text{-}P}$ = 39.9 Hz, C-P), 21.6 (Ar-CH_3). ^{31}P NMR (122 MHz, $CDCl_3$): δ = 18.87. HRMS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_{21}H_{18}N_2OPS^+$: 377.0872; found: 377.0875.

[5-(4-Fluoro-3-phenoxyphenyl)-1,3,4-thiadiazol-2-yl]diphenylphosphine oxide (3c).[S1] The general procedure **A** was followed using 37 mg of 4-fluoro-3-phenoxybenzaldehyde. Product was obtained as a pale yellow solid (59 mg, 83% yield), m.p. 101 - 102 °C, R_f 0.46 ($CHCl_3$ – MeOH, 50 : 1). IR (KBr) 3060, 3047, 3014, 2924, 2853, 1589, 1515, 1489, 1422, 1390, 1277, 1203, 1164, 1122, 1104, 1073, 898, 726, 700, 688, 623, 553, 516 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.93 – 7.89 (m, 1H, CH), 7.88 – 7.79 (m, 5H, 5 × CH), 7.70 (dd, $J_{H\text{-}H}$ = 7.7 Hz, 2H, 2 × CH), 7.66 – 7.58 (m, 5H, 5 × CH), 7.42 (dd, $J_{H\text{-}H}$ = 7.9 Hz, 2H, 2 × CH), 7.19 (dd, $J_{H\text{-}H}$ = 7.9 Hz, 1H, CH), 7.09 (d, $J_{H\text{-}H}$ = 7.9 Hz, 2H, 2 × CH). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 170.2 (C), 166.7 (d, $^1J_{C\text{-}P}$ = 111.7 Hz, C-P), 156.1 (C), 155.5 (d, $J_{C\text{-}F}$ = 253.6 Hz, C-F), 144.0 (d, $^2J_{C\text{-}F}$ = 12.1 Hz, C), 133.2 (2 × CH), 131.3 (d, $^2J_{C\text{-}P}$ = 11.1 Hz, 4 × CH), 130.4 (d, $^1J_{C\text{-}P}$ = 109.7 Hz, 2 × C), 130.2 (2 × CH), 129.1 (d, $^3J_{C\text{-}P}$ = 13.1 Hz, 4 × CH), 126.0 (C), 125.8 (d, $^3J_{C\text{-}F}$ = 8.0 Hz, CH), 124.0 (CH), 120.6 (CH), 118.5 (d, $^2J_{C\text{-}F}$ = 19.1 Hz, CH), 117.6 (2 × CH). ^{31}P NMR (162 MHz, DMSO-d_6): δ = 17.70. ^{19}F NMR (376 MHz, DMSO-d_6): δ = -126.65. HRMS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_{26}H_{19}FN_2O_2PS^+$: 473.0883; found: 473.0877.

[5-(4-(Benzyloxy)phenyl)-1,3,4-thiadiazol-2-yl]diphenylphosphine oxide (3d).[S1] The general procedure **A** was followed using 36 mg of 4-(benzyloxy)benzaldehyde. Product was obtained as a pale yellow solid (44 mg, 63% yield), m.p. 155 - 156 °C, R_f 0.61 ($CHCl_3$ – MeOH, 25 : 1). IR (KBr) 3060, 3031, 2936, 2875, 1604, 1517, 1438, 1383, 1259, 1198, 1174, 1121, 1006, 830, 756, 728, 699, 640, 575, 546, 532 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.05 – 7.97 (m, 2H, 2 × CH), 7.91 – 7.82 (m, 4H, 4 × CH), 7.74 – 7.67 (m, 2H, 2 × CH), 7.66 – 7.59 (m, 4H, 4 × CH), 7.50 – 7.43 (m, 2H, 2 × CH), 7.43 – 7.37 (m, 2H, 2 × CH), 7.37 – 7.31 (m, 1H, CH), 7.23 – 7.16 (m, 2H, 2 × CH), 5.21 (s, 2H, CH_2). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 171.7 (C), 165.2 (d, $^1J_{C\text{-}P}$ = 113.3 Hz, C-P), 161.4 (C), 136.4 (C), 133.2 (2 × CH), 131.4 (d, $^2J_{C\text{-}P}$ = 10.4 Hz, 4 × CH), 130.6 (d, $^1J_{C\text{-}P}$ = 109.7 Hz, 2 × C), 130.2 (2 × CH), 129.1 (d, $^3J_{C\text{-}P}$ = 12.5 Hz, 4 × CH), 128.5 (2 × CH), 128.0 (CH), 127.8 (2 × CH), 121.3 (C), 115.8 (2 × CH), 69.6 (CH_2). ^{31}P NMR (162 MHz, DMSO-d_6): δ = 17.59. HRMS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_{27}H_{22}N_2O_2PS^+$: 469.1134; found: 469.1128.

***P*-[5-(Phenyl)-1,3,4-thiadiazol-2-yl]-*N,N'*-di-*tert*-butylphosphonic diamide (3e).**[S1] The general procedure **A** was followed using 18 mg of benzaldehyde. Product was obtained as a pale yellow solid (50 mg, 95% yield), m.p. 200 - 210 °C, R_f 0.33 ($CHCl_3$ – MeOH, 50 : 1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.10 – 7.96 (m, 2H, 2 × CH), 7.59 – 7.44 (m, 3H, 3 × CH), 3.51 (br. s, 2H, 2 × NH), 1.37 (s, 18H, 6 × CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.0 (d, $^3J_{C\text{-}P}$ = 3.3 Hz, C-P), 169.3 (d, $^1J_{C\text{-}P}$ = 168.7 Hz, C-P), 131.6 (C), 130.0 (CH), 129.3 (2 × CH), 128.3 (2 × CH), 52.6 (2 × C), 31.9 (d, $^3J_{C\text{-}P}$ = 4.5 Hz, 6 × CH_3). ^{31}P NMR (122 MHz, $CDCl_3$): δ = 1.21. HRMS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_{16}H_{26}N_4OPS^+$: 353.1559; found: 353.1562.

***P*-[5-(*p*-Tolyl)-1,3,4-thiadiazol-2-yl]-*N,N'*-di-*tert*-butylphosphonic diamide (3f).** The general procedure **A** was followed using 20 mg of *p*-tolualdehyde. Product was obtained as a pale yellow solid (44 mg, 80% yield), m.p. 181 – 185 °C, R_f 0.34 ($CHCl_3$ – MeOH, 50 : 1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.89 (d, J_{H-H} = 8.1 Hz, 2H, 2 × CH), 7.29 (d, J_{H-H} = 8.0 Hz, 2H, 2 × CH), 3.22 (d, J_{H-P} = 10.1 Hz, 2H, NH-P), 2.42 (s, 3H, CH_3), 1.36 (s, 18H, 6 × CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.1 (d, J_{C-P} = 3.3 Hz, C-P), 168.8 (d, J_{C-P} = 168.8 Hz, C-P), 142.1 (CH), 130.0 (2 × CH), 128.2 (2 × CH), 126.9 (C), 52.6 (C), 31.9 (d, J_{C-P} = 4.5 Hz, C-P), 21.6 (Ar-CH_3). ^{31}P NMR (122 MHz, $CDCl_3$): δ = 1.38. HRMS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_{16}H_{26}N_4OPS^+$: 353.1568; found: 353.1559.

***P*-[5-(4-Ethoxyphenyl)-1,3,4-thiadiazol-2-yl]-*N,N'*-diphenylphosphonic diamide (3g).**[S1] The general procedure **B** was followed using 117 mg of 4-ethoxybenzaldehyde. Product was obtained as a pale yellow solid (48 mg, 72% yield), m.p. 174 - 175 °C, R_f 0.53 ($CHCl_3$ – MeOH, 50 : 1). IR (KBr) 3154, 2085, 2978, 2894, 1603, 1498, 1398, 1282, 1259, 1239, 1177, 1080, 1034, 952, 924, 751, 693, 537, 535, 506 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.60 (d, J_{H-P} = 11.0 Hz, 2H, 2 × NH), 8.03 – 7.93 (m, 2H, 2 × CH), 7.27 – 7.18 (m, 8H, 8 × CH), 7.13 – 7.05 (m, 2H, 2 × CH), 6.95 – 6.86 (m, 2H, 2 × CH), 4.13 (q, J_{H-H} = 7.0 Hz, 2H, CH_2), 1.36 (t, J_{H-H} = 7.0 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 171.3 (C), 163.5 (d, $^1J_{C-P}$ = 181.6 Hz, C-P), 161.4 (C), 140.5 (2 × C), 129.9 (2 × CH), 128.9 (4 × CH), 121.4 (2 × CH), 121.1 (C), 118.4 (d, $^3J_{C-P}$ = 7.1 Hz, 4 × CH), 115.3 (2 × CH), 63.5 (CH_2), 14.4 (CH_3). ^{31}P NMR (162 MHz, DMSO-d_6): δ = -3.76. HRMS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_{22}H_{22}N_4O_2PS^+$: 437.1196; found: 437.1189.

***P*-[5-(4-Acetoxy-3-methoxyphenyl)-1,3,4-thiadiazol-2-yl]-*N,N'*-diphenylphosphonic diamide (3h).**[S1] The general procedure **B** was followed using 151 mg of 4-acetoxy-3-methoxybenzaldehyde. Product was obtained as a pale yellow solid (54 mg, 75% yield), m.p. 116 - 117 °C, R_f 0.39 ($CHCl_3$ – MeOH, 50 : 1). IR (KBr) 3158, 3086, 2963, 2928, 1769, 1601, 1498, 1414, 1370, 1280, 1211, 1196, 1168, 1125, 1080, 1034, 1005, 956, 929, 861, 823, 752, 693, 657, 627, 505 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.64 (d, J_{H-P} = 11.0 Hz, 2H, 2 × NH), 7.72 (d, J_{H-H} = 2.0 Hz, 1H, CH), 7.64 (dd, J_{H-H} = 8.2, 2.0 Hz, 1H, CH), 7.29 (d, J_{H-H} = 8.2 Hz, 1H, CH), 7.26 – 7.17 (m, 8H, 8 × CH), 6.94 – 6.88 (m, 2H, 2 × CH), 3.88 (s, 3H, CH_3), 2.29 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 170.8 (C), 168.2 (C=O), 164.9 (d, $^1J_{C-P}$ = 179.7 Hz, C-P), 151.5 (C), 142.1 (C), 140.4 (2 × C), 129.0 (4 × CH), 127.5 (C), 124.1 (CH), 121.5 (2 × CH), 121.1 (CH), 118.4 (d, $^3J_{C-P}$ = 7.2 Hz, 4 × CH), 112.1 (CH), 56.2 (CH_3), 20.4 (CH_3). ^{31}P NMR (162 MHz, DMSO-d_6): δ = -3.88. HRMS (ESI): *m*/*z* $[M+H]^+$calcd for $C_{23}H_{22}N_4O_4PS^+$: 481.1094; found: 481.1084.

***P*-[5-(2,3,4-Trimethoxyphenyl)-1,3,4-thiadiazol-2-yl]-*N,N'*-diphenylphosphonic diamide (3i).**[S1] The general procedure **B** was followed using 152 mg of 2,3,4-trimethoxybenzaldehyde. Product was obtained as a pale yellow solid (50 mg, 70% yield), m.p. 170 - 171 °C, R_f 0.50 ($CHCl_3$ – MeOH, 35 : 1). IR (KBr) 3258, 3120, 3082, 2969, 2898, 2842 1595, 1498, 1470, 1423, 1381, 1297, 1226, 1186, 1101, 1072, 1032, 985, 928, 858, 806, 770, 751, 695, 644, 518, 482, 424 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.55 (d, J_{H-P} = 11.0 Hz, 2H, 2 × NH), 8.08 (d, J_{H-H} = 9.0 Hz, 1H, CH), 7.27 – 7.18 (m, 8H, 8 × CH), 7.06 (d, J_{H-H} = 9.0 Hz, 1H, CH), 6.90 (tt, J_{H-H} = 6.9, 1.6 Hz, 2H, 2 × CH), 3.97 (s, 3H, CH_3), 3.90 (s, 3H, CH_3), 3.82 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 164.8 (C), 163.9 (d, $^1J_{C-P}$ = 180.5 Hz, C-P), 156.4 (CH), 150.8 (C), 141.5 (C), 140.6 (2 × C), 128.9 (4 × CH), 122.9 (CH), 121.3 (2 × CH), 118.4 (d, $^3J_{C-P}$ = 7.1 Hz, 4 × CH), 115.2 (C), 108.9 (C), 60.7 (CH_3), 60.6 (CH_3), 56.2 (CH_3). ^{31}P NMR (162 MHz, DMSO-d_6): δ = -3.08. HRMS (ESI): *m*/*z* $[M+H]^+$ calcd for $C_{23}H_{24}N_4O_4PS^+$: 483.1250; found: 483.1244.

***P*-[5-(4-Bromophenyl)-1,3,4-thiadiazol-2-yl]-*N,N'*-diphenylphosphonic diamide (3j).**[S1] The general procedure **B** was followed using 144 mg of 4-bromobenzaldehyde. Product was obtained as a pale yellow solid (61 mg, 86% yield), m.p. 189 – 190 °C, R_f 0.47 ($CHCl_3$ – MeOH, 25 : 1). IR (KBr) 3131, 3085, 2964, 2884, 1600, 1497, 1396, 1383, 1281, 1221, 1072, 957, 828, 752, 693, 537, 503 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.66 (d, J_{H-P} = 11.1 Hz, 2H, 2 × NH), 8.00 (d, J_{H-H} = 7.8 Hz, 2H, 2 × CH), 7.78 (d, J_{H-H} = 7.8 Hz, 2H, 2 × CH), 7.31 – 7.11 (m, 8H, Ph), 7.00 – 6.82 (m, 2H, Ph). ^{13}C NMR (150 MHz, DMSO-d_6): δ = 170.5 (C), 165.0 (d, $^1J_{C-P}$ = 181.0 Hz, C), 140.4 (2 × C), 132.6 (2 × CH),

130.1 (2 × CH), 129.0 (4 × CH), 128.0 (C), 125.6 (C), 121.5 (2 × CH), 118.4 (d, $^{3}J_{C\text{-}P}$ = 7.5 Hz, 4 × CH). ^{31}P NMR (121 MHz, DMSO-d_6): δ = -3.71. HRMS (ESI): *m*/*z* [M+Na]$^{+}$ calcd for $C_{20}H_{16}NaBrN_4OPS^{+}$: 492.9858; found: 492.9855.

***P*-[5-(Antracene-9-yl)-1,3,4-thiadiazol-2-yl]-*N,N'*-diphenylphosphonic diamide (3k).**[S1] The general procedure **B** was followed using 161 mg of anthracene-9-carbaldehyde. Product was obtained as a pale yellow solid (49 mg, 67% yield), m.p. 229 - 230 ^{o}C, R_f 0.26 ($CHCl_3$ – MeOH, 25 : 1). IR (KBr) 3139, 3083, 2971, 2894, 1600, 1497, 1418, 1373, 1283, 1216, 1180, 1128, 1081, 1032, 956, 930, 752, 736, 692, 502 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.93 (s, 1H, CH), 8.75 (d, $J_{H\text{-}P}$ = 11.1 Hz, 2H, 2 × NH), 8.24 (dd, $J_{H\text{-}H}$ = 1.8, 7.5 Hz, 2H, CH), 7.66 – 7.54 (m, 4H, 4 × CH), 7.47 (dd, $J_{H\text{-}H}$ = 1.9, 7.5 Hz, 2H, 2 × CH), 7.35 – 7.23 (m, 8H, 8 × CH), 6.95-7.01 (m, 2H, CH). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 167.6 (d, $^{3}J_{C\text{-}P}$ = 2.2 Hz, C), 167.5 (d, $^{1}J_{C\text{-}P}$ = 178.2 Hz, C-P), 140.5 (2 × C), 130.5 (CH), 130.5 (3 × C), 130.4 (2 × C), 129.0 (4 × CH), 128.8 (2 × CH), 127.7 (2 × CH), 125.8 (2 × CH), 124.3 (2 × CH), 121.6 (2 × CH), 118.7 (d, $^{3}J_{C\text{-}P}$ = 6.9 Hz, 4 × CH). ^{31}P NMR (162 MHz, DMSO-d_6): δ = -3.85. HRMS (ESI): *m*/*z* [M+H]$^{+}$ calcd for $C_{28}H_{22}N_4OPS^{+}$: 493.1246; found: 493.1240.

***P*-[5-(5-Methylthiophen-2-yl)-1,3,4-thiadiazol-2-yl]-*N,N'*-diphenylphosphonic diamide (3l).**[S1] The general procedure **B** was followed using 100 mg of 5-methylthiophene-2-carbaldehyde. Product was obtained as a pale yellow solid (42 mg, 68% yield), m.p. 213 - 214 ^{o}C, R_f 0.38 ($CHCl_3$ – MeOH, 50 : 1). IR (KBr) 3163, 3086, 2972, 2894, 1601, 1497, 1478, 1390, 1283, 1224, 1076, 1032, 952, 799, 751, 692, 624, 505 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.60 (d, $J_{H\text{-}P}$ = 11.1 Hz, 2H, 2 × NH), 7.72 (d, $J_{H\text{-}H}$ = 3.8 Hz, 1H, CH), 7.25 – 7.17 (m, 8H, 8 × CH), 6.96 (dd, $J_{H\text{-}H}$ = 1.2, 3.8 Hz, 1H, CH), 6.93 – 6.87 (m, 2H, 2 × CH), 2.52 (d, $J_{H\text{-}H}$ = 1.2 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 165.3 (d, $^{3}J_{C\text{-}P}$ = 2.4 Hz, C), 163.2 (d, $^{1}J_{C\text{-}P}$ = 180.8 Hz, C-P), 145.8 (C), 140.4 (2 × C), 132.7 (CH), 128.9 (4 × CH), 128.0 (C), 127.5 (CH), 121.4 (2 × CH), 118.4 (d, $^{3}J_{C\text{-}P}$ = 7.2 Hz, 4 × CH), 15.1 (CH_3). ^{31}P NMR (162 MHz, DMSO-d_6): δ = -4.01. HRMS (ESI): *m*/*z* [M+H]$^{+}$ calcd for $C_{19}H_{18}N_4OPS_2^{+}$: 413.0650; found: 413.0647.

***P*-[5-(Pyridine-3-yl)-1,3,4-thiadiazol-2-yl]-*N,N'*-diphenylphosphonic diamide (3m).**[S1] The general procedure **A** was followed using 83 mg of nicotinaldehyde. Product was obtained as a pale yellow solid (51 mg, 86% yield), m.p. 217 - 218 ^{o}C, R_f 0.42 ($CHCl_3$ – MeOH, 20 : 1). IR (KBr) 3125, 3069, 2957, 2873, 2808, 1596, 1489, 1405, 1379, 1283, 1221, 1087, 1029, 968, 930, 812, 760, 698, 682, 620, 527, 506 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 9.22 (d, $J_{H\text{-}H}$ = 3.4 Hz, 1H, CH), 8.77 (dd, $J_{H\text{-}H}$ = 2.7, 4.7 Hz, 1H, CH), 8.71 (d, $J_{H\text{-}P}$ = 11.2 Hz, 2H, 2 × NH), 8.49 – 8.38 (m, 1H, CH), 7.61 (dd, $J_{H\text{-}H}$ = 8.4 Hz, *J* = 4.7 Hz, 1H, CH), 7.29 – 7.17 (m, 8H, 8 × CH), 6.97 – 6.86 (m, 2H, 2 × CH). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 168.7 (d, $^{3}J_{C\text{-}P}$ = 2.4 Hz, C), 165.4 (d, $^{1}J_{C\text{-}P}$ = 178.9 Hz, C-P), 152.4 (CH), 148.6 (CH), 140.4 (2 × C), 135.7 (CH), 129.0 (4 × CH), 125.2 (C), 124.4 (CH), 121.5 (2 × CH), 118.5 (d, $^{3}J_{C\text{-}P}$ = 7.2 Hz, 4 × CH). ^{31}P NMR (162 MHz, DMSO-d_6): δ = -4.00. HRMS (ESI): *m*/*z* [M+H]$^{+}$ calcd for $C_{19}H_{17}N_5OPS^{+}$: 394.0886; found: 394.0879.

***P*-[5-(1-Phenylethyl)-1,3,4-thiadiazol-2-yl]-*N,N'*-diphenylphosphonic diamide (3n).**[S1] The general procedure **A** was followed using 105 mg of 2-phenylpropanal. Product was obtained as a pale yellow solid (40 mg, 64% yield), m.p. 65 - 66 ^{o}C, R_f 0.45 ($CHCl_3$ – MeOH, 50 : 1). IR (KBr) 3158, 2974, 2894, 1601, 1497, 1453, 1399, 1281, 1224, 1079, 1032, 1002, 950, 751, 694, 630, 503 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.54 (d, $J_{H\text{-}P}$ = 10.6 Hz, 2H, 2 × NH), 7.41 – 7.33 (m, 4H, 4 × CH), 7.32 – 7.23 (m, 1H, CH), 7.23 – 7.09 (m, 8H, 8 × CH), 6.95 – 6.80 (m, 2H, 2 × CH), 4.81 (q, $J_{H\text{-}H}$ = 7.1 Hz, 1H, CH), 1.74 (d, $J_{H\text{-}H}$ = 7.1 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 178.6 (d, $^{3}J_{C\text{-}P}$ = 1.9 Hz, C), 164.9 (d, $^{1}J_{C\text{-}P}$ = 180.7 Hz, C-P), 142.9 (C), 140.6 (2 × C), 129.0 (3 × CH), 128.9 (4 × CH), 127.4 (2 × CH), 121.4 (2 × CH), 118.3 (d, $^{3}J_{C\text{-}P}$ = 7.0 Hz, 4 × CH), 40.1 (CH), 21.5 (CH_3). ^{31}P NMR (162 MHz, DMSO-d_6): δ = -3.78. HRMS (ESI): *m*/*z* [M+H]$^{+}$ calcd for $C_{22}H_{22}N_4OPS^{+}$: 421.1246; found: 421.1241.

Pharmacology/Biology

Evaluation of Antiproliferative Activity

The antiproliferative properties of the compounds were assessed *in vitro* using the MTT assay [S3] in the MCF-7 cell line (ATCC). MCF-7 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; PanEco) supplemented with 10% fetal bovine serum (FBS; HyClone), 50 U/mL penicillin, and 50 µg/mL streptomycin (PanEco). Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

Antiproliferative activity was determined using a modified MTT assay as described in [S4]. Stock solutions of the compounds (10 mM) were prepared in DMSO (AppliChem) and stored at -20 °C. Cells were seeded into 24-well plates, and after 24 h, the compounds were added at concentrations ranging from 3.1 to 50 µM. After 72 h of exposure, MTT reagent was added, and the resulting formazan crystals were dissolved in DMSO. Absorbance was measured at 571 nm using a MultiSkan FC reader (Thermo Fisher Scientific). IC_{50} values were calculated in GraphPad Prism 8 using nonlinear regression analysis. All experiments were performed in triplicate, and the data are presented as mean ± S.D.

Determination of the Synergistic Effect of the Studied Compounds in Therapeutic Combinations

MCF-7 cells were seeded into 24-well plates (Corning) at a density of 4×10^4 cells/well. After 24 h of incubation, test compound **3j** and the chemotherapeutic agents, either alone or in combination, were added to the cells based on the respective IC_{50} values obtained in the antiproliferative assay. The concentrations of the test compounds were prepared by serial dilution from the maximum concentrations indicated in Figure 3. For everolimus, for which reliable half-maximal inhibitory concentration data were unavailable, a maximum concentration of 10 µM was selected.

Cell viability was assessed colorimetrically 72 h after drug treatment using the MTT assay described above. The open-source program SynergyFinder+[S5] was used to calculate synergy scores for combinations of standard chemotherapeutic drugs with the test compound using the HSA model. This model identifies concentration ranges in which the observed combined effect exceeds the expected effect of the individual drugs administered separately. Based on the calculated values, heat maps were generated to evaluate the therapeutic significance of the drug combinations. In these heat maps, red regions indicate synergistic effects within the selected concentration range, and the therapeutic significance increases proportionally with both color intensity and numerical score values.

