# Supporting Information

## Mitochondria-targeted biotin-conjugated BODIPYs for cancer imaging and therapy

Dhiraj Dutta[a,b], Rajshree R. Nair[c,d], Kashmiri Neog[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.*

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

[c]*Cancer Research Program 4, Rajiv Gandhi Centre for Biotechnology, Trivandrum 695014, Kerala, India.*

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

*E-mail: gogoipranj@yahoo.co.uk; gogoipranj@gmail.com; sasha@rgcb.res.in*

**Corresponding author.*

### Table of content

*1. General Information*

All reagents and solvents were purified according to standard procedures or were obtained from commercial sources from Aldrich, TCI, Merck, Thermo-fisher, Invitrogen and Spectrochem. Reactions were monitored by thin-layer chromatography (TLC) on aluminium-backed silica gel $60F_{254}$ plates (0.2 mm thickness). Merck silica gel (Kieselgel 60; 230−400 mesh) was used for chromatography columns. Flash chromatography was performed on redisep silica gel columns. NMR spectra were recorded at 500 MHz on a Bruker Advance spectrometer and 400 MHz Jeol ECZ 400R spectrophotometer. HRMS data were recorded by electron spray ionization with a Q-TOF mass analyzer. Purity of our final compounds were analyzed by HPLC (Waters advanced fully automated HPLC system, 2545 binary gradient module) with a flow rate of 1 mL/min using a C18 column (SunFire® C18 5µm, 250 × 4.6 $mm^2$). A solution of compounds in methanol were injected, and a mixture of 0.1% TFA (v/v) in milli-Q water and HPLC-grade methanol with a gradient of 95% was used as the mobile phase. All our final compounds are >95% pure by HPLC analysis.

*2) Synthesis of BODIPY core:*

*a) Synthesis of 4-(prop-2-yn-1-yloxy)benzaldehyde **2a**:*[1]

Compound **2a** was synthesized using reported methods. In a round bottomed flask flushed with argon, 4-hydroxy benzaldehyde **1a** (1 g, 8.19 mmol, 1 eq) and $K_2CO_3$ (3.4 g, 24.6 mmol, 3 eq) were added. After degassing with argon, anhydrous DMF was added and the solution was allowed to stir for 30 mins at 60 $^oC$ under argon atmosphere. The mixture was then cooled to room temperature followed by addition of propargyl bromide (0.745 mL, 9.83 mmol, 1.2 eq) and stirred at rt for 4 h. After completion of the reaction, the mixture was poured into ice cold water with stirring. After filtration, off white solid was obtained as product **2a** (0.764 g, 58.24%) which was used for the next step without any further purification.

*b) Synthesis of propargylated BODIPY **3**:*[1]

In a round bottomed flask flushed with argon, a mixture of **2a** (3 g, 18.73 mmol, 1 eq) and 2, 4-dimethylpyrrole **2b** (3.8 mL, 37.46 mmol, 2 eq) was stirred in anhydrous DCM at room temperature. After that TFA (0.4 mL) was added to the mixture in an ice bath, and the reaction was allowed to stir at rt overnight. After that DDQ (1 eq) dissolved in THF (40 mL) was added to the mixture and allowed to stir for 7 h. The reaction mixture was then cooled in ice water bath and TEA (18 mL) was added dropwise for 30 min followed by addition of $BF_3.OEt_2$ (18 mL) and stirred overnight. The solution was then concentrated under reduced pressure and residue was added to water and stirred for 24 h. The solution was then extracted with DCM and purified by flash column chromatography using hexane/ethyl acetate (9:1) as eluent to give **3** as red solid (0.956 g, 13.5%); m.p.: 215.8 −218.5 ºC; **$^1$H NMR (500 MHz, $CDCl_3$):** δ 7.21 – 7.17 (m, 2H), 7.11 – 7.07 (m, 2H), 5.98 (s, 2H), 4.76 (d, *J* = 2.3 Hz, 2H), 2.58 – 2.53 (m, 7H), 1.42 (s, 6H); **$^{13}$C NMR (126 MHz, $CDCl_3$):** δ 158.0, 155.3, 143.1, 141.4, 131.7, 129.1, 127.9, 121.1, 115.5, 77.9, 75.8, 55.9, 29.6, 14.5; **HRMS (+ESI) Calcd for $C_{22}H_{21}BF_2N_2O^+$ $[M+H]^+$:** 379.1793; found: 379.1788.

*c) Synthesis of iodinated BODIPY **4**:*[1]

To a solution of **3** (0.05 g, 0.132 mmol, 1 eq) in a mixture of chloroform and acetic acid (20 mL, 3:1) as solvents, *N*-iodosuccinimide (0.074 g, 0.33 mmol, 2.5 eq) was added and stirred at room temperature for 10 h. After the completion of the reaction, the reaction mixture was quenched with sodium thiosulphate solution and extracted with chloroform (2 ×10 mL). The organic layer was washed with sodium bicarbonate solution and dried over $Na_2SO_4$. After removing the organic solvent under reduced pressure, the residue was purified by flash column chromatography on silica gel using hexane/ethyl acetate (9:1) as eluent to give **4** as red solid (0.067 g; 80.56% yield); m.p.: 252.3-253.7 ºC; **$^1$H NMR (500 MHz, $CDCl_3$):** δ 7.18 – 7.10 (m, 4H), 4.78 (d, *J* = 2.4 Hz, 2H), 2.64 (s, 6H), 1.44 (s, 6H); **$^{13}$C NMR (126 MHz, $CDCl_3$):** δ 158.4,

156.6, 145.3, 141.1, 131.6, 129.0, 127.5, 115.9, 56.0, 29.6, 17.1, 15.9; **HRMS (+ESI) Calcd for $C_{22}H_{19}BF_2I_2N_2O^+$ $[M+H]^+$:** 630.9726; found: 630.9837.

*3) Synthesis of AzBiotins:*

**AzBiotin1** has been synthesized using our previously reported method.[2] **AzBiotin2** has been procured from TCI.

*4) Synthesis of BODIPY-biotin conjugates*

The BODIPY-biotin conjugates were synthesized according to scheme 1. General procedure for **KDP1** and **KDP2** is described below.

To the solution of AzBiotin (1.2 eq) in a mixture of $CHCl_3$, EtOH and water (6:1:1, 8 mL) as solvents, propargylated BODIPY (1 eq), sodium ascorbate (40 mol%), $CuSO_4.5H_2O$ (23 mol%) were added and stirred at room temperature for 2 h. After completion of the reaction, the reaction mixture was quenched with water and extracted with $CHCl_3$ (3 × 15 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 to afford the product.

