Super-Photostable Phosphole-Based Dye for Multiple-Acquisition Stimulated Emission Depletion Imaging

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Table S1. Photophysical Data of C-Naphox, **1**, and **2** in Various Organic Solvents

Cmpd	Solvent	Absorption		Fluorescence				
		λ_{abs} /nm[a]	ε /10^4 $M^{-1}cm^{-1}$	λ_{em} /nm	Φ_F[b]	τ /ns	k_r /10^8 s^{-1}	k_{nr} /10^8 s^{-1}
C-Naphox	toluene	443	2.40	499	0.93	4.3	2.2	0.16
	$CHCl_3$	434	2.47	530	0.94	5.6	1.7	0.11
	CH_2Cl_2	431	2.48	543	0.93	6.1	1.5	0.11
	CH_3CN	424	2.45	570	0.88	6.8	1.3	0.18
1	toluene	377	2.54	454	0.87	3.2	2.7	0.41
	$CHCl_3$	373	2.38	460	0.84	4.3	2.0	0.37
	CH_2Cl_2	373	2.41	457	0.83	4.4	1.9	0.39
	CH_3CN	371	2.44	455	0.84	4.3	2.0	0.37
2	toluene	420	1.32	515	0.80	6.3	1.3	0.32
	$CHCl_3$	423	1.24	525	0.85	8.4	1.0	0.18
	CH_2Cl_2	421	1.27	523	0.82	8.3	0.99	0.22
	CH_3CN	418	1.26	520	0.82	8.2	1.0	0.22

[a]The longest absorption maximum wavelengths. [b]Absolute fluorescence quantum yields determined by a calibrated integrating sphere system within ±3% error.

Table S2. Photophysical Data of PB430, PB430-Antibpdy Conjugate, and Some Representative Fluorophores with Similar Absorption and Emission Wavelengths in PBS Buffer (pH = 7.4)

Probe	λ_{abs} /nm	λ_{em} /nm	Stokes shift /cm^{-1}	ε /M^{-1} cm^{-1}	Φ_F[f]	brightness ($\varepsilon \times \Phi_F$) /$M^{-1}$ cm^{-1}	τ / ns	relative photostability[g]
PB430	426	542	5020	10400	0.66	6900	10.6	37
PB430-antibody conjugate	427	539	4870	---	0.67	---	10.1	---
Alexa Fluor 430[a,b]	431	541	4720	16000	0.55	8800	3.3	1
Lucifer Yellow CH[c]	428	540	4850	11300	0.21	2400	5.3	n.d.
Pacific Orange[d]	404	553	6670	25000	n.d.	n.d.	n.d.	n.d.
BD Horizon V550[d]	405	535	6000	20500	0.68	14000	n.d.	n.d.
Star 440SXP[b]	436	515	3520	22700	0.68	15000	3.3	1.3
Atto 425[b]	436	484	2270	45000	0.65	29000	4.0	5.3
Star 470SXP[b]	472	624	5160	29000	0.12	3500	0.8	n.d.
Alexa Fluor 488[e]	495	519	935	73000	0.92	67000	4.1	2.3

[a]Nizamov, S. *et al. Chem. Eur. J.*, **18**, 16339–16348 (2012); Al Attar, H. A. *et al. Biomacromolecules*, **10**, 1077–1083, (2009). [b]Sednev, M. V. *et al. Methods Appl. Fluoresc.* **3**, 042004 (2015). [c]data in water: http://omlc.org/spectra/PhotochemCAD/html/065.html; Kristoffersen, A. S. *J. Fluoresc.*, **24**, 1015–1024, (2014). [d]Abrams, B. *et al. Cytometry*, **83A**, 752–762 (2013). [e]https://www.thermofisher.com/jp/ja/home/references/molecular-probes-the-handbook.html. [f]The fluorescence quantum yields of PB430 and PB430-antibody conjugate were determined using an absolute PL quantum yield spectrometer C9920-02 (Hamamatsu Photonics K.K) calibrated integrating sphere system equipped with a multichannel spectrometer (PMA-11); the quantum yields of other dyes are referred to the corresponding literatures. [g]Relative photostability is defined as the degree to which the fluorescence signal remains under the irradiation with a confocal laser at 470 nm. The value for Alexa Fluor 430 was referred as to 1.

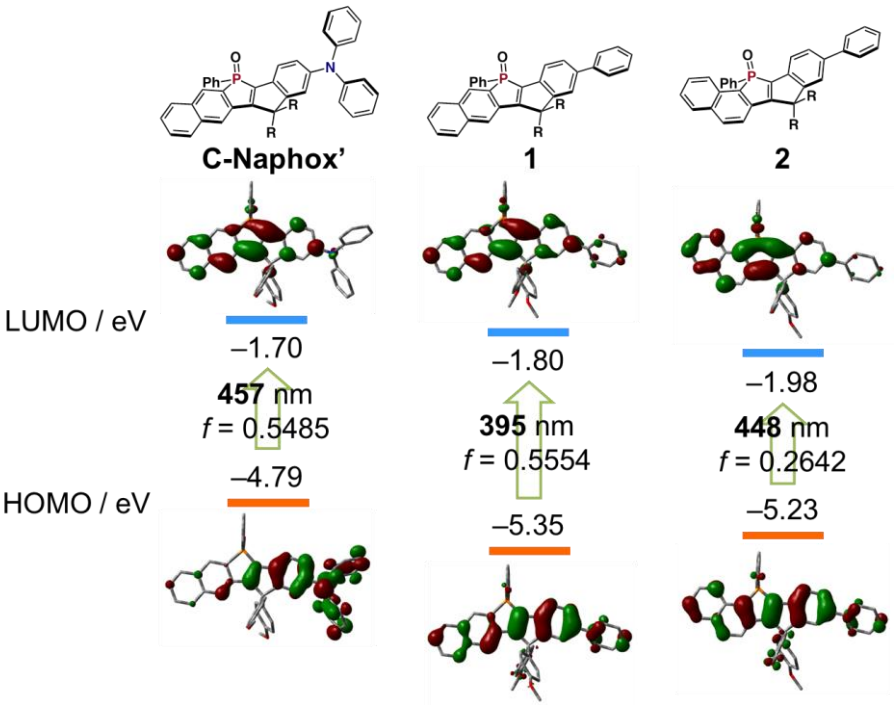


Figure S1. Energy diagrams, Kohn-Sham HOMOs and LUMOs, and the TD-DFT vertical excitation wavelengths and oscillator strengths (*f*) for a series of naphthophosphole *P*-oxide-based fluorophores C-Naphox′, **1**, and **2** calculated at the B3LYP/6-31G(d) level of theory. C-Naphox′ is a model compound of C-Naphox, of which substituents on the carbon bridge are simplified to 4-methoxyphenyl groups. R represents a 4-methoxyphenyl group.

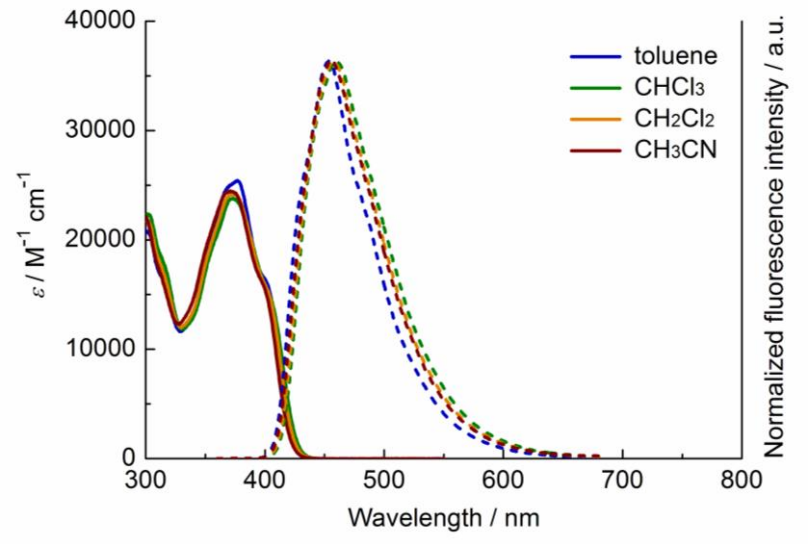


Figure S2. UV-vis absorption (solid line) and fluorescence (dot line) spectra of **1** in various solvents.

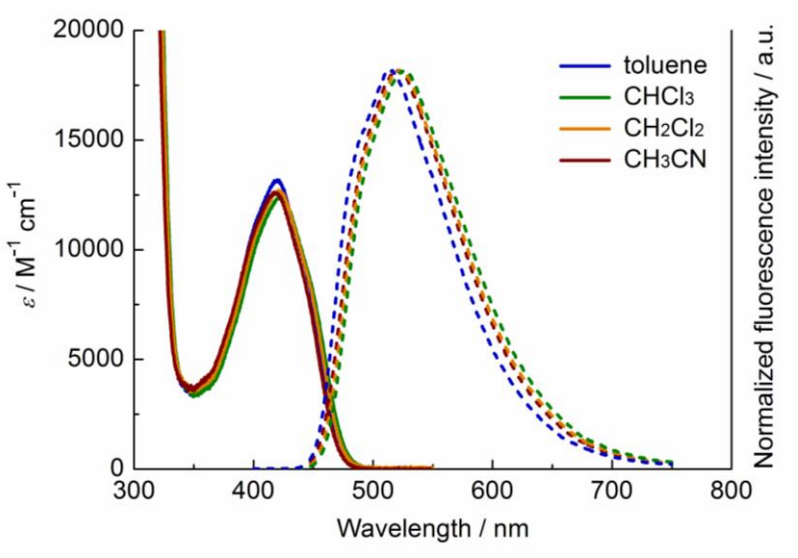


Figure S3. UV-vis absorption (solid line) and fluorescence (dot line) spectra of **2** in various solvents.

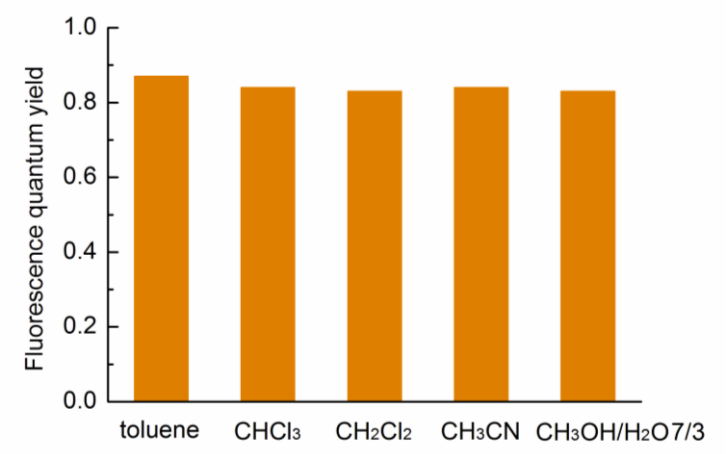


Figure S4. Fluorescence quantum yields of **1** in various organic solvents and aqueous medium of CH_3OH/H_2O (v/v = 7/3).

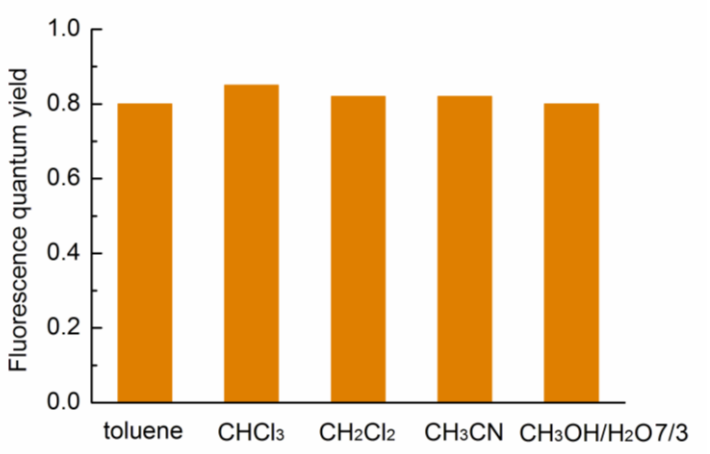


Figure S5. Fluorescence quantum yields of **2** in various organic solvents and aqueous medium of CH_3OH/H_2O (v/v = 7/3).

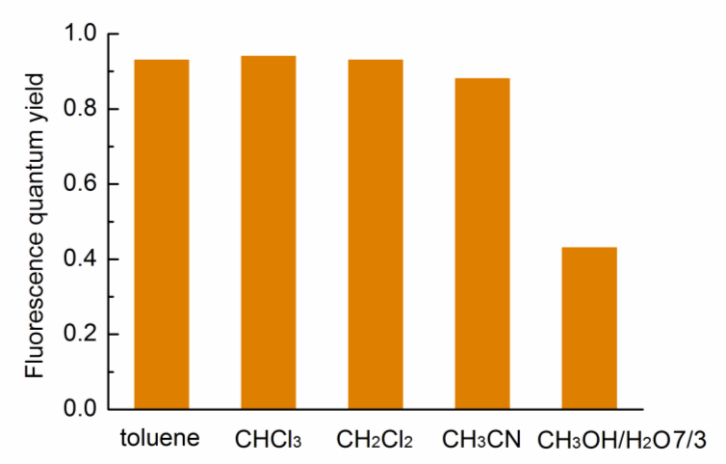


Figure S6. Fluorescence quantum yields of C-Naphox in various organic solvents and aqueous medium of CH_3OH/H_2O (v/v = 7/3).

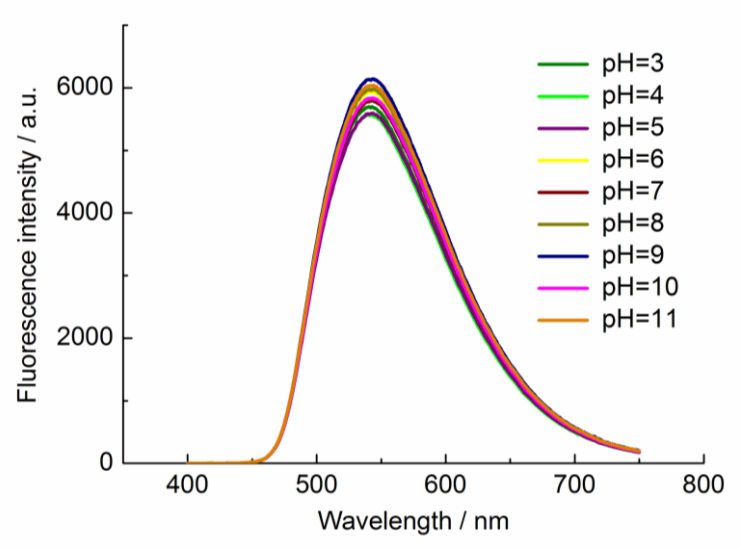


Figure S7. Fluorescence spectra of PB430 in various pH buffer solutions.

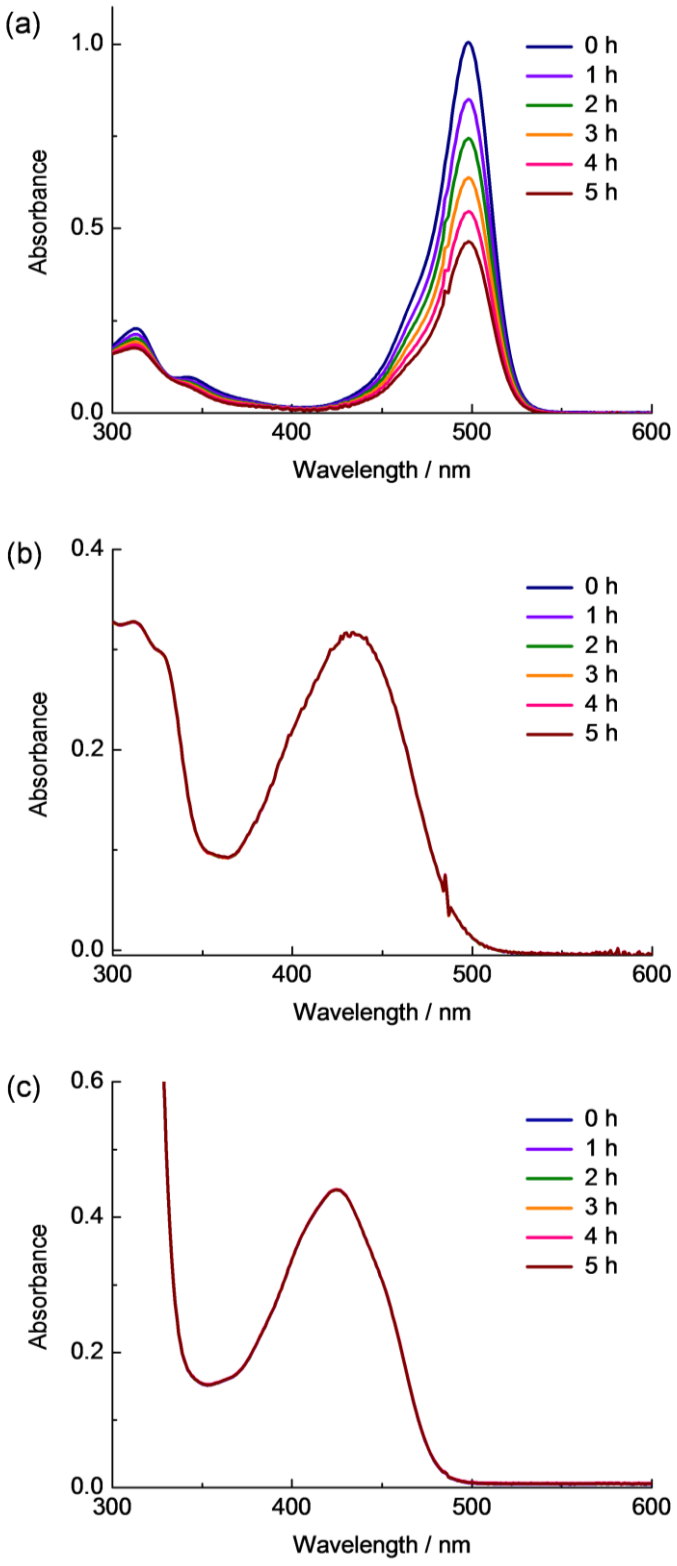


Figure S8. Absorption spectra of (a) Alexa Fluor 488, (b) C-Naphox, and (c) PB430 under the varied irradiation times with a Xe lamp (300 W) equipped with a 460 nm band-pass filter (a half peak width of 11 nm) in DMSO/HEPES buffer (v/v = 7/3, pH = 7.3). Solution concentrations were adjusted to be comparable to one another in terms of the optical density (0.22 of Alexa Fluor 488, 0.22 of C-Naphox, and 0.21 of PB430) at the excitation wavelength of 460 nm.

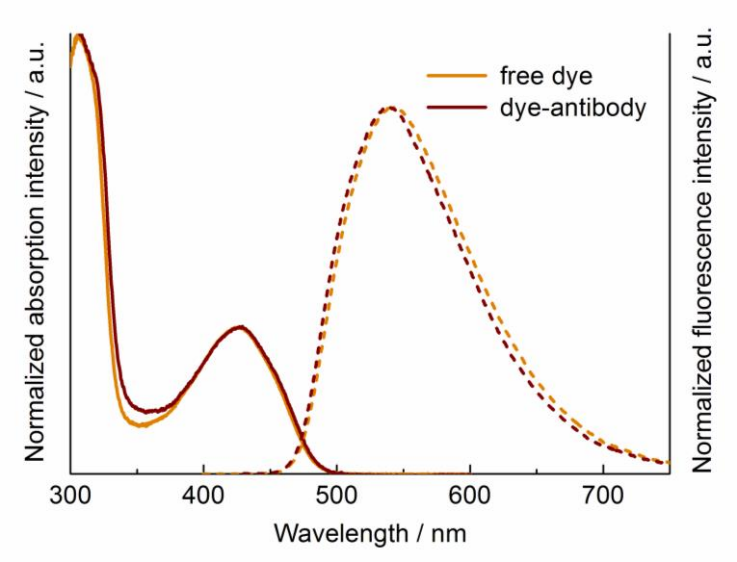


Figure S9. UV-vis absorption (solid line) and fluorescence (broken line) spectra of PB430 and PB430-antibody conjugate in PBS buffer solution (pH = 7.4).

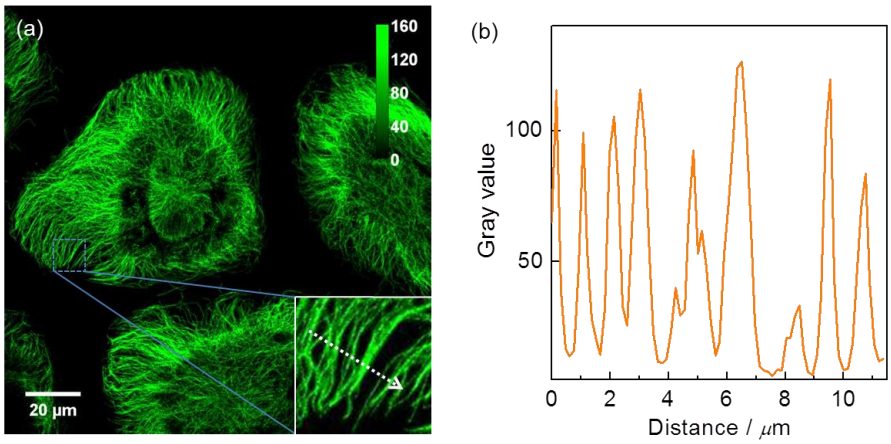


Figure S10. (a) Confocal microscopy image of microtubules immunolabeled with PB430 in fixed HeLa cells. Magnified views of the selected area are shown in insets. (b) Line profile taken across the filaments was marked by the arrow in the zoomed view.

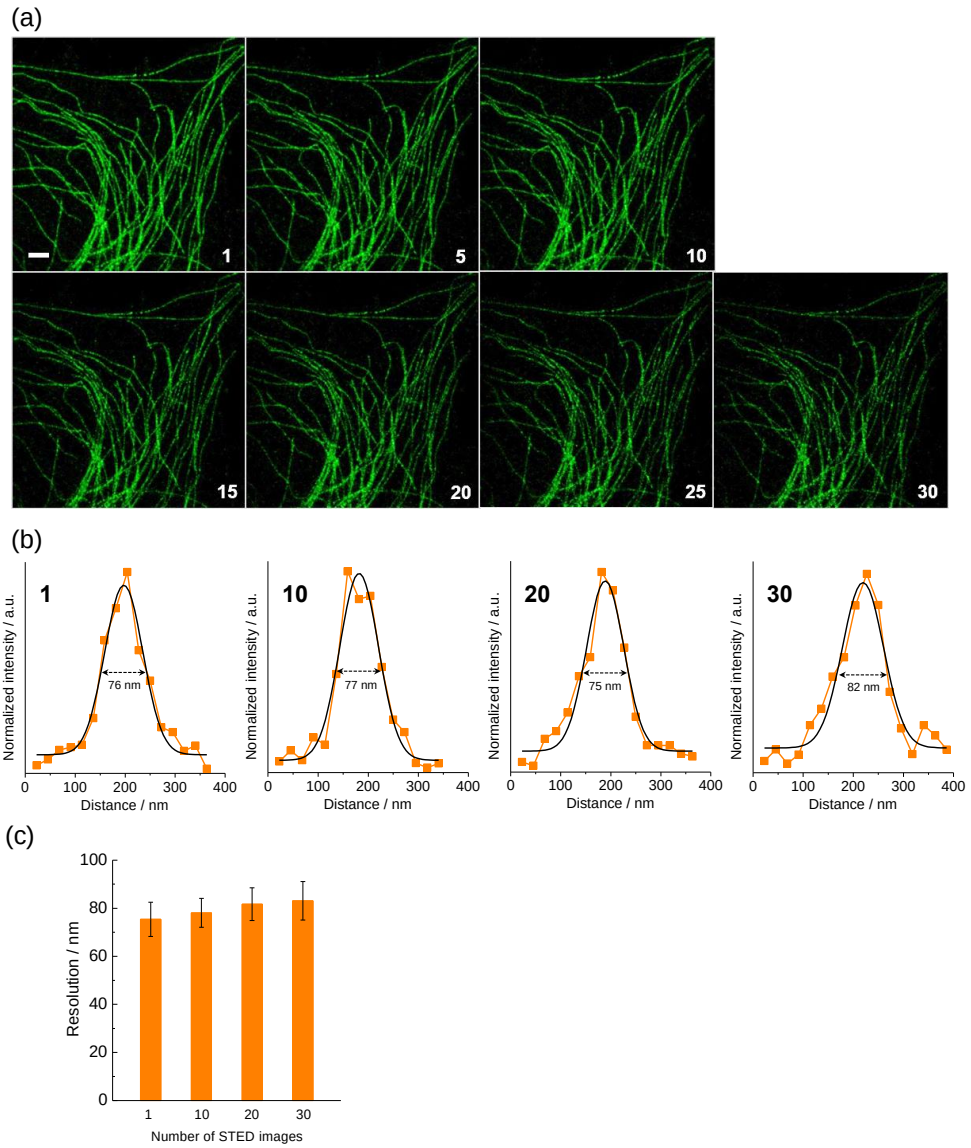


Figure S11. Repeated scanning of the same area of the cells (scale bar: 2 μm) under the STED conditions. Microtubules of fixed HeLa cells were immunolabeled with PB430. Images were recorded with the excitation at 470 nm (WLL, 5 μW), depletion at 592 nm (CW-STED, 30 mW), and gated detection at T_g = 0.5 ns. (a) The repeated STED microscopy images. Each number indicates the number of flames. (b) The typical intensity profiles of PB430-labeled microtubules at each image (the flame numbers are 1, 10, 20, and 30, respectively). The full width at half maximum (FWHM) was calculated by Resolutions of microtubules were calculated from a Gaussian fit. (c) Statistical analysis of FWHM obtained with Gaussian fit of 10 individual emitting spots.

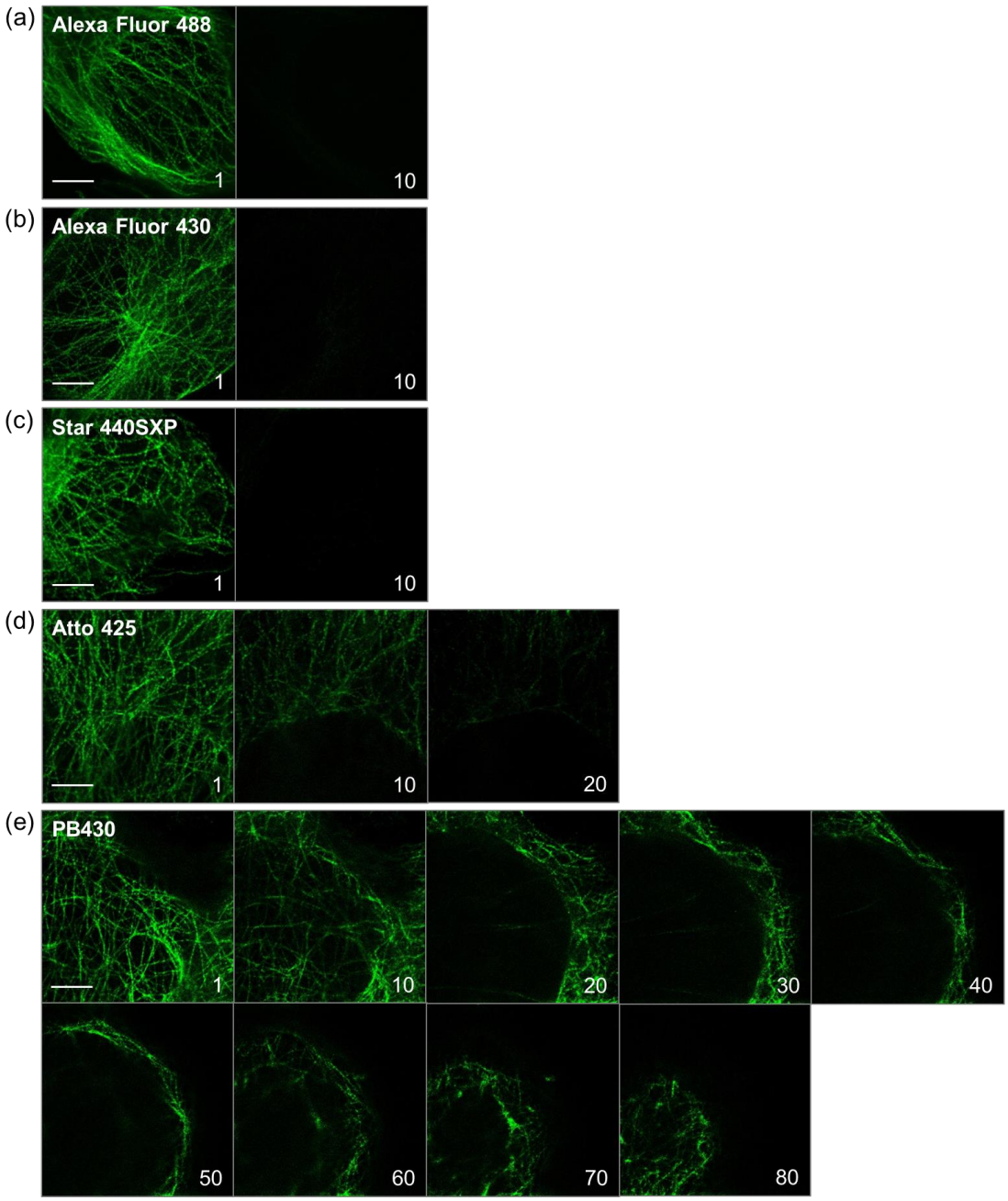


Figure S12. Comparison of the z-scan STED images of microtubules immunolabeled with (a) Alexa Fluor 488, (b) Alexa Fluor 430, (c) Star 440SXP, (d) Atto 425, and (e) PB430. Each number indicates the number of recorded z-scan images. The z-scan imaging was conducted with a step of 50 nm, an excitation of 470 nm and a depletion laser of 592 nm (CW-STED, 30 mW; STED 3D z donut, 50%). Scale bars indicate 2 μm.

