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Supporting Information

Avidin triggered turn-on NIR-fluorescent aza-BODIPY-biotin self-assemblies for cancer cell imaging

Dhiraj Dutta[a,b], Rajshree R. Nair[c,d], Nasib Kayastha,[a] S. Asha Nair[c], and Pranjal Gogoi[a,b]*

[a]*Applied Organic Chemistry Group, Chemical Science and Technology Division, CSIR-North East Institute of Science and Technology (CSIR-NEIST), Assam, Jorhat- 785006, India E-mail: gogoipranj@yahoo.co.uk; gogoipranj@gmail.com*

[b]*Academy of Scientific and Innovative Research (AcSIR), Ghaziabad- 201002, India*

[c]*Cancer Research Program, Rajiv Gandhi Centre for Biotechnology, Trivandrum- 695014, Kerala, India*

[d]*Manipal Academy of Higher Education, Manipal- 576104, Karnataka, India*

***Corresponding Author**

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1. Synthesis of core-aza-BODIPYs

The core-aza-BODIPYs were synthesized as described in scheme 1. Aza-BODIPYs **1c** and **2c** were synthesized according to the reported procedures[1].

*1.1. 5,5-Difluoro-3,7-diphenyl-1,9-bis(4-(prop-2-yn-1-yloxy)phenyl)-5H-4l4,5l4-dipyrrolo [1,2-c:2',1'-f][1,3,5,2]triazaborinine (**1d**)*

To a solution of **1c** (0.5 g, 0.95 mmol) in DMF (30 mL), K_2CO_3 (0.39 g, 2.83 mmol), propargyl bromide (0.337 g, 2.83 mmol, 0.21 mL) were added and the reaction mixture was stirred at room temperature for 24 h. After completion of the reaction, the reaction mixture was quenched with water and extracted with DCM (3 × 20 mL). The organic layer was dried over Na_2SO_4 and removed under reduced pressure. The residue was purified by flash column chromatography on silica gel using hexanes/ethyl acetate (4:1) as eluent to give **1d** as red solid (0.41 g; 71% yield); **^{1}H NMR (500 MHz, $CDCl_3$):** δ 7.98-7.93 (m, 8 H), 7.41-7.38 (m, 6 H), 6.98 (d, *J* = 8.93 Hz, 4 H), 6.85 (s, 2 H), 4.69 (d, *J* = 2.4 Hz, 4 H), 2.53 (t, *J* = 2.36 Hz, 2 H); **^{13}C NMR (126 MHz, $CDCl_3$):** δ 159.1, 158.7, 145.4, 143.4, 131.7, 130.8, 130.7, 129.5, 128.5, 126.0, 117.7, 115.0, 78.2, 76.0, 55.8; **IR ($CHCl_3$):** 2122, 1602, 1225 cm^{-1} ; **HRMS (+ESI) Calcd for $C_{38}H_{27}BF_2N_3O_2^+$ $[M+H]^+$:** 606.2164; found: 606.2173.

*1.2. 5,5-Difluoro-1,9-diphenyl-3,7-bis(4-(prop-2-yn-1-yloxy)phenyl)-5H-4λ^4,5λ^4-dipyrrolo [1,2-c:2',1'-f][1,3,5,2]triazaborinine (**2d**)*

To a solution of **2c** (0.5 g, 0.945 mmol) in DMF (30 mL), K_2CO_3 (0.392 g, 2.834 mmol), propargyl bromide (0.337 g, 2.834 mmol, 0.22 mL) were added and the mixture was stirred at room temperature for 24 h. After completion of the reaction, the reaction mixture was quenched with water and extracted with DCM (3 × 20 mL). The organic layer was dried over Na_2SO_4 and removed under reduced pressure. The residue was purified by flash column

chromatography on silica gel using hexanes/ethyl acetate (4:1) as eluent to give **2d** as red solid (0.42 g; 73% yield); **^{1}H NMR (500 MHz, $CDCl_3$):** δ 8.10-8.04 (m, 8 H), 7.48-7.40 (m, 6 H), 7.10-7.07 (m, 4 H), 7.04 (s, 2 H), 4.76 (d, *J* = 2.4 Hz, 4 H), 2.57 (t, *J* = 2.4 Hz, 2 H); **^{13}C NMR (126 MHz, $CDCl_3$):** δ 159.8, 158.1, 145.4, 143.4, 132.4, 131.6, 131.5, 131.5, 129.3, 128.6, 124.9, 118.7, 115.0, 78.0, 76.0, 55.8; **IR ($CHCl_3$):** 2124, 1601, 1234 cm^{-1}; **HRMS (+ESI) Calcd for $C_{38}H_{26}BF_2N_3O_2$ $[M+H]^+$:** 606.2164; found: 606.2136.

2. Synthesis of azide functionalized biotin

The azide functionalized biotin was synthesized according to scheme 2.

*2.1. Tetra-(ethyleneglycol)di-p-toluene sulfonate (**3a**)*

Aqueous NaOH solution (4.2 g, 102.97 mmol, 15 mL) was added to a solution of tetraethylene glycol (8 g, 41.189 mmol) in THF (10 mL). After cooling the mixture to 0 °C in an ice/water bath, TsCl (15.71 g, 82.38 mmol) in THF (20 mL) was added dropwise. The reaction mixture was allowed to stir overnight at room temperature. After completion of the reaction, the organic solvent was removed and the aqueous layer was extracted with ethyl acetate (3 × 30 mL). The ethyl acetate layer was dried over Na_2SO_4 and removed under reduced pressure. The residue was purified by flash column chromatography on silica gel using hexanes/ethyl acetate (3:2) as eluent to give **3a** as colorless liquid; (14 g; 68% yield); **^{1}H NMR (500 MHz, $CDCl_3$):** δ 7.81-7.77 (m, 4 H), 7.34 (d, *J* = 7.9 Hz, 4 H), 4.17-4.12 (m, 4 H), 3.70-3.66 (m, 4 H), 3.59-3.54 (m, 8 H), 2.45 (s, 6 H); **^{13}C NMR (126 MHz, $CDCl_3$):** δ 144.8, 132.8, 129.8, 127.9, 70.6, 70.4, 69.2, 68.6, 21.6; **IR ($CHCl_3$):** 1353, 1175 cm^{-1}; **HRMS (+ESI) Calcd for $C_{22}H_{31}O_9S_2^+$ $[M+H]^+$:** 503.1409; found: 503.1405.

*2.2. 1,11-Diazido-3,6,9-trioxaundecane (**3b**)*

To a solution of compound **3a** (14 g, 27.86 mmol) in DMF (30 mL), NaN_3 (7.25 g, 111.42 mmol) was added and the reaction mixture was allowed to stir at 80 °C for 18 h. After cooling

to room temperature, water (50 mL) was added and extracted with ethyl acetate (3 × 40 mL). The organic solvent was dried over Na_2SO_4 and removed under reduced pressure. The residue was purified by flash column chromatography on silica gel using hexanes/ethyl acetate (2:1) as eluent to give **3b** as colorless oil; (6.2 g; 91% yield); **^{1}H NMR (500 MHz, $CDCl_3$):** δ 3.70-3.65 (m, 12 H), 3.41-3.38 (m, 4 H); **^{13}C NMR (126 MHz, $CDCl_3$):** δ 70.5, 70.5, 69.9, 50.5; **IR ($CHCl_3$):** 2923, 2866, 2098, 1439, 1283, 1119 cm^{-1}; **HRMS (+ESI) Calcd for $C_8H_{17}N_4O_3^+$ $[M+H-N_2]^+$:** 217.1301; found: 217.1298.

*2.3. 1-Amino-11-azido-3,6,9-trioxaundecane (**3c**)*

Diazide **3b** (4.1 g, 16.79 mmol) was added to a solution of H_3PO_4 (60 mL, 0.65 M) at room temperature followed by another solution of PPh_3 (3.96 g, 15.11 mmol) in Et_2O (30 mL) was added dropwise using a dropping funnel. The reaction mixture was stirring at room temperature for 24 h under argon atmosphere. After completion of the reaction, Et_2O was removed using a separating funnel, the separated aqueous layer was washed with Et_2O and KOH (3.2 g) was added to the aqueous layer. The aqueous mixture was kept at 4 ^oC for 16 h and filtered. The filtrate was extracted with DCM for several times. The combine DCM layer was dried over Na_2SO_4 and removed under reduced pressure. The residue was purified by flash column chromatography on silica gel using DCM/MeOH (9:1) as eluent to give **3c** as amber oil (2.9 g; 79% yield); **^{1}H NMR (500 MHz, $CDCl_3$):** δ 6.70 (br, 2 H), 3.83 (t, *J* = 4.26 Hz. 2 H), 3.75-3.60 (m, 10 H), 3.48-3.42 (m, 2 H), 3.24 (br, 2 H); **^{13}C NMR (126 MHz, $CDCl_3$):** δ 70.3, 70.2, 70.1, 69.9, 69.7, 66.9, 50.5, 39.6; **IR ($CHCl_3$):** 2917, 2877, 2109, 1630, 1299, 1117 cm^{-1}; **HRMS (+ESI) Calcd for $C_8H_{19}N_4O_3$ $[M+H]^+$:** 219.1457; found: 219.1458.

*2.4. Biotinated tetraethyleneglycol linker (**AzBiotin**)*

EDC.HCl (0.878 g, 4.58 mmol) and DMAP (0.28 g, 2.29 mmol) were added to a solution of biotin (1 g, 4.58 mmol) in DMF (20 mL) followed by compound **3c** (1 g, 4.58 mmol) in DMF

(10 mL) was added and stirred for 8 h. The reaction mixture was quenched with water and extracted with ethyl acetate (3 × 25 mL). The organic layer was dried over Na_2SO_4 and removed under reduced pressure. The residue was purified by flash column chromatography on silica gel using DCM/MeOH (9:1) as eluent to give **AzBiotin** as off-white solid (1.55 g; 76% yield); **^{1}H NMR (500 MHz, $CDCl_3$):** δ 6.73 (t, J = 5.3 Hz, 1 H), 6.38 (s, 1 H), 5.38 (s, 1 H), 4.51 (q, J_1 = 4.9 Hz, J_2 = 7.6 Hz, 1 H), 4.32 (q, J_1 = 4.5 Hz, J_2 = 7.2 Hz, 1 H), 3.72-3.61 (m, 10 H), 3.57 (t, J = 5.0 Hz, 2 H), 3.50-3.35 (m, 4 H), 3.15 (td, J_1 = 4.6 Hz, J_2 = 7.3 Hz, J_3 = 7.3 Hz, 1 H), 2.96-2.89 (m, 1 H), 2.75 (d, J = 12.7 Hz, 1 H), 2.23 (td, J_1 = 2.7 Hz, J_2 = 7.3 Hz, J_3 = 7.2 Hz, 2 H), 1.80-1.67 (m, 4 H), 1.50-1.40 (m, 2 H); **^{13}C NMR (126 MHz, $CDCl_3$):** δ 173.3, 163.7, 70.6, 70.5, 70.4, 70.1, 70.0, 69.9, 61.7, 60.1, 55.4, 50.6, 40.5, 39.0, 35.9, 28.1, 28.0, 25.5; **IR ($CHCl_3$):** 3292, 2928, 2858, 2106, 1705, 1645, 1555, 1461, 1120 cm^{-1}; **HRMS (+ESI) Calcd for $C_{18}H_{33}N_6O_5S^+$ $[M+H]^+$:** 445.2233; found: 445.2234.

Hydrophobic-hydrophilic characteristic tests. In tubes, 1 mL of n-octanol and 1 mL of water were combined, and then 40 µL of the samples (1 mM in DMSO) were added. The tubes were shaken for 30 minutes and then allowed to rest for 24 hours. After extraction from the two phases, the octanol/water partition coefficients of the samples were estimated using fluorescence intensity.

(a) **(b)**

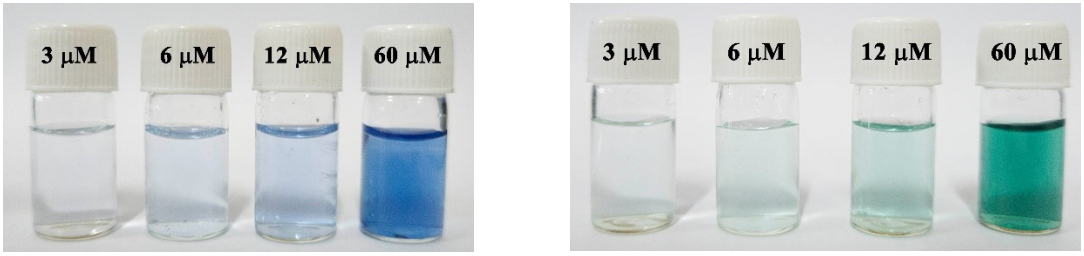


Figure S1. Pictures showing the possibilities of nanoparticle constructions of (a) **DPR1a** and (b) **DPR1b**. Each were dissolved in DMSO first and then diluted into water with different concentrations and underwent aging for 2 h.

