Supporting Information

A Ni-NTA-based Red Fluorescence Probe for Protein Labelling in Live Cells†

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Synthesis of Compound 1–7

Compound 1: **1** is synthesized based a previous report[1] with modifications. In a 100 mL reaction flask, 4-formylbenzoic acid (500 mg, 3.3 mmol), 2,4-dimethylpyrrole (634 mg, 6.7 mmol, 2 molar equiv.) and DCM (dichloromethane, 25 mL) were added. The mixture was stirred for 20 min at room temperature under nitrogen. Trifluoroacetic acid (26 μL) was added and stirred overnight. To the reaction mixture, chloranil (1.638 g, 6.6 mmol, 2 molar equiv. in 5 ml DCM) was added with continuous stirring. After 4 hrs, trimethylamine (4 mL, 28 mmol, 8.6 molar equiv.) was added and stirred for 30 min. Boron trifluoride (4 mL, 17 mmol, 5 molar equiv.) was added and stirred for another 4 hrs. The mixture was diluted with water (50 mL) and extracted with DCM (6 × 20 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and filtered. The solvent of the filtrate was removed via vacuum evaporation. The compound was purified by column chromatography with DCM/EA (2/1 v/v) with 0.2% acetic acid to afford **5** (210 mg, 18% yield) as brown solid. ^{1}H NMR (300 MHz, $CDCl_3$+0.5%TFA) δ 8.26 (d, J = 8.1 Hz, 2H), 7.49 (d, J = 8.1 Hz, 2H), 6.02 (s, 2H), 2.55 (s, 6H), 1.37 (s, 6H). ESI-MS (m/z): $[M-H]^-$ calcd. 367.2, obsd. 367.3.

Compound 2-a and 2-b: **1** (110 mg, 0.3 mmol) and 4-acetamidobenzaldehyde (for compound **2-a**: 48.9 mg, 0.3 mmol; for compound **2-b**: 114.2 mg, 0.7 mmol) were dissolved in toluene (12 mL). Piperidine (0.32 mL) and acetic acid (0.2 mL) were added to the solution and refluxed overnight in a Dean-Stark apparatus (Reflux at 180 °C for compound **2-b** and at 140–160 °C for compound **2-a**). The solvent was evaporated under reduced pressure, and the residue was extracted with ethyl acetate. The combined organic layer was washed with water and dried over anhydrous Na_2SO_4. The product was concentrated by evaporation and purified by column chromatography on silica gel with DCM/MeOH (20/1 v/v to 10/1 v/v with 0.1 % acetic acid for compound **2-b)** or ethyl acetate/DCM (1/1 v/v with 0.2 % acetic acid for compound **2-a**) eluent, yielding **2-a** (22 mg, 14.3 %) and **2-b** (88 mg, 46.4%). **2-a**: ESI-MS (*m/z*): $[M-H]^-$calcd. 512.3, obsd. 512.3; **2-b**: ESI-MS (*m/z*): $[M-H]^-$calcd. 657.5, obsd. 657.3.

Compound 3: The azide formation was modified based on a previous report[2]. Boc-Phe(4-NH_2)-OH (200 mg, 0.71 mmol) was dissolved in a mixture of water (1.4 mL) and THF (0.6 mL). HCl (12 M, 0.2 mL) was added at 4 °C, followed by $NaNO_2$ (74 mg, 1.07 mmol) in 0.6 mL of water. The mixture was stirred at this temperature for 30 min. NaN_3 (232 mg, 3.56 mmol) in water (0.6 mL) was then added. The stirring continued for another 30 min. The reaction mixture was extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4, and concentrated to afford **3** (Yellow

product, 0.16 g, 84%). ^{1}H NMR (300 MHz, MeOH) δ 7.24 (d, J = 7.8 Hz, 2H), 6.95 (d, J = 7.8 Hz, 2H), 4.35 (dd, J = 8.0, 4.9 Hz, 1H), 3.14 (dd, J = 13.7, 4.6 Hz, 1H), 2.89 (dd, J = 13.5, 9.0 Hz, 1H), 1.38 (s, 9H). ESI-MS (*m/z*): $[M+Na]^+$calcd. 329.1, obsd. 329.1.

Compound 4: 4 was synthesized based on our previous report[3].

Compound 5: **4** (110 mg, 0.359 mmol) and HATU (273 mg, 0.719 mmol) was dissolved in 5 mL DCM, followed by the addition of DIEA (188 μL, 1.078 mmol) and **3** (115 mg, 0.377 mmol) in 10 mL DCM and 0.2 mL DMF. The reaction was left for 1–2 hrs. TLC (10% Methanol in DCM) was used to check the reaction. The reaction mixture was then diluted with DCM and extracted by 5% acetic acid and 10% $NaHCO_3$. The organic phase was dried by Na_2SO_4, filtered and rotary-evaporated to afford **5**. Flash chromatography (eluent: 3% methanol in DCM with 0.2% acetic acid) was used to purify the product (160 mg, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.17 (d, J = 8.1 Hz, 2H), 6.90 (d, J = 8.4 Hz, 2H), 5.40 (d, J = 8.3 Hz, 1H), 4.41 – 4.23 (m, 1H), 3.70 – 3.61 (m, 10H), 3.58 (s, 4H), 3.42 – 3.30 (m, 1H), 3.22 – 3.11 (m, 2H), 3.10 – 2.99 (m, 1H), 2.98 – 2.84 (m, 1H), 1.70 – 1.56 (m, 2H), 1.39 (s, 4H), 1.32 (s, 9H). ESI-MS (*m/z*): $[M+H]^+$calcd. 615.3, obsd. 615.3.

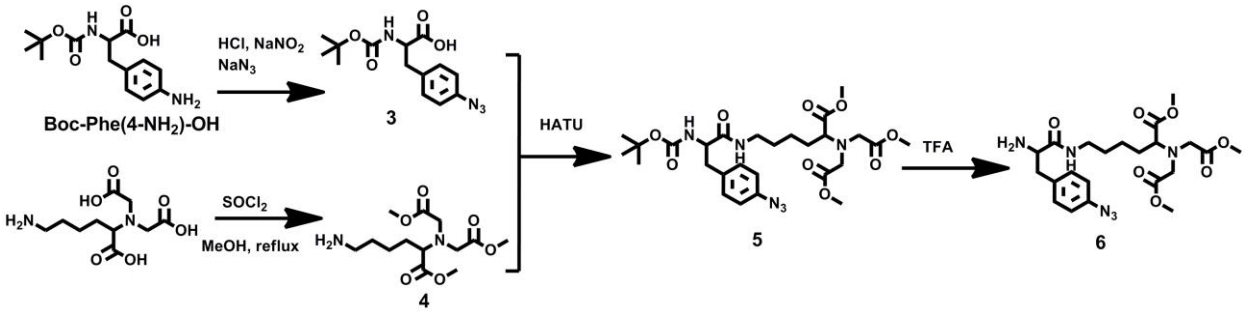
Compound 6: **5** (50 mg, 0.102 mmol) was dissolved in 9 mL of DCM and 3 mL of TFA under stirring for about 1 hr. Then diethyl ether was added and all the solvent and TFA was blown away by condensed air to afford **6** (about 100% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (s, 1H), 7.23 (d, *J* = 6.6 Hz, 2H), 6.97 (d, *J* = 7.3 Hz, 2H), 4.38 (s, 1H), 3.69 (s, 3H), 3.68 (s, 6H), 3.63 (s, 1H), 3.56 – 3.46 (m, 2H), 3.40 (s, 2H), 3.25 – 3.08 (m, 2H), 3.08 – 2.94 (m, 1H), 1.88 – 1.71 (m, 1H), 1.68 – 1.54 (m, 1H), 1.51 – 1.38 (m, 2H), 1.36 – 1.27 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.27, 172.68, 168.46, 139.53, 130.99, 119.38, 77.42, 77.10, 76.78, 64.18, 55.01, 52.88, 51.99, 51.59, 38.93, 36.84, 29.72, 29.40, 27.80, 22.33. ESI-MS (*m/z*): $[M+H]^+$calcd. 493.2, obsd. 493.4.