**KDP1**: Using the general procedure, **AzBiotin1** (0.120 g, 0.27 mmol), **4** (0.142 g, 0.225 mmol), sodium ascorbate (10.3 mg, 0.0517 mmol), $CuSO_4.5H_2O$ (22.5 mg, 0.09 mmol) in a mixture solvent system of $CHCl_3$, EtOH and water (6:1:1, 8 mL), compound **KDP1** was obtained as red solid (152 mg, 62.87% yield) after purification by flash column chromatography using DCM/MeOH (9:1) as eluent; m.p.: 93.8-94.5 $^oC$; **$^1$H NMR (500 MHz, $CDCl_3$):** δ 7.91 (s, 1H), 7.20-7.12 (br, 4H), 6.88 (t, *J* = 5.3 Hz, 1H), 6.69 (s, 1H), 5.76 (s, 1H), 5.26 (s, 2H), 4.61 (t, *J* = 5.04 Hz, 2H), 4.48 (dd, *J* = 7.6, 5.0 Hz, 1H), 4.30 (dd, *J* = 7.3, 4.6 Hz, 1H), 3.93 (t, *J* = 5.1 Hz, 2H), 3.67-3.59 (m, 8H), 3.55 (t, *J* = 5.2 Hz, 2H), 3.45-3.38 (m, 2H), 3.13 (td, *J* = 7.4, 4.5 Hz, 1H), 2.88 (dd, *J* = 12.8, 4.9 Hz, 1H), 2.73 (d, *J* = 12.8 Hz, 1H), 2.64 (br, 6H), 2.21 (t, *J* = 7.6 Hz, 2H), 1.78 – 1.60 (m, 4H), 1.43 (s, 8H); **$^{13}$C NMR (126 MHz, $CDCl_3$):** δ 173.4, 164.0, 159.1, 156.5, 145.1, 143.0, 141.2, 131.5, 129.0, 127.1, 124.2, 115.6, 85.5, 70.3, 70.2, 70.2, 69.9, 69.8,

69.3, 61.8, 61.6, 60.0, 55.5, 50.2, 40.4, 38.9, 35.8, 28.1, 27.9, 25.5, 17.0, 15.9; **HRMS (+ESI) Calcd for $C_{40}H_{51}BF_2I_2N_8O_6S^+$ $[M+H]^+$:** 1075.1881; found: 1075.1909.

**KDP2:** Using the general procedure, **AzBiotin2** (0.050 g, 0.1 mmol), **4** (0.052 g, 0.083 mmol), sodium ascorbate (7 mg, 0.033 mmol), $CuSO_4.5H_2O$ (6 mg, 0.019 mmol) in a mixture solvent system of $CHCl_3$, EtOH and water (6:1:1, 8 mL), compound **KDP2** was obtained as red solid (68 mg, 73.24% yield) after purification by flash column chromatography using DCM/MeOH (9:1) as eluent; m.p.: 94.4-95.6 $^oC$; **$^1H$ NMR (500 MHz, $CDCl_3$):** δ 8.13-7.83 (br, 1H), 7.20 – 7.09 (m, 4H), 7.05-6.87 (br, 1H), 5.32-5.19 (m, 2H), 4.59 (s, 2H), 4.49 (s, 1H), 4.30 (s, 1H), 3.90 (s, 2H), 3.70-3.46 (m, 15H), 3.40 (s, 2H), 3.22-2.68 (m, 4H), 2.64-2.57 (m, 6H), 2.27-2.11 (m, 2H), 1.78-1.52 (br, 4H), 1.45-1.34 (m, 8H); **$^{13}C$ NMR (126 MHz, $CDCl_3$):** δ 173.3, 159.1, 156.4, 145.1, 141.1, 131.4, 128.9, 127.0, 126.9, 115.5, 85.4, 70.3, 70.2, 69.8, 69.2, 61.8, 61.7, 61.6, 60.1, 60.1, 55.6, 55.5, 55.4, 55.3, 50.3, 40.4, 38.9, 35.7, 30.8, 29.5, 28.1, 27.9, 25.4; **HRMS (+ESI) Calcd for $C_{42}H_{55}BF_2I_2N_8O_7S^+$ $[M+H]^+$:** 1119.2143; found: 1119.2147.

***5)*** *Hydrophobic-hydrophilic characteristic tests.*

The octanol/water partition coefficient of our PSs was evaluated by adding *n*-octanol (1 mL), water (1 mL), and the sample (40 μL; 1 mM in DMSO) in a glass vial. The whole mixture was vigorously shaken for 30 minutes, then allowed to rest, and kept for 24 hours. The concentrations of PSs in the two phases were estimated by evaluating their fluorescence intensities.

*6) DLS profile of **KDP1** and **KDP2** in DMEM*

The stability of our self-assemblies in DMEM was checked by incubating them at 37 $^oC$ for 24 h using the DLS profile. The DLS profile shows that **KDP1** was less aggregated as compared to **KDP2** in DMEM, which may result in less photocytotoxicity of **KDP2**.

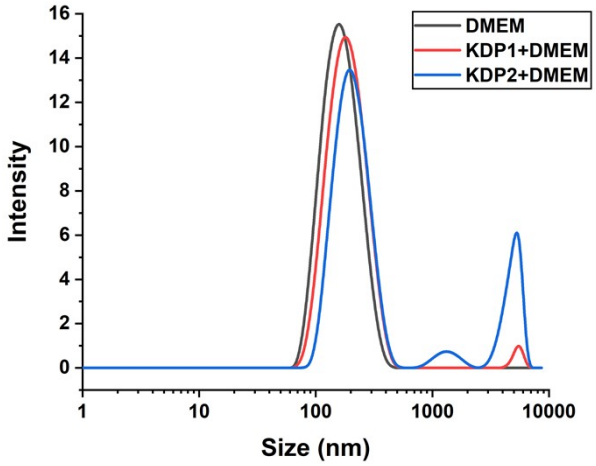



**Figure S1.** DLS profile of **DMEM**, **KDP1,** and **KDP2** in **DMEM** after 24 h of incubation at 37 °C. (DMEM, pH = 8.3)

