Electronic Supplementary Information

Bioorthogonal activation and mitochondrial targeting of a near-infrared-emitting iridium(III) nitrone complex *via* cyclooctynylated phosphonium cations for enhanced cellular imaging and photodynamic therapy

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Experimental

Materials and instrumentation

Experiments conducted at Imperial College London

All commercially available reagents were used as received from suppliers without further purification. Solvents used were of laboratory grade, and anhydrous solvents were obtained from departmental solvent towers and stored over 3 Å molecular sieves. Tris(3,5-dimethylphenyl)phosphine (**Phos-5**)[1] and tris(4-(2-(2-methoxyethoxy)-ethoxy)phenyl)phosphine (**Phos-7**)[2] were prepared according to literature procedures. Moisture-sensitive reactions were carried out by Schlenk-line techniques, under an inert atmosphere of nitrogen. For **BCN-Phos-*n*** analogous, the reaction was carried out with the protection from light (wrapped in foil). Thin-layer chromatography was performed on silica (Merk Art 5554) and visualised under UV irradiation and iodine staining. Automated column chromatography was performed using a Biotage Isolera Four Flash Chromatography System and a 10 G KP-SIL cartridge. ^{1}H, ^{13}C and ^{31}P nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AV-400 spectrometer at 298 K. Chemical shifts are reported in parts per million (ppm) and coupling constants in Hertz (Hz). Peak multiplicities are abbreviated as: s = singlet, d = doublet, t = triplet, m = multiplet and br = broad. Electrospray ionisation (ESI) mass spectrometry analyses were conducted by the Mass Spectrometry Service, Imperial College London.

Experiments conducted at City University of Hong Kong

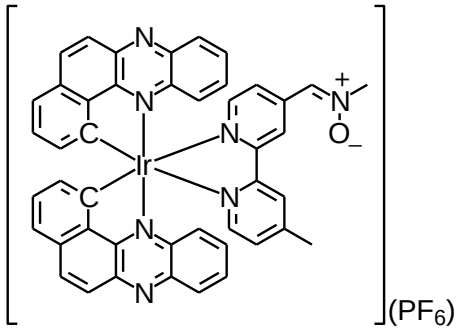
All solvents were of analytical reagent grade and purified according to standard procedures.[3] *N*-Methylhydroxylamine hydrochloride, Na_2CO_3, $MgSO_4$, $NaHCO_3$, *o*-phenylenediamine and KPF_6 were purchased from Acros. 4,4'-Dimethyl-2,2'-bipyridine,

SeO_2, $Na_2S_2O_5$, 1,2-naphthoquinone, $IrCl_3 \cdot 3H_2O$, (1*R*,8*S*,9*s*)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (BCN-OH) and Neutral Red were purchased from Aldrich. All these chemicals were used without further purification. 4-((Methyl(oxido)imino)methyl)-4'-methyl-2,2'-bipyridine (bpy-nitrone),[4] benzo[*a*]phenazine (Hbpz)[5] and the iridium(III) dimer $[Ir_2(bpz)_4Cl_2]$[6] were prepared according to literature procedures. All buffer components were of biological grade and used as received. Autoclaved Milli-Q water was used for the preparation of the aqueous solutions. HeLa cells were obtained from American Type Culture Collection. Dulbecco's Modified Eagle Medium (DMEM), phosphate-buffered saline (PBS), fetal bovine serum (FBS), trypsin-EDTA, penicillin/streptomycin and MitoTracker Green were purchased from Invitrogen. The growth medium for cell culture contained DMEM with 10% FBS and 1% penicillin/streptomycin.

^1H and ^{13}C NMR spectra were recorded on a Bruker 600 MHz AVANCE III spectrometer at 298 K using deuterated solvents. Chemical shifts (δ, ppm) were reported relative to tetramethylsilane (TMS). Positive-ion ESI mass spectra were recorded on a SCIEX API-3200 Triple-Q MS/MS mass spectrometer at 298 K. High-resolution ESI (HR-ESI) mass spectra were recorded on a Bruker micrOTOF-QII. Infrared (IR) spectra of the samples in KBr pellets were recorded in the range of 4000 – 400 cm^{-1} using a Perkin Elmer FTIR–1600 spectrophotometer. Elemental analyses were carried out on an Elementar Analysensysteme GmbH Vario MICRO elemental analyser.

Synthesis

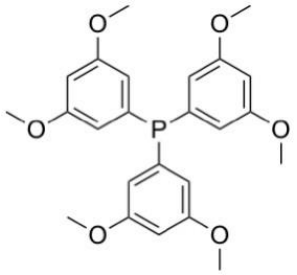
[Ir(bpz)$_2$(bpy-nitrone)](PF$_6$) (**1**)



A mixture of [Ir$_2$(bpz)$_4$Cl$_2$][6] (127 mg, 0.09 mmol) and bpy-nitrone[4] (42 mg, 0.18 mmol) in CH_2Cl_2/MeOH (20 mL) (1:1, v/v) was stirred under an inert atmosphere of nitrogen in the dark for 18 h. The mixture was further stirred for 2 h after addition of solid KPF_6 (34 mg, 0.18 mmol). The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel using CH_2Cl_2/acetone (10:1, v/v) as the eluent. The solvent was removed under reduced pressure to give a black solid. Subsequent recrystallisation of the solid from CH_2Cl_2/Et_2O afforded the complex as black crystals. Yield: 129 mg (68%). ^{1}H NMR (600 MHz, acetone-d_6, 298 K, TMS): δ 9.36 (s, 1H, H3 of bpy), 8.44 (s, 1H, H3’ of bpy), 8.41 – 8.37 (m, 2H, H7 of bpz), 8.31 – 8.28 (m, 2H, H6 of bpz), 8.05 (d, *J* = 6.0 Hz, 1H, H6 of bpy), 8.02 – 7.99 (m, 3H, H5 of bpz and bpy-C*H*=N), 7.94 – 7.88 (m, 3H, H8 of bpz and H5 of bpy), 7.75 – 7.74 (m, 3H, H4 of bpz and H6’ of bpy), 7.62 – 7.49 (m, 4H, H9 and H10 of bpz), 7.40 – 7.39 (d, *J* = 5.7 Hz, 1H, H5’ of bpy), 7.19 (t, *J* = 7.6 Hz, 2H, H3 of bpz), 6.63 – 6.60 (m, 2H, H2 of bpz), 3.94 (s, 3H, CH_3 of nitrone), 2.53 (s, 3H, CH_3 of bpy). ^{13}C NMR (150 MHz, acetone-d_6, 298 K, TMS): δ 156.6, 155.7, 155.1, 153.1, 150.6, 150.4, 149.4, 148.6, 145.7, 143.8, 141.42, 141.36, 141.2, 141.1, 140.8, 135.9, 135.8, 134.5,

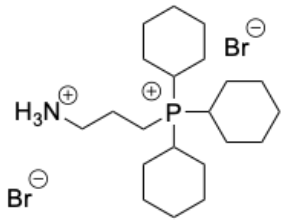
132.7, 132.5, 131.8, 131.7, 131.6, 131.4, 131.0, 130.1, 130.0, 129.7, 126.4, 125.4, 125.2, 123.0, 122.8, 121.77, 121.75, 120.7, 55.1, 20.4. IR (KBr) $\tilde{\nu}$/cm^{-1}: 1615 (C=N), 1560 (C=N), 1195 (N–O), 845 (PF_6^-). HR-ESI-MS (positive-ion mode, *m*/*z*): $[M - PF_6^-]^+$ calcd for $IrC_{45}N_7OH_{31}$: 878.2219, found 878.2227. Anal. calcd for $IrC_{45}H_{31}N_7O_1PF_6 \cdot 2H_2O$: C 51.04, H 3.33, N 9.26, found: C 50.99, H 3.26, N 9.03%.

Tris(3,5-dimethoxyphenyl)phosphine (**Phos-6**)



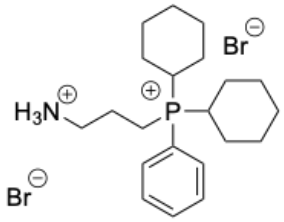
A solution of 1-bromo-3,5-dimethoxybenzene (3.41 g, 15.7 mmol) in anhydrous THF (9 mL) was added to magnesium turnings (0.39 g, 16.0 mmol) dropwise over 10 min while maintaining reflux. The resulting suspension was stirred under an inert atmosphere of nitrogen. After 2 h, a solution of PCl_3 (0.5 mL, 5.73 mmol) in anhydrous THF (12 mL) was added dropwise over 15 min, and the resulting solution was stirred at room temperature under an inert atmosphere of nitrogen for additional 2 h. The reaction was then quenched with water (10 mL). The organic phase was separated and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (gradient from *n*-hexane to *n*-hexane/CH_2Cl_2 (1:1, v/v)) to afford the title compound as a white solid. Yield: 841.1 mg (36%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 6.49 – 6.43 (m, 9H), 3.72 (s, 18H). ^{13}C NMR (101 MHz, $CDCl_3$, 298 K): δ 160.8 (d, $^3J_{C–P}$ = 9.8 Hz), 139.0 (d, $^2J_{C–P}$ = 11.3 Hz), 111.6 (d, $^1J_{C–P}$ = 21.7 Hz), 101.2 (d, $^4J_{C–P}$ = 9.1 Hz), 55.5. ^{31}P NMR (162 MHz, $CDCl_3$, 298 K): δ 0.75. ESI-MS (positive-ion mode, *m*/*z*): 443.2 $[C_{24}H_{28}O_6P]^+$. HR-ESI-MS (positive-ion mode, *m*/*z*): calcd for $[C_{24}H_{28}O_6P]^+$: 443.1624, found: 443.1622.

(3-Ammoniopropyl)tricyclohexylphosphonium dibromide (**H_2N-Phos-1**)



3-Bromopropylamine hydrobromide (970.0 mg, 4.43 mmol) was added to a solution of tricyclohexylphosphine (**Phos-1**) (1.10 g, 3.92 mmol) in *n*-butanol (7 mL). The mixture was heated at 120°C under an inert atmosphere of nitrogen. After 3 d, the reaction mixture was cooled to room temperature and *n*-hexane (35 mL) was added to give an off-white gum. The solid was isolated by centrifugation (4,500 rpm for 5 min) and purified by recrystallisation from isopropanol/Et_2O to afford the title compound as a pale-yellow gum which was used in the next step without further purification. ESI-MS (positive-ion mode, *m*/*z*): 338.3 $[C_{21}H_{41}NP]^+$. HR-ESI-MS (positive-ion mode, *m*/*z*): calcd for $[C_{21}H_{41}NP]^+$: 338.2977, found: 338.2975.

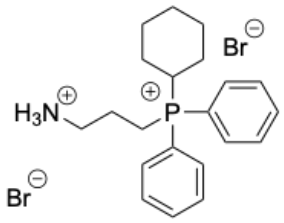
(3-Ammoniopropyl)dicyclohexyl(phenyl)phosphonium dibromide (**H_2N-Phos-2**)



3-Bromopropylamine hydrobromide (762.8 mg, 3.48 mmol) was added to a solution of dicyclohexyl(phenyl)phosphine (**Phos-2**) (880.3 mg, 3.21 mmol) in *n*-butanol (8 mL). The mixture was heated at 120°C under an inert atmosphere of nitrogen. After 3 d, the reaction mixture was cooled to room temperature and *n*-hexane (50 mL) was added to give an off-white solid. The solid was isolated by centrifugation (4,500 rpm for 5 min) and purified by recrystallisation from isopropanol/Et_2O to afford the title compound as a colourless oil that slowly solidified to a white solid. Yield: 823.4 mg (52 %). ^{1}H NMR (400 MHz, CD_3OD, 298 K): δ 8.01 – 9.96 (m, 2H), 7.88 – 7.85 (m, 1H), 7.80 – 7.76 (m, 2H), 3.28 – 3.24 (m, 2H), 3.07 – 2.90 (m, 4H), 2.19 – 2.07 (m, 4H), 1.95 – 1.84 (m, 6H), 1.79 – 1.75 (m, 2H), 1.81 – 1.31 (m, 10H). ^{13}C NMR (101 MHz, CD_3OD, 298 K): δ 135.8 (d, $^4J_{C–P}$ = 3.0 Hz), 134.4 (d, $^3J_{C–P}$ = 7.7 Hz), 131.4 (d, $^2J_{C–P}$ = 11.3 Hz), 115.6 (d, $^1J_{C–P}$ = 75.7 Hz), 41.0 (d, $^2J_{C–P}$ = 19.2 Hz), 30.8 (d, $^1J_{C–P}$ = 44.2 Hz), 27.1 – 27.0*, 26.4, 22.2 (d, $^4J_{C–P}$ = 3.5 Hz), 14.1 (d, $^1J_{C–P}$ = 48.4 Hz). ^{31}P NMR (162 MHz, CD_3OD, 298 K): δ 32.3. ESI-MS (positive-ion mode, *m*/*z*): 332.3 $[C_{21}H_{35}NP]^+$. HR-ESI-MS (positive-ion mode, *m*/*z*): calcd for $[C_{21}H_{35}NP]^+$: 332.2507, found: 332.2509.

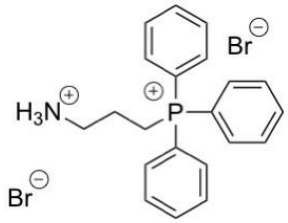
* Broad peak, two ^{13}C environments are present.

(3-Ammoniopropyl)cyclohexyl(diphenyl)phosphonium dibromide (**H_2N-Phos-3**)



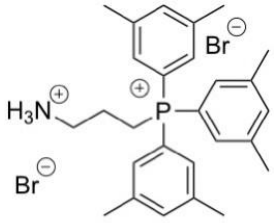
3-Bromopropylamine hydrobromide (770.0 mg, 3.52 mmol) was added to a solution of cyclohexyl(diphenyl)phosphine (**Phos-3**) (888.7 mg, 3.31 mmol) in *n*-butanol (7 mL). The mixture was heated at 120°C under an inert atmosphere of nitrogen. After 3 d, the reaction mixture was cooled to room temperature and *n*-hexane (35 mL) was added to give an off-white solid. The solid was isolated by centrifugation (4,500 rpm for 5 min) and purified by recrystallisation from isopropanol/Et_2O to afford the title compound as a white solid. Yield: 821.6 mg (51%). ^{1}H NMR (400 MHz, CD_3OD, 298 K): δ 7.95 – 7.90 (m, 6H), 7.84 – 7.78 (m, 4H), 3.58 – 3.45 (m, 1H), 3.23 – 3.10 (m, 4H), 2.12 – 2.08 (m, 2H), 1.90 – 1.74 (m, 5H), 1.68 – 1.55 (m, 2H), 1.22 – 1.06 (m, 3H). ^{13}C NMR (101 MHz, CD_3OD, 298 K): δ 136.4 (d, $^4J_{C–P}$ = 3.2 Hz), 135.2 (d, $^3J_{C–P}$ = 8.8 Hz), 131.5 (d, $^2J_{C–P}$ = 11.8 Hz), 116.4 (d, $^1J_{C–P}$ = 81.3 Hz), 40.6 (d, $^2J_{C–P}$ = 20.0 Hz), 31.8 (d, $^1J_{C–P}$ = 46.8 Hz), 26.7 (br s), 26.5 (d, $^2J_{C–P}$ = 21.8 Hz), 21.4 (d, $^4J_{C–P}$ = 3.1 Hz), 18.3 (d, $^1J_{C–P}$ = 51.6 Hz). ^{31}P NMR (162 MHz, CD_3OD, 298 K): δ 30.5. ESI-MS (positive-ion mode, *m*/*z*): 326.2 $[C_{21}H_{29}NP]^+$. HR-ESI-MS (positive-ion mode, *m*/*z*): calcd for $[C_{21}H_{29}NP]^+$: 326.2038, found: 326.2031.

(3-Ammoniopropyl)triphenylphosphonium dibromide (**H_2N-Phos-4**)



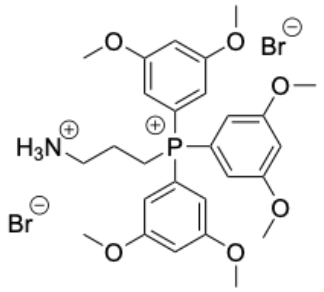
3-Bromopropylamine hydrobromide (1.04 g, 4.75 mmol) was added to a solution of triphenylphosphine (**Phos-4**) (1.16 g, 4.43 mmol) in *n*-butanol (20 mL). The mixture was heated at 120°C under an inert atmosphere of nitrogen. After 3 d, the reaction mixture was cooled to room temperature and *n*-hexane (50 mL) was added to give a white precipitate. The solid was isolated by centrifugation (4,500 rpm for 5 min) and purified by recrystallisation from isopropanol/Et_2O to afford the title compound as a white solid. Yield: 1.19 g (56%). ^{1}H NMR (400 MHz, CD_3OD, 298 K): δ 7.95 – 7.76 (m, 15H), 3.70 – 3.63 (m, 2H), 3.25 – 3.21 (m, 2H), 2.10 – 2.00 (m, 2H). ^{13}C NMR (101 MHz, CD_3OD, 298 K): δ 136.6 (d, $^4J_{C–P}$ = 3.1 Hz), 134.9 (d, $^3J_{C–P}$ = 10.0 Hz), 131.7 (d, $^2J_{C–P}$ = 12.6 Hz), 119.2 (d, $^1J_{C–P}$ = 87.2 Hz), 40.7 (d, $^2J_{C–P}$ = 21.6 Hz), 21.9 (d, $^3J_{C–P}$ = 2.7 Hz), 20.7 (d, $^1J_{C–P}$ = 54.7 Hz). ^{31}P NMR (162 MHz, CD_3OD, 298 K): δ 23.7. ESI-MS (positive-ion mode, *m*/*z*): 320.2 $[C_{21}H_{29}NP]^+$. HR-ESI-MS (positive-ion mode, *m*/*z*): calcd for $[C_{21}H_{29}NP]^+$: 320.1568, found: 320.1570.