Compound 7: A solution of **2-b** (29 mg, 0.044 mmol), EDC·HCl (67 mg, 0.35 mmol), HOBt (47.0 mg, 0.35 mmol), DIPEA (67 ml, 0.5 mmol, 10 molar equiv.), and **6** (53 mg, 0.087 mmol, 2.0 molar equiv.) was stirred in 1 mL DMF at room temperature under argon atmosphere. The reaction mixture was stirred for overnight until **2-b** was consumed. The reaction mixture was diluted with 50 mL DCM and washed with 10% $NaHCO_3$, water, 2% HCl and brine. The organic phase was collected, dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by 3–5% MeOH in DCM with 0.2% acetic acid to afford **7** (35 mg, 70% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (s, 2H), 7.92 (d, *J* = 7.9 Hz, 2H), 7.60 – 7.48 (m, 6H), 7.48 – 7.39 (m, 4H), 7.32 (d, *J* = 7.8 Hz, 2H), 7.26 (d, *J* = 4.1 Hz, 2H), 7.16 (d, *J* = 16.1 Hz, 2H), 6.94 (d, *J* = 8.3 Hz, 2H), 6.80 – 6.69 (m, 1H), 6.61 (s, 2H), 4.96 (dd, *J* = 14.0, 7.0 Hz, 1H), 3.70 (d, *J* = 1.3 Hz, 9H), 3.65 – 3.49 (m, 5H), 3.40 (t, *J* = 7.6 Hz, 1H), 3.32 – 3.08 (m, 4H), 2.07 (s, 9H), 1.66 (dd, *J* = 6.2 Hz, 3H), 1.54 – 1.39 (m, 4H), 1.35 (s, 6H), 1.30 – 1.27 (m, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 173.22, 172.12, 170.81, 168.99, 166.26, 153.02, 141.88, 139.26, 138.84, 138.70, 136.58, 134.52, 133.58, 132.91, 132.04, 130.91, 129.02, 128.42, 127.99, 119.82, 119.16, 118.26, 117.55, 77.40, 77.29, 77.09, 76.77, 64.21, 55.08, 52.65, 51.92, 51.61, 39.25, 38.49, 29.44, 28.01, 24.38, 22.73, 14.92, 14.18. ESI-MS (*m/z*): $[M+Na]^+$calcd. 1155.5, obsd. 1155.3.

Reference

(1) A. Singh, W. T. Yip and R. L. Halterman, *Org Lett*, 2012, **14**, 4046.

(2) Y. S. Wang, X. Q. Fang, H.Y. Chen, B. Wu, Z. Y. U. Wang, C. Hilty and W. S. R. Liu, *ACS Chem Biol*, 2013, **8**, 405.
(3) Y. T. Lai, Y. Y. Chang, L. G. Hu, Y. Yang, A. L. Chao, Z. Y. Du, J. A. Tanner, M. L. Chye, C. M. Qian, K. M. Ng, H. Y. Li and H. Sun, *Proc Natl Acad Sci USA*, 2015, **112**, 2948.



Scheme S1. Coupling of the photoactive cross-linker with NTA moiety.

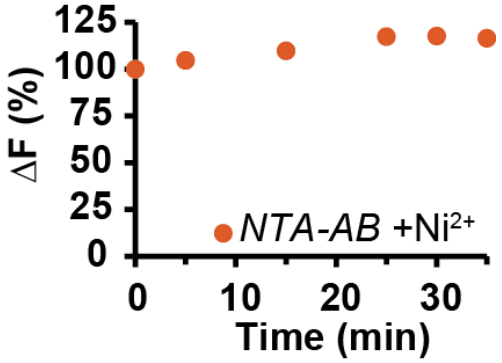


Fig. S1. The normalized fluorescence changes of *NTA-AB* upon the addition of Ni^{2+} (as $NiSO_4$). The formation of Ni-*NTA-AB* accomplished within 40 min.

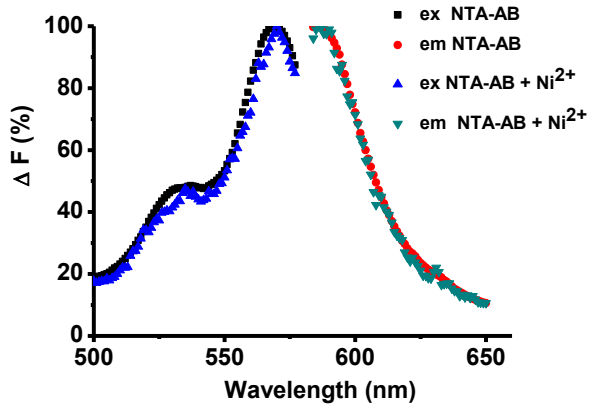


Fig. S2. The normalized excitation and emission spectra of *NTA-AB* (5 μM) and Ni-*NTA-AB* (5 μM). Both *NTA-AB* and Ni-*NTA-AB* exhibit the excitation wavelength of 570 nm and the emission wavelength of 590 nm.

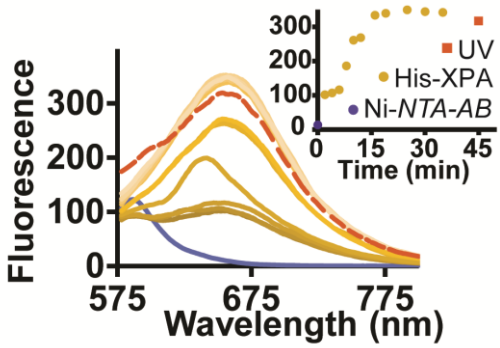


Fig. S3. The fluorescence responses of Ni-*NTA-AB* for His-XPA122 addition over time. The samples were then subjected to UV irradiation at 365 nm for 10 min.

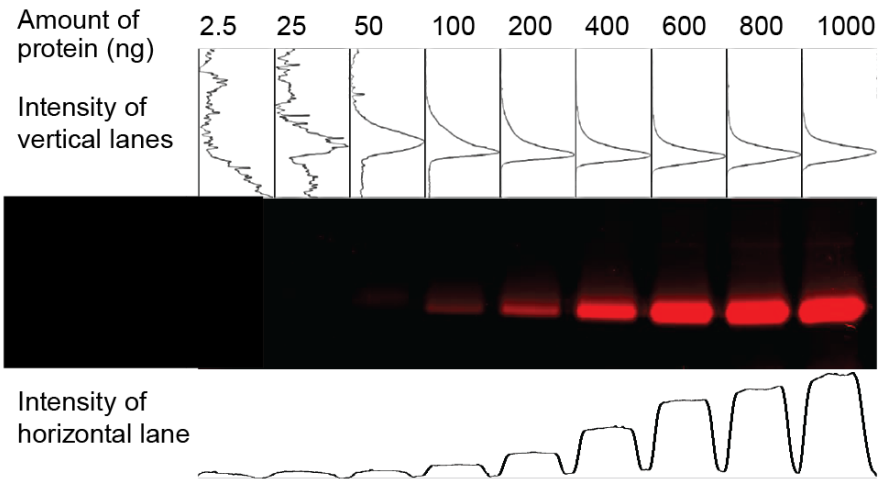


Fig. S4. Determination the detection limit of Ni-*NTA-AB* and quantification of the fluorescence protein bands in SDS-PAGE by ImageJ.