Table S1. Combined effect of the synthesized thiadiazole derivative **3j** with AKT Inhibitor X on the MCF-7 cell line (cell viability, %).

Comp. 3j, µM \ **AKT Inhibitor X, µM**	**0**	**0.22**	**0.44**	**0.88**	**1.8**	**3.5**
0	100	94	80	75	73	71
2.8	100	89	75	68	80	83
5.6	92	86	76	72	78	80
11.2	86	74	75	75	77	77
22.4	74	56	52	48	45	44

Table S2. Combined effect of the synthesized thiadiazole derivative **3j** with everolimus on the MCF-7 cell line (cell viability, %).

	0	**0.63**	**1.25**	**2.5**	**5**	**10**
0	100	89	85	83	82	76
2.8	100	82	76	70	78	74
5.6	92	72	69	72	77	73
11.2	86	73	69	73	75	71
22.4	74	68	63	61	59	59

Everolimus, μM

Comp. 3j, μM

Table S3. Combined effect of the synthesized thiadiazole derivative **3j** with doxorubicin on the MCF-7 cell line (cell viability, %).

	0	**0.02**	**0.04**	**0.08**	**0.16**	**0.24**
0	100	80	74	72	64	65
2.8	100	74	63	57	52	64
5.6	92	71	58	56	56	59
11.2	86	66	61	55	57	50
22.4	74	57	48	45	45	43

Doxorubicin, μM

Comp. 3j, μM

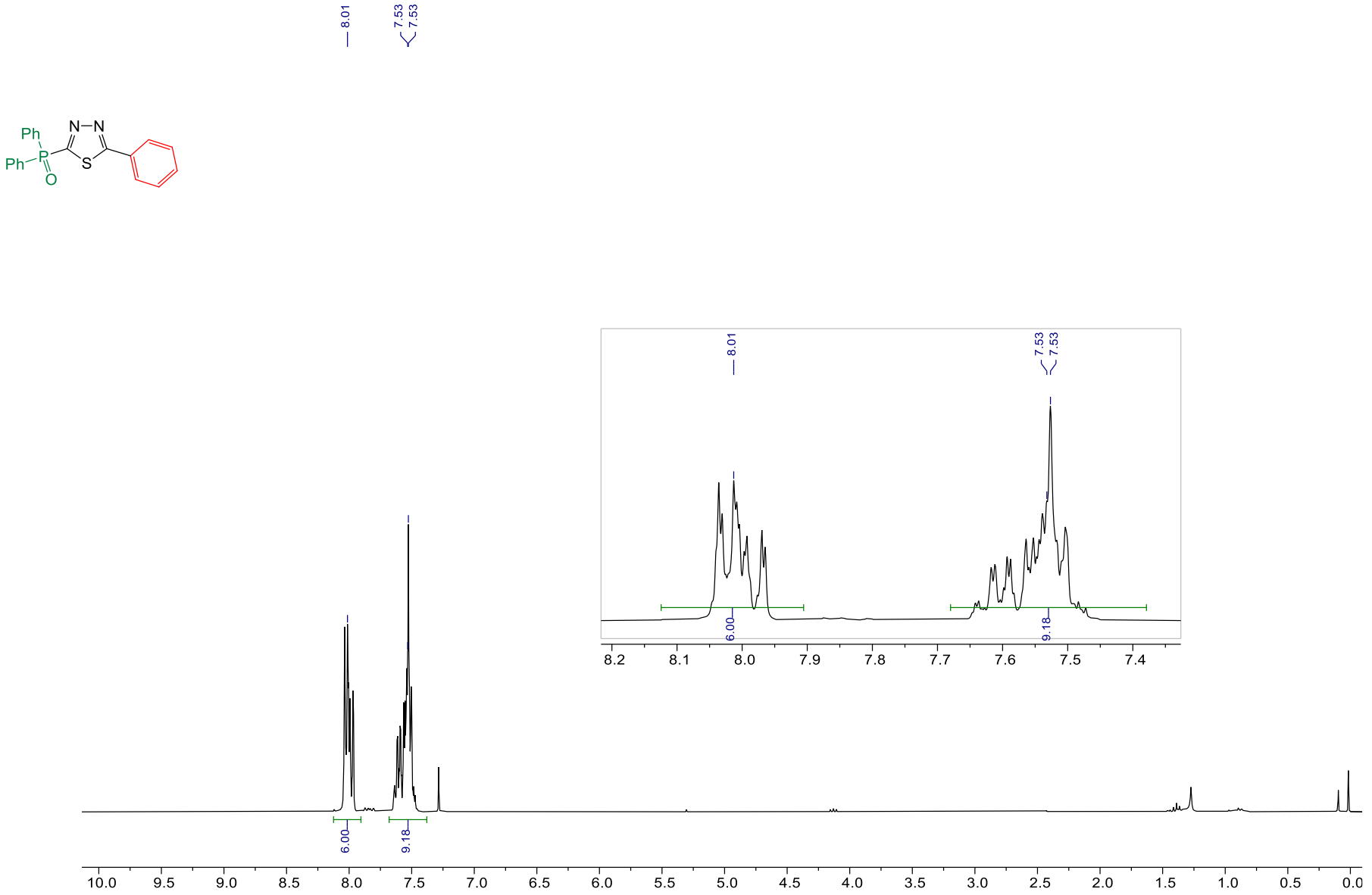
Table S4. Combined effect of the synthesized thiadiazole derivative **3j** with 4-hydroxytamoxifen on the MCF-7 cell line (cell viability, %).

	0	**0.4**	**0.8**	**1.6**	**3.2**	**6.4**
0	100	105	97	95	85	49
2.8	100	94	93	85	83	47
5.6	92	93	80	83	87	42
11.2	86	84	79	78	85	40
22.4	74	60	56	52	48	25

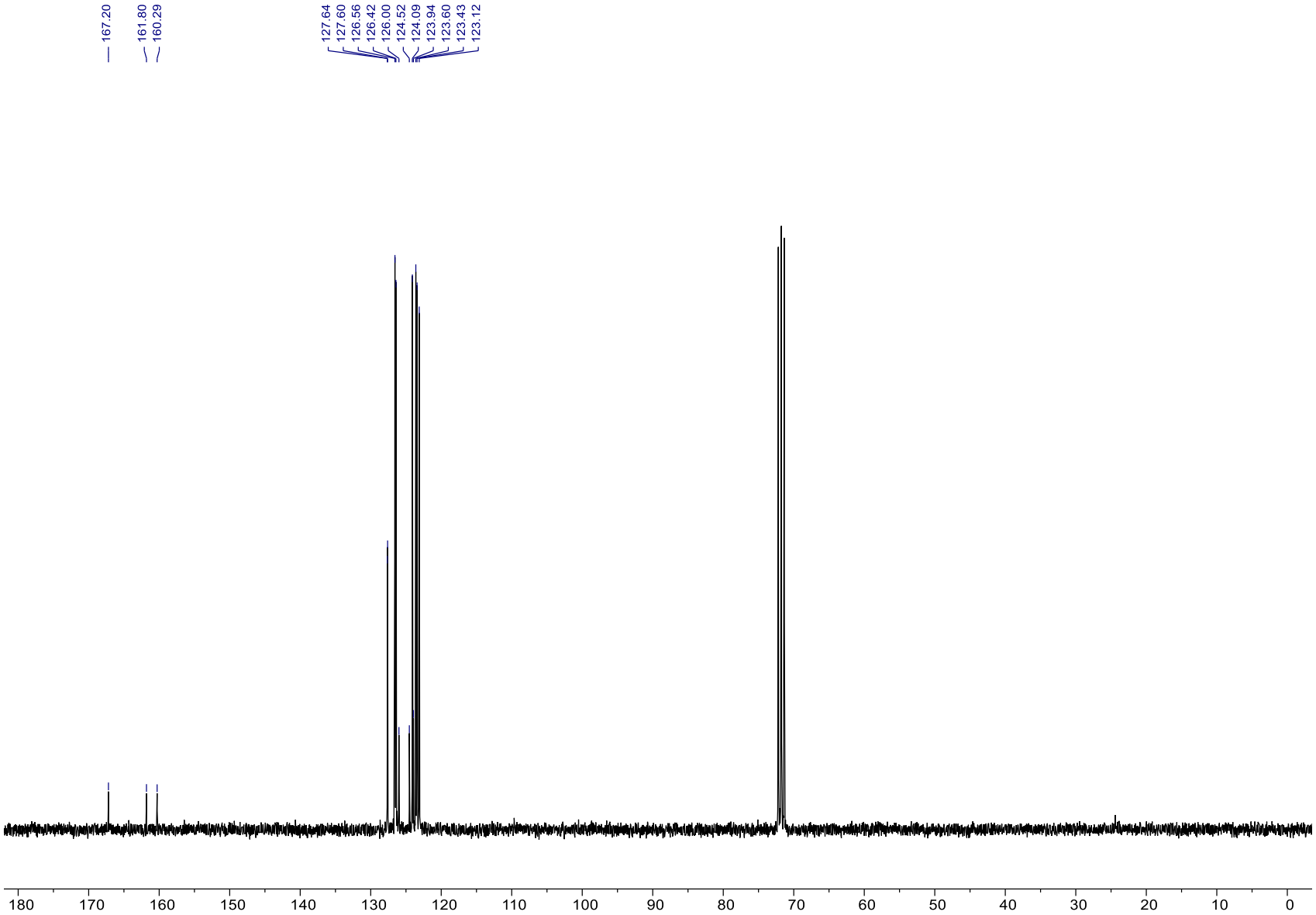
4-Hydroxytamoxifen, μM

Comp. 3j, μM

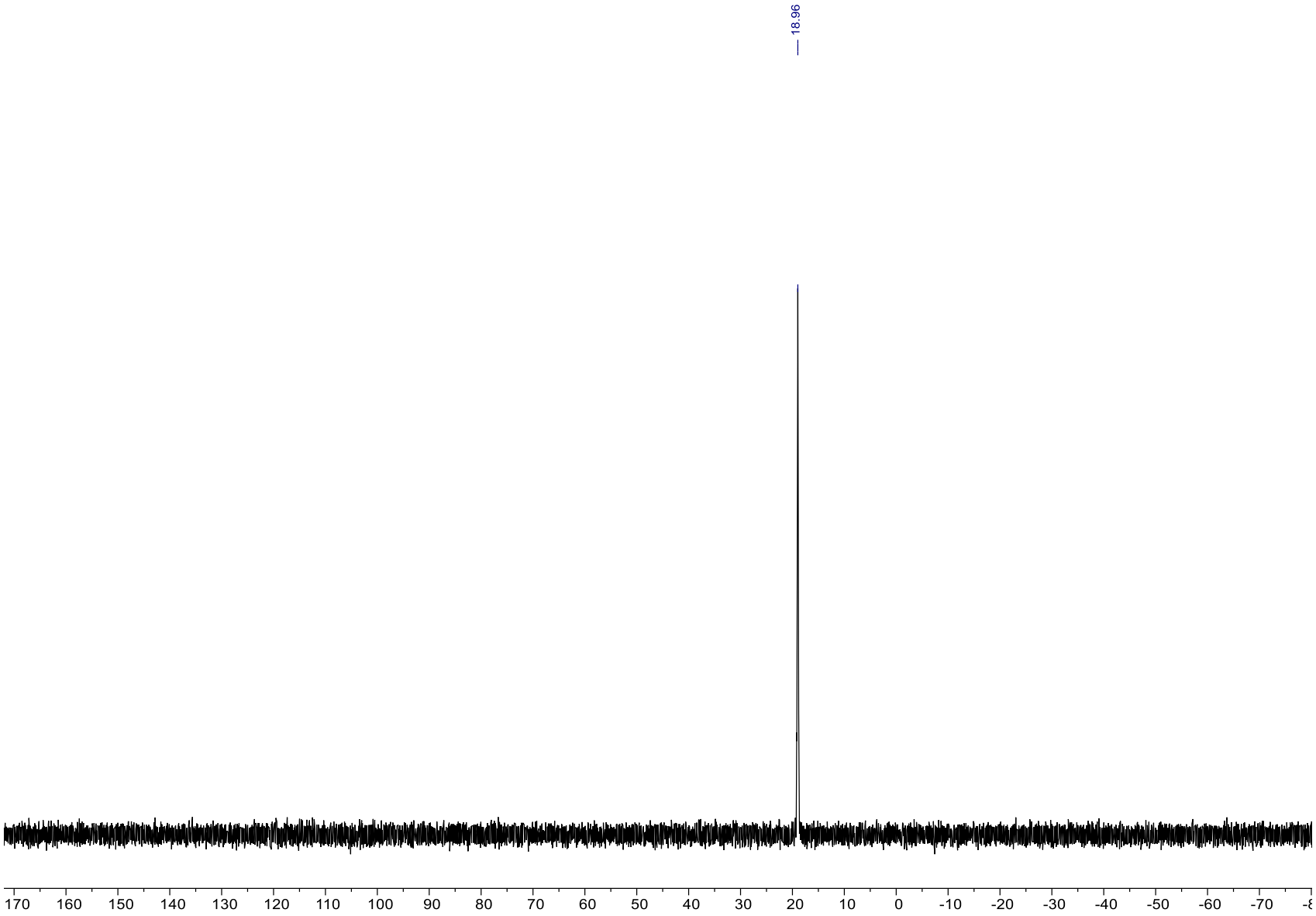
^{1}H NMR (300 MHz, $CDCl_3$) of diphenyl(5-phenyl-1,3,4-thiadiazol-2-yl)phosphine oxide (3a)



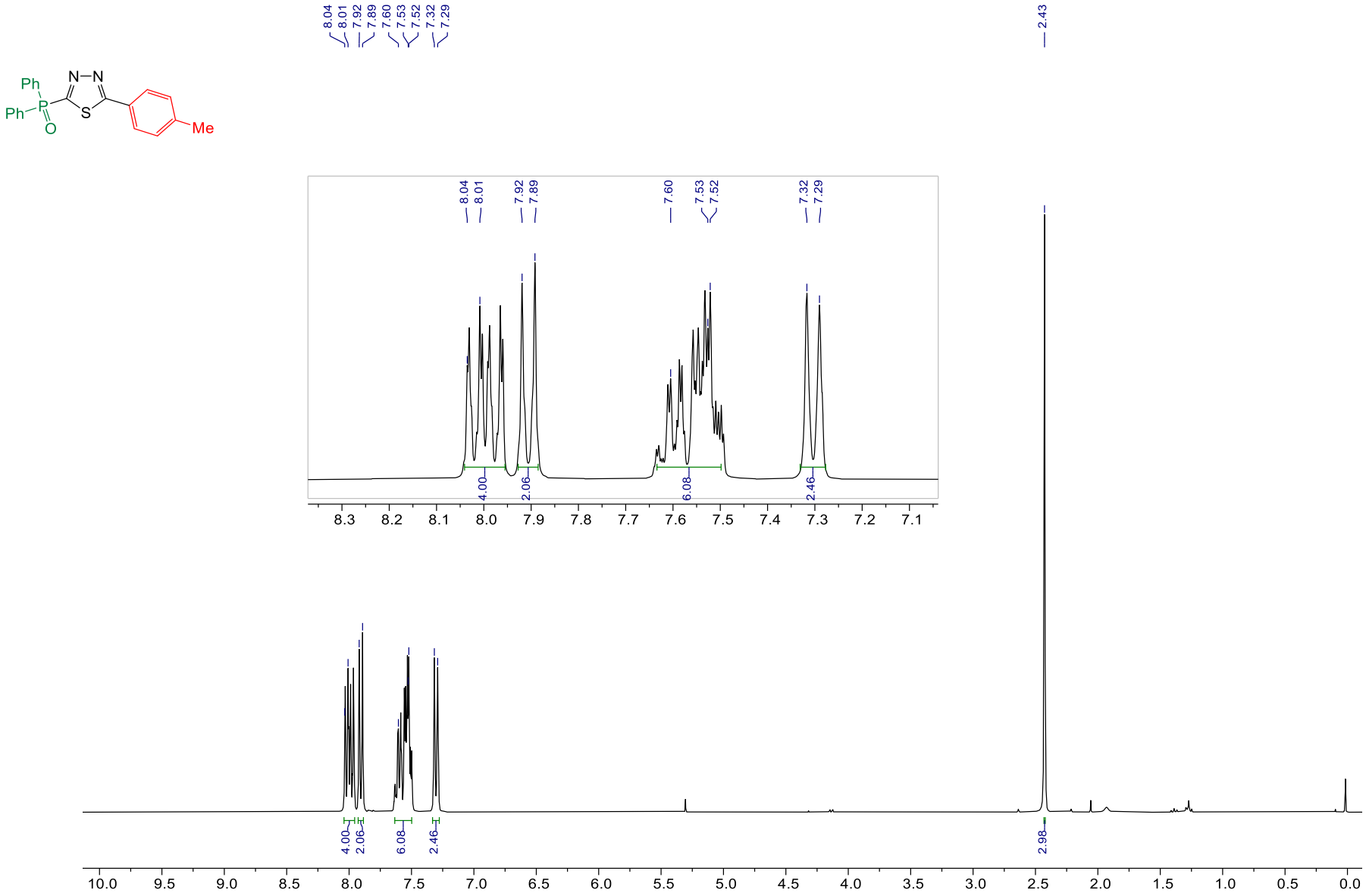
^{13}C NMR (75 MHz, $CDCl_3$) of diphenyl(5-phenyl-1,3,4-thiadiazol-2-yl)phosphine oxide (3a)



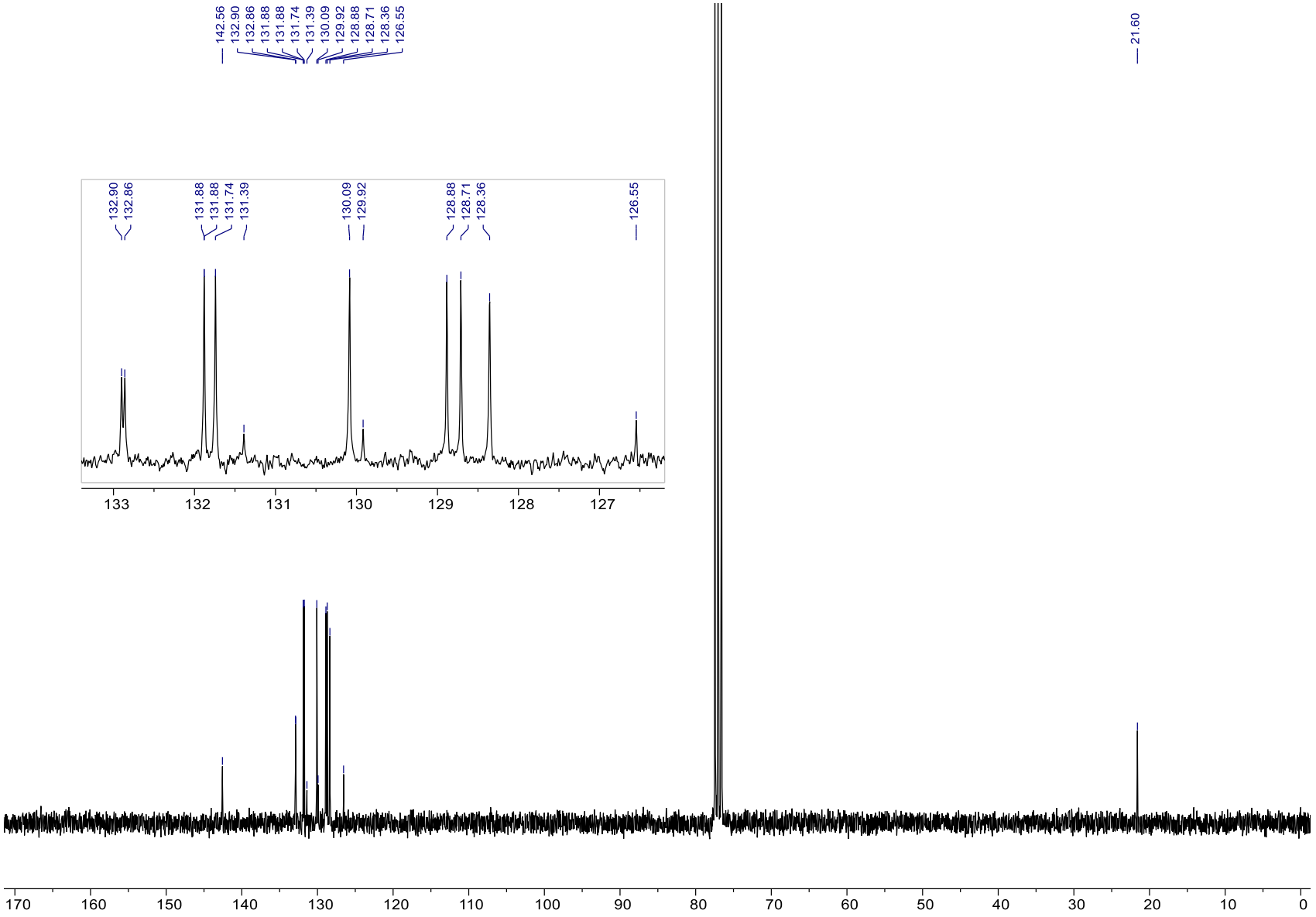
^{31}P NMR (122 MHz, $CDCl_3$) of diphenyl(5-phenyl-1,3,4-thiadiazol-2-yl)phosphine oxide (3a)



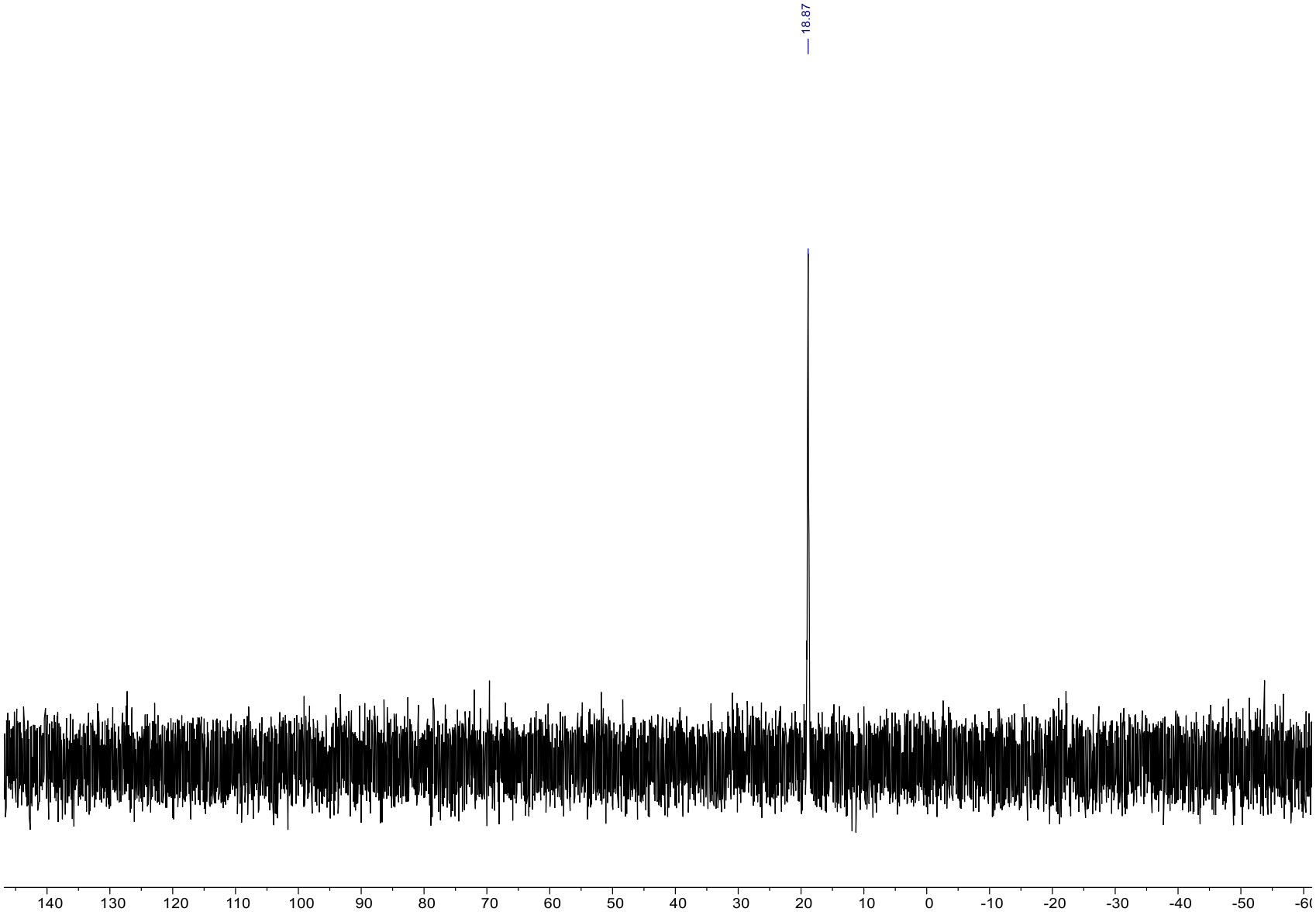
^{1}H NMR (300 MHz, $CDCl_3$) of diphenyl(5-tolyl-1,3,4-thiadiazol-2-yl)phosphine oxide (3b)