Photostability of DPR1a, DPR1b in DMSO and NSA-DPR1a, NSA-DPR1b in water:

We irradiated our monomer dye in DMSO and self-assembled dye in water for 1 h and the fluorescence intensity was monitored. It was good to find that both the monomer units were stable after irradiation with 680 nm light source for 1 h as well as the self-assemblies were also intact as no increase in fluorescence intensity was observed.

(a) **(b)**

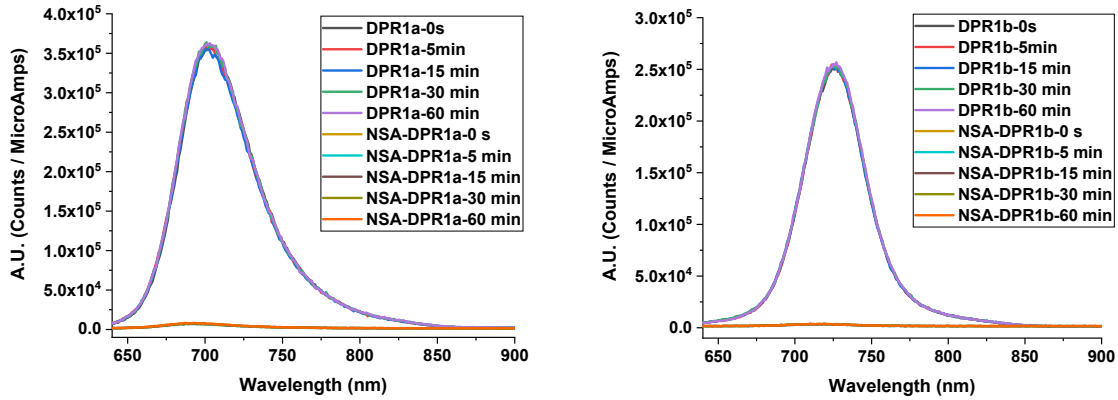


Figure S2: (a) Fluorescence intensity of **NSA-DPR1a** (in water) and **DPR1a** (in DMSO); **(b) NSA-DPR1b** (in water) and **DPR1b** (in DMSO) after irradiating it with 630 nm (700 mW/cm^{-1}) light source for 1 h.

Stability and photostability of NSA-DPR1a and NSA-DPR1b in DMEM:

We checked the stability of our self-assemblies in DMEM after 24 h of incubation at 37 ^{o}C using the DLS profile as well as the fluorescence spectrum. The DLS profile shows that NSA-DPR1a is stable in DMEM, however, more aggregation was observed for **NSA-DPR1b**. Fluorescence spectrum shows that both the self-assemblies don't disintegrate into its monomer in DMEM after irradiation with 630 nm (700 mW/cm^{-1}) light source and 24 h of incubation at 37 ^{o}C.

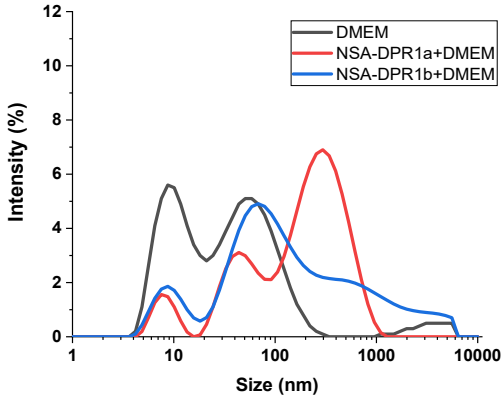


Figure S3: DLS profile of **DMEM**, **NSA-DPR1a** and **NSA-DPR1b** in **DMEM** after 24 h of incubation at 37 °C. (DMEM, pH = 8.4)

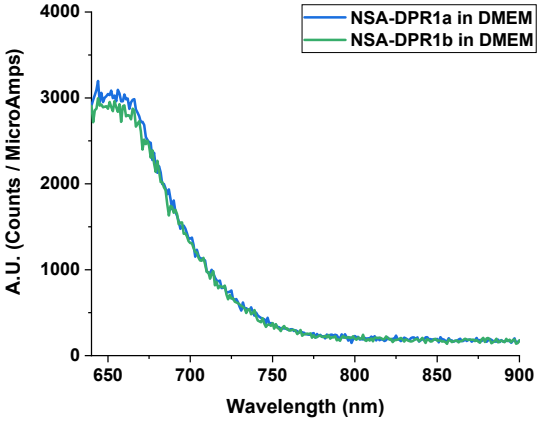


Figure S4: Florescence spectrum of **NSA-DPR1a** and **NSA-DPR1b** in **DMEM** after 24 h of incubation at 37 °C. (DMEM, pH = 8.4)

Mechanism of partial disassembly process through DLS profile

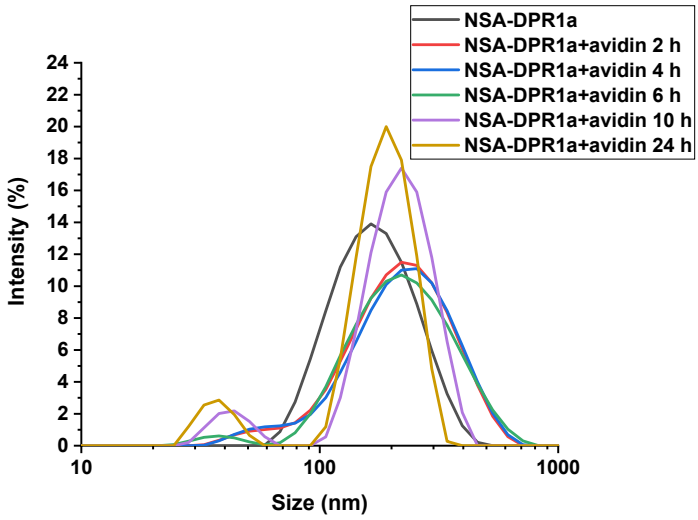


Figure S5: DLS profile of **NSA-DPR1a** after addition of avidin. DLS measurements were done at time intervals of 2, 4, 6, 10 and 24 h. (at 25 °C)

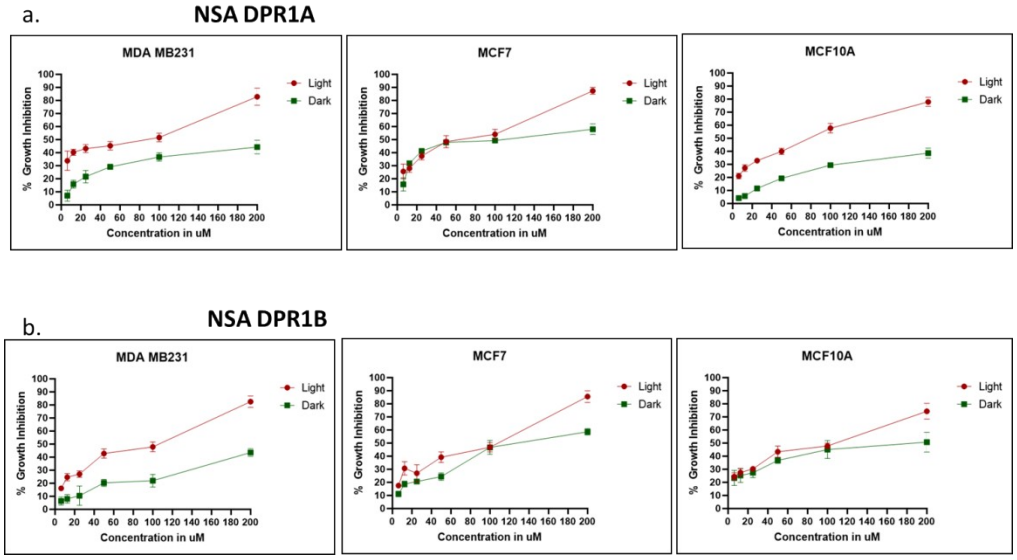


Figure S6: Photocytotoxicity of (a) **NSA-DPR1a** and (b) **NSA-DPR1b** in MDA-MB-231, MCF7, and MCF10A breast cell line. The analyses were carried out in triplicates and data are shown as a standard deviation from the mean n=3.

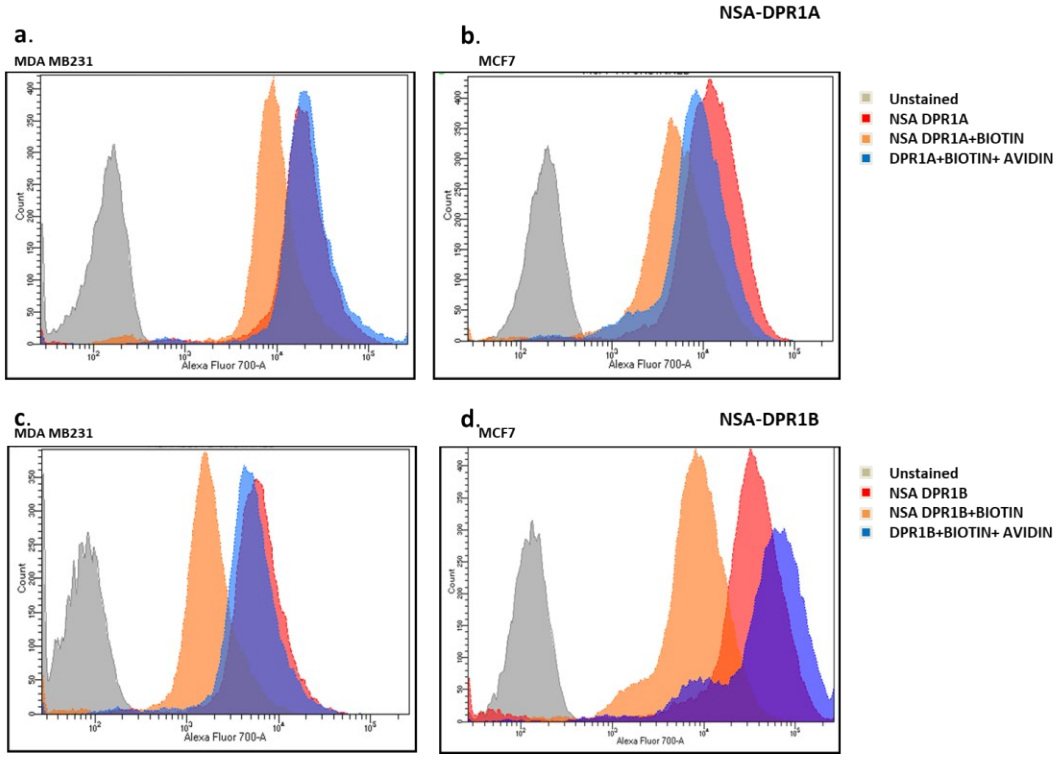


Figure S7: Flow cytometry data shows the uptake of **NSA-DPR1a** and **NSA-DPR1b** in MDA-MB-231 and MCF7. The graph shows quenching of fluorescence upon biotin addition and regaining of fluorescence after avidin addition. (a) MDA-MB-231 with **NSA-DPR1a**; (b) MCF7 with **NSA-DPR1a**; (c) MDA-MB-231 with **NSA-DPR1b**; (d) MCF7 with **NSA-DPR1b**.