(3-Ammoniopropyl)tris(3,5-dimethylphenyl)phosphonium dibromide (**H_2N-Phos-5**)



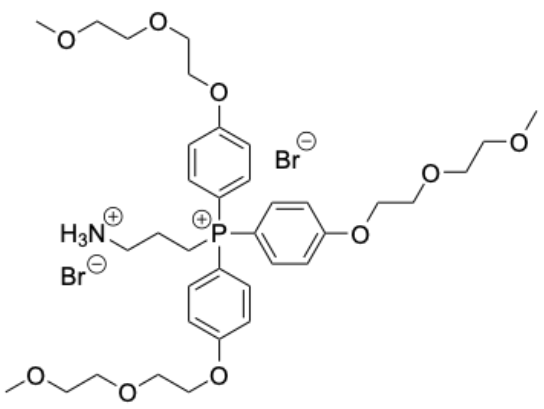
3-Bromopropylamine hydrobromide (518.9 mg, 2.37 mmol) was added to a solution of **Phos-5**[1] (787.3 mg, 2.27 mmol) in *n*-butanol (14 mL). The mixture was heated at 120°C under an inert atmosphere of nitrogen. After 3 d, the reaction mixture was cooled to room temperature and *n*-hexane (35 mL) was added to give a white precipitate. The solid was isolated by centrifugation (4,500 rpm for 5 min) and purified by recrystallisation from isopropanol/Et_2O to afford the title compound as a white solid. Yield: 654.4 mg (51%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 7.34 – 7.31 (m, 9H), 3.76 – 3.68 (m, 2H), 3,42 – 3.36 (m, 2H), 2.41 (m, 18H), 2.08 – 2.05 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$, 298 K): δ 140.7 (d, $^2J_{C–P}$ = 13.2 Hz), 137.0 (d, $^4J_{C–P}$ = 3.2 Hz), 131.0 (d, $^3J_{C–P}$ = 10.1 Hz), 117.9 (d, $^1J_{C–P}$ = 85.1 Hz), 40.2 (d, $^2J_{C–P}$ = 19.2 Hz), 21.6 (d, $^1J_{C–P}$ = 53.4 Hz), 21.6, 20.1. ^{31}P NMR (162 MHz, $CDCl_3$, 298 K): δ 23.4. ESI-MS (positive-ion mode, *m*/*z*): 404.3 $[C_{27}H_{35}NP]^+$. HR-ESI-MS (positive-ion mode, *m*/*z*): calcd for $[C_{27}H_{35}NP]^+$: 404.2507, found: 404.2512.

(3-Ammoniopropyl)tris(3,5-dimethoxyphenyl)phosphonium dibromide (**H_2N-Phos-6**)



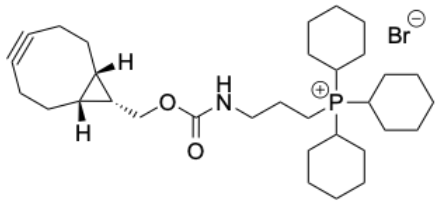
3-Bromopropylamine hydrobromide (462.3 mg, 2.11 mmol) was added to a solution of **Phos-6** (860.5 mg, 1.94 mmol) in *n*-butanol (10 mL). The mixture was heated at 120°C under an inert atmosphere of nitrogen. After 3 d, the reaction mixture was cooled to room temperature and *n*-hexane (35 mL) was added to give a white precipitate. The solid was isolated by centrifugation (4,500 rpm for 5 min) and purified by recrystallisation from isopropanol/Et_2O to afford the title compound as a white solid. Yield: 425.7 mg (44%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 6.80 – 6.74 (m, 9H), 4.15 – 4.01 (m, 2H), 3.86 (s, 18H), 3.41 (t, $^3J_{H–H}$ = 6.1 Hz, 2H), 2.32 (br s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$, 298 K): δ 162.0 (d, $^3J_{C–P}$ = 19.0 Hz), 119.4 (d, $^1J_{C–P}$ = 87.0 Hz), 111.2 (d, $^2J_{C–P}$ = 11.6 Hz), 106.9, 56.6, 39.9 (d, $^2J_{C–P}$ = 18.8 Hz), 21.0 (d, $^1J_{C–P}$ = 52.4 Hz), 20.2. ^{31}P NMR (162 MHz, $CDCl_3$, 298 K): δ 27.5. ESI-MS (positive-ion mode, *m*/*z*): 500.2 $[C_{27}H_{35}NO_6P]^+$. HR-ESI-MS (positive-ion mode, *m*/*z*): calcd for $[C_{27}H_{35}NO_6P]^+$: 500.2202, found: 500.2210.

(3-Ammoniopropyl)tris(4-(2-(2-methoxyethoxy)ethoxy)phenyl)phosphonium dibromide (**H_2N-Phos-7**)



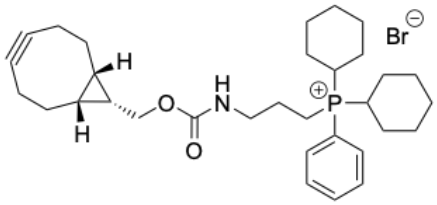
3-Bromopropylamine hydrobromide (236.3 mg, 1.08 mmol) was added to a solution of **Phos-7**[2] (608.3 mg, 0.99 mmol) in *n*-butanol (2.5 mL). The reaction mixture was heated at 120°C under an inert atmosphere of nitrogen. After 3 d, the reaction mixture was cooled to room temperature and *n*-hexane (35 mL) was added to give an off-white solid. The solid was isolated by centrifugation (4,500 rpm for 5 min) and purified by recrystallisation from isopropanol/Et_2O to afford the title compound as a pale-yellow oily solid (512.9 mg, 62 %). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 7.68 – 7.62 (m, 6H), 7.17 – 7.14 (dd, $^3J_{H–H}$ = 9.0 Hz and $^4J_{H–P}$ = 2.6 Hz, 6H), 4.23 (t, $^3J_{H–H}$ = 4.4 Hz, 6H), 3.78 – 3.68 (m, 8H), 3.56 – 3.54 (m, 6H), 3.39 – 3.36 (m, 11H), 2.26 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$, 298 K): δ 164.0, 135.7 (d, $^3J_{C–P}$ = 11.4 Hz), 116.9 (d, $^2J_{C–P}$ = 13.4 Hz), 108.9 (d, $^1J_{C–P}$ = 94.4 Hz), 72.0, 70.8, 69.4, 68.1, 40.1 (d, $^2J_{C–P}$ = 18.6 Hz), 22.0 (d, $^1J_{C–P}$ = 56.5 Hz), 20.2. ^{31}P NMR (162 MHz, $CDCl_3$, 298 K): δ 21.7. ESI-MS (positive-ion mode, *m*/*z*): 674.3 $[C_{36}H_{53}NO_9P]^+$. HR-ESI-MS (positive-ion mode, *m*/*z*): calcd for $[C_{36}H_{53}NO_9P]^+$: 674.3452, found: 674.3461.

BCN-Phos-1



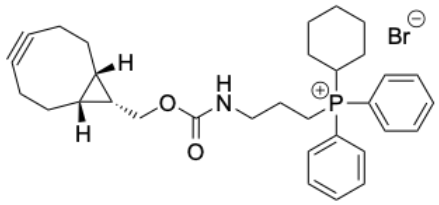
Triethylamine (11 μL) was added to a solution of (1*R*,8*S*,9*s*)-bicyclo[6.1.0]non-4-yn-9-ylmethyl-*N*-succinimidyl carbonate (BCN-NHS) (15.5 mg, 0.053 mmol) and **H_2N-Phos-1** (26.3 mg, 0.052 mmol) in anhydrous CH_2Cl_2 (7 mL). The mixture was stirred at room temperature in the dark under an inert atmosphere of nitrogen for 24 h. Then the reaction mixture was diluted with CH_2Cl_2 (20 mL), and the organic layer was washed with water (10 mL), dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (gradient from CH_2Cl_2 to CH_2Cl_2/MeOH (10:1, v/v)) to afford the title compound as a colourless oil. Yield: 14.2 mg (45%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 6.83 (t, $^3J_{H–H}$ = 6.2 Hz, 1H), 4.09 (d, $^3J_{H–H}$ = 8.0 Hz, 2H), 3.44 – 3.39 (m, 2H), 2.70 – 2.63 (m, 2H), 2.42 – 2.15 (m, 8H), 2.00 – 1.80 (m, 19H), 1.56 – 1.24 (m, 17H), 0.90 (t, $^3J_{H–H}$ = 9.7 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$, 298 K): δ 157.6, 99.0, 62.6, 41.0 (d, $^2J_{C–P}$ = 15.0 Hz), 30.2 (d, $^1J_{C–P}$ = 40.4 Hz), 29.2, 27.2 (d, $^3J_{C–P}$ = 4.0 Hz), 26.7 (d, $^2J_{C–P}$ = 11.8 Hz), 25.5, 22.9, 21.6, 20.3, 18.0, 13.8 (d, $^1J_{C–P}$ = 44.2 Hz). ^{31}P NMR (162 MHz, $CDCl_3$, 298 K): δ 32.8. ESI-MS (positive-ion mode, *m*/*z*): 514.4 $[C_{32}H_{53}NO_2P]^+$. HR-ESI-MS (positive-ion mode, *m*/*z*): calcd for $[C_{32}H_{53}NO_2P]^+$: 514.3814, found: 514.3801.

BCN-Phos-2



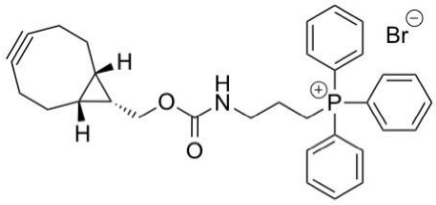
Triethylamine (36 μL) was added to a solution of BCN-NHS (15.6 mg, 0.053 mmol) and **H_2N-Phos-2** (25.2 mg, 0.051 mmol) in anhydrous CH_2Cl_2 (7 mL). The mixture was stirred at room temperature in the dark under an inert atmosphere of nitrogen for 24 h. Then the reaction mixture was diluted with CH_2Cl_2 (20 mL), and the organic layer was washed with water (10 mL), dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (gradient from CH_2Cl_2 to CH_2Cl_2/MeOH (10:1, v/v)) to afford the title compound as a colourless oil. Yield: 20.7 mg (69%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 7.90 – 7.86 (m, 2H), 7.76 – 7.67 (m, 3H), 6.91 (t, $^3J_{H–H}$ = 6.3 Hz, 1H), 4.10 (d, $^3J_{H–H}$ = 7.8 Hz, 2H), 3.50 – 3.46 (m, 2H), 3.16 – 3.06 (m, 2H), 2.71 – 2.63 (m, 2H), 2.29 – 2.02 (m, 8H), 1.99 – 1.74 (m, 12H), 1.38 – 1.17 (m, 14H), 0.92 – 0.79 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$, 298 K): δ 157.6, 134.6, 133.1 (d, $^3J_{C–P}$ = 7.6 Hz), 130.5 (d, $^2J_{C–P}$ = 11.3 Hz), 114.8 (d, $^1J_{C–P}$ = 75.2 Hz), 99.0, 62.6, 40.8 (d, $^2J_{C–P}$ = 14.8 Hz), 30.3 (d, $^1J_{C–P}$ = 44.1), 29.8, 29.2, 26.3 (d, $^2J_{C–P}$ = 7.0 Hz), 26.2 (d, $^3J_{C–P}$ = 7.0 Hz), 26.0, 25.4, 21.6, 20.2, 18.0, 13.9 ($^1J_{C–P}$ = 46.4 Hz). ^{31}P NMR (162 MHz, $CDCl_3$, 298 K): δ 32.42. ESI-MS (positive-ion mode, *m*/*z*): 508.3 $[C_{32}H_{49}NO_2P]^+$. HR-ESI-MS (positive-ion mode, *m*/*z*): calcd for $[C_{32}H_{49}NO_2P]^+$: 508.3339, found: 508.3339.

BCN-Phos-3



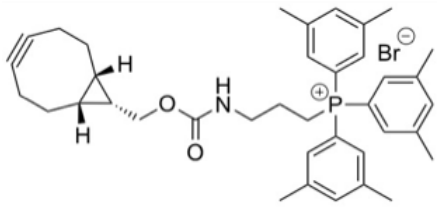
Triethylamine (19 μL) was added to a solution of BCN-NHS (16.2 mg, 0.056 mmol) and **H_2N-Phos-3** (22.2 mg, 0.046 mmol) in anhydrous CH_2Cl_2 (6 mL). The mixture was stirred at room temperature in the dark under an inert atmosphere of nitrogen for 24 h. Then the reaction mixture was diluted with CH_2Cl_2 (20 mL), and the organic layer was washed with water (10 mL), dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (gradient from CH_2Cl_2 to CH_2Cl_2/MeOH (10:1, v/v)) to afford the title compound as a colourless oil. Yield: 13.1 mg (49%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 7.90 – 7.67 (m, 10H), 6.61 (t, $^3J_{H–H}$ = 6.3 Hz, 1H), 4.11 – 4.03 (m, 2H), 3.39 – 3.28 (m, 4H), 2.30 – 2.03 (m, 7H), 1.87 – 1.47 (m, 10H), 1.37 – 1.19 (m, 2H), 1.14 – 0.99 (m, 3H), 0.92 – 0.81 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$, 298 K): δ 157.5, 135.0, 133.9 (d, $^3J_{C–P}$ = 8.7 Hz), 130.4 (d, $^2J_{C–P}$ = 11.8 Hz), 115.8 (d, $^1J_{C–P}$ = 79.9 Hz), 99.0, 62.6, 40.5 (d, $^2J_{C–P}$ = 15.8 Hz), 31.4 (d, $^1J_{C–P}$ = 46.7 Hz), 31.0, 29.2, 25.7, 25.7, 25.5 (d, $^1J_{C–P}$ = 49.9 Hz), 25.3, 21.6, 20.2, 17.9. ^{31}P NMR (162 MHz, $CDCl_3$, 298 K): δ 31.6. ESI-MS (positive-ion mode, *m*/*z*): 502.3 $[C_{32}H_{41}NO_2P]^+$. HR-ESI-MS (positive-ion mode, *m*/*z*): calcd for $[C_{32}H_{41}NO_2P]^+$: 502.2869, found: 502.2876.

BCN-Phos-4



Triethylamine (14 μL) was added to a solution of BCN-NHS (12.7 mg, 0.044 mmol) and **H_2N-Phos-4** (19.2 mg, 0.040 mmol) in anhydrous CH_2Cl_2 (6.0 mL). The mixture was stirred at room temperature in the dark under an inert atmosphere of nitrogen for 24 h. Then the reaction mixture was diluted with CH_2Cl_2 (20 mL), and the organic layer was washed with water (10 mL), dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (gradient from CH_2Cl_2 to CH_2Cl_2/MeOH (10:1, v/v)) to afford the title compound as an oily white solid. Yield: 13.2 mg (59%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 7.80 – 7.65 (m, 15H), 6,96 (t, $^3J_{H–H}$ = 6.6 Hz, 1H), 4.07 (d, $^3J_{H–H}$ = 8.3 Hz, 2H), 3.86 – 3.82 (m, 2H), 3.50 – 3.47 (m, 2H), 2.26 – 2.14 (m, 6H), 1.88 – 1.83 (m, 4H), 1.34 (t, $^3J_{H–H}$ = 8.3 Hz, 1H), 1.26 – 1.21 (m, 2H), 0.92 – 0.85 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$, 298 K): δ 157.5, 135.2, 133.7 (d, $^2J_{C–P}$ = 10.3 Hz), 130.6 (d, $^3J_{C–P}$ = 13.4 Hz), 118.4 (d, $^1J_{C–P}$ = 86.4 Hz), 99.0, 62.6, 40.3 (d, $^2J_{C–P}$ = 17.2 Hz), 29.2, 23.0, 21.6, 20.9 (d, $^1J_{C–P}$ = 52.2 Hz), 20.2, 18.0. ^{31}P NMR (162 MHz, $CDCl_3$, 298 K): δ 24.6. ESI-MS (positive-ion mode, *m*/*z*): 496.2 $[C_{32}H_{35}NO_2P]^+$. HR-ESI-MS (positive-ion mode, *m*/*z*): calcd for $[C_{32}H_{35}NO_2P]^+$: 496.2405, found: 496.2402.

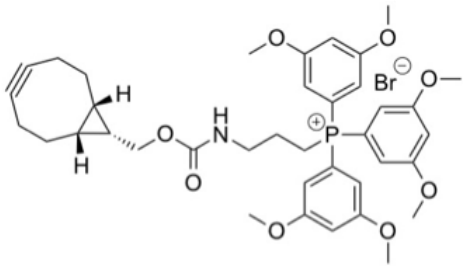
BCN-Phos-5



Triethylamine (45 μL) was added to a solution of BCN-NHS (12.7 mg, 0.044 mmol) and **H_2N-Phos-5** (32.7 mg, 0.067 mmol) in anhydrous CH_2Cl_2 (10 mL). The mixture was stirred at room temperature in the dark under an inert atmosphere of nitrogen for 24 h. Then the reaction mixture was diluted with CH_2Cl_2 (20 mL), and the organic layer was washed with water (10 mL), dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (gradient from CH_2Cl_2 to CH_2Cl_2/MeOH (10:1, v/v)) to afford the title compound as an oily white solid. Yield: 30.9 mg (78%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 7.34 – 7.28 (m, 9H), 7.19 (t, $^3J_{H–H}$ = 6.4 Hz, 1H), 4.11 (d, $^3J_{H–H}$ = 7.9 Hz, 2H), 3.63 – 3.55 (m, 2H), 3.50 – 3.45 (m, 2H), 2.40 (s, 18H), 2.28 – 2.14 (m, 6H), 1.84 – 1.72 (m, 4H), 1.38 – 1.30 (m, 1H), 0.93 – 0.87 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$, 298 K): δ 157.6, 140.6 (d, $^2J_{C–P}$ = 13.1 Hz), 136.8 (d, $^4J_{C–P}$ = 3.3 Hz), 130.8 (d , $^3J_{C–P}$ = 9.7 Hz), 118.6 (d, $^1J_{C–P}$ = 85.1 Hz), 99.1, 62.4, 40.4 (d, $^2J_{C–P}$ = 17.6 Hz), 29.2, 23.2, 21.6, 21.1*, 20.2, 18.0. ^{31}P NMR (162 MHz, $CDCl_3$, 298 K): δ 23.45. ESI-MS (positive-ion mode, *m*/*z*): 580.3 $[C_{38}H_{47}NO_2P]^+$. HR-ESI-MS (positive-ion mode, *m*/*z*): calcd for $[C_{38}H_{47}NO_2P]^+$: 580.3344, found: 580.3344.

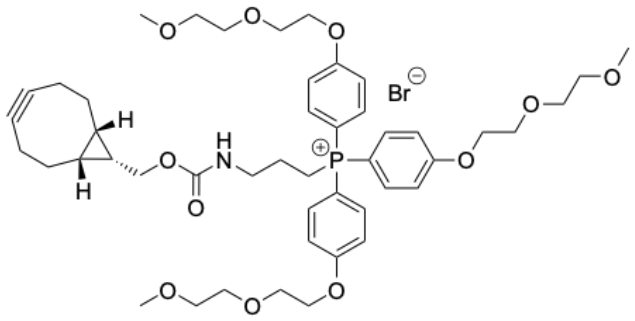
* $^1J_{C–P}$ doublet underneath peak at 21.6 ppm.

