Supporting Information

A sensitive fluorescent probe for alkaline phosphatase and activity assay based on the aggregation-induced emission effect

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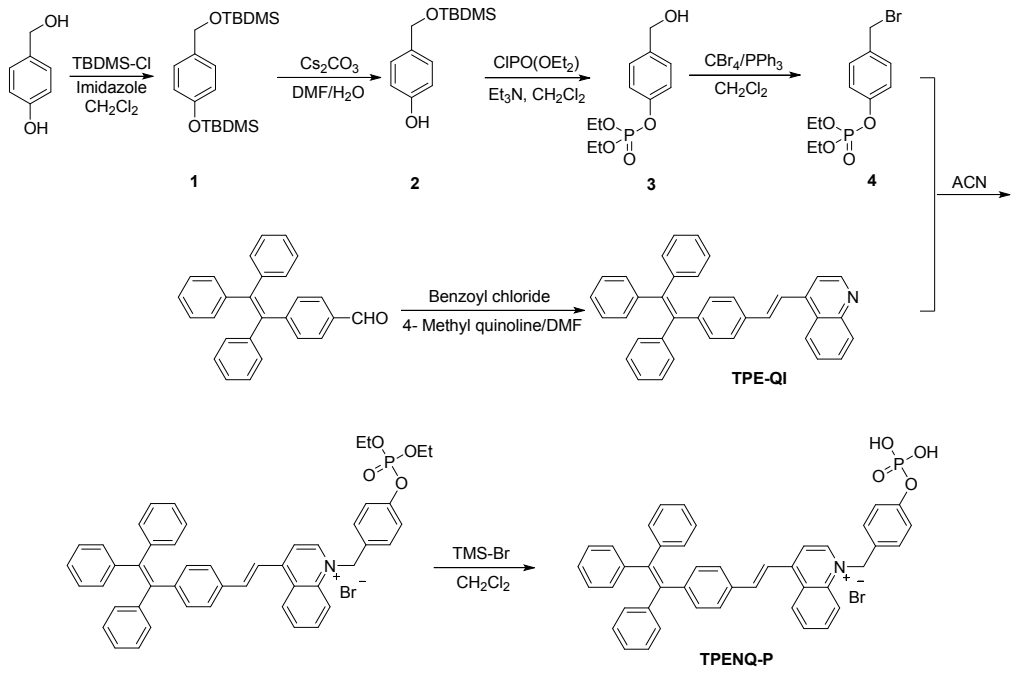

Scheme S1 The synthetic routes for TPEQN-P.

Synthesis of 1: A solution contain 4-hydroxybenzyl alcohol (1.00 g, 8.05 mmol), imidazole (2.19 g, 32.22 mmol), and *tert*-butyldimethylsilyl chloride (3.03 g, 20.13 mmol) in dry CH_2Cl_2 (40 mL) was heated at reflux overnight. The solution was then cooled to room temperature and diluted with CH_2Cl_2. Then the solution was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the product as colorless oil which used directly for the next step without further purification. ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 7.08 (d, 2H, J = 8.1 Hz), 6.72 (d, 2H, J = 8.1 Hz), 4.58 (s, 2H), 0.89 (s, 9H), 0.85 (s, 9H), 0.09 (s, 6H), 0.00 (s, 6H).

Synthesis of 2: Compound **1** (1.00 g, 2.84 mmol) and Cs_2CO_3 (0.46 g, 1.42 mmol) was dissolved in DMF/H_2O (10/1, 11 mL) and stirred at room temperature. After the reaction was completed (monitored by TLC), the reaction mixture was diluted with Et_2O (100 mL). The resultant mixture was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the resulting residue was purified by column chromatography to give the desired product in 81% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.06 (d, 2H, J = 8.3 Hz), 6.63 (d, 2H, J = 8.3 Hz), 4.56 (s, 2H), 0.83 (s, 9H), 0.00 (s, 6H).

Synthesis of 3: Compound **2** (1.17 g, 5.00 mmol), freshly distilled Et_3N (3.47 mL, 25.00 mmol) and chlorophosphoric acid diethyl ester (1.45 mL, 10.00 mmol) was dissolved in dry chloroform (10 mL) and stirred at room temperature under N_2 overnight. The reaction mixture was then concentrated under reduced pressure. And the resulting residue was purified by column chromatography to give the intermediate. Then the solution of HCl/EtOH (4/50, 54 mL) was added into above intermediate and the mixture was stirred at room temperature for 20 minutes. After that the pH of reaction mixture was adjusted 7.0 using saturated $NaHCO_3$ solution. EtOH was removed under reduced pressure and the product was extracted using Et_2O (30

mL, 3 times). Combined organic phase was dried over anhydrous Na_2SO_4 and purified by column chromatography to afford the desired product in 42% yield. ^{1}H NMR (400 MHz, MeOD) δ (ppm): 7.38 (d, 2H, J = 8.4 Hz), 7.19 (d, 2H, J = 8.2 Hz), 4.59 (s, 2H), 4.22 (q, 4H, J = 7.4 Hz), 1.35 (t, 6H, J = 7.0 Hz).

Synthesis of 4: PPh_3 (1.40 g, 5.35 mmol) was added to the pre-cooled CH_2Cl_2 solution (25 mL, 0 °C) that contained compound **3** (1.16 g, 4.46 mmol) and CBr_4 (1.77 g, 5.35 mmol). The resultant mixture was stirred for 2 hours until the reaction was finished as indicated by TLC. Then the solvent was removed under reduced pressure and residue was purified by column chromatography to give the desired product in 57% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.36 (d, 2H, J = 8.5 Hz), 7.19 (d, 2H, J = 8.3 Hz), 4.47 (s, 2H), 4.17 (q, 4H), 1.35 (t, 6H, J = 7.0 Hz).

Synthesis of TPE-QI: The 4-methyl quinoline (79.6 mg, 1.12 mmol) was dissolved in dry DMF (10 mL) and then benzoyl chloride (78.8 mg, 0.56 mmol, 0.06 mL) was added. The resultant mixture was stirred for 20 minutes at room temperature. After that, TPE-CHO (201.8 mg, 0.56 mmol) was added and the solution was refluxed for another 5 hours at 160 ^{o}C. When the reaction was completed according to TLC, the reaction mixture was cooled to room temperature, diluted with H_2O and extracted with CH_2Cl_2 for three times. Combined organic layers were washed by saturated sodium thiosulfate solution and dried by anhydrous Na_2SO_4. Then solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography to give the desired product in 63% yield. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.91 (d, J = 4.0 Hz, 1H), 8.20 (d, J = 8.0 Hz, 1H), 8.15 (d, J = 8.0 Hz, 1H), 7.82-7.72 (m, 2H), 7.62-7.58 (m, 2H), 7.41 (d, J = 8.0 Hz, 2H), 7.30 (d, J = 4.0 Hz, 1H), 7.26-7.09 (m, 16H).