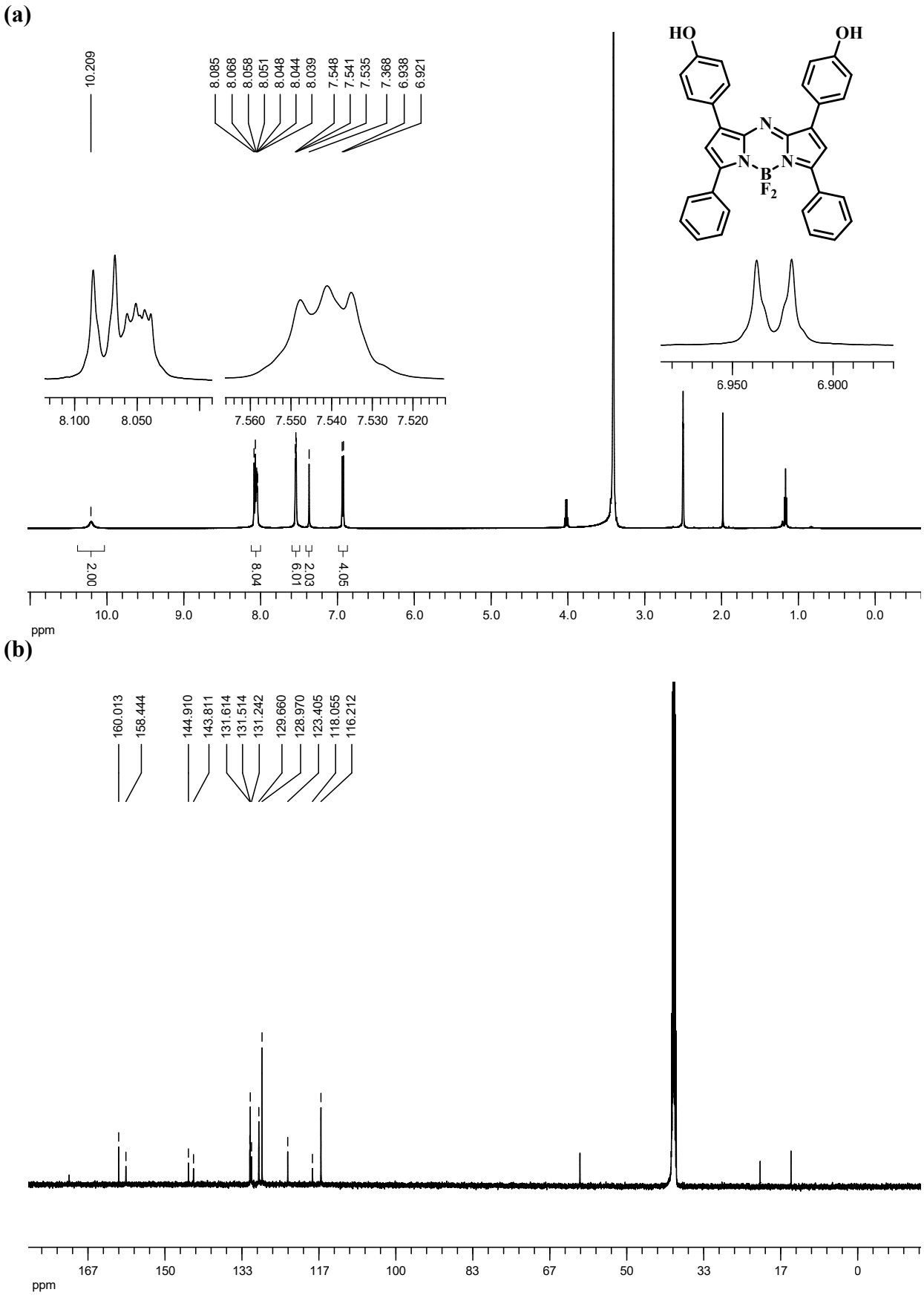
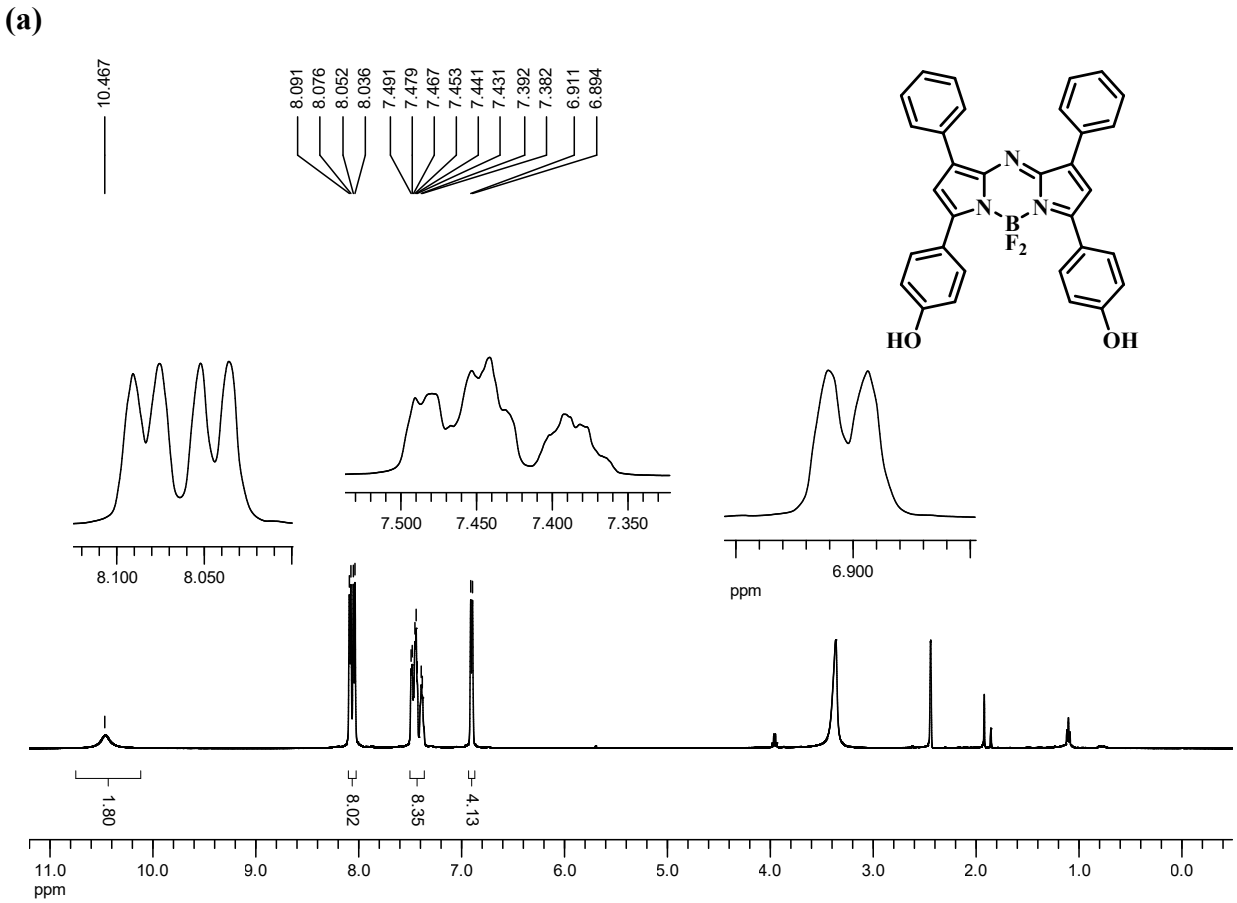


Figure S8: (**a**) ^{1}H-NMR (500 MHz) and (**b**) ^{13}C-NMR (126 MHz) of compound **1c** in DMSO-d_6.



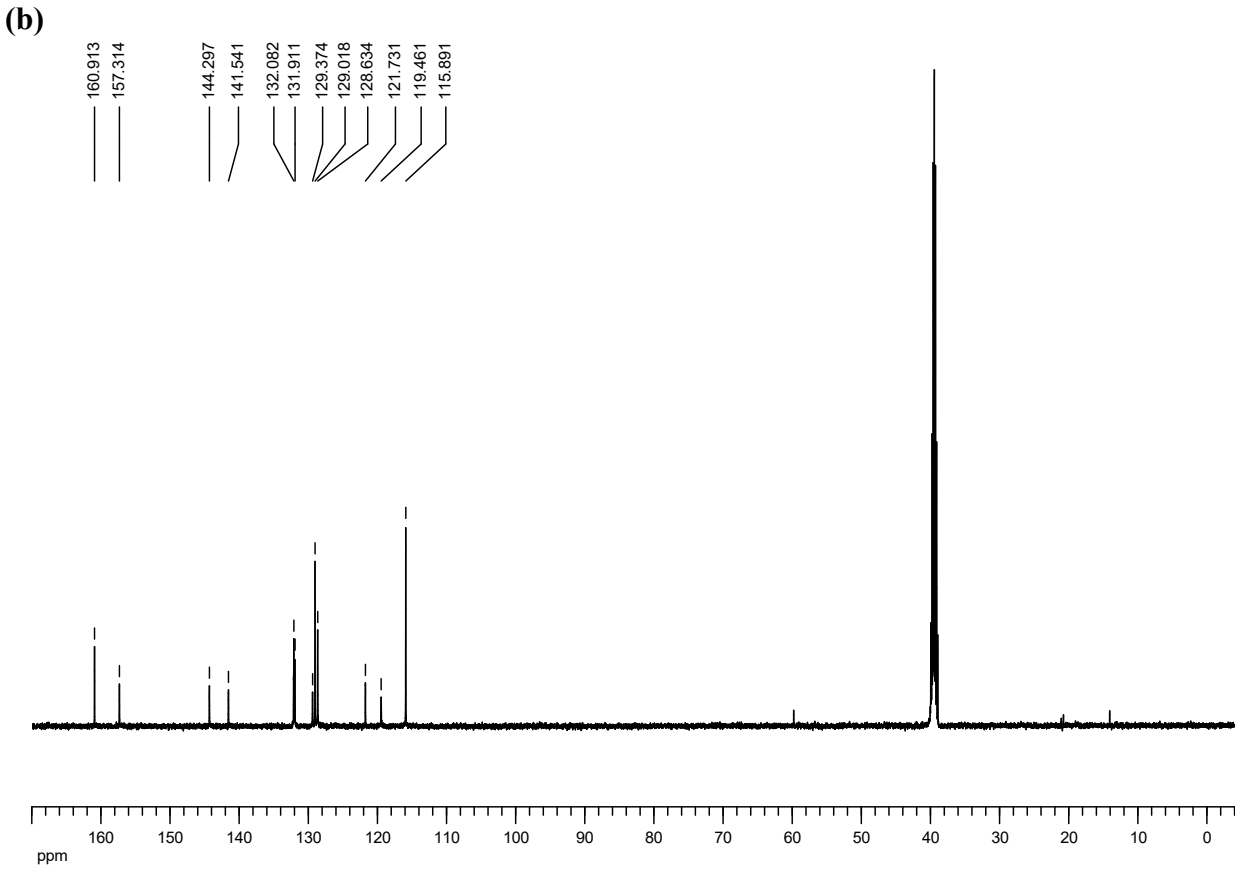


Figure S9: (**a**) ^{1}H-NMR (500 MHz) and (**b**) ^{13}C-NMR (126 MHz) of compound **2c** in DMSO-d_6.