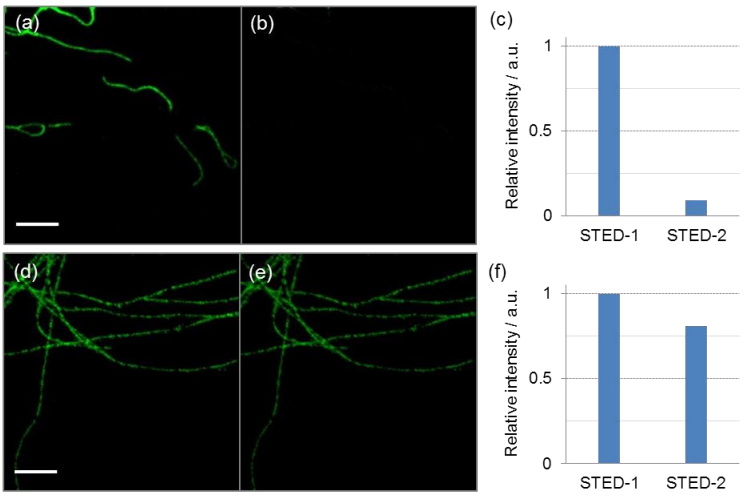


Figure S13. Comparison of the photostability between Alexa Fluor 430 (a–c) and PB430 (d–f) under the STED conditions. The first and second STED images of Alexa Fluor 430-labled vimentins (a, b) and PB430-labled tubulins (d, e) were taken continuously (scale bar: 2 μm), and their relative fluorescence intensity were compared (c, f). The same imaging conditions were used for both: excitation at 470 nm (WLL, 10 μW), depletion at 592 nm (CW-STED, 30 mW), gated detection at T_g = 0.5 ns.

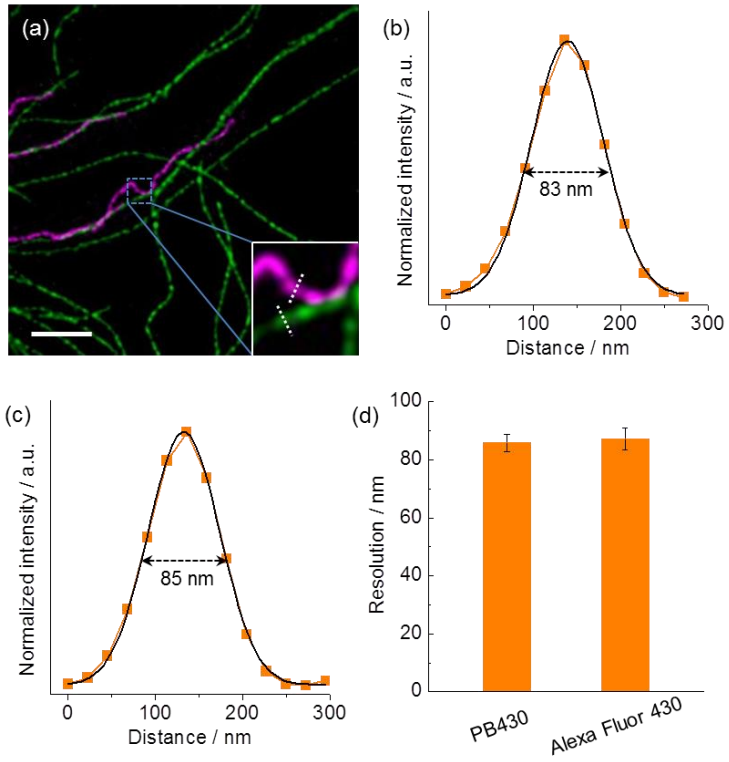


Figure S14. Determination of the resolution of distinct photostability-based two-color STED imaging. (a) A two-color STED microscopy image (deconvoluted data), in which microtubules (green) and vimentin filaments (magenta) were separately immunolabeled with PB430 and Alexa Fluor 430, respectively. Plots of the intensity profiles along with the dotted lines crossed (b) microtubules and (c) vimentin filaments in the inset of the two-color STED image. The profiles were fitted to Gaussian distributions. (d) The statistical analysis (n = 10) of the full width at half maximum (FWHM) resolution.

Supplementary Note

Experimental detail for the chemical synthesis of phosphole oxide-based fluorescent dyes

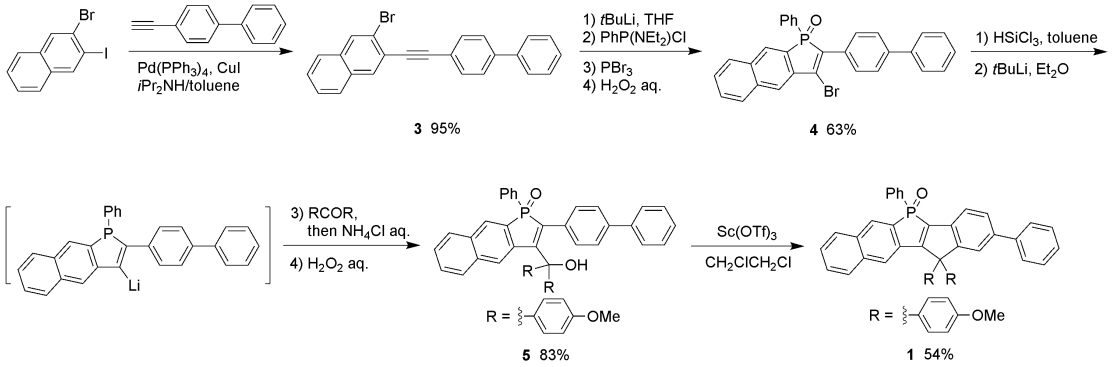
General. Melting points (mp) or decomposition temperatures were determined with a Yanaco MP-S3 instrument. ^{1}H, $^{13}C\{^{1}H\}$, and $^{31}P\{^{1}H\}$ NMR spectra were recorded with a JEOL AL-400 spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C, and 162 MHz for ^{31}P) or a JEOL A-600 spectrometer (600 MHz for ^{1}H) in $CDCl_3$ or DMSO-d_6. The chemical shifts in ^{1}H NMR spectra are reported in δ ppm using the residual protons of the solvents as an internal standard ($CHCl_3$ δ 7.26 and DMSO δ 2.50), and those in ^{13}C NMR spectra are reported using the solvent signals as an internal standard ($CDCl_3$ δ 77.16 and DMSO δ 39.52). The chemical shifts in ^{31}P NMR spectra are reported using H_3PO_4 (δ 0.00) as an external standard. Mass spectra were measured with a Bruker micrOTOF Focus spectrometry system with the ionization method of APCI. Thin layer chromatography (TLC) was performed on glass plates coated with 0.25 mm thickness of silica gel $60F_{254}$ (Merck). Column chromatography was performed using neutral silica gel PSQ100B (Fuji Silysia Chemicals). Preparative HPLC was performed using LC-918 (Japan Analytical Industry Co.) equipped with reverse phase column (Wakosil-II 5C18 HG Prep) or YMC LC-forte/R equipped with reverse phase column (YMC-DispoPackAT ODS). All reactions were performed under a N_2 atmosphere, unless stated otherwise. Commercially available solvents and reagents were used without further purification unless otherwise mentioned. Anhydrous THF, toluene, Et_2O, and CH_2Cl_2 were purchased from Kanto Chemicals and further purified by Glass Contour Solvent Systems. 2-Bromo-3-iodonaphthalene,[1] 1-bromonaphthalen-2-yl triflate,[2] and (4-trimethylsilylphenyl)acetylene[3] were synthesized according to the literatures.

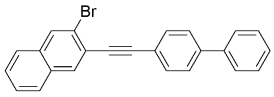
References

1 Cottet, F. *et al. Synthesis*, **5**, 798–803 (2005).

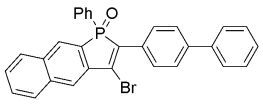
2 Weimar, M. *et al. Org. Lett.*, **15**, 1706–1709 (2013).

3 Wu, J. *et al. J. Org. Chem.*, **69**, 8194–8204 (2004).



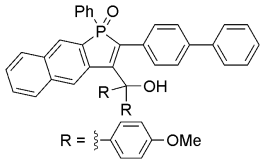


2-([1,1′-biphenyl]-4-ylethynyl)-3-bromonaphthalene (3). The degassed solvents of toluene (10 mL) and *i*Pr$_2$NH (5 mL) were added to the solids of 2-bromo-3-iodonaphthalene (999 mg, 3.00 mmol), 4-ethynylbiphenyl (561 mg, 3.15 mmol), Pd(PPh$_3$)$_4$ (116 mg, 0.10 mmol) and CuI (19 mg, 0.10 mmol). After stirring at room temperature for 16 h, CH_2Cl_2 (200 mL) was added to the mixture in order to dissolve the solid product. The solution was washed with H_2O (30 mL), and then dried over anhydrous Na_2SO_4, and filtered. After concentration of the filtrate under reduced pressure, the resulting solids were purified by recrystallization from EtOH (30 mL) to afford 1.091 g (2.85 mmol, 95%) of **3** as white solids. Mp: 180.5–181.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (s, 1H), 8.11 (s, 1H), 7.81–7.78 (m, 1H), 7.76–7.72 (m, 3H), 7.65–7.63 (m, 4H), 7.54–7.46 (m, 4H), 7.39 (t, J = 7.2 Hz, 1H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 141.5 (C), 140.5 (C), 133.8 (C), 133.1 (CH), 132.3 (CH), 131.9 (C), 131.2 (CH), 129.0 (CH), 127.9 (CH), 127.8 (CH), 127.7 (CH), 127.22 (CH), 127.19 (CH), 127.0 (CH), 122.8 (C), 122.2 (C), 122.0 (C), 93.8 (C), 89.2 (C). One signal of a CH carbon may be overlapped. HRMS (APCI): *m/z* calcd. for $C_{24}H_{16}Br$: 383.0430 ([M+H]$^+$); found. 383.0447.

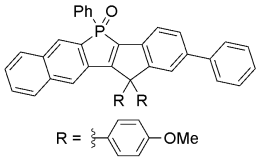


2-([1,1′-biphenyl]-4-yl)-3-bromo-1-phenylnaphtho[2,3-*b*]phosphole-*P*-oxide (4). To a solution of **3** (600 mg, 1.57 mmol) in anhydrous THF (20 mL) was slowly added *t*BuLi in pentane (1.65 M, 2.00 mL, 3.30 mmol) dropwise at −78 °C. After stirring at −78 °C for 1 h, the suspension was warmed slowly to −50 °C in 2 h. After cooling the mixture to −78 °C, PhP(NEt$_2$)Cl (0.300 mL, 1.56 mmol) was added in 5 min and the mixture was warmed slowly to 0 °C in 0.5 h. Cooled the mixture to −78 °C again, PBr_3 (0.150 mL, 1.58 mmol) was added in 5 min and the resulting mixture was allowed to warm to room temperature. After stirring at 35 °C for 12 h , the reaction mixture was oxidized using an aqueous solution of H_2O_2 (1 mL, 30 %) at 0 °C and stirred for 1 h. After quenching the reaction with an aqueous solution of Na_2SO_3 (20 mL, 10 %) at 0 °C, the mixture was extracted with $CHCl_3$ (150 mL). The organic layer was washed with H_2O (30 mL), dried over anhydrous Na_2SO_4, and then filtered. After concentration of the filtrate under reduced pressure, the resulting

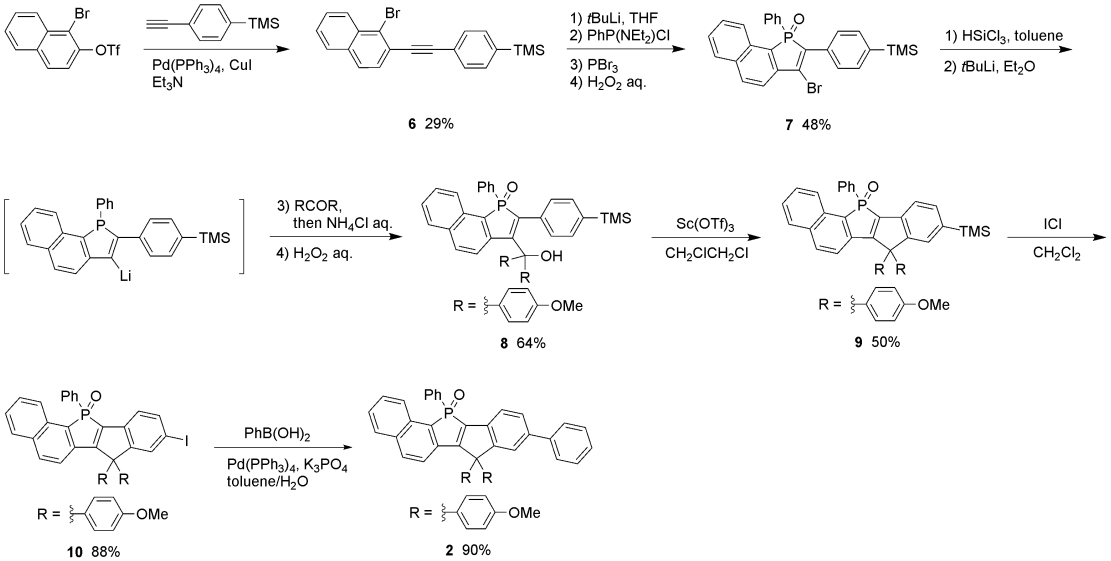
solids were purified by silica gel column chromatography (CH_2Cl_2 to 10/1 CH_2Cl_2/EtOAc, R_f = 0.45 in 10/1 CH_2Cl_2/EtOAc) and recrystallization from EtOH (20 mL) to afford 502 mg (0.989 mmol, 63%) of **4** as white solids. Mp: 287.0–288.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15–8.12 (m, 2H), 7.98 (d, J = 7.6 Hz, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.83–7.80 (m, 2H), 7.76–7.71 (m, 2H), 7.66–7.55 (m, 6H), 7.52–7.47 (m, 1H), 7.45–7.33 (m, 5H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$, 50°C): δ 141.8 (s, C), 140.5 (s, C), 137.9 (d, J = 23.0 Hz, C), 137.7 (d, J = 87.2 Hz, C), 135.6 (d, J = 0.9 Hz, C), 133.8 (d, J = 12.4 Hz, C), 133.3 (d, J = 32.1 Hz, C), 132.6 (d, J = 3.3 Hz, CH), 131.4 (d, J = 8.2 Hz, C), 131.3 (d, J = 10.7 Hz, CH), 130.7 (d, J = 8.3 Hz, CH), 129.9 (d, J = 103.7 Hz, C), 129.3 (s, CH), 129.2 (s, CH), 129.0 (d, J = 11.5 Hz, CH), 129.0 (d, J = 107.0 Hz, C), 129.0 (s, CH), 128.9 (s, CH), 128.0 (s, CH), 127.7 (s, CH), 127.3 (s, CH), 127.1 (s, CH), 124.8 (d, J = 9.1 Hz, CH). One singlet signal of a CH carbon may be overlapped. ^{31}P{^{1}H} NMR (162 MHz, $CDCl_3$): δ 30.7. HRMS (APCI): *m/z* calcd. for $C_{30}H_{21}BrOP$: 507.0508 ([M+H]$^+$); found. 507.0516.

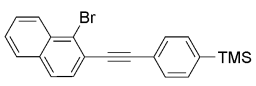


Compound 5. To a suspension of **4** (200 mg, 0.394 mmol) in anhydrous toluene (5 mL) was added $HSiCl_3$ (0.200 mL, 1.98 mmol) in one portion. After stirring at room temperature for 1 h, all volatiles were removed under reduced pressure. Then, CH_2Cl_2 (5 mL) was added and the resulting suspension was filtered through a plug of Celite® under a N_2 atmosphere and rinsed with CH_2Cl_2 (10 mL). After concentration of the filtrate under reduced pressure, the resulting white solids were suspended in anhydrous Et_2O (10 mL). To this suspension was added *t*BuLi in pentane (1.65 M, 0.540 mL, 0.891 mmol) over 10 min at –78 °C. After stirring for 2 h, 4,4′-dimethoxybenzophenone (121 mg, 0.500 mmol) was added, and resulting mixture was warmed slowly to room temperature for overnight. Then, the reaction was quenched with a saturated aqueous solution of NH_4Cl (5 mL), followed by the oxidation with an aqueous solution of H_2O_2 (1 mL, 30%), and stirred for 1 h at room temperature. After quenching the reaction with a 10% Na_2SO_3 aqueous solution (20 mL) at 0 °C, the mixture was extracted with CH_2Cl_2 (300 mL). The organic layer was washed with water (30 mL), and then dried over anhydrous Na_2SO_4, and filtered. After concentration of the filtrate under reduced pressure, the resulting crude product was purified by recrystallization from toluene (30 mL) to afford 220 mg (0.328 mmol, 83%) of **5** as white solids. Mp: >300 °C. ^{1}H NMR (400 MHz, DMSO-d_6, 80 °C): δ 8.16 (d, J = 8.0 Hz, 1H), 8.08 (d, J = 3.6 Hz, 1H), 7.96–7.93 (m, 1H), 7.65–7.45 (m, 10H), 7.40 (t, J = 7.6 Hz, 2H), 7.31 (t, J = 7.6 Hz, 1H), 7.24 (d, J = 8.4 Hz, 4H), 7.09 (d, J = 8.0 Hz, 2H), 6.71–6.66 (m, 6H), 6.58 (s, 1H), 3.60 (s, 3H), 3.58 (s, 3H). Satisfying ^{13}C{^{1}H} NMR was not obtained duo to the poor solubility. ^{31}P{^{1}H} NMR (162 MHz, DMSO-d_6, 80 °C): δ 33.5. HRMS (APCI): *m/z* calcd. for $C_{45}H_{36}O_4P$: 671.2346 ([M+H]$^+$); found. 671.2333.



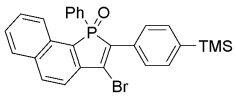
Compound 1. The mixture of compound **5** (120 mg, 0.179 mmol) and $Sc(OTf)_3$ (88.1 mg, 0.179 mmol) in 1,2-dichloroethane (5 mL) was refluxed at 90 °C for 6 h. After cooling, H_2O (20 mL) was added to quench the reaction. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (50 mL). The combined organic layer was washed with H_2O (20 mL), and dried over anhydrous Na_2SO_4, and filtered. After concentration of the filtrate under reduced pressure, the resulting solids were purified by silica gel column chromatography (CH_2Cl_2 to 5/1 CH_2Cl_2/EtOAc, R_f = 0.23 in 10/1 CH_2Cl_2/EtOAc) and recrystallization from EtOH (5 mL) to afford 63.0 mg (0.0965 mmol, 54%) of **1** as cyan solids. Mp: 186.5–188.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (d, J = 12.0 Hz, 1H), 7.87–7.82 (m, 2H), 7.76 (d, J = 8.0 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.61 (s, 1H), 7.58–7.28 (m, 17H), 6.88–6.83 (m, 4H), 3.78 (s, 3H), 3.77 (s, 3H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 168.9 (d, J = 18.9 Hz, C), 159.1 (s, C), 159.0 (s, C), 158.5 (d, J = 9.9 Hz, C), 141.2 (s, C), 140.8 (s, C), 139.6 (d, J = 103.6 Hz, C), 136.3 (d, J = 11.6 Hz, C), 135.2 (d, J = 1.7 Hz, C), 133.9 (d, J = 20.5 Hz, C), 133.3 (s, C), 132.9 (d, J = 12.3 Hz, C), 132.8 (s, C), 132.5 (d, J = 3.3 Hz, CH), 131.4 (d, J = 9.9 Hz, CH), 131.1 (d, J = 11.6 Hz, CH), 130.6 (d, J = 105.3 Hz, C), 130.0 (s, CH), 129.9 (s, CH), 129.2 (s, CH), 129.1 (d, J = 13.2 Hz, CH), 129.1 (s, CH), 128.8 (s, CH), 128.5 (s, CH), 127.5 (s, CH), 127.4 (s, CH), 127.3 (s, CH), 126.9 (s, CH), 123.7 (s, CH), 123.1 (d, J = 8.2 Hz, CH), 122.5 (s, CH), 114.2 (s, CH), 114.1 (s, CH), 65.5 (d, J = 9.9 Hz, C), 55.4 (s, CH), 55.3 (s, CH). One of the doublet signals of a quaternary carbon paired with the signal at 135.16 ppm may be overlapped. ^{31}P{^{1}H} NMR (162 MHz, $CDCl_3$): δ 21.2. HRMS (APCI): *m/z* calcd. for $C_{45}H_{34}O_3P$: 653.2240 ($[M+H]^+$); found. 653.2211.





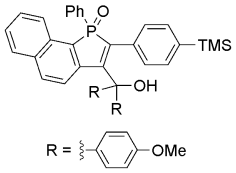
1-Bromo-2-(4-trimethylsilylphenylethynyl)naphthalene (6). The solution of 1-bromonaphthalen-2-yl triflate (99.44 g, 280 mmol),

4-trimethylsilylphenyl acetylene (48.80 g, 280 mmol) in Et_3N (500 mL) was degassed by N_2 bubbling for 20 min. After adding $Pd(PPh_3)_4$ (3.24 g, 2.80 mmol) and CuI (0.533 g, 2.80 mmol), the mixture was stirred at 60 °C for 20 h. After cooling, the inorganic salt was removed by filtration followed by washing with toluene (200 mL). After concentration of the filtrate under reduced pressure, the resulting solids were purified by silica gel column chromatography (hexane, R_f = 0.24) and recrystallization from MeOH (100 mL) to afford 30.50 g (80.4 mmol, 29%) of **6** as white powders. Mp: 95.0–95.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.33 (d, J = 8.8 Hz, 1H), 7.82 (d, J = 8.0 Hz, 1H), 7.77 (d, J = 8.4 Hz, 1H), 7.64–7.59 (m, 4H), 7.56–7.52 (m, 3H), 0.31 (s, 9H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 141.8 (C), 133.8 (C), 133.4 (CH), 132.4 (C), 130.9 (CH), 129.1 (CH),.128.3 (CH), 128.0 (CH), 127.9 (CH), 127.6 (CH), 127.3 (CH), 126.6 (C), 123.6 (C), 123.4 (C), 95.0 (C), 89.9 (C), −1.1 (CH). HRMS (APCI): *m/z* calcd. for $C_{21}H_{20}BrSi$: 379.0512 ([M+H]$^+$); found. 379.0513.

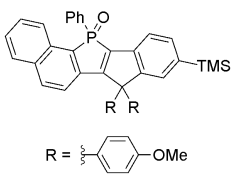


3-Bromo-1-phenyl-2-(4-trimethylsilylphenyl)naphtho[1,2-*b*]phosphole-*P*-oxide (7). To a solution of **6** (30.50 g, 80.4 mmol) in anhydrous THF (400 mL) was slowly added *t*BuLi in pentane (1.60 M, 100.5 mL, 160.8 mmol) dropwise at −78 °C. After stirring at −78 °C for 1 h, the suspension was warmed slowly to −40 °C in 2 h. After cooling the mixture to −78 °C, $PhP(NEt_2)Cl$ (15.5 mL, 80.5 mmol) was added in 0.5 h and the mixture was warmed slowly to 0 °C in 0.5 h. Cooled the mixture to −78 °C again, PBr_3 (7.64 mL, 80.4 mmol) was added in 0.5 h and the resulting mixture was warmed to room temperature. After distilling most of the pentane (about 100 mL), the mixture was refluxed at 80 °C for 48 h. After cooling, most of the volatiles were evaporated under reduced pressure. The residue was diluted by EtOAc (100 mL) followed by oxidizing with an aqueous solution of H_2O_2 (10 mL, 30%) at 0 °C for 1 h. After quenching the reaction with an aqueous solution of Na_2SO_3 (100 mL, 10%) at 0 °C, the mixture was extracted with EtOAc (300 mL) two times. The combined organic layer was washed with brine (100 mL), and then dried over anhydrous Na_2SO_4, and filtered. After concentration of the filtrate under reduced pressure, the resulting solids were purified by silica gel column chromatography (CH_2Cl_2 to 10/1 CH_2Cl_2/EtOAc, R_f = 0.65 in 10/1 CH_2Cl_2/EtOAc) and recrystallization from MeOH (100 mL) to afford 19.48 g (38.7 mmol, 48%) of **7** as yellow solids. Mp: 119.5–120.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 (d, J = 8.4 Hz, 1H), 8.06–8.04 (m, 1H), 7.91–7.87 (m, 2H), 7.82–7.71 (m, 4H), 7.54–7.43 (m, 5H), 7.36 (td, J = 7.6 Hz, J = 3.2 Hz, 2H), 0.26 (s, 9H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 141.8 (s, C), 140.8 (d, J = 21.4 Hz, C), 136.9 (d, J = 87.4 Hz, C), 134.3 (d, J = 1.6 Hz, CH), 134.1 (d, J = 8.2 Hz, C), 133.6 (s, CH), 132.6 (d, J = 3.3 Hz, CH), 132.4 (d, J = 8.3 Hz, C), 132.1 (d, J = 37.2 Hz, C), 131.1 (d, J = 9.0 Hz, C), 130.9 (d, J = 10.7 Hz, CH), 129.2 (d, J = 12.3 Hz, CH), 129.1 (d, J = 99.8 Hz, C), 128.92 (s, CH), 128.87 (s, CH), 127.7 (d, J = 5.0 Hz, CH), 127.4 (s, CH), 127.0 (d, J = 103.1

Hz, C), 125.8 (d, J = 5.0 Hz, CH), 121.8 (d, J = 11.6 Hz, CH), −1.1 (s, CH). $^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$): δ 35.6. HRMS (APCI): *m/z* calcd. for $C_{27}H_{25}BrOPSi$: 503.0590 ($[M+H]^+$); found. 503.0609.

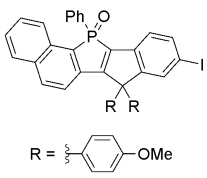


Compound 8. To a suspension of **7** (10.07 g, 20.0 mmol) in anhydrous toluene (30 mL) was added $HSiCl_3$ (8.07 mL, 80.0 mmol) in one portion. After stirring at 50 °C for 1 h, all volatiles were removed under reduced pressure. Then, toluene (20 mL) was added and the resulting suspension was filtered through a plug of Celite® under a N_2 atmosphere and rinsed with toluene (10 mL). After concentration of the filtrate under reduced pressure, the resulting white solids were suspended in anhydrous Et_2O (100 mL). To this suspension was added *t*BuLi in pentane (1.60 M, 25.0 mL, 40.0 mmol) over 30 min at –78 °C. After stirring at –78 °C for 2 h, 4,4′-dimethoxybenzophenone (4.85 g, 20.0 mmol) was added, and resulting mixture was warmed slowly to room temperature for overnight. Then, the reaction was quenched with a saturated aqueous solution of NH_4Cl (50 mL), followed by the oxidation with an aqueous solution of H_2O_2 (10 mL, 30%), and stirred for 0.5 h at room temperature. After quenching the reaction with a 10% Na_2SO_3 aqueous solution (100 mL) at 0 °C, the mixture was extracted with $CHCl_3$ (2000 mL). The organic layer was washed with brine (200 mL), and then dried over anhydrous Na_2SO_4, and filtered. After concentration of the filtrate under reduced pressure, the resulting crude product was purified by recrystallization from toluene (200 mL) two times to afford 8.50 g (12.7 mmol, 64%) of **8** as white solids. Mp: 269.0–270.0 °C (dec.). ^{1}H NMR (600 MHz, DMSO-d_6, 100 °C): δ 7.90–7.83 (m, 4H), 7.66–7.62 (m, 2H), 7.52 (t, J = 7.2 Hz, 1H), 7.48–7.43 (m, 4H), 7.24 (d, J = 8.4 Hz, 2H), 7.19 (d, J = 8.4 Hz, 2H), 6.97 (d, J = 7.8 Hz, 2H), 6.67–6.62 (m, 6H), 6.51 (s, 1H), 3.68 (s, 6H), 0.17 (s, 9H). Satisfying $^{13}C\{^{1}H\}$ NMR was not obtained duo to the poor solubility. $^{31}P\{^{1}H\}$ NMR (162 MHz, DMSO-d_6, 100 °C): δ 39.2. HRMS (APCI): *m/z* calcd. for $C_{42}H_{40}O_4PSi$: 667.2428 ($[M+H]^+$); found. 667.2395.