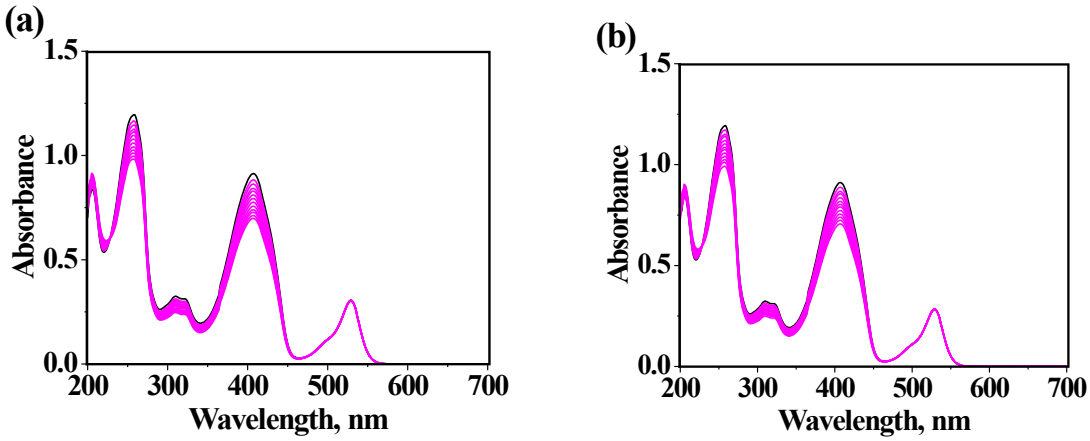



**Figure S2.** Absorption spectra of DPBF after irradiation at 540 nm in the presence of (a) **KDP1** and (b) **KDP2** for 120 s (interval of 10 s) in MeOH.

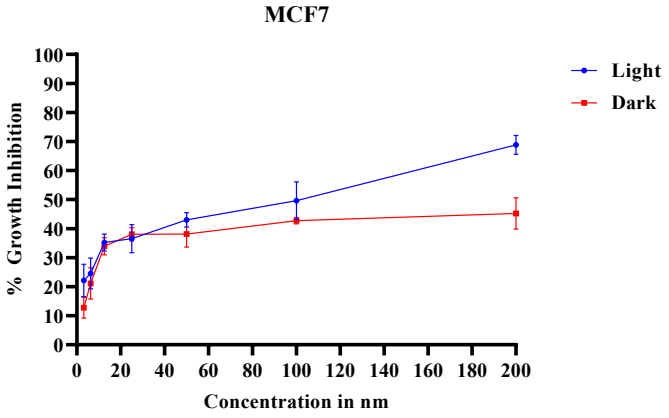
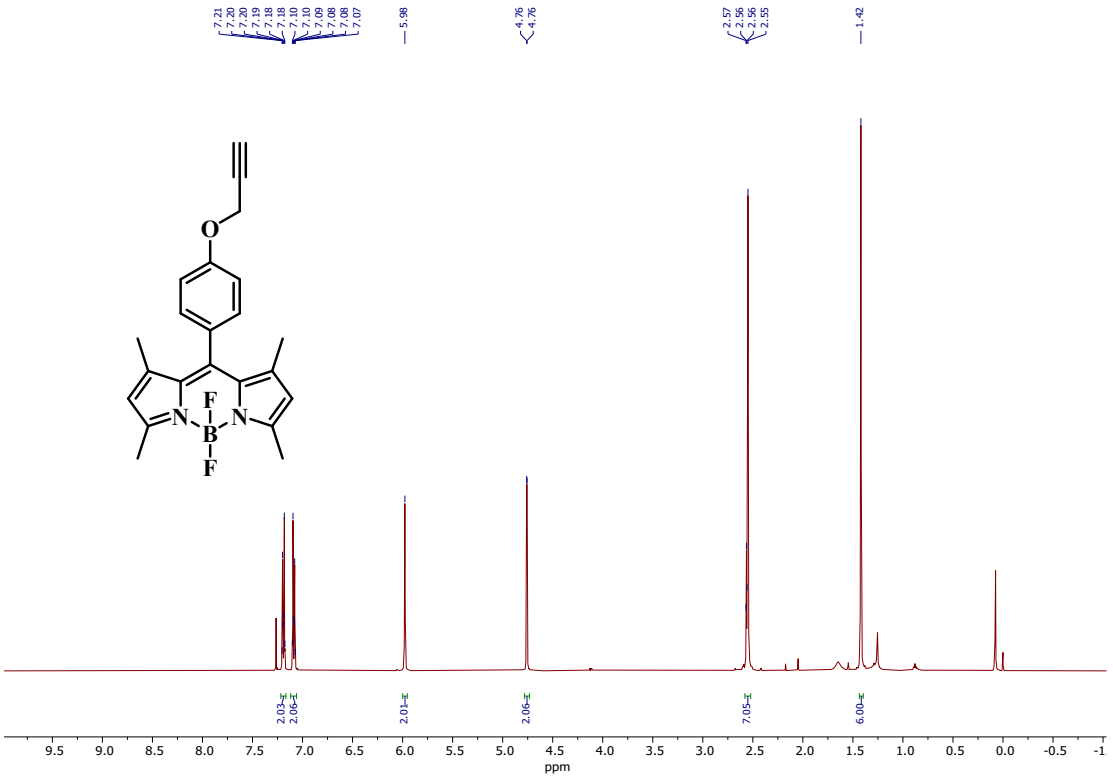