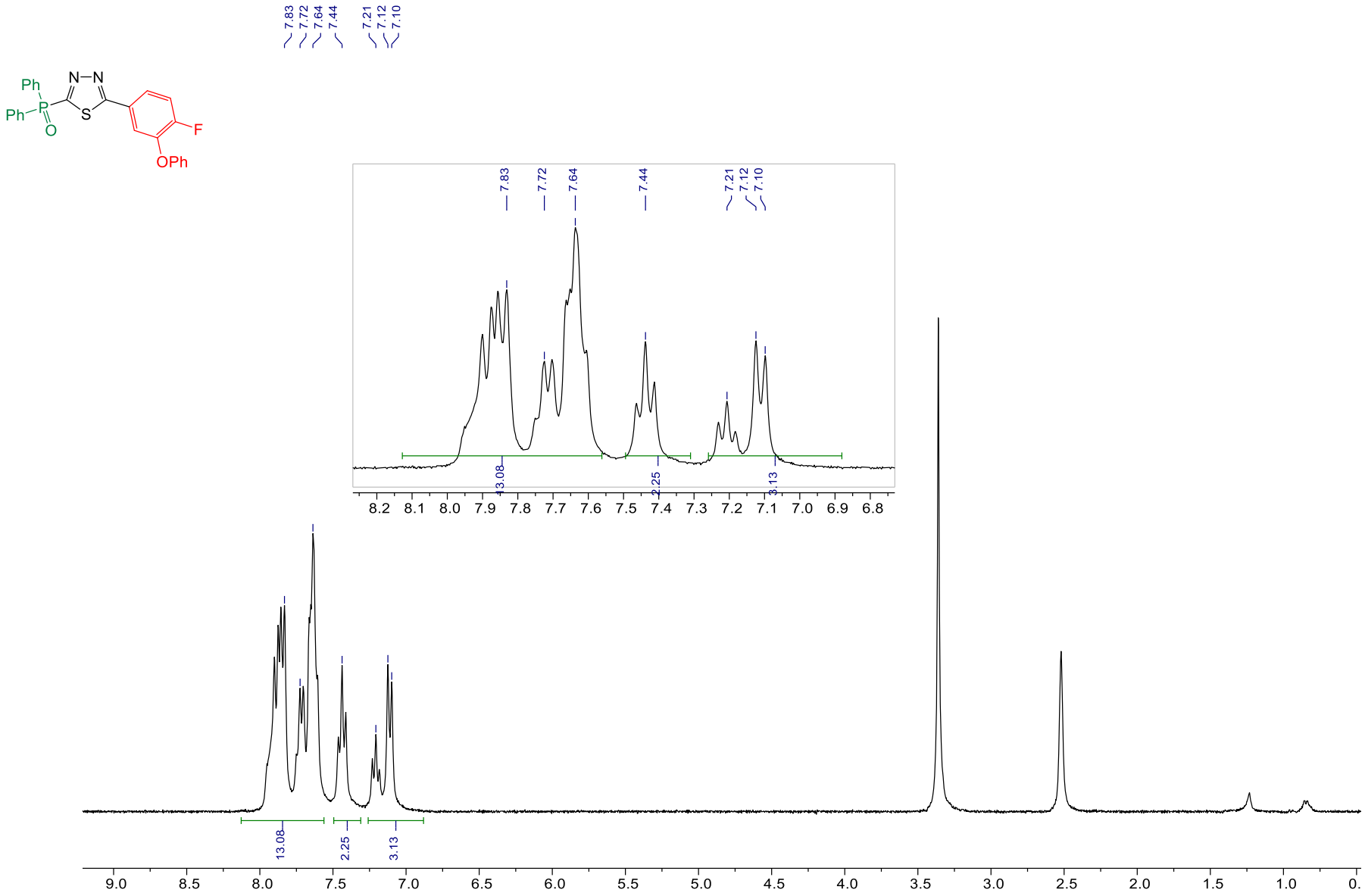
^{13}C NMR (75 MHz, $CDCl_3$) of diphenyl(5-tolyl-1,3,4-thiadiazol-2-yl)phosphine oxide (3b)



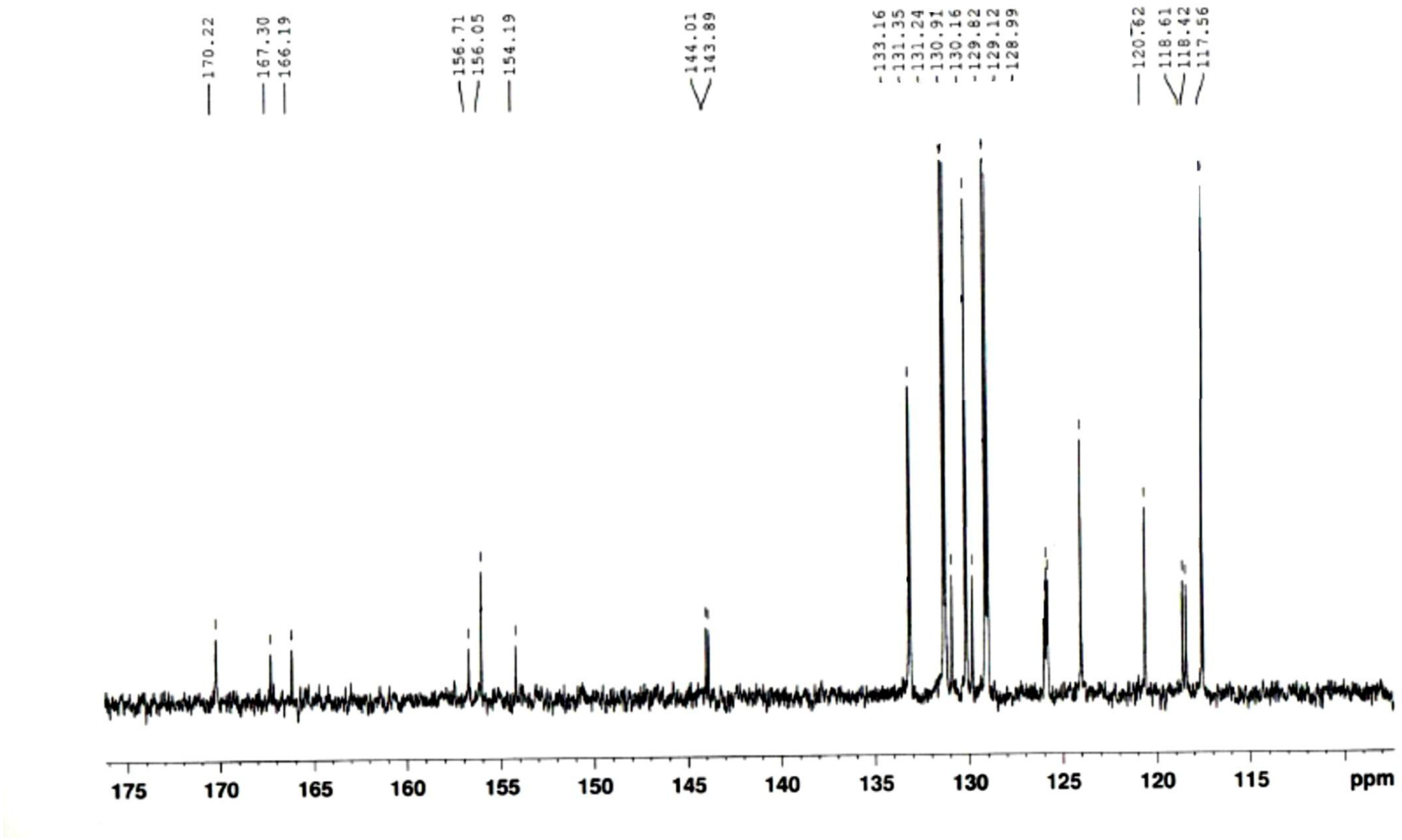
^{31}P NMR (122 MHz, $CDCl_3$) of diphenyl(5-tolyl-1,3,4-thiadiazol-2-yl)phosphine oxide (3b)



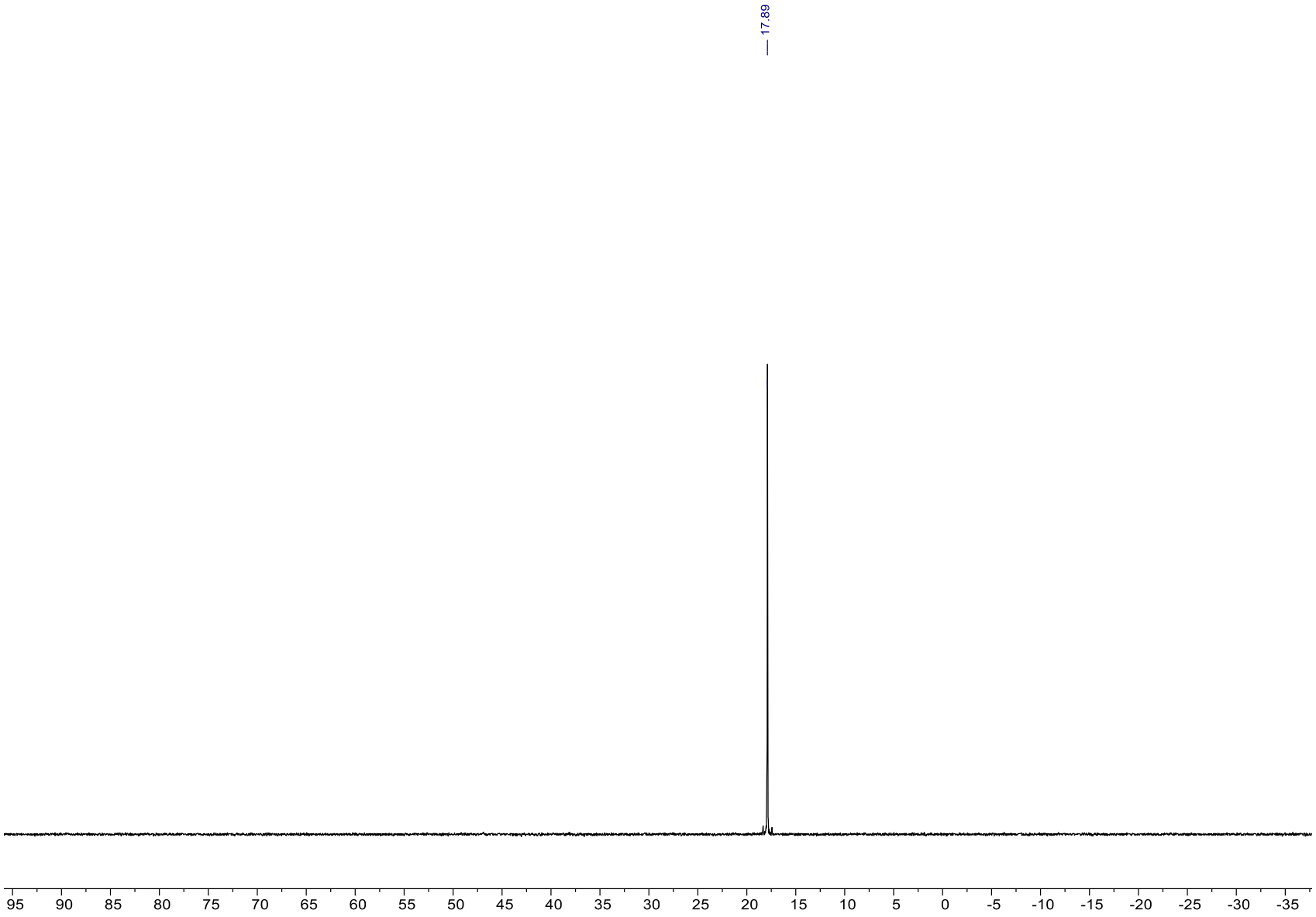
^{1}H NMR (400 MHz, DMSO-d_6) of (5-(4-fluoro-3-phenoxyphenyl)-1,3,4-thiadiazol-2-yl)diphenylphosphine oxide (3c)



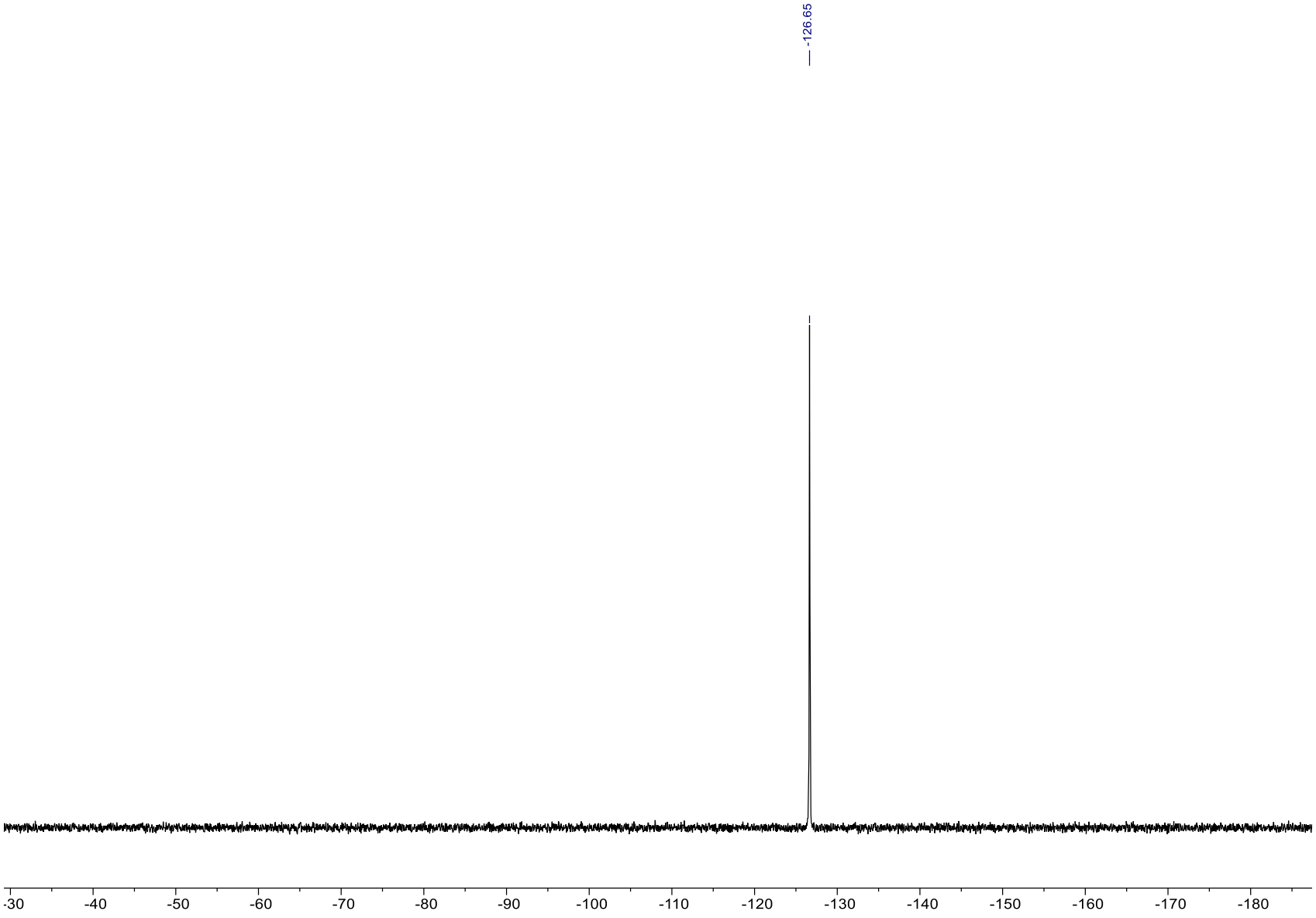
^{13}C NMR (100 MHz, DMSO-d_6) of (5-(4-fluoro-3-phenoxyphenyl)-1,3,4-thiadiazol-2-yl)diphenylphosphine oxide (3c)



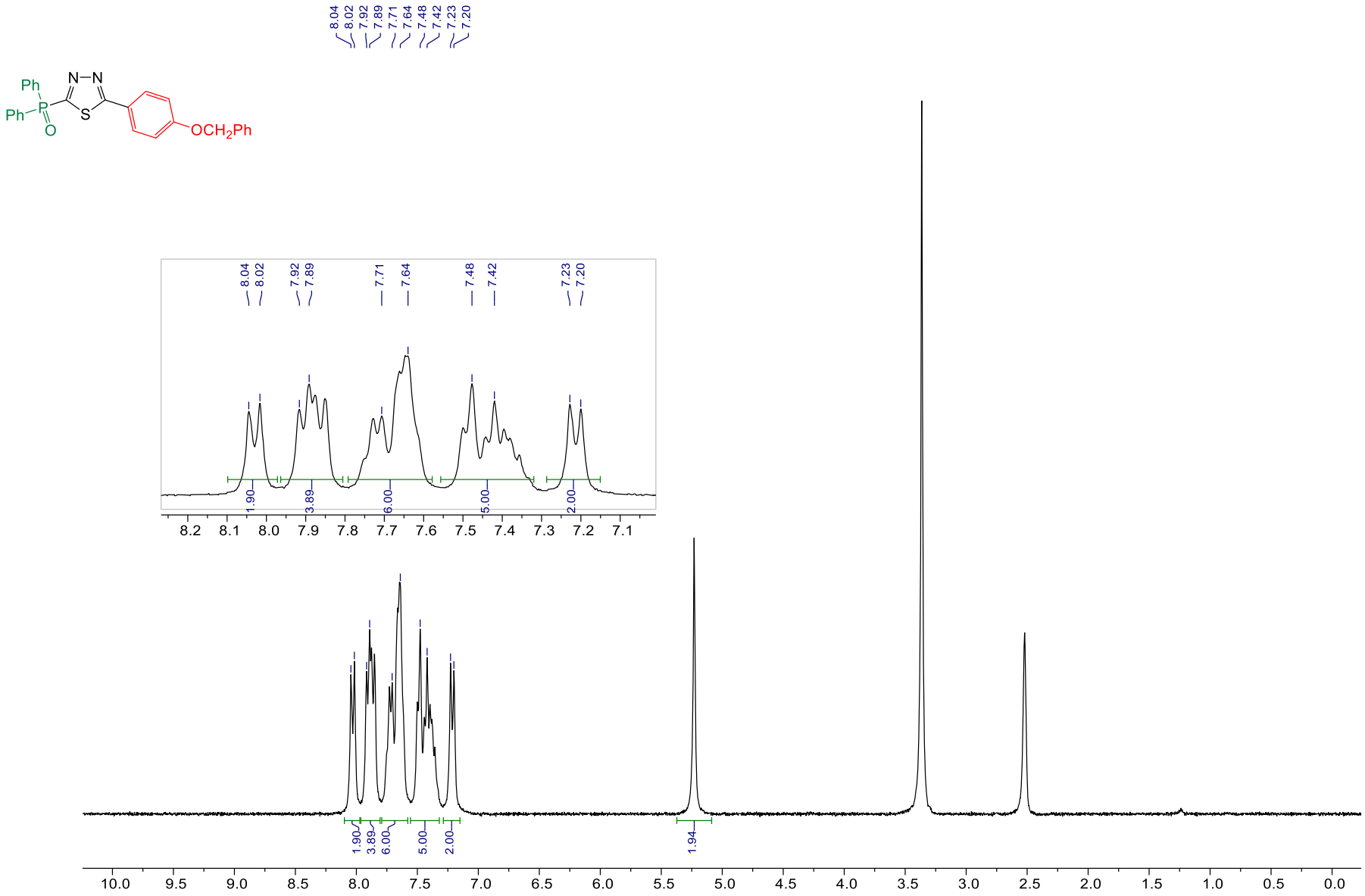
^{31}P NMR (162 MHz, DMSO-d_6) of (5-(4-fluoro-3-phenoxyphenyl)-1,3,4-thiadiazol-2-yl)diphenylphosphine oxide (3c)



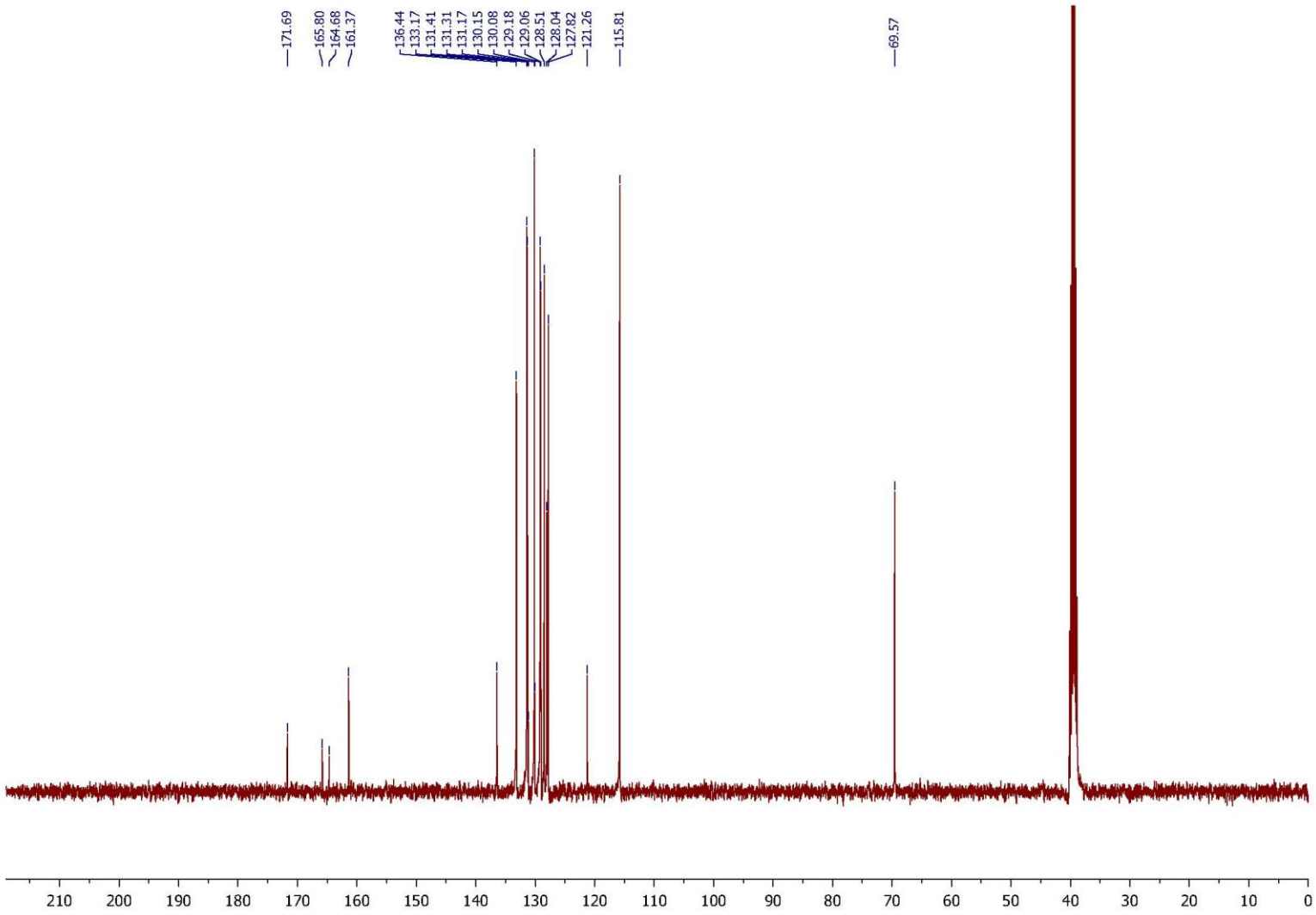
^{19}F NMR (376 MHz, DMSO-d_6) of (5-(4-fluoro-3-phenoxyphenyl)-1,3,4-thiadiazol-2-yl)diphenylphosphine oxide (3c)