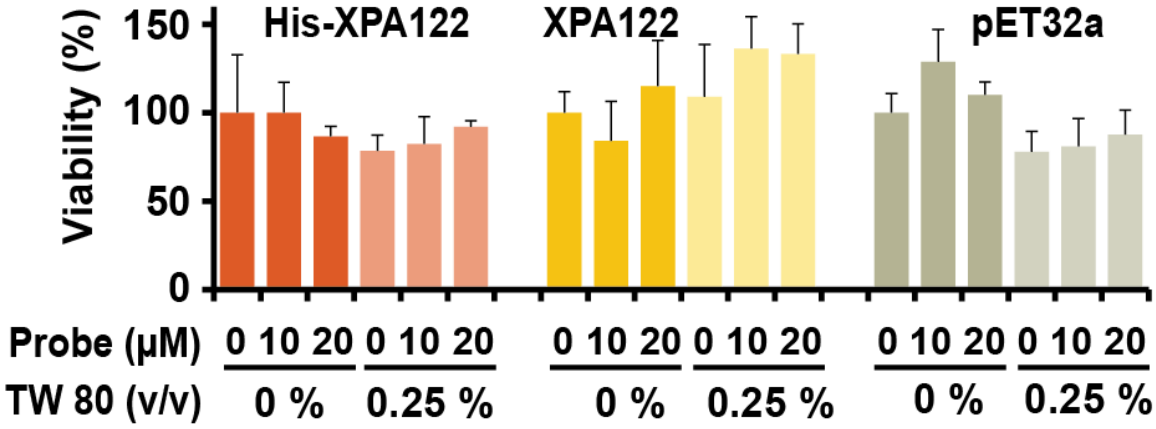


Fig. S5. Toxicity of Ni-*NTA-AB* in *E. coli* cells. Ni-*NTA-AB* (10 and 20 μM, with or without 0.25 % Tween 80) was incubated with *E. coli* cells. The toxicity was tested by counting the number of CFU after plating (n= 3).

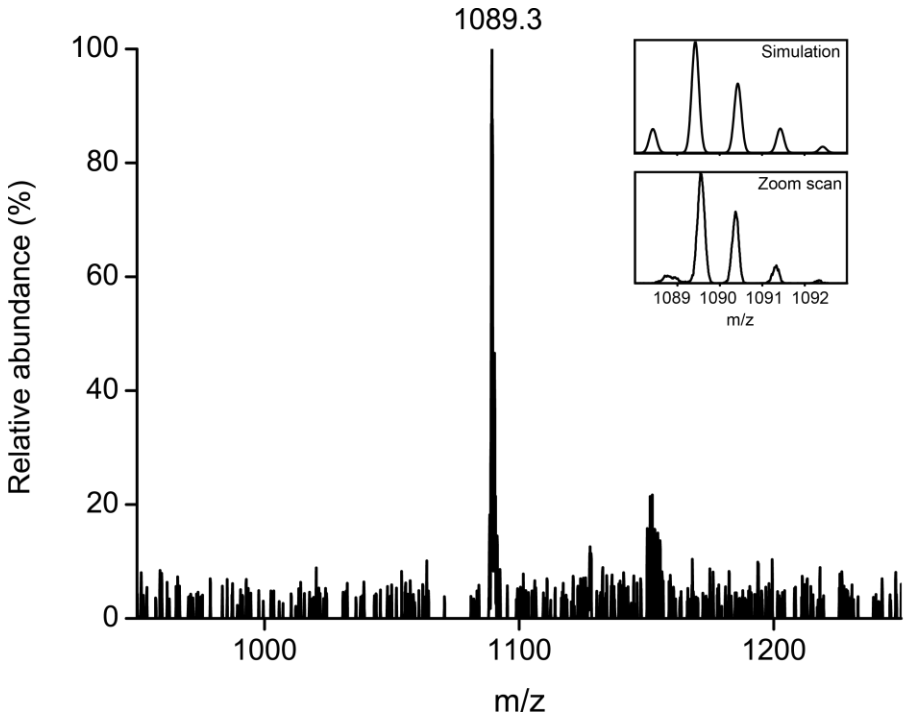


Fig. S6. ESI-MS of *NTA-AB*. The ion at m/z 1089.3 corresponding to $[M\text{-}H]^-$(cald. 1089.4). *Inset*: the pattern of isotopic distribution was identical to the simulation results.

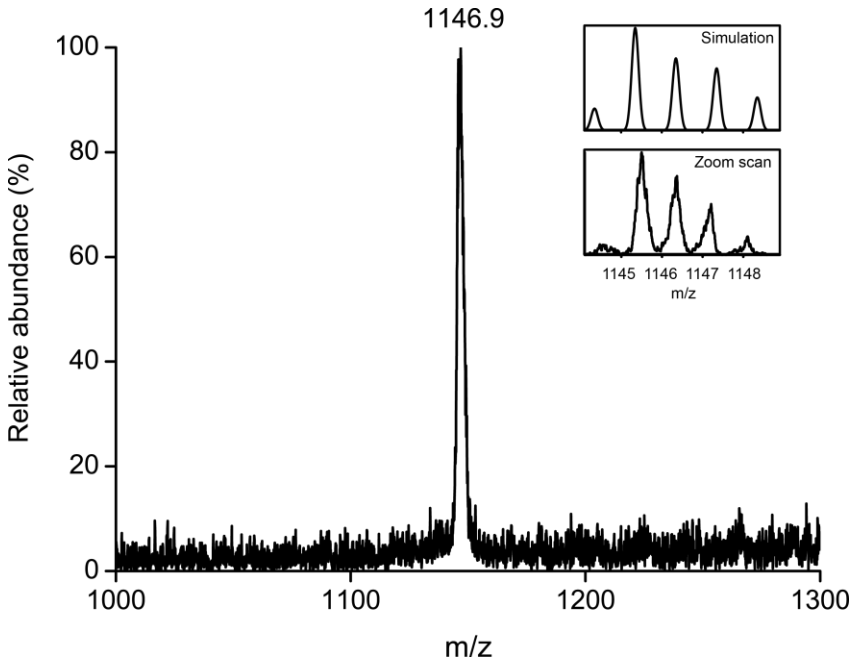


Fig. S7. ESI-MS of Ni-*NTA-AB*. The ion at m/z 1146.9 corresponding to $[M\text{-}3H+Ni^{2+}]^-$(cald. 1145.3). *Inset*: the pattern of isotopic distribution was identical to the simulation results.