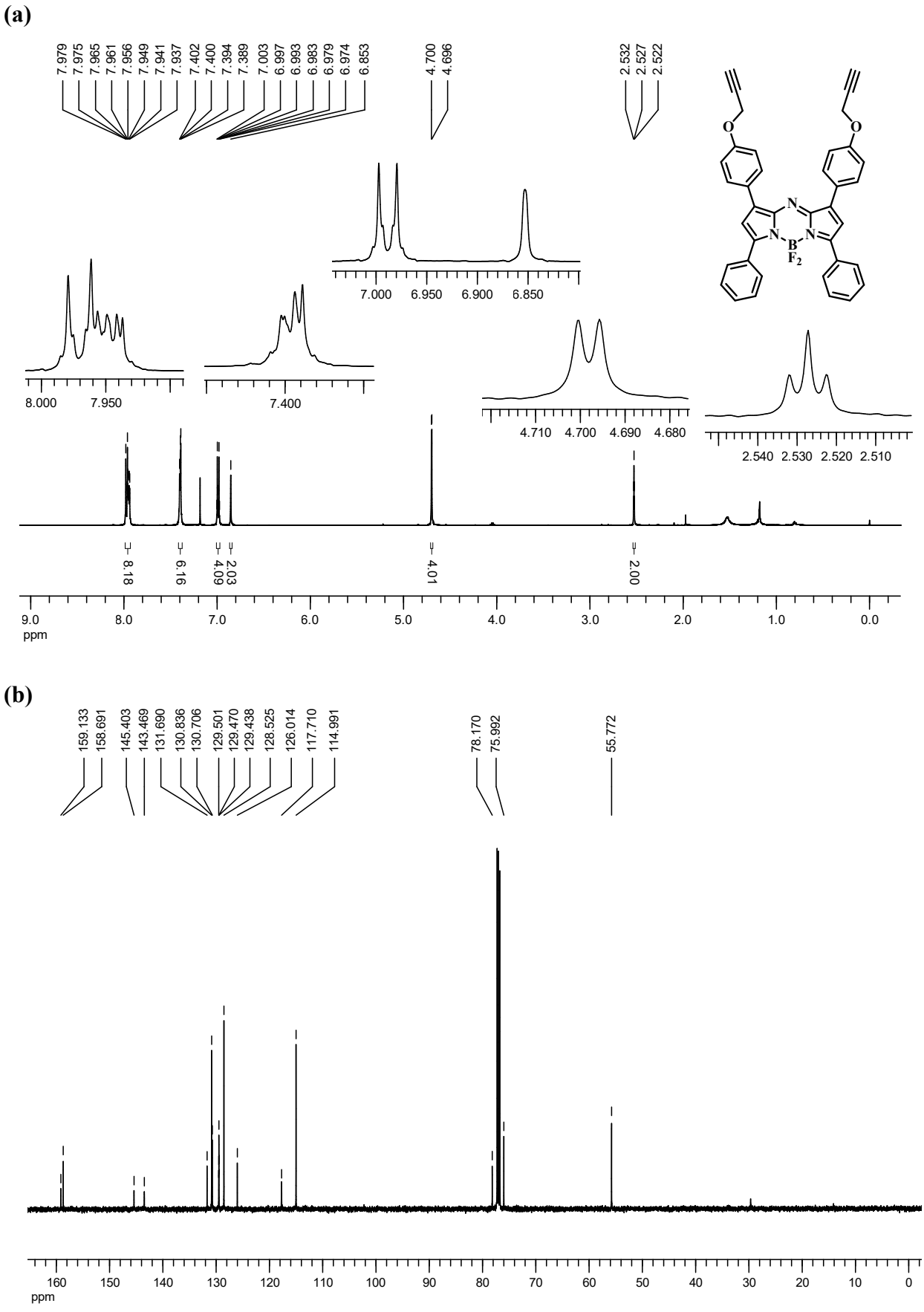
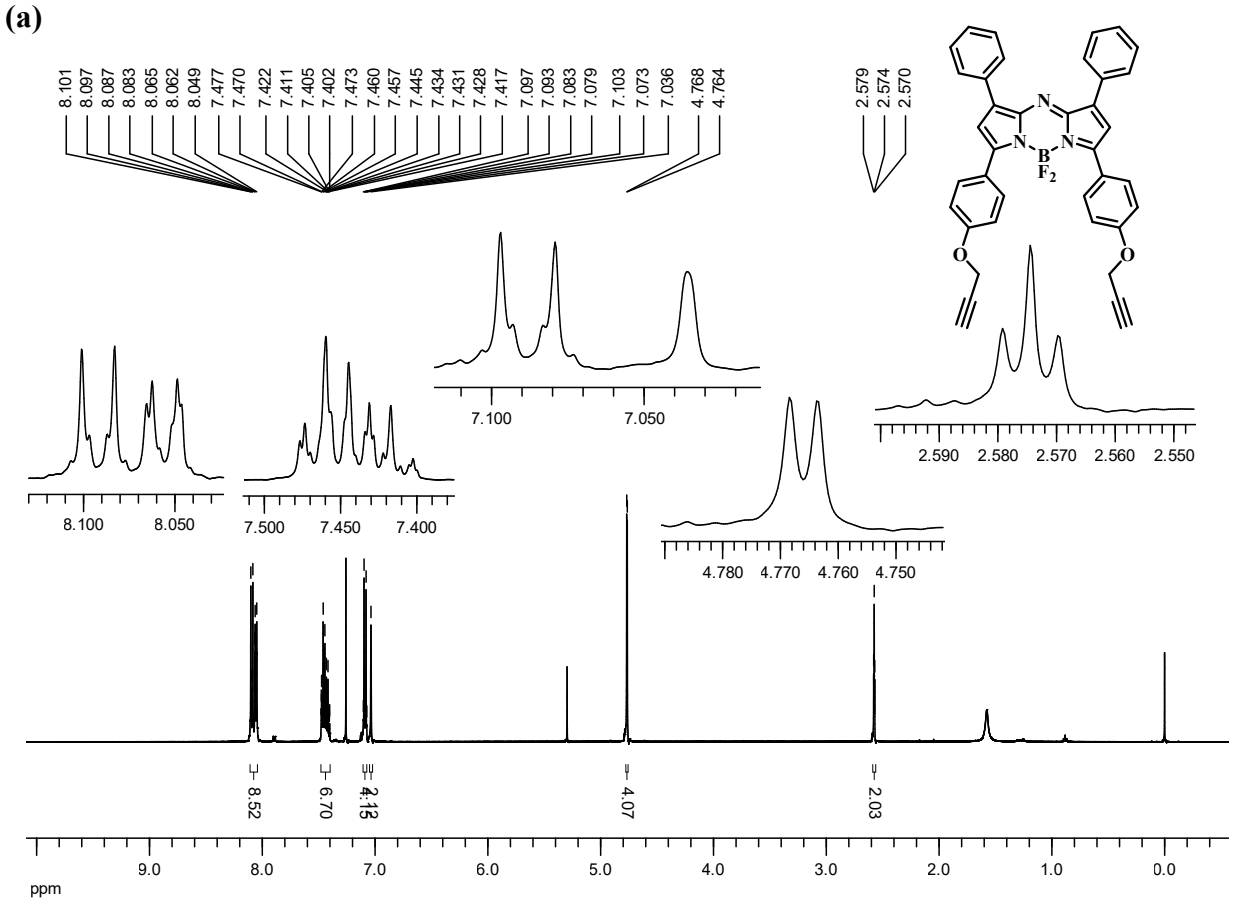


Figure S10: (**a**) ^{1}H-NMR (500 MHz) and (**b**) ^{13}C-NMR (126 MHz) of compound **1d** in $CDCl_3$.



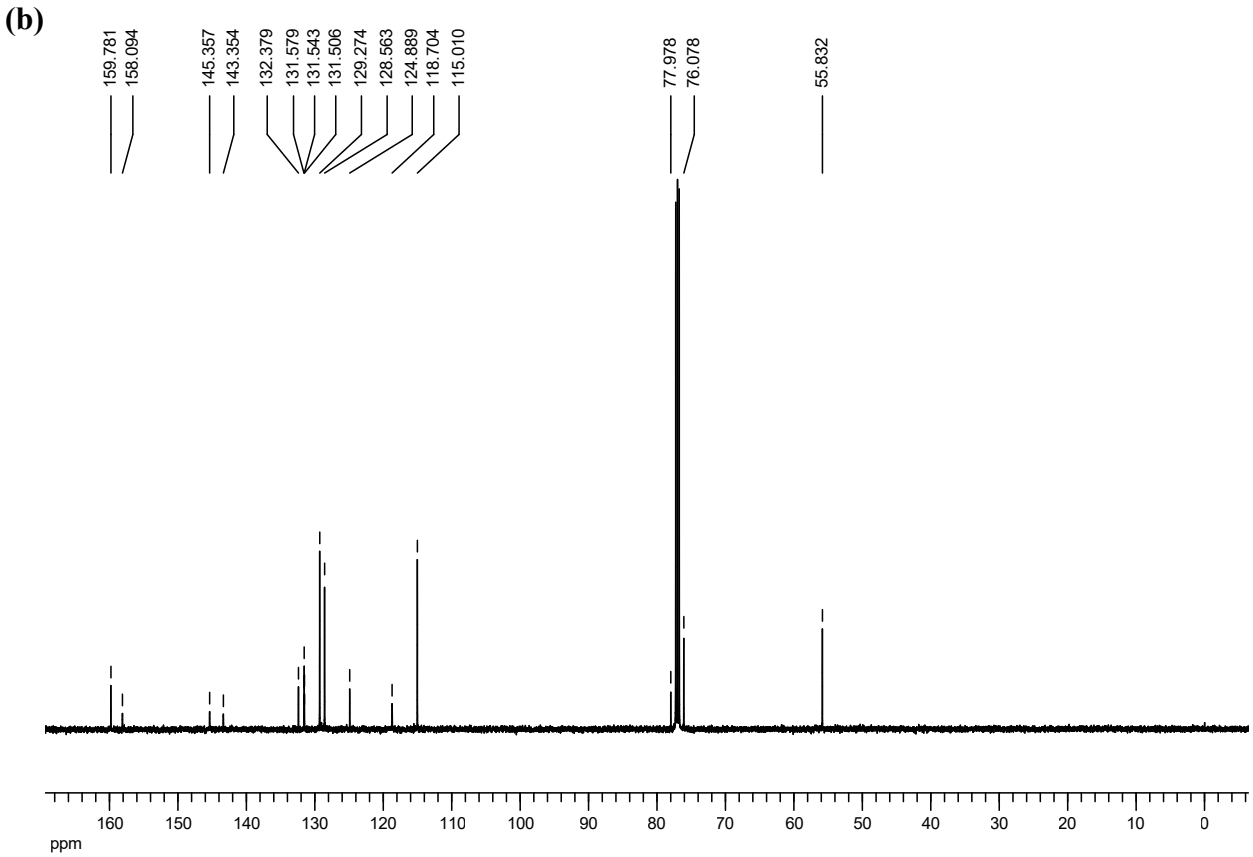


Figure S11: (**a**) ^{1}H-NMR (500 MHz) and (**b**) ^{13}C-NMR (126 MHz) of compound **2d** in $CDCl_3$.

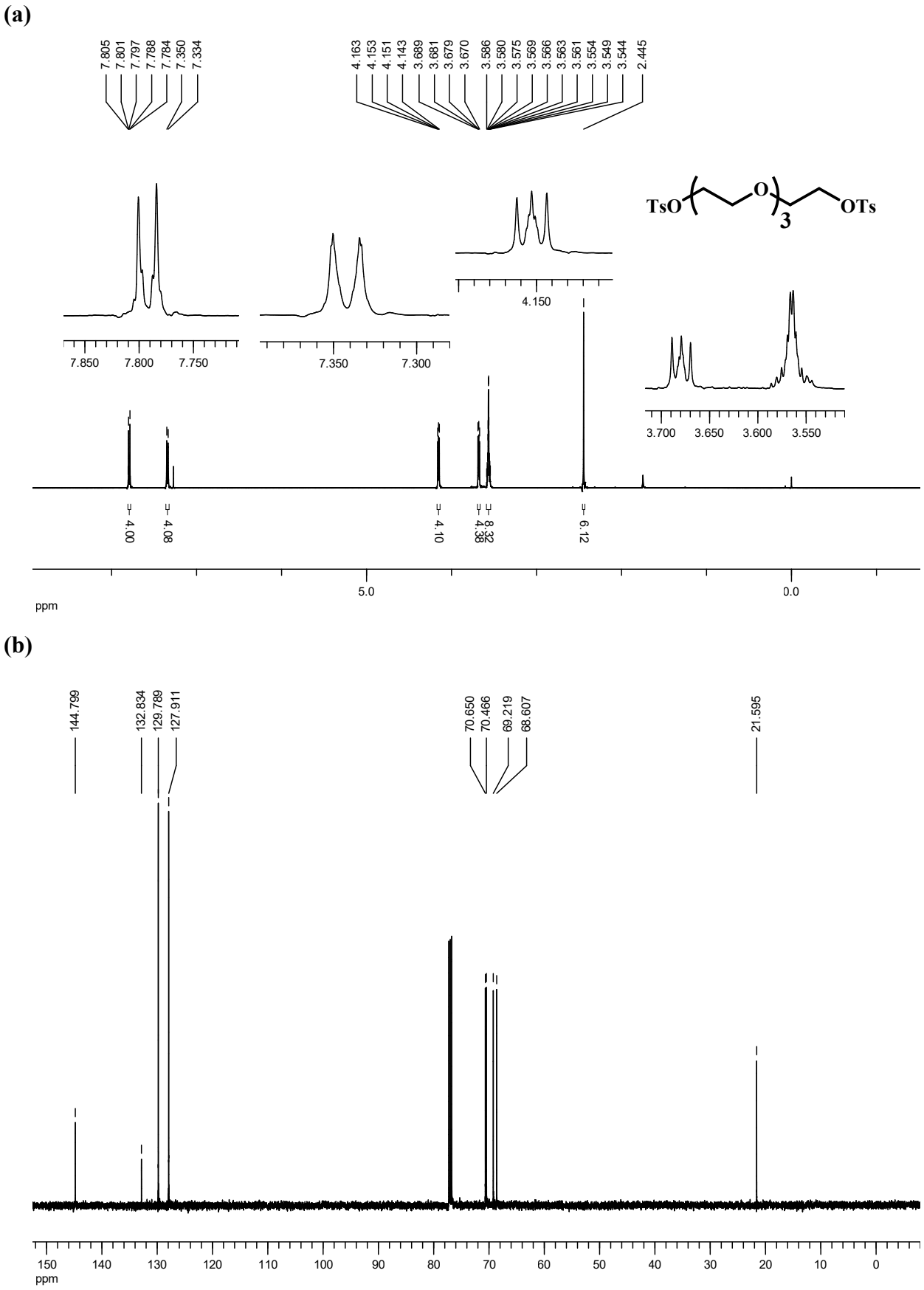


Figure S12: (**a**) ^{1}H-NMR (500 MHz) and (**b**) ^{13}C-NMR (126 MHz) of compound **3a** in $CDCl_3$.