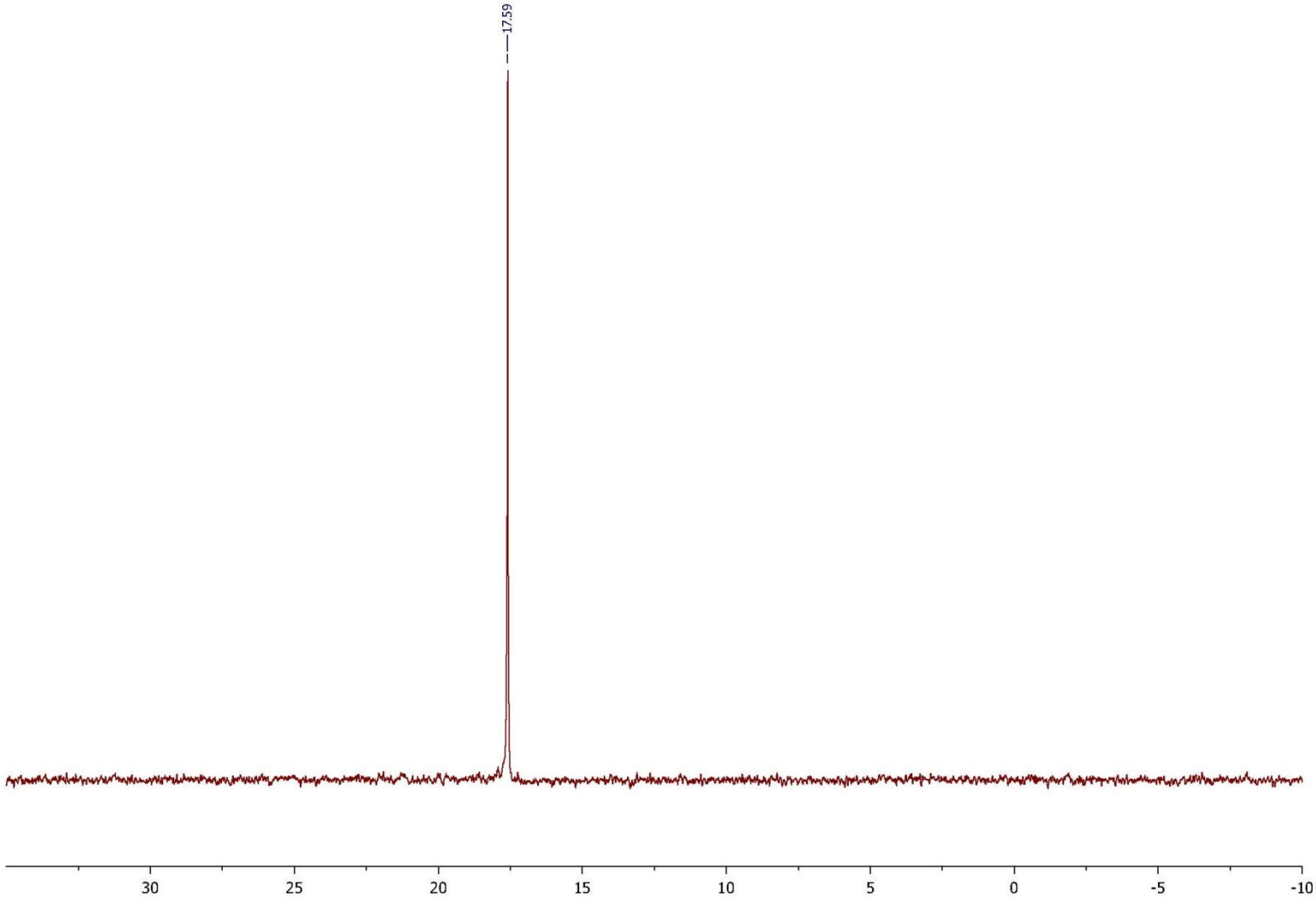
^{1}H NMR (400 MHz, DMSO-d_6) of (5-(4-(benzyloxy)phenyl)-1,3,4-thiadiazol-2-yl)diphenylphosphine oxide (3d)



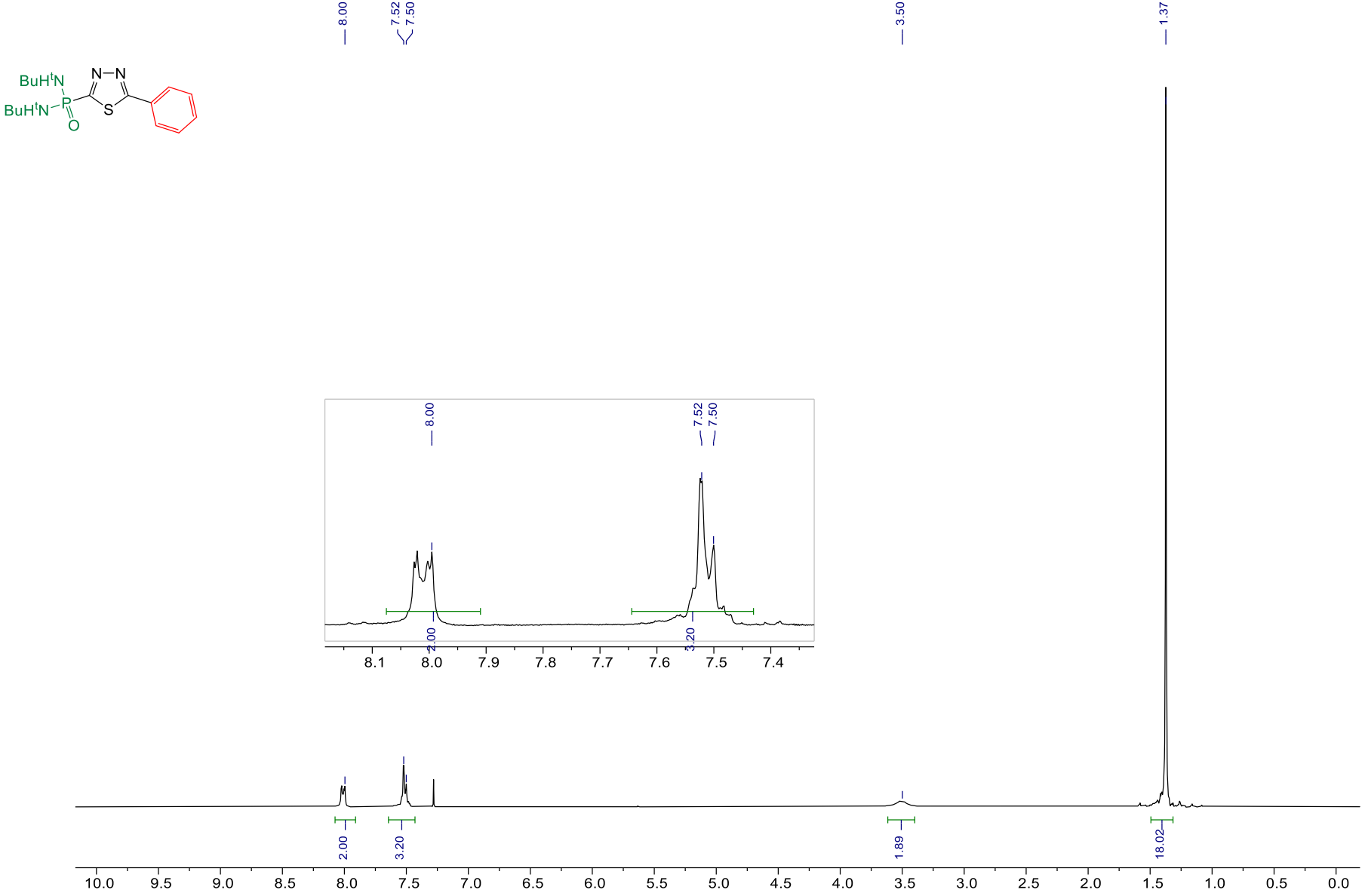
^{13}C NMR (101 MHz, DMSO-d_6) of (5-(4-(benzyloxy)phenyl)-1,3,4-thiadiazol-2-yl)diphenylphosphine oxide (3d)



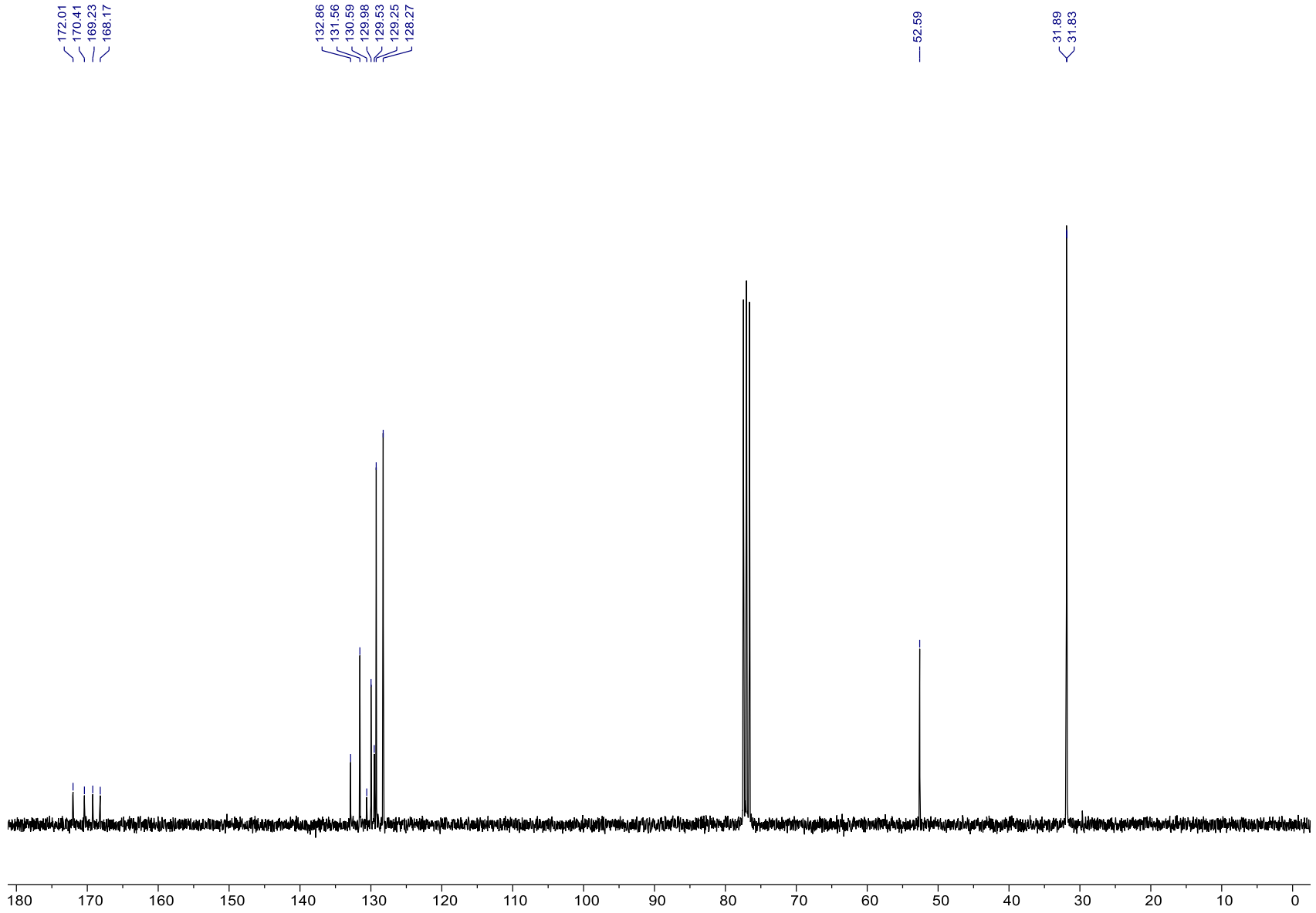
^{31}P NMR (162 MHz, DMSO-d_6) of (5-(4-(benzyloxy)phenyl)-1,3,4-thiadiazol-2-yl)diphenylphosphine oxide (3d)



^{1}H NMR (300 MHz, $CDCl_3$) of *P*-[5-(phenyl)-1,3,4-thiadiazol-2-yl]-*N,N′*-di-*tert*-butylphosphonic diamide (3e)



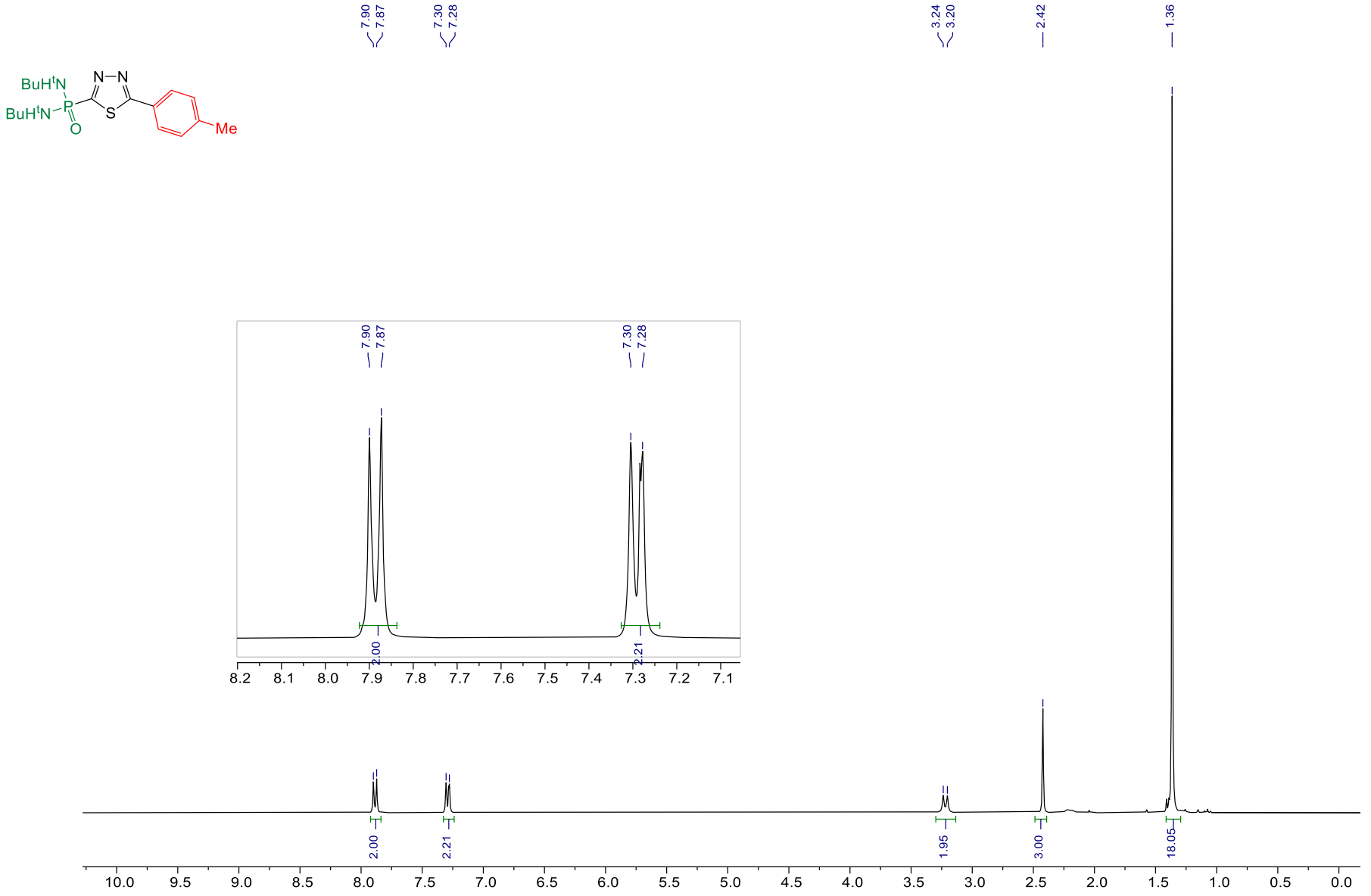
^{13}C NMR (75 MHz, $CDCl_3$) of *P*-[5-(phenyl)-1,3,4-thiadiazol-2-yl]-*N,N'*-di-*tert*-butylphosphonic diamide (3e)



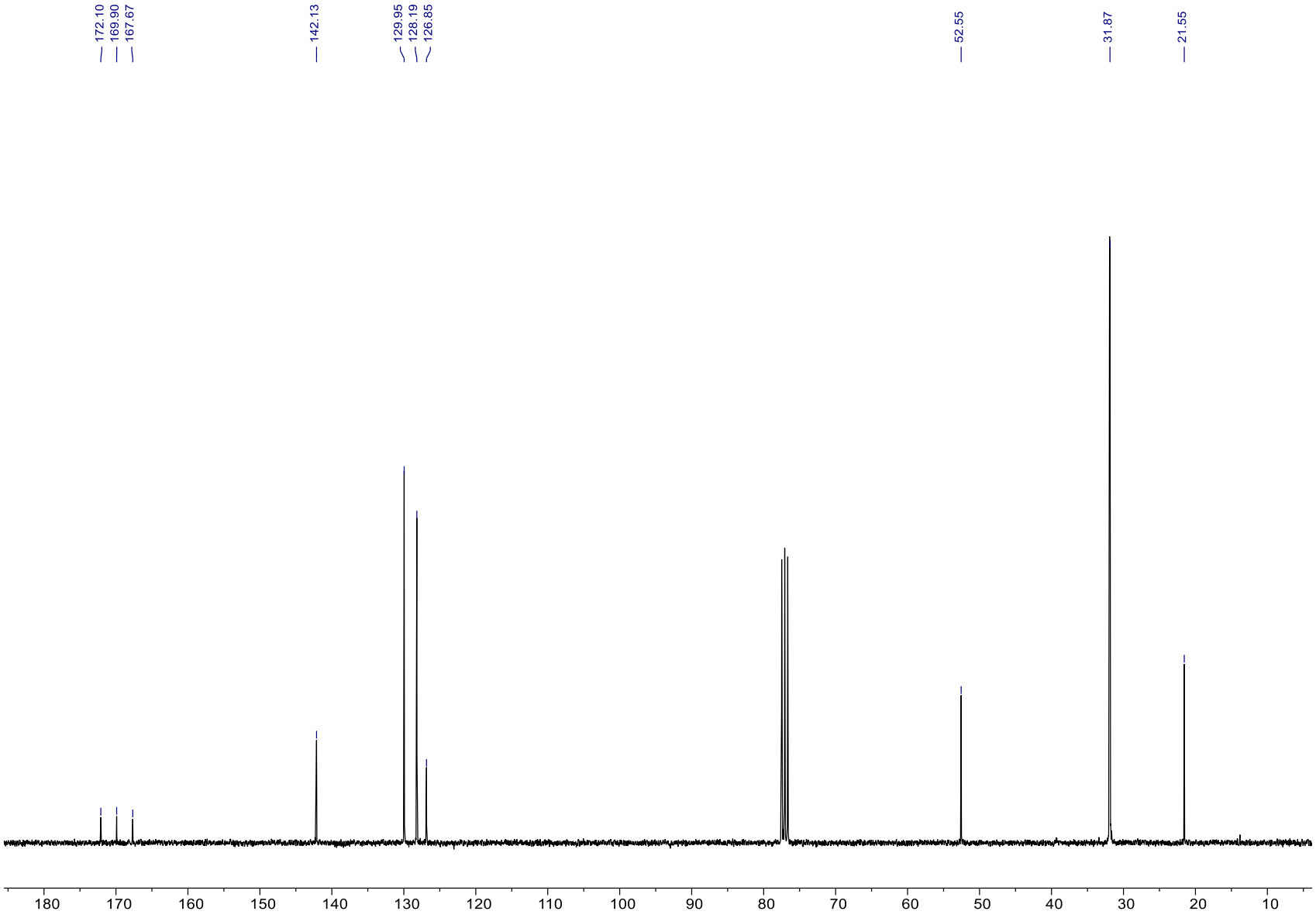
^{31}P NMR (122 MHz, $CDCl_3$) of *P*-[5-(phenyl)-1,3,4-thiadiazol-2-yl]-*N,N'*-di-*tert*-butylphosphonic diamide (3e)



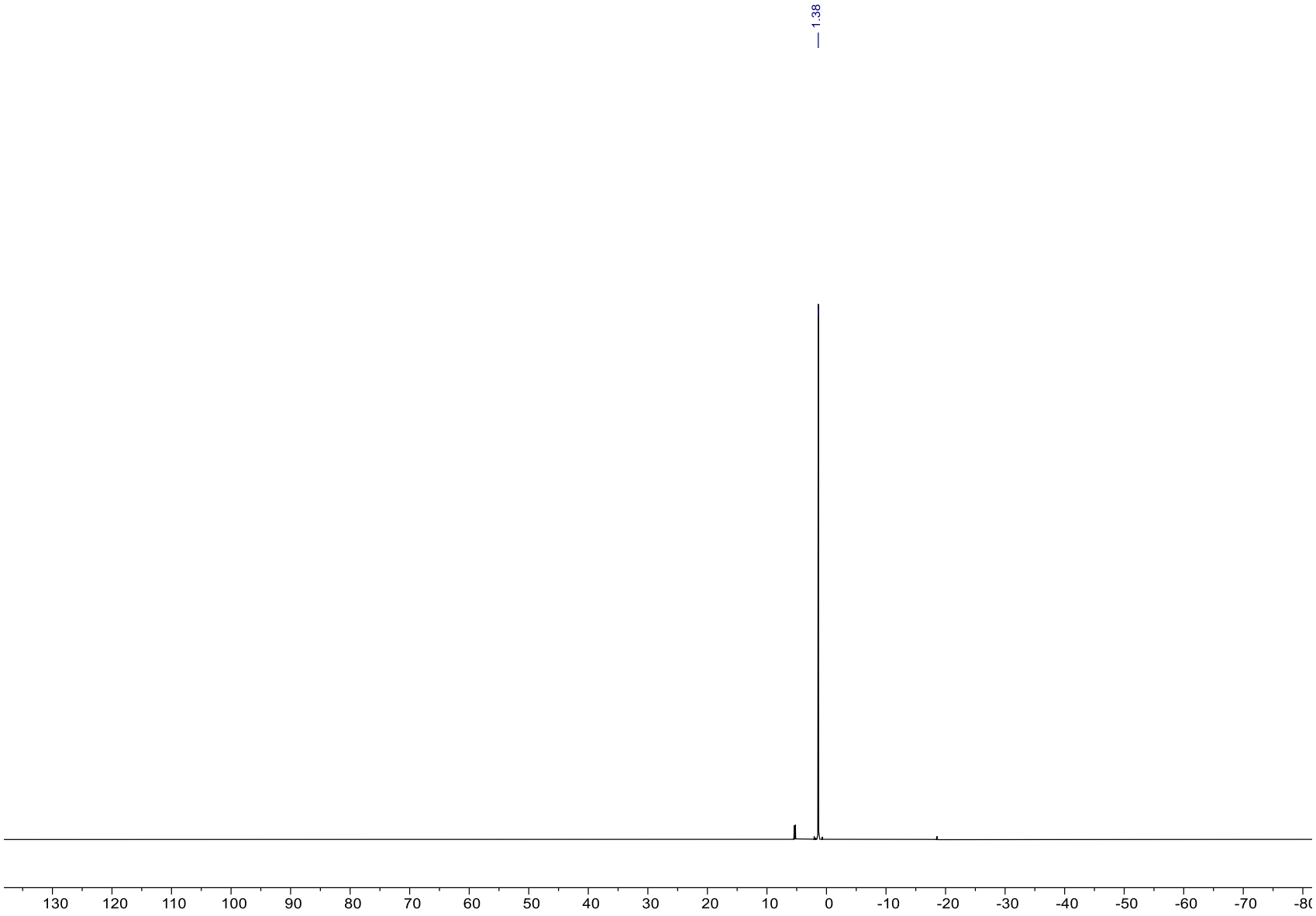
^{1}H NMR (300 MHz, $CDCl_3$) of *P*-[5-(*p*-tolyl)-1,3,4-thiadiazol-2-yl]-*N,N'*-di-*tert*-butylphosphonic diamide (3f)