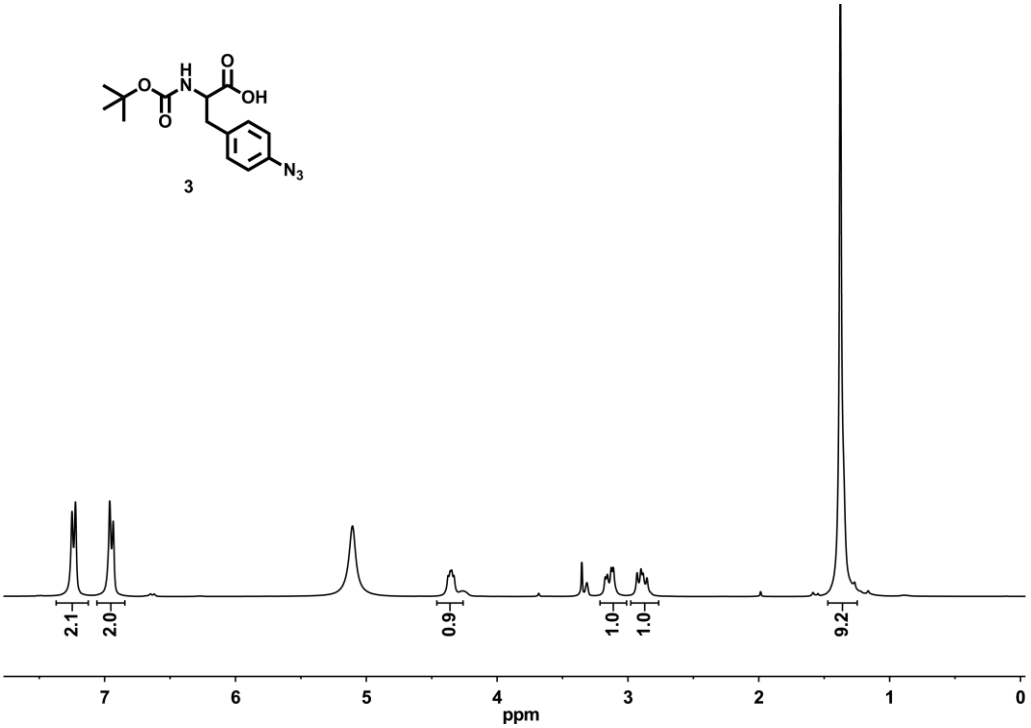


Fig. S8. ^{1}H NMR spectrum of **3.**

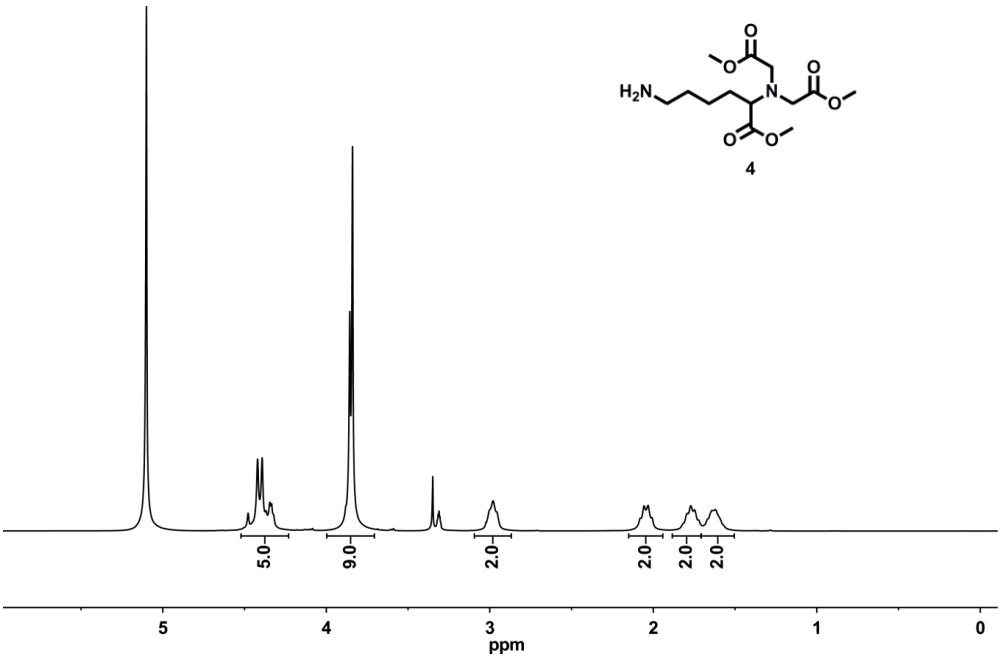


Fig. S9. ^{1}H NMR spectrum of **4.**

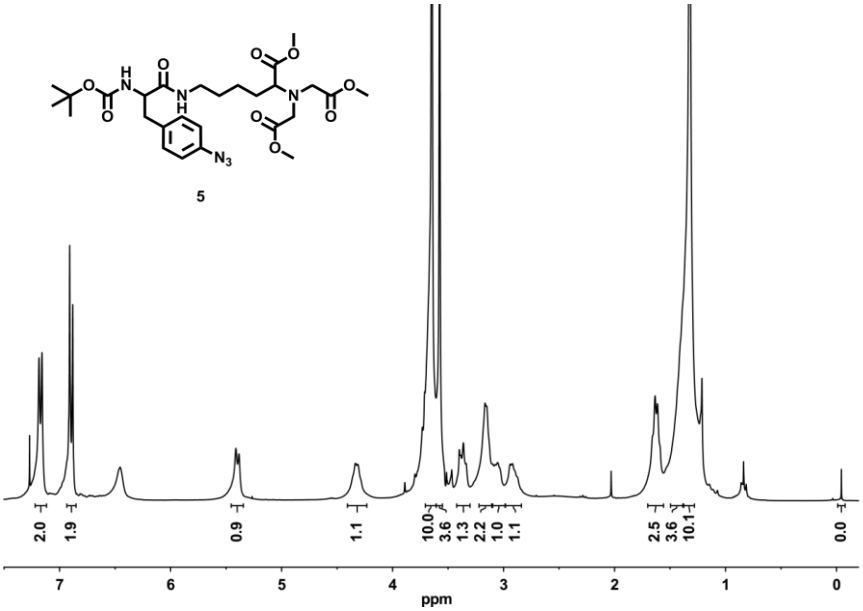


Fig. S10. ^{1}H NMR spectrum of **5.**