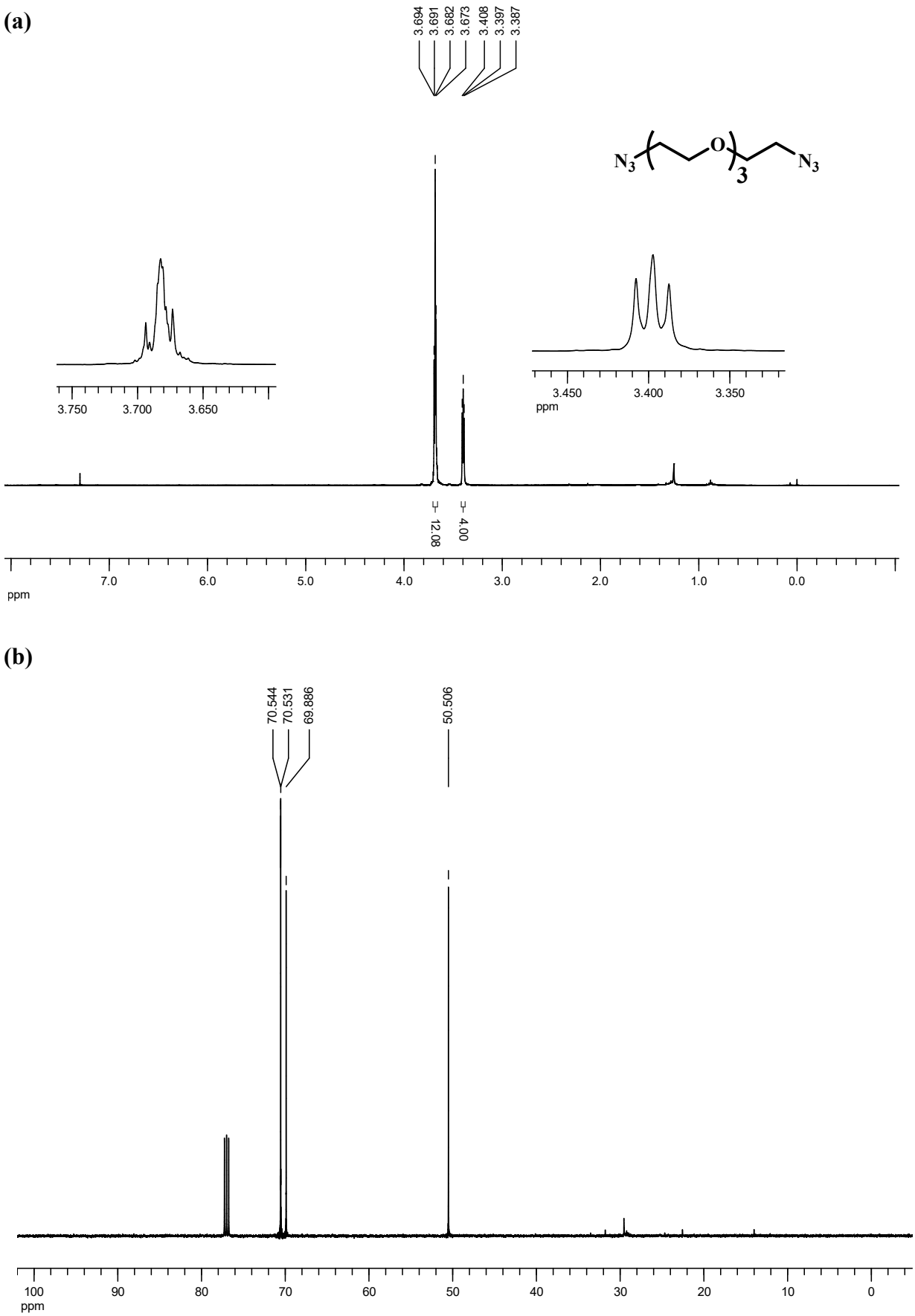


Figure S13: (**a**) ^{1}H-NMR (500 MHz) and (**b**) ^{13}C-NMR (126 MHz) of compound **3b** in $CDCl_3$.

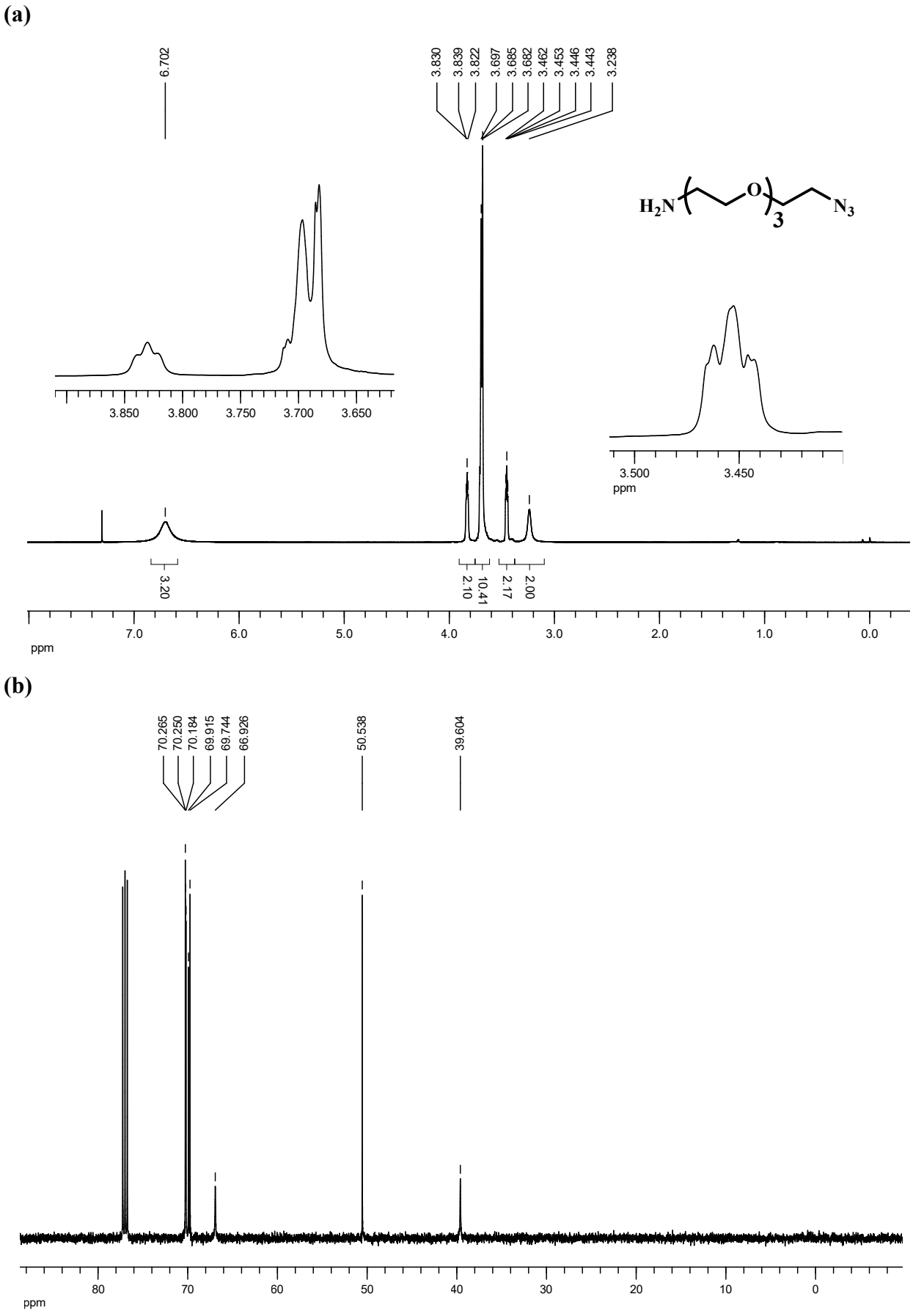


Figure S14: (**a**) ^{1}H-NMR (500 MHz) and (**b**) ^{13}C-NMR (126 MHz) of compound **3c** in $CDCl_3$.

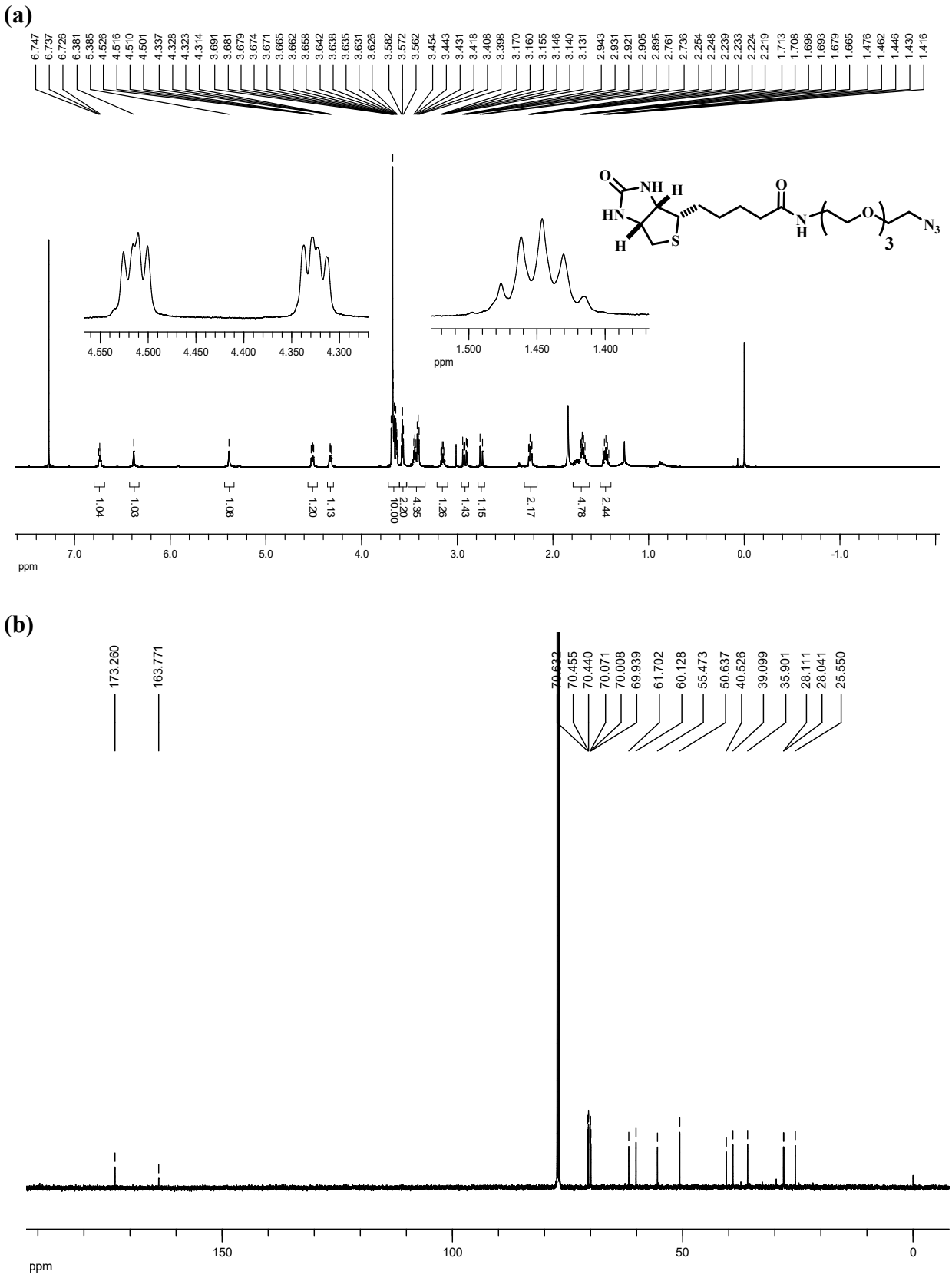
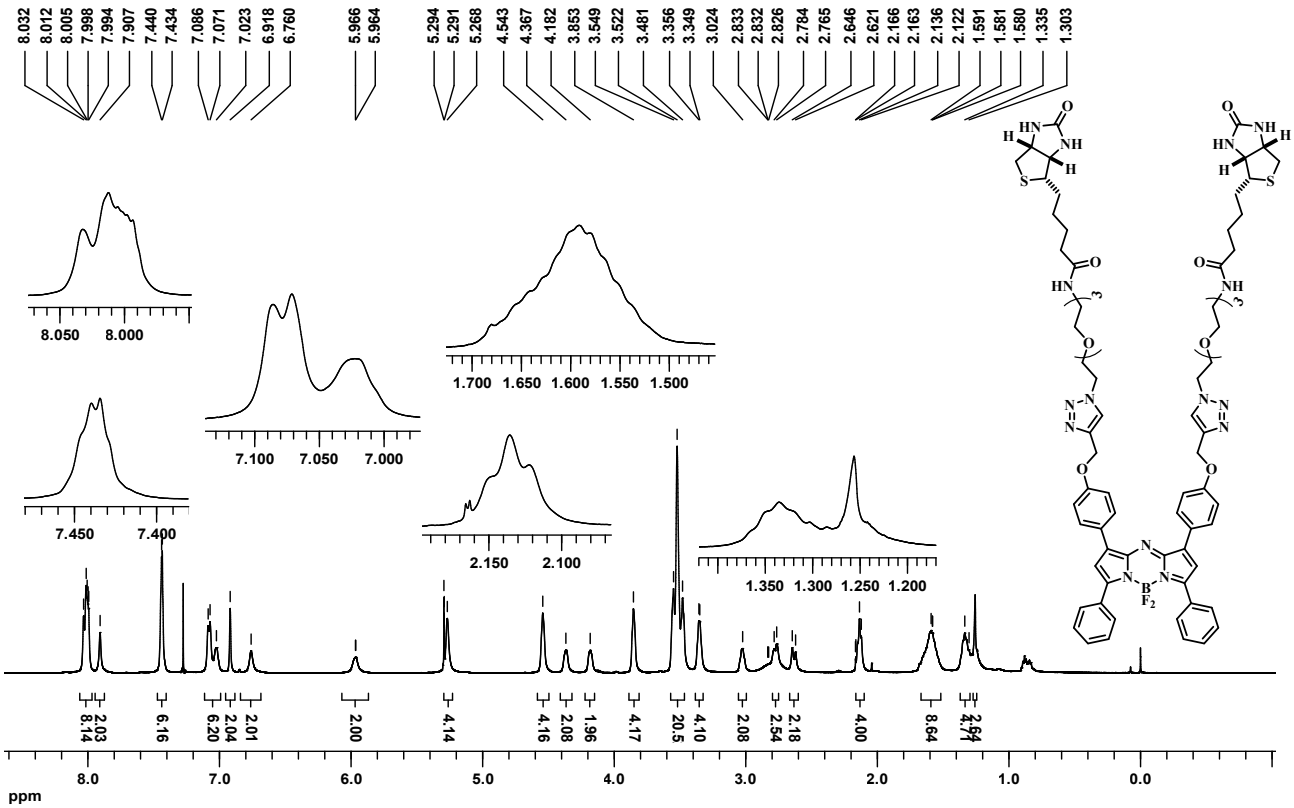