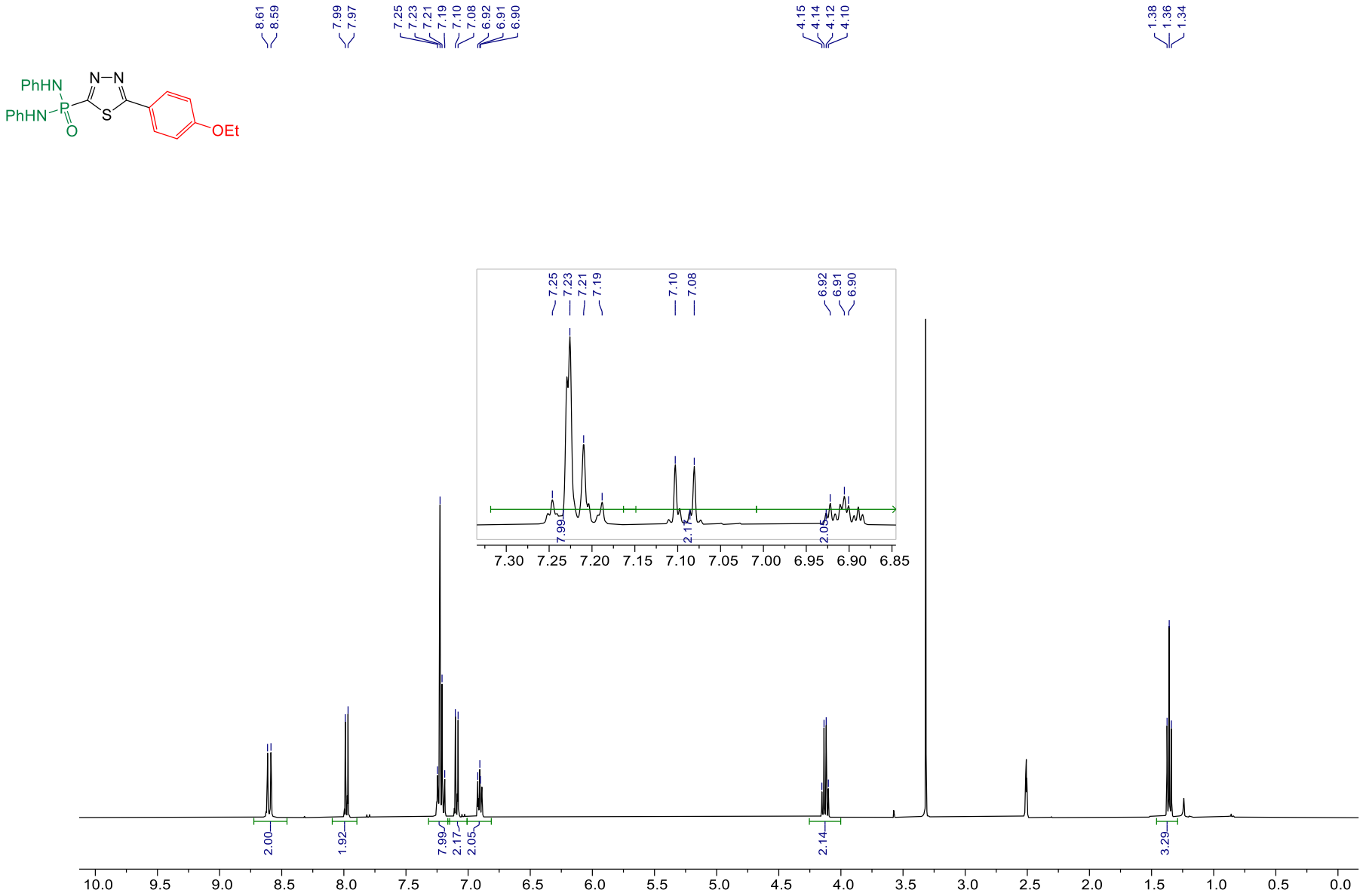
^{13}C NMR (75 MHz, $CDCl_3$) of *P*-[5-(*p*-tolyl)-1,3,4-thiadiazol-2-yl]-*N,N'*-di-*tert*-butylphosphonic diamide (3f)



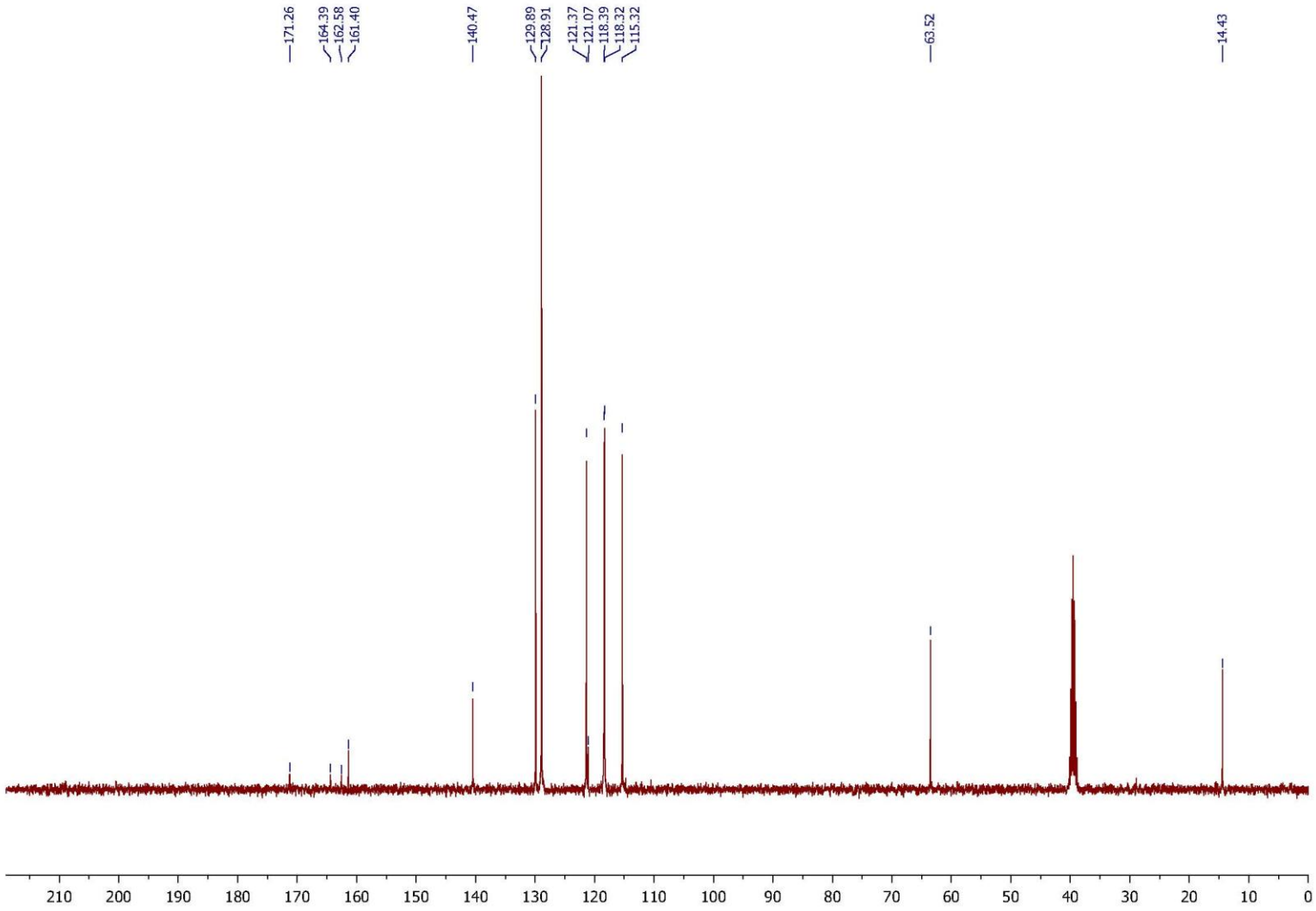
^{31}P NMR (122 MHz, $CDCl_3$) of *P*-[5-(*p*-tolyl)-1,3,4-thiadiazol-2-yl]-*N,N′*-di-*tert*-butylphosphonic diamide (3f)



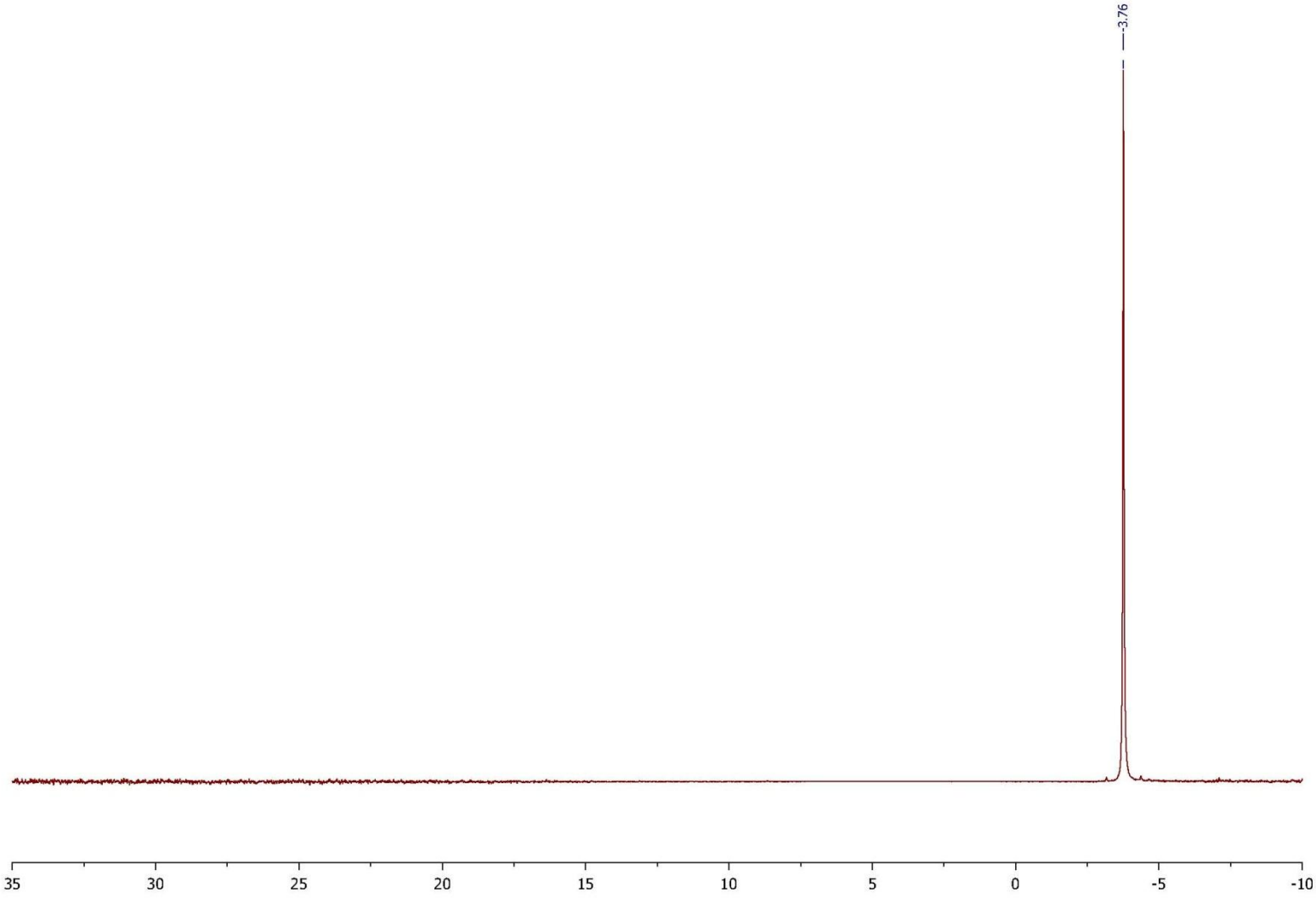
^{1}H NMR (400 MHz, DMSO-d_6) of *P*-[5-(4-ethoxyphenyl)-1,3,4-thiadiazol-2-yl]-*N,N′*-diphenylphosphonic diamide (3g)



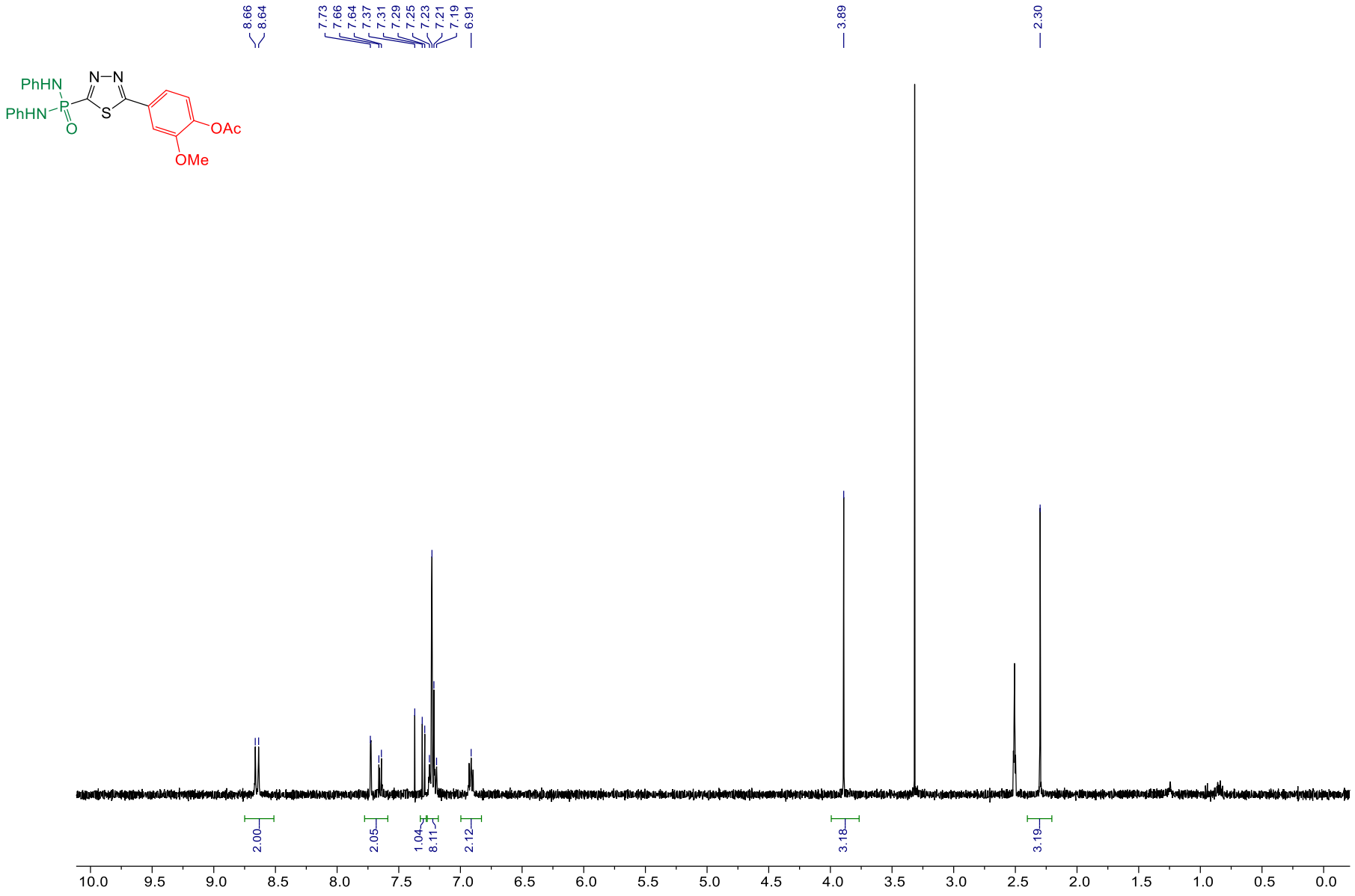
^{13}C NMR (101 MHz, DMSO-d_6) of *P*-[5-(4-ethoxyphenyl)-1,3,4-thiadiazol-2-yl]-*N,N'*-diphenylphosphonic diamide (3g)



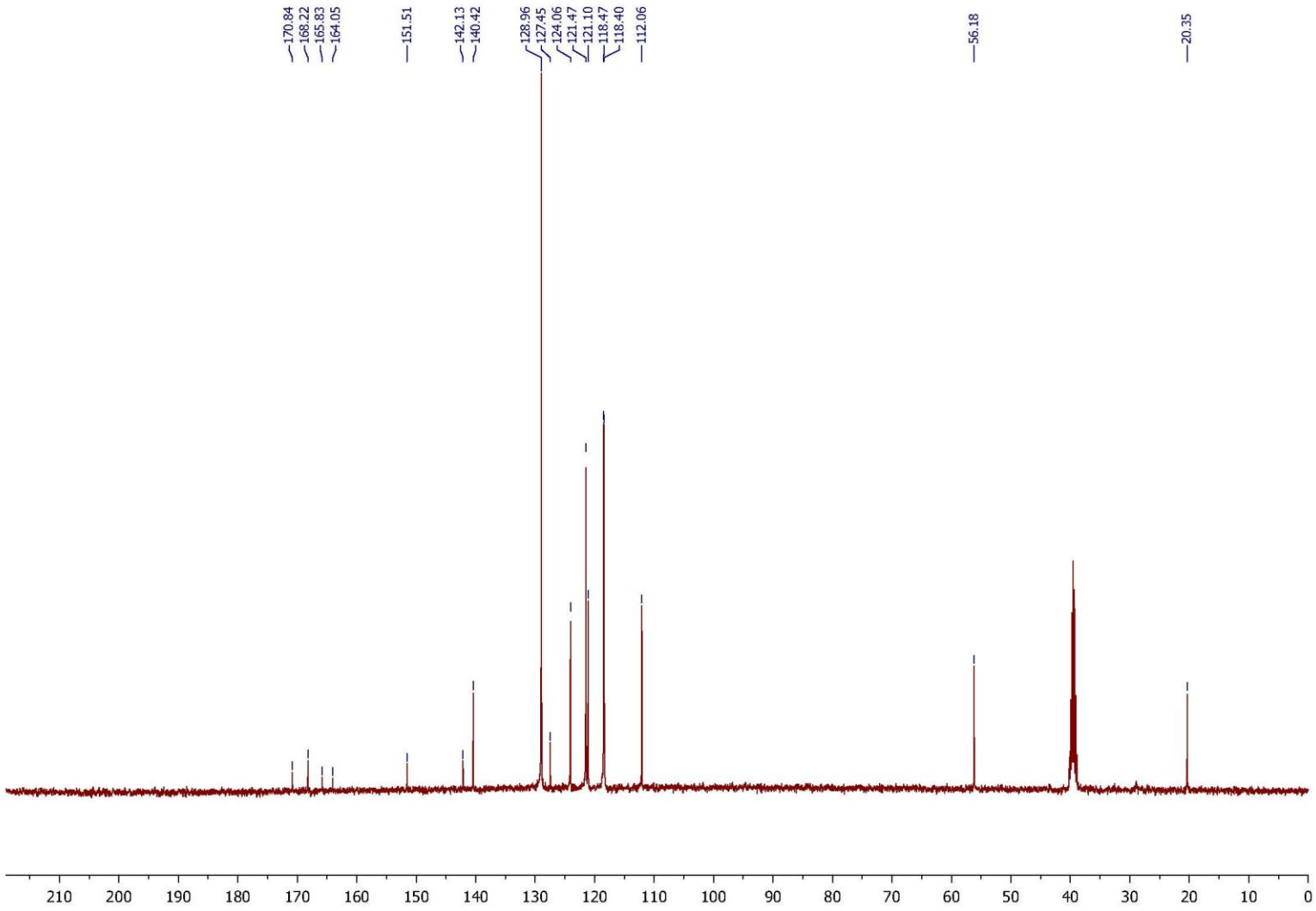
^{31}P NMR (162 MHz, DMSO-d_6) of *P*-[5-(4-ethoxyphenyl)-1,3,4-thiadiazol-2-yl]-*N,N'*-diphenylphosphonic diamide (3g)



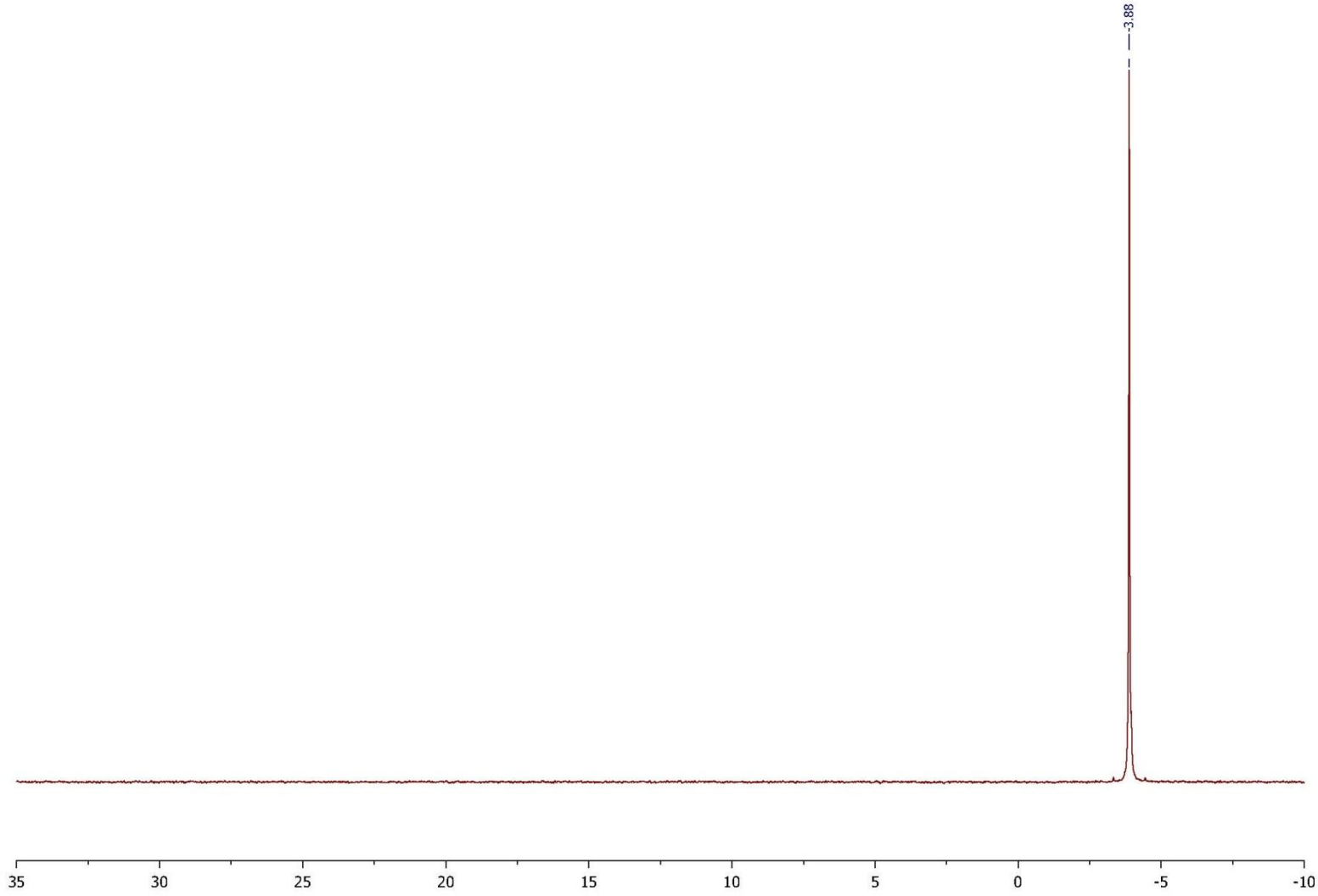
^{1}H NMR (400 MHz, DMSO-d_6) of *P*-[5-(4-acetoxy-3-methoxyphenyl)-1,3,4-thiadiazol-2-yl]-*N,N′*-diphenylphosphonic diamide (3h)