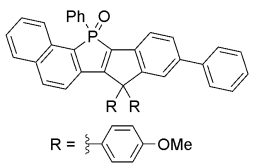
Compound 9. The mixture of compound **8** (8.180 g, 12.27 mmol) and $Sc(OTf)_3$ (6.039 g, 12.27 mmol) in 1,2-dichloroethane (300 mL) was stirred at room temperature for 48 h. Then, H_2O (200 mL) was added to quench the reaction. The organic phase was separated and the aqueous phase was extracted with $CHCl_3$ (500 mL). The combined organic layer was washed with H_2O (200 mL), and dried over anhydrous Na_2SO_4, and filtered. After concentration of the filtrate under reduced pressure, the resulting solids were purified by silica gel column chromatography (CH_2Cl_2 to 10/1 CH_2Cl_2/EtOAc, R_f = 0.31 in 10/1 CH_2Cl_2/EtOAc) and recrystallization from EtOH (20 mL) to afford 3.99 g (6.15 mmol, 50%) of **9** as yellow solids. Mp: 239.0–240.5 °C. ^{1}H NMR (400

MHz, $CDCl_3$): δ 8.12 (d, J = 7.6 Hz, 1H), 7.86–7.80 (m, 3H), 7.75 (d, J = 8.0 Hz, 1H), 7.53–7.38 (m, 11H), 7.24 (d, J = 8.8 Hz, 2H), 6.86 (d, J = 8.8 Hz, 2H), 6.81 (d, J = 8.8 Hz, 2H), 3.78 (s, 3H), 3.77 (s, 3H), 0.20 (s, 9H). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 168.1 (d, J = 22.2 Hz, C), 159.01 (s, C), 158.95 (s, C), 156.4 (d, J = 9.9 Hz, C), 139.8 (s, C), 138.8 (d, J = 103.9 Hz, C), 137.7 (d, J = 12.4 Hz, C), 133.4 (d, J = 8.3 Hz, C), 133.3 (d, J = 2.5 Hz, CH), 133.0 (s, C), 132.8 (s, CH), 132.7 (d, J = 106.4 Hz, C), 132.6 (d, J = 9.0 Hz, C), 132.5 (s, C), 130.9 (d, J = 11.6 Hz, CH), 130.1 (d, J = 102.3 Hz, C), 129.91 (s, CH), 129.85 (s, CH), 129.2 (d, J = 12.4 Hz, CH), 129.1 (s, CH), 128.8 (s, CH), 128.3 (s, CH), 126.8 (s, CH), 125.3 (d, J = 5.0 Hz, CH), 121.3 (s, CH), 114.1 (s, CH), 114.0 (s, CH), 65.2 (d, J = 11.5 Hz, C), 55.33 (s, CH), 55.31 (s, CH), −0.9 (s, CH). One of the doublet signals of a quaternary carbon paired with the signal at 138.07 ppm, one of the doublet signals of a CH carbon paired with the signal at 132.45 ppm, and one of the doublet signals of a CH carbon paired with the signal at 121.23 ppm may be overlapped. $^{31}P\{^1H\}$ NMR (162 MHz, $CDCl_3$): δ 23.7. HRMS (APCI): *m/z* calcd. for $C_{42}H_{38}O_3PSi$: 649.2322 ($[M+H]^+$); found. 649.2325.

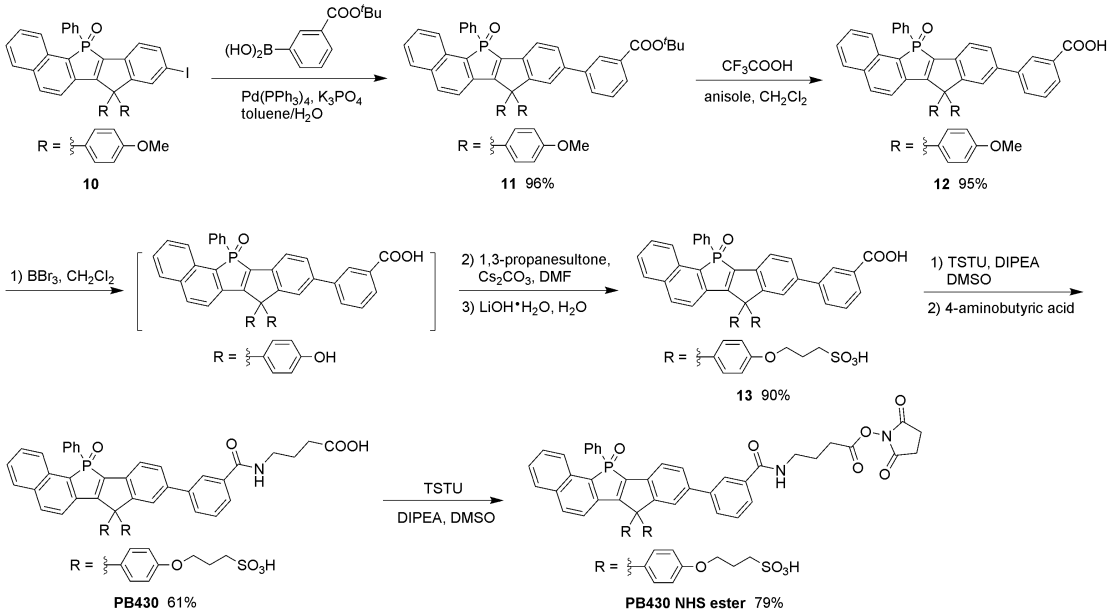


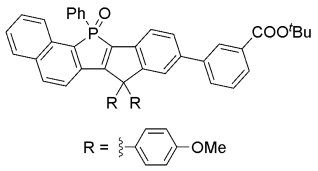
Compound 10. To a solution of **9** (3.945 g, 6.08 mmol) in anhydrous CH_2Cl_2 (50 mL) was added ICl in CH_2Cl_2 (1.00 M, 12.16 mL, 12.16 mmol) at 0 °C, and the resulting solution was stirred at room temperature for 2 h. After quenching the reaction with a 5% $Na_2S_2O_3$ aqueous solution (100 mL), the organic phase was separated and the aqueous phase was extracted with $CHCl_3$ (200 mL). The combined organic layer was washed with H_2O (50 mL), and dried over anhydrous Na_2SO_4, and filtered. After concentration of the filtrate under reduced pressure, the resulting solids were purified by silica gel column chromatography (CH_2Cl_2 to 10/1 CH_2Cl_2/EtOAc, R_f = 0.29 in 10/1 CH_2Cl_2/EtOAc) and recrystallization from EtOH (20 mL) to afford 3.782 g (5.38 mmol, 88%) of **10** as yellow solids. Mp: > 300 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 8.11 (d, J = 8.4 Hz, 1H), 7.83–7.78 (m, 3H), 7.74 (d, J = 8.0 Hz, 1H), 7.69 (s, 1H), 7.57 (dd, J = 8.0 Hz, J = 1.6 Hz, 1H), 7.52–7.34 (m, 8H), 7.25–7.20 (m, 3H), 6.86 (d, J = 8.8 Hz, 2H), 6.82 (d, J = 8.8 Hz, 2H), 3.78 (s, 3H), 3.77 (s, 3H). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 168.2 (d, J = 21.4 Hz, C), 159.2 (s, C), 159.2 (d, J = 9.1 Hz, C), 159.1 (s, C), 137.9 (d, J = 103.9 Hz, C), 137.6 (d, J = 18.2 Hz, C), 136.8 (s, CH), 136.6 (d, J = 11.5 Hz, C), 133.8 (s, CH), 133.5 (s, CH), 132.8 (d, J = 107.2 Hz, C), 132.6 (d, J = 2.5 Hz, CH), 132.5 (d, J = 9.9 Hz, C), 131.9 (s, C), 131.4 (s, C), 130.8 (d, J = 11.6 Hz, CH), 129.8 (s, CH), 129.7 (s, CH), 129.7 (d, J = 102.3 Hz, C), 129.3 (d, J = 12.4 Hz, CH), 128.8 (s, CH), 128.4 (s, CH), 127.0 (s, CH), 125.2 (d, J = 5.8 Hz, CH), 123.4 (s, CH), 121.2 (d, J = 10.7 Hz, CH), 114.4 (s, CH), 114.2 (s, CH), 92.8 (s, C), 65.3 (d, J = 10.7 Hz, C), 55.4 (s, CH), 55.3 (s, CH). One of the doublet signals of a quaternary carbon paired with the signal at 133.57 ppm may be

overlapped. $^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$): δ 23.4. HRMS (APCI): *m/z* calcd. for $C_{39}H_{29}IO_3P$: 703.0894 ($[M+H]^+$); found. 703.0890.



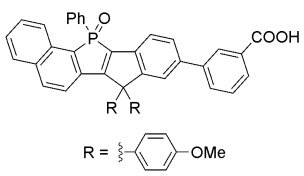
Compound 2. The degassed solvents of toluene (2 mL) and H_2O (0.5 mL) were added to the solids of compound **10** (50.0 mg, 0.0712 mmol), phenylboronic acid (17.4 mg, 0.143 mmol), $Pd(PPh_3)_4$ (10.0 mg, 0.0087 mmol), and K_3PO_4 (90.4 mg, 0.426 mmol), and the resulting mixture was stirred at 80 °C for 4 h. After cooling, the organic phase was separated and the aqueous phase was extracted with EtOAc (20 mL). The combined organic layer was washed with H_2O (5 mL), brine (5 mL), and then dried over anhydrous Na_2SO_4, and filtered. After concentration of the filtrate under reduced pressure, the resulting solids were purified by silica gel column chromatography (1/1 to 1/2 hexane/EtOAc, R_f = 0.35 in 2/3 hexane/EtOAc) to afford 42 mg (0.064 mmol, 90%) of **2** as yellow solids. Mp: 181.0–182.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (d, *J* = 8.0 Hz, 1H), 7.91–7.83 (m, 3H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.63 (s, 1H), 7.59 (d, *J* = 8.0 Hz, 1H), 7.53–7.28 (m, 16H), 6.88 (d, *J* = 8.8 Hz, 2H), 6.85 (d, *J* = 9.2 Hz, 2H), 3.78 (s, 3H), 3.77 (s, 3H). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$): δ 168.1 (d, *J* = 22.2 Hz, C), 159.1 (s, C), 159.0 (s, C), 158.1 (d, *J* = 9.9 Hz, C), 141.1 (s, C), 140.5 (s, C), 138.5 (d, *J* = 104.7 Hz, C), 138.1 (d, *J* = 18.2 Hz, C), 136.3 (d, *J* = 12.4 Hz, C), 133.4 (d, *J* = 9.1 Hz, C), 133.4 (s, CH), 132.8 (s, C), 132.7 (d, *J* = 107.2 Hz, C), 132.6 (d, *J* = 9.1 Hz, C), 132.5 (d, *J* = 2.4 Hz, CH), 132.1 (s, C), 130.9 (d, *J* = 10.8 Hz, CH), 130.1 (d, *J* = 101.5 Hz, C), 129.91 (s, CH), 129.86 (s, CH), 129.2 (d, *J* = 12.4 Hz, CH), 128.79 (s, CH), 128.75 (d, *J* = 1.6 Hz, CH), 128.3 (s, CH), 127.4 (s, CH), 127.2 (s, CH), 126.8 (s, CH), 125.3 (d, *J* = 5.0 Hz, CH), 123.6 (s, CH), 122.2 (s, CH), 121.2 (d, *J* = 10.7 Hz, CH), 114.3 (s, CH), 114.1 (s, CH), 65.4 (d, *J* = 11.5 Hz, C), 55.33 (s, CH), 55.31 (s, CH). One singlet signal of a CH carbon may be overlapped. $^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$): δ 23.7. HRMS (APCI): *m/z* calcd. for $C_{45}H_{34}O_3P$: 653.2240 ($[M+H]^+$); found. 653.2248.



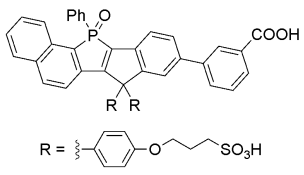


Compound 11. The degassed solvents of toluene (24 mL) and H_2O (6 mL) were added to the solids of compound **10** (1.405 g, 2.00 mmol), 3-(*tert*-butoxycarbonyl)phenylboronic acid (0.888 g, 4.00 mmol), $Pd(PPh_3)_4$ (0.116 g, 0.100 mmol), and K_3PO_4 (2.547 g, 12.0 mmol), and the resulting mixture was stirred at 80 °C for 12 h. After cooling, the organic phase was separated and the aqueous phase was extracted with EtOAc (50 mL). The combined organic layer was washed with H_2O (20 mL), brine (20 mL), and then dried over anhydrous Na_2SO_4, and filtered. After concentration of the filtrate under reduced pressure, the resulting solids were purified by silica gel column chromatography (2/1 to 1/1 hexane/EtOAc, R_f = 0.29 in 1/1 hexane/EtOAc) to afford 1.444 g (1.92 mmol, 96%) of **11** as yellow solids. Mp: 177.0–179.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16–8.13 (m, 2H), 7.94–7.83 (m, 4H), 7.76 (d, J = 8.4 Hz, 1H), 7.66–7.64 (m, 2H), 7.60 (d, J = 7.6 Hz, 1H), 7.54–7.38 (m, 10H), 7.31 (d, J = 8.8 Hz, 2H), 6.87 (d, J = 8.8 Hz, 2H), 6.84 (d, J = 8.4 Hz, 2H), 3.77 (s, 3H), 3.76 (s, 3H), 1.61 (s, 9H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$): δ 168.4 (d, J = 21.4 Hz, C), 165.7 (s, C), 159.1 (s, C), 159.0 (s, C), 158.3 (d, J = 9.1 Hz, C), 141.2 (s, C), 139.5 (s, C), 138.3 (d, J = 103.9 Hz, C), 138.0 (d, J = 18.2 Hz, C), 136.7 (d, J = 11.6 Hz, C), 133.4 (d, J = 9.0 Hz, C), 133.4 (d, J = 1.7 Hz, CH), 132.6 (s, C), 132.6 (d, J = 3.3 Hz, CH), 132.5 (s, C), 132.2 (s, C), 131.2 (s, CH), 130.8 (d, J = 10.7 Hz, CH), 129.9 (d, J = 101.4 Hz, C), 129.9 (s, CH), 129.8 (s, CH), 129.2 (d, J = 12.4 Hz, CH), 128.8 (s, CH), 128.7 (s, CH), 128.4 (s, CH), 128.3 (s, CH), 128.2 (s, CH), 126.9 (s, CH), 125.3 (d, J = 5.0 Hz, CH), 123.5 (s, CH), 122.3 (s, CH), 121.2 (d, J = 10.7 Hz, CH), 114.3 (s, CH), 114.1 (s, CH), 81.2 (s, C), 65.4 (d, J = 10.7 Hz, C), 55.32 (s, CH), 55.30 (s, CH), 28.2 (s, CH). One of the doublet signals of a quaternary carbon paired with the signal at 133.20 ppm, one of the doublet signals of a quaternary carbon paired with the signal at 132.61 ppm, and one singlet signal of a CH carbon may be

overlapped. $^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$): δ 23.7. HRMS (APCI): *m/z* calcd. for $C_{50}H_{42}O_5P$: 753.2764 ($[M+H]^+$); found. 753.2749.

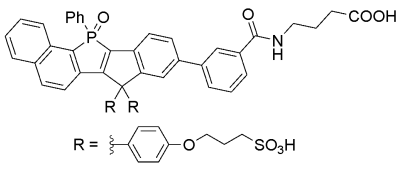


Compound 12. To a solution of **11** (1.400 g, 1.86 mmol) in CH_2Cl_2 (20 mL) was added anisole (2 mL) and TFA (5 mL) sequentially, and the resulting mixture was stirred at room temperature for 4 h. After evaporating all volatiles under reduced pressure, the resulting solids were purified by silica gel column chromatography ($CHCl_3$ to 5/1 $CHCl_3$/EtOAc, R_f = 0.04 in 5/1 $CHCl_3$/EtOAc) and recrystallization from EtOAc (10 mL) to afford 1.230 g (1.77 mmol, 95%) of **12** as yellow solids. Mp: 231.0–233.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (s, 1H), 8.16 (d, *J* = 8.0 Hz, 1H), 8.05 (d, *J* = 7.6 Hz, 1H), 7.94–7.84 (m, 3H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.68–7.63 (m, 3H), 7.54–7.38 (m, 10H), 7.31 (d, *J* = 9.2 Hz, 2H), 6.87–6.83 (m, 4H), 3.77 (s, 3H), 3.74 (s, 3H). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$): δ 170.2 (s, C), 168.8 (d, *J* = 21.5 Hz, C), 159.2 (s, C), 159.1 (s, C), 158.3 (d, *J* = 9.9 Hz, C), 141.4 (s, C), 139.4 (s, C), 138.2 (d, *J* = 18.9 Hz, C), 137.9 (d, *J* = 104.8 Hz, C), 136.7 (d, *J* = 11.5 Hz, C), 133.6 (s, CH), 133.5 (d, *J* = 9.1 Hz, C), 132.8 (d, *J* = 1.6 Hz, CH), 132.6 (d, *J* = 9.1 Hz, C), 132.6 (s, C), 132.3 (d, *J* = 107.3 Hz, C), 132.07 (s, C), 132.04 (s, CH), 131.0 (d, *J* = 10.7 Hz, CH), 130.7 (s, C), 129.91 (s, CH), 129.87 (s, CH), 129.4 (d, *J* = 12.3 Hz, CH), 129.0 (s, CH), 128.9 (s, CH), 128.8 (s, CH), 128.7 (s, CH), 128.5 (s, CH), 127.02 (s, CH), 126.98 (s, CH), 125.3 (d, *J* = 5.8 Hz, CH), 123.5 (s, CH), 122.4 (s, CH), 121.3 (d, *J* = 10.7 Hz, CH), 114.4 (s, CH), 114.2 (s, CH), 65.5 (d, *J* = 10.7 Hz, C), 55.36 (s, CH), 55.34 (s, CH). One doublet signals of a quaternary carbon may be overlapped. $^{31}P\{^{1}H\}$ NMR (162 MHz, $CDCl_3$): δ 25.0. HRMS (APCI): *m/z* calcd. for $C_{46}H_{34}O_5P$: 697.2138 ($[M+H]^+$); found. 697.2140.

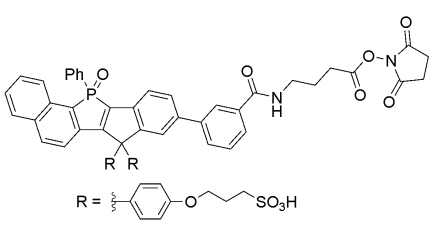


Compound 13. To a solution of **12** (200 mg, 0.287 mmol) in anhydrous CH_2Cl_2 (10 mL) was added BBr_3 (0.55 mL, 5.7 mmol) dropwise at –78 °C, and resulting mixture was warmed slowly to room temperature for overnight. After quenching the reaction with H_2O (5 mL) at 0 °C, the mixture was extracted with EtOAc (200 mL). The organic layer was washed with brine (30 mL) three times, and then dried over anhydrous Na_2SO_4, and filtered. After concentration of the filtrate under reduced pressure, the resulting solids were dissolved in dry DMF (5 mL). Then, Cs_2CO_3 (2.22 g, 6.81 mmol) and 1,3-propanesultone (0.500 mL, 5.69 mmol) were added to the solution, and the resulting mixture was stirred at room temperature for 2 h. After removing all volatiles of the mixture under reduced pressure, the resulting solids were dissolved in H_2O (10 mL). Then, $LiOH{\bullet}H_2O$ (1.00 g, 23.8 mmol) was added, and the resulting solution was stirred at room temperature for 2 h. After acidizing by concentrate HCl solution, the mixture was purified by reverse phase HPLC (H_2O to 1/1 H_2O/CH_3CN, + 0.1% TFA) to afford

235 mg (0.257 mmol, 90%) of **13** as orange solids. Mp: > 300 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.11–8.06 (m, 2H), 7.98–7.77 (m, 7H), 7.65–7.28 (m, 12H), 7.15 (d, J = 51.2 Hz, 1H), 6.97–6.95 (m, 4H), 4.05 (br, 4H), 2.74 (br, 4H), 2.05 (br, 4H). ^{13}C{^{1}H} NMR (100 MHz, DMSO-d_6): δ 168.4 (d, J = 20.7 Hz, C), 167.4 (s, C), 158.6 (d, J = 9.1 Hz, C), 158.2 (s, C), 140.3 (s, C), 138.9 (s, C), 137.7 (d, J = 103.1 Hz, C), 137.5 (d, J = 18.2 Hz, C), 136.3 (d, J = 11.6 Hz, C), 134.1 (s, CH), 133.2 (s, CH), 131.9 (s, C), 131.8 (s, C), 131.7 (s, C), 131.5 (s, CH), 130.4 (d, J = 10.8 Hz, CH), 129.9 (d, J = 12.4 Hz, CH), 129.7 (d, J = 100.6 Hz, C), 129.70 (s, CH), 129.66 (s, CH), 129.56 (s, CH), 129.4 (s, CH), 128.9 (s, CH), 128.6 (s, CH), 127.6 (s, CH), 127.3 (s, CH), 127.1 (s, CH), 124.4 (s, CH), 123.3 (s, CH), 121.9 (s, CH), 121.3 (d, J = 11.6 Hz, CH), 115.1 (s, CH), 115.0 (s, CH), 66.6 (s, CH), 65.2 (d, J = 9.9 Hz, C), 48.2 (s, CH), 25.2 (s, CH). One singlet signal of a quaternary carbon, one of the doublet signals of a quaternary carbon paired with the signal at 133.23 ppm, one of the doublet signals of a quaternary carbon paired with the signal at 132.90 ppm, one of the doublet signals of a quaternary carbon paired with the signal at 131.73 ppm, and three singlet signals of alkyl carbons may be overlapped. ^{31}P{^{1}H} NMR (162 MHz, DMSO-d_6): δ 21.8. HRMS (APCI): *m/z* calcd. for $C_{50}H_{41}NaO_{11}PS_2$: 935.1720 ([M+Na]$^+$); found. 935.1737.



PB430. To a mixture of **13** (14.0 mg, 0.0153 mmol) and DIPEA (0.10 mL, 0.61 mmol) in anhydrous DMSO (1 mL) was added TSTU (9.2 mg, 0.031 mmol). After stirring at room temperature for 1 h, 4-aminobutyric acid (10.3 mg, 0.100 mmol) was added to the mixture. After stirring at room temperature for 12 h, the mixture was purified by reverse phase HPLC (6/4 H_2O/CH_3CN, + 0.1% TFA) to afford 9.3 mg (0.0093 mmol, 61%) of PB430 as yellow solids. Mp: 197.0–199.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.60 (t, J = 6.0 Hz, 1H), 8.08 (d, J = 8.4 Hz, 1H), 7.97–7.95 (m, 3H), 7.84 (s, 1H), 7.79–7.48 (m, 12H), 7.42 (dd, J = 8.4 Hz, 2.8Hz, 1H), 7.37 (d, J = 8.8 Hz, 2H), 7.25 (d, J = 8.8 Hz, 2H), 6.95–6.90 (m, 4H), 4.03–3.98 (m, 4H), 3.31–3.26 (m, 2H), 2.60–2.54 (m, 4H), 2.29 (t, J = 7.2 Hz, 2H), 2.00–1.94 (m, 4H), 1.79–1.74 (m, 2H). Satisfying ^{13}C{^{1}H} NMR was not obtained duo to the small amount of product and the splitting of carbon signal. ^{31}P{^{1}H} NMR (162 MHz, DMSO-d_6): δ 21.5. HRMS (APCI): *m/z* calcd. for $C_{54}H_{49}NO_{12}PS_2$: 998.2428 ([M+H]$^+$); found. 998.2427.



PB430 NHS ester. To a mixture of PB430 (5.0 mg, 5.0 μmol) and DIPEA (17 μL, 100 μmol) in anhydrous DMSO (0.5 mL) was added TSTU (4.5 mg, 15 μmol). After stirring at room temperature for 1 h, a solution of TFA (17 μL, 220 μmol) in H_2O (2 mL) was added to

quench the reaction, and the mixture was purified by reverse phase HPLC (55/45 H_2O/CH_3CN, + 0.1% TFA) to afford 4.3 mg (3.9 μmol, 79%) of PB430 NHS ester as yellow solids. Mp: 195.5–197.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.65 (s, 1H), 8.08 (d, J = 8.8 Hz, 1H), 7.98–7.95 (m, 3H), 7.84–7.35 (m, 16H), 7.25 (d, J = 8.0 Hz, 2H), 6.94–6.89 (m, 4H), 4.03–3.98 (m, 4H), 3.36–3.31 (m, 2H), 2.82 (s, 4H), 2.78 (t, J = 7.6 Hz, 2H), 1.98–1.87 (m, 6H). One set of alkyl multiple signals was overlapped in DMSO residual peak. Satisfying $^{13}C\{^1H\}$ NMR was not obtained duo to the small amount of product and the splitting of carbon signal. $^{31}P\{^1H\}$ NMR (162 MHz, DMSO-d_6): δ 25.0. HRMS (APCI): *m/z* calcd. for $C_{58}H_{52}N_2O_{14}PS_2$: 1095.2592 ($[M+H]^+$); found. 1095.2600.

Determination of the degree of labeling (DOL) value

Firstly, the correction factor CF_{280} of PB430 was calculated according to the following equation:

$$CF_{280} = \frac{\varepsilon_{280}}{\varepsilon_{\max}} = \frac{19900\ \mathrm{M^{-1}\ cm^{-1}}}{10400\ \mathrm{M^{-1}\ cm^{-1}}} = 1.91$$

where ε_{280} and $\varepsilon_{\max}$ represent the absorption coefficients of dye at 280 nm and at the absorption maximum (426 nm), respectively.

Next, the degree of labeling (DOL, dye-to-protein ratio) value of PB430-antibody conjugate was calculated according to the following equation:

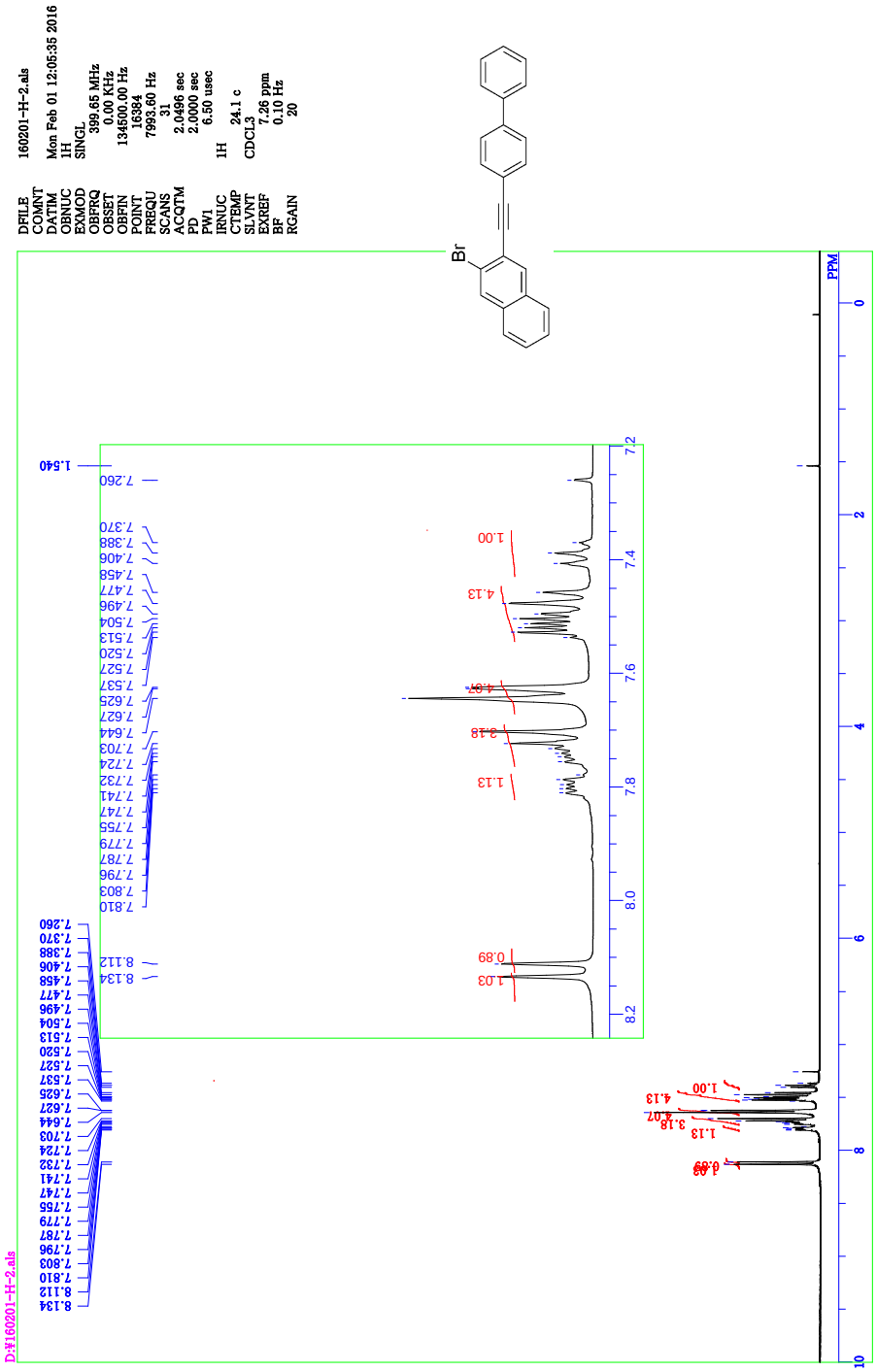
$$\mathrm{DOL} = \frac{A_{\max}/\varepsilon_{\max}}{A_{\mathrm{protein}}/\varepsilon_{\mathrm{protein}}} = \frac{A_{\max}/\varepsilon_{\max}}{(A_{280} - A_{\max} \times CF_{280})/\varepsilon_{\mathrm{protein}}}$$
$$= \frac{0.0362/(10400\ \mathrm{M^{-1}\ cm^{-1}})}{(0.333 - 0.0362 \times 1.91)/(210000\ \mathrm{M^{-1}\ cm^{-1}})} = 2.8$$

where $A_{\max}$ and A_{280} respectively represent the absorbance of dye-antibody conjugate at the absorption maximum of dye (426 nm) and at 280 nm, the difference between A_{280} and $A_{\max} \times CF_{280}$ means the absorbance of antibody itself (A_{protein}) at 280 nm, $\varepsilon_{\mathrm{protein}}$ represents the absorption coefficient of antibody itself at 280 nm.