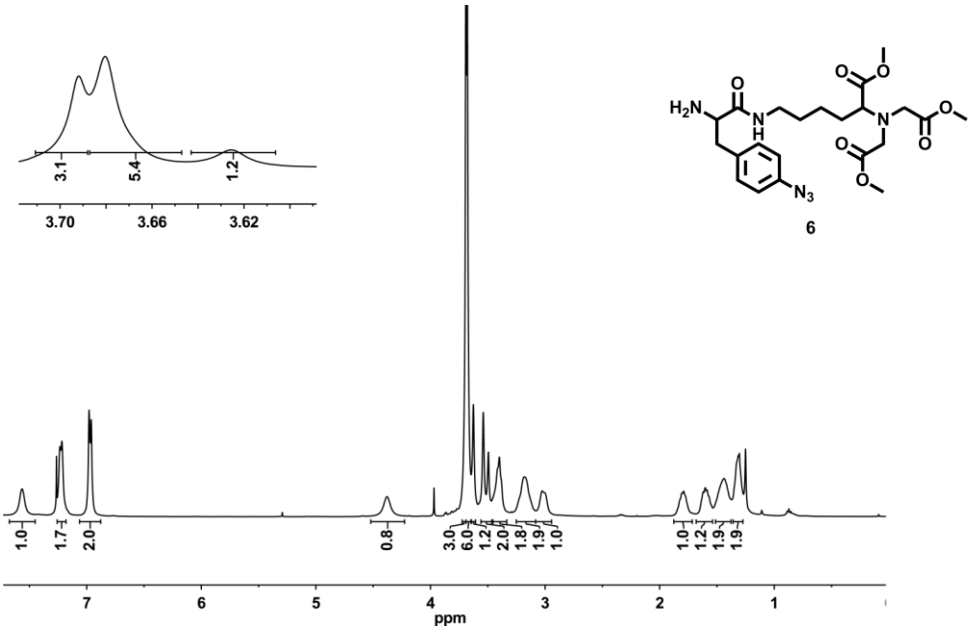


Fig. S11. ^{1}H NMR spectrum of **6.**

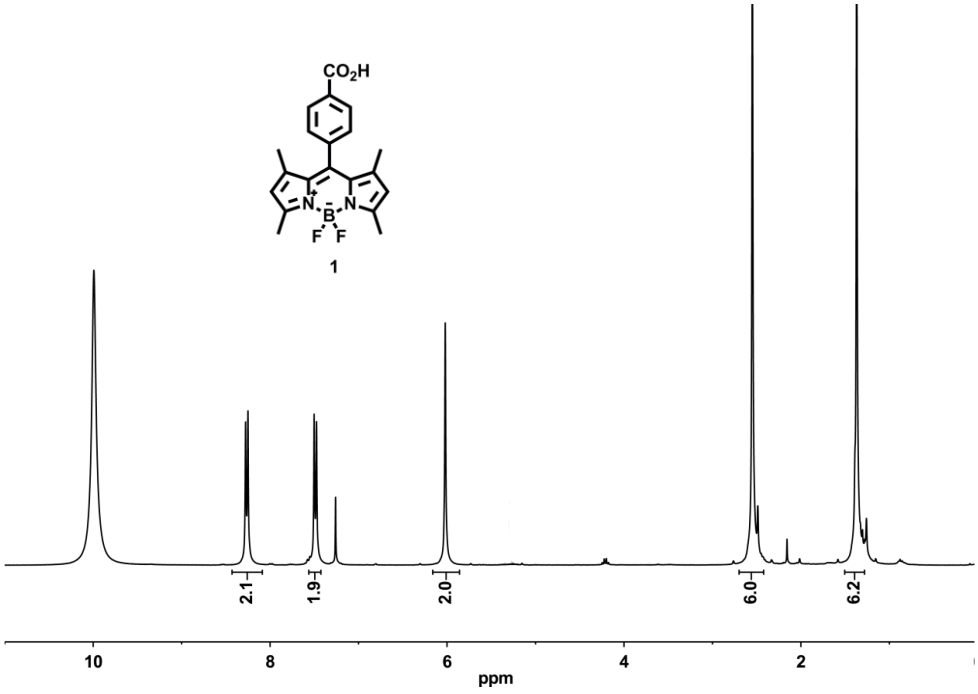


Fig. S12. ^{1}H NMR spectrum of **1.**

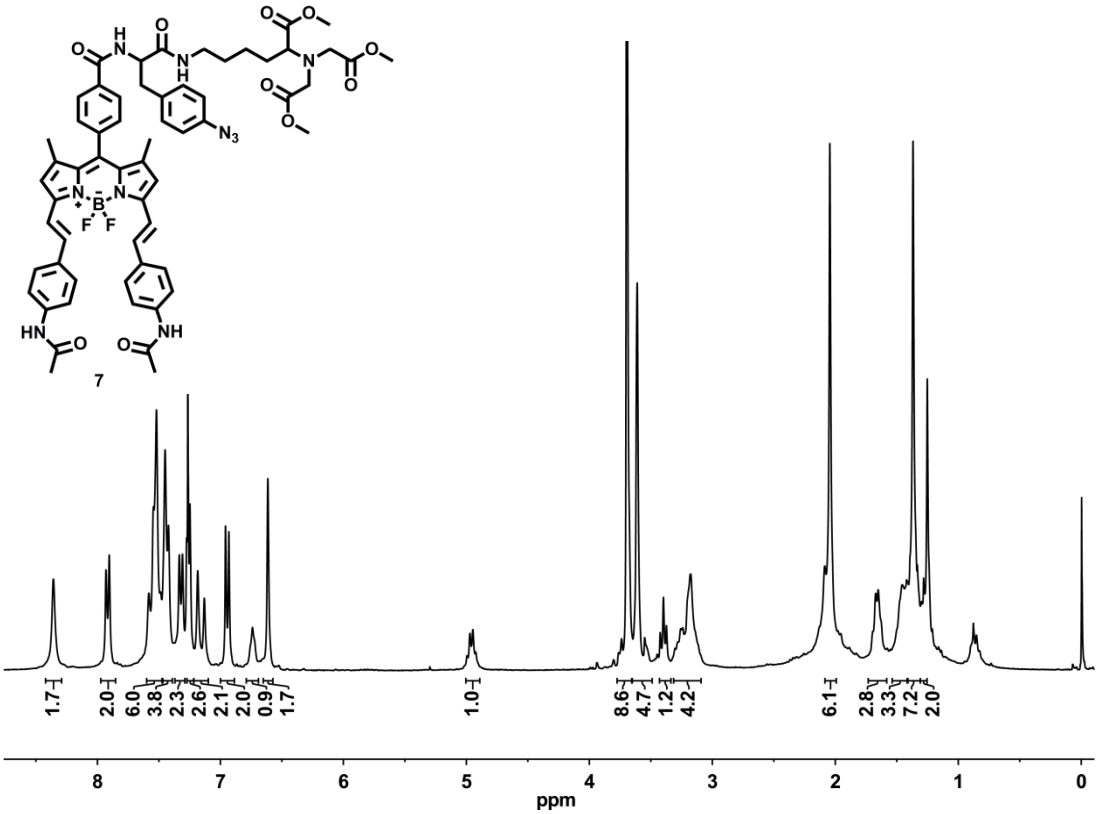


Fig. S13. ^{1}H NMR spectrum of **7.**