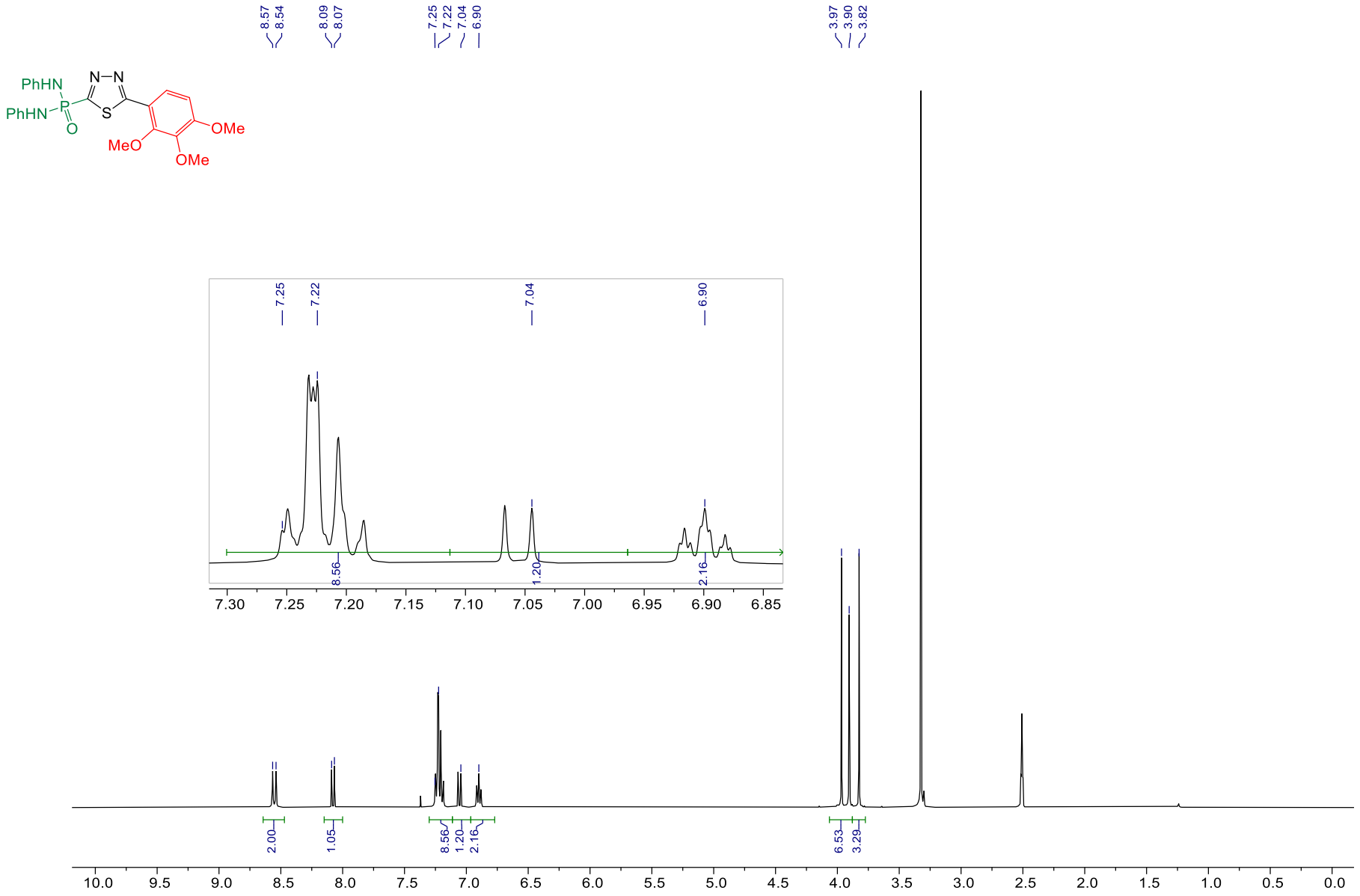
^{13}C NMR (101 MHz, DMSO-d_6) of *P*-[5-(4-acetoxy-3-methoxyphenyl)-1,3,4-thiadiazol-2-yl]-*N,N'*-diphenylphosphonic diamide (3h)



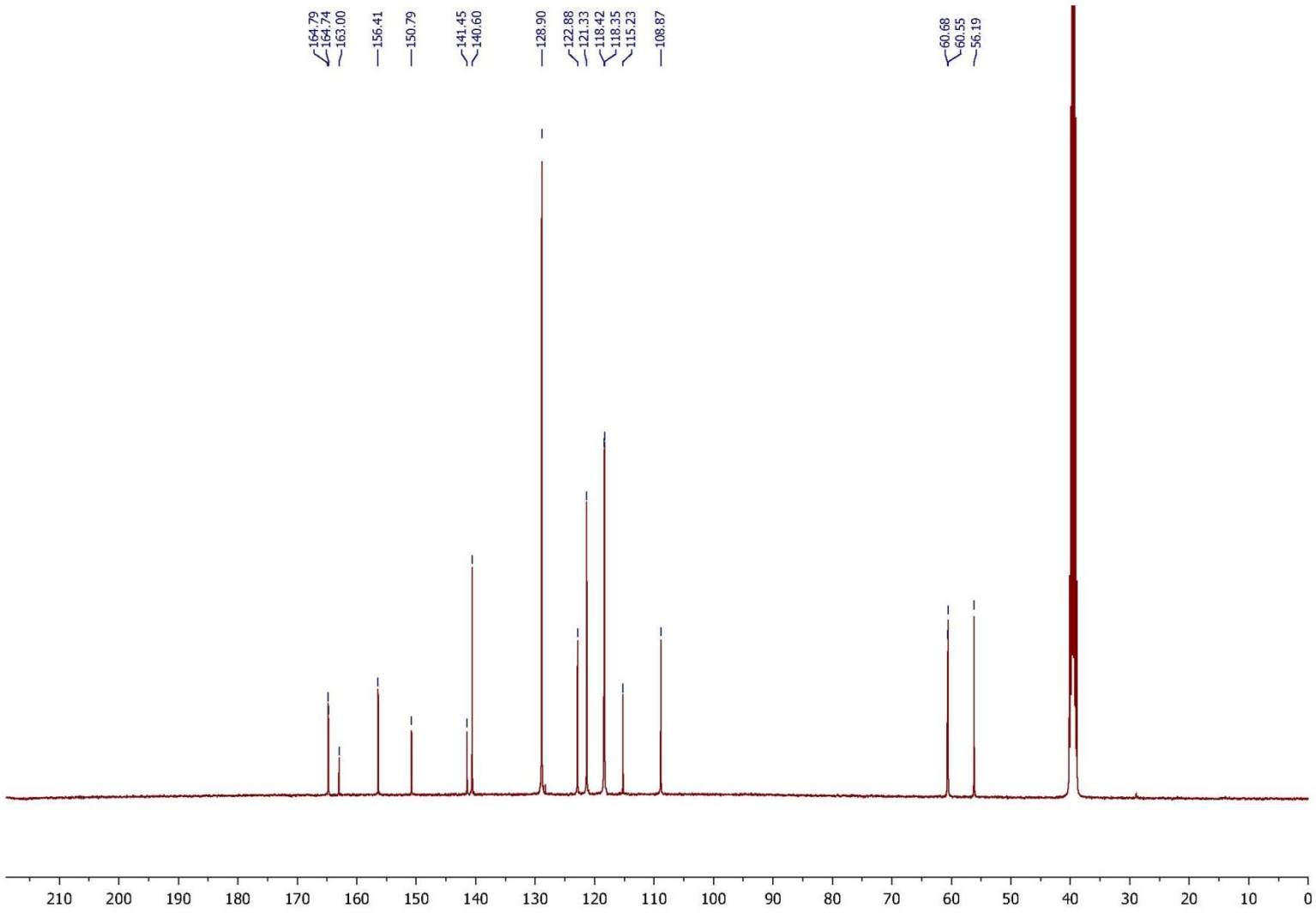
^{31}P NMR (162 MHz, DMSO-d_6) of *P*-[5-(4-acetoxy-3-methoxyphenyl)-1,3,4-thiadiazol-2-yl]-*N,N'*-diphenylphosphonic diamide (3h)



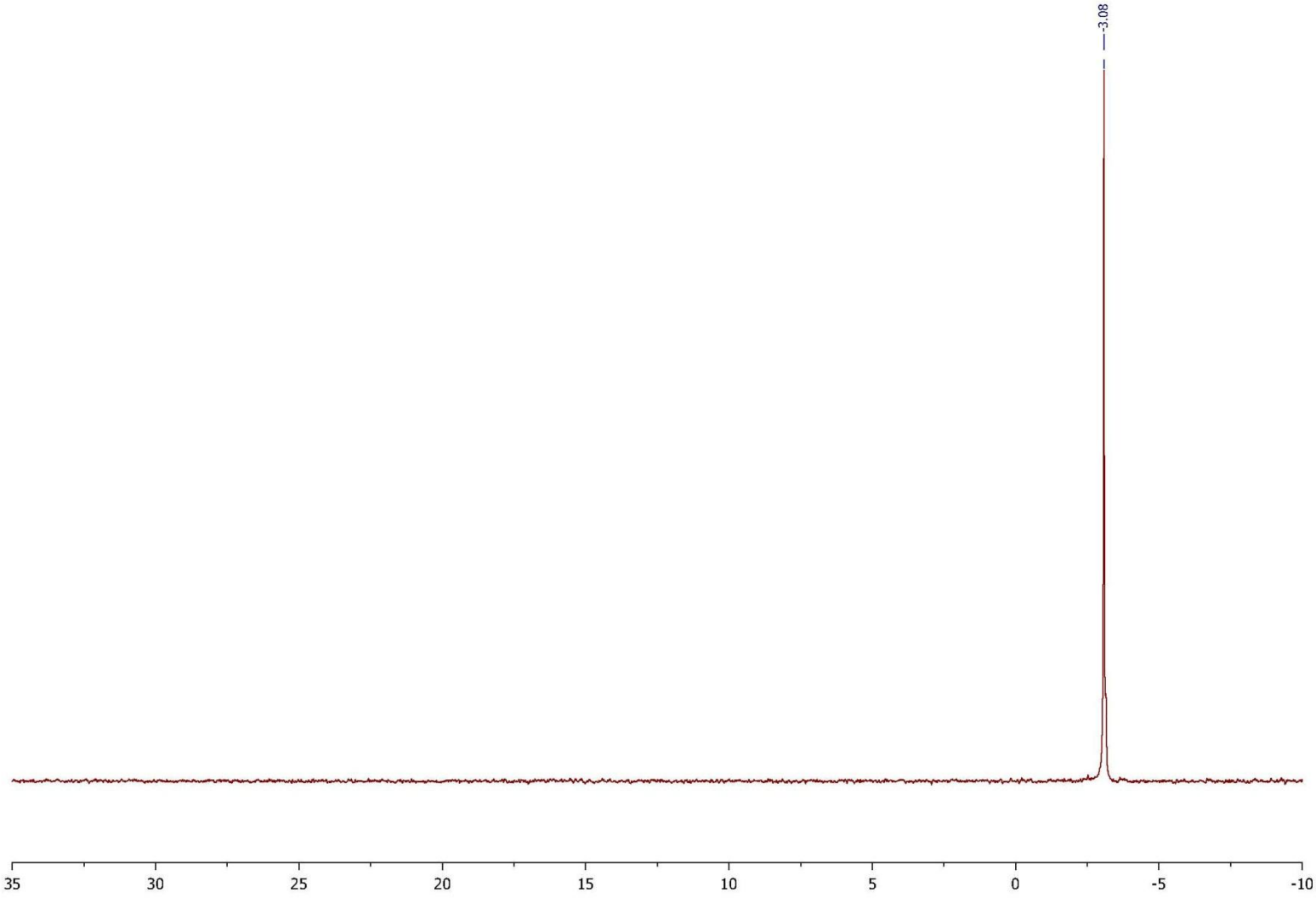
^{1}H NMR (400 MHz, DMSO-d_6) of *P*-[5-(2,3,4-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl]-*N,N′*-diphenylphosphonic diamide (3i)



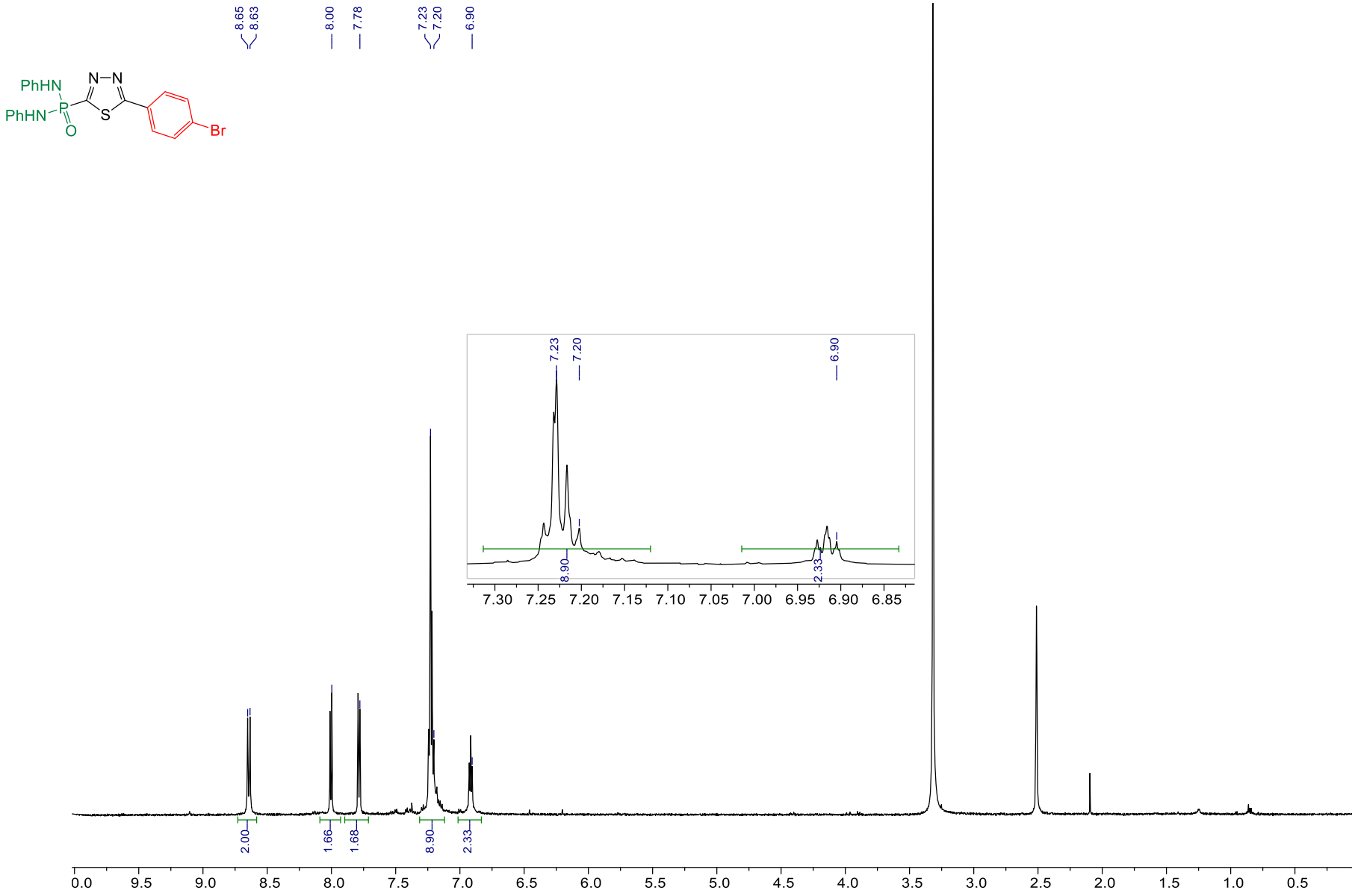
^{13}C NMR (101 MHz, DMSO-d_6) of *P*-[5-(2,3,4-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl]-*N,N′*-diphenylphosphonic diamide (3i)



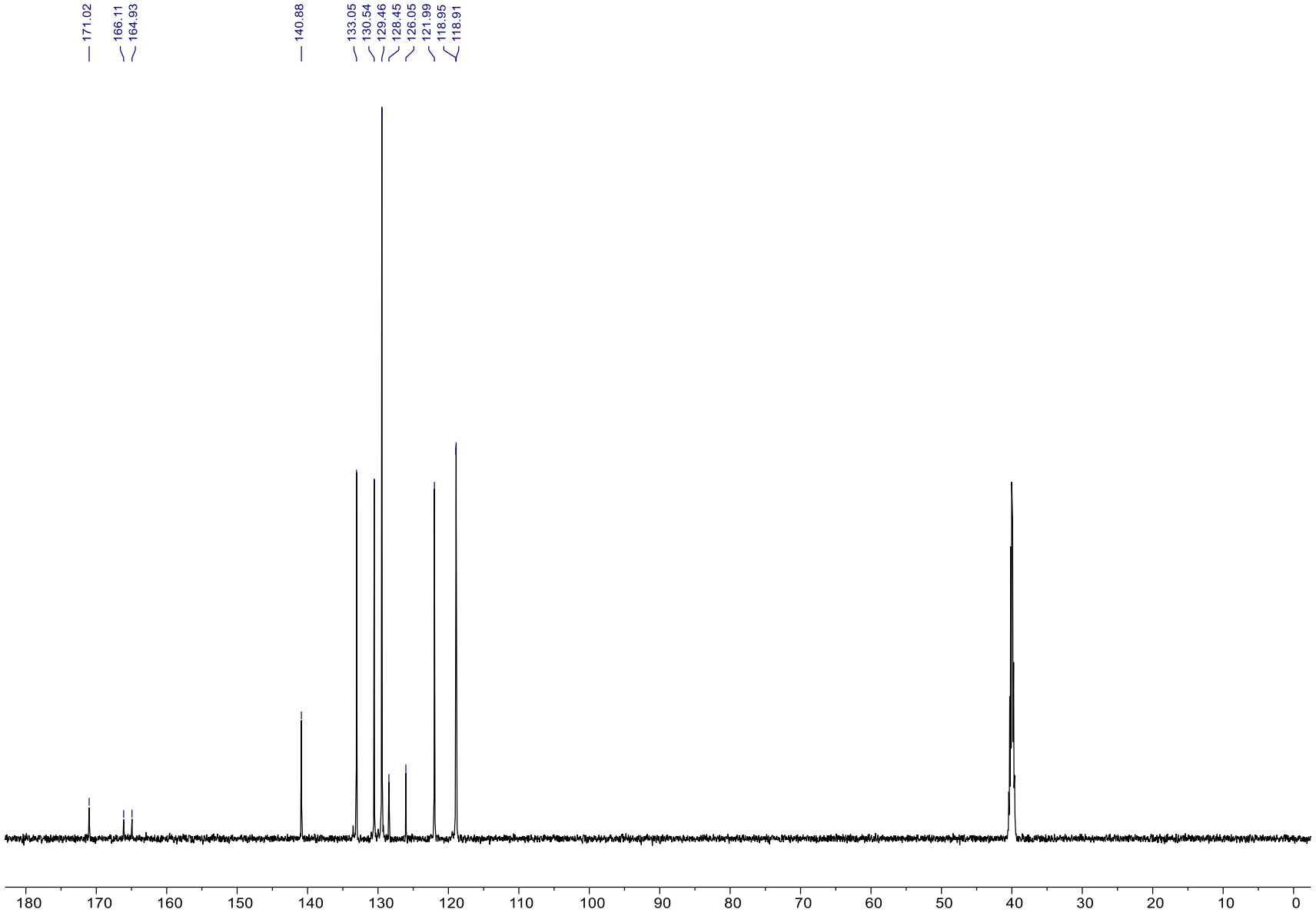
^{31}P NMR (162 MHz, DMSO-d_6) of *P*-[5-(2,3,4-trimethoxyphenyl)-1,3,4-thiadiazol-2-yl]-*N,N'*-diphenylphosphonic diamide (3i)



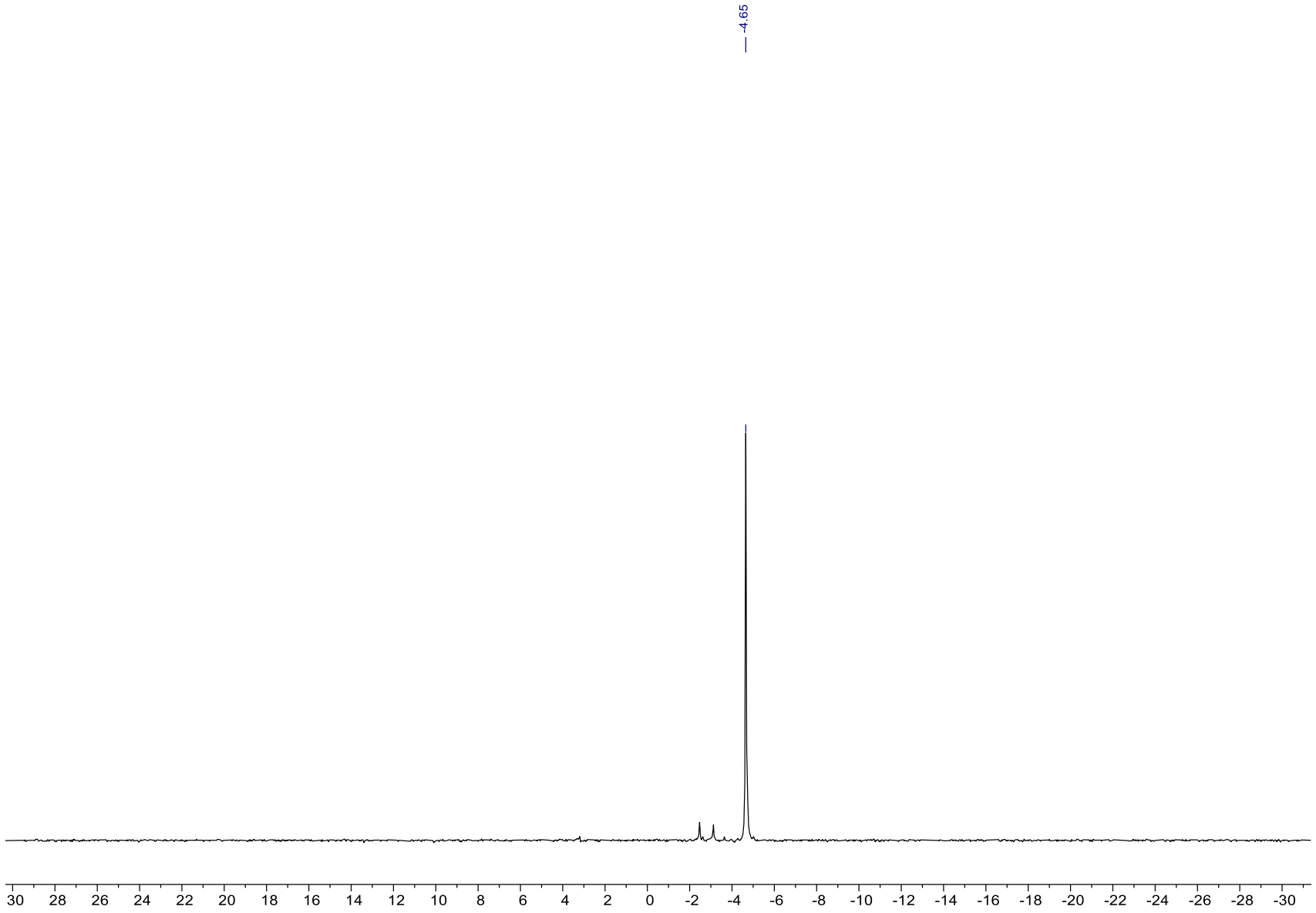
^{1}H NMR (300 MHz, DMSO-d_6) of *P*-[5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl]-*N,N'*-diphenylphosphonic diamide (3j)