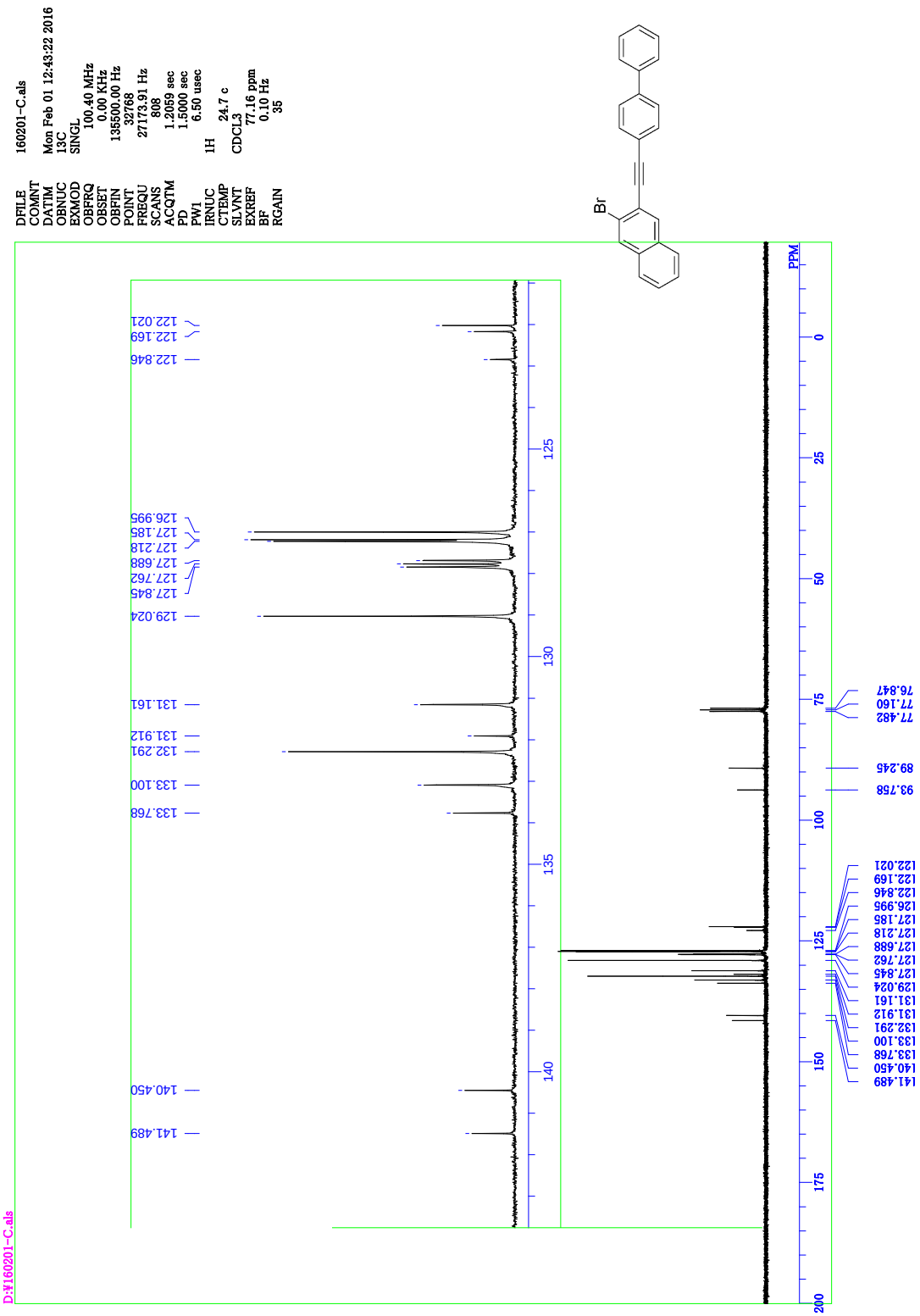
The conjugations between PB430 NHS ester and antibody were conducted three times under the same condition. All the DOL values were comparable.

Entry	$\boldsymbol{A_{280}}$	$\boldsymbol{A_{max}}$	**DOL**
1	0.333	0.0362	2.77
2	0.803	0.0793	2.46
3	0.815	0.0827	2.54

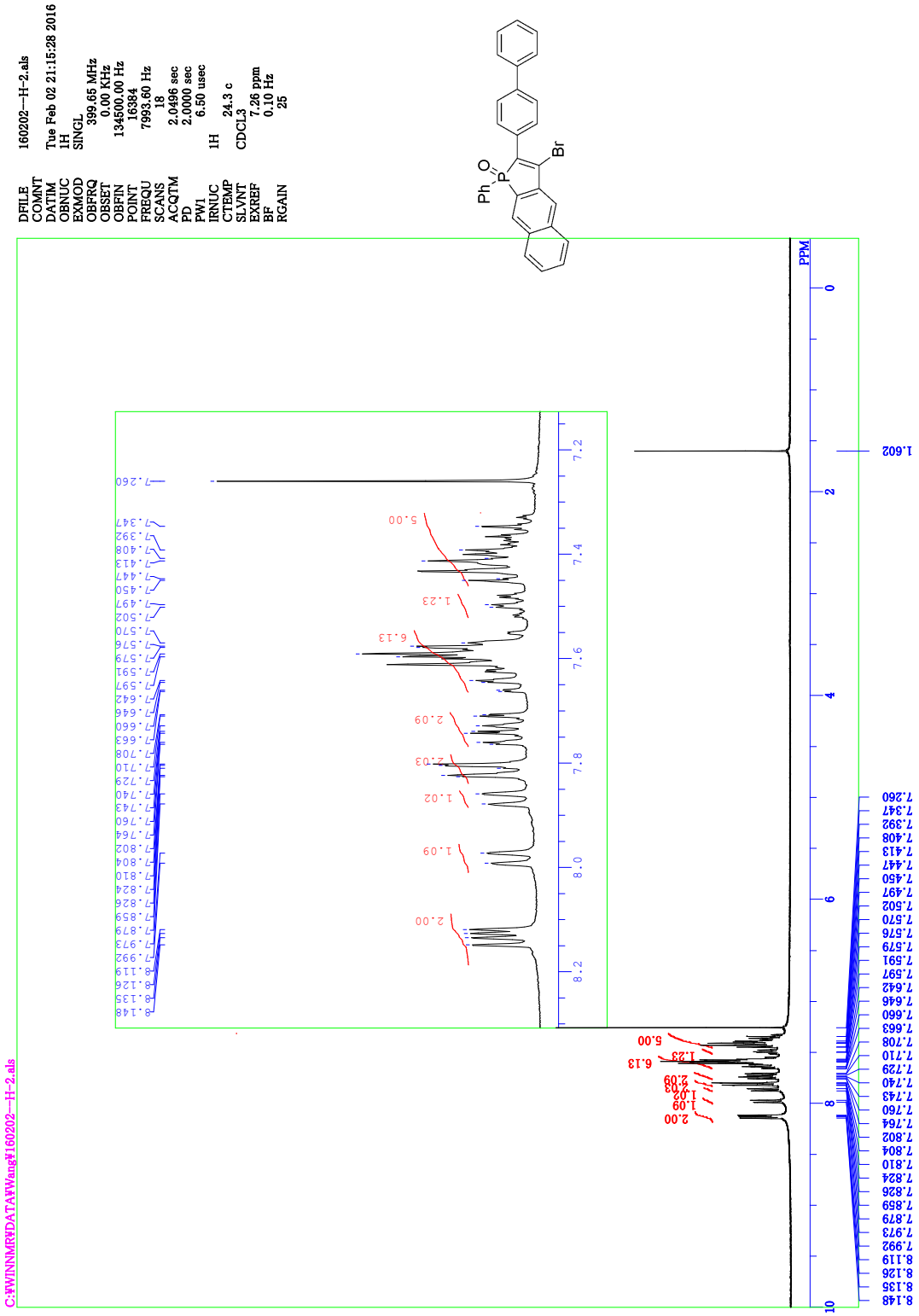
NMR data for newly synthesized compounds



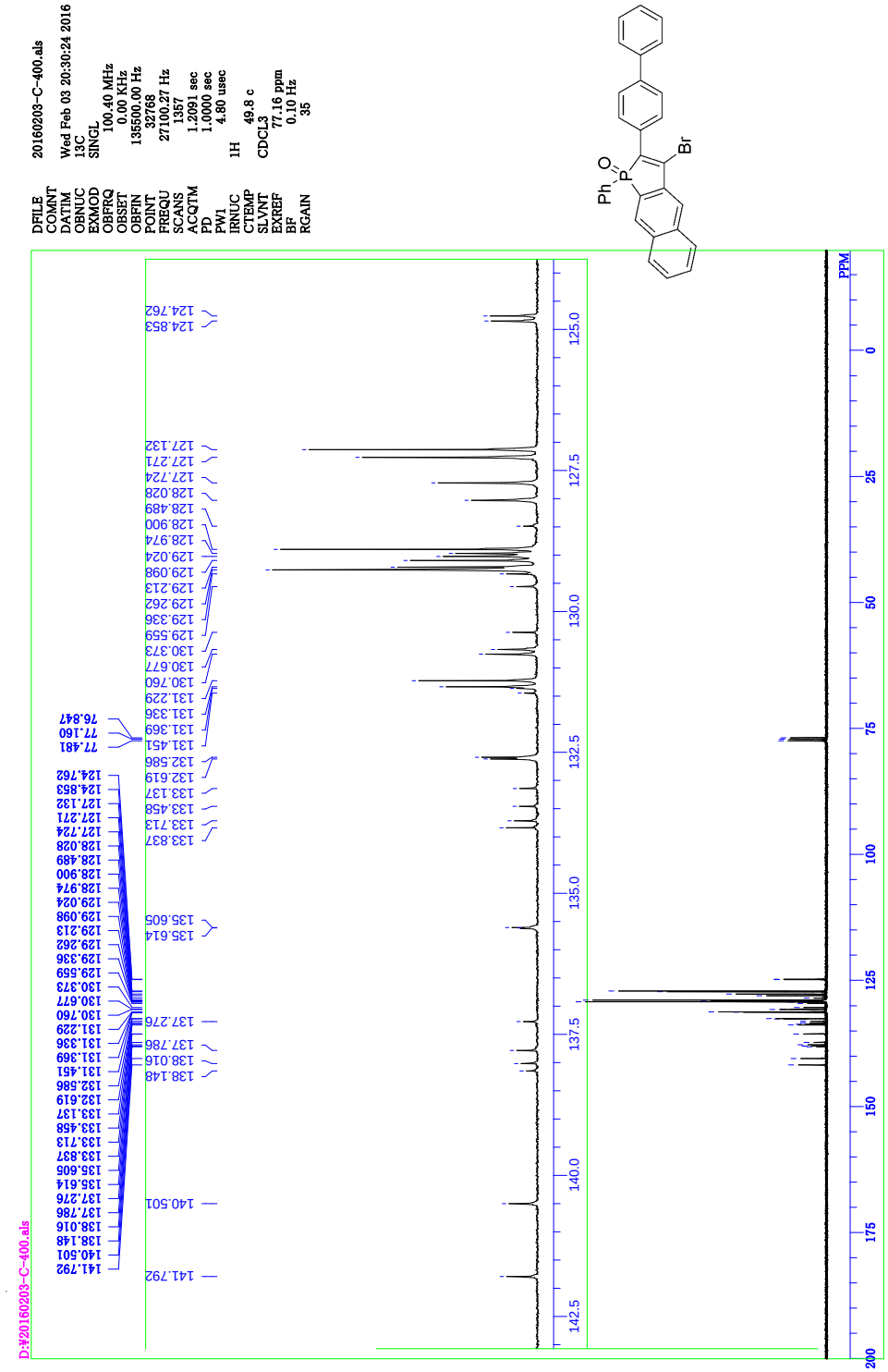
NMR-1. ^{1}H NMR spectrum of **3** (400 MHz, $CDCl_3$).



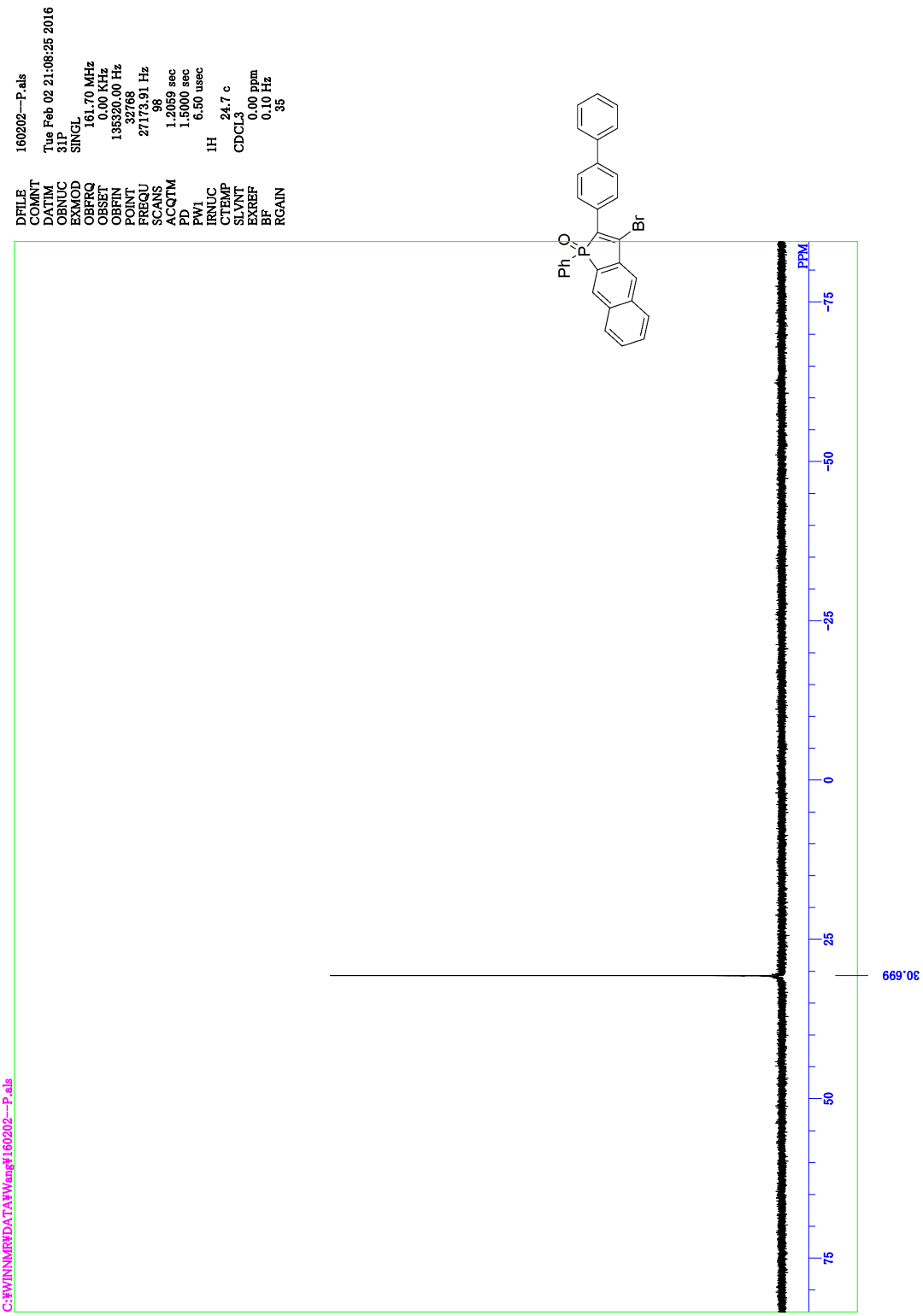
NMR-2. $^{13}C\{^{1}H\}$ NMR spectrum of **3** (100 MHz, $CDCl_3$).



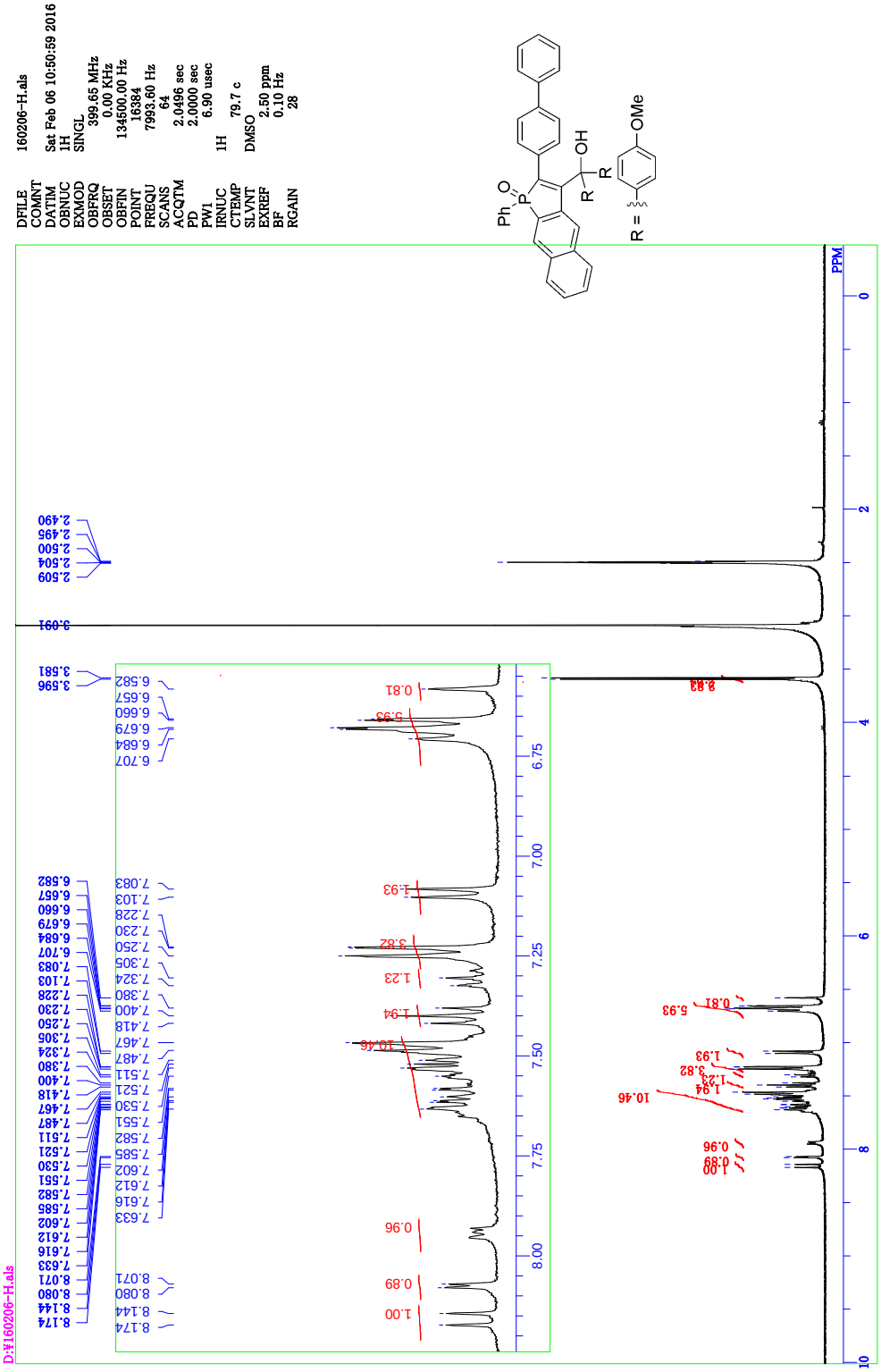
NMR-3. ^{1}H NMR spectrum of **4** (400 MHz, $CDCl_3$).



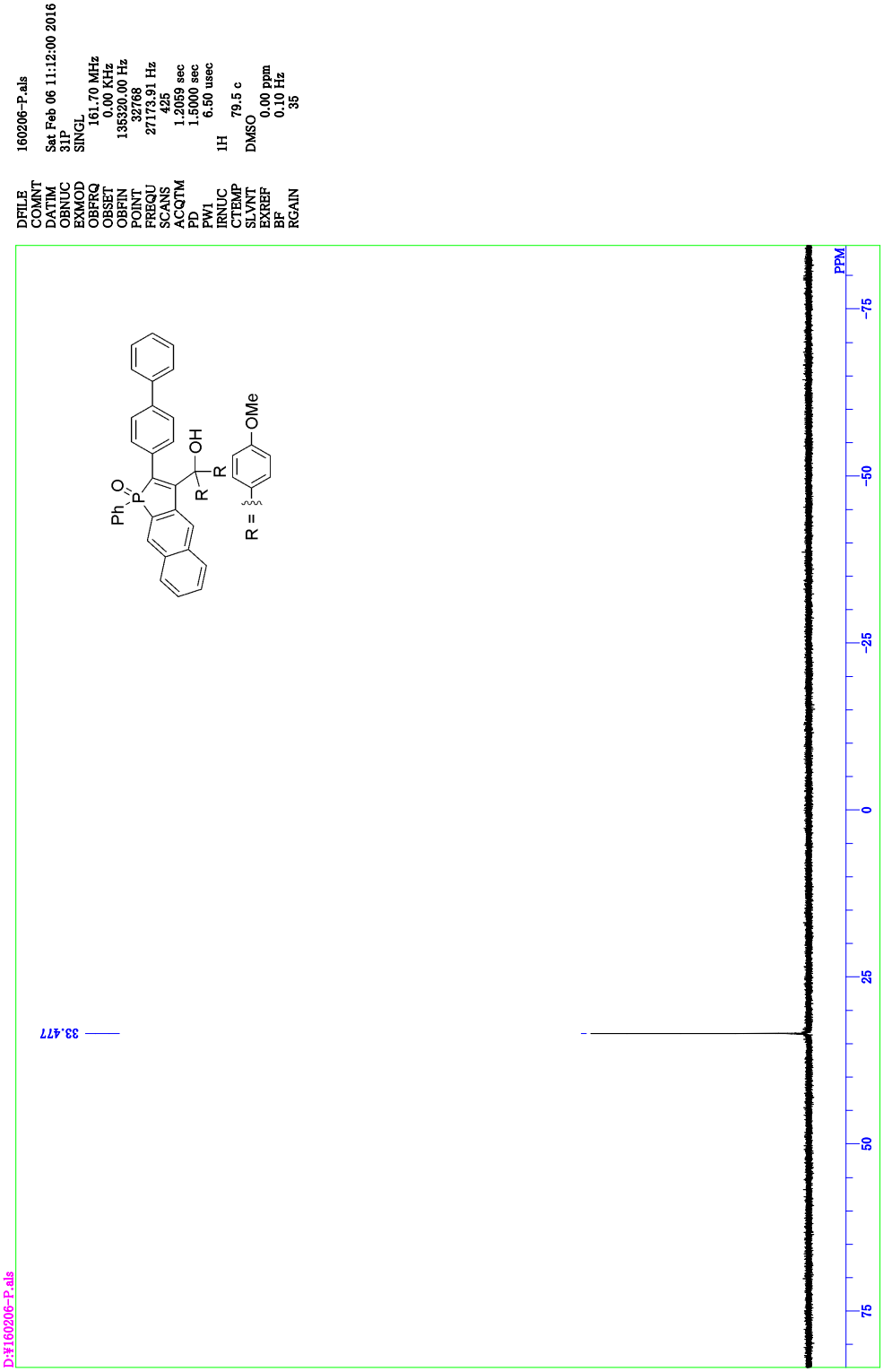
NMR-4. $^{13}C\{^{1}H\}$ NMR spectrum of **4** (100 MHz, $CDCl_3$, 50 °C).



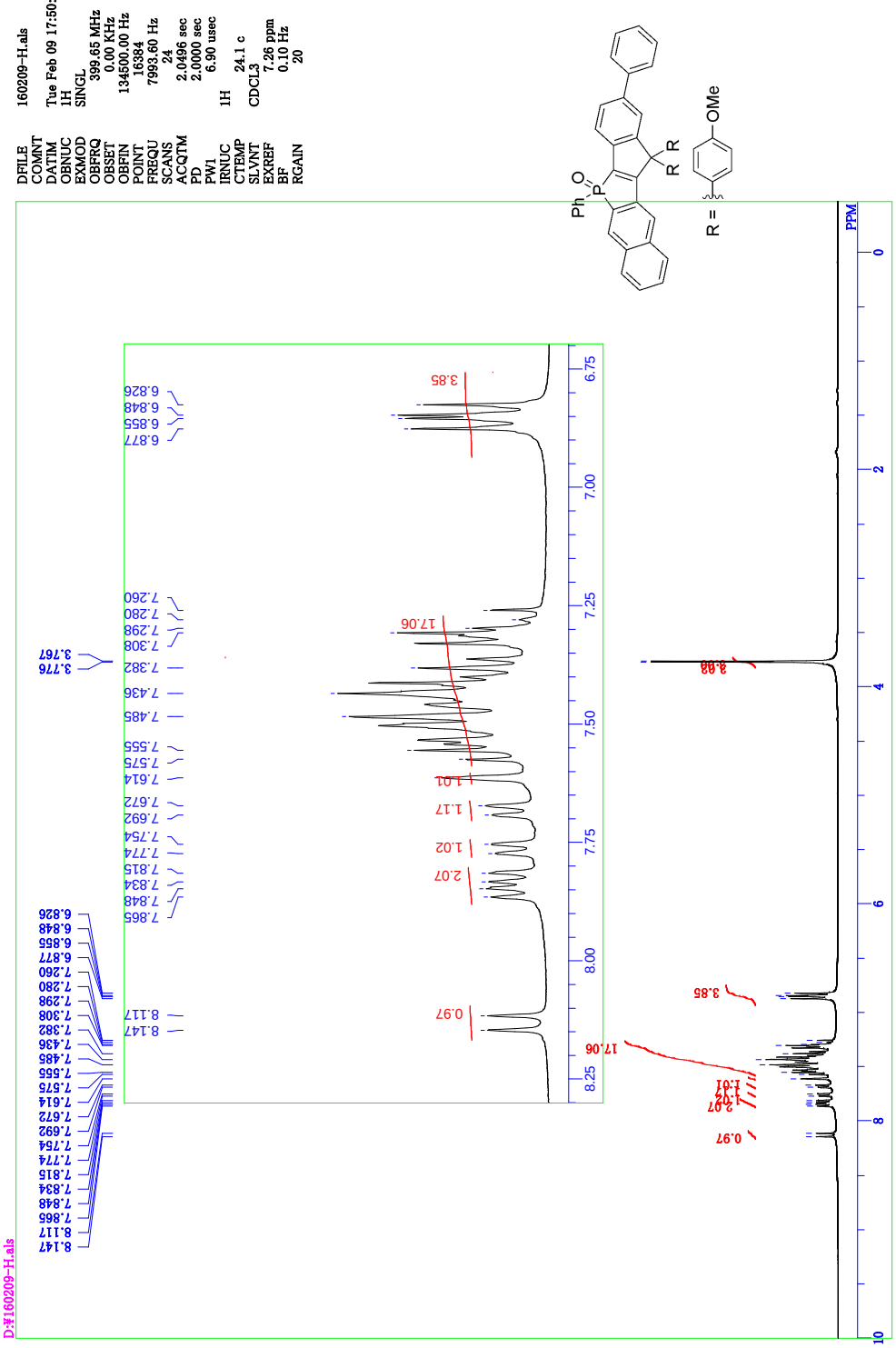
NMR-5. $^{31}P\{^{1}H\}$ NMR spectrum of **4** (162 MHz, $CDCl_3$).