Synthesis of TPEQN-P: A solution of **TPE-QI** (230.0 mg, 0.47 mmol) and compound **4** (151.90 mg, 0.47 mmol) in acetonitrile (5 mL) was heated to reflux overnight. After the reaction was completed, the reaction mixture was cooled to room temperature and solvent was removed under reduced pressure. The resulting residue was purified by column chromatography. Obtained product (140 mg, 0.17 mmol) was dissolved in anhydrous CH_2Cl_2 (5 mL), and trimethylbromosilane (10 equiv, 0.23 mL) was added dropwise. Then the mixture was stirred overnight at room temperature under N_2. After the reaction was completed, the solvent was removed under reduced pressure. The residue was dissolved in methanol (2 mL) and stirred for another 30 minutes. After that, hexane (3 mL) was added, the precipitate was filtrated off and the resultant solution was evaporated to afford the pure product as a red powder in 79% yield. ^{1}H NMR (400 MHz, MeOD) δ (ppm): 9.29 (d, 1H, J = 6.4 Hz), 8.84 (d, 1H, J = 8.5 Hz), 8.40 (d, 2H, J = 7.4 Hz), 8.15 (t, 2H, J = 12.9 Hz), 7.99 (dd, J = 16.5, 2H, 12.1 Hz), 7.67 (d, 2H, J = 8.0 Hz), 7.47 - 6.95 (m, 21H), 6.19 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ (ppm): 156.11 , 153.79, 153.73, 148.61 ,148.44, 145.06, 144.82, 144.69, 144.58 ,141.64,139.85, 136.58, 135.06, 133.26 , 132.41, 132.31 , 130.74, 130.66, 129.94 , 129.42 , 128.98, 128.80 , 127.98 , 127.87, 122.35, 122.31, 120.51 , 120.46, 117.72 , 60.75. HRMS (ESI-TOF) m/z: $[M - Br]^+$ calcd for 672.2298, found, 672.2292.

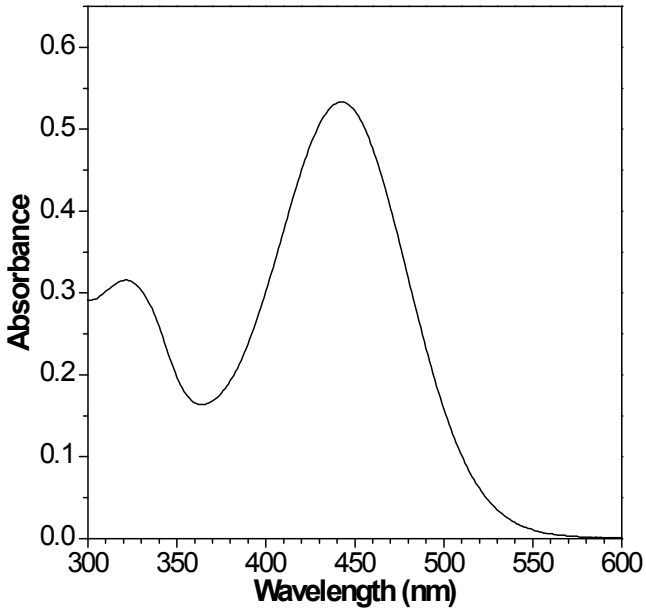

Fig. S1 Absorption spectra of TPEQN-P (10 μM) in DMSO.

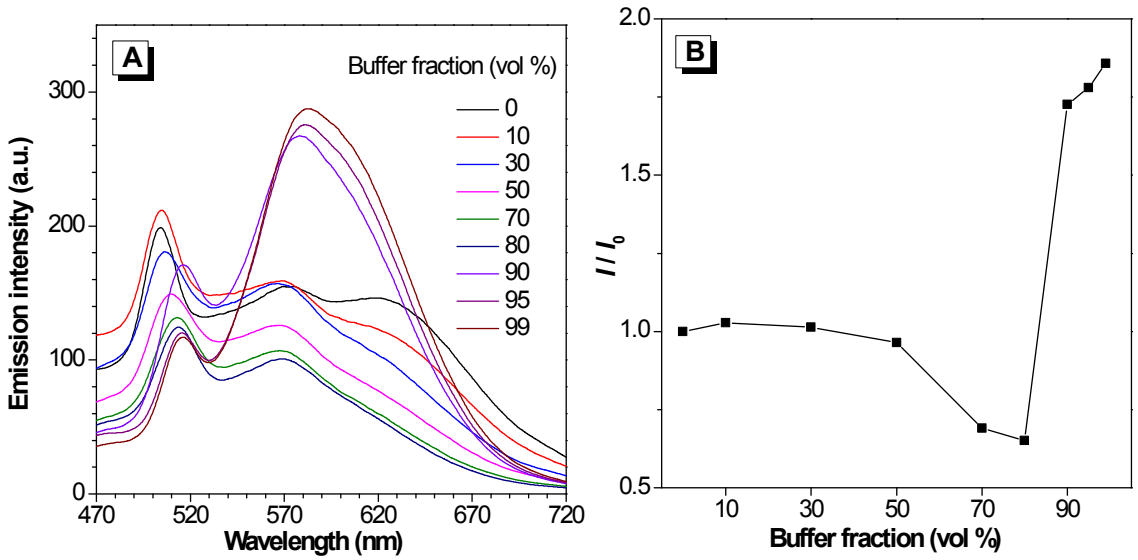

Fig. S2 (A) Emission spectra of TPEQN-P (10 μM) in DMSO and DMSO/Tris–HCl buffer (10 mM, pH = 9.0) mixtures with different buffer fractions. (B) Plots of emission intensity at 590 nm versus the composition of the buffer mixtures of TPEQN-P, where I_0 is the emission intensity at 590 nm of TPEQN-P in pure DMSO.