BCN-Phos-6



Triethylamine (33 μL) was added to a solution of BCN-NHS (14.6 mg, 0.049 mmol) and **H_2N-Phos-6** (30.6 mg, 0.046 mmol) in anhydrous CH_2Cl_2 (7 mL). The mixture was stirred at room temperature in the dark under an inert atmosphere of nitrogen for 24 h. Then the reaction mixture was diluted with CH_2Cl_2 (20 mL), and the organic layer was washed with water (10 mL), dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (gradient from CH_2Cl_2 to CH_2Cl_2/MeOH (10:1, v/v)) to afford the title compound as a colourless oil. Yield: 24.7 mg (70%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 7.18 (t, $^3J_{H–H}$ = 6.3 Hz, 1H), 6.82 – 6.72 (m, 9H), 4.07 (d, $^3J_{H–H}$ = 7.9 Hz, 2H), 3.86 (s, 18H), 3.51 – 3.47 (m, 2H), 2.29 – 2.14 (m, 6H), 1.85 – 1.78 (m, 4H), 1.37 – 1.31 (m, 1H), 1.24 (br s, 2H), 0.92 – 0.87 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$, 298 K): δ 162.0 (d, $^3J_{C–P}$ = 19.1 Hz), 157.4, 120.0 (d, $^1J_{C–P}$ = 86.8 Hz), 111.1 (d, $^2J_{C–P}$ = 11.5 Hz), 106.5, 98.9, 62.3, 56.4, 40.0 (d, $^2J_{C–P}$ = 17.3 Hz), 29.7, 29.1, 23.6, 21.5, 20.9 (d, $^1J_{C–P}$ = 50.6 Hz), 20.1, 17.9. ^{31}P NMR (162 MHz, $CDCl_3$, 298 K): δ 27.69. ESI-MS (positive-ion mode, *m*/*z*): 676.3 $[C_{38}H_{47}NO_8P]^+$. HR-ESI-MS (positive-ion mode, *m*/*z*): calcd for $[C_{38}H_{47}NO_8P]^+$: 676.3039, found: 676.3050.

BCN-Phos-7



Triethylamine (10 μL) was added to a solution of BCN-NHS (11.3 mg, 0.038 mmol) and **H_2N-Phos-7** (30.3 mg, 0.036 mmol) in anhydrous CH_2Cl_2 (6.5 mL). The mixture was stirred at room temperature in the dark under an inert atmosphere of nitrogen for 24 h. Then the reaction mixture was diluted with CH_2Cl_2 (20 mL), and the organic layer was washed with water (10 mL), dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (gradient from CH_2Cl_2 to CH_2Cl_2/MeOH (10:1, v/v)) to afford the title compound as a colourless oil. Yield: 16.9 mg (50%). ^{1}H NMR (400 MHz, $CDCl_3$, 298 K): δ 7.62 – 7.57 (m, 6H), 7.12 (dd, $^4J_{H–P}$ 2.8 Hz and $^4J_{H–H}$ = 9.1 Hz, 6H), 6.89 (t, $^3J_{H–H}$ = 6.4 Hz, 1H), 4.21 (t, $^3J_{H–H}$ = 4.7 Hz, 6H), 4.07 (d, $^3J_{H–H}$ = 8.0 Hz, 2H), 3.87 (t, $^3J_{H–H}$ = 4.7 Hz, 6H), 3.71 – 3.68 (m, 6H), 3.57 – 4.42 (m, 10H), 3.37 (s, 9H), 2.28 – 1.98 (m, 8H), 1.81 (m, 2H), 1.42 (t, $^3J_{H–H}$ = 7.3 Hz, 1H), 0.88 (t, $^3J_{H–H}$ = 9.9 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$, 298 K): δ 163.9 (d, $^4J_{C–P}$ = 2.5 Hz), 157.53, 135.4 (d, $^3J_{C–P}$ = 12.0 Hz), 116.7 (d, $^2J_{C–P}$ = 13.5 Hz), 109.4 (d, $^1J_{C–P}$ = 94.3 Hz), 99.0, 72.0, 70.9, 69.4, 68.1, 62.5, 59.2, 40.6 (d, $^2J_{C–P}$ = 18.5 Hz), 29.2, 23.0, 22.1*, 21.6, 20.2, 17.9. ^{31}P NMR (162 MHz, $CDCl_3$, 298 K): δ 22.0. ESI-MS (positive-ion mode, *m*/*z*): 850.4 $[C_{47}H_{65}NO_{11}P]^+$. HR-ESI-MS (positive-ion mode, *m*/*z*): calcd for $[C_{47}H_{65}NO_{11}P]^+$:

850.4295, found: 850.4307.

* $^{1}J_{C–P}$ doublet underneath peak at 21.6 ppm.

Photophysical measurements

Electronic absorption spectra were recorded on an Agilent 8453 diode array spectrophotometer. Steady-state emission spectra were recorded on a HORIBA JOBIN YVON Fluorolog TCSPC spectrofluorometer. Unless specified otherwise, all solutions for photophysical studies were degassed with no fewer than four successive freeze-pump-thaw cycles and stored in a 10-cm^3 round bottomed flask equipped with a side-arm 1-cm fluorescence cuvette and sealed from the atmosphere by a Rotaflo HP6/6 quick-release Teflon stopper.

Emission quantum yields were measured by the optically dilute method[7] using an aerated aqueous solution of $[Ru(bpy)_3]Cl_2$ (Φ_{em} = 0.040, λ_{ex} = 455 nm) as the standard solution.[8] The concentrations of the standard and sample solutions were adjusted until the absorbance at 455 nm was 0.1. The quantum yields of the samples were calculated according to the following equation, where the subscripts *s* and *r* refer to the sample and reference solutions, respectively:

$$\Phi_s = \Phi_r \left(\frac{I_r}{I_s}\right)\left(\frac{B_r}{B_s}\right)\left(\frac{n_s}{n_r}\right)^2\left(\frac{D_s}{D_r}\right)$$

where Φ is luminescence quantum yield, *I* is excitation intensity, *B* is $1 - 10^{-AL}$, *A* is absorbance at the excitation wavelength, *L* is path length in cm, *n* is refractive index of the solvent and *D* is integrated emission intensity.

The emission lifetimes were measured on an Edinburgh Instruments LP920 laser flash photolysis spectrometer using the third harmonic output (355 nm; 6 – 8 ns fwhm pulse width) of a Spectra-Physics Quanta-Ray Q-switched LAB-150 pulsed Nd:YAG laser (10 Hz) as the excitation source.

Determination of singlet oxygen ($^1O_2$) generation quantum yields (Φ_Δ)

The $^1O_2$ generation quantum yields (Φ_Δ) of the complexes were measured by the optically dilute method[7] using $[Ru(bpy)_3]Cl_2$ in aerated CH_3CN (Φ_Δ = 0.57) as a reference.[9] An air-equilibrated CH_3CN solution (2 mL) containing the complex was introduced to a quartz cuvette of 1-cm path length. The concentrations of the reference and sample solutions were adjusted until absorbance at the excitation wavelength (450 nm) was *ca.* 0.15. The solutions were excited at λ = 450 nm and the emission spectra of $^1O_2$ at 1200 – 1350 nm were recorded on an Edinburgh Instruments FLS980 spectrometer equipped with an R5509-73 NIR photomultiplier tube and C9940-02 exclusive coolers. The Φ_Δ values of the complexes were determined using the following equation, where the subscripts *s* and *r* refer to the sample and reference solutions, respectively:

$$\Phi_s = \Phi_r \left(\frac{I_r}{I_s}\right)\left(\frac{B_r}{B_s}\right)\left(\frac{n_s}{n_r}\right)^2\left(\frac{D_s}{D_r}\right)$$

where Φ is $^1O_2$ generation quantum yield, *I* is excitation intensity, *B* is $1 - 10^{-AL}$, *A* is absorbance at the excitation wavelength, *L* is path length in cm, *n* is refractive index of the solvent and *D* is integrated emission intensity.

Kinetics studies for the reactions of complex 1 with BCN-OH

The reaction kinetics of the strain-promoted alkyne–nitrone cycloaddition reaction was studied by electronic absorption spectroscopy. The second-order rate constant (k_2) of complex **1** was measured under pseudo first order conditions with a 50- to 200-fold excess of BCN-OH in MeOH at 298 K. The reaction process was followed by monitoring the exponential decay of the absorbance at 322 nm. Stock solutions of BCN-OH (1, 2, 2.5, 3, 3.5 and 4 mM) in MeOH were prepared. The changes of

absorbance of complex **1** (20 μM) in MeOH were measured immediately after the addition of an equal volume of the prepared BCN-OH solutions. The final concentration of complex **1** was 10 μM and those of BCN-OH ranged from 0.5 to 2 mM. The data were fitted to a single-exponential equation to give the observed rate constants (k_{obs}), which were plotted against the concentrations of BCN-OH to obtain the k_2 from the slope of the plots.

Phosphorogenic response of complex 1 towards BCN substrates

Complex **1** (10 μM) was incubated with BCN-OH or **BCN-Phos-*n*** (250 μM) in aerated PBS (pH 7.4)/ MeOH (9:1, v/v) at 298 K for 24 h. The emission spectra were recorded on a HORIBA FluoroMax-4 spectrofluorometer and the emission lifetimes were measured on an Edinburgh Instruments LP920 laser flash photolysis spectrometer.

Live-cell confocal imaging

HeLa cells in growth medium were seeded on a sterilised coverslip in a 35-mm tissue culture dish and grown at 37°C under a 5% CO_2 atmosphere for 48 h. The growth medium was removed and replaced with either fresh growth medium or **BCN-Phos-*n*** (5 μM) in growth medium/DMSO (99:1, v/v) at 37°C under a 5% CO_2 atmosphere for 2 h. The medium was removed and the cells were thoroughly washed with PBS (1 mL × 3). The cells were then incubated with complex **1** (5 μM) in growth medium/DMSO (99:1, v/v) at 37°C under a 5% CO_2 atmosphere for 2 h. The medium was removed and the cells were thoroughly washed with PBS (1 mL × 3). The coverslip was mounted onto a sterilised glass slide and then imaging was performed using a Leica TCS SPE confocal microscope with an oil immersion 63× objective and an excitation wavelength at 488 nm. For the co-staining experiments, after treatment with the complex, the

medium was removed and the cells were thoroughly washed with PBS (1 mL × 3). The cells were then incubated with MitoTracker Green (100 nM) in growth medium at 37°C under a 5% CO_2 atmosphere for 30 min. The medium was then removed and the cells were thoroughly washed with PBS (1 mL × 3) prior to imaging. The excitation wavelength of MitoTracker Green was 488 nm. The Pearson's correlation coefficients were determined using the program ImageJ (Version 1.4.3.67).

Flow cytometry

HeLa cells were grown in a 35-mm tissue culture dish and incubated at 37°C under a 5% CO_2 atmosphere for 48 h. The growth medium was removed and replaced with either fresh growth medium or **BCN-Phos-*n*** (5 μM) in growth medium/DMSO (99:1, v/v) at 37°C under a 5% CO_2 atmosphere for 2 h. The medium was removed and the cells were thoroughly washed with PBS (1 mL × 3). The cells were then incubated with complex **1** (5 μM) in growth medium/DMSO (99:1, v/v) at 37°C under a 5% CO_2 atmosphere for 2 h. The medium was removed and the cells were thoroughly washed with PBS (1 mL × 3). The cells were trypsinised and harvested with PBS. The resultant solution (2 mL) was analysed by a FACSCalibur flow cytometer (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) with an excitation wavelength at 488 nm. The number of cells analysed for each sample was between 9,000 and 10,000.

Cellular uptake measurements

HeLa cells were grown in a 35-mm tissue culture dish and incubated at 37°C under a 5% CO_2 atmosphere for 48 h. The growth medium was removed and replaced with either fresh growth medium or **BCN-Phos-*n*** (5 μM) in growth medium/DMSO (99:1, v/v) at 37°C under a 5% CO_2 atmosphere for 2 h. The medium was removed and the

cells were thoroughly washed with PBS (1 mL × 3). The cells were then incubated with complex **1** (5 μM) in growth medium/DMSO (99:1, v/v) at 37°C under a 5% CO_2 atmosphere for 2 h. The medium was removed and the cells were thoroughly washed with PBS (1 mL × 3). The cells were then trypsinised and harvested with PBS. The resultant solution (2 mL) was heated with 65% HNO_3 (2 mL) at 70°C for 2 h, cooled to room temperature and analysed using a NexION 2000 ICP-MS (PerkinElmer SCIEX Instruments).

(Photo)cytotoxicity assays

HeLa cells were seeded in two 96-well flat-bottomed microplates (*ca*. 10,000 cells per well) in growth medium (100 μL) and incubated at 37°C under a 5% CO_2 atmosphere for 24 h. The growth medium was removed and replaced with either fresh growth medium or **BCN-Phos-*n*** (5 μM) in growth medium/DMSO (99:1, v/v) at 37°C under a 5% CO_2 atmosphere for 2 h. The medium was removed and the cells were thoroughly washed with PBS (1 mL × 3). The cells were then incubated with complex **1** (5 μM) in growth medium/DMSO (99:1, v/v) at 37°C under a 5% CO_2 atmosphere for 2 h. After treatment, the medium was removed, the cells were washed with PBS (100 μL × 3) and phenol red-free growth medium (100 μL) was added to each well. One of the microplates was irradiated at λ = 525 nm (10 mW cm^{-2}) for 5 min with a LED cellular photocytotoxicity irradiator (PURI Materials, Shenzhen, China) whereas the other microplate was kept in the dark. The cells were then replenished with fresh growth medium (100 μL) and further incubated at 37°C under a 5% CO_2 atmosphere for 20 h. The medium in each well was then replaced with fresh medium (90 μL) and Neutral Red in PBS (10 μL, 0.4 mg mL^{-1}) was added to each well. The microplates were

incubated at 37°C under a 5% CO_2 atmosphere for 2 h. The growth medium was then removed and a destain solution (EtOH/H_2O/AcOH (glacial), 50/49/1, v/v/v) (200 μL) was added to each well. The absorbance of the solutions at 540 nm was measured with an Epoch 2 microplate spectrophotometer (BioTek., Santa Clara, CA). The IC_{50} values of the complex were determined from dose dependence of surviving cells after exposure to the treatment.

Table S1 Electronic absorption spectral data of complex **1** at 298 K.

Complex	Solvent	λ_{abs}/nm (ε/dm^3 mol^{-1} cm^{-1})
1	CH_2Cl_2	275 (59,370), 300 sh (37,670), 334 (20,215), 383 sh (15,270), 431 sh (11,290), 456 sh (7,610), 550 (5,825)
	CH_3CN	274 (79,490), 299 sh (47,235), 342 (28,245), 374 sh (22,510), 427 sh (15,430), 456 sh (9,750), 545 (7,160)

Table S2 Flow cytometric results of HeLa cells incubated with complex **1** (5 μM) for 2 h without or with pretreatment of **BCN-Phos-*n*** (5 μM) for 2 h.

Entry	Relative intensity/A.U.
1	230.8
1 + **BCN-Phos-1**	252.4
1 + **BCN-Phos-2**	214.9
1 + **BCN-Phos-3**	250.5
1 + **BCN-Phos-4**	242.4
1 + **BCN-Phos-5**	495.7
1 + **BCN-Phos-6**	678.9
1 + **BCN-Phos-7**	264.9
Blank	7.3

Table S3 Cellular uptake of complex **1** in HeLa cells without or with pretreatment with **BCN-Phos-*n*** derivatives.

Entry	Amount of iridium/fmol[a]
1	1.83 ± 0.44
1 + **BCN-Phos-1**	1.74 ± 0.26
1 + **BCN-Phos-2**	1.75 ± 0.18
1 + **BCN-Phos-3**	1.77 ± 0.31
1 + **BCN-Phos-4**	1.88 ± 0.09
1 + **BCN-Phos-5**	1.77 ± 0.08
1 + **BCN-Phos-6**	1.74 ± 0.21
1 + **BCN-Phos-7**	1.70 ± 0.02

[a] Amount of iridium associated with an average HeLa cell upon incubation with complex **1** (5 μM, 2 h) without or with pretreatment with **BCN-Phos-*n*** derivatives (5 μM, 2 h) at 37°C, as determined by ICP-MS.

Table S4 (Photo)cytotoxicity of complex **1** towards HeLa cells in the dark and upon irradiation at 525 nm (10 mW cm^{-2}) for 5 min. Photocytotoxicity index (PI) = $IC_{50,dark}/IC_{50,light}$.

– **BCN-Phos-*n***			+ **BCN-Phos-5**[a]			+ **BCN-Phos-6**[b]		
$IC_{50,dark}/\mu M$	$IC_{50,light}/\mu M$	PI	$IC_{50,dark}/\mu M$	$IC_{50,light}/\mu M$	PI	$IC_{50,dark}/\mu M$	$IC_{50,light}/\mu M$	PI
18 ± 1	0.37 ± 0.02	49	9.6 ± 0.3	0.14 ± 0.01	69	6.3 ± 0.5	0.22 ± 0.01	29

[a] Cells were treated with **BCN-Phos-5** (5 μM) for 2 h, washed with PBS (100 μL × 3), and then incubated with complex **1** for 2 h.

[b] Cells were treated with **BCN-Phos-6** (5 μM) for 2 h, washed with PBS (100 μL × 3), and then incubated with complex **1** for 2 h.

Fig. S1 Pseudo first-order kinetics for the reaction of complex **1** (10 μM) with different concentrations of BCN-OH (0.5 – 2 mM) in MeOH at 298 K. The slope of the plot corresponds to the k_2 value of the reaction.

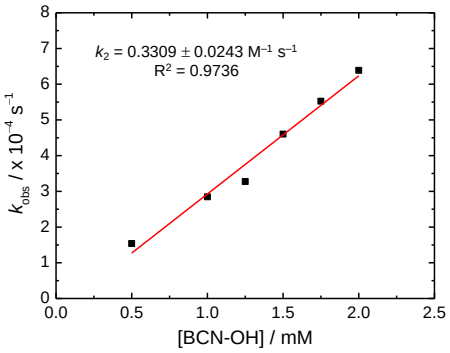


Fig. S2 ESI mass spectra of complex **1** (10 μM) in the absence (top) and presence (bottom) of BCN-OH (250 μM) in MeOH at 298 K for 24 h.