**Figure S3**: Photocytotoxicity of compound 4 in MDA-MB-231 breast cancer cell line

**(a)**




**(b)**

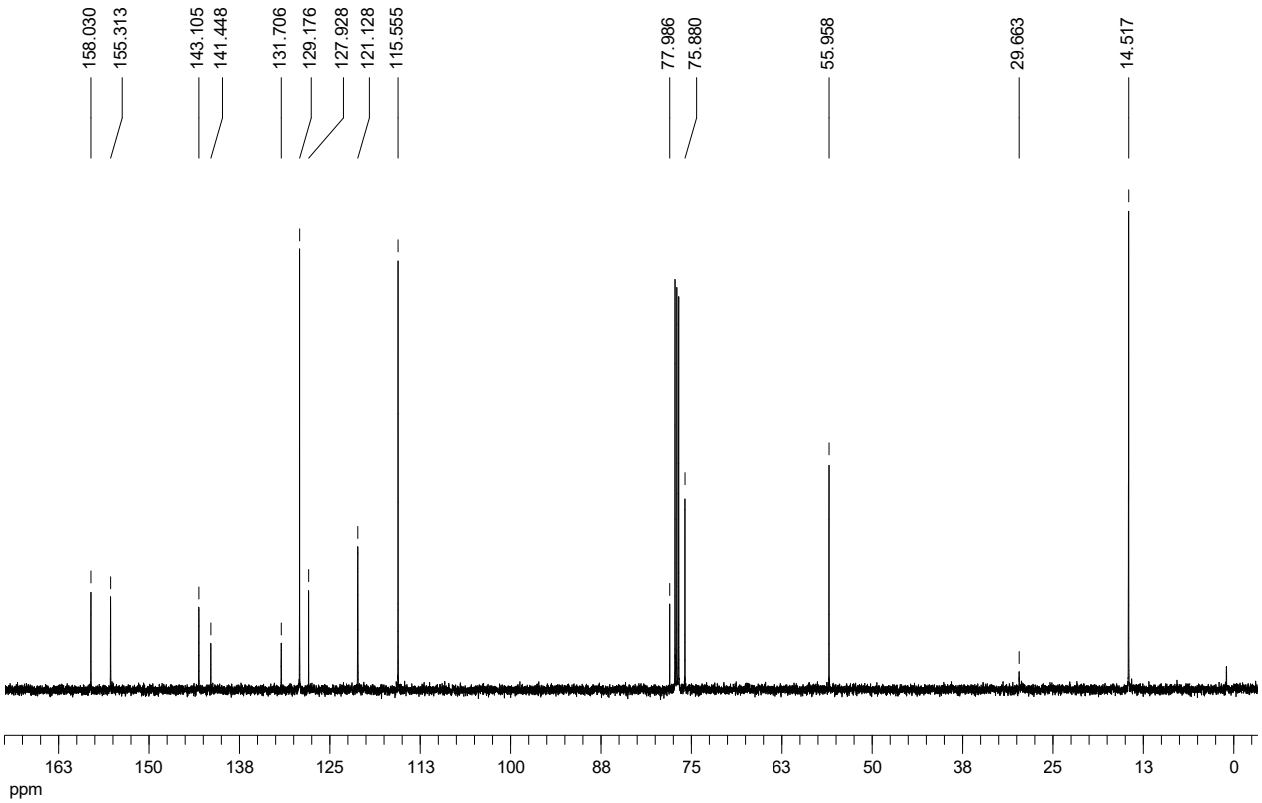
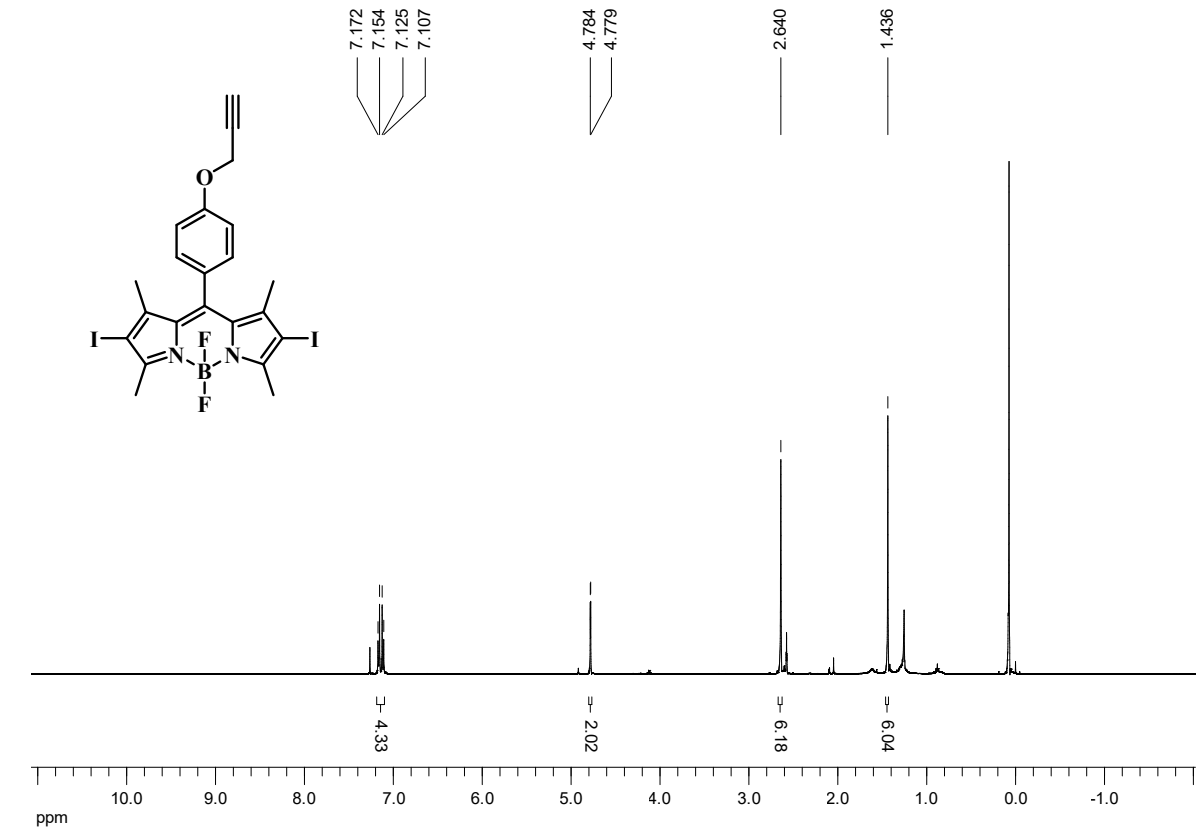