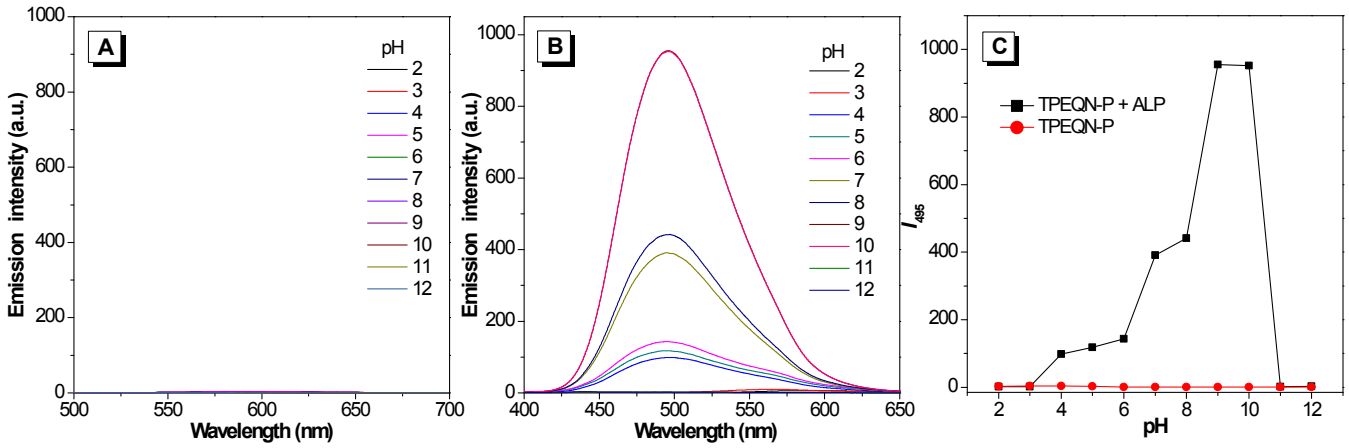

Fig. S3 Emission spectra of TPEQN-P (10 μM) without (A) and with ALP (100 mU mL^{-1}) (B) under different pH. (C) Plots of emission intensity at 590 nm without and with ALP versus the pH.

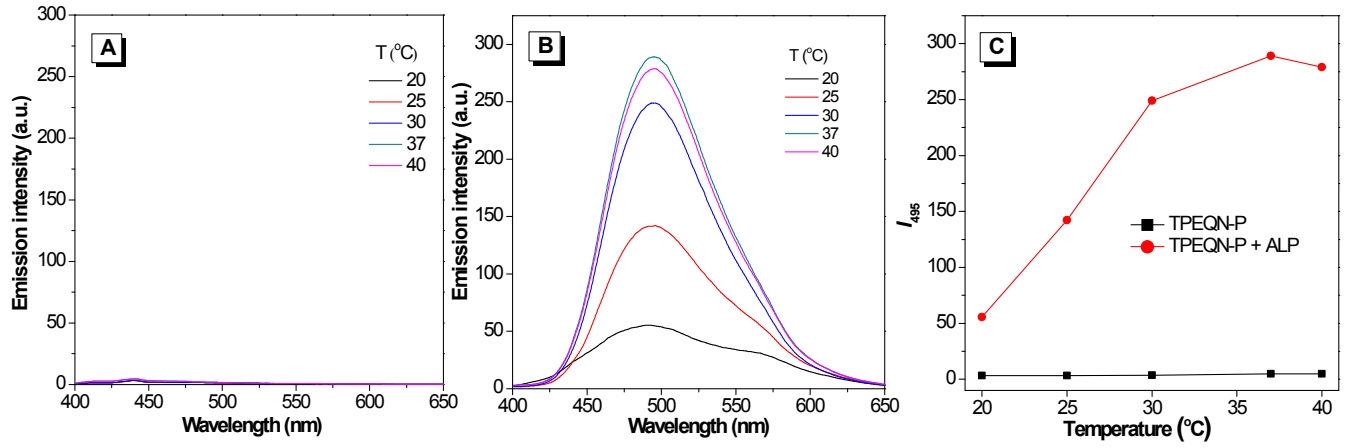

Fig. S4 Emission spectra of TPEQN-P (10 μM) without (A) and with ALP (100 mU mL^{-1}) (B) under different temperature. (C) Plots of emission intensity at 590 nm without and with ALP versus the temperature.

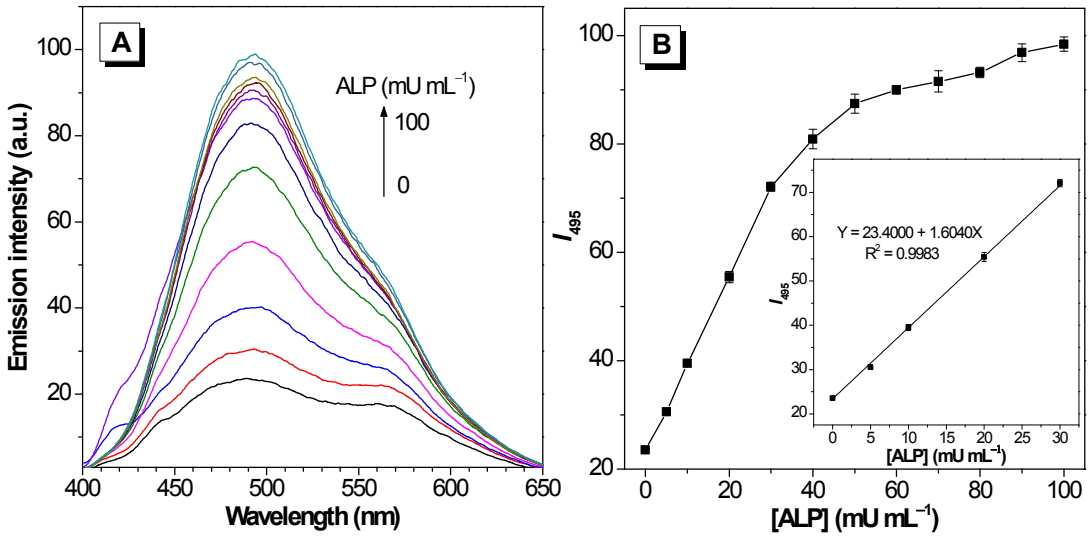

Fig. S5 (A) Emission spectra of TPEQN-P (10 μM) after addition of ALP with different concentrations. (B) Plots of emission intensity at 495 nm versus the concentration of ALP. Inset in B: the linear relationship between intensity at 495 nm and the concentration of ALP (0-30 mU mL^{-1}).