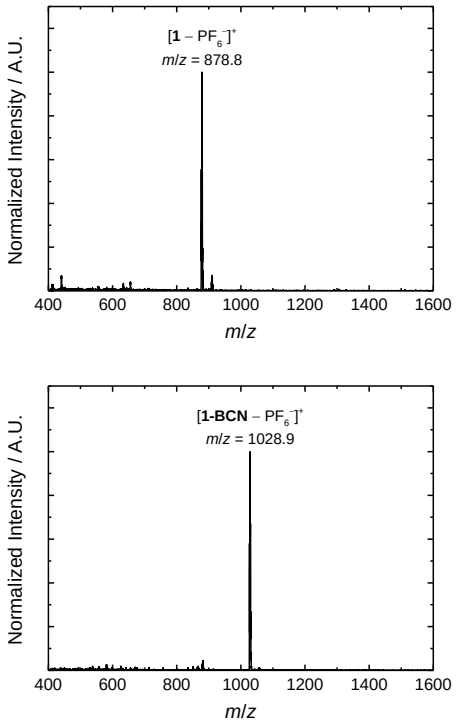


Fig. S3 Flow cytometric results of HeLa cells under different treatment. The cells were treated with blank medium (2 h) (grey); complex **1** (5 μM, 2 h) (black); **BCN-Phos-1** (5 μM, 2 h) and then complex **1** (5 μM, 2 h) (red); **BCN-Phos-2** (5 μM, 2 h) and then complex **1** (5 μM, 2 h) (blue); **BCN-Phos-3** (5 μM, 2 h) and then complex **1** (5 μM, 2 h) (magenta); **BCN-Phos-4** (5 μM, 2 h) and then complex **1** (5 μM, 2 h) (olive); and **BCN-Phos-7** (5 μM, 2 h) and then complex **1** (5 μM, 2 h) (navy) at 37°C.

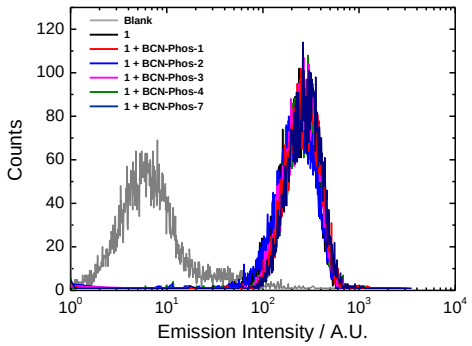


Fig. S4 ^{1}H NMR spectrum of complex **1** in acetone-d_6 at 298 K.

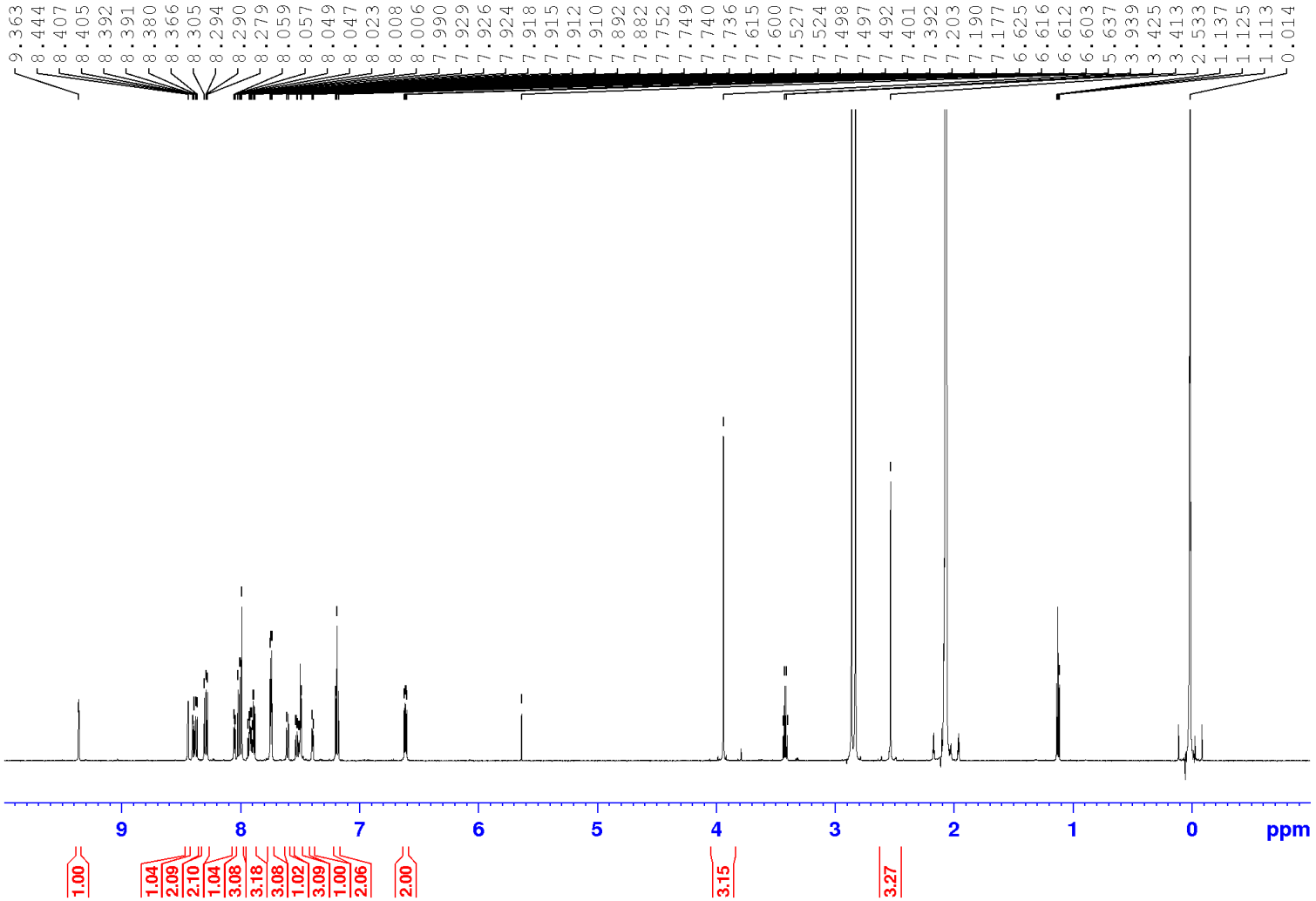


Fig. S5 ^{13}C NMR spectrum of complex **1** in acetone-d_6 at 298 K.

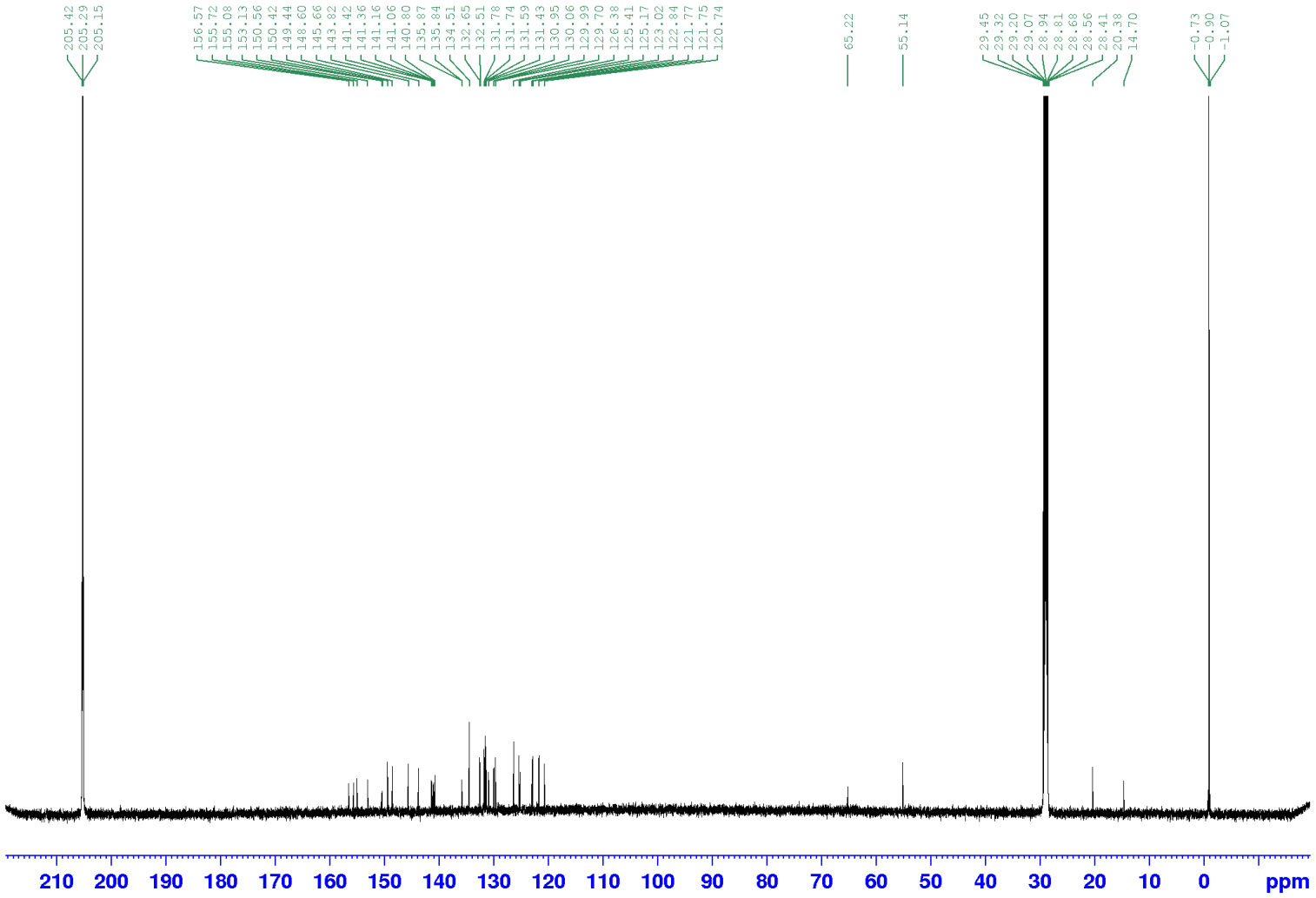


Fig. S6 (a) Experimental and (b) simulated HR-ESI mass spectra of complex **1** in MeOH at 298 K.

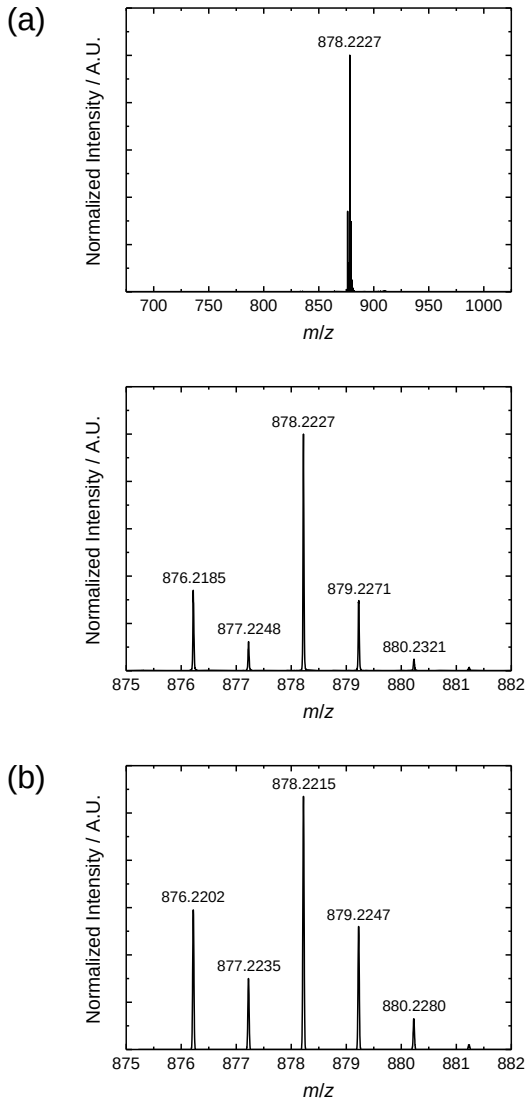


Fig. S7 ^{1}H NMR spectrum of **BCN-Phos-1** in $CDCl_3$ at 298 K.

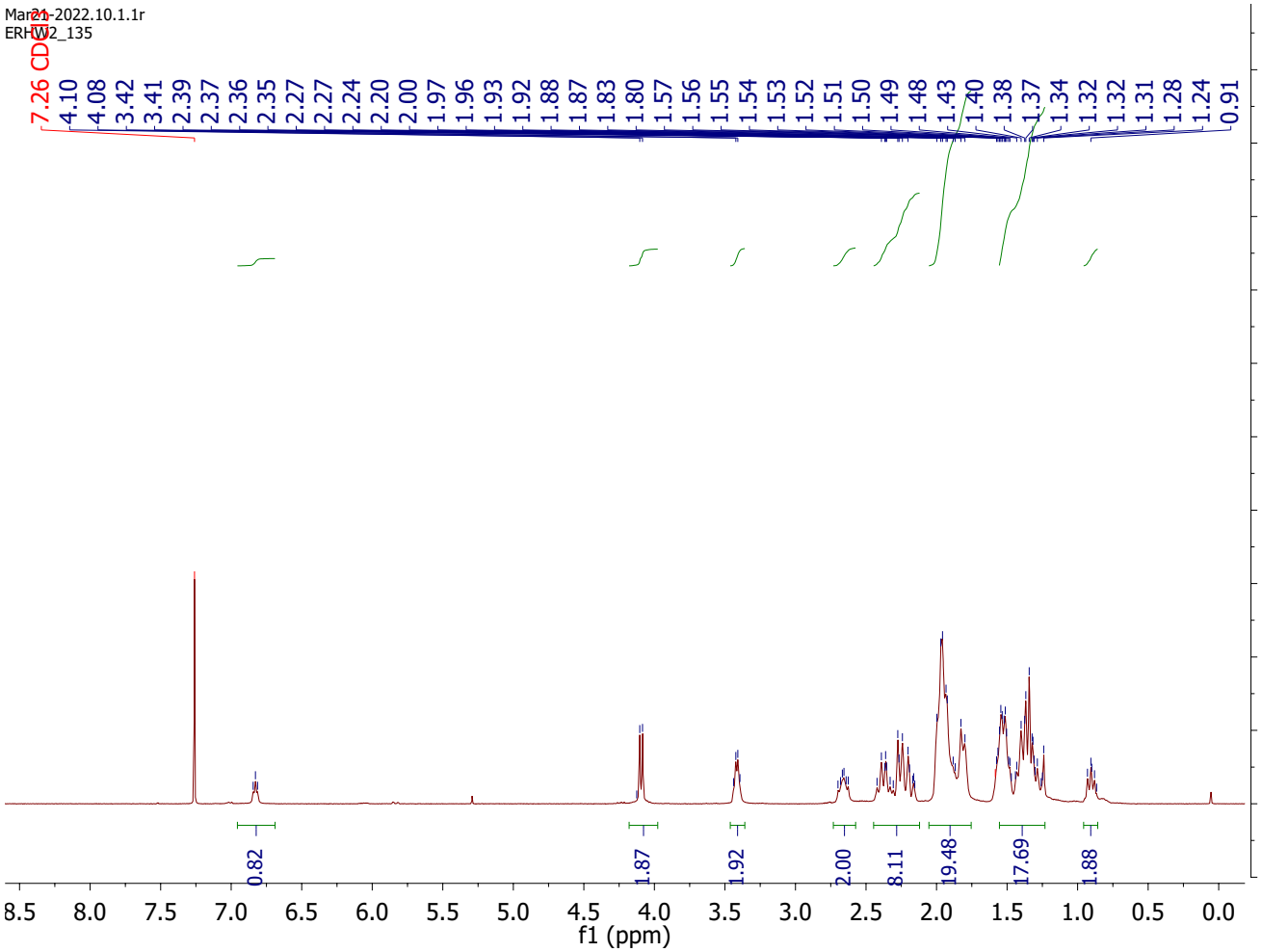


Fig. S8 ^{13}C NMR spectrum of **BCN-Phos-1** in $CDCl_3$ at 298 K.

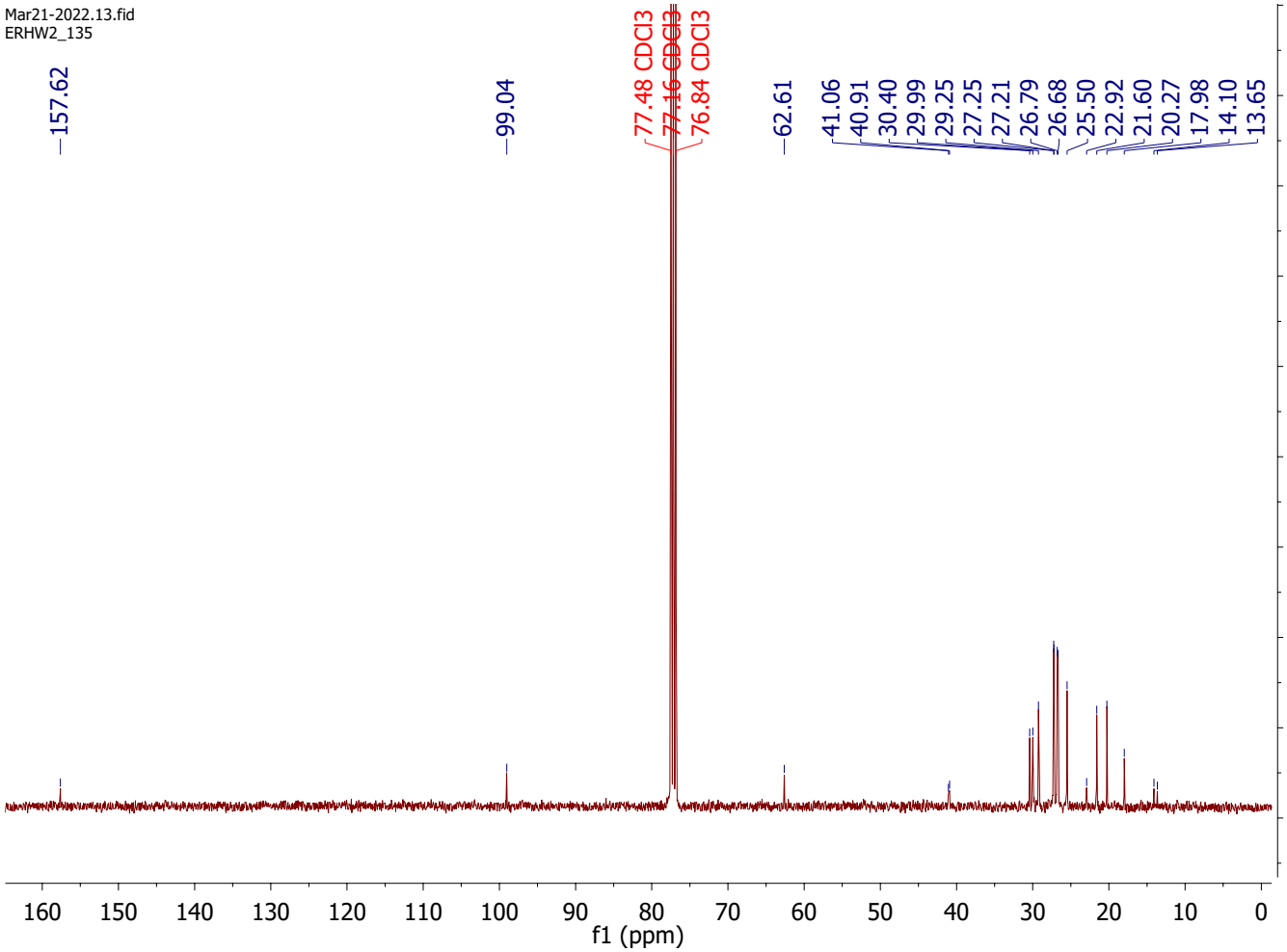


Fig. S9 ^{31}P NMR spectrum of **BCN-Phos-1** in $CDCl_3$ at 298 K.