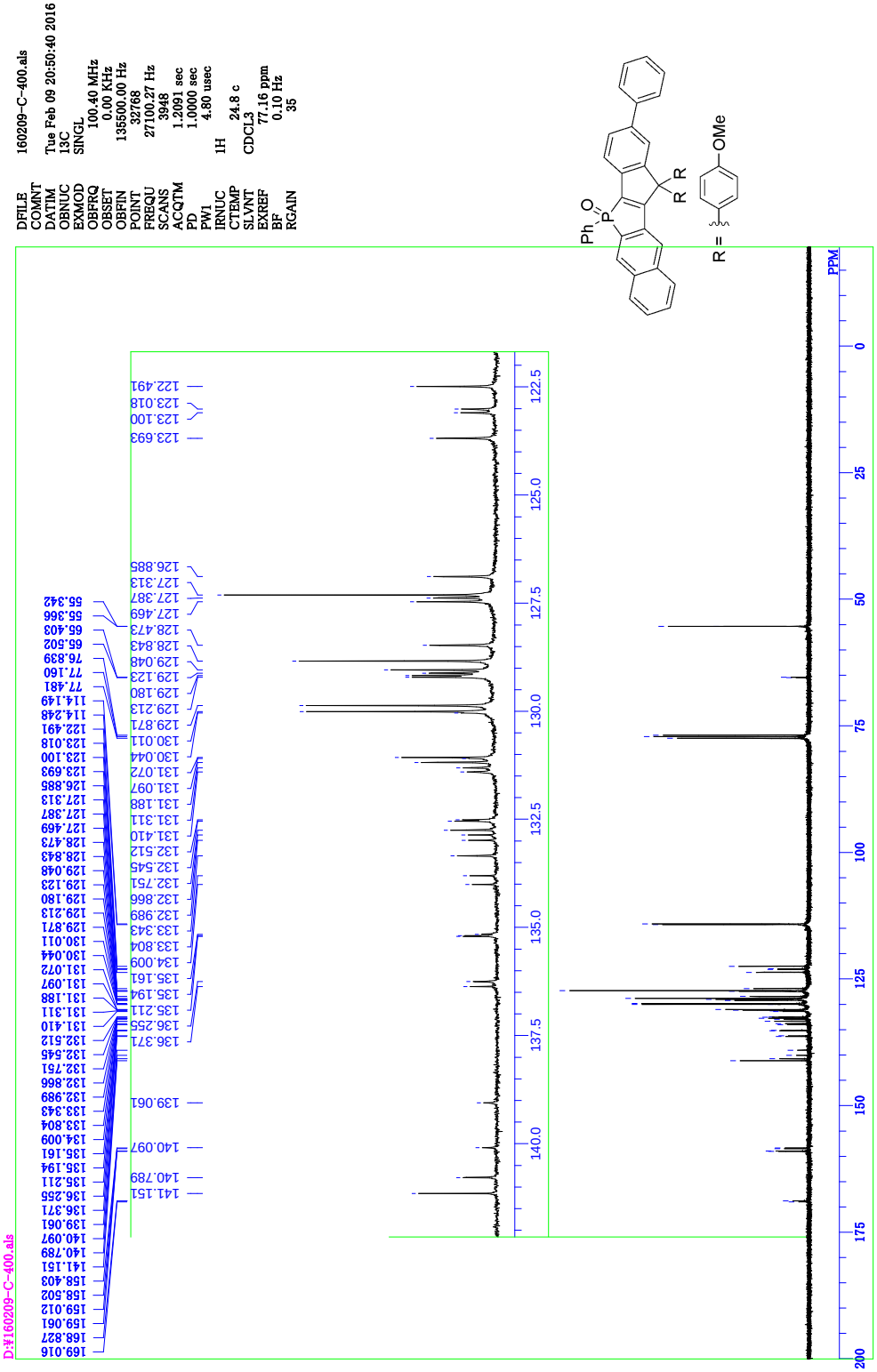
NMR-6. ^{1}H NMR spectrum of **5** (400 MHz, DMSO-d_6, 80 °C).



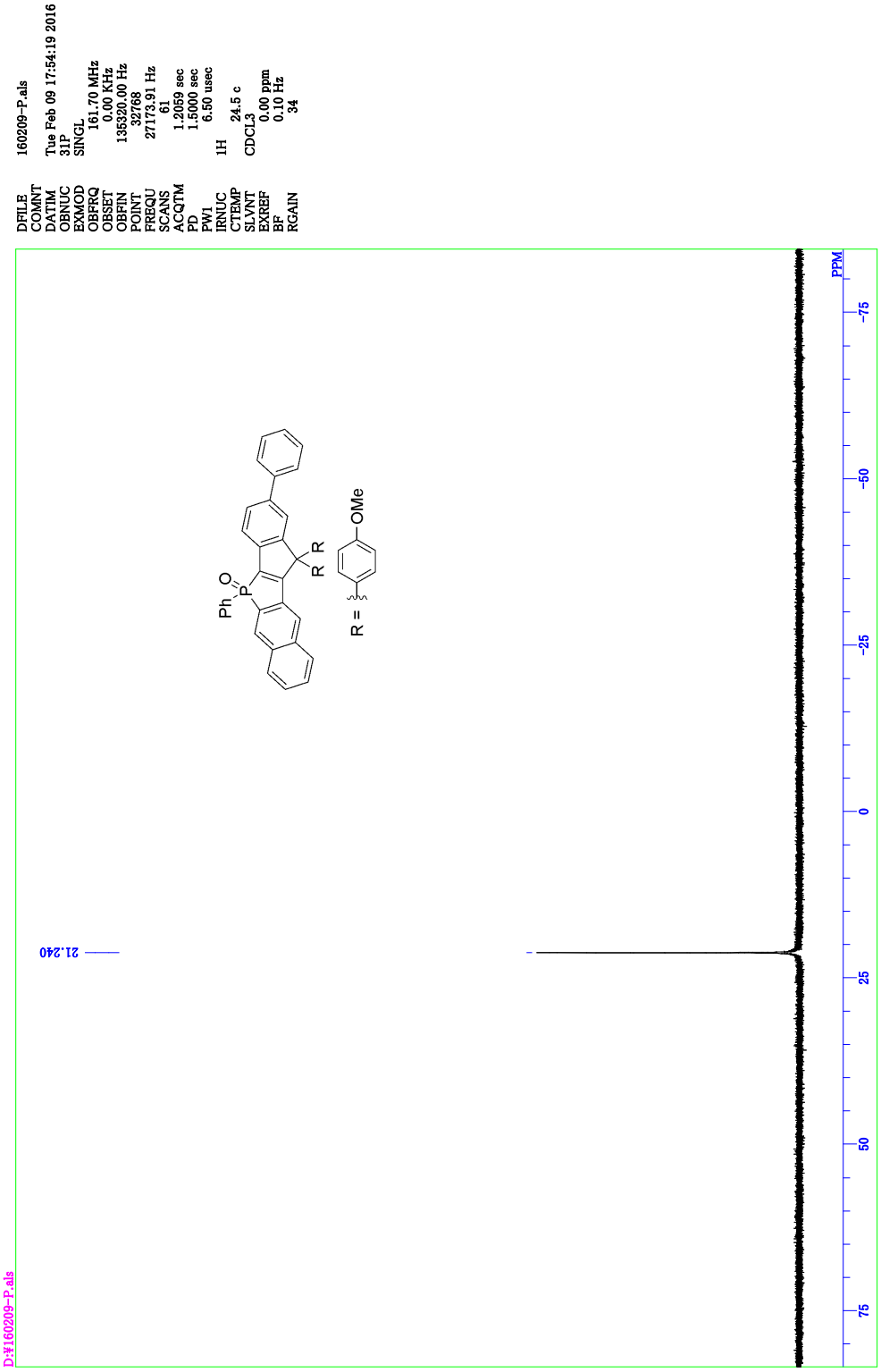
NMR-7. $^{31}P\{^{1}H\}$ NMR spectrum of **5** (162 MHz, DMSO-d_6, 80 °C).



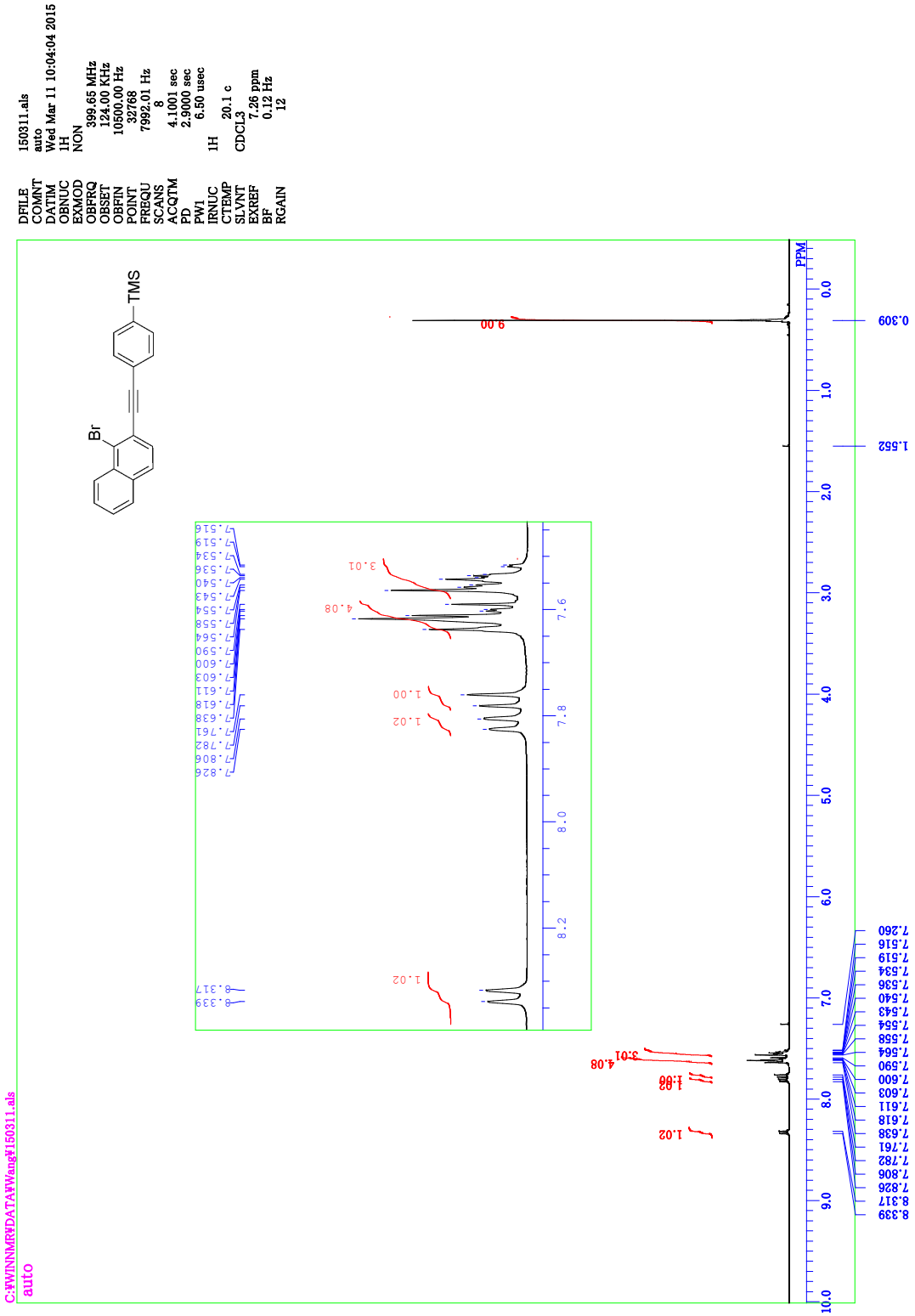
NMR-8. ^{1}H NMR spectrum of **1** (400 MHz, $CDCl_3$).



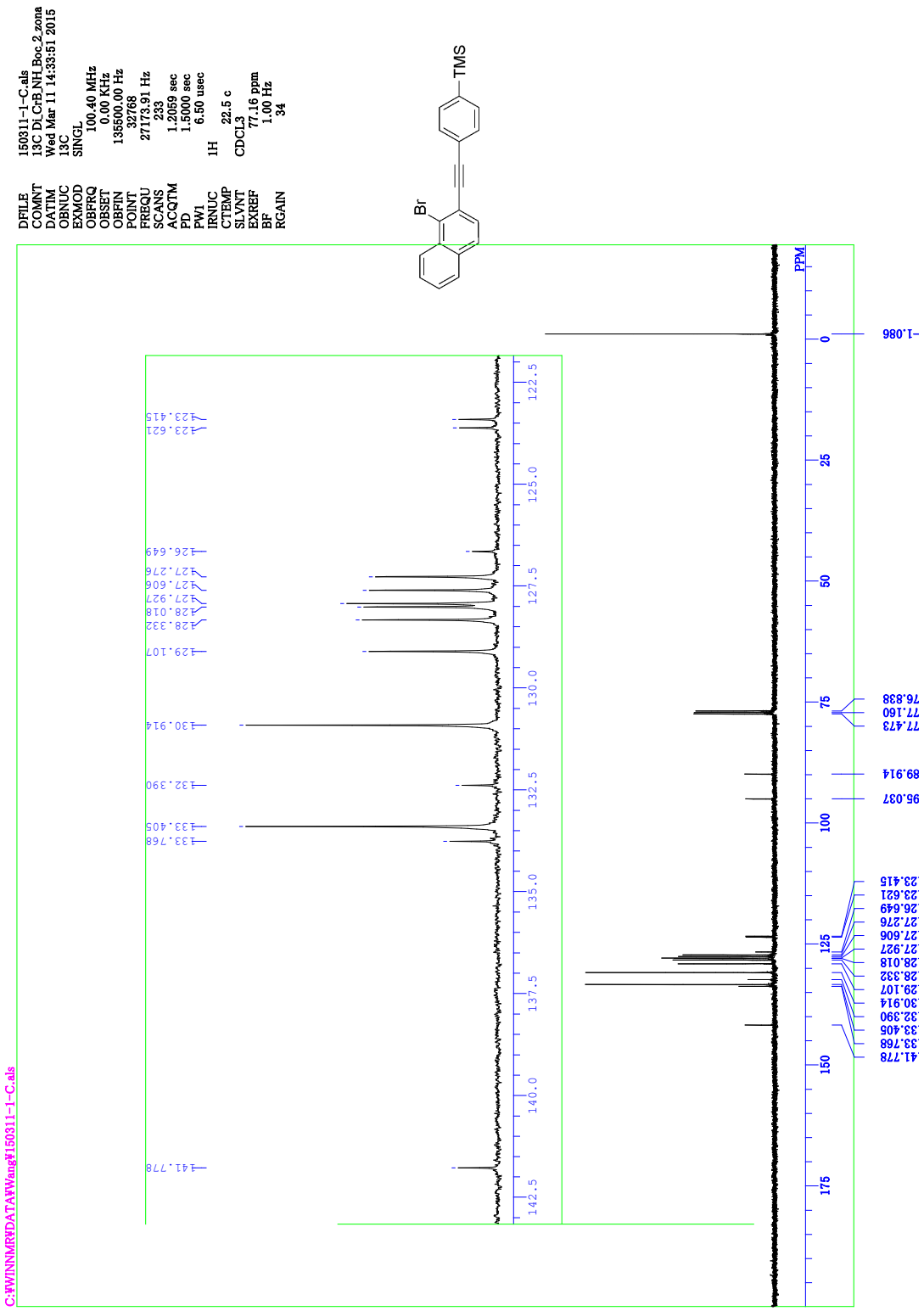
NMR-9. $^{13}C\{^{1}H\}$ NMR spectrum of **1** (100 MHz, $CDCl_3$).



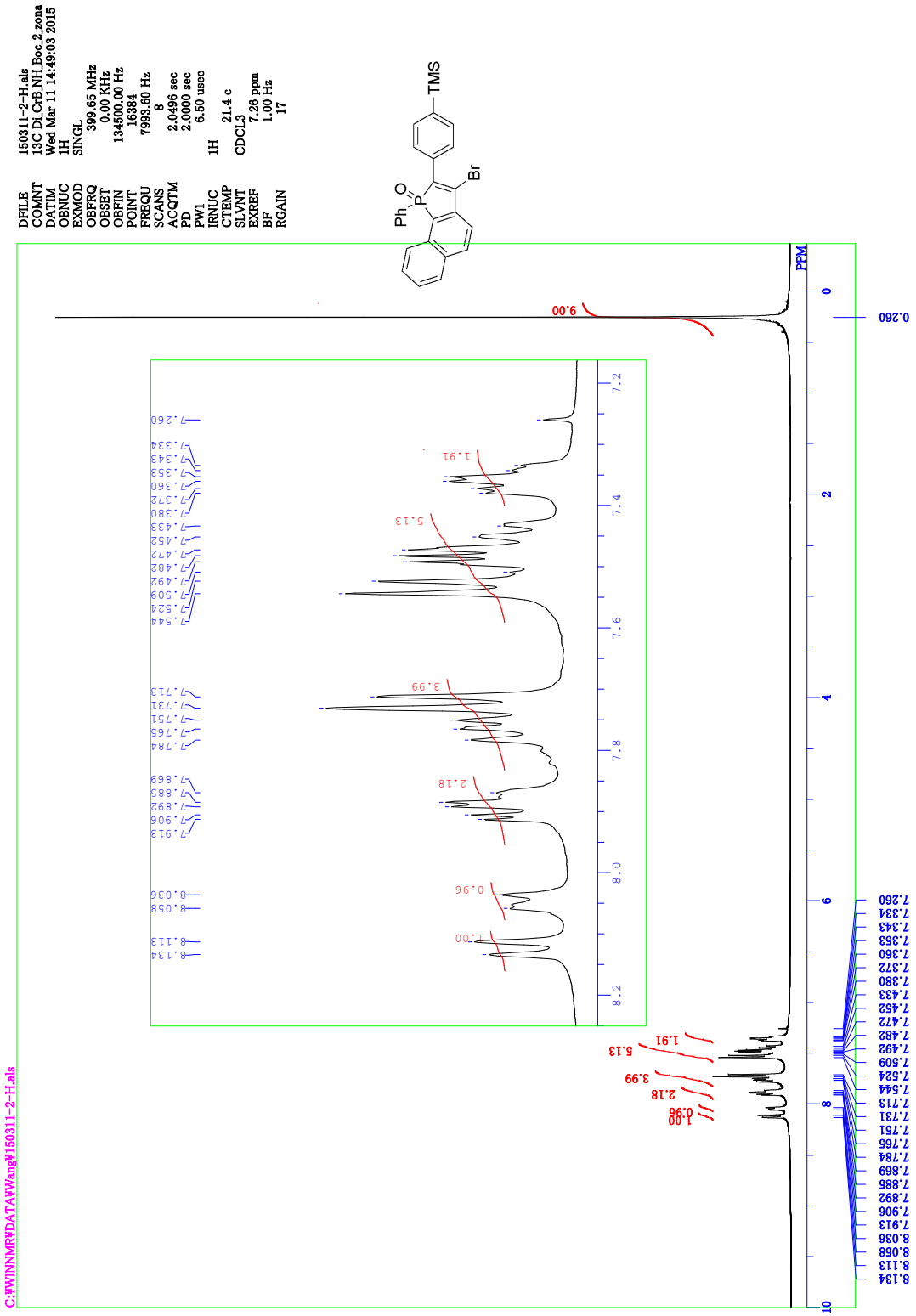
NMR-10. $^{31}P\{^{1}H\}$ NMR spectrum of **1** (162 MHz, $CDCl_3$).



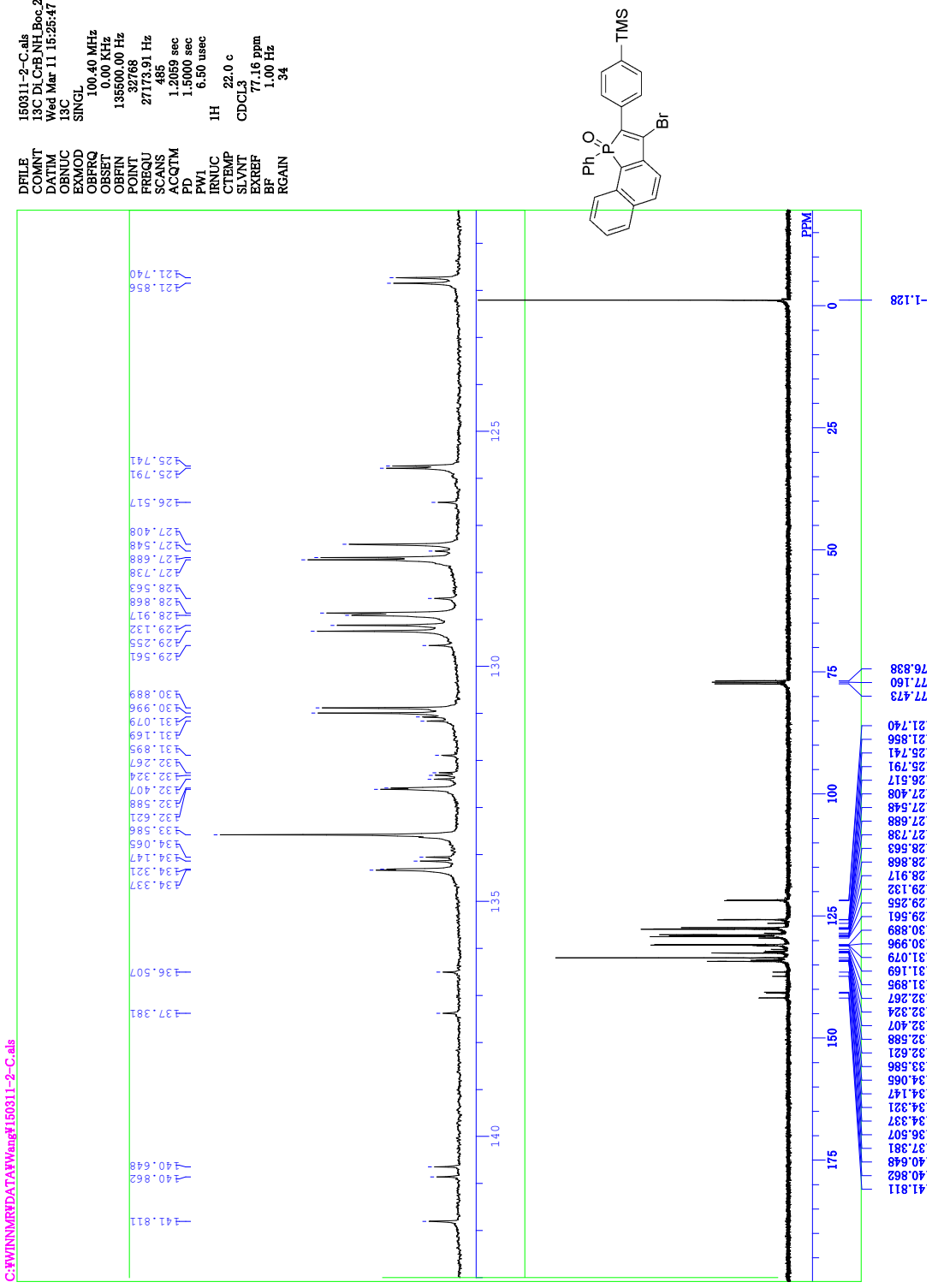
NMR-11. ^{1}H NMR spectrum of **6** (400 MHz, $CDCl_3$).



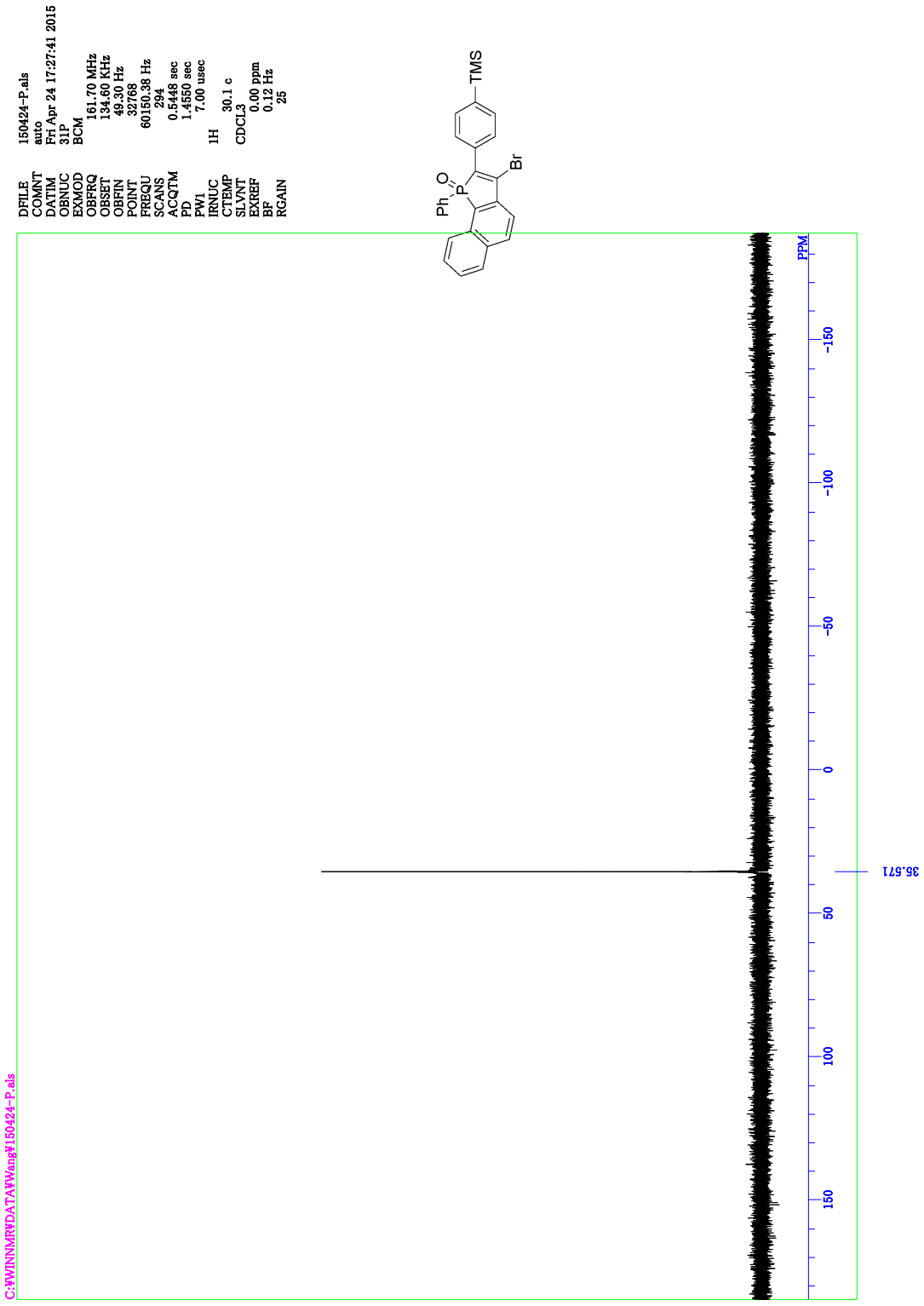
NMR-12. $^{13}C\{^{1}H\}$ NMR spectrum of **6** (100 MHz, $CDCl_3$).



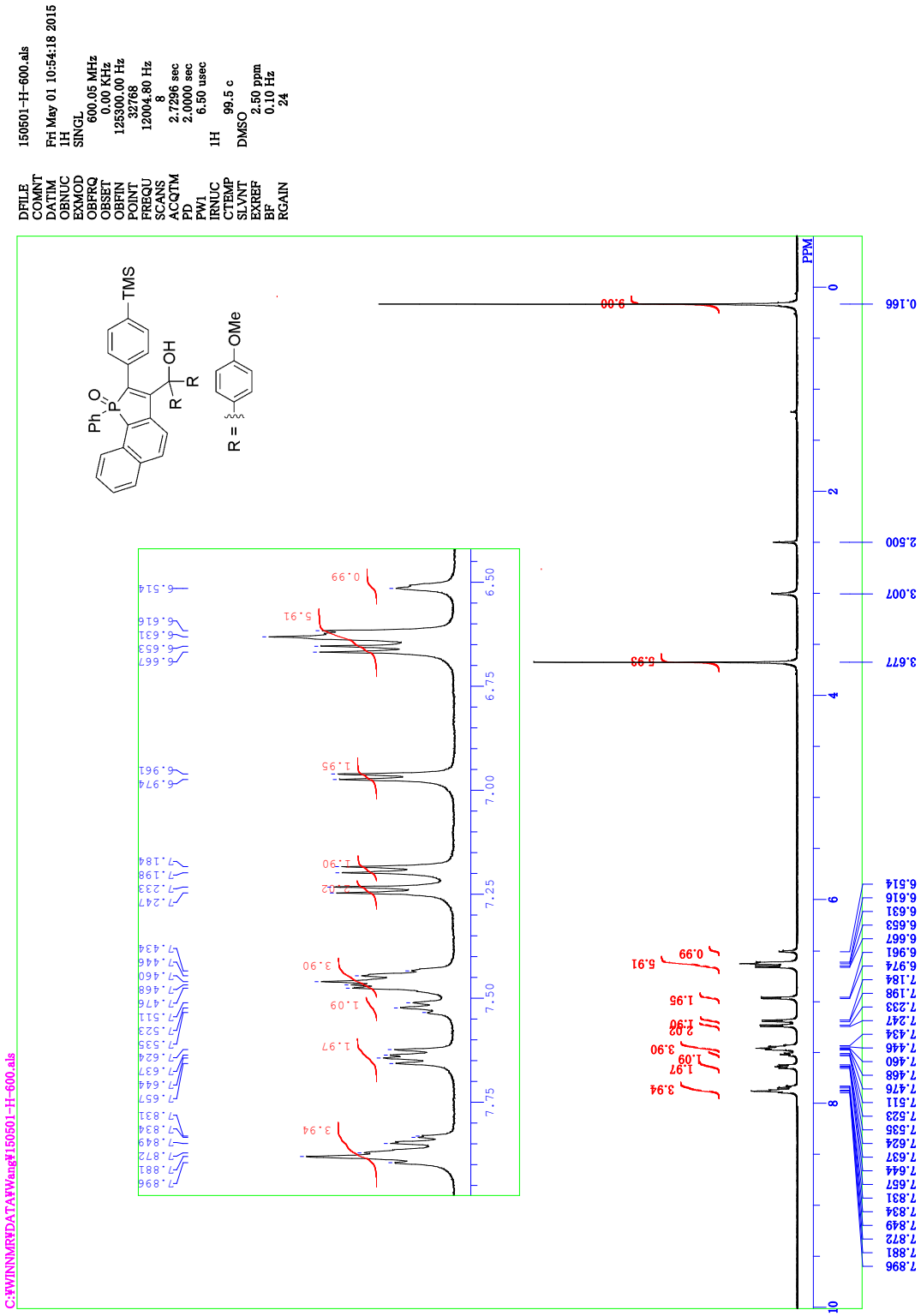
NMR-13. ^{1}H NMR spectrum of **7** (400 MHz, $CDCl_3$).