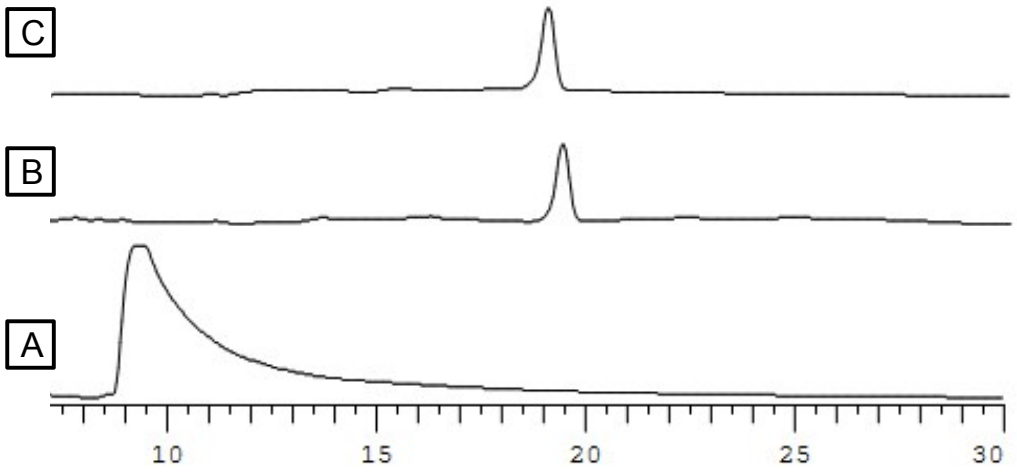

Fig. S6 HPLC chromatograms of (A) TPEQN-P, (B) TPEQN-P incubated with ALP (100 mU mL^{-1}) for 60 min and (C) TPE-QI.

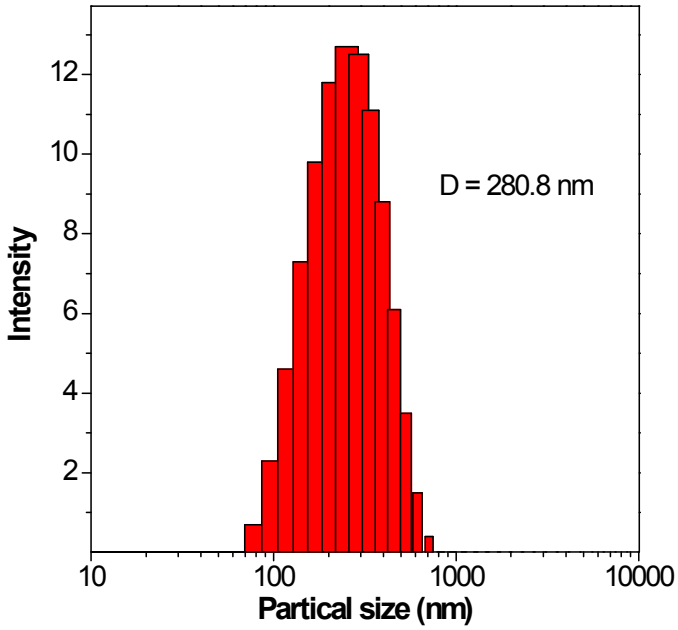

Fig. S7 Size distribution of TPEQN-P (10 μM) after incubated with ALP (100 mU mL^{-1}).

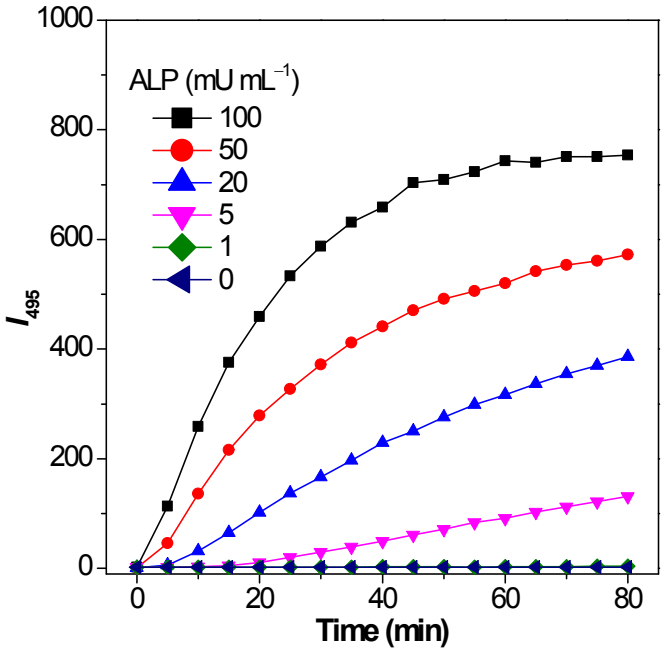

Fig. S8 Time-dependent fluorescence intensity of ALP at different concentrations (0-100 mU mL^{-1}) incubated with TPEQN-P (10 μM).

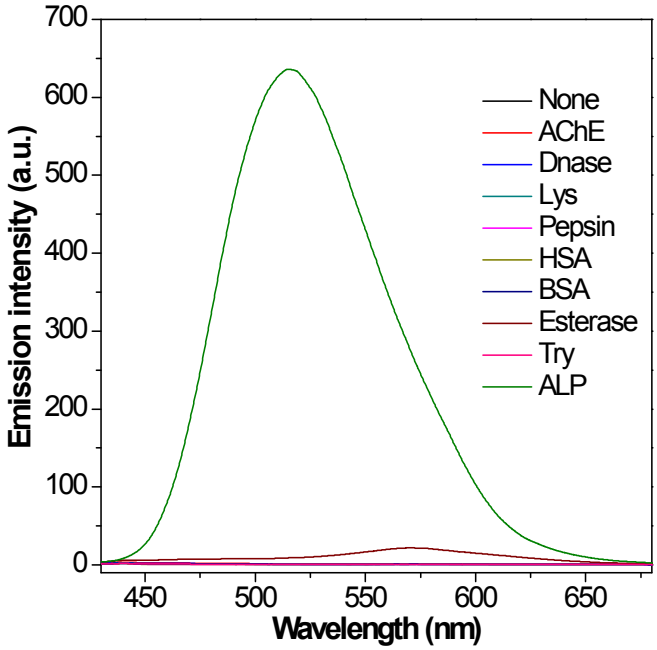

Fig. S9 Emission spectra of TPEQN-P (10 μM) without and with different proteins.

Table S1 Comparison of different probes for the detection of ALP.

No.	Mechanism	Linear range ($mU\ mL^{-1}$)	LOD of ALP ($U\ L^{-1}$)	Reference
1	AIE	0-30	0.0077	Present work
2	ESIPT	5-100	1.36	S1
3	ICT	0-100	0.07	S2
4	Complexation/PET	0.1-1000	0.08	S3
5	Reduction	0-120	5.4	S4
6	ICT	50-200	3.8	S5
7	ICT	0-5	0.38	S6
8	PET	0.01-1000	1.09	S7
9	Complexation	0.1-10	0.1	S8
10	ESIPT+AIE	0-150	0.15	S9
11	AIE	3-526	0.2	S10
12	AIE	0-100	18	S11
13	AIE	0.3-7.5	0.3	S12

References:

S1. H. W. Liu, K. Li, X. X. Hu, L. M. Zhu, Q. M. Rong, Y. C. Liu, X. B. Zhang, J. Hasserodt, F. L. Qu and W. H. Tan, *Angew. Chem. Int. Ed.*, 2017, **56**, 11788-11792.