**Figure S4:** (**a**) $^{1}$H-NMR (400 MHz) and (**b**) $^{13}$C-NMR (101 MHz) of compound **3** in $CDCl_3$.

**(a)**




**(b)**

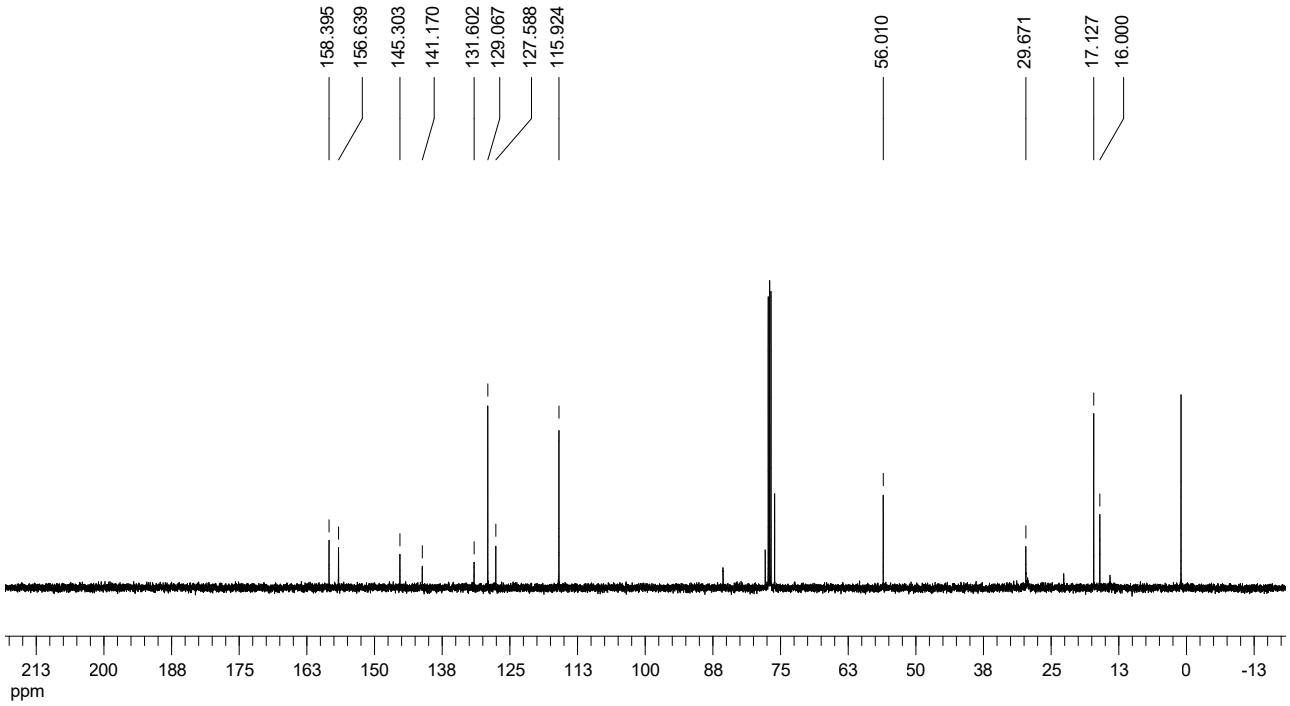
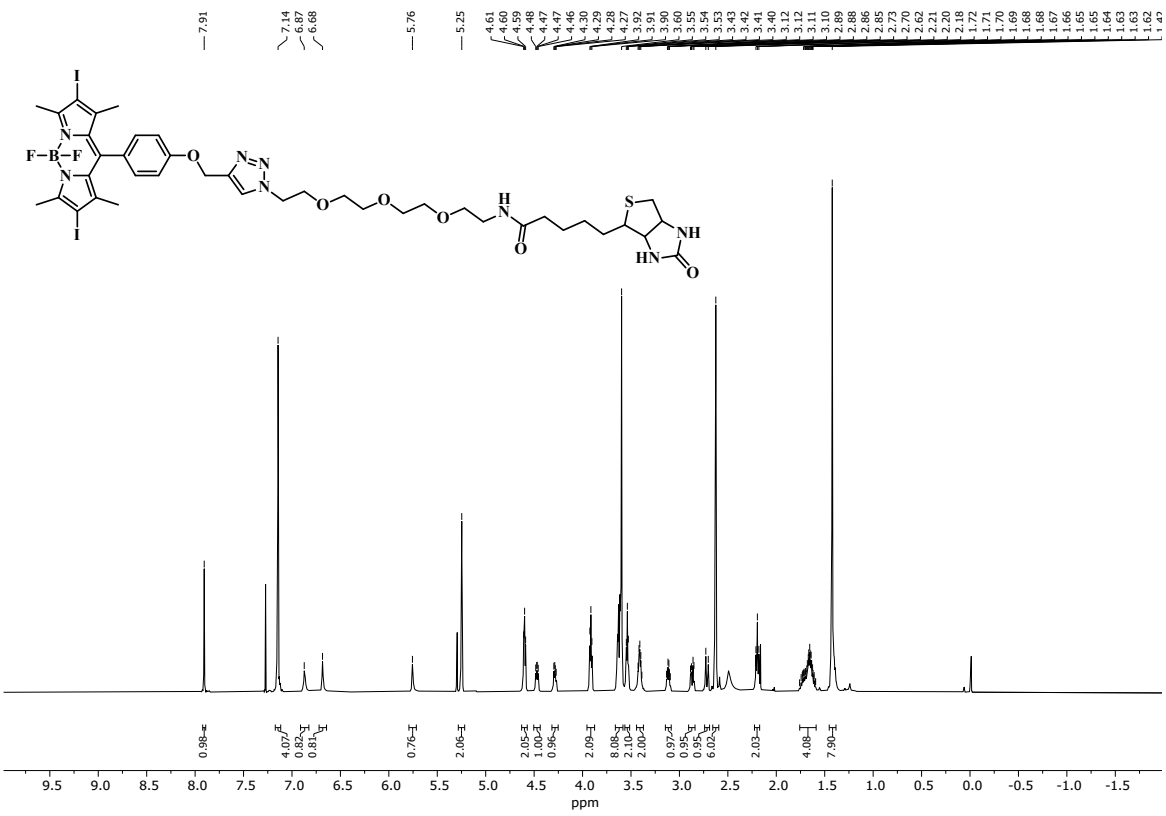