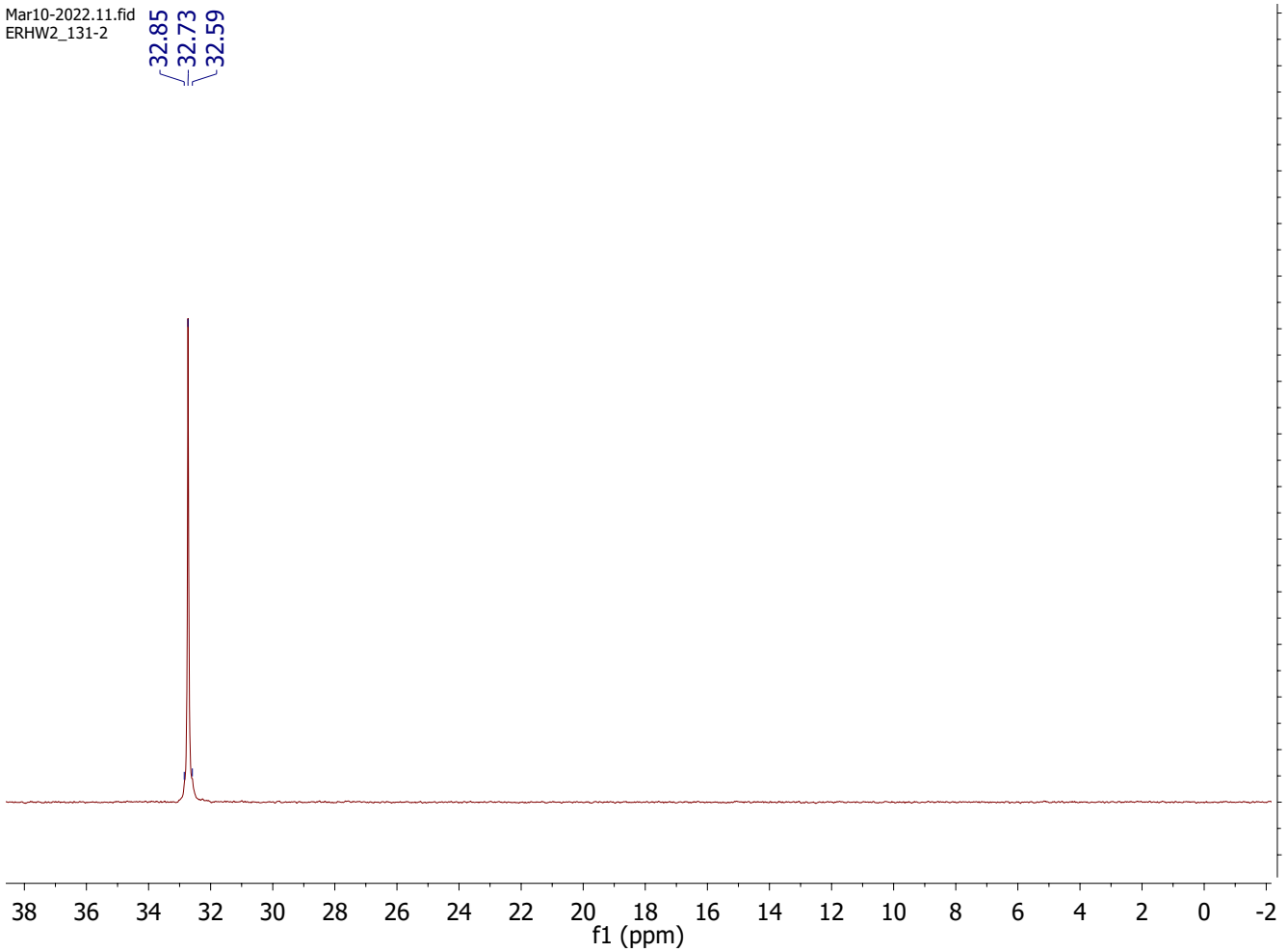


Fig. S10 ^{1}H NMR spectrum of **BCN-Phos-2** in $CDCl_3$ at 298 K. * Trace amounts of triethylamine salts.

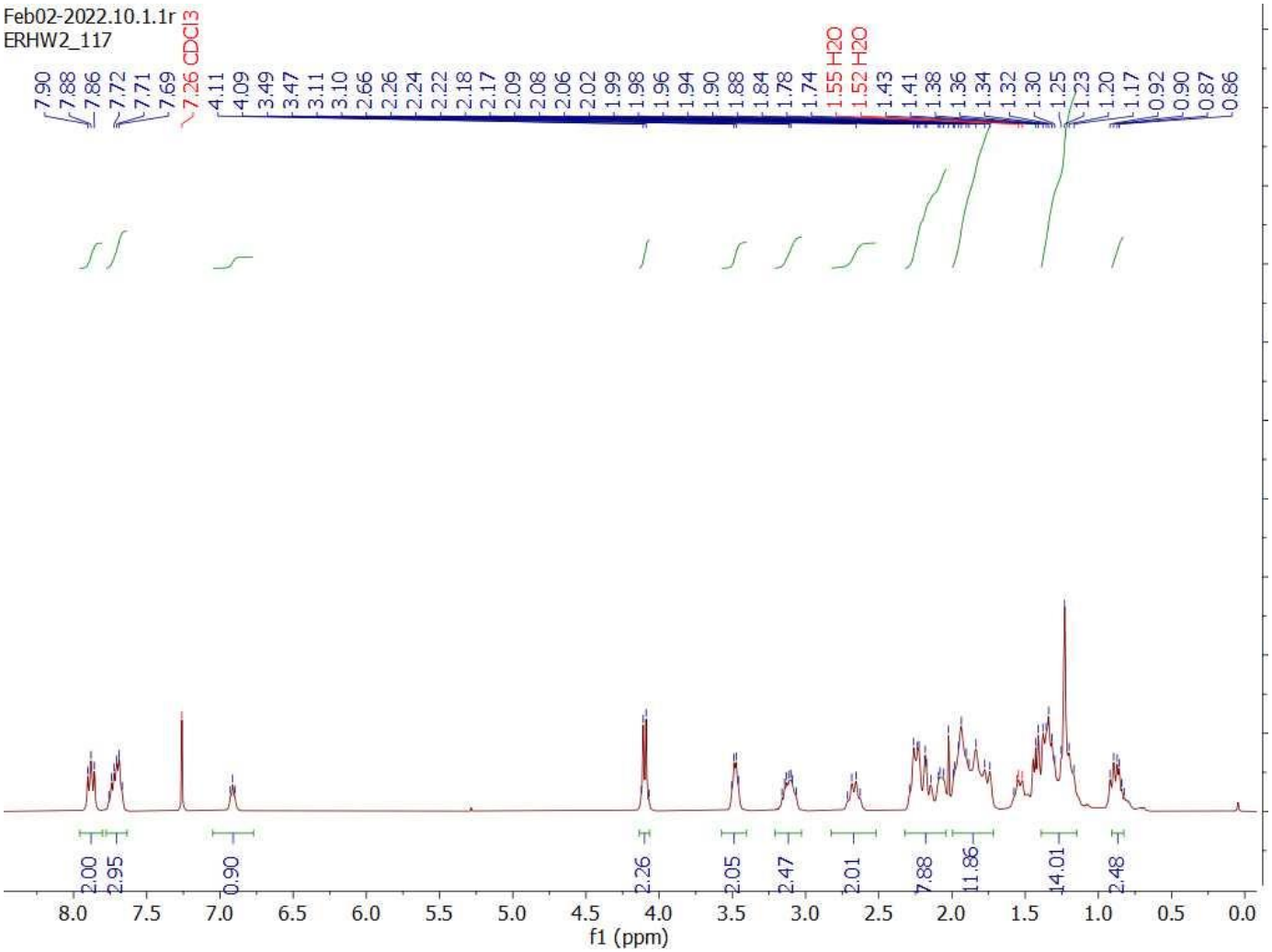


Fig. S11 ^{13}C NMR spectrum of **BCN-Phos-2** in $CDCl_3$ at 298 K.

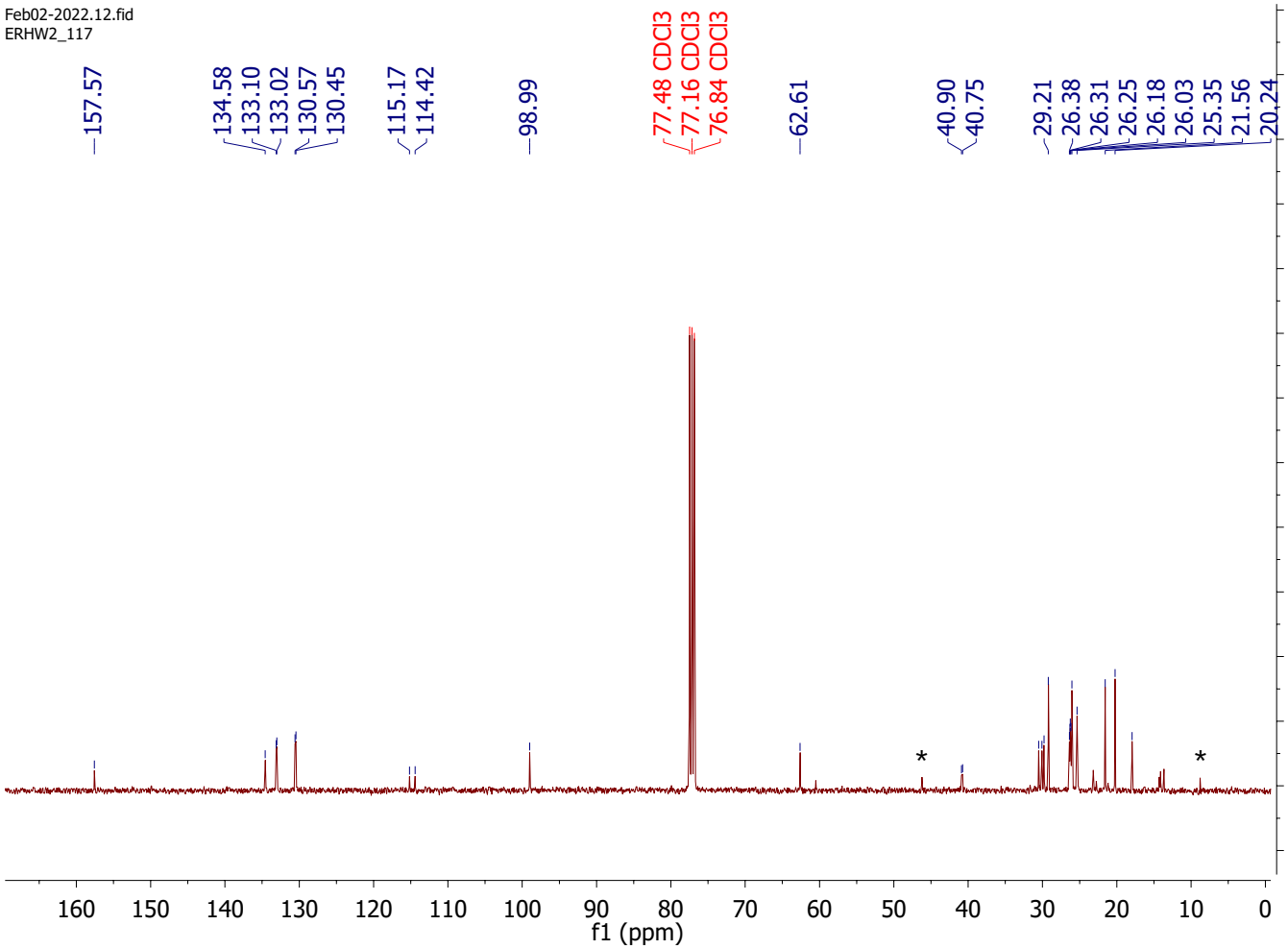


Fig. S12 ^{31}P NMR spectrum of **BCN-Phos-2** in $CDCl_3$ at 298 K.

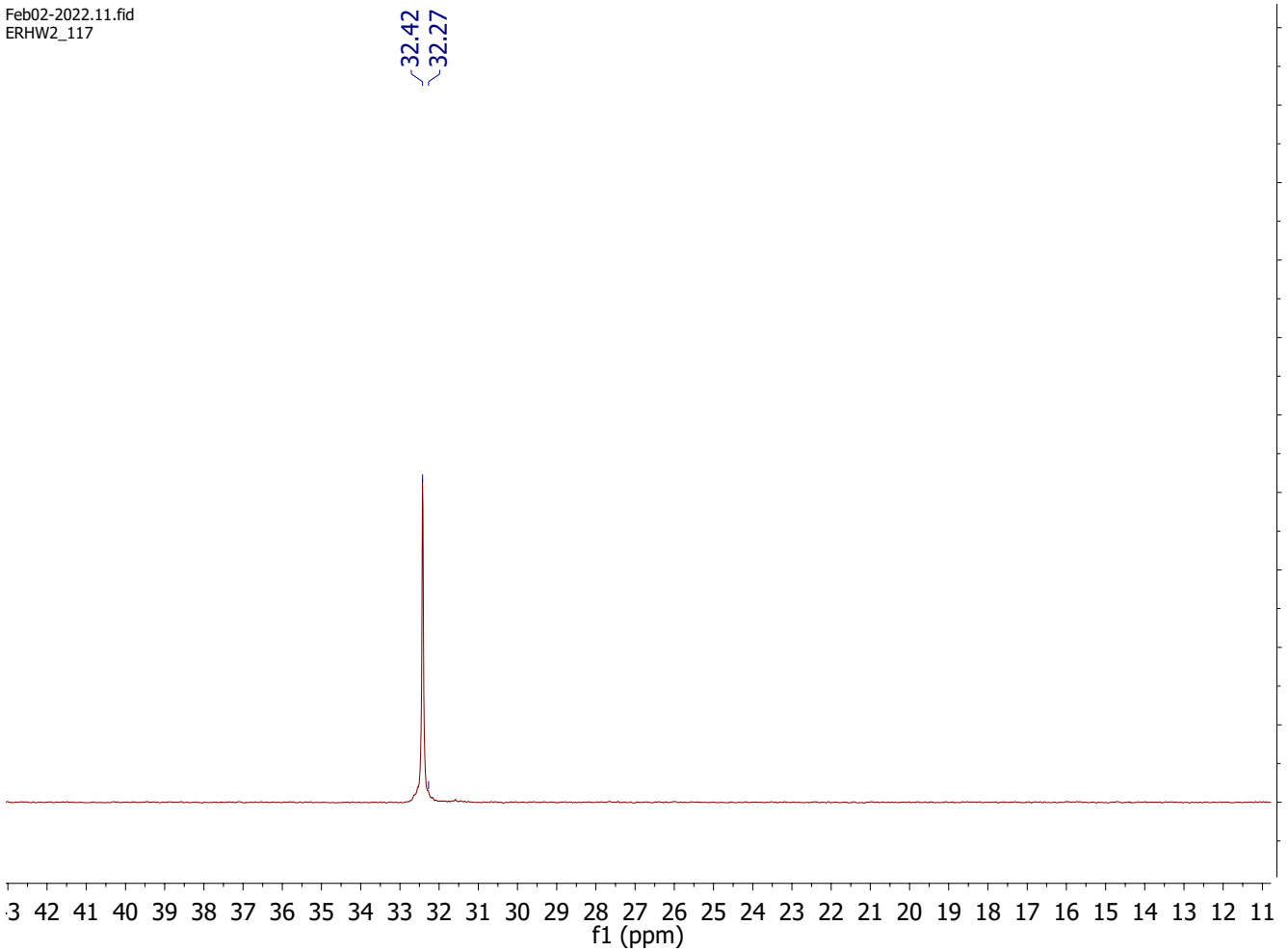


Fig. S13 ^{1}H NMR spectrum of **BCN-Phos-3** in $CDCl_3$ at 298 K. * Trace amounts of triethylamine salts.

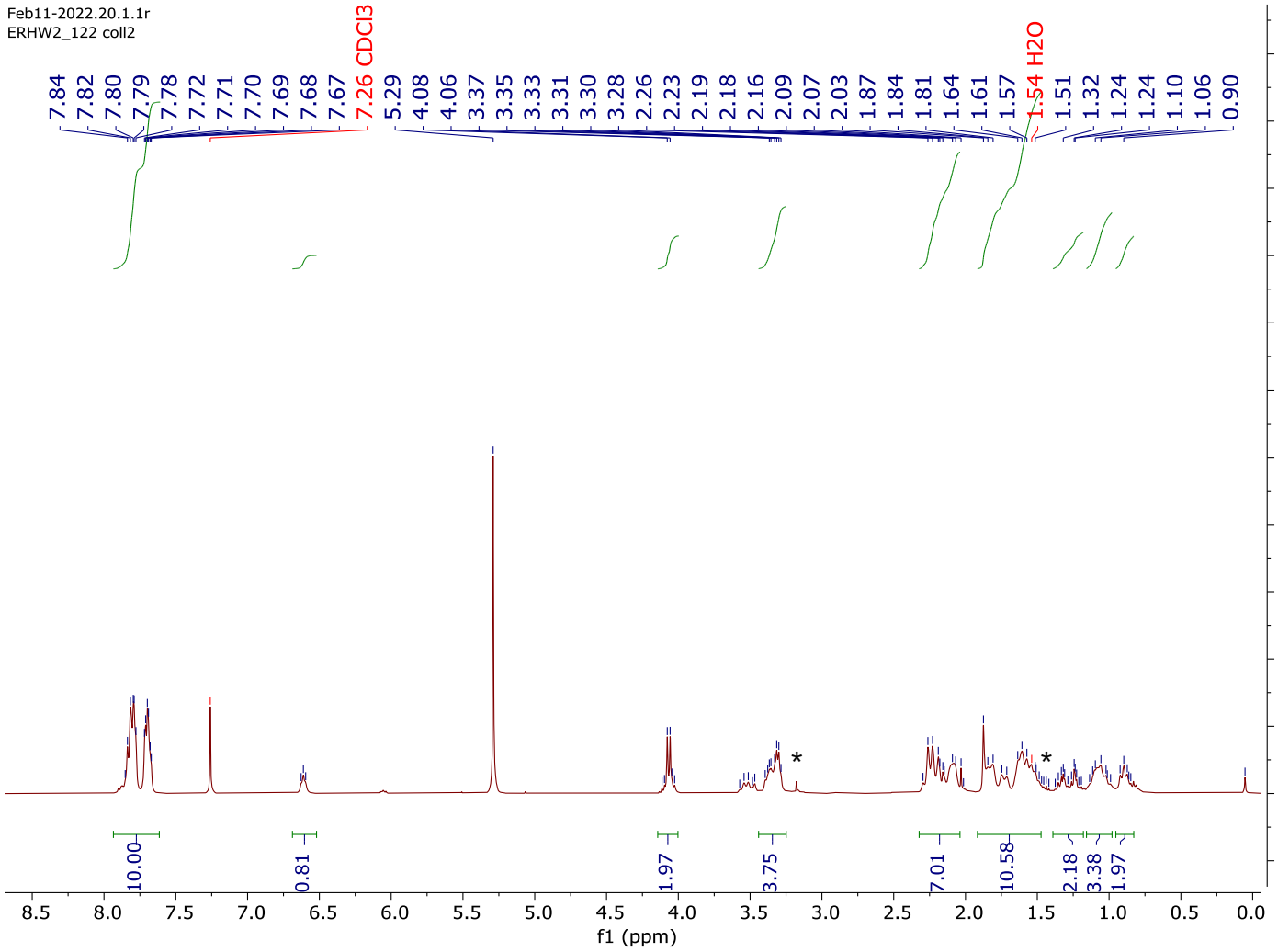


Fig. S14 ^{13}C NMR spectrum of **BCN-Phos-3** in $CDCl_3$ at 298 K.