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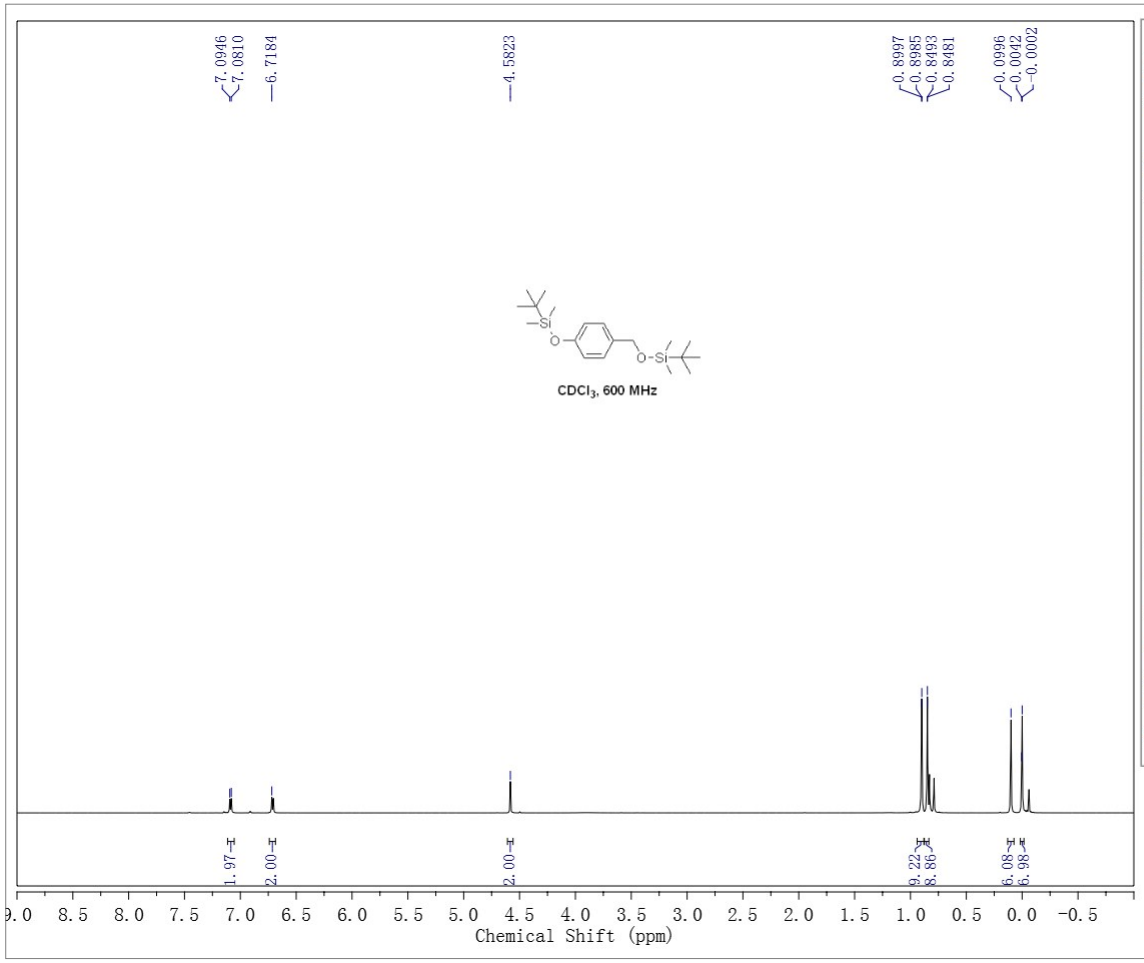
S12. L. L. Zhang, J. J. Zhao, M. Duan, H. Zhang, J. H. Jiang, and R. Q. Yu, *Anal. Chem.,* 2013, **85**, 3797-3801.



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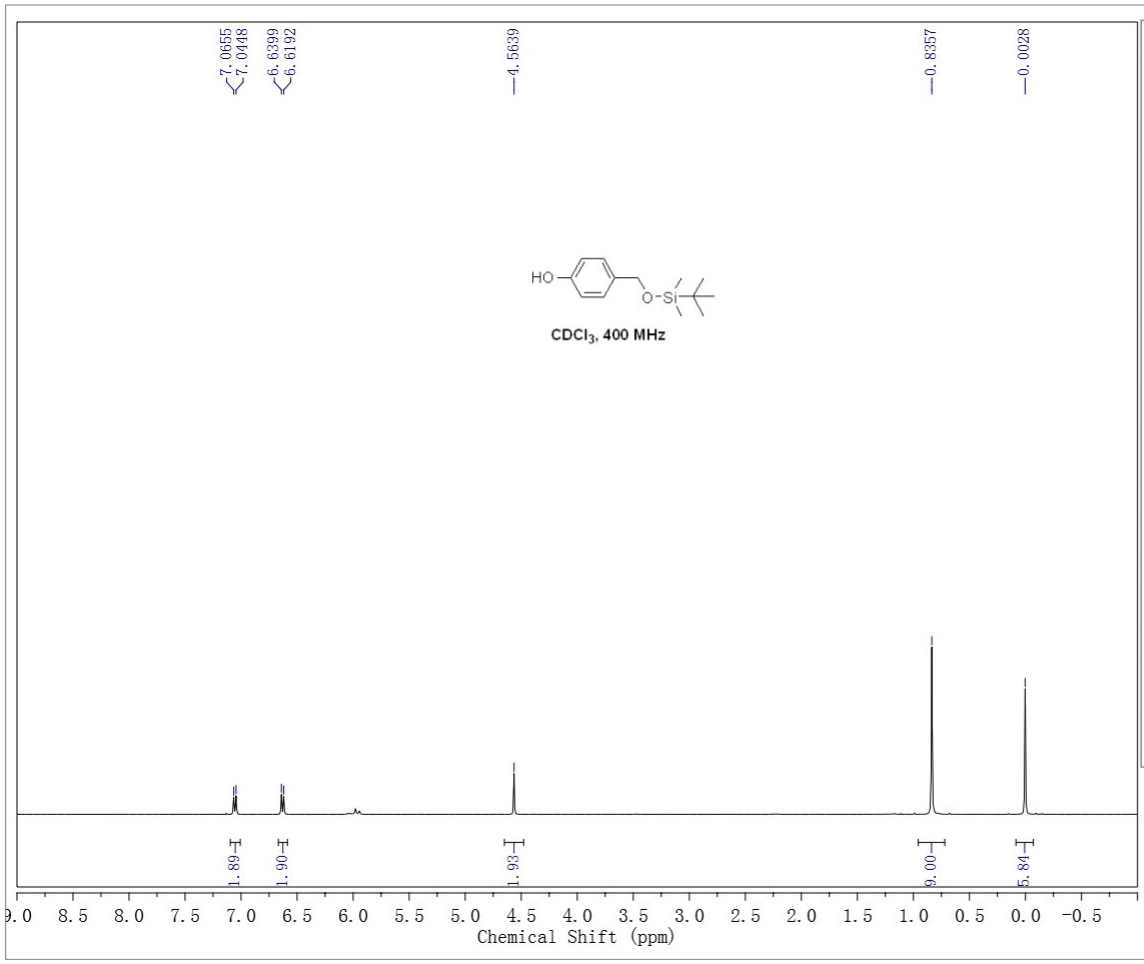