Figure S15: (**a**) ^{1}H-NMR (500 MHz) and (**b**) ^{13}C-NMR (126 MHz) of compound **AzBiotin** in $CDCl_3$.

(a)



(b)

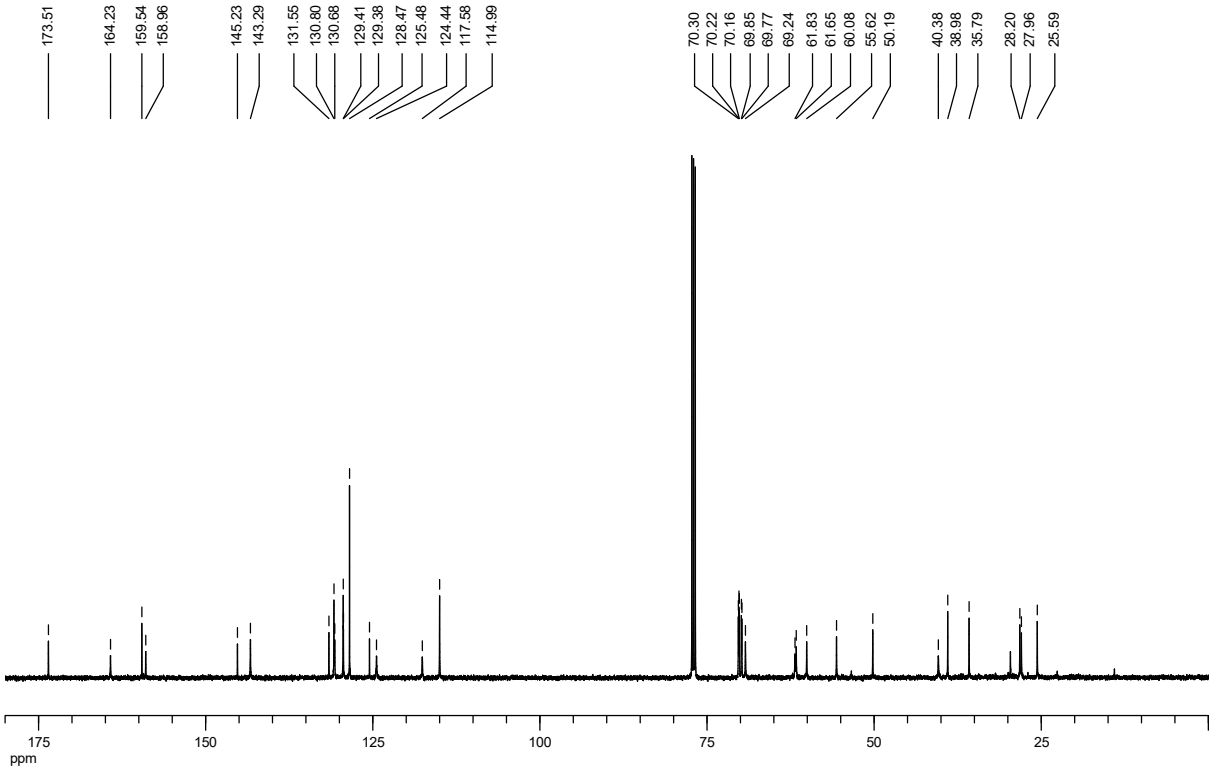


Figure S16: (**a**) ^{1}H-NMR (500 MHz) and (**b**) ^{13}C-NMR (126 MHz) of compound **DPR1a** in $CDCl_3$.

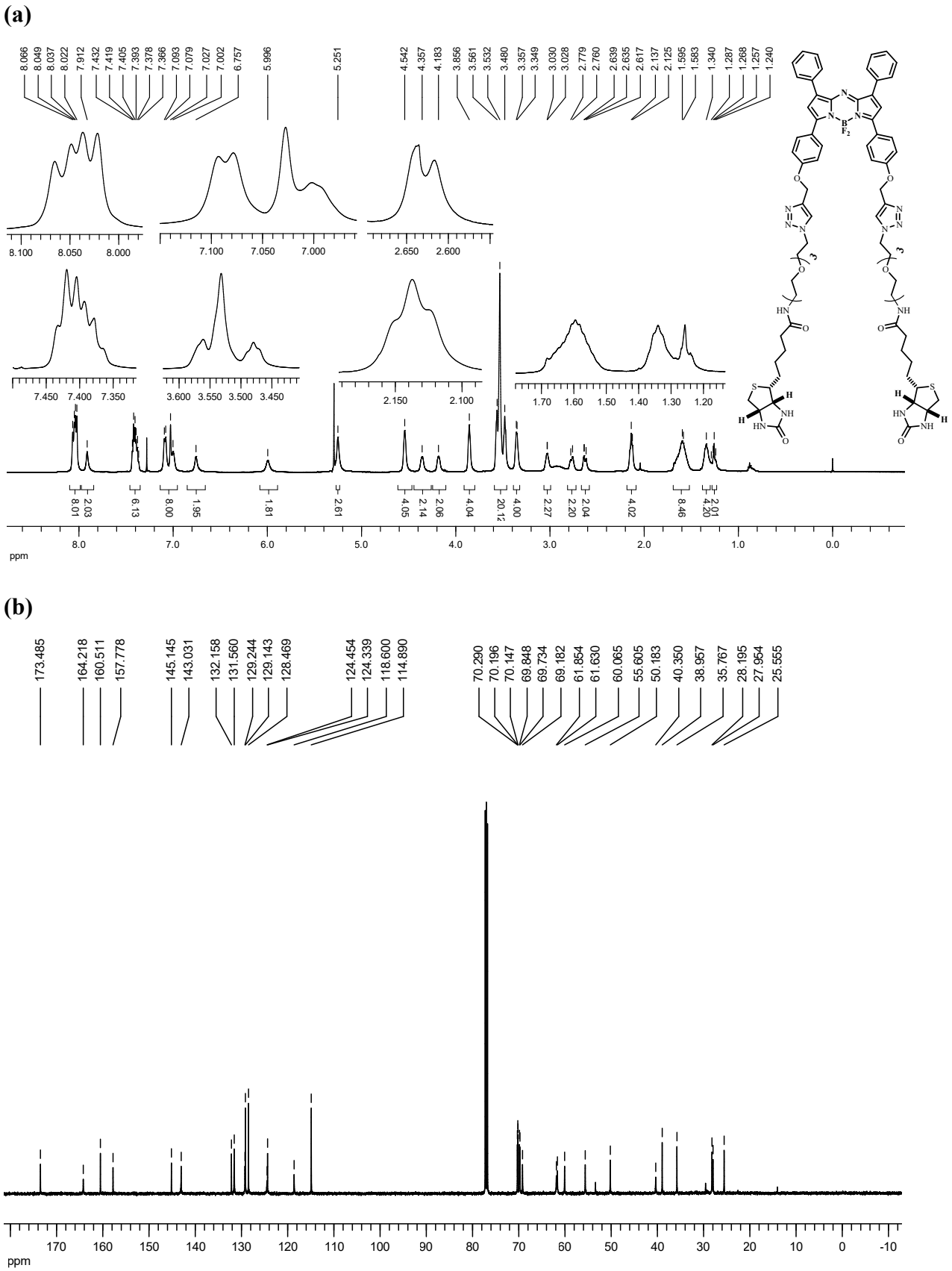


Figure S17: (**a**) ^{1}H-NMR (500 MHz) and (**b**) ^{13}C-NMR (126 MHz) of compound **DPR1b** in $CDCl_3$.