**Figure S5:** (**a**) $^{1}$H-NMR (500 MHz) and (**b**) $^{13}$C-NMR (126 MHz) of compound **4** in $CDCl_3$.

**(a)**




**(b)**

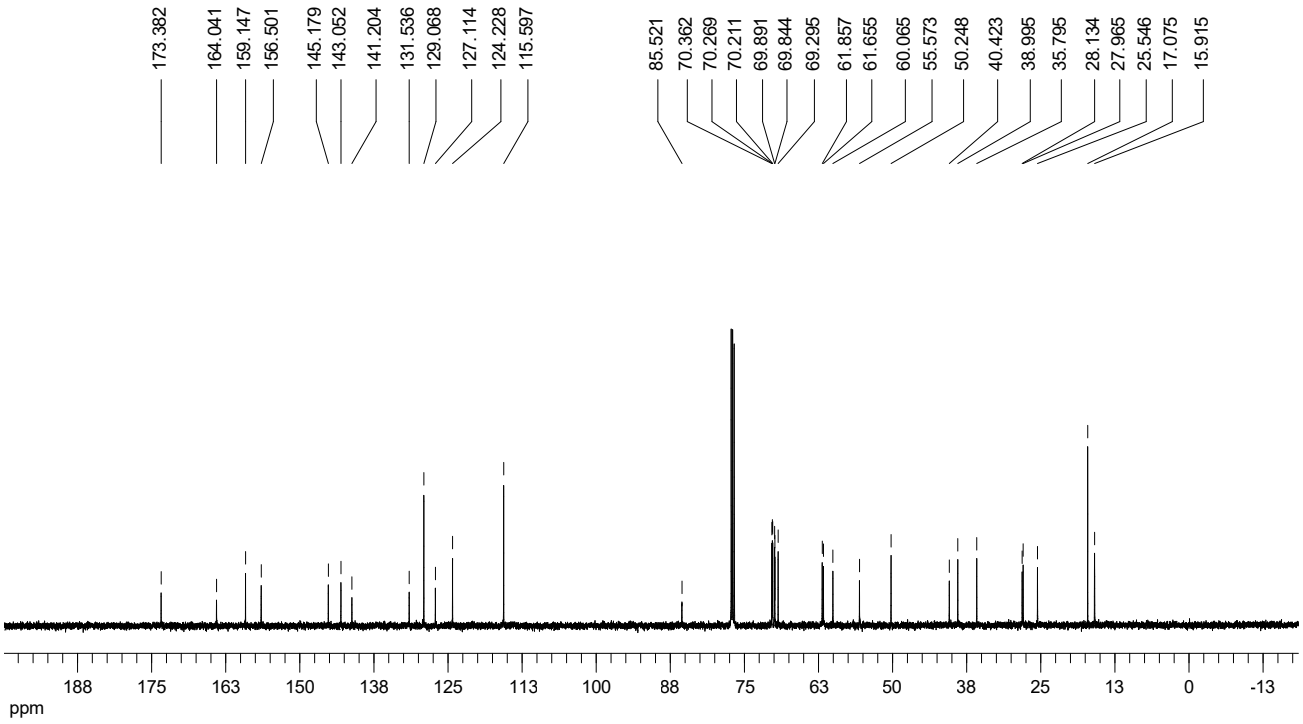
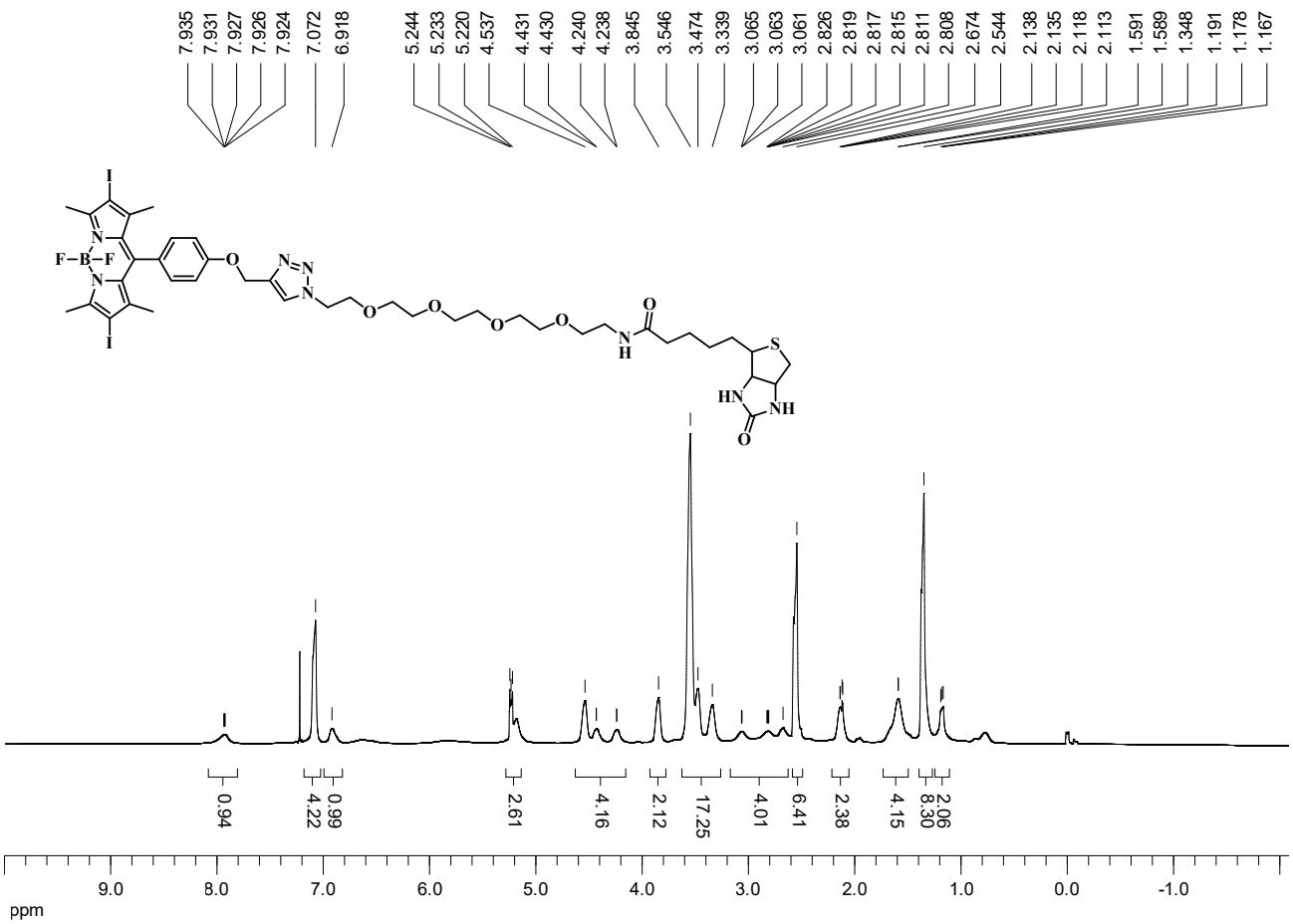