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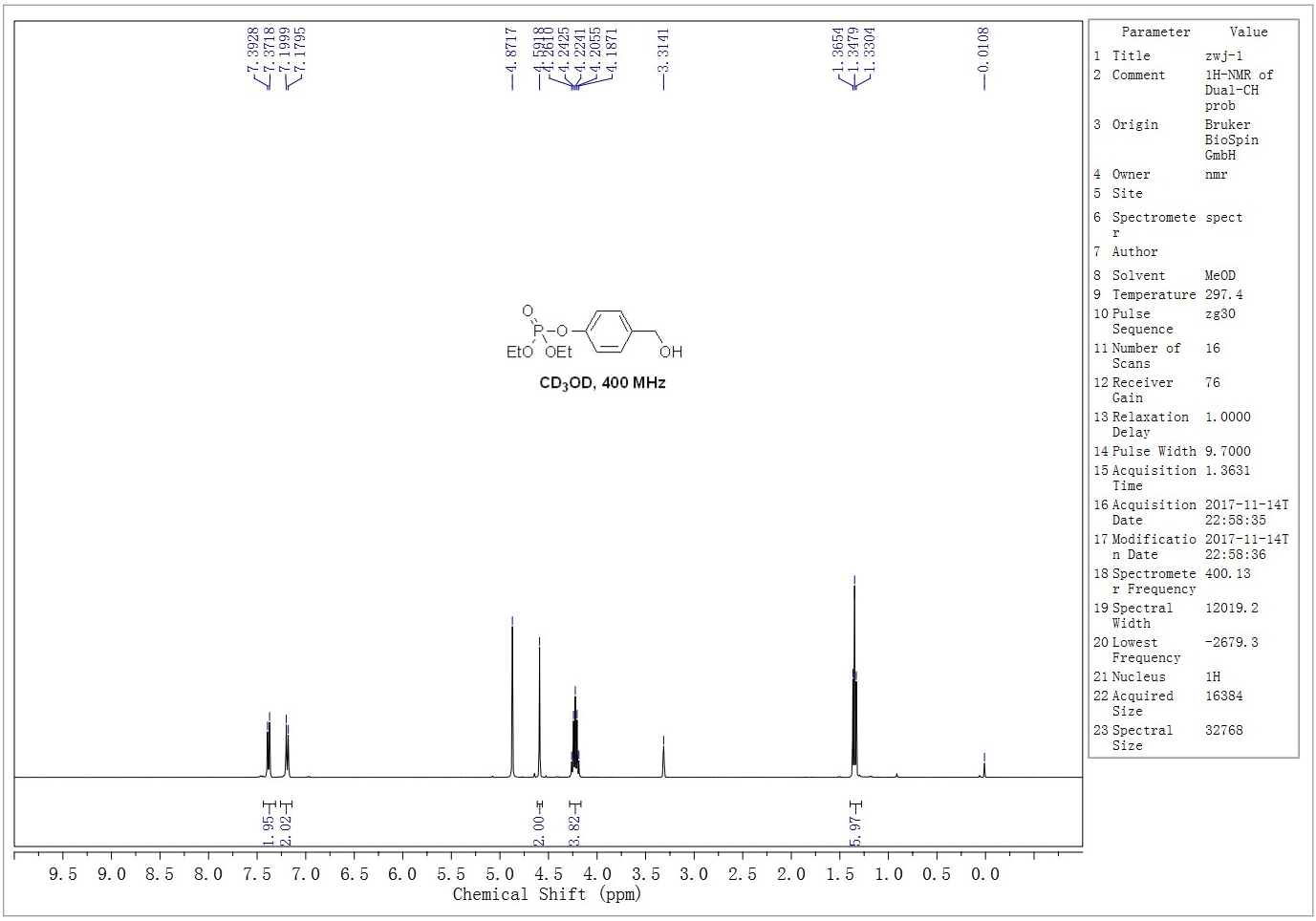