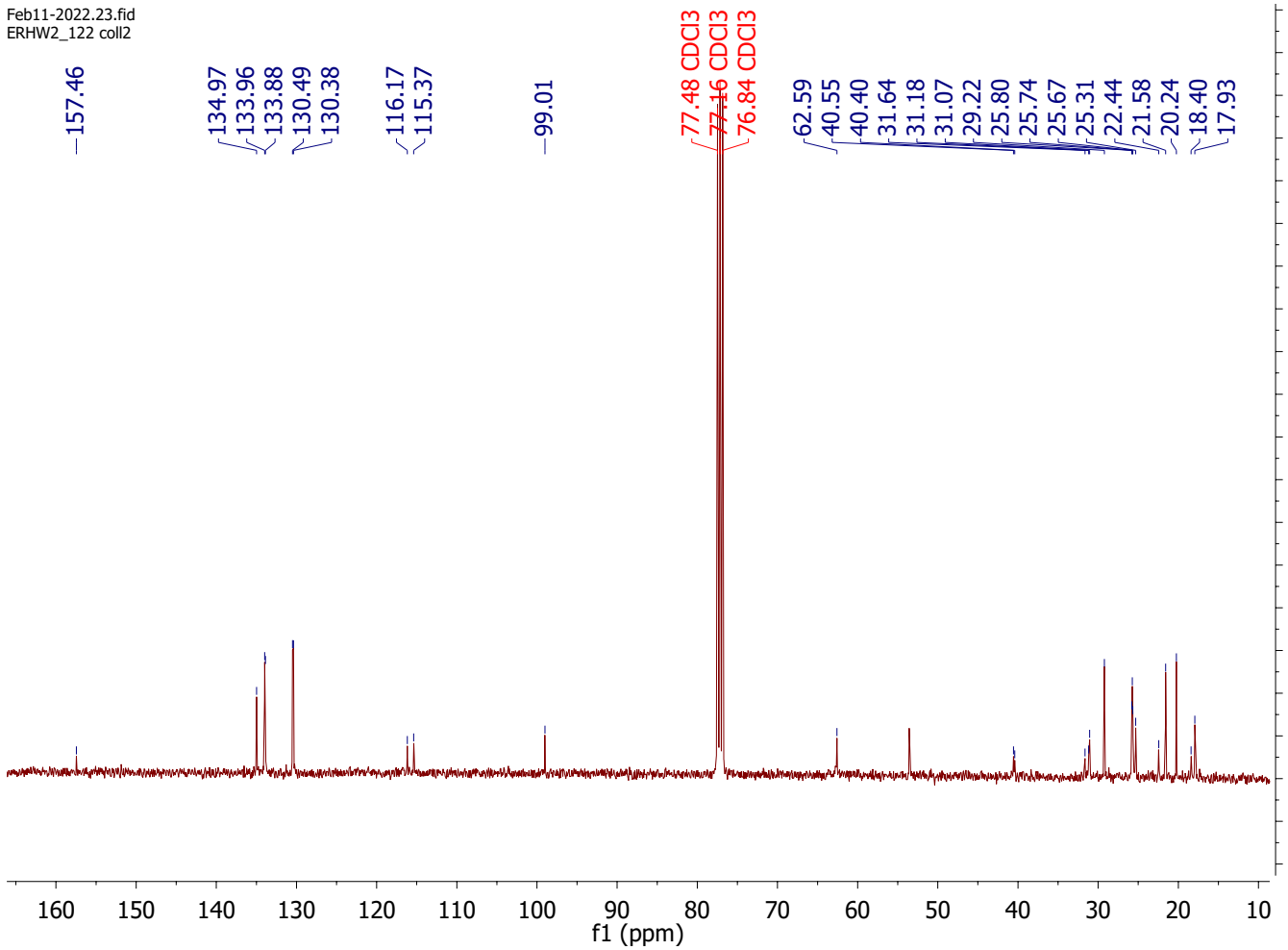


Fig. S15 ^{31}P NMR spectrum of **BCN-Phos-3** in $CDCl_3$ at 298 K.

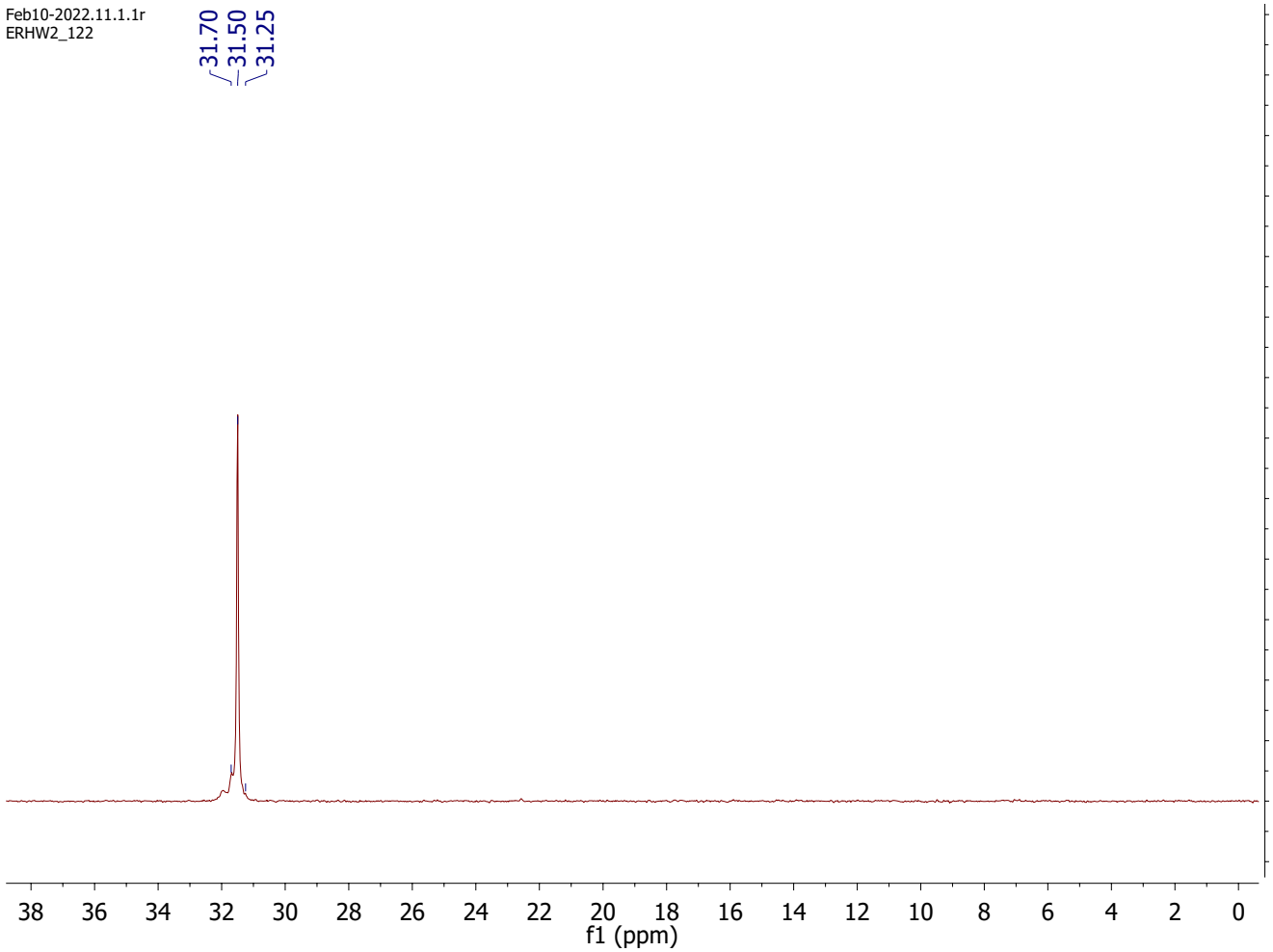


Fig. S16 ^{1}H NMR spectrum of **BCN-Phos-4** in $CDCl_3$ at 298 K. ~ Trace amounts of triethylamine salts.

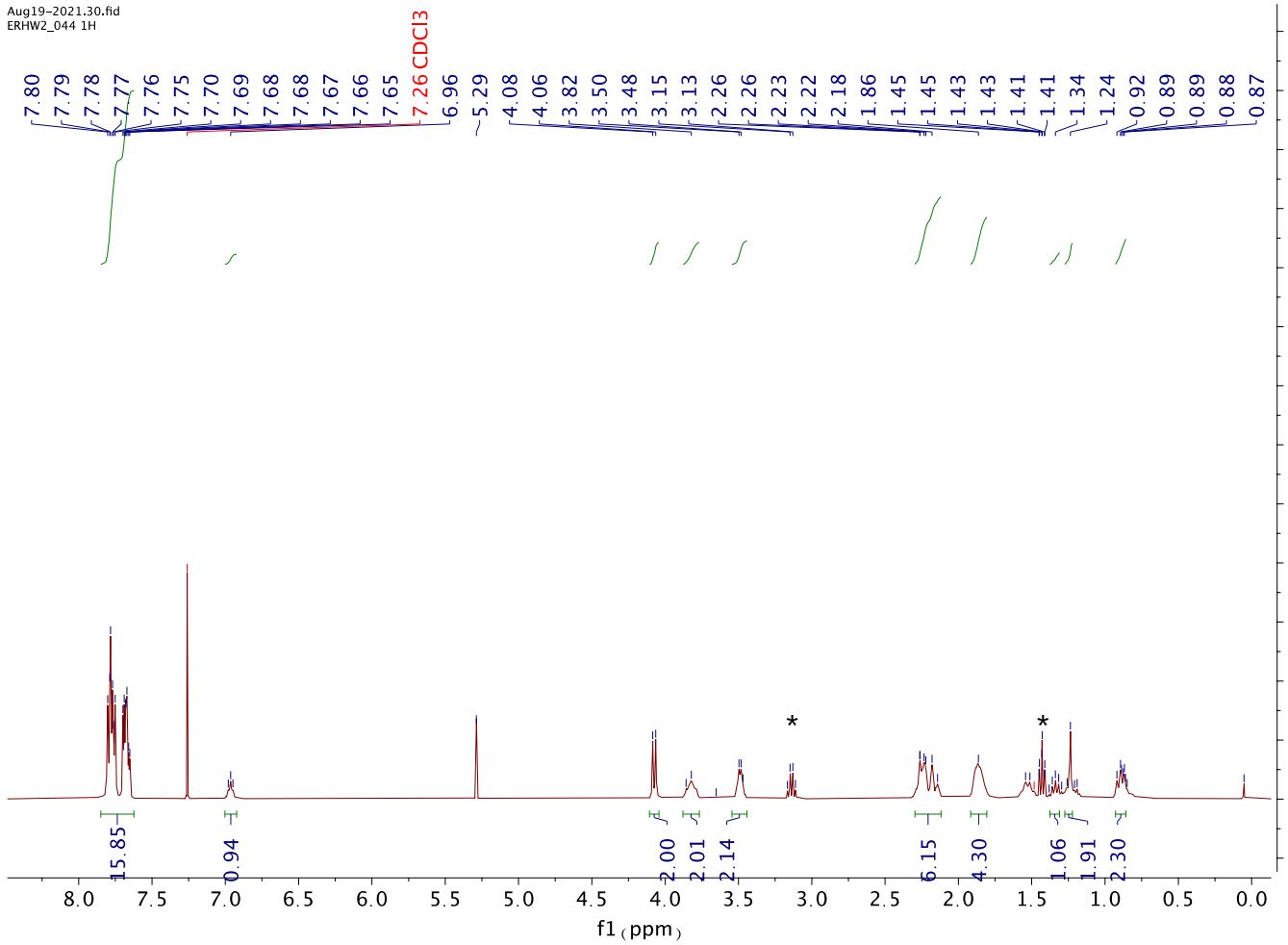


Fig. S17 ^{13}C NMR spectrum of **BCN-Phos-4** in $CDCl_3$ at 298 K. * Trace amounts of triethylamine salts.

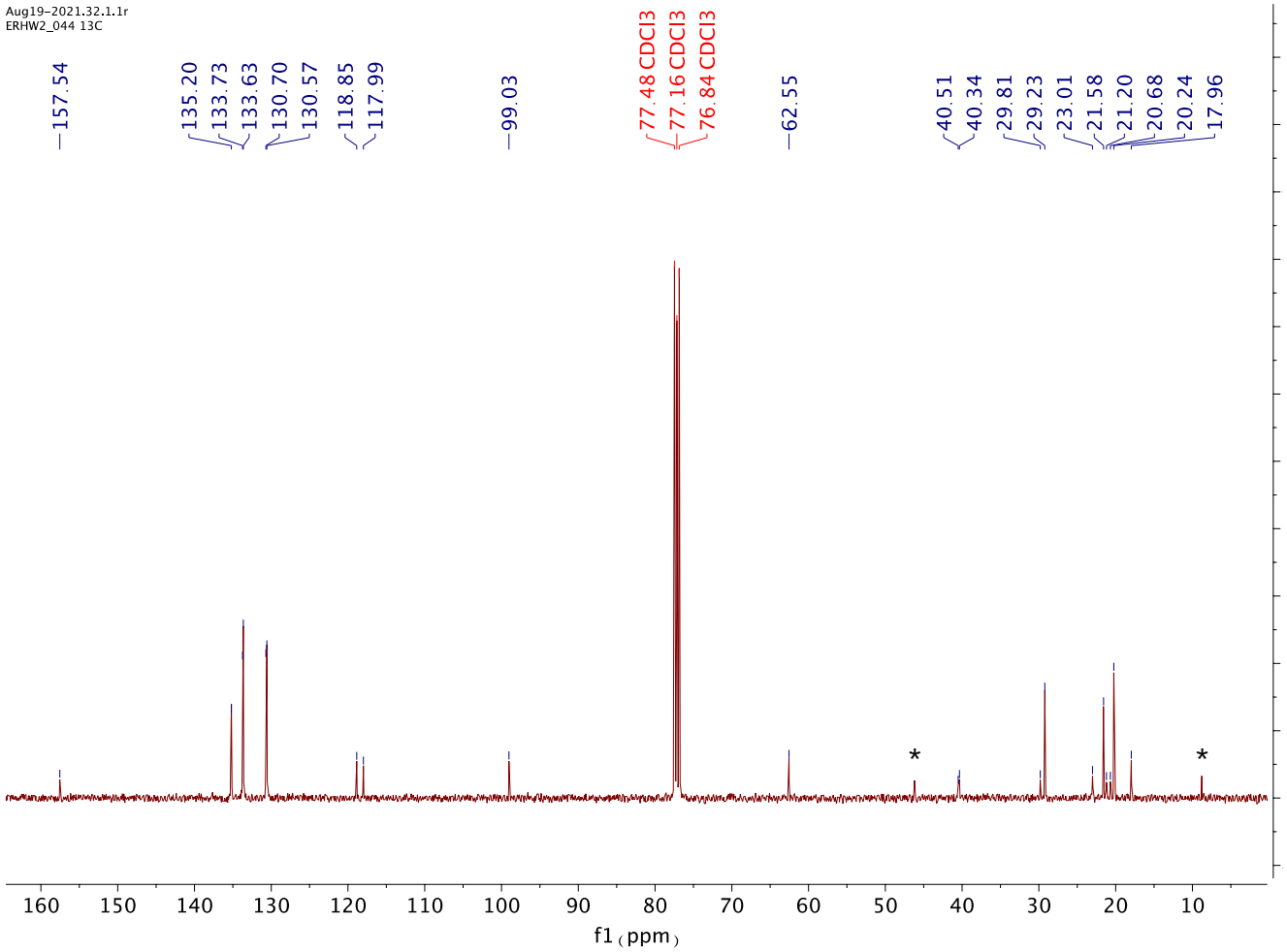


Fig. S18 ^{31}P NMR spectrum of **BCN-Phos-4** in $CDCl_3$ at 298 K.