**Figure S6:** (**a**) $^{1}$H-NMR (500 MHz) and (**b**) $^{13}$C-NMR (126 MHz) of **KDP1** in $CDCl_3$.

**(a)**




**(b)**

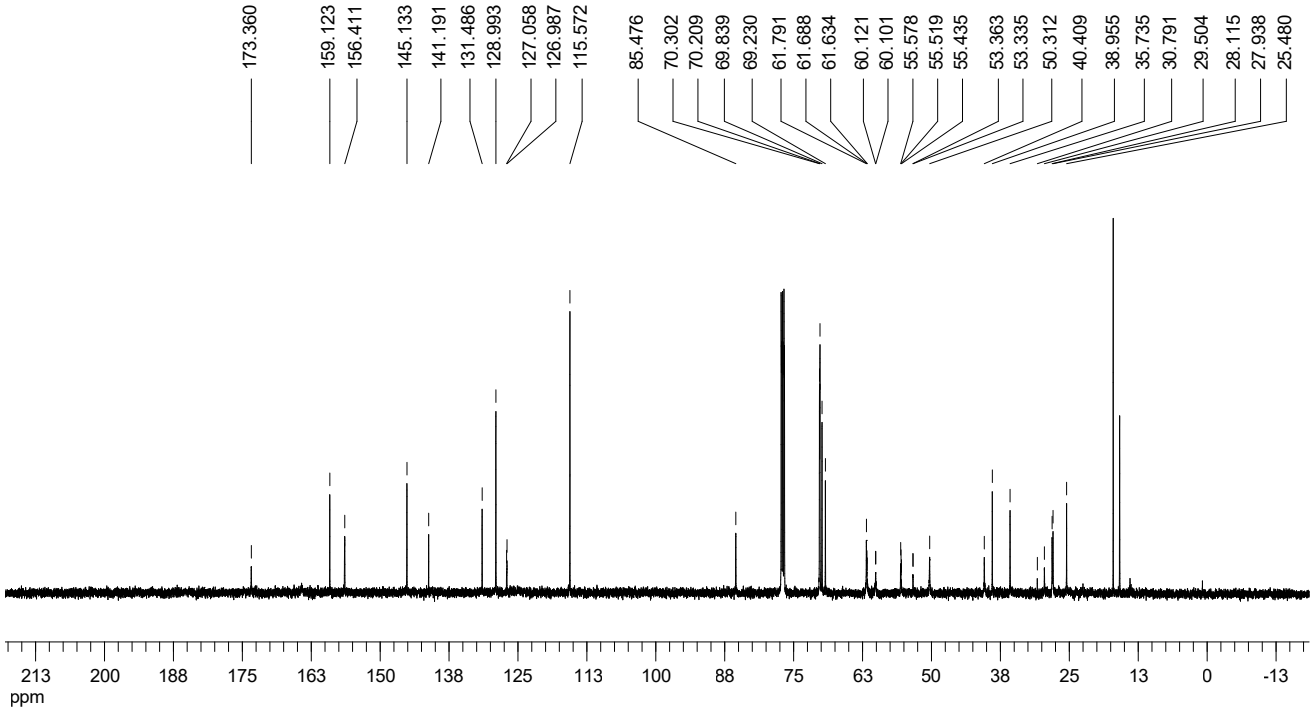



**Figure S7:** (**a**) $^{1}$H-NMR (500 MHz) and (**b**) $^{13}$C-NMR (126 MHz) of **KDP2** in $CDCl_3$.