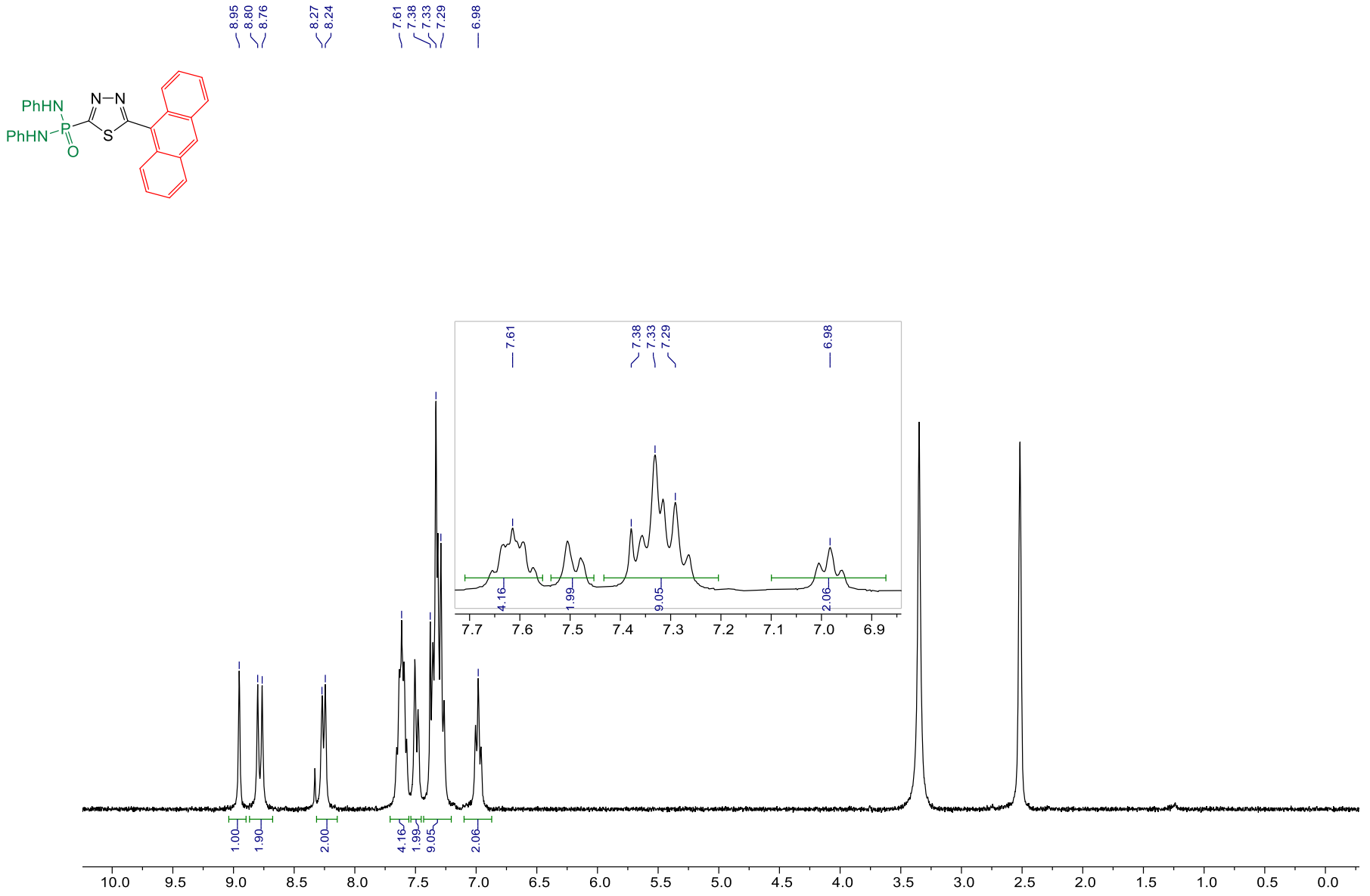
^{13}C NMR (150 MHz, DMSO-d_6) of *P*-[5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl]-*N,N′*-diphenylphosphonic diamide (3j)



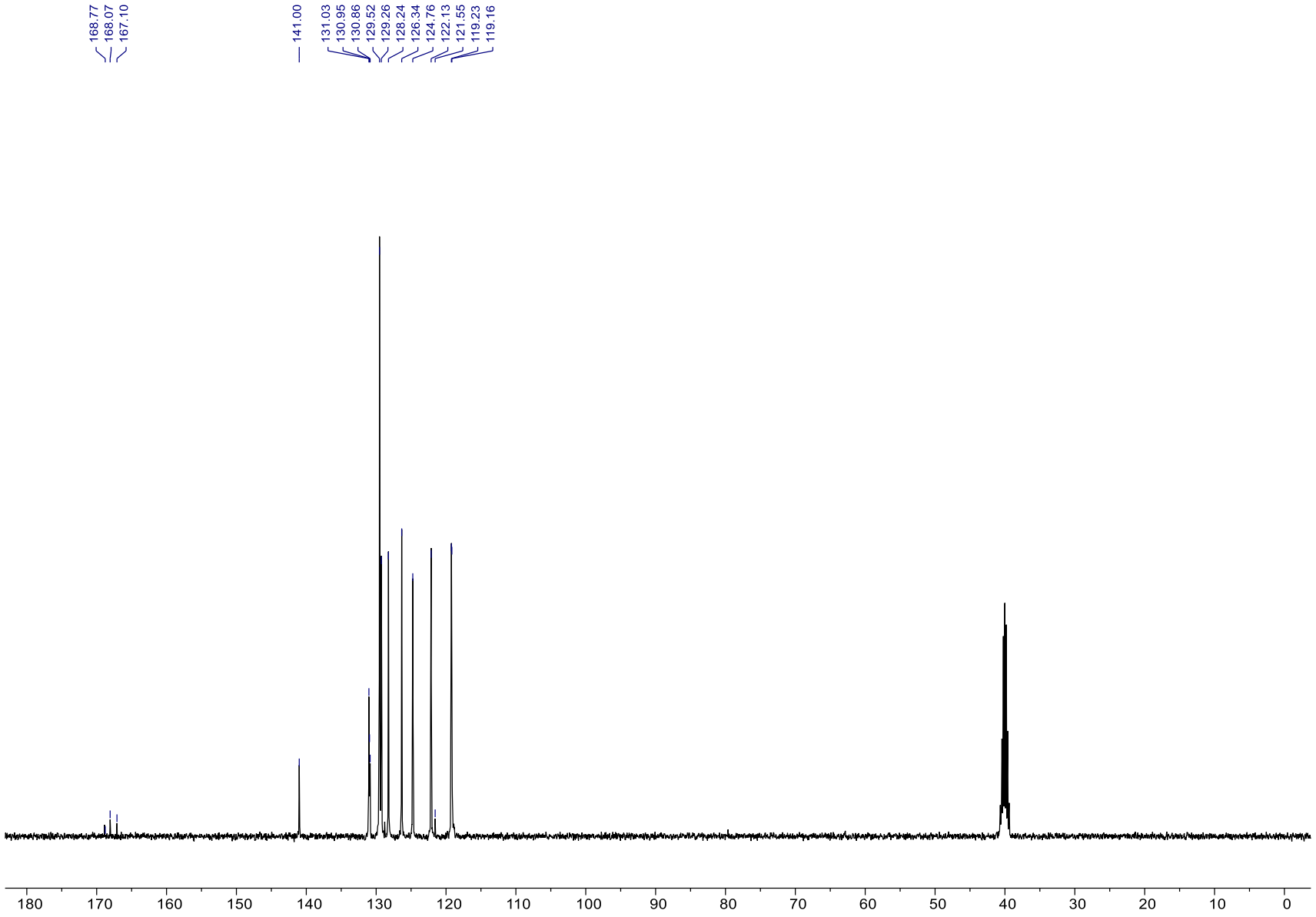
^{31}P NMR (121 MHz, DMSO-d_6) of *P*-[5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl]-*N,N′*-diphenylphosphonic diamide (3j)



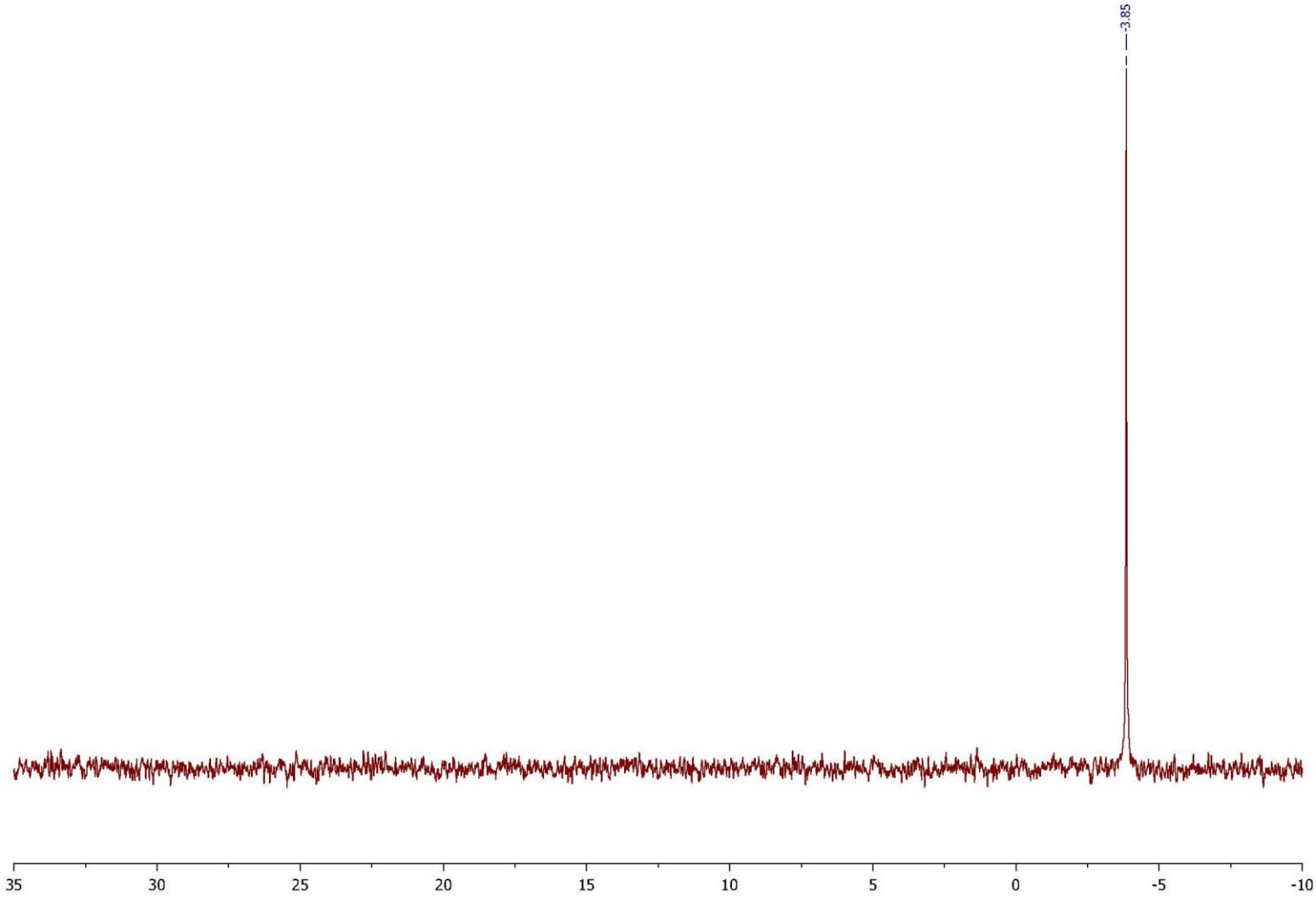
^{1}H NMR (400 MHz, DMSO-d_6) of *P*-[5-(antracene-9-yl)-1,3,4-thiadiazol-2-yl]-*N,N'*-diphenylphosphonic diamide (3k)



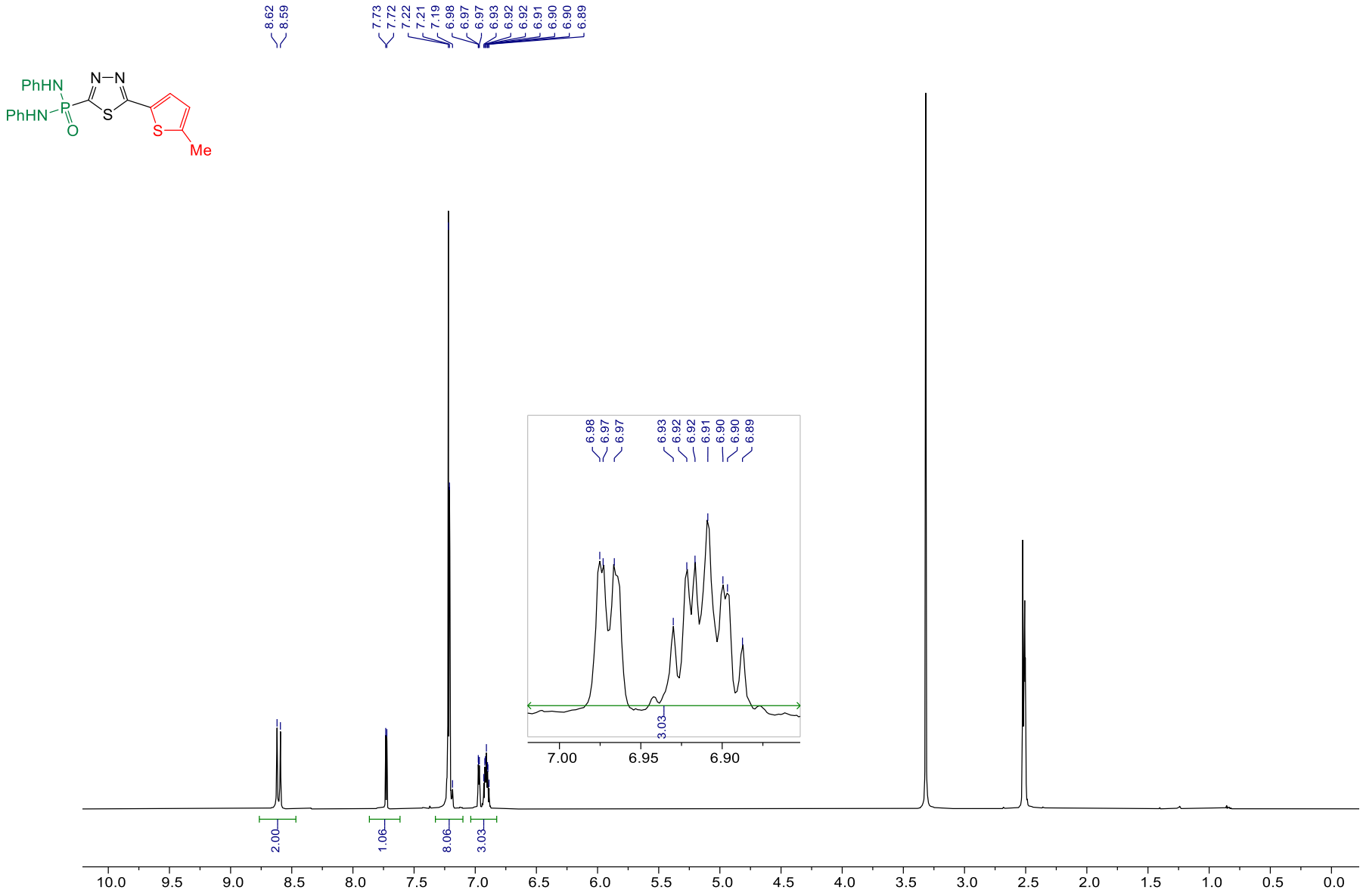
^{13}C NMR (101 MHz, DMSO-d_6) of *P*-[5-(antracene-9-yl)-1,3,4-thiadiazol-2-yl]-*N,N'*-diphenylphosphonic diamide (3k)



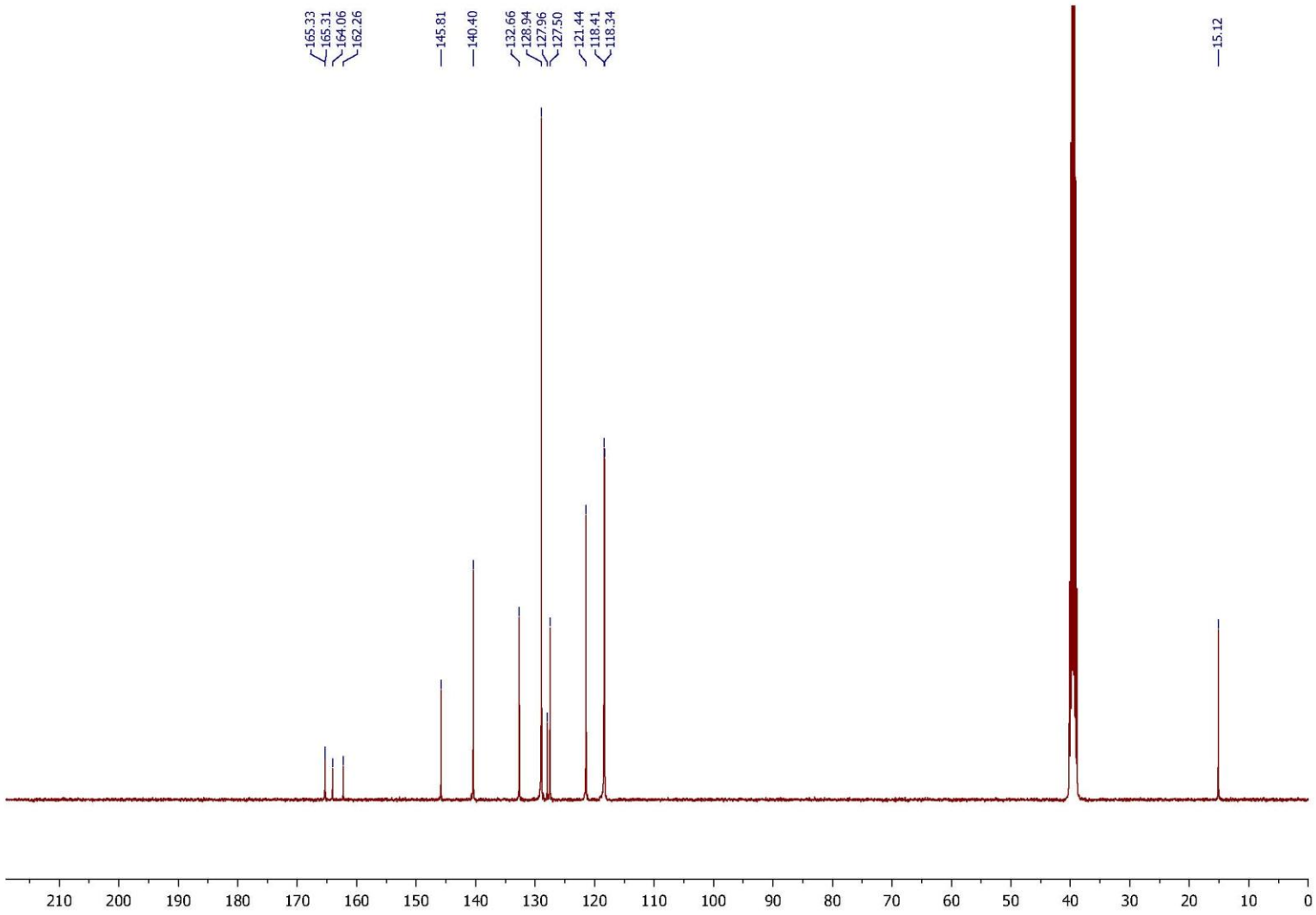
^{31}P NMR (162 MHz, DMSO-d_6) of *P*-[5-(antracene-9-yl)-1,3,4-thiadiazol-2-yl]-*N,N'*-diphenylphosphonic diamide (3k)



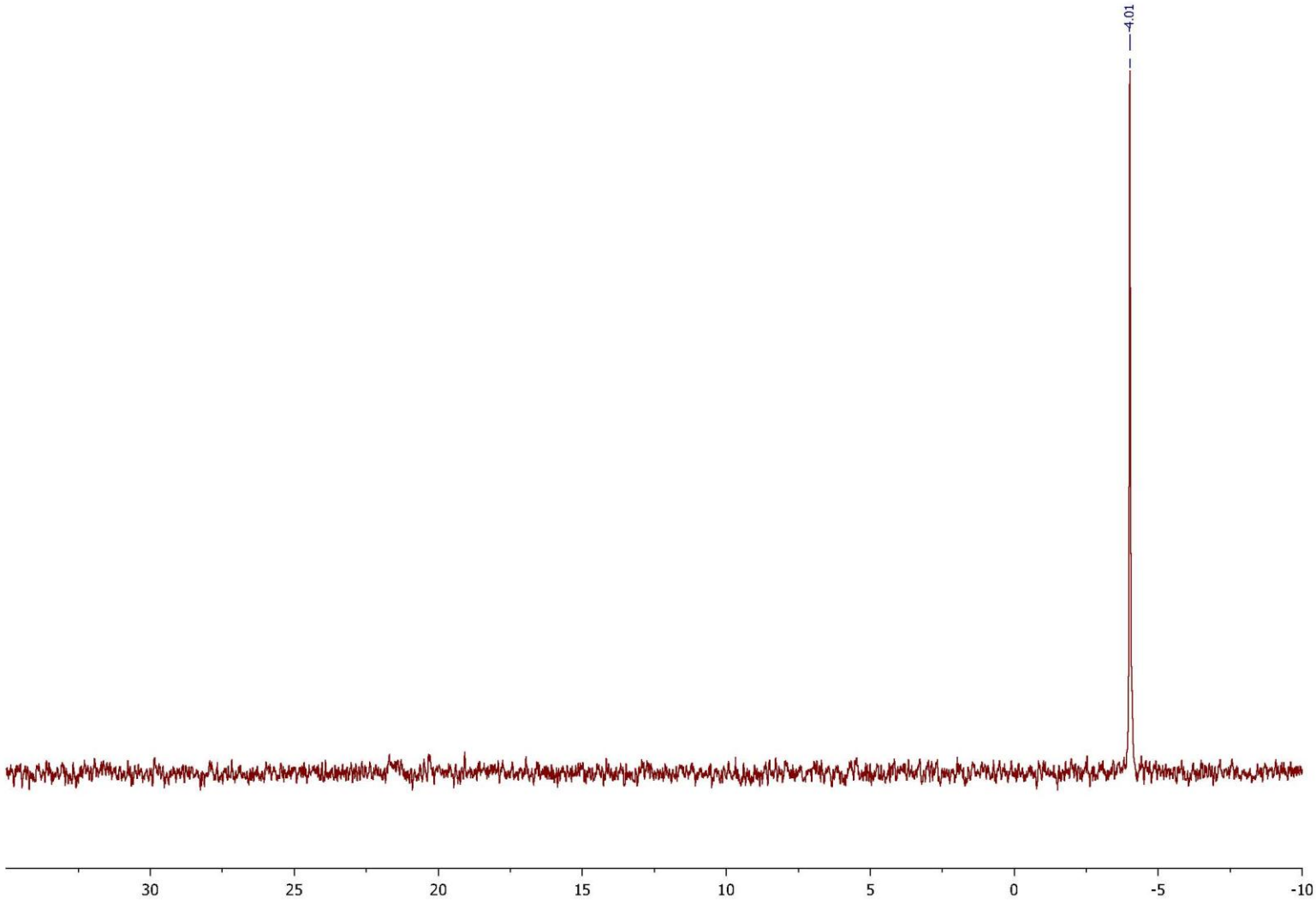
^{1}H NMR (400 MHz, DMSO-d_6) of *P*-[5-(5-methylthiophen-2-yl)-1,3,4-thiadiazol-2-yl]-*N,N′*-diphenylphosphonic diamide (3l)