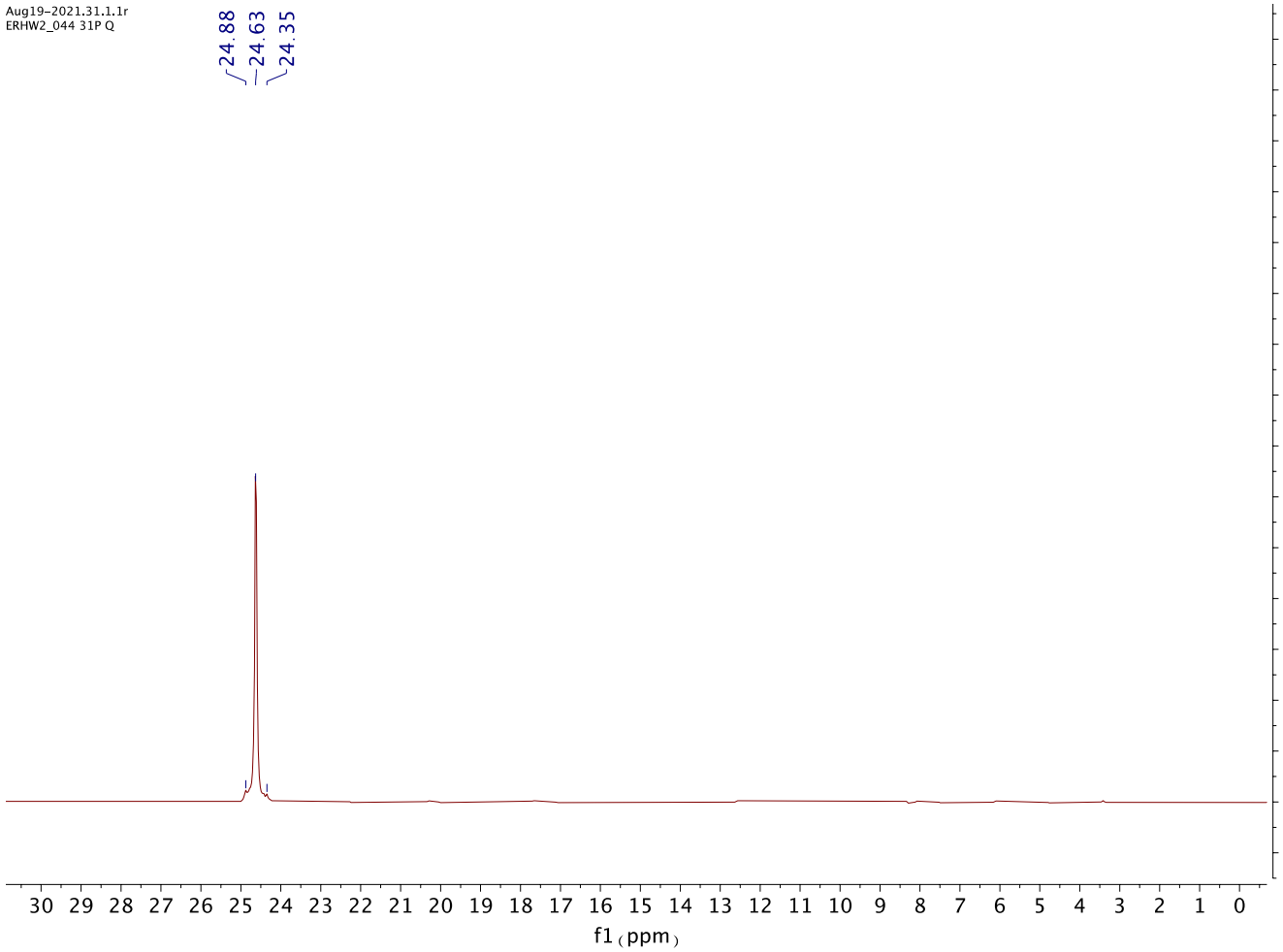


Fig. S19 ^{1}H NMR spectrum of **BCN-Phos-5** in $CDCl_3$ at 298 K. * Trace amounts of triethylamine salts. ~ Trace amounts of CH_2Cl_2.

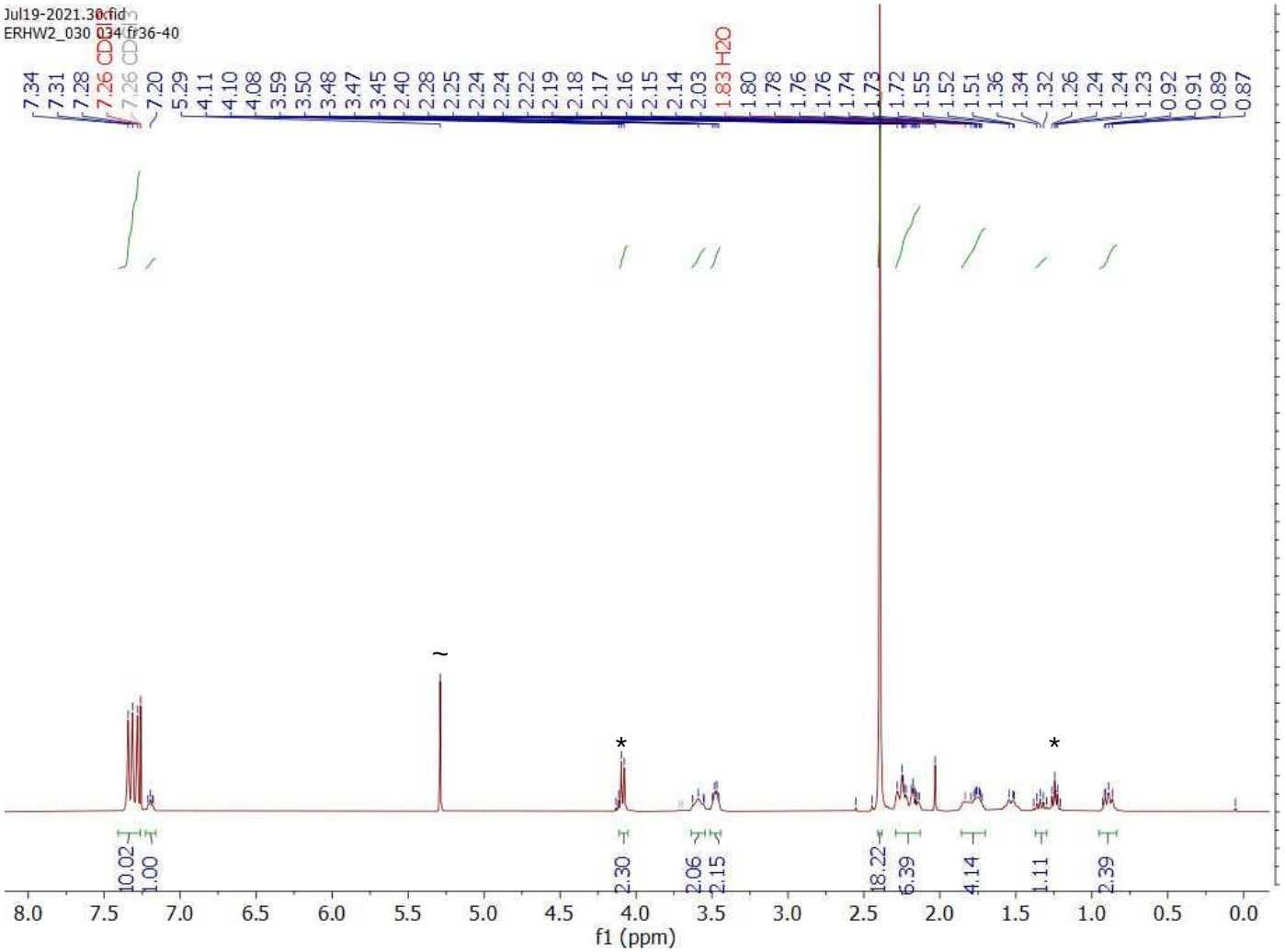


Fig. S20 ^{13}C NMR spectrum of **BCN-Phos-5** in $CDCl_3$ at 298 K. ~ Trace amounts of CH_2Cl_2.

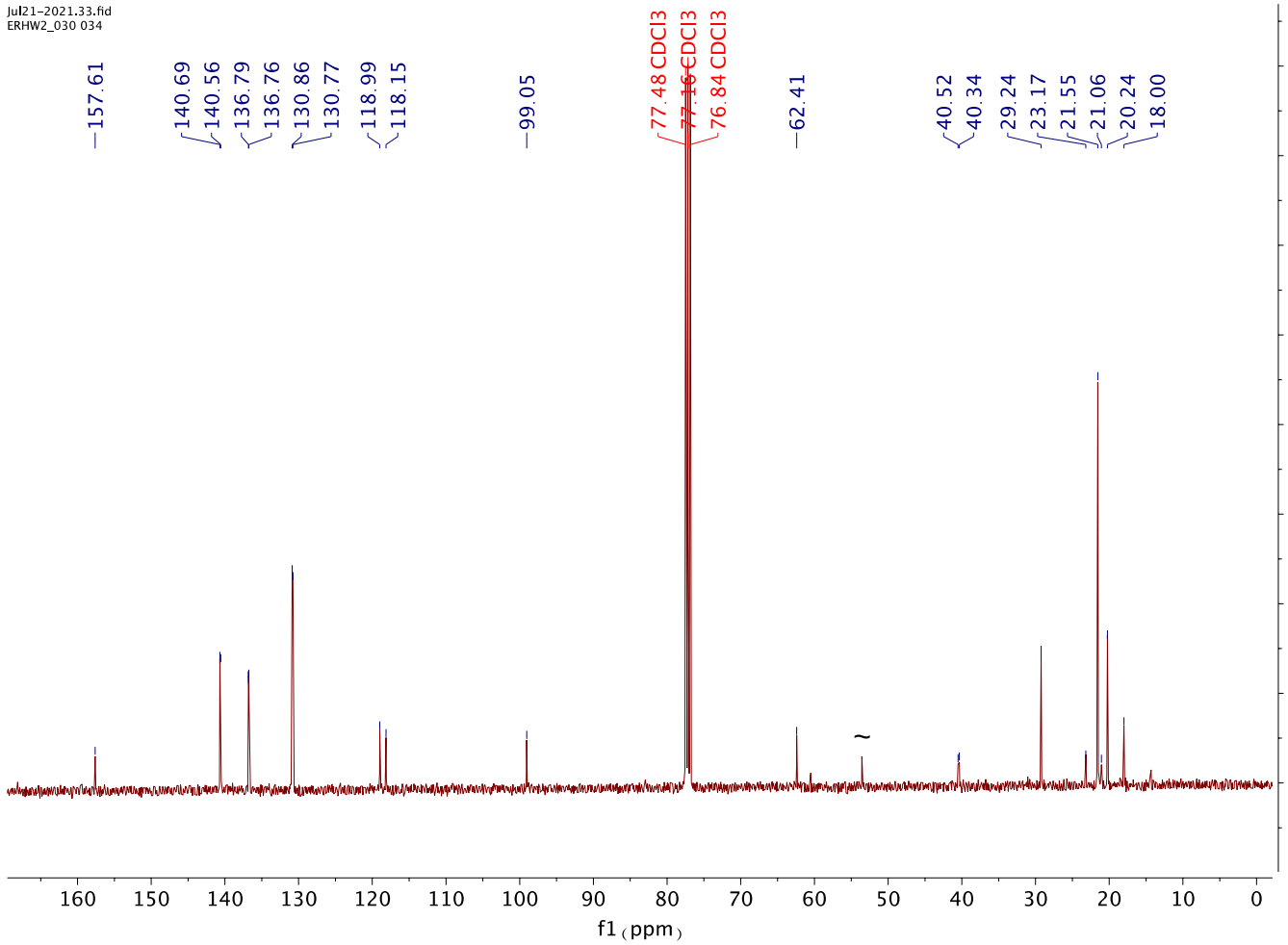


Fig. S21 ^{31}P NMR spectrum of **BCN-Phos-5** in $CDCl_3$ at 298 K.

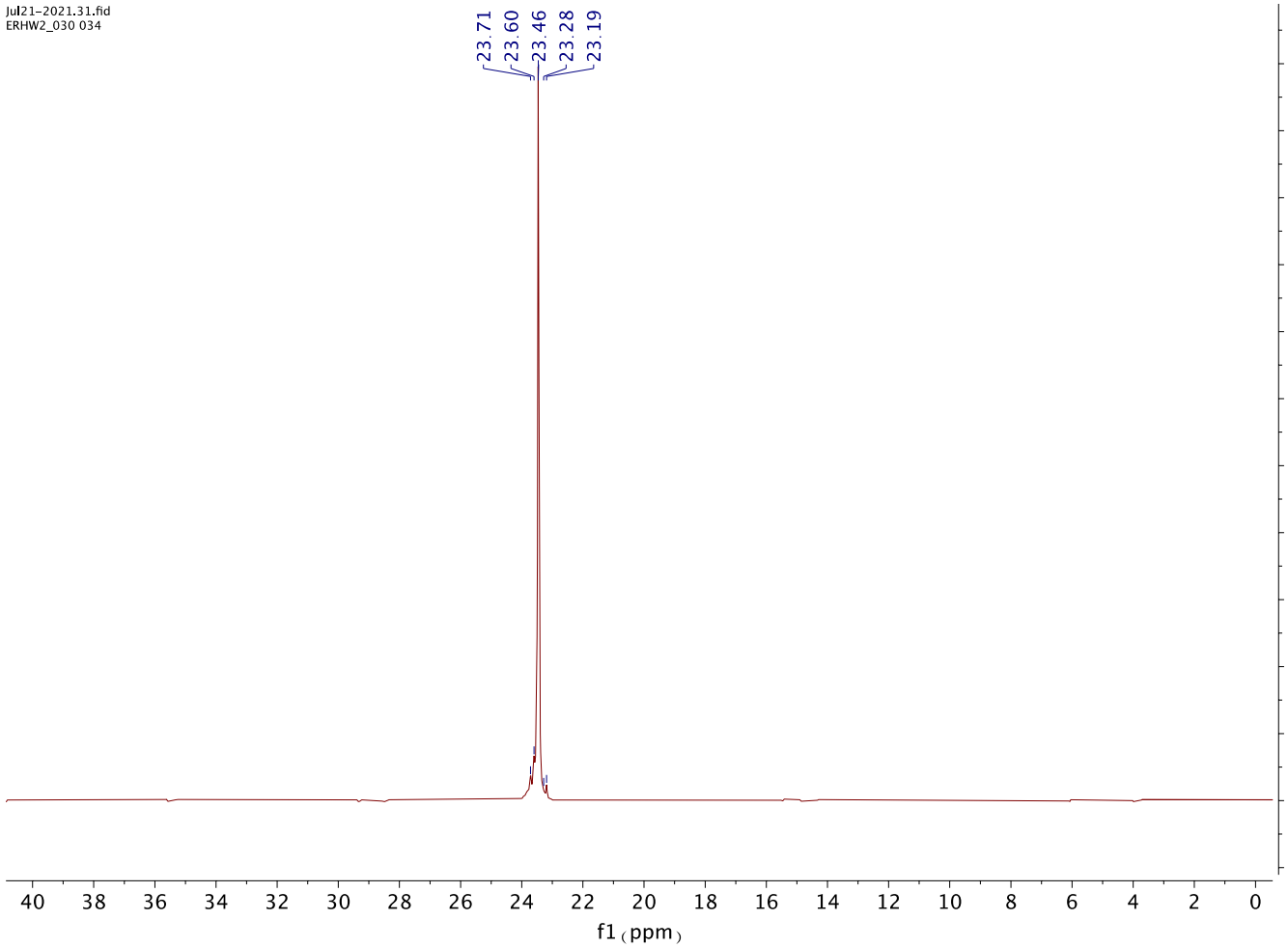


Fig. S22 ^{1}H NMR spectrum of **BCN-Phos-6** in $CDCl_3$ at 298 K. ~ Trace amounts of CH_2Cl_2.

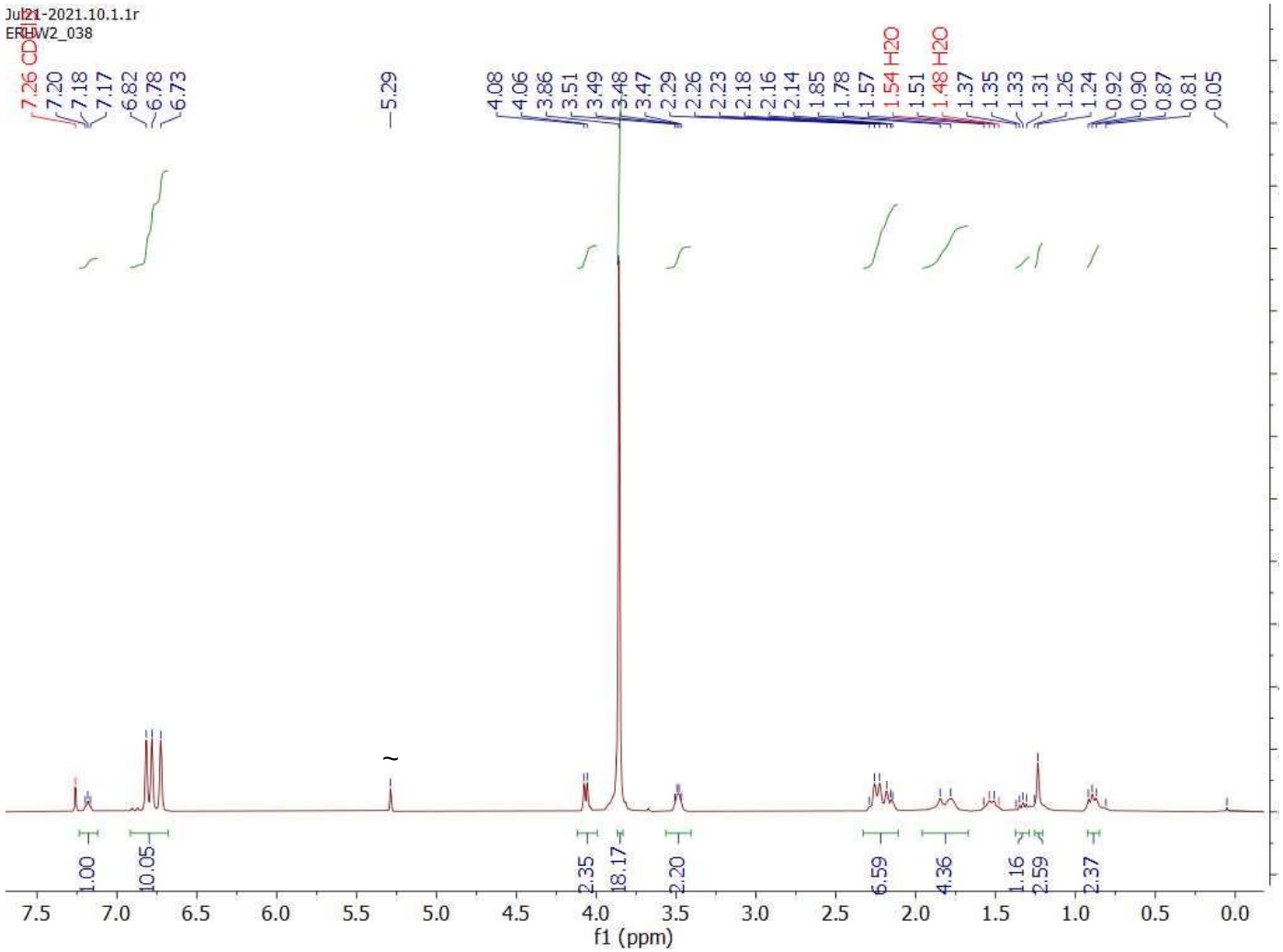


Fig. S23 ^{13}C NMR spectrum of **BCN-Phos-6** in $CDCl_3$ at 298 K.