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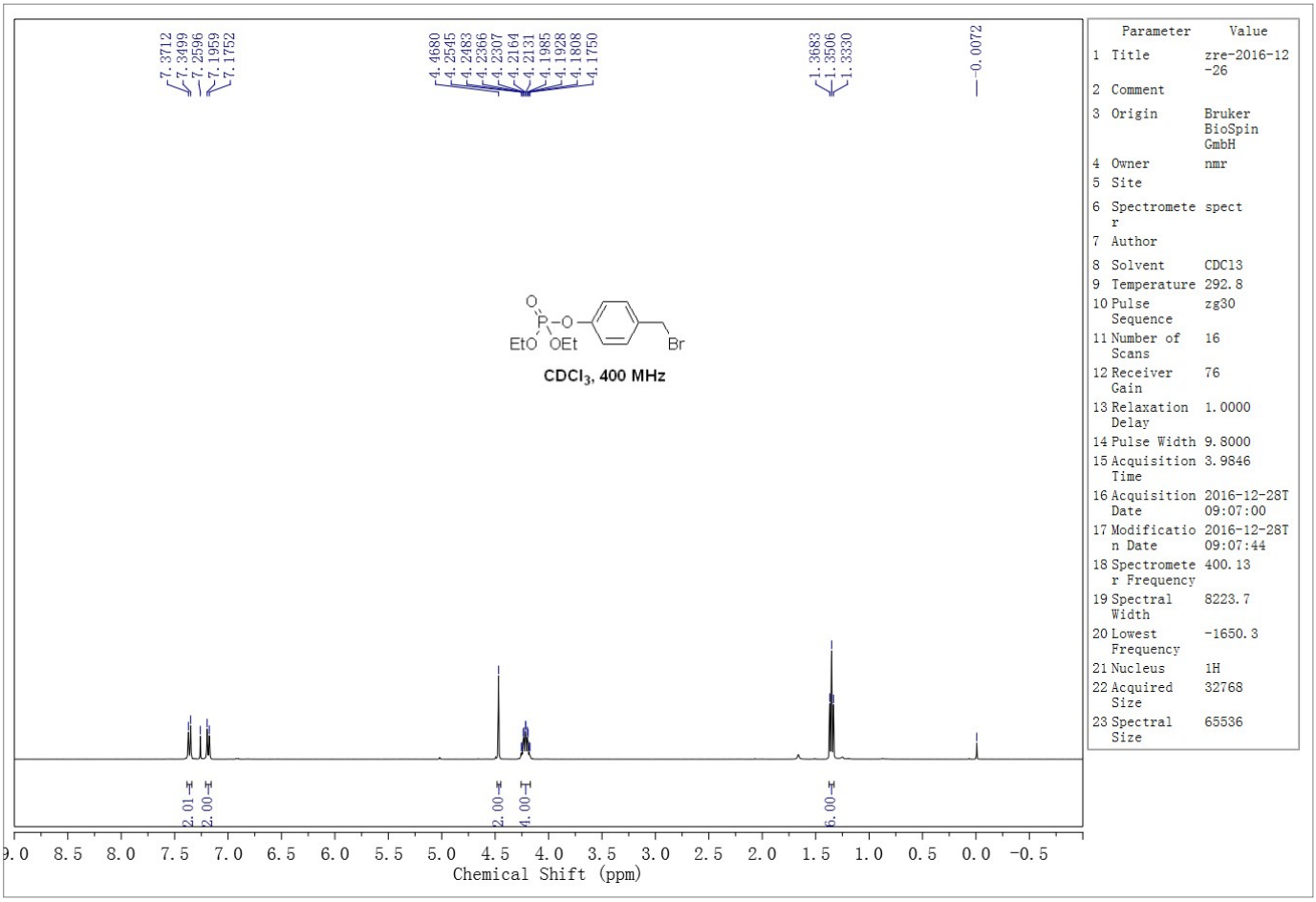

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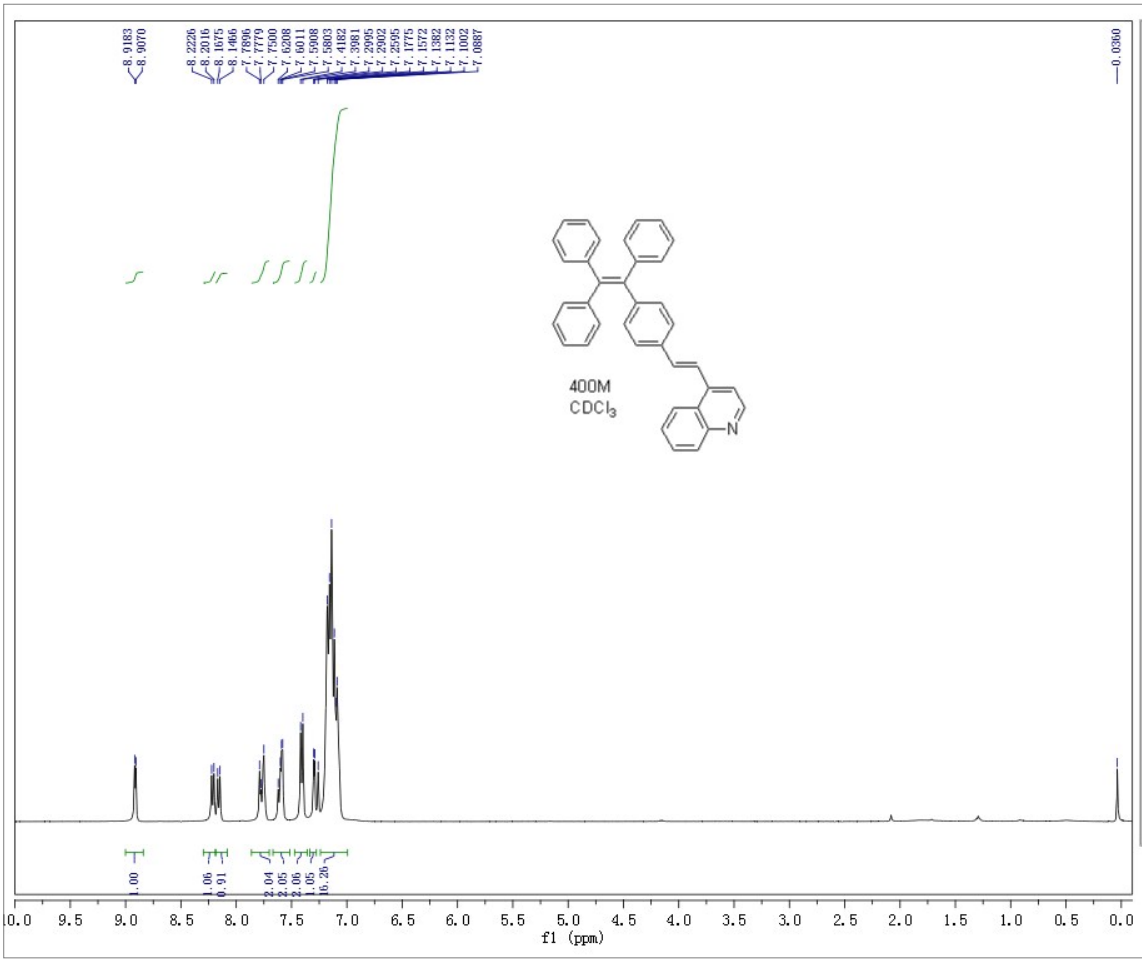