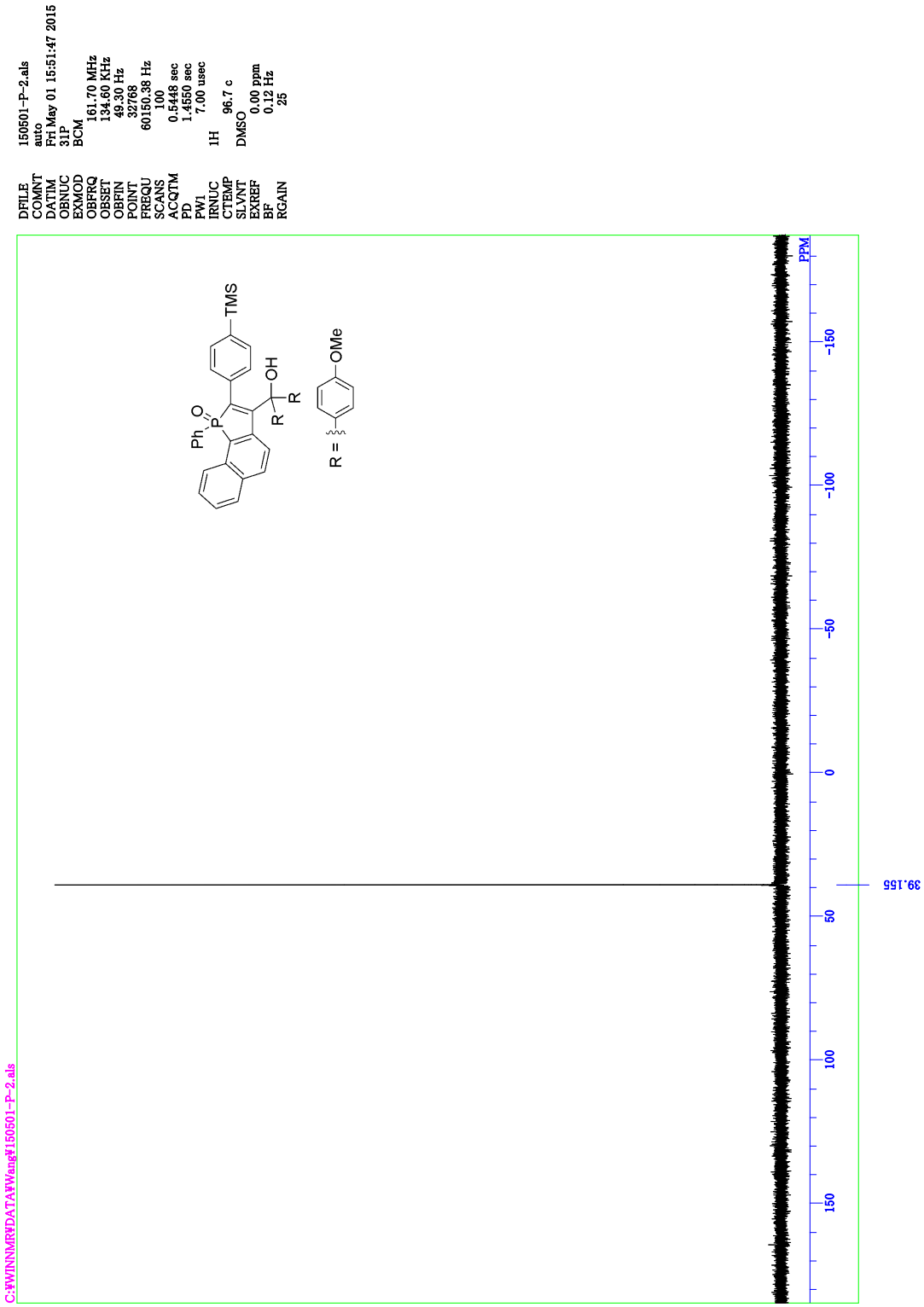
NMR-14. $^{13}C\{^{1}H\}$ NMR spectrum of **7** (100 MHz, $CDCl_3$).



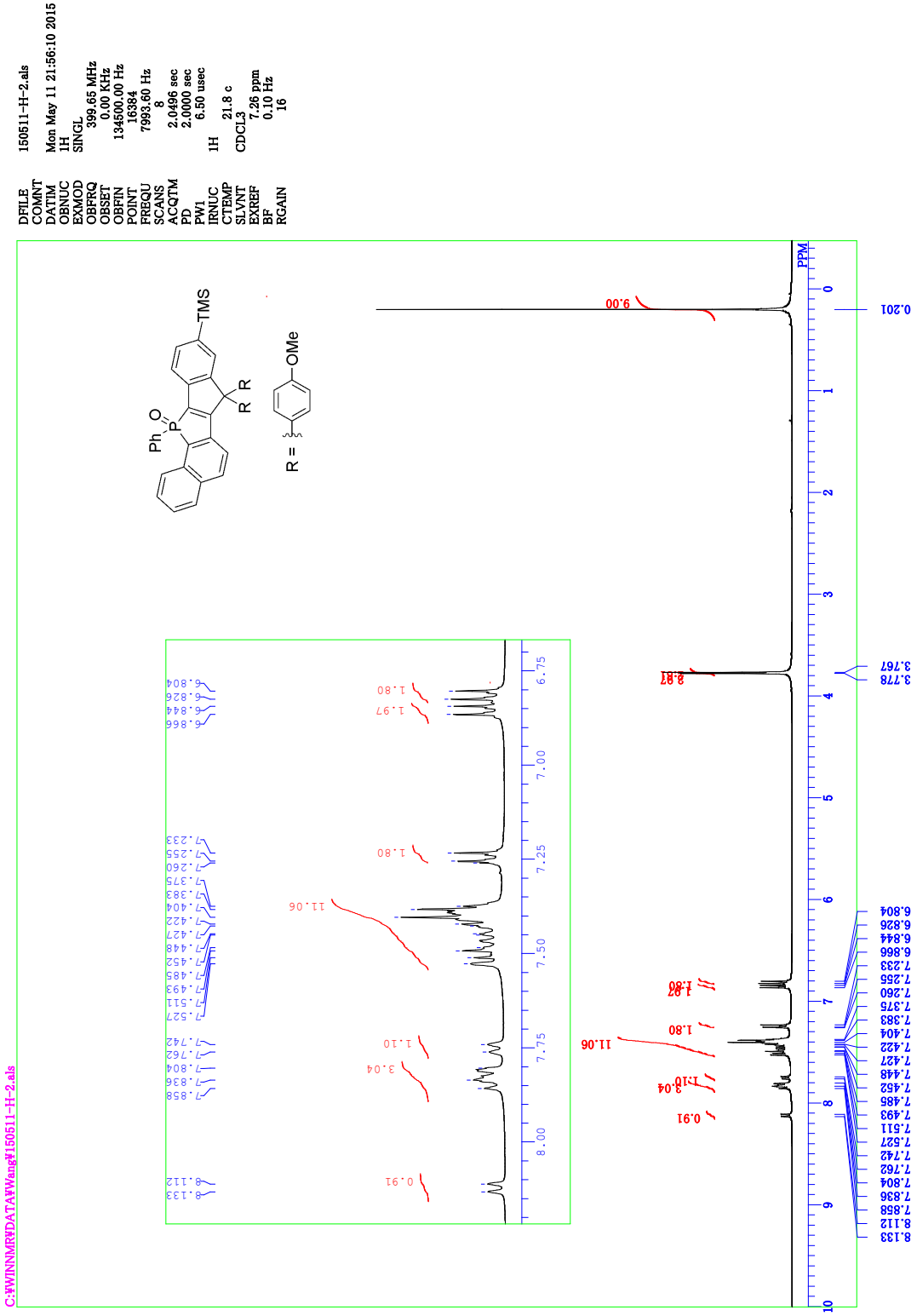
NMR-15. $^{31}P\{^{1}H\}$ NMR spectrum of **7** (162 MHz, $CDCl_3$).



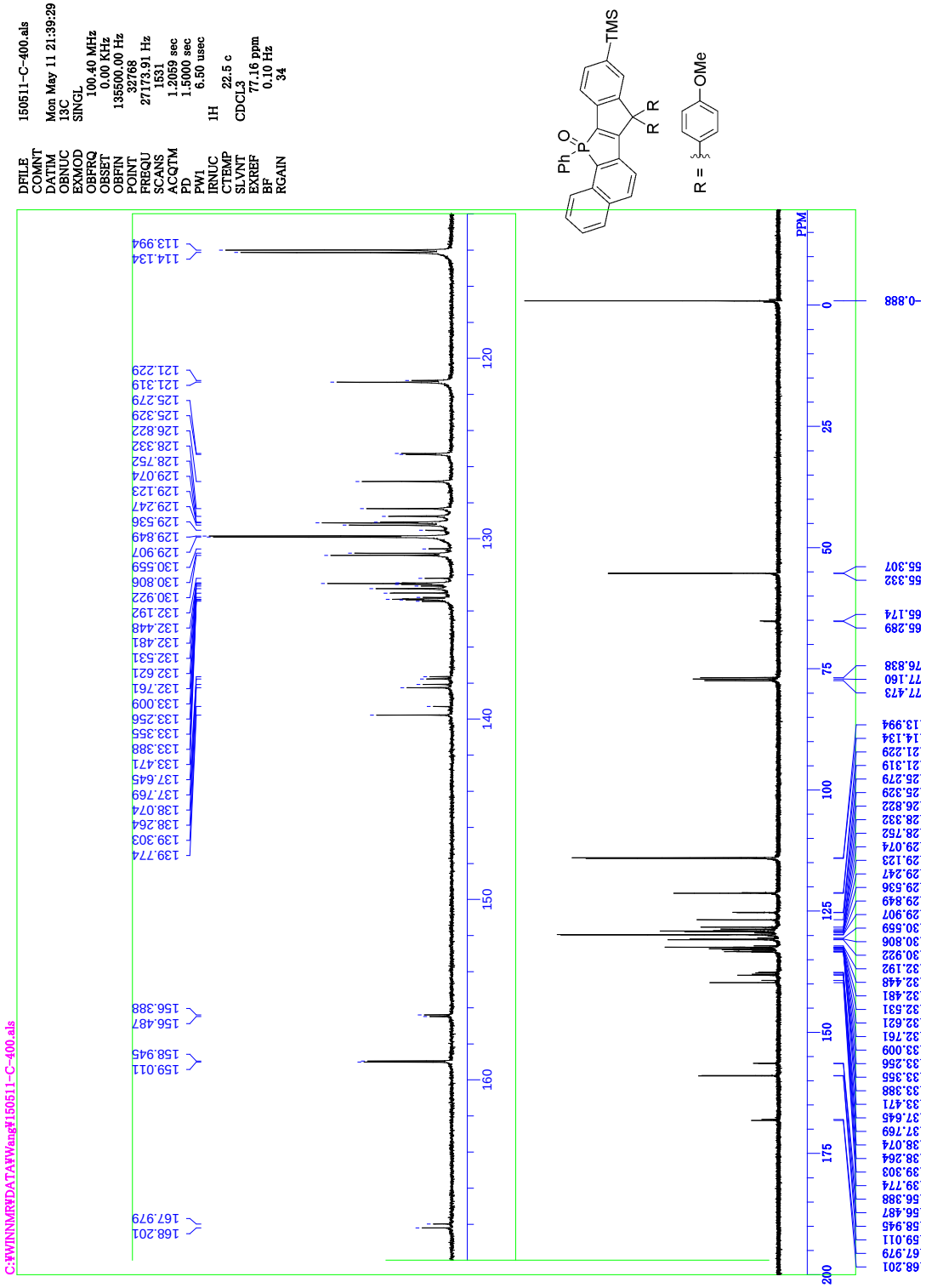
NMR-16. ^{1}H NMR spectrum of **8** (600 MHz, DMSO-d_6, 100 °C).



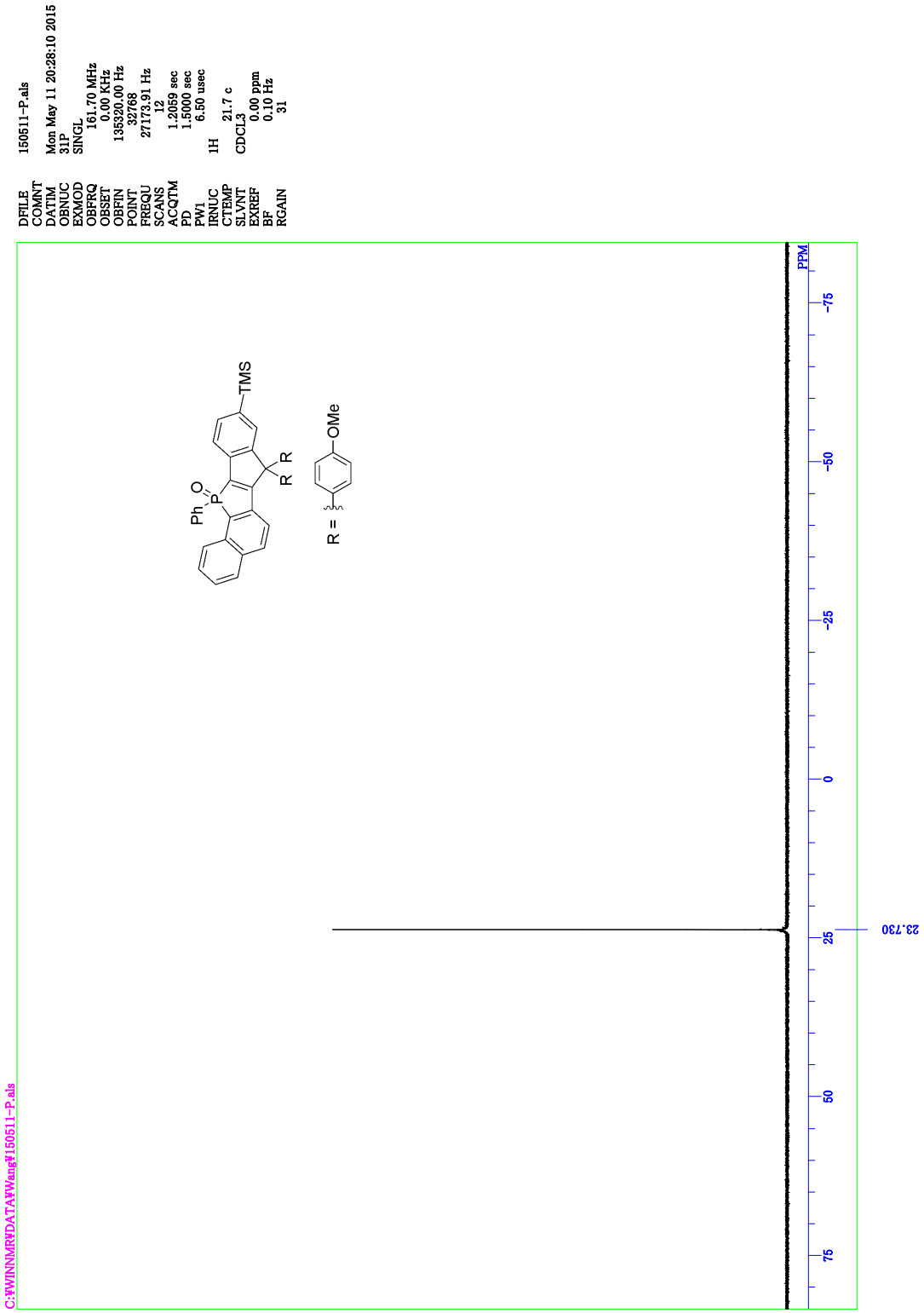
NMR-17. $^{31}P\{^{1}H\}$ NMR spectrum of **8** (162 MHz, DMSO-d_6, 100 °C).



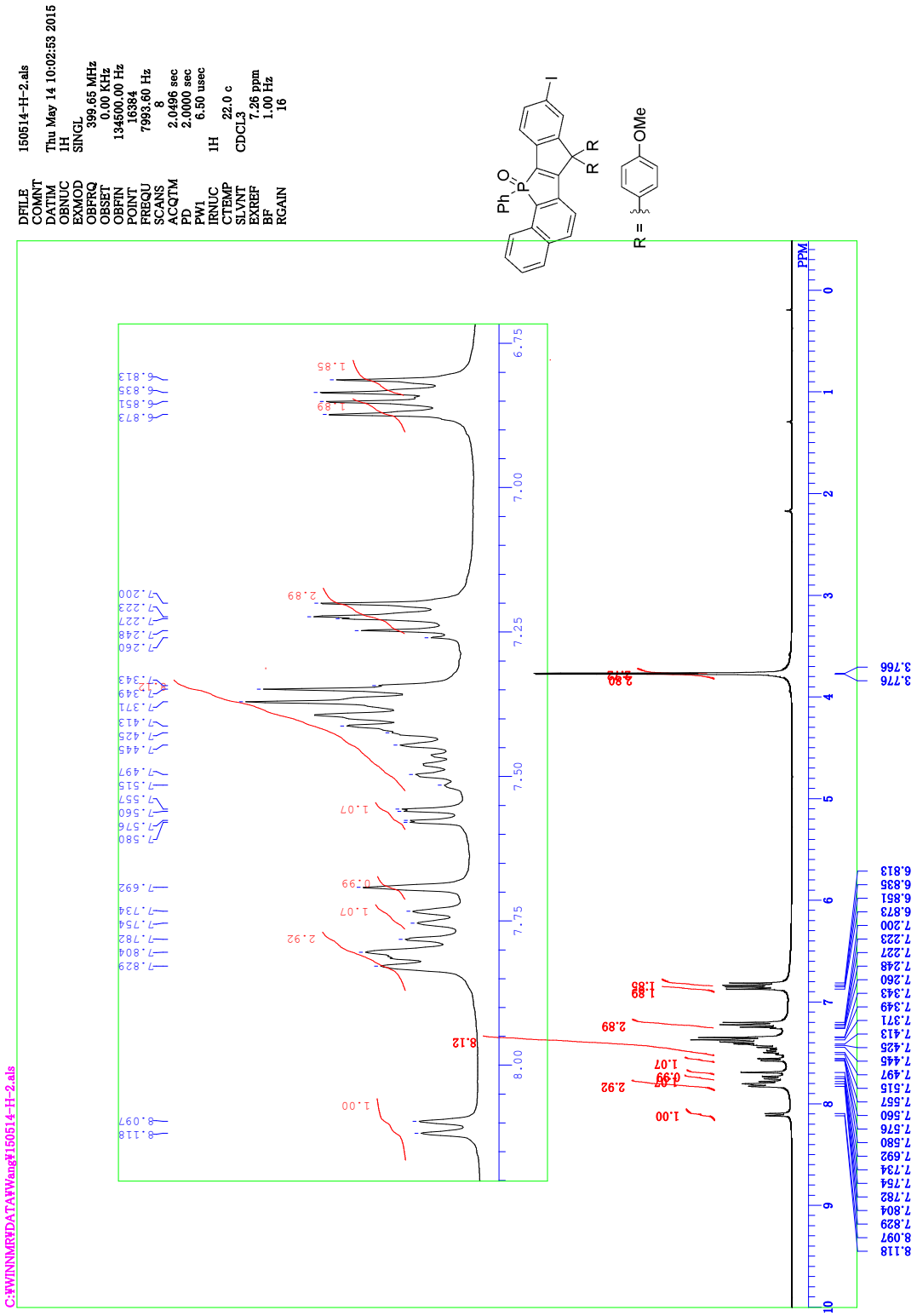
NMR-18. ^{1}H NMR spectrum of **9** (400 MHz, $CDCl_3$).



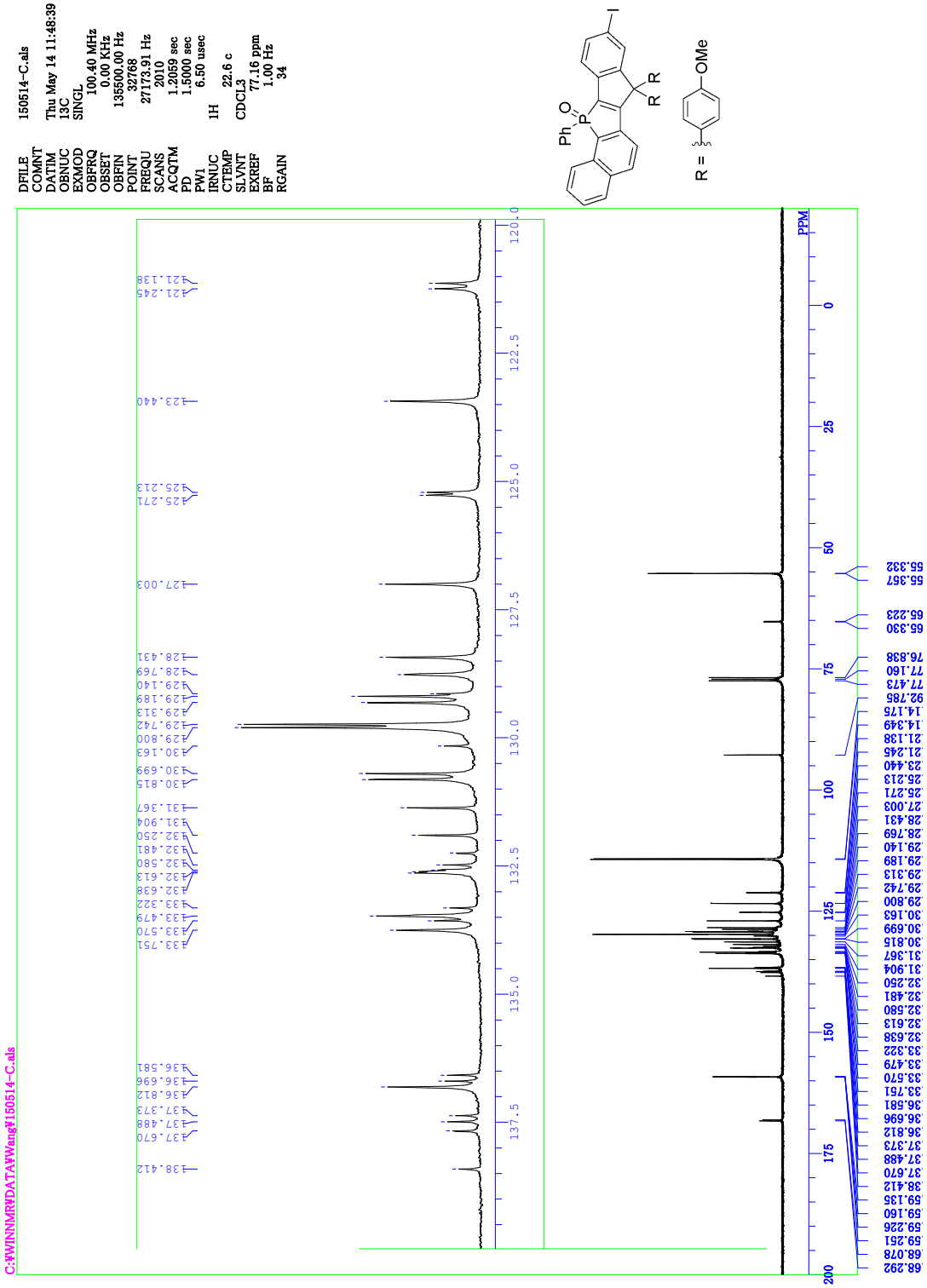
NMR-19. $^{13}C\{^{1}H\}$ NMR spectrum of **9** (100 MHz, $CDCl_3$).



NMR-20. $^{31}P\{^{1}H\}$ NMR spectrum of **9** (162 MHz, $CDCl_3$).

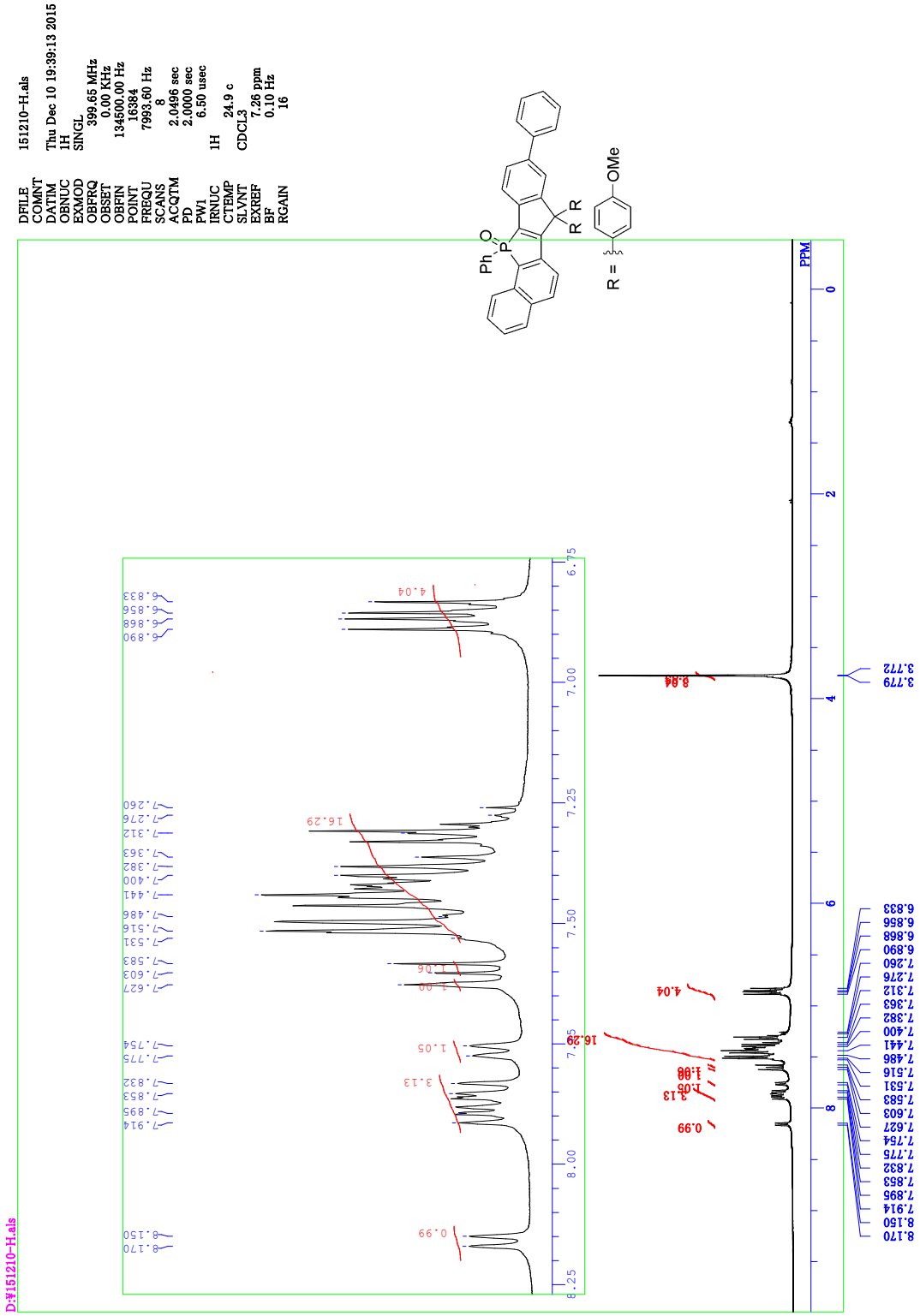


NMR-21. ^{1}H NMR spectrum of **10** (400 MHz, $CDCl_3$).