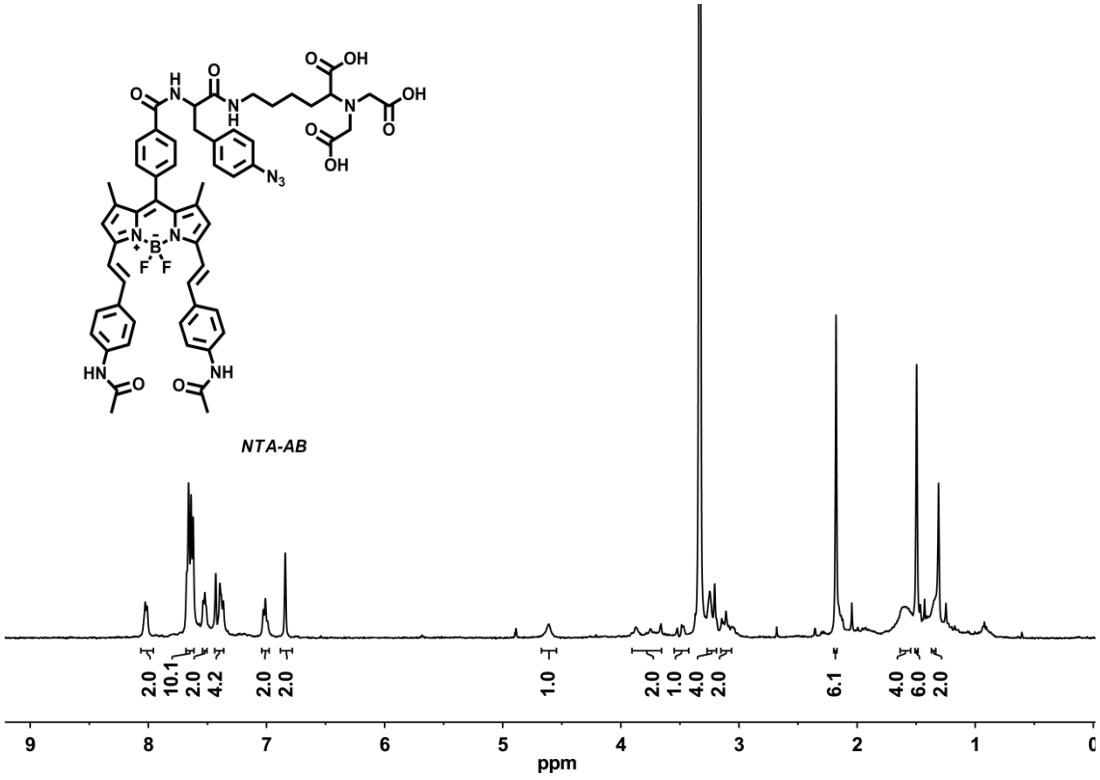


Fig. S14. ^{1}H NMR spectrum of compound *NTA-AB* in deuterated methanol, water signal was suppressed.

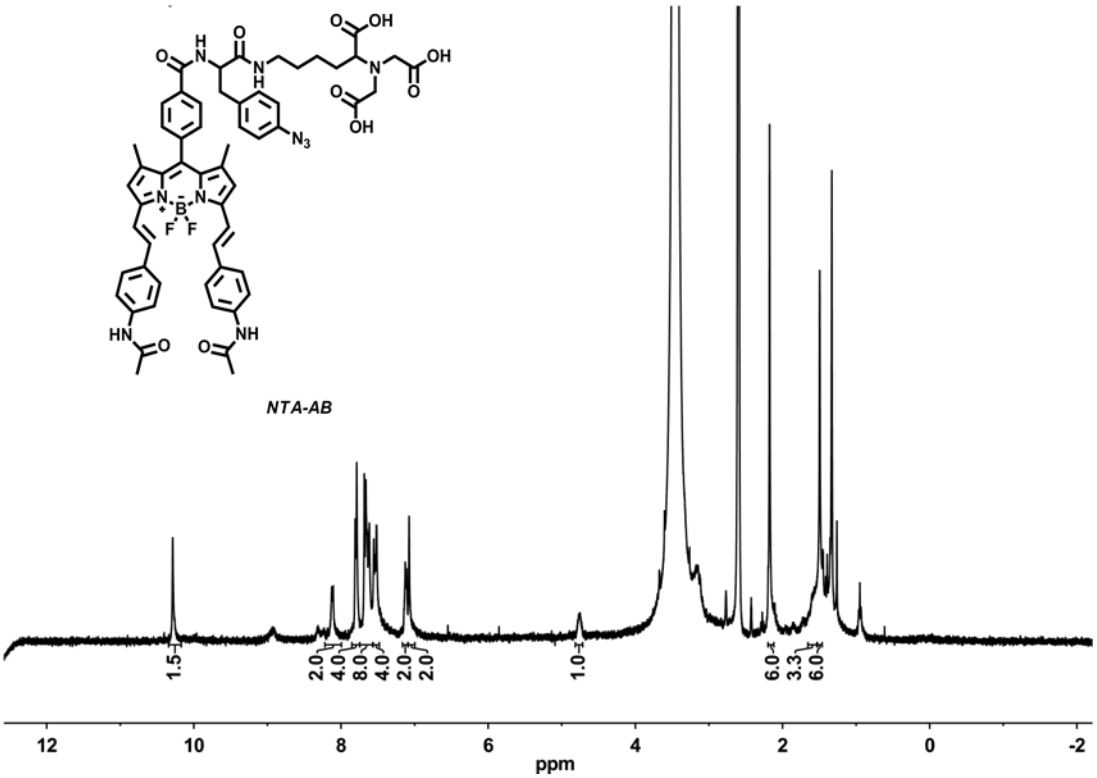


Fig. S15. ^{1}H NMR spectrum of compound *NTA-AB* in deuterated DMSO.