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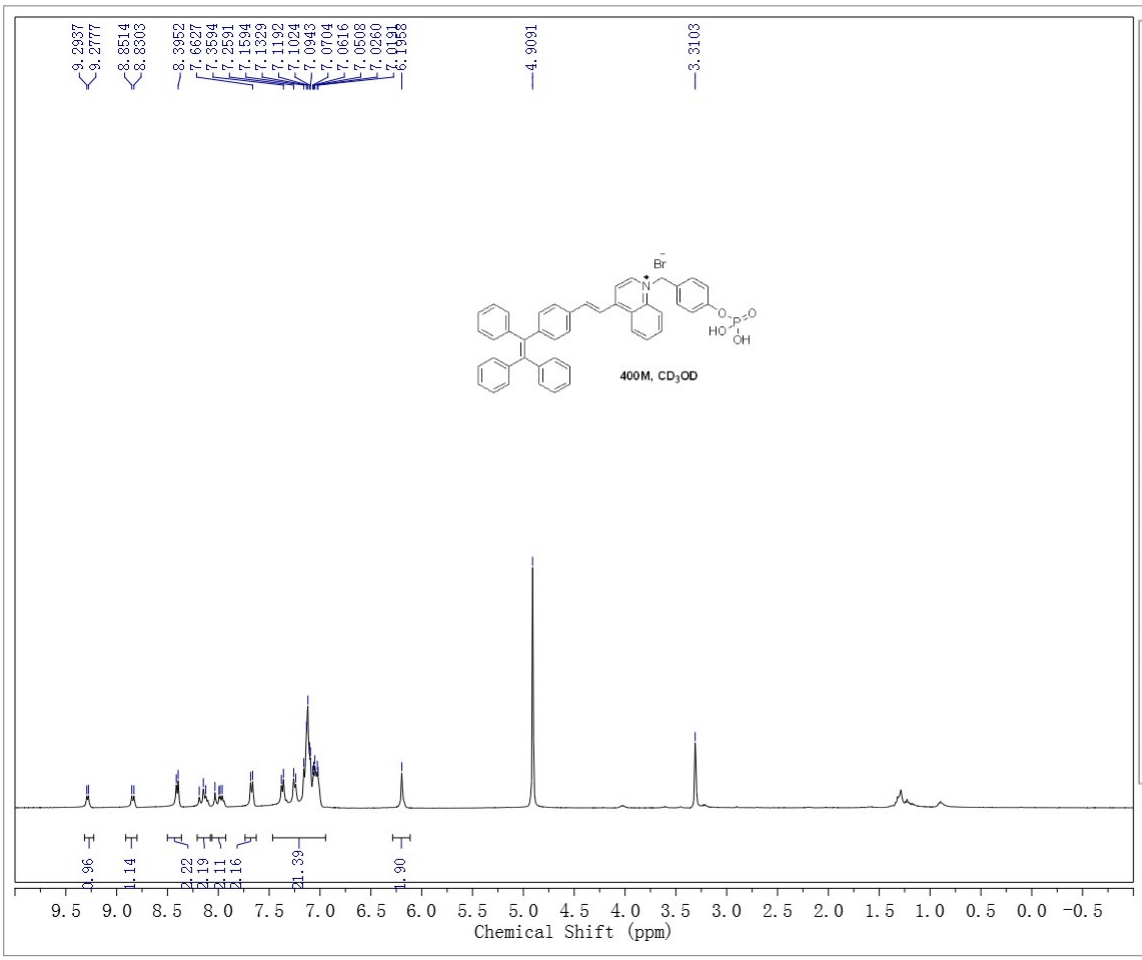

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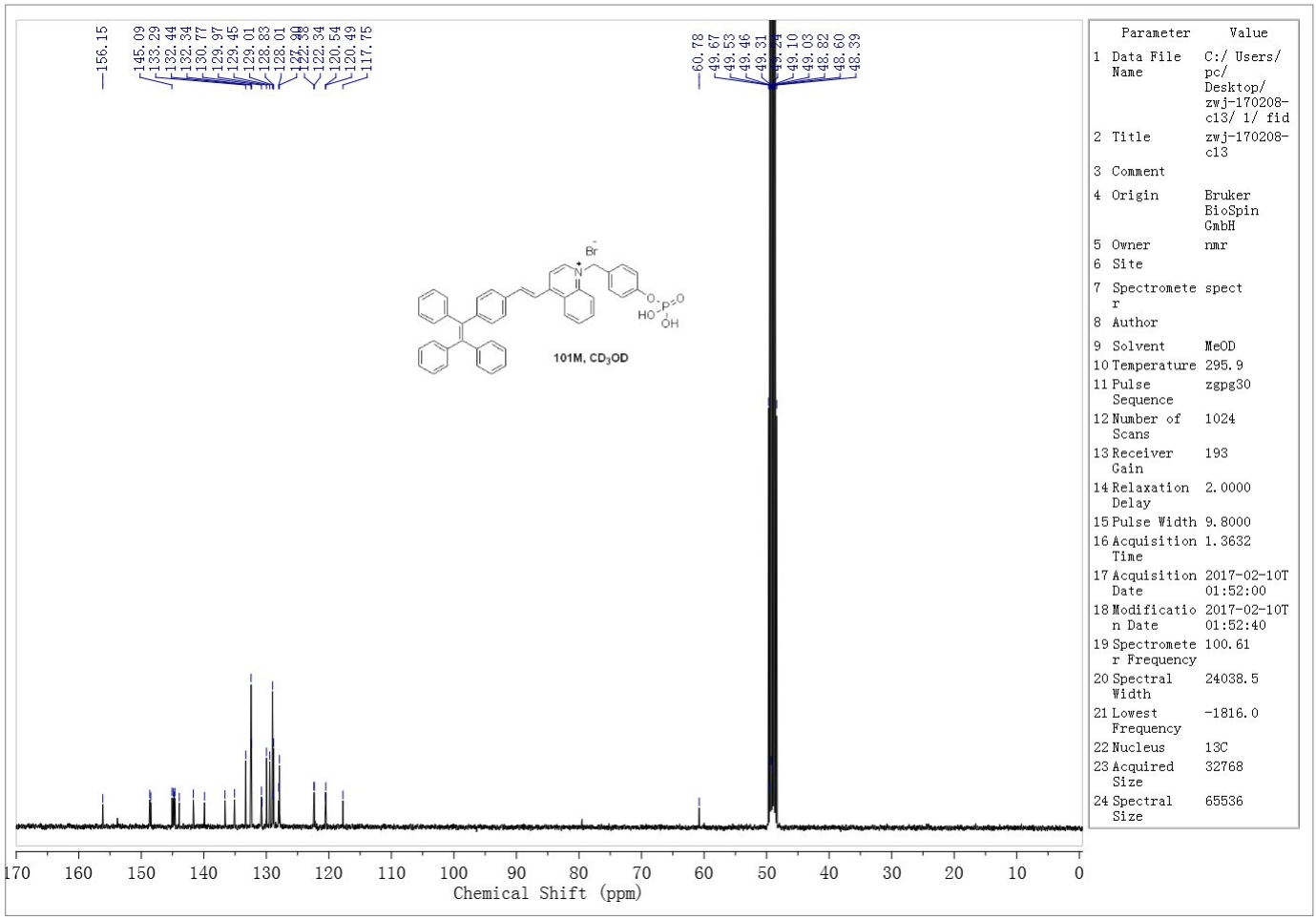

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