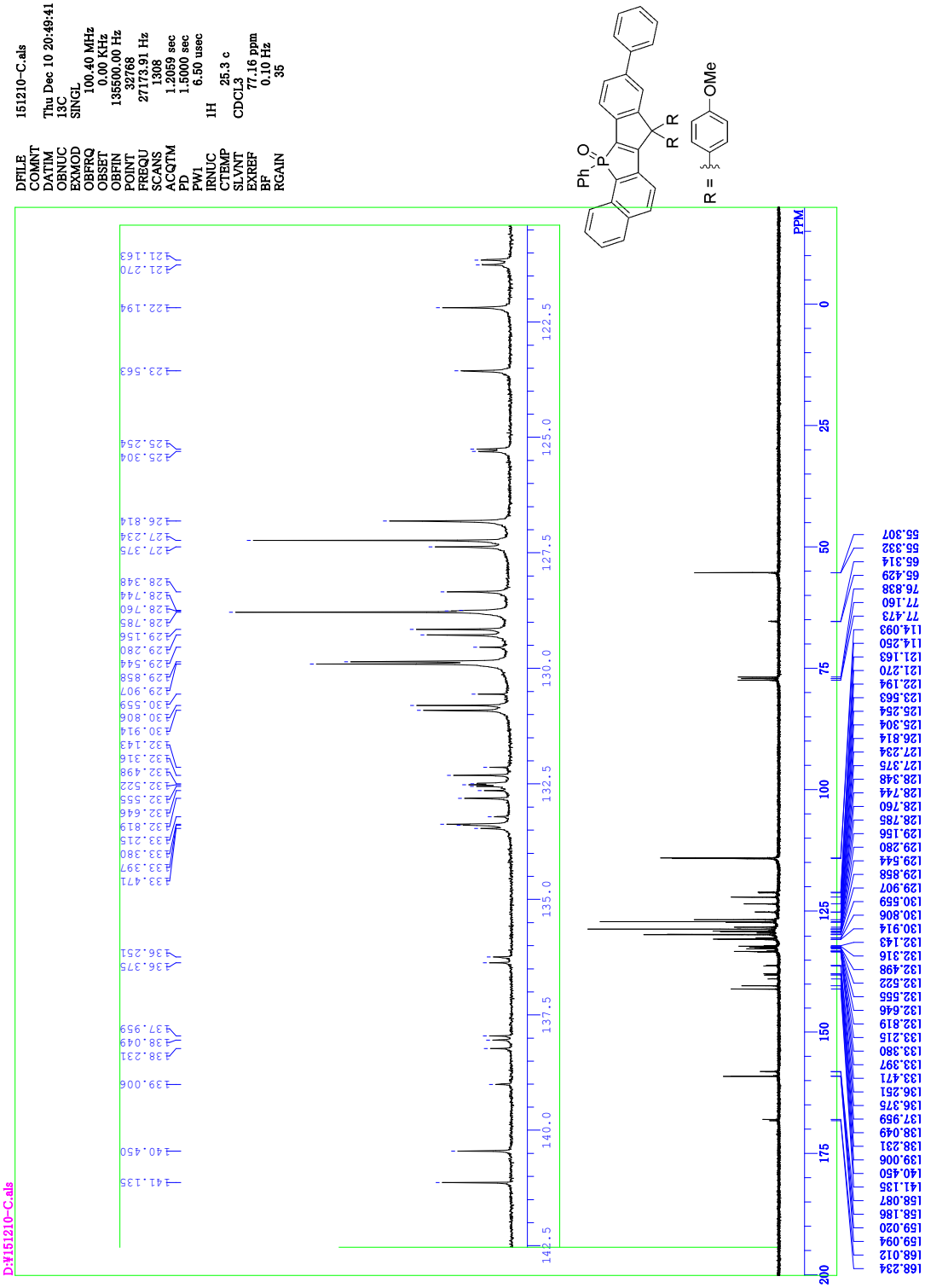


NMR-22. $^{13}C\{^{1}H\}$ NMR spectrum of **10** (100 MHz, $CDCl_3$).

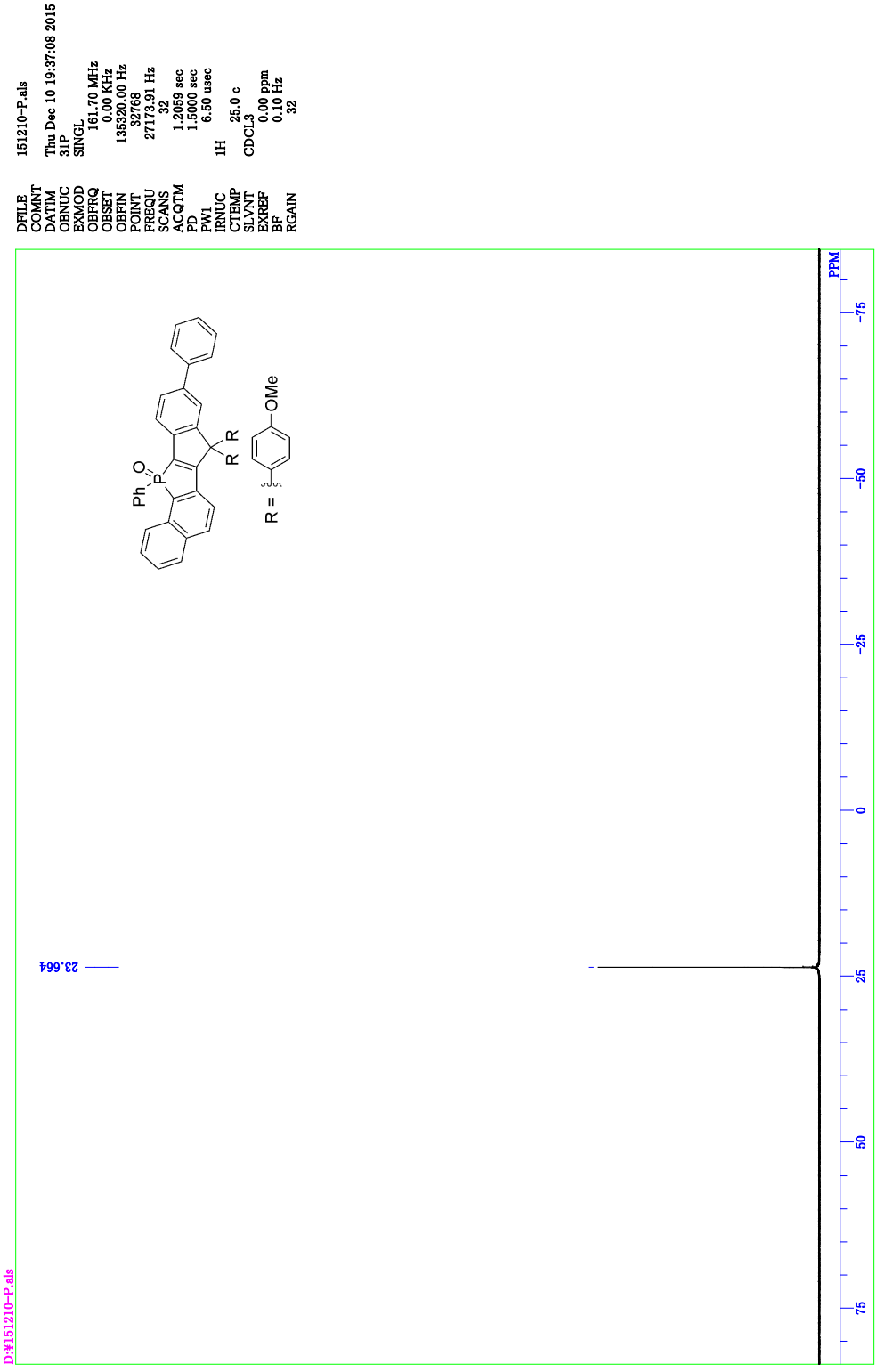
NMR-23. $^{31}P\{^{1}H\}$ NMR spectrum of **10** (162 MHz, $CDCl_3$).



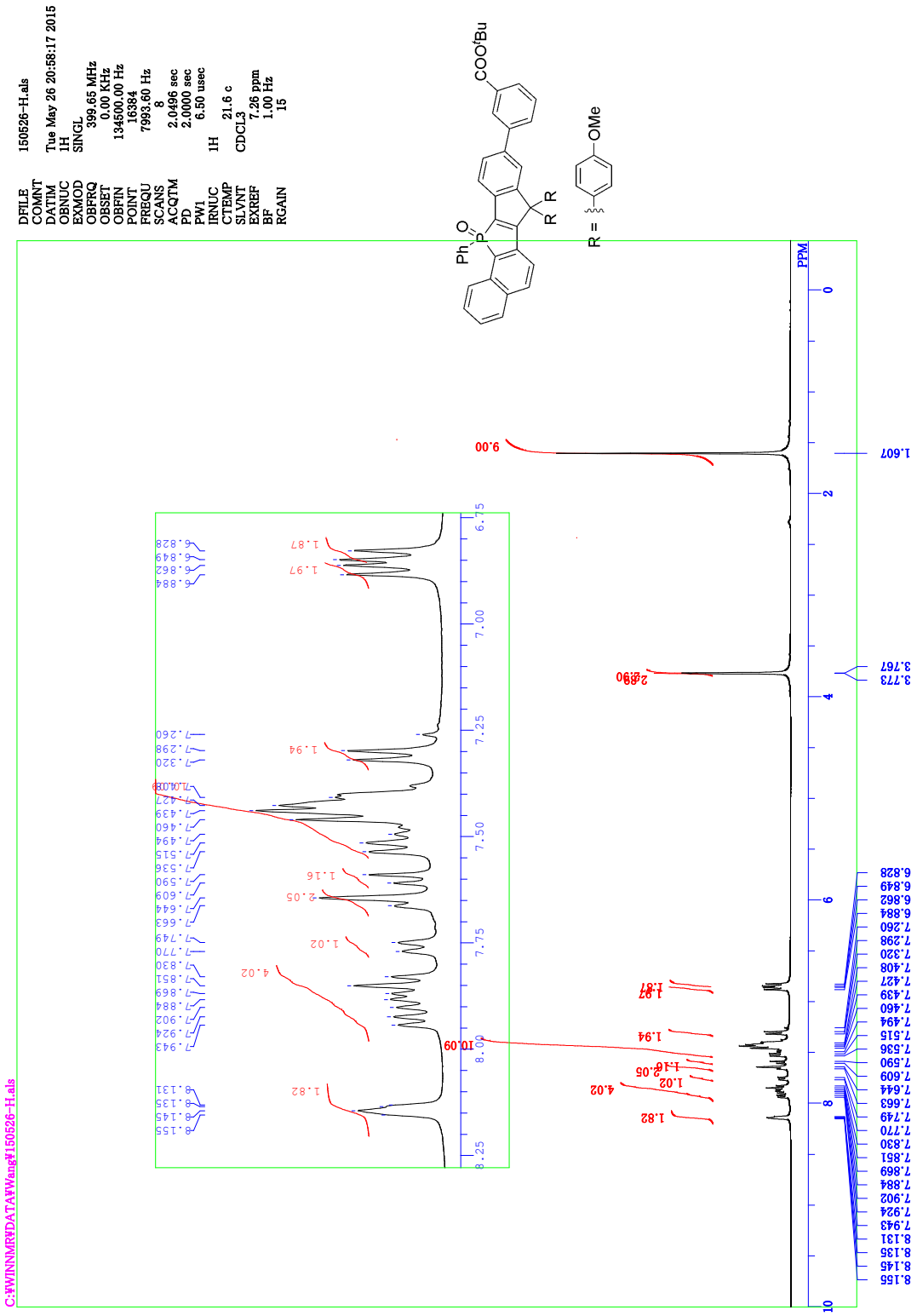
NMR-24. ^{1}H NMR spectrum of **2** (400 MHz, $CDCl_3$).



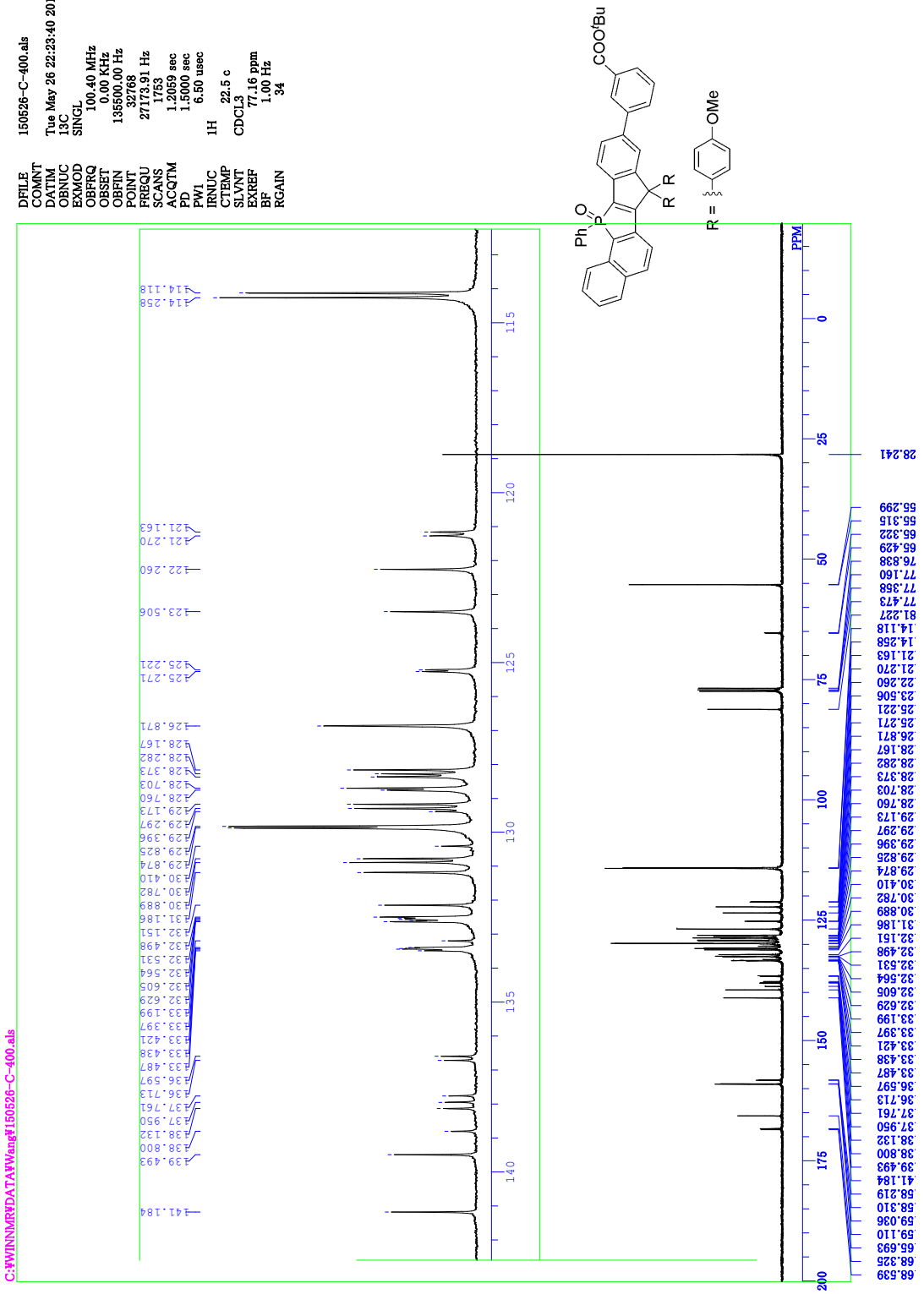
NMR-25. $^{13}C\{^{1}H\}$ NMR spectrum of **2** (100 MHz, $CDCl_3$).



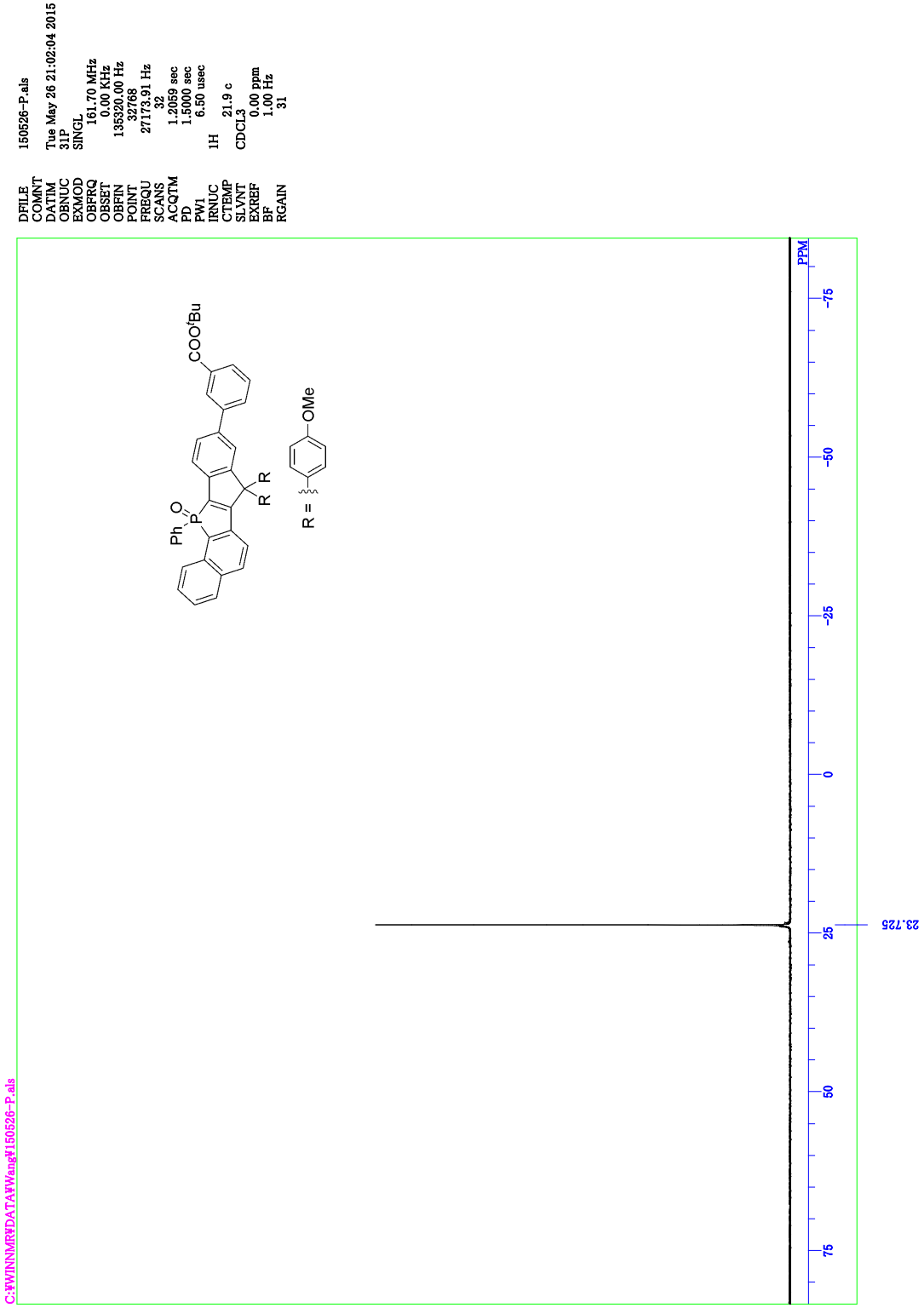
NMR-26. $^{31}P\{^{1}H\}$ NMR spectrum of **2** (162 MHz, $CDCl_3$).



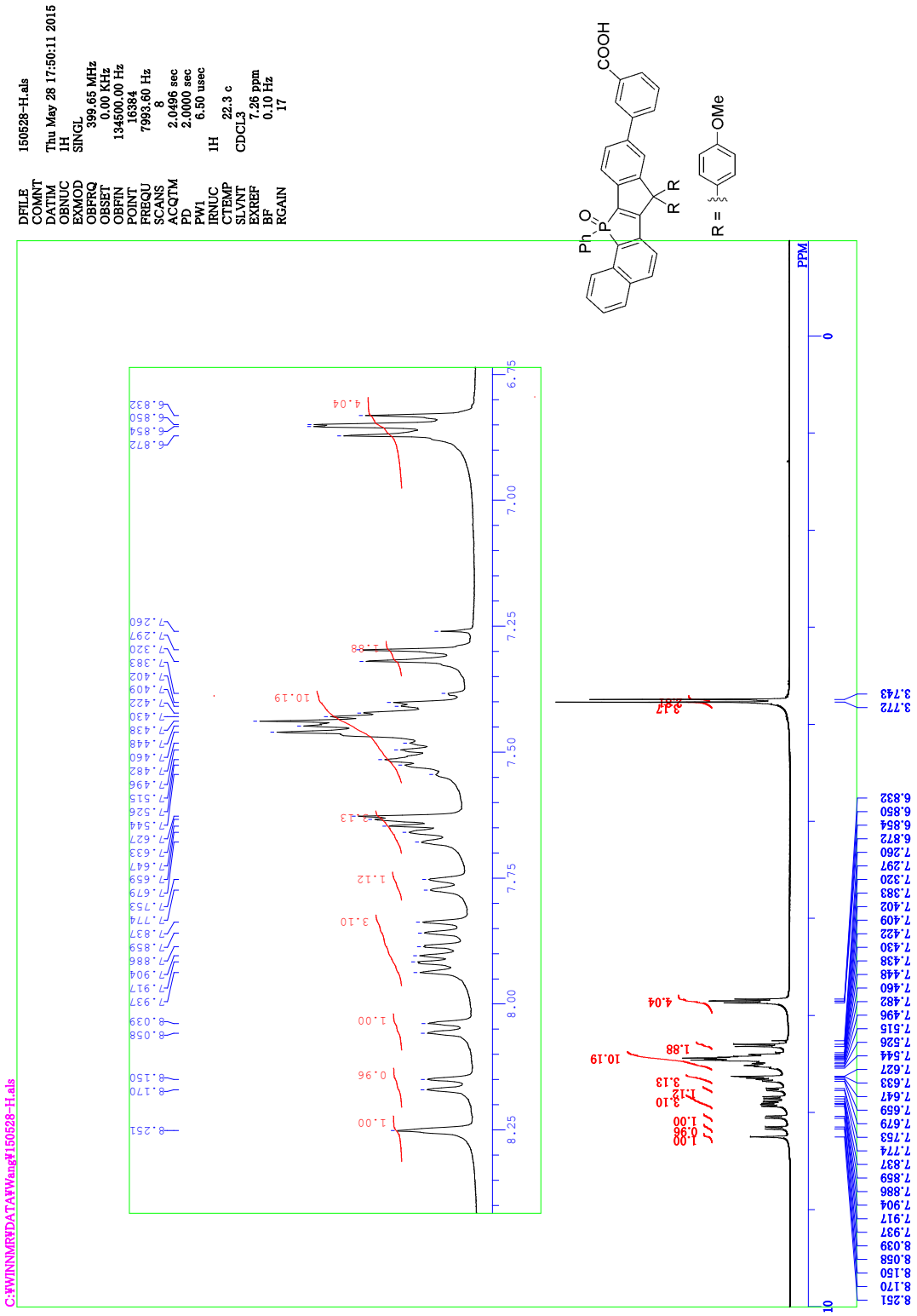
NMR-27. ^{1}H NMR spectrum of **11** (400 MHz, $CDCl_3$).



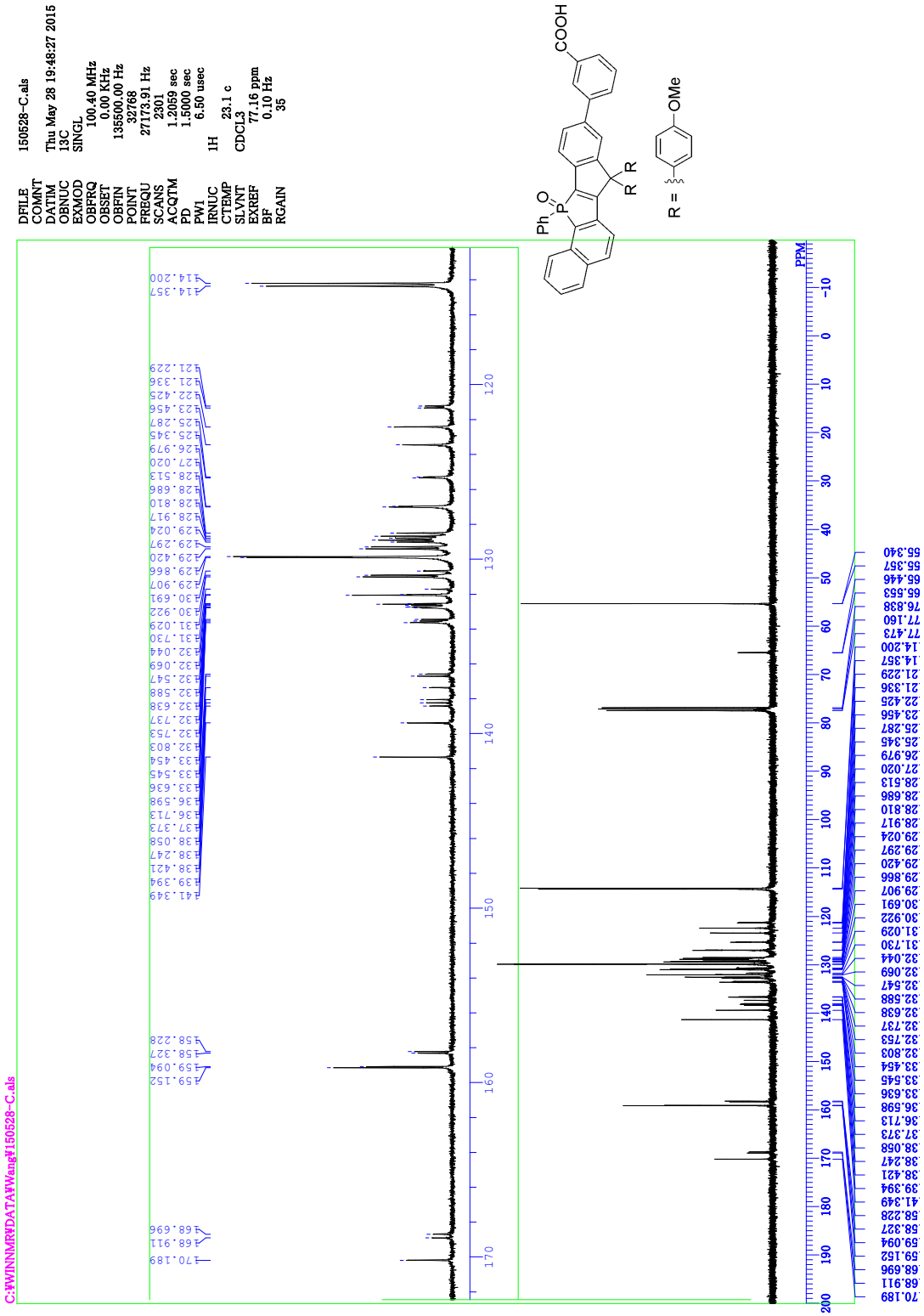
NMR-28. $^{13}C\{^{1}H\}$ NMR spectrum of **11** (100 MHz, $CDCl_3$).



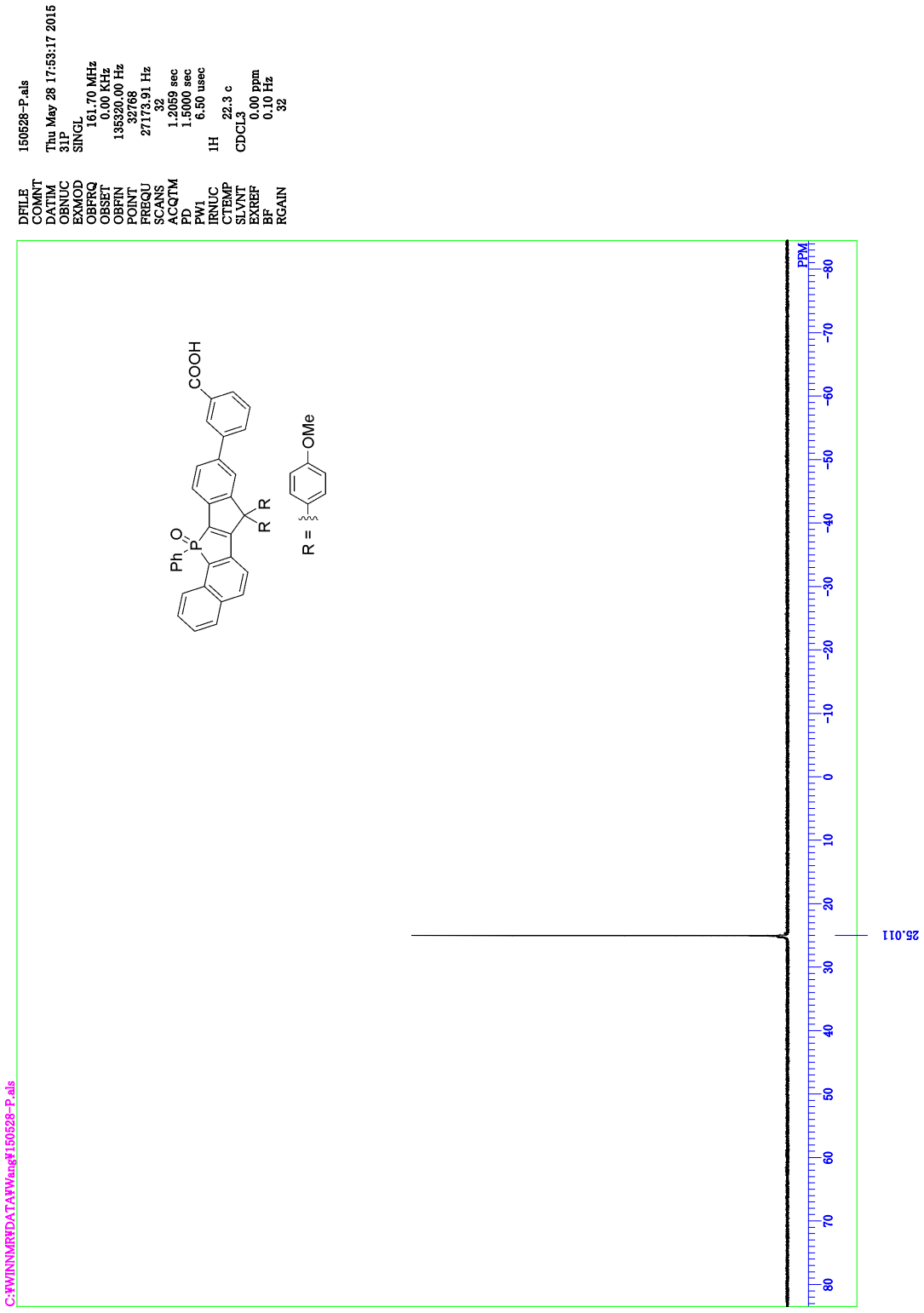
NMR-29. $^{31}P\{^{1}H\}$ NMR spectrum of **11** (162 MHz, $CDCl_3$).