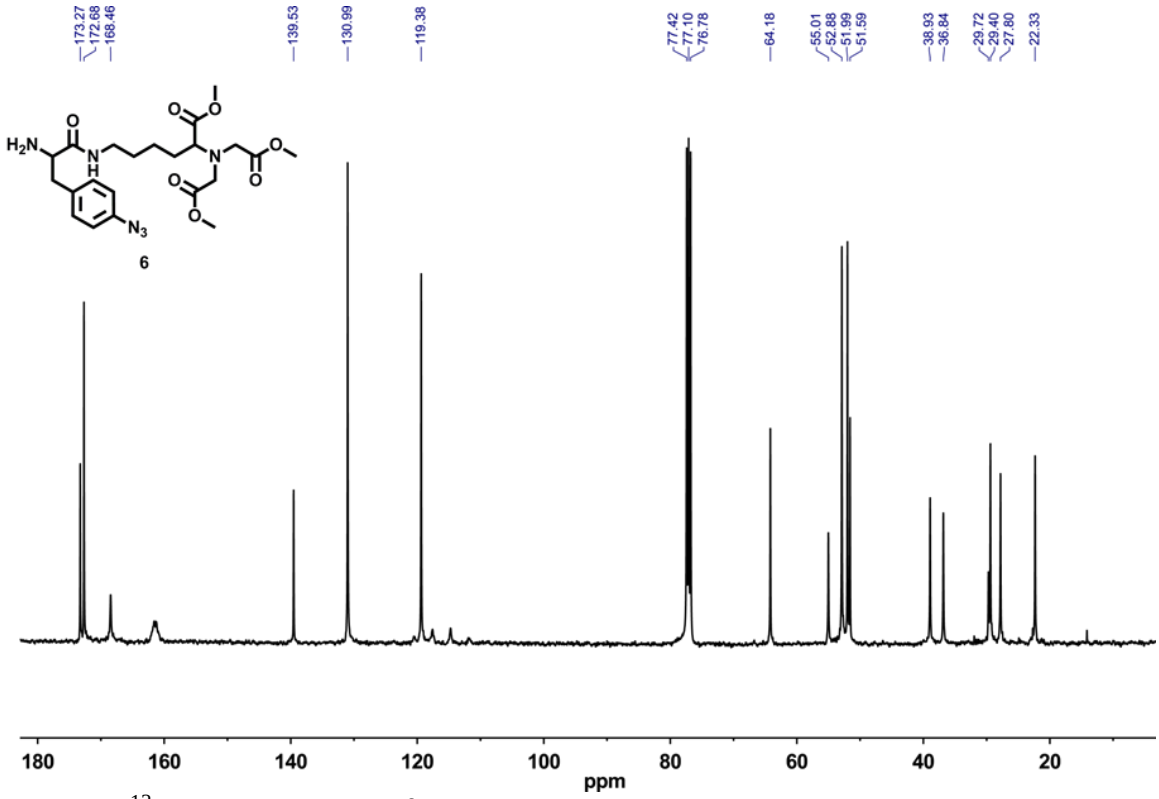


Fig. S16. ^{13}C NMR spectrum of **6.**

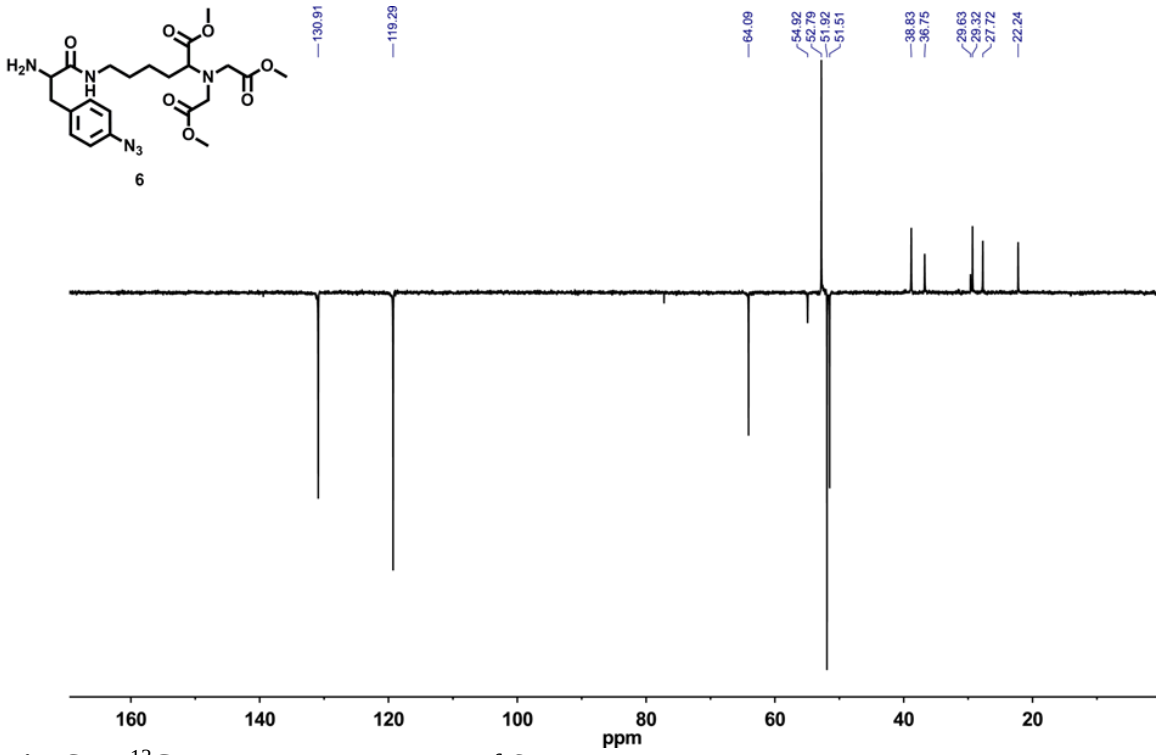


Fig. S17. ^{13}C NMR DEPT spectrum of **6.**

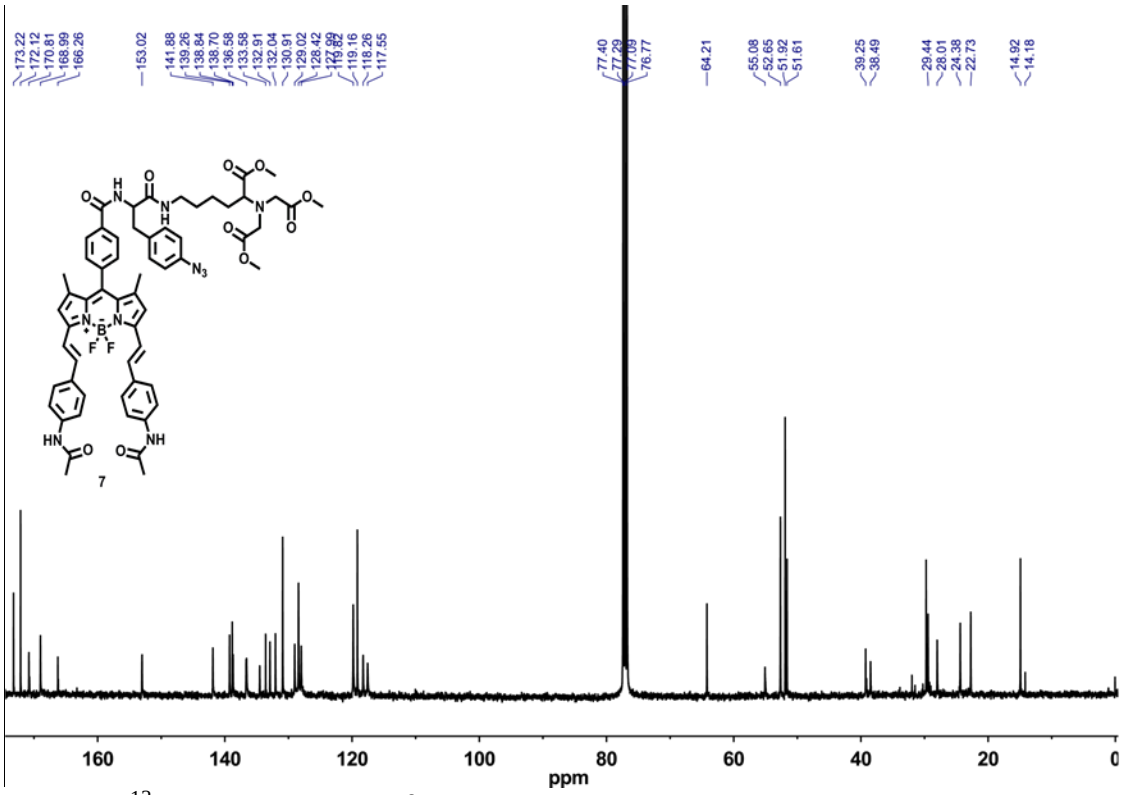


Fig. S18. ^{13}C NMR spectrum of **7.**