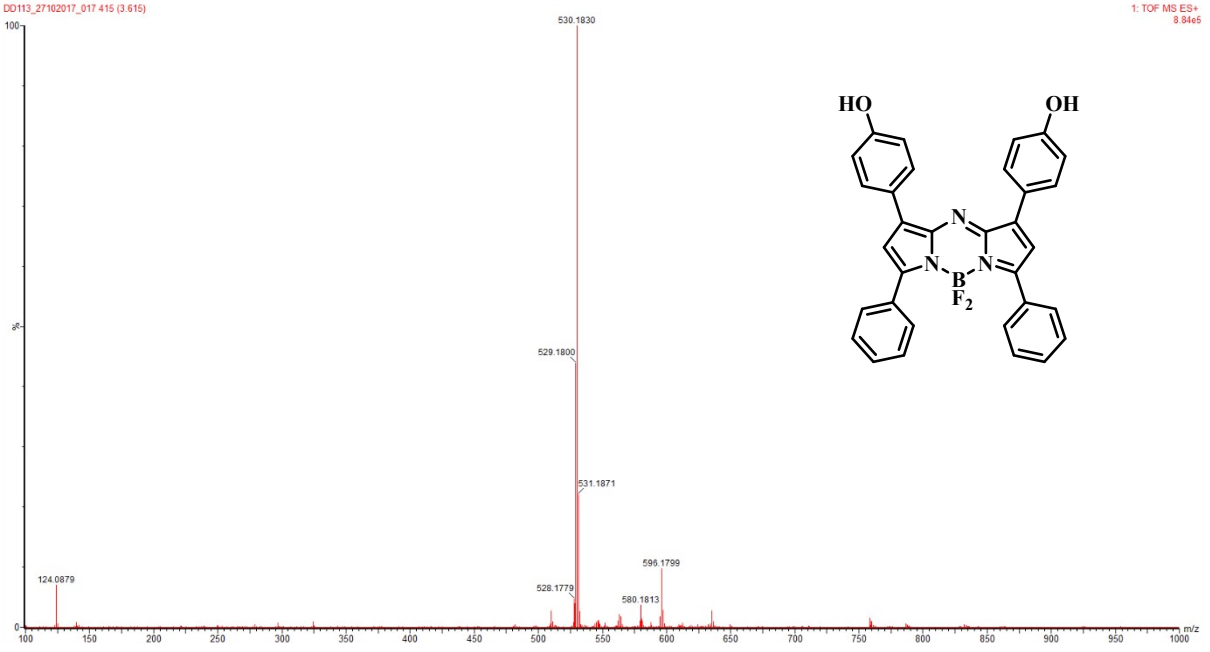


Figure S18: HRMS spectrum of compound **1c**

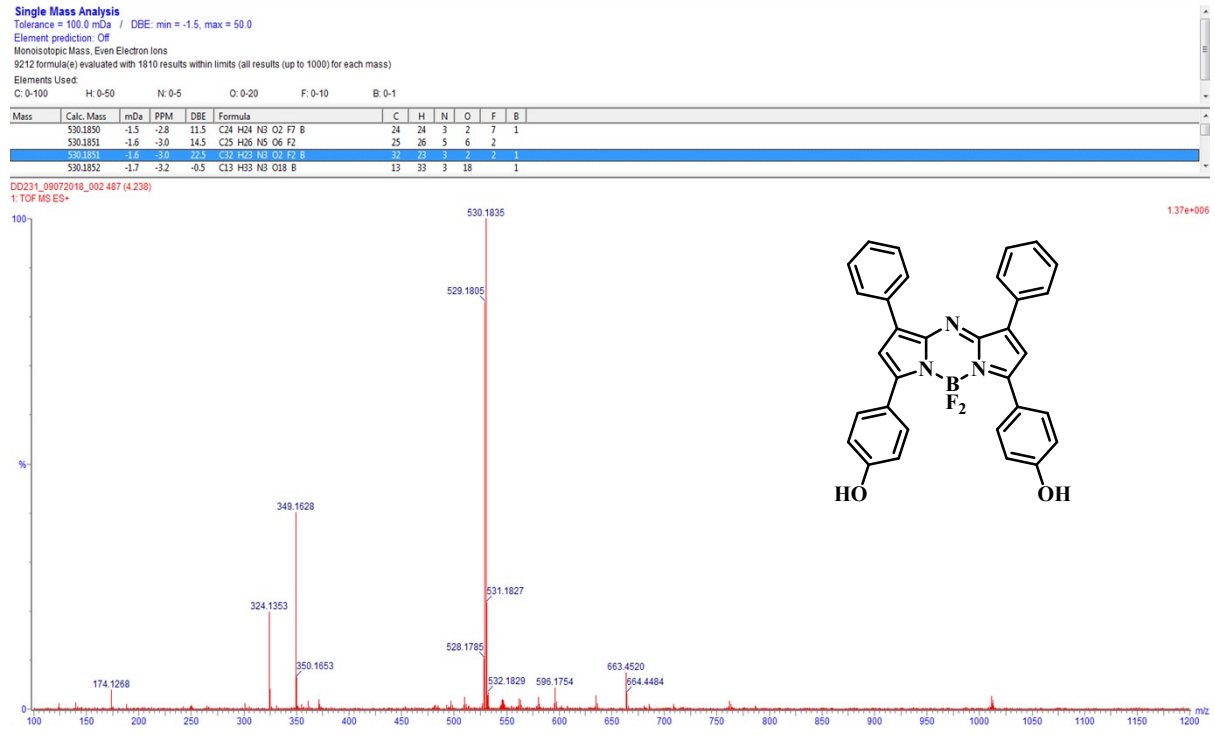


Figure S19: HRMS spectrum of compound **2c**

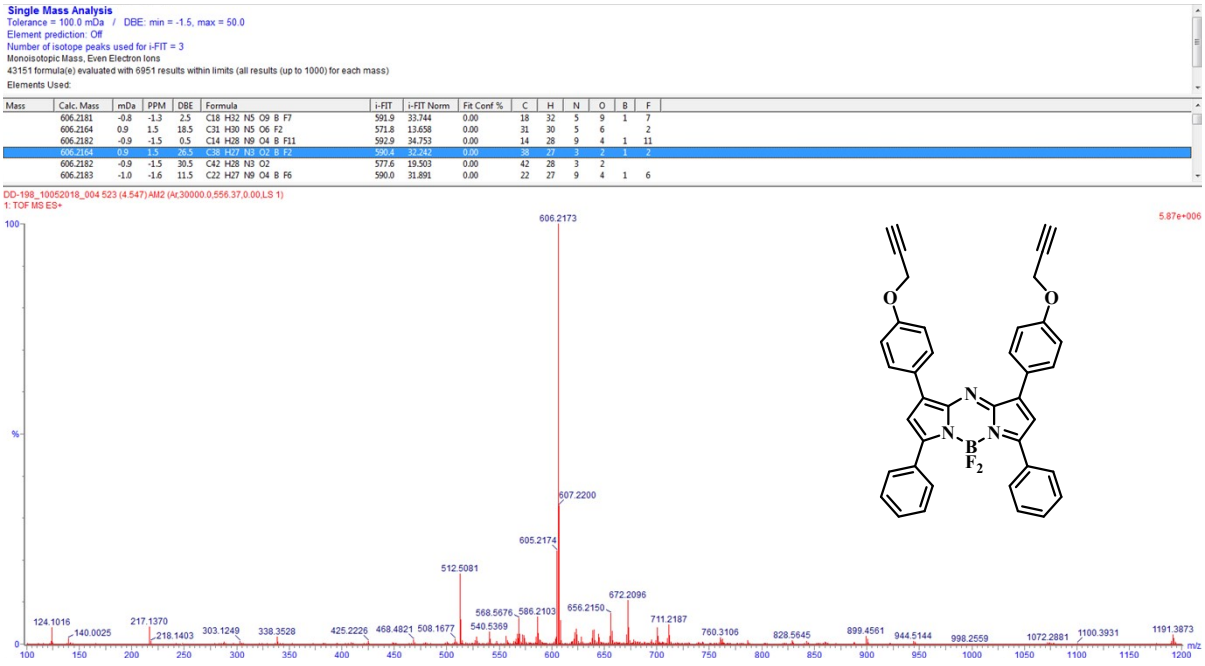


Figure S20: HRMS spectrum of compound **1d**

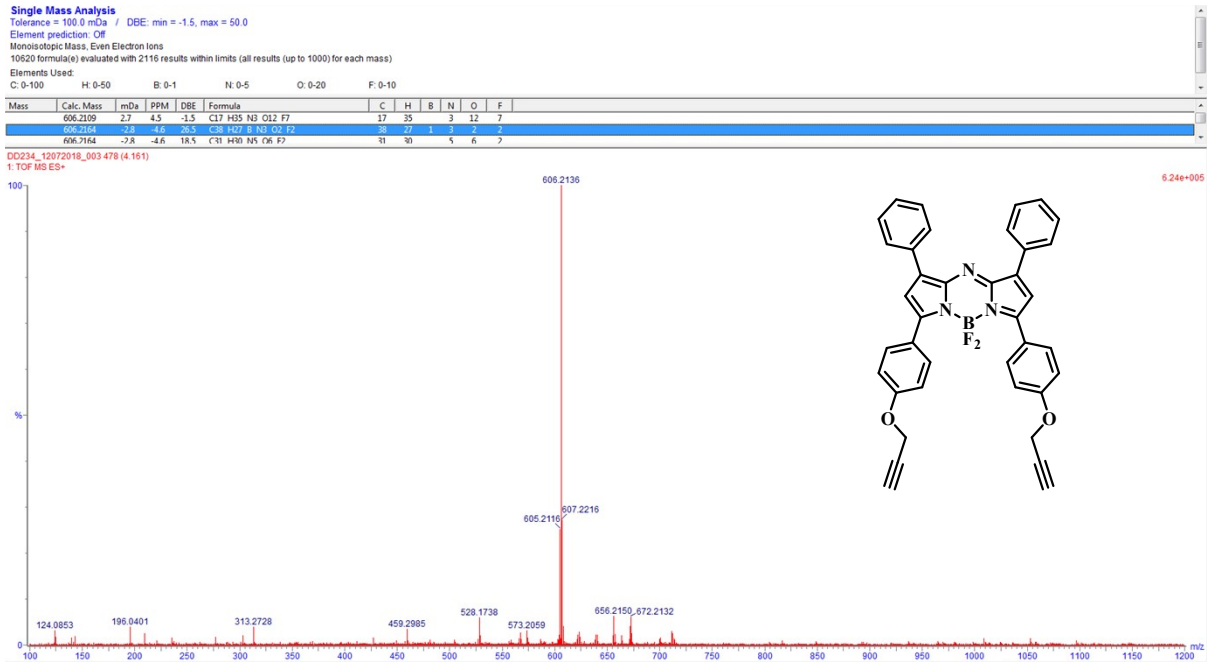


Figure S21: HRMS spectrum of compound **2d**

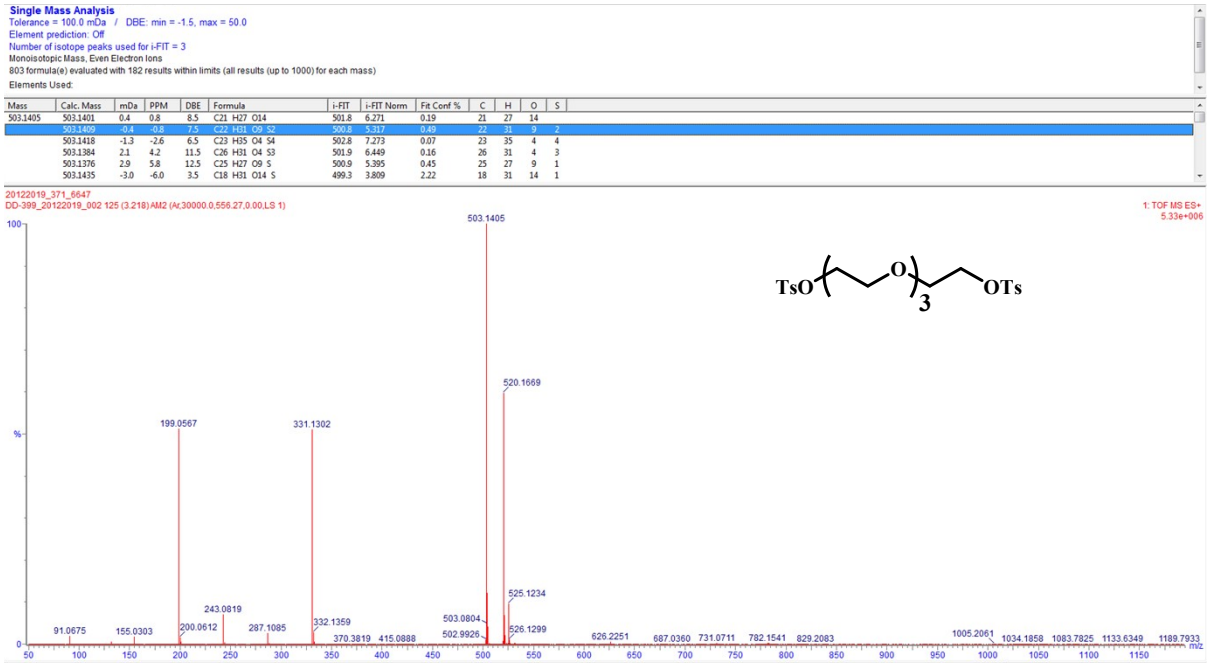


Figure S22: HRMS spectrum of compound **3a**

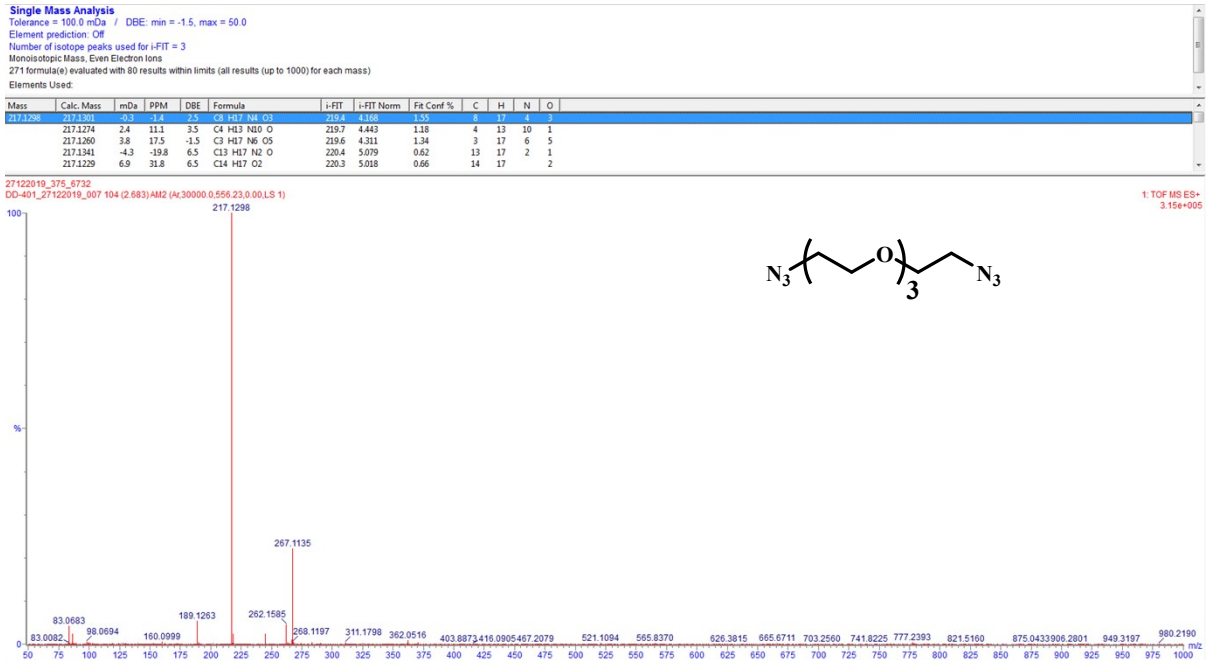


Figure S23: HRMS spectrum of compound **3b**