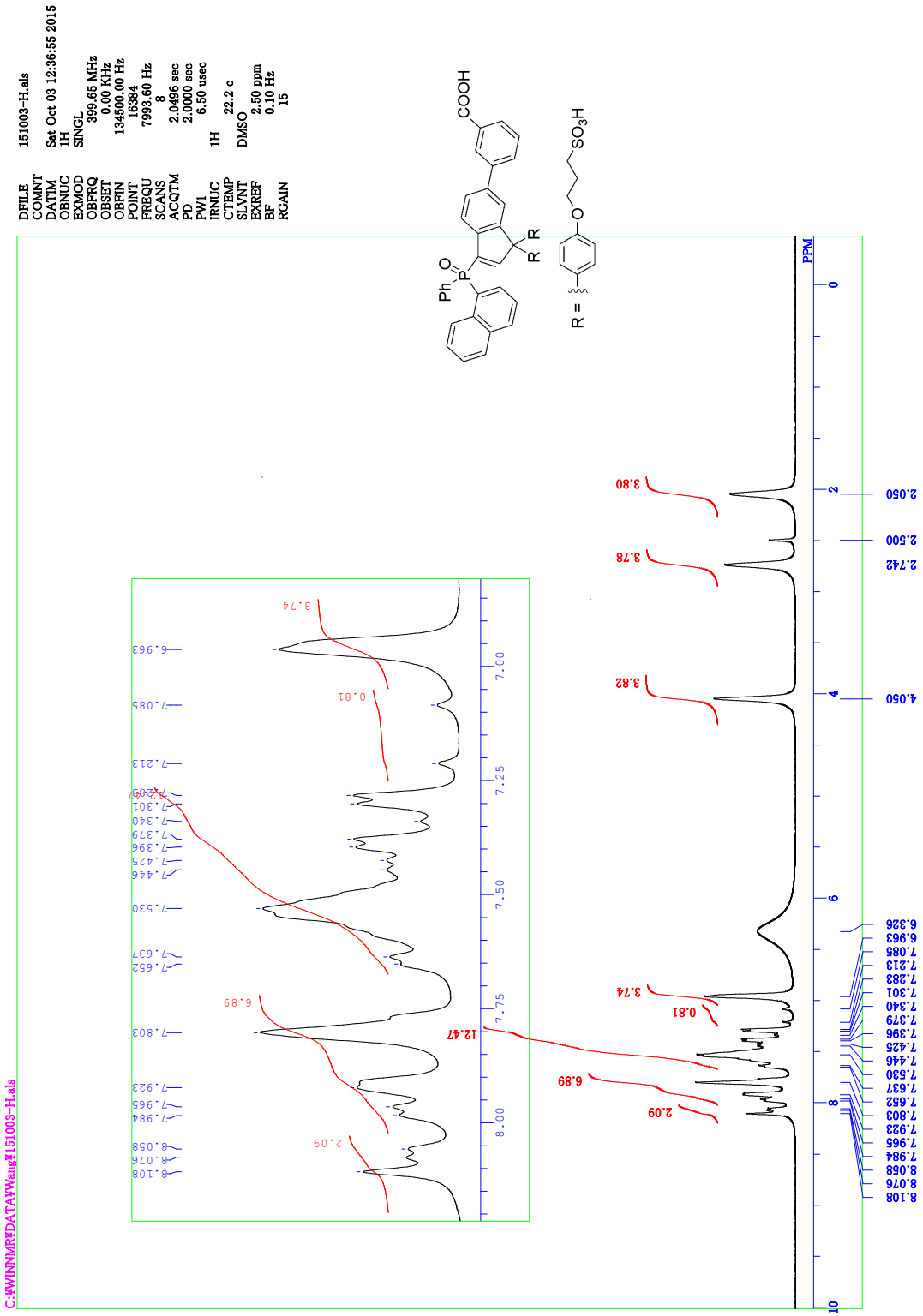
NMR-30. ^{1}H NMR spectrum of **12** (400 MHz, $CDCl_3$).



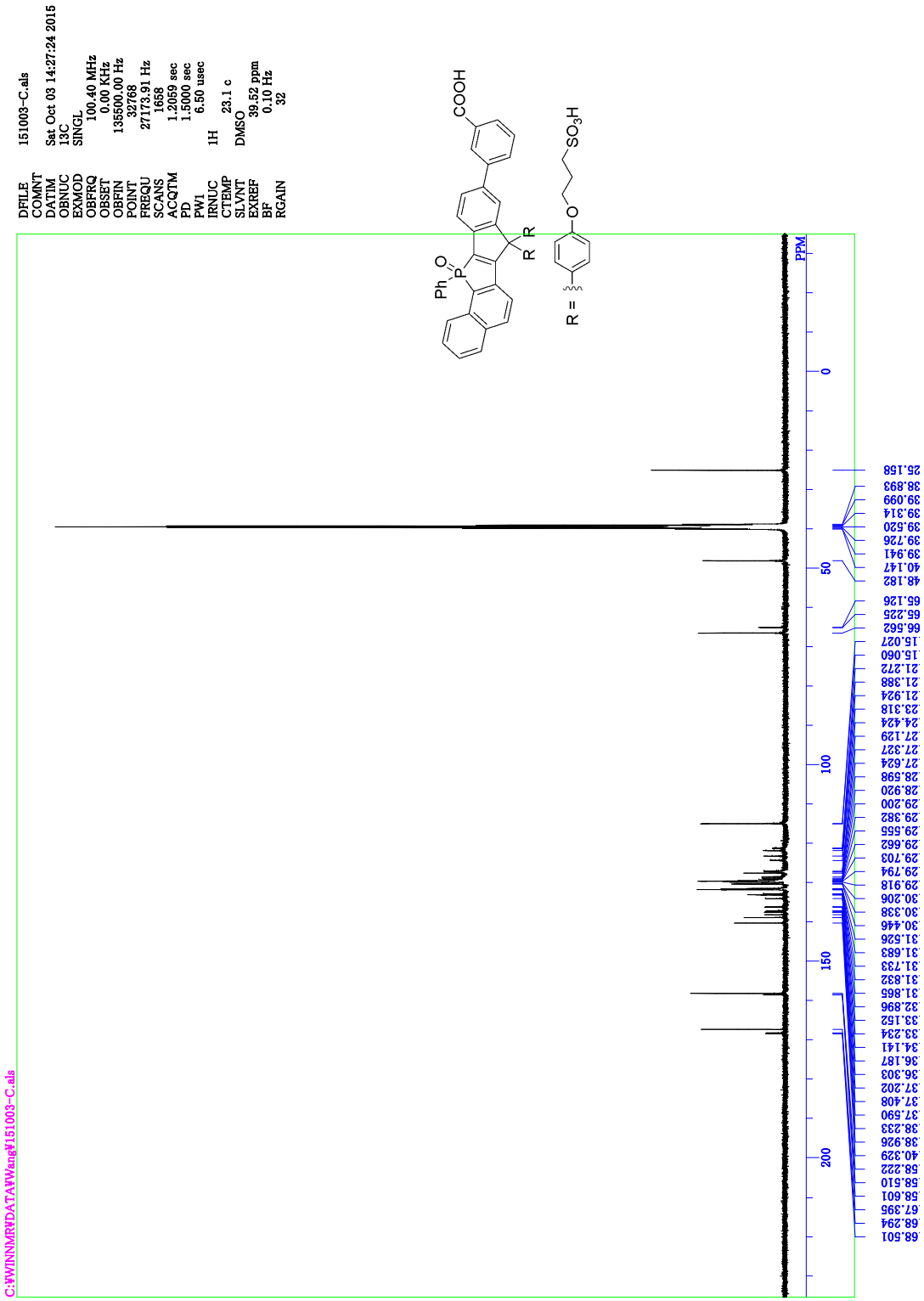
NMR-31. $^{13}C\{^{1}H\}$ NMR spectrum of **12** (100 MHz, $CDCl_3$).



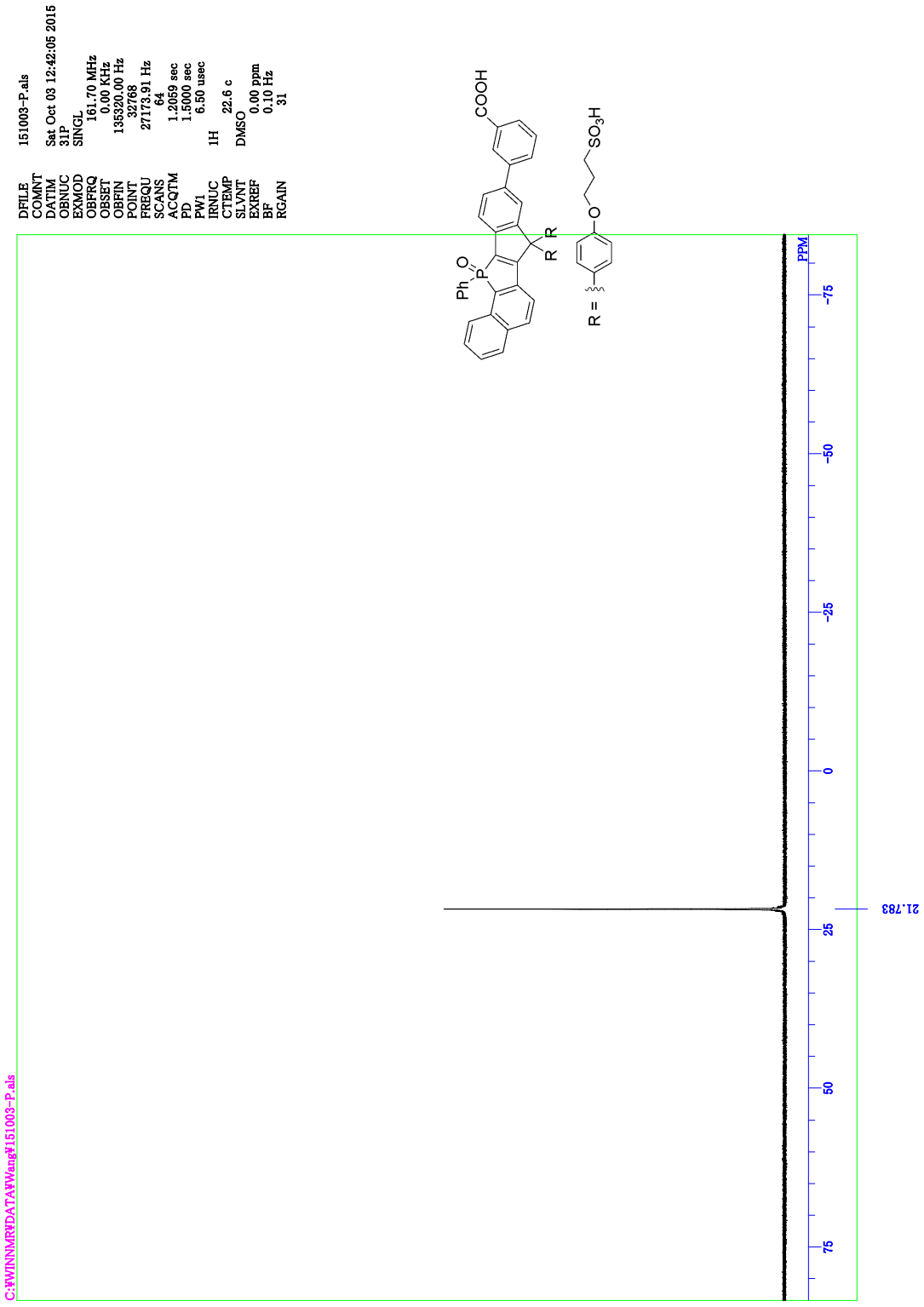
NMR-32. $^{31}P\{^{1}H\}$ NMR spectrum of **12** (162 MHz, $CDCl_3$).



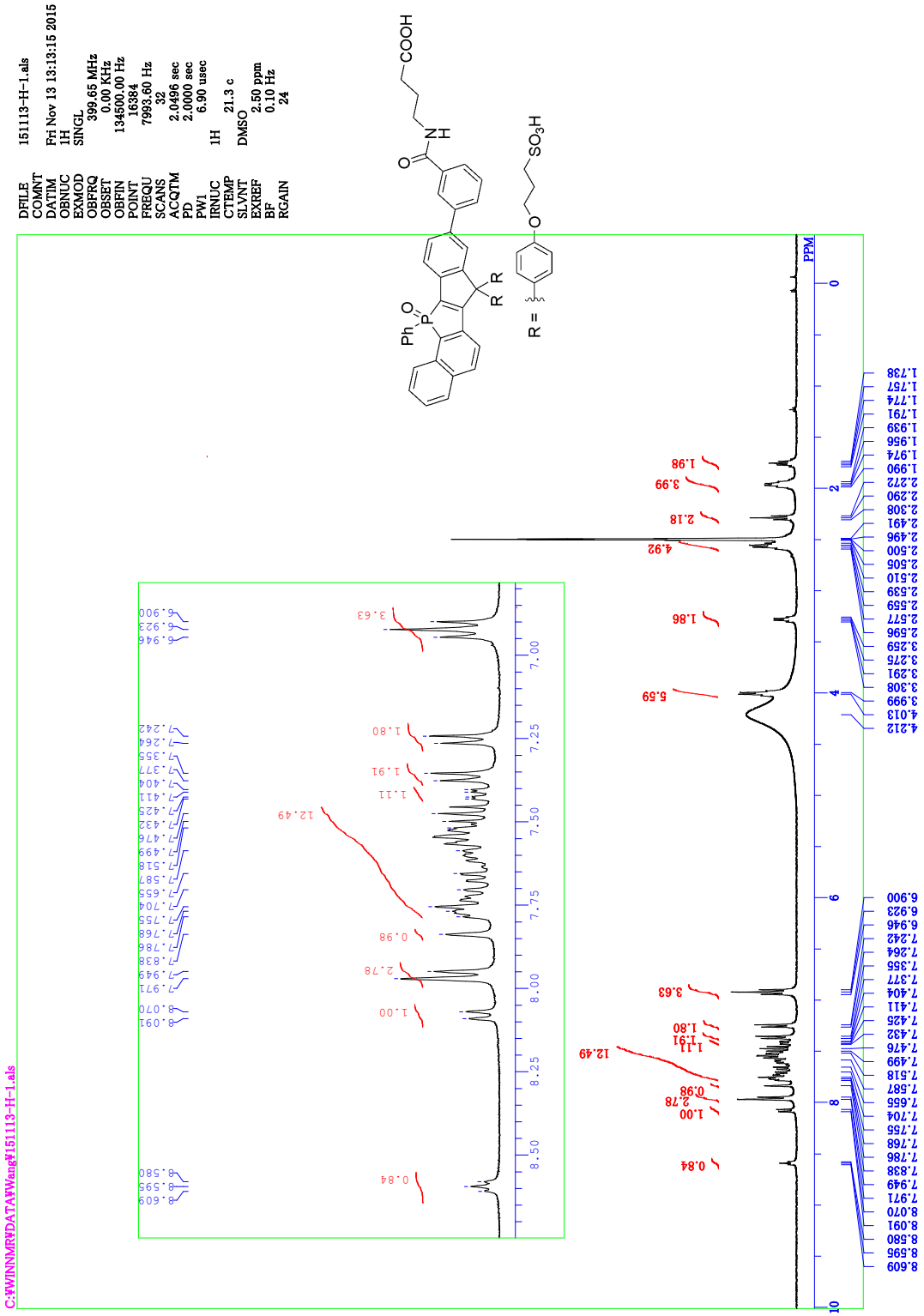
NMR-33. ^{1}H NMR spectrum of **13** (400 MHz, DMSO-d_6).



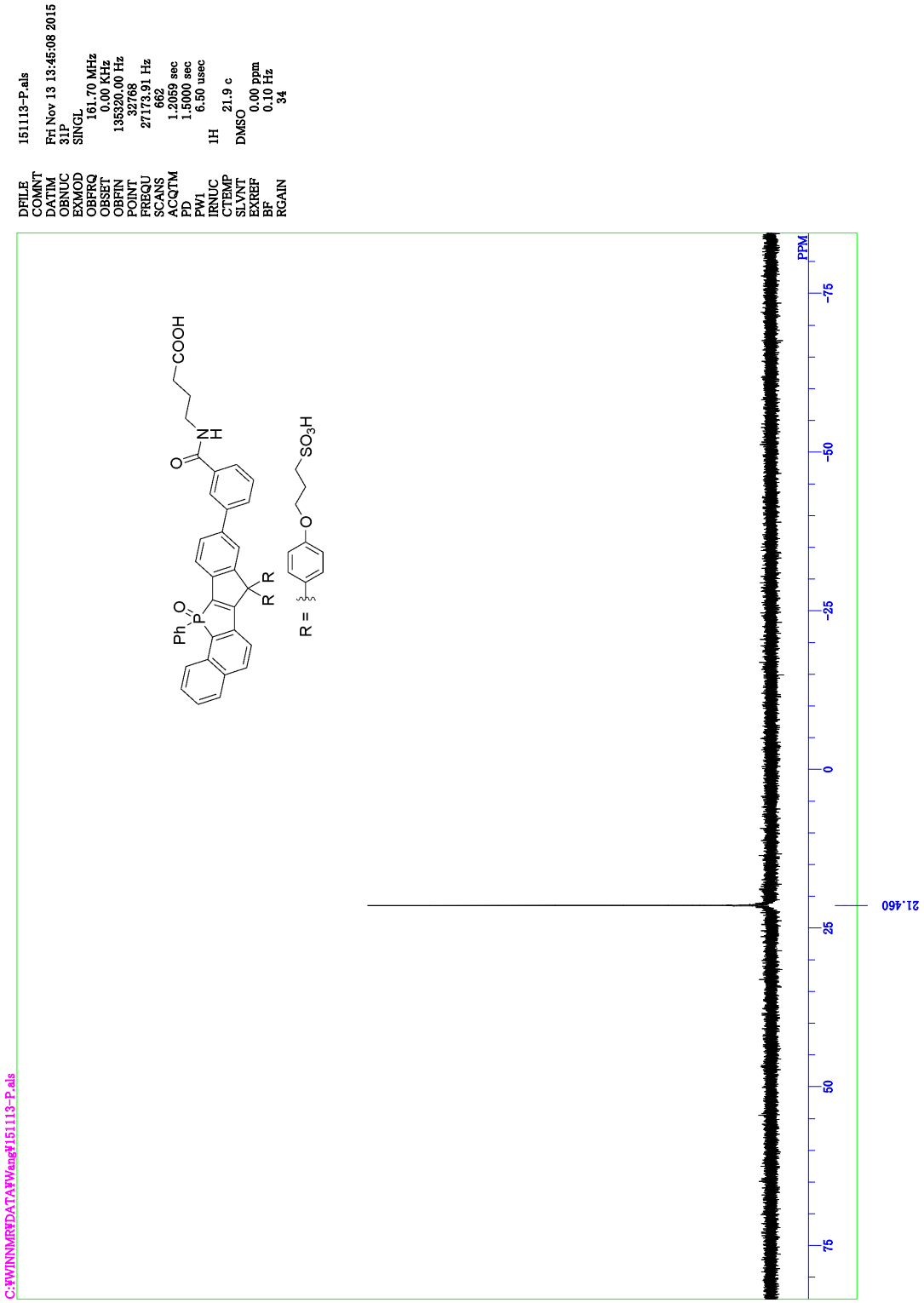
NMR-34. $^{13}C\{^{1}H\}$ NMR spectrum of **13** (100 MHz, DMSO-d_6).



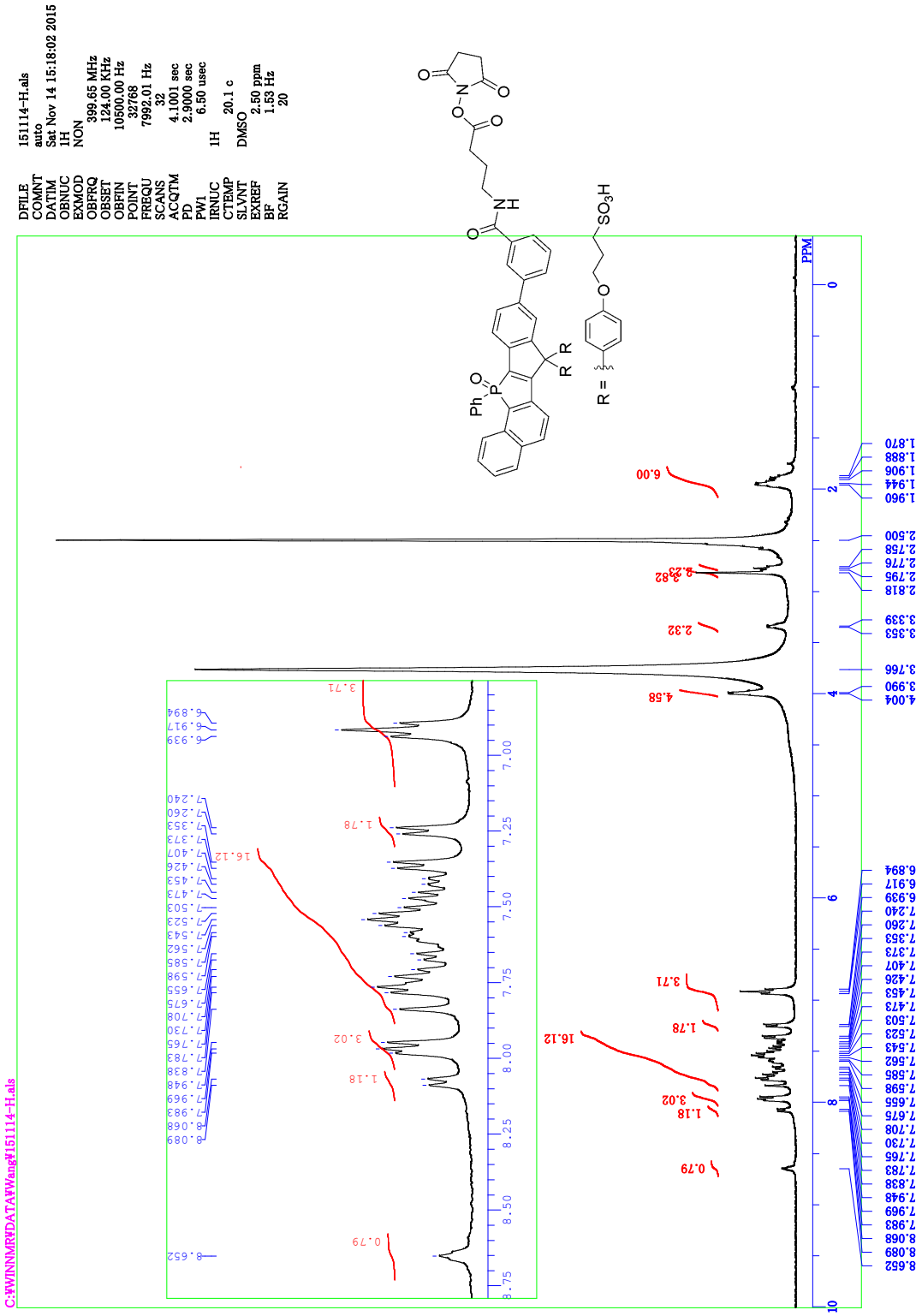
NMR-35. $^{31}P\{^{1}H\}$ NMR spectrum of **13** (162 MHz, DMSO-d_6).



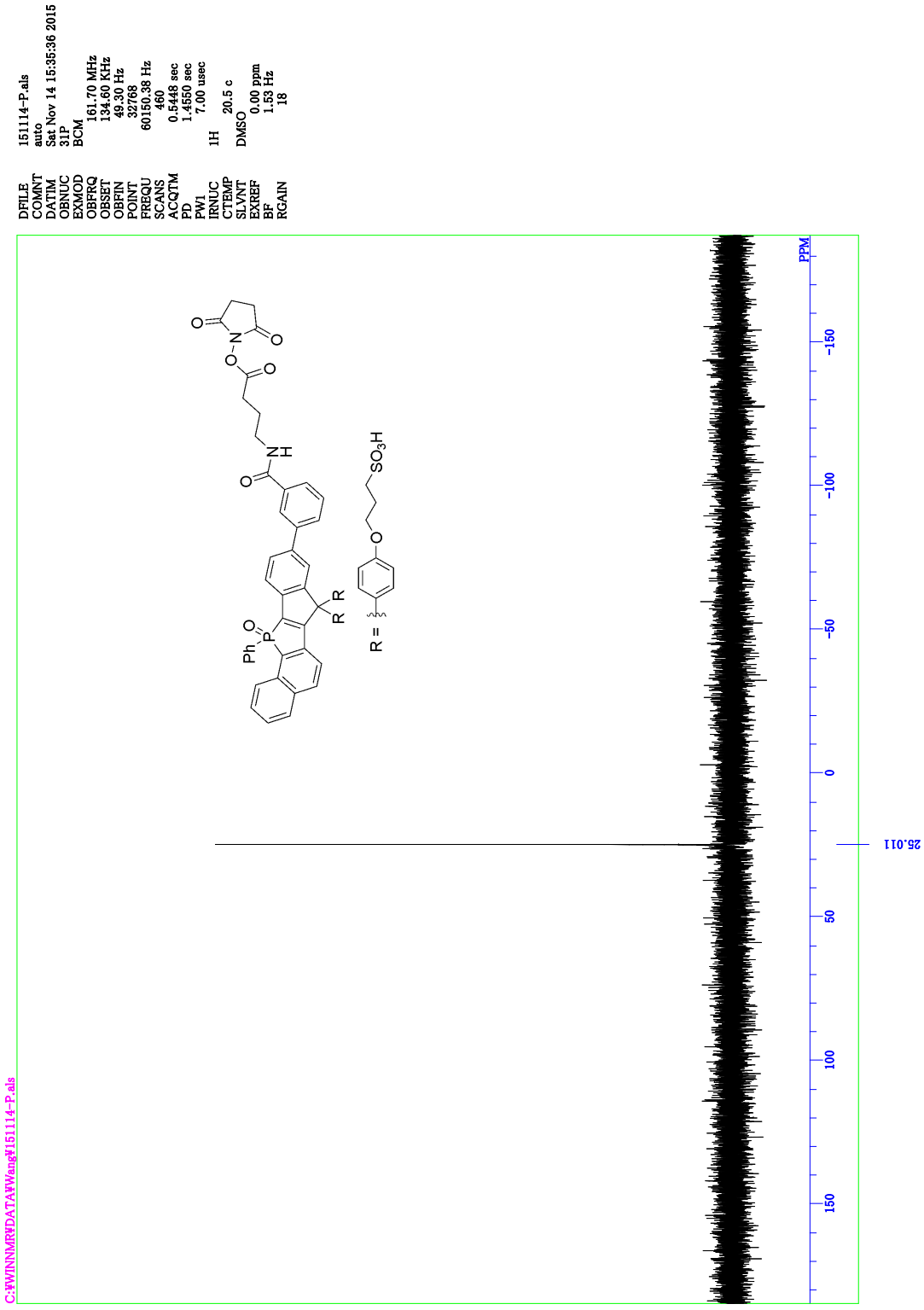
NMR-36. ^{1}H NMR spectrum of PB430 (400 MHz, DMSO-d_6).



NMR-37. $^{31}P\{^{1}H\}$ NMR spectrum of PB430 (162 MHz, DMSO-d_6).



NMR-38. ^{1}H NMR spectrum of PB430 NHS ester (400 MHz, DMSO-d_6).



NMR-39. $^{31}P\{^{1}H\}$ NMR spectrum of PB430 NHS ester (162 MHz, DMSO-d_6).