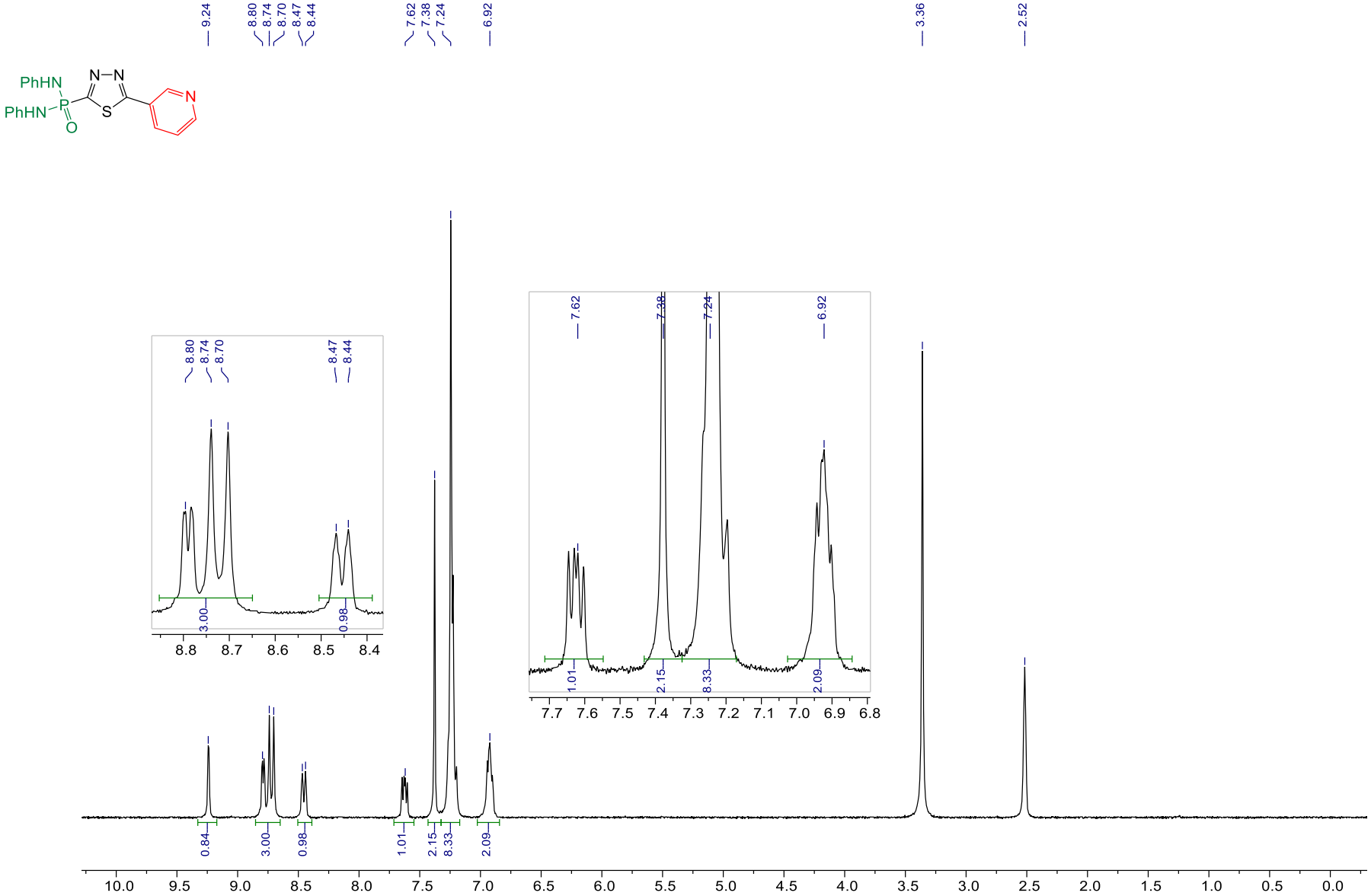
^{13}C NMR (101 MHz, DMSO-d_6) of *P*-[5-(5-methylthiophen-2-yl)-1,3,4-thiadiazol-2-yl]-*N,N′*-diphenylphosphonic diamide (3l)



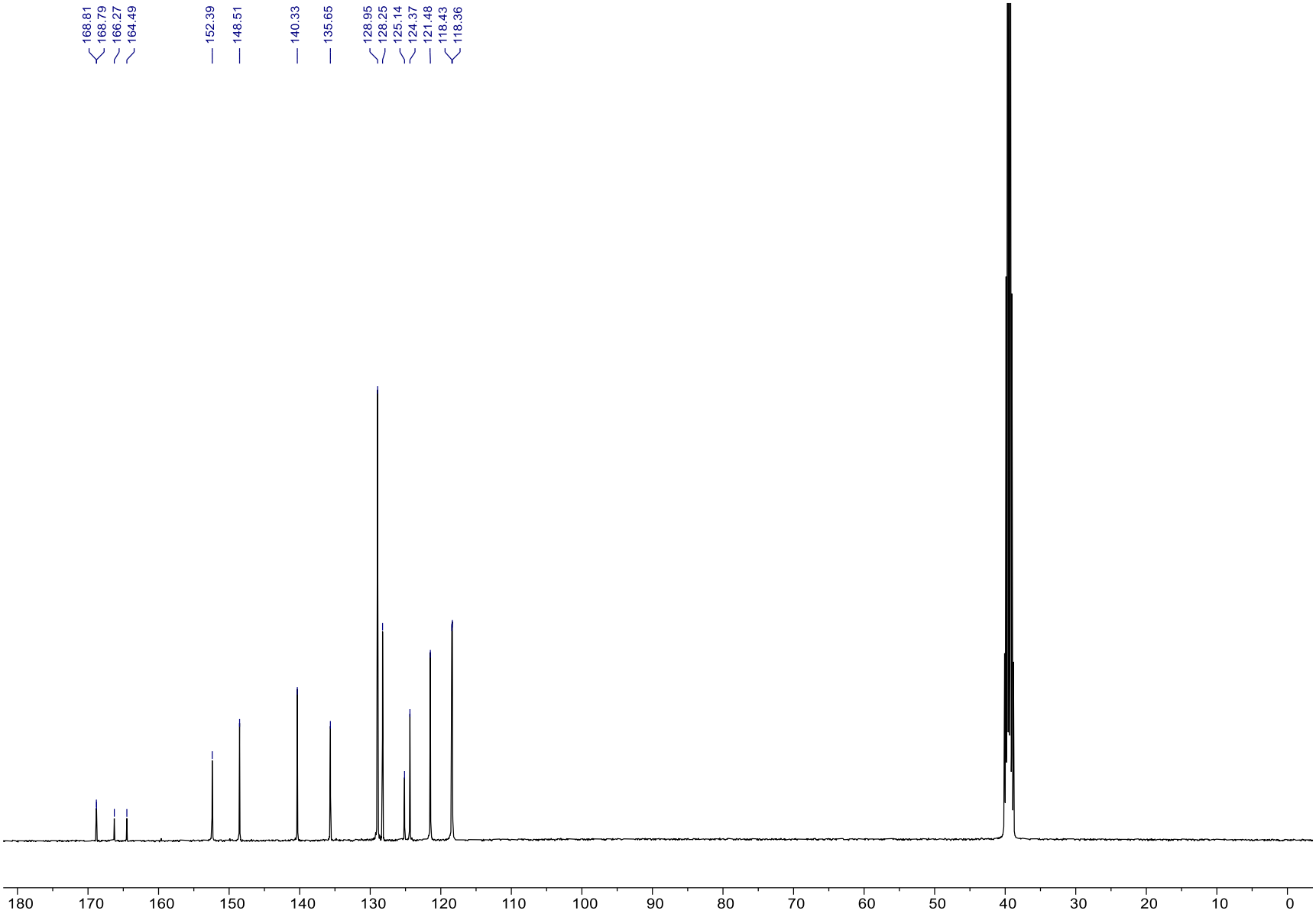
^{31}P NMR (162 MHz, DMSO-d_6) of *P*-[5-(5-methylthiophen-2-yl)-1,3,4-thiadiazol-2-yl]-*N,N′*-diphenylphosphonic diamide (3l)



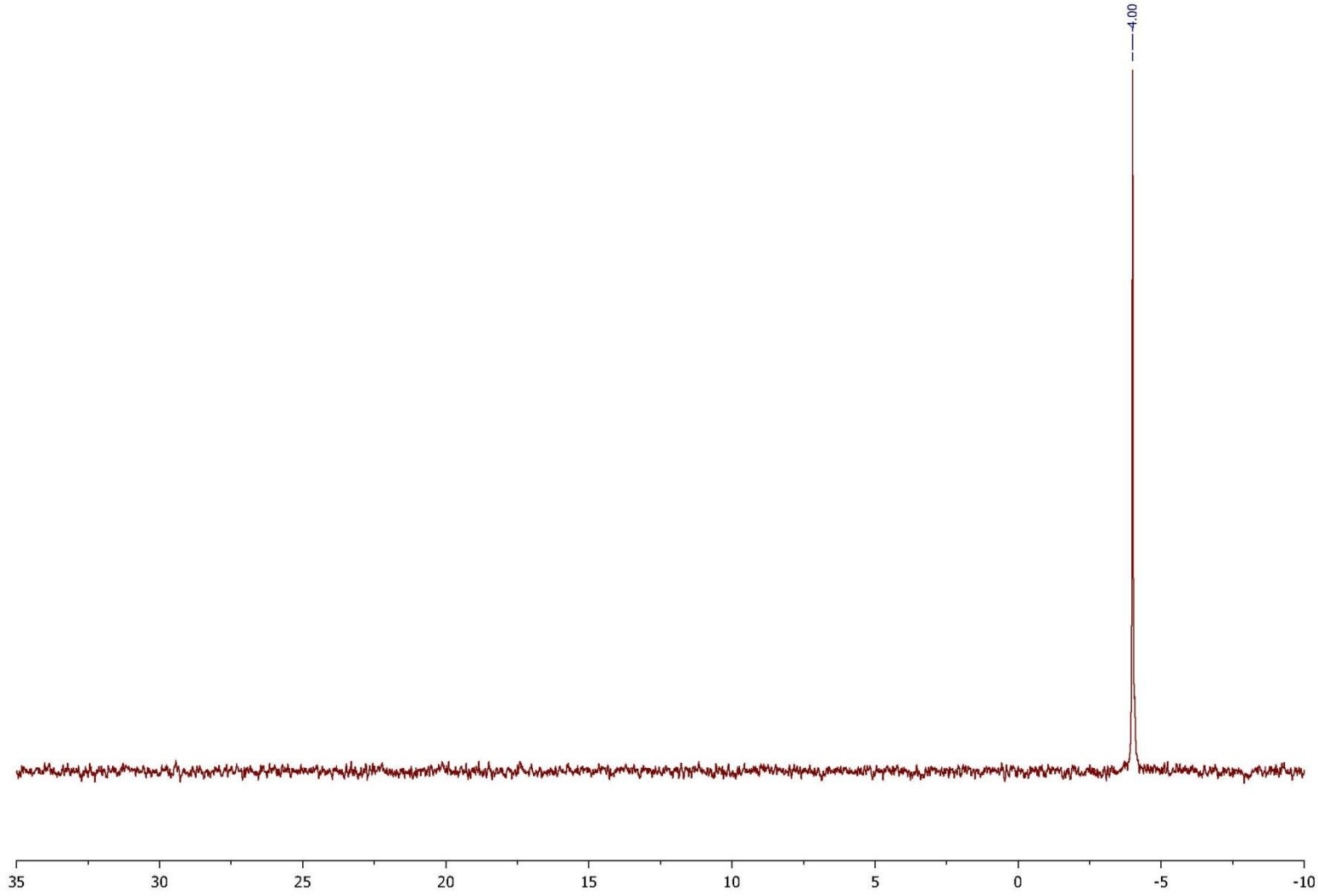
^{1}H NMR (400 MHz, DMSO-d_6) of *P*-[5-(pyridine-3-yl)-1,3,4-thiadiazol-2-yl]-*N,N′*-diphenylphosphonic diamide (3m)



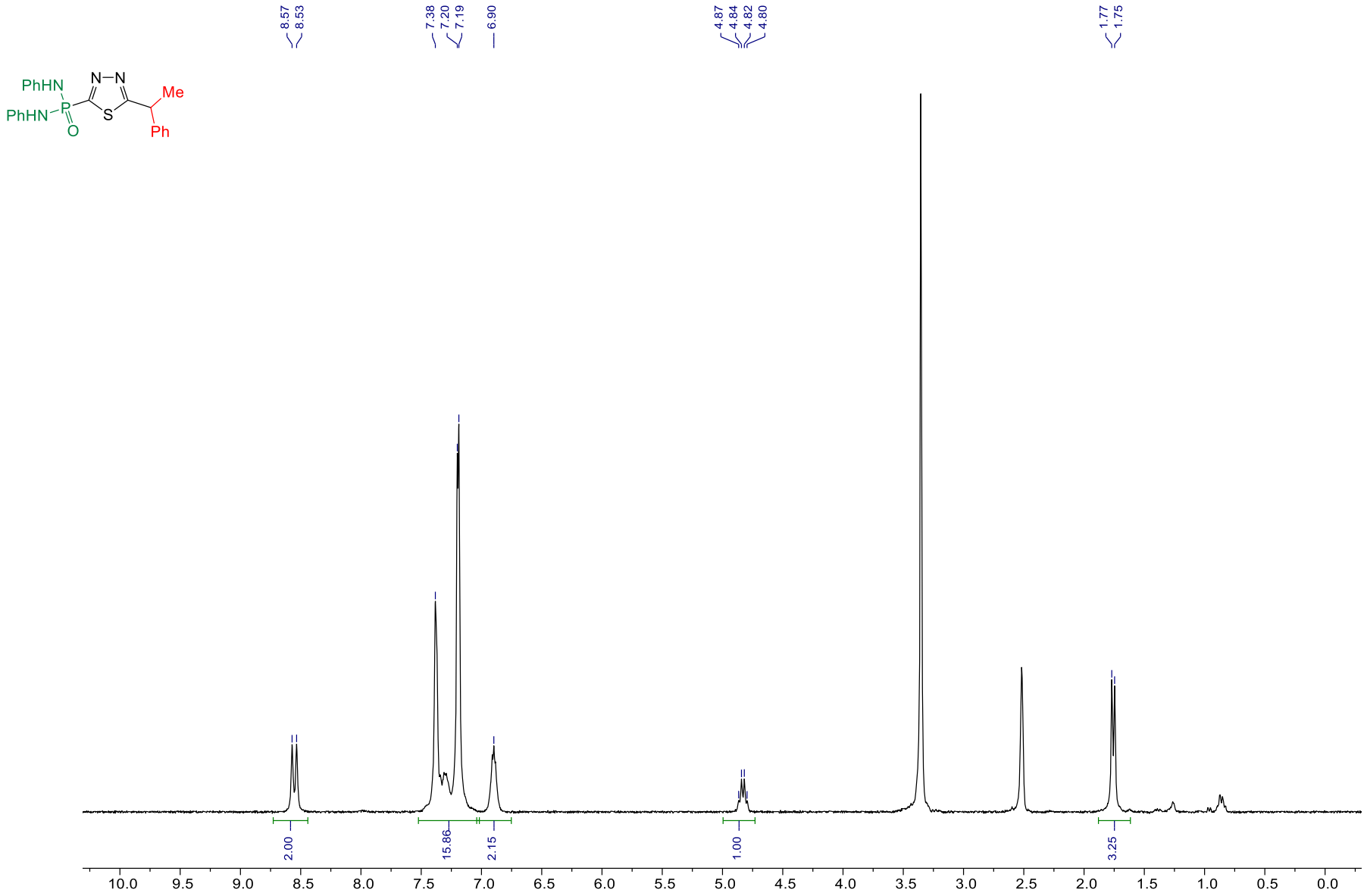
^{13}C NMR (101 MHz, DMSO-d_6) of *P*-[5-(pyridine-3-yl)-1,3,4-thiadiazol-2-yl]-*N,N'*-diphenylphosphonic diamide (3m)



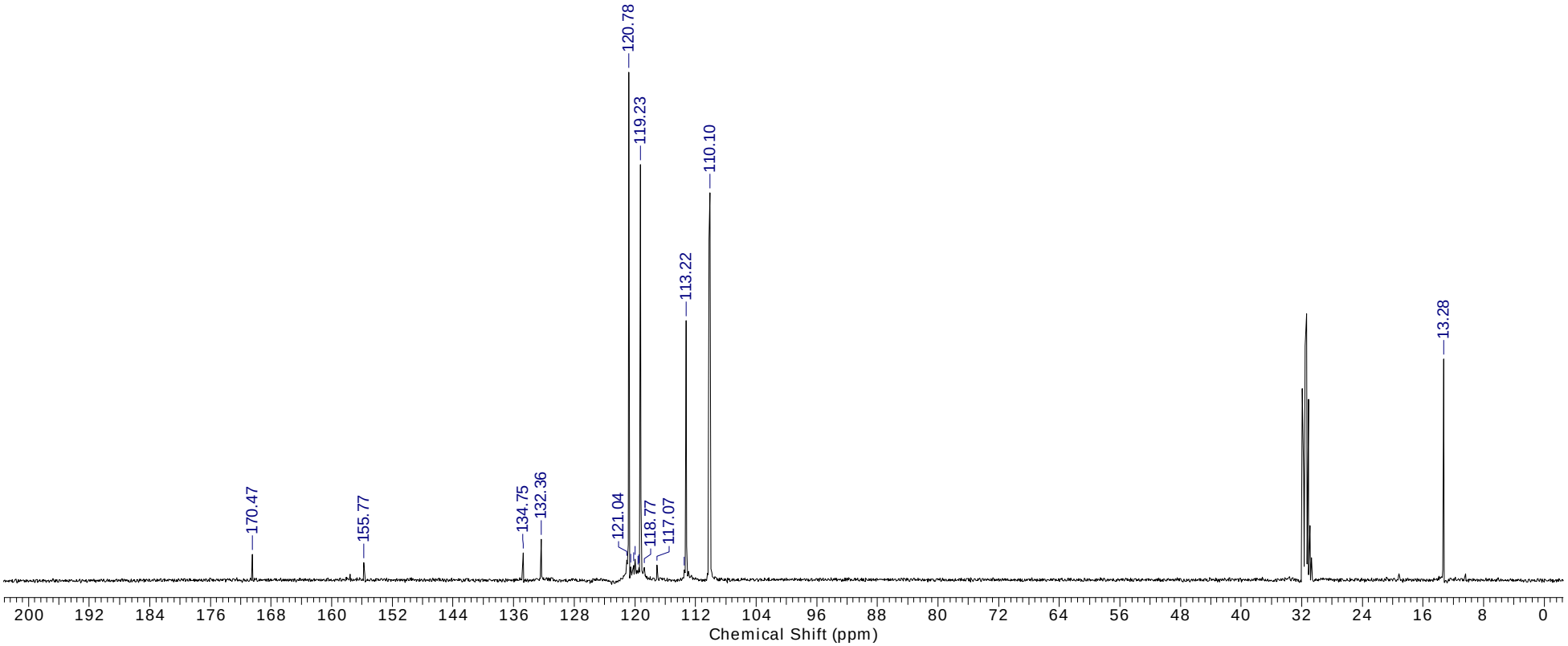
^{31}P NMR (162 MHz, DMSO-d_6) of *P*-[5-(pyridine-3-yl)-1,3,4-thiadiazol-2-yl]-*N,N'*-diphenylphosphonic diamide (3m)



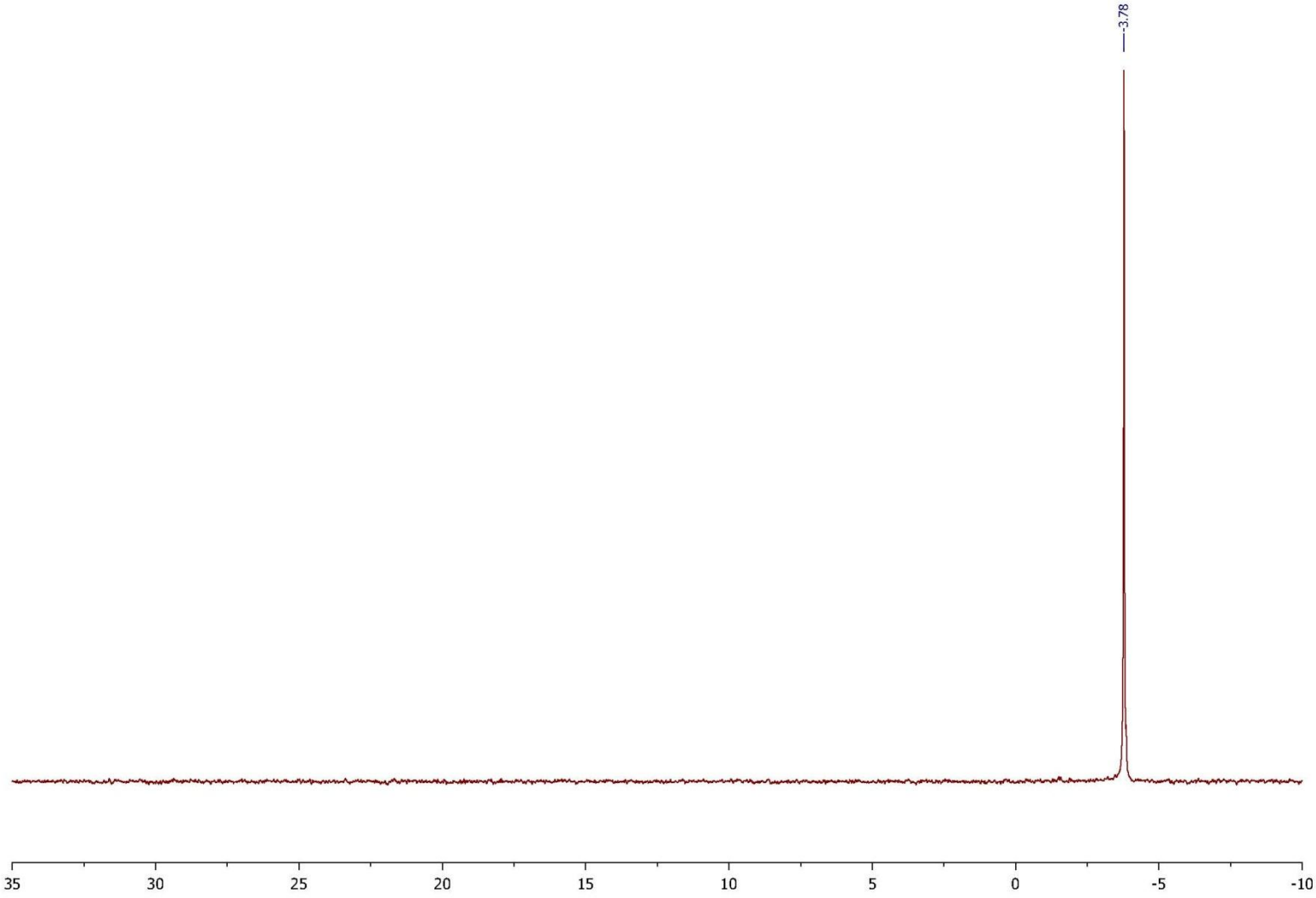
^{1}H NMR (400 MHz, DMSO-d_6) of *P*-[5-(1-phenylethyl)-1,3,4-thiadiazol-2-yl]-*N,N′*-diphenylphosphonic diamide (3n)



^{13}C NMR (101 MHz, DMSO-d_6) of *P*-[5-(1-phenylethyl)-1,3,4-thiadiazol-2-yl]-*N,N'*-diphenylphosphonic diamide (3n)



^{31}P NMR (162 MHz, DMSO-d_6) of *P*-[5-(1-phenylethyl)-1,3,4-thiadiazol-2-yl]-*N,N'*-diphenylphosphonic diamide (3n)



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