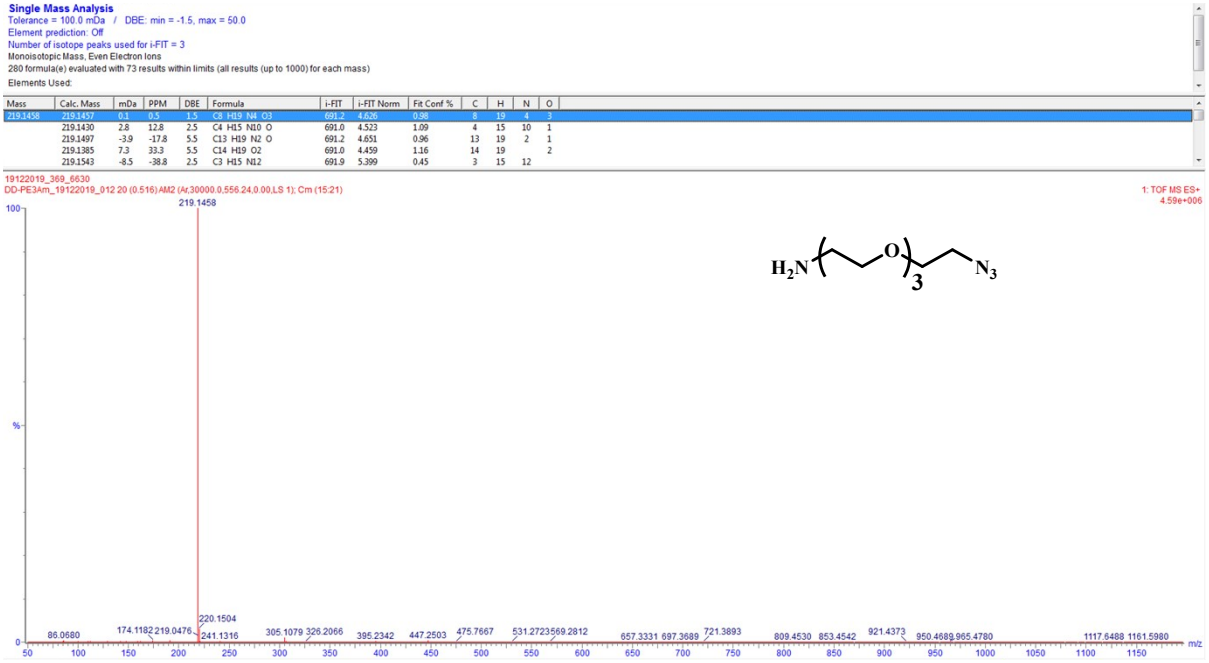


Figure S24: HRMS spectrum of compound **3c**

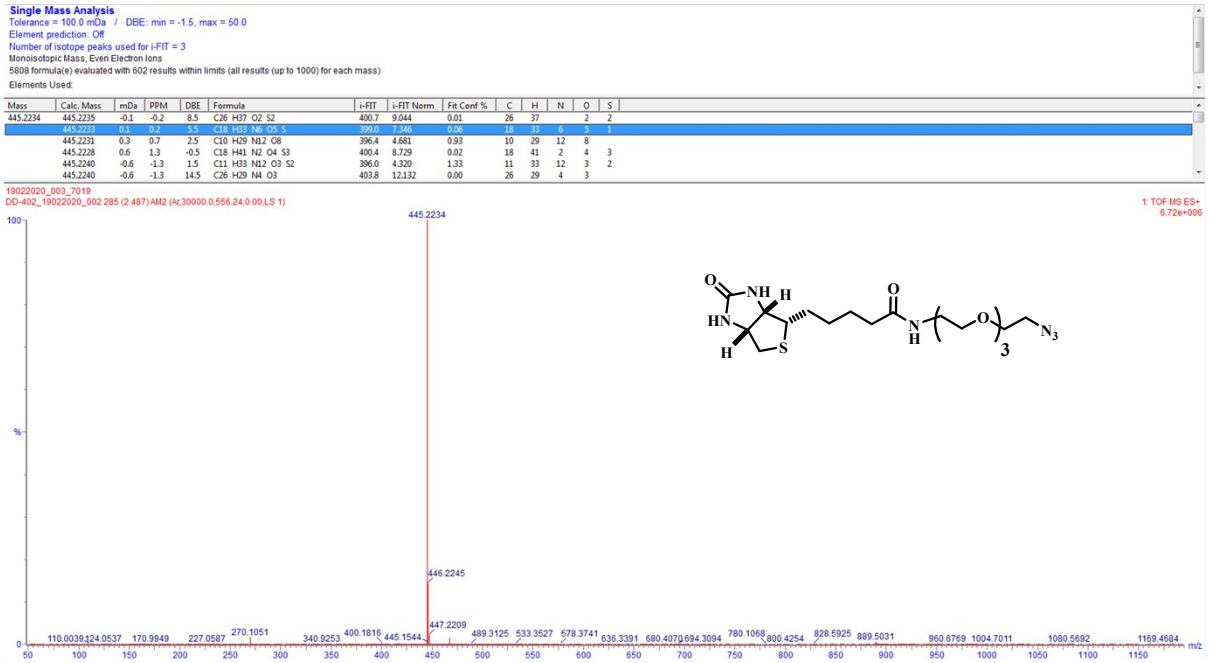


Figure S25: HRMS spectrum of compound **AzBiotin**

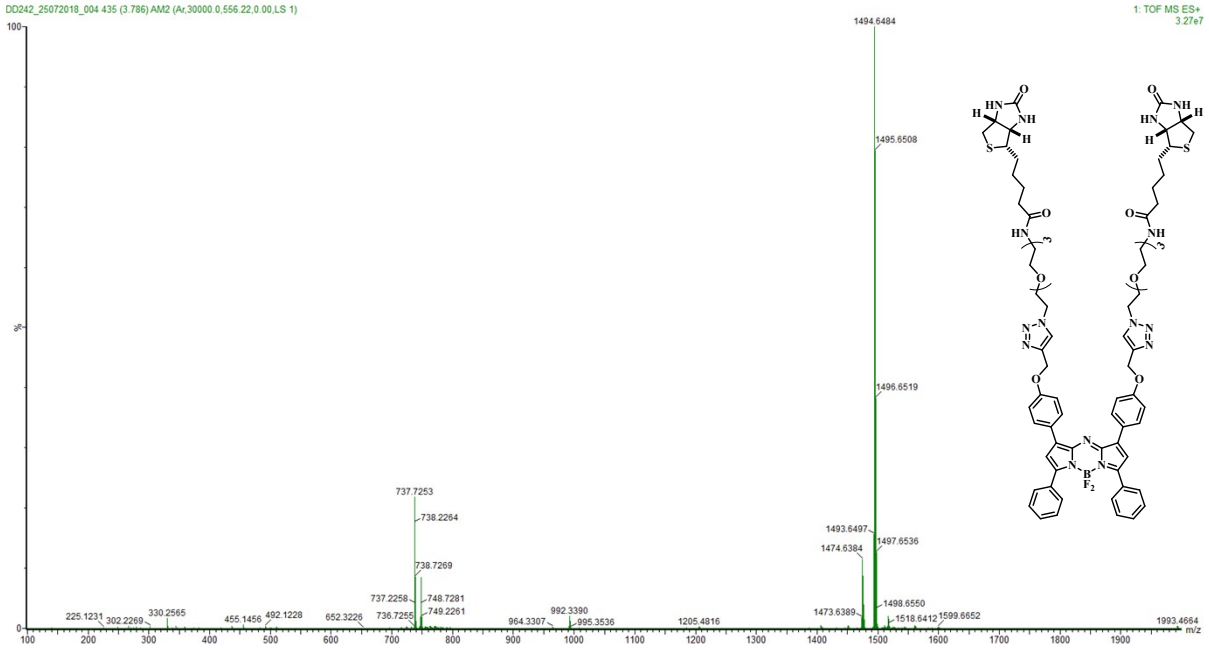


Figure S26: HRMS spectrum of compound **DPR1a**

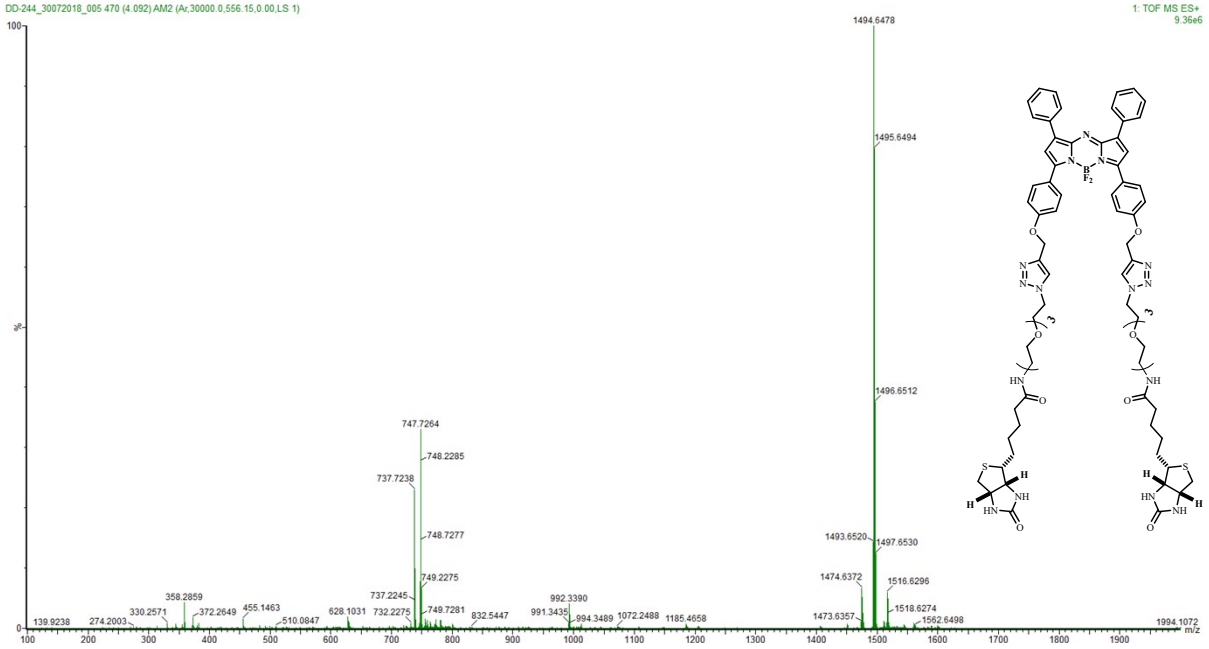


Figure S27: HRMS spectrum of compound **DPR1b**

Reference

1 V. Bandi, M. E. El-Khouly, K. Ohkubo, V. N. Nesterov, M. E. Zandler, S. Fukuzumi and F. D'Souza, *Chemistry – A European Journal*, 2013, **19**, 7221–7230.