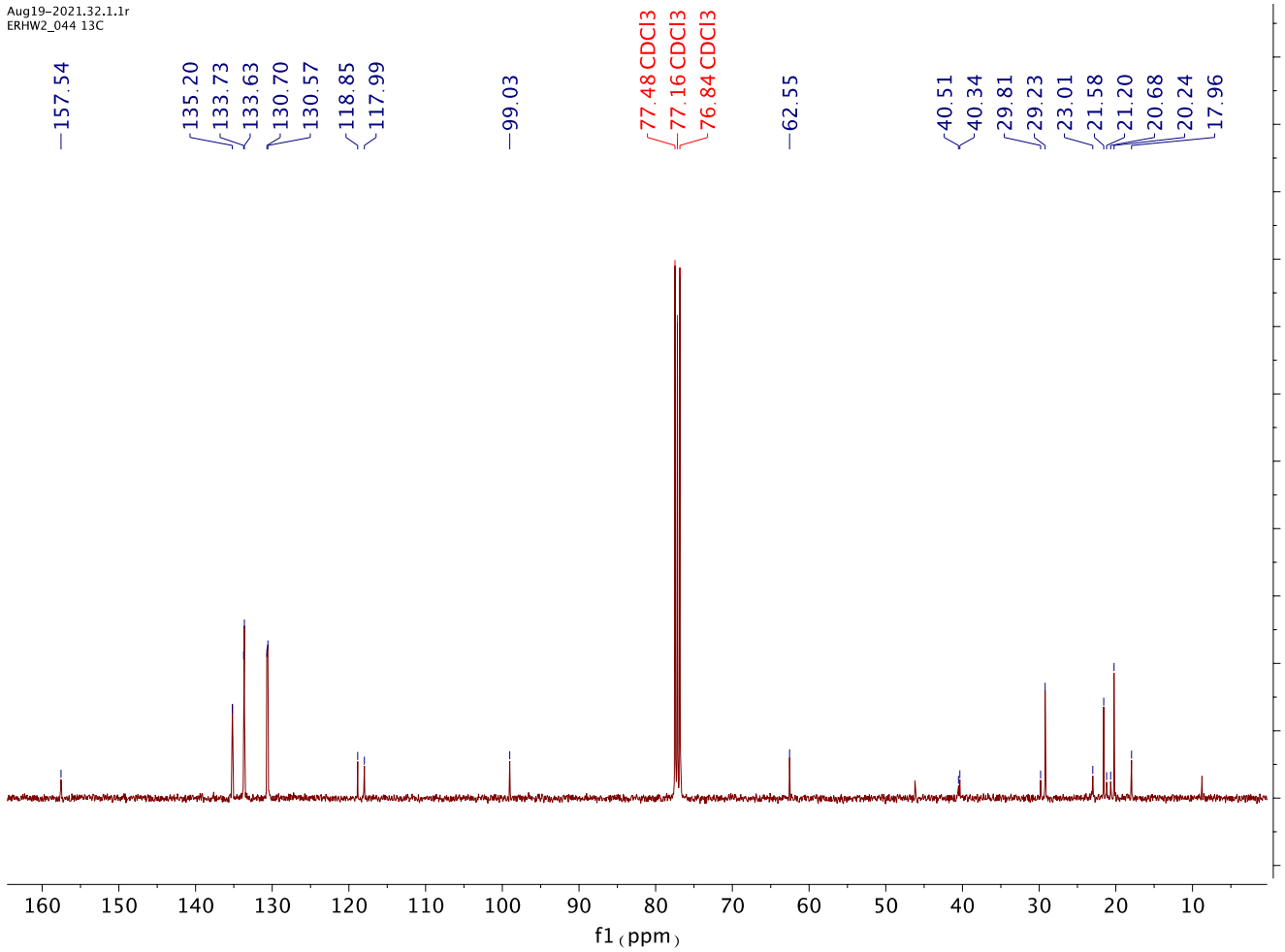


Fig. S24 ^{31}P NMR spectrum of **BCN-Phos-6** in $CDCl_3$ at 298 K.

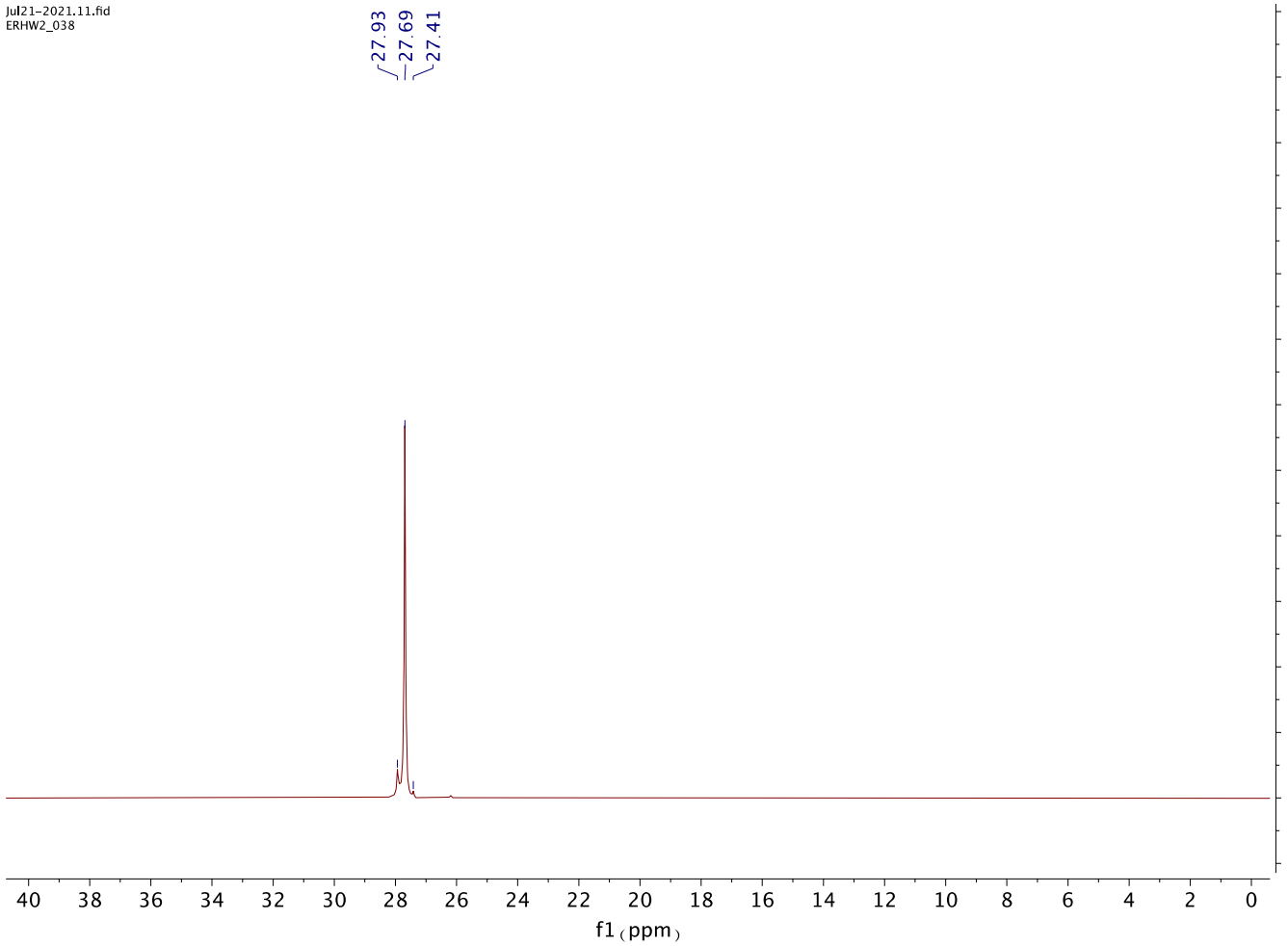


Fig. S25 ^{1}H NMR spectrum of **BCN-Phos-7** in $CDCl_3$ at 298 K.

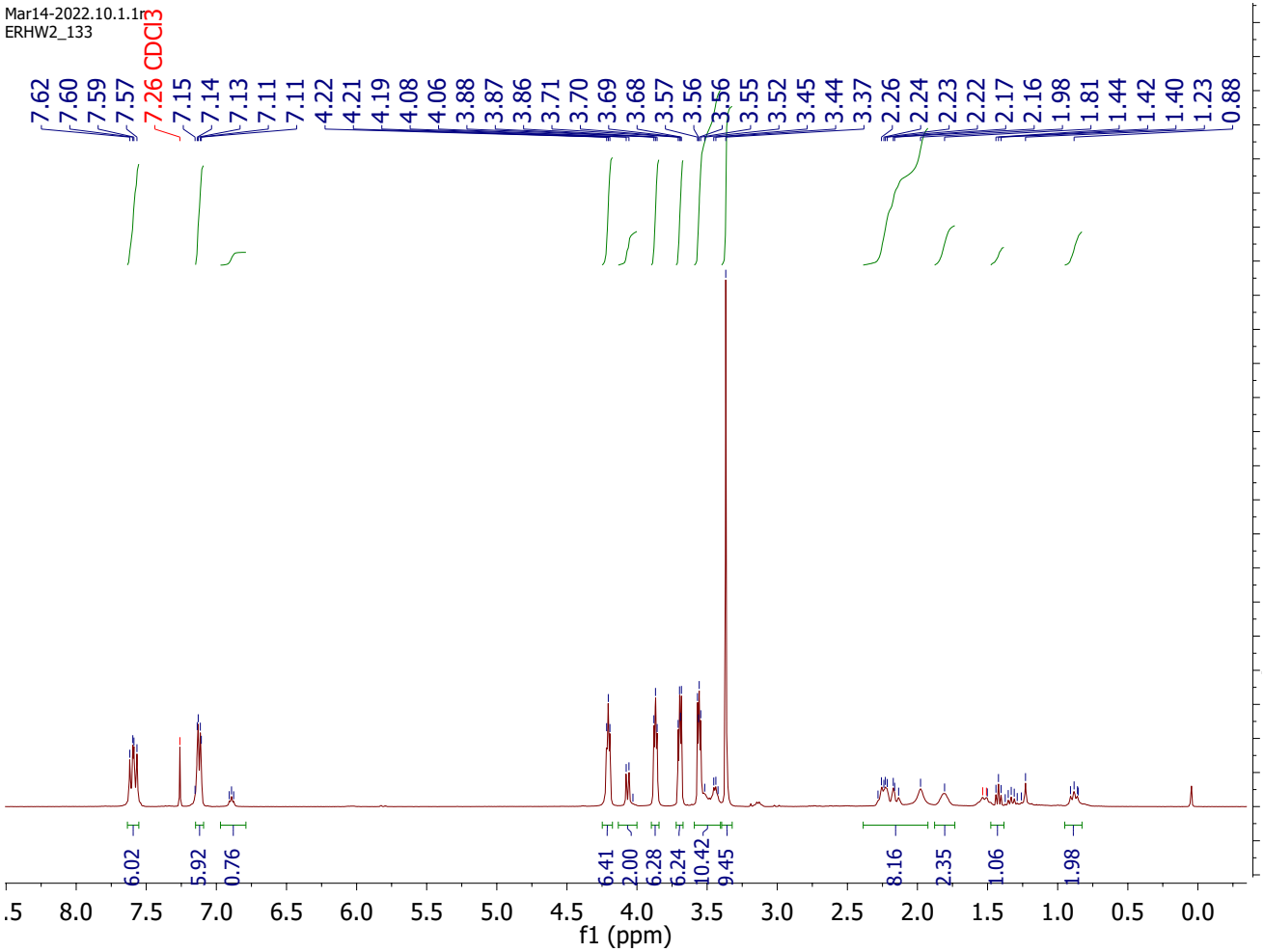


Fig. S26 ^{13}C NMR spectrum of **BCN-Phos-7** in $CDCl_3$ at 298 K.

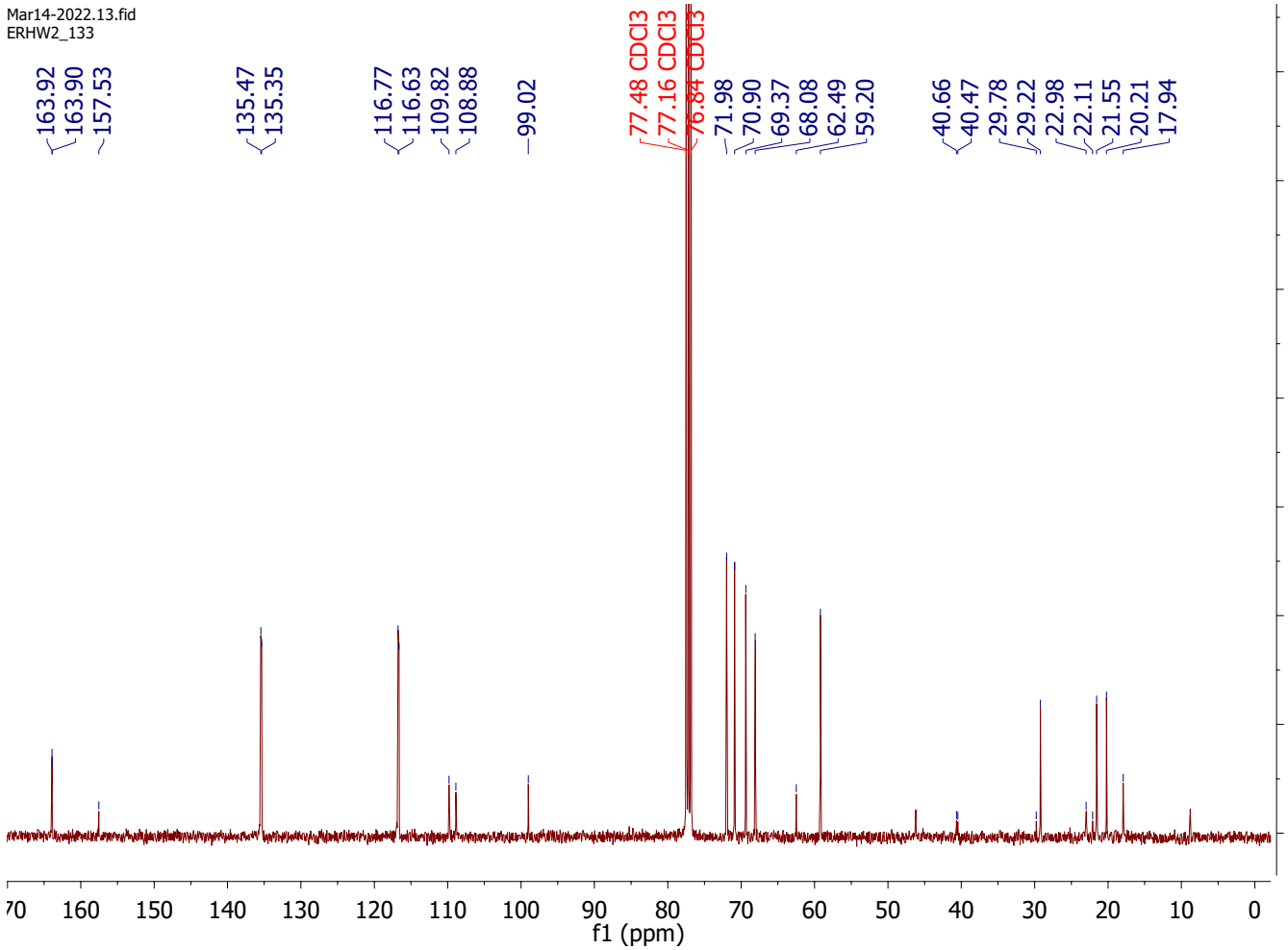
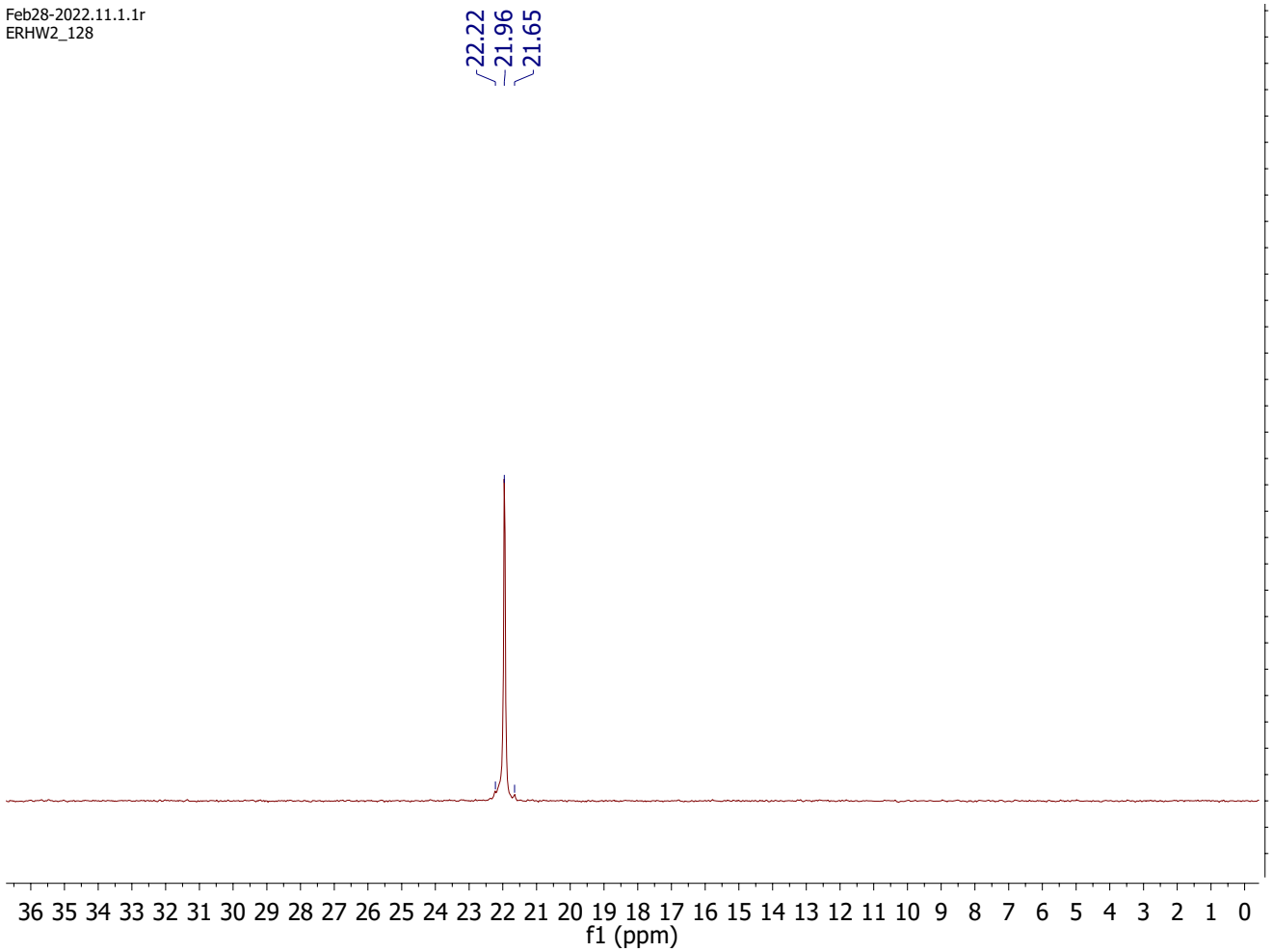


Fig. S27 ^{31}P NMR spectrum of **BCN-Phos-7** in $CDCl_3$ at 298 K.



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