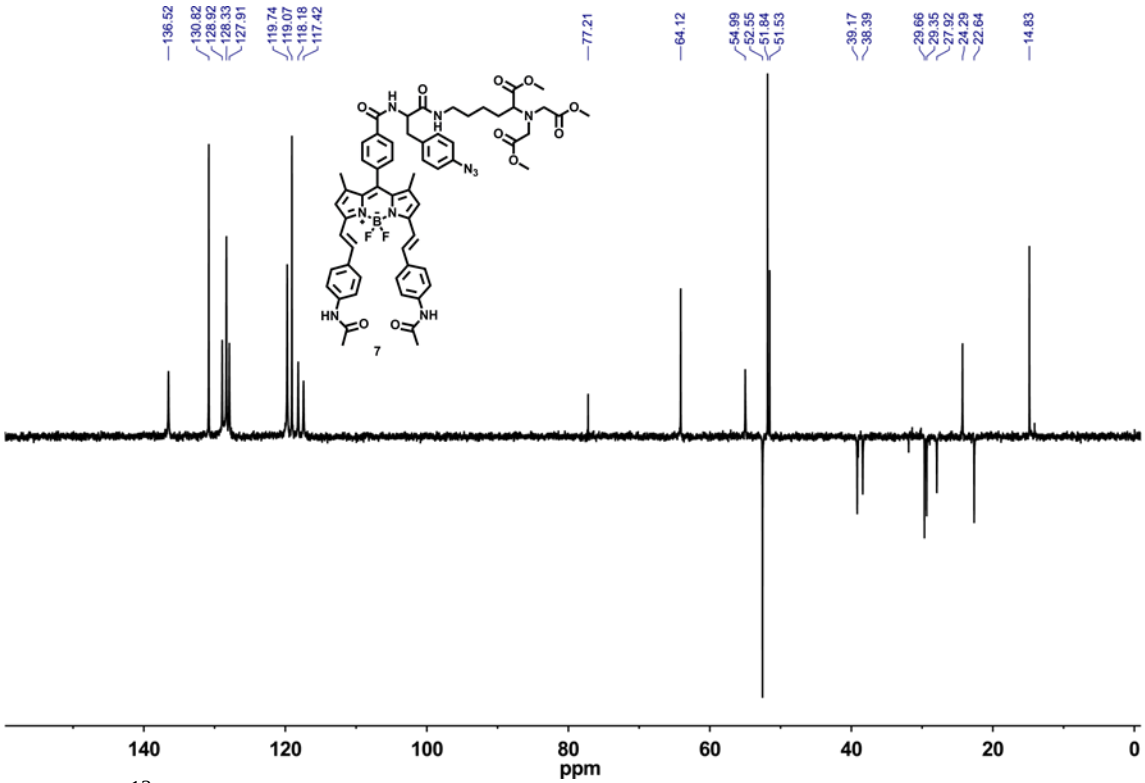


Fig. S19. ^{13}C NMR DEPT spectrum of **7.**

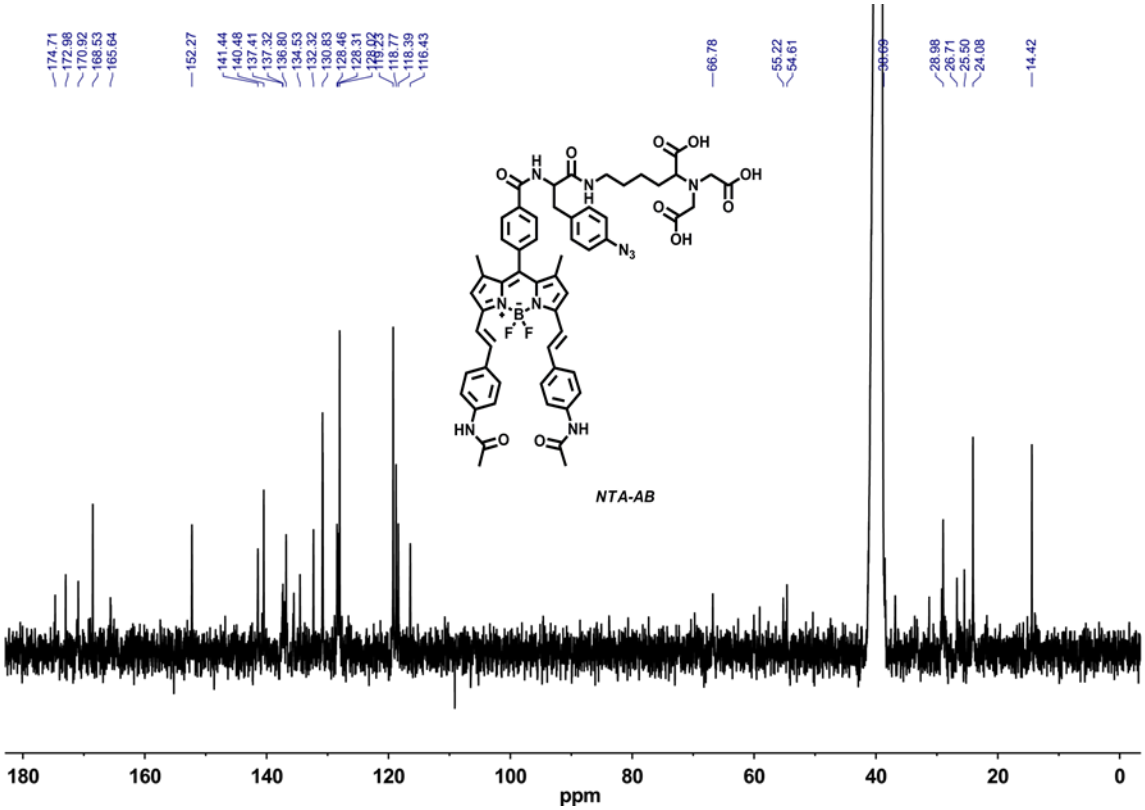


Fig. S20. ^{13}C NMR spectrum of *NTA-AB*.

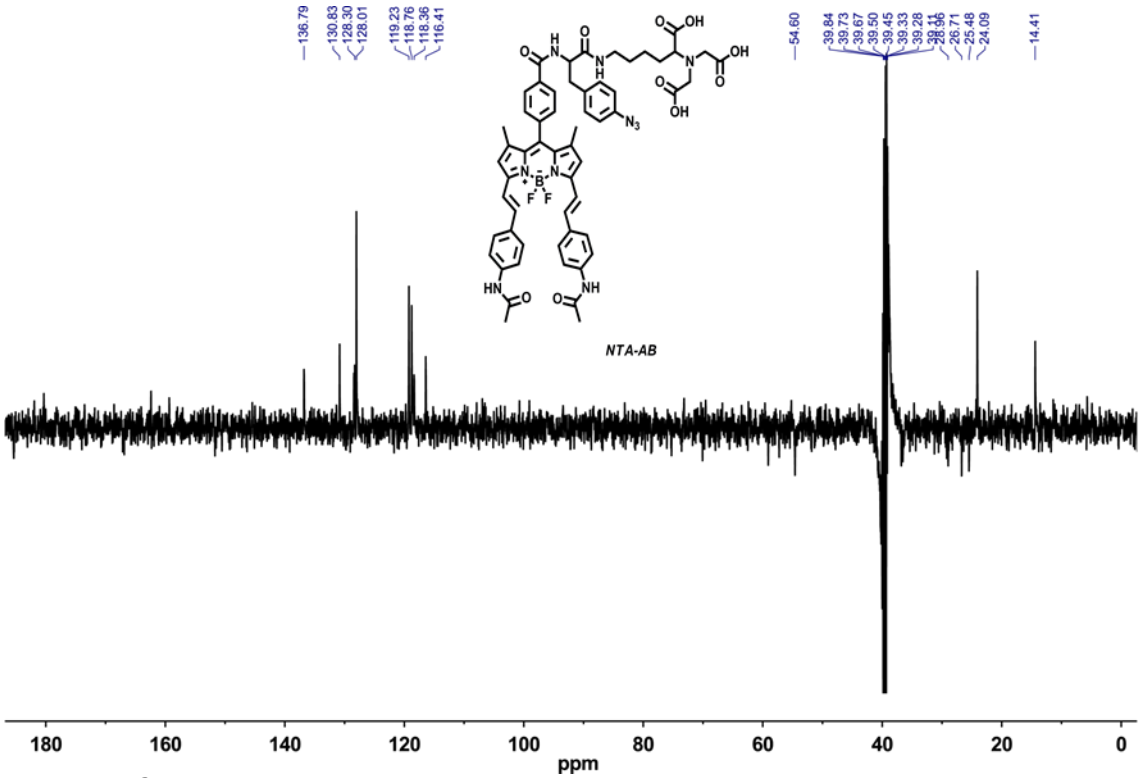


Fig. S21. ^{13}C NMR DEPT spectrum of *NTA-AB*.