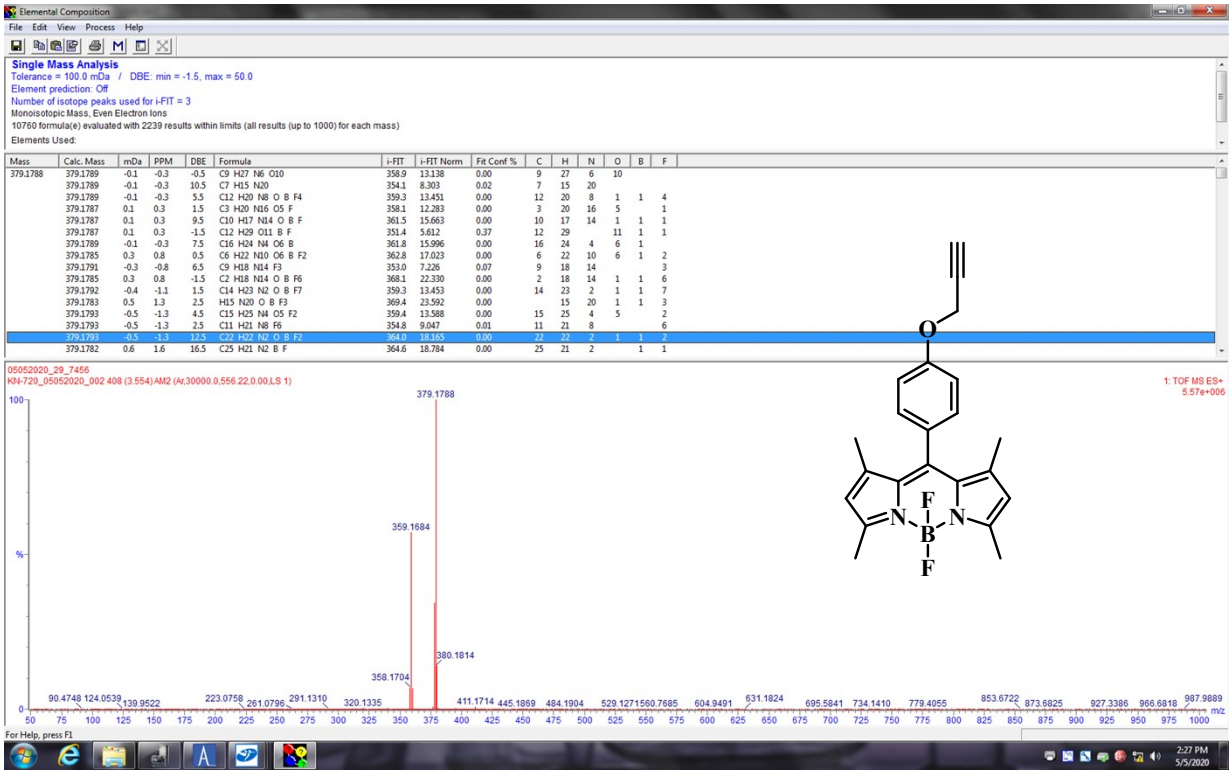



**Figure S8:** HRMS Spectrum of compound **3**

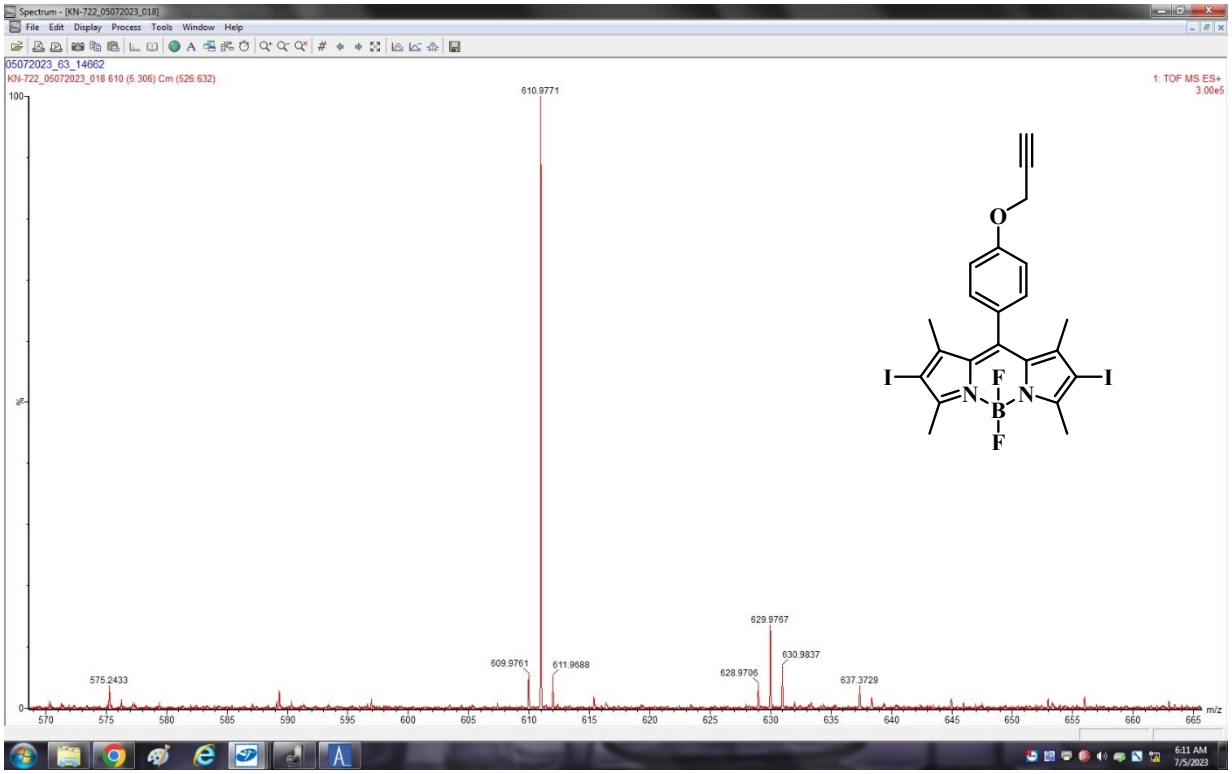



**Figure S9:** HRMS Spectrum of compound **4**

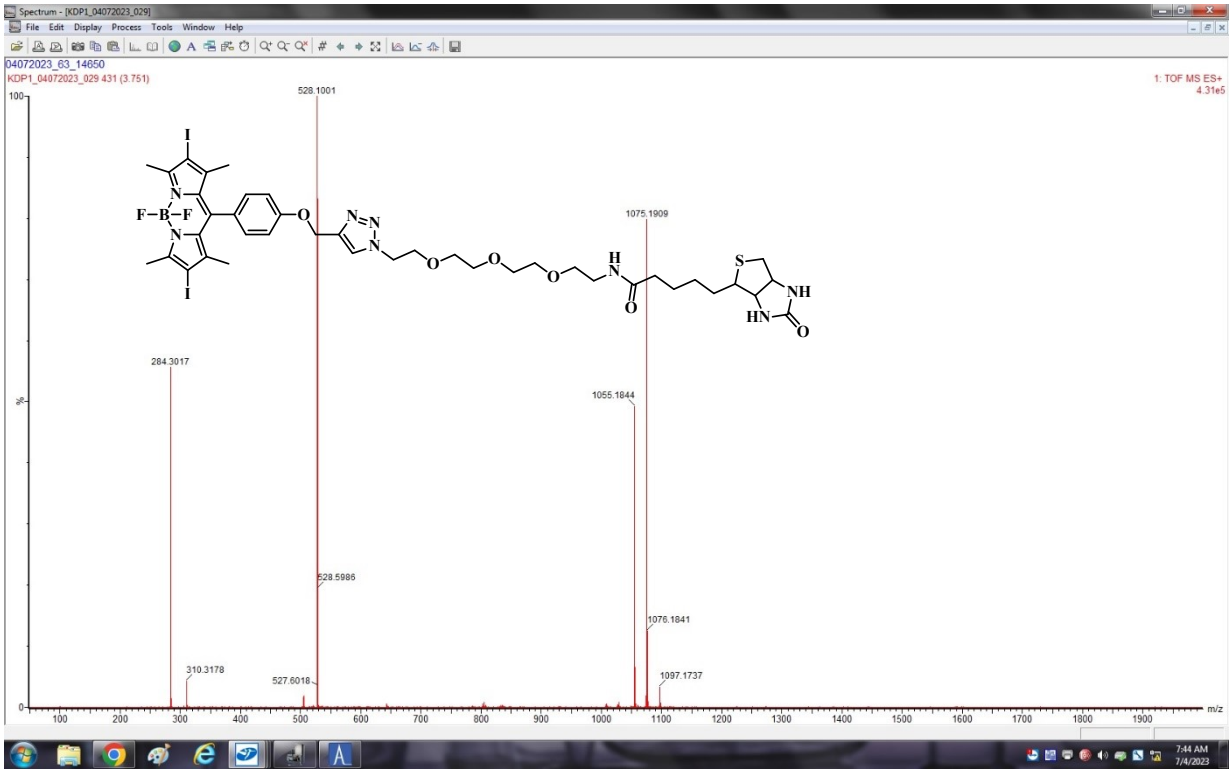



**Figure S10:** HRMS Spectrum of **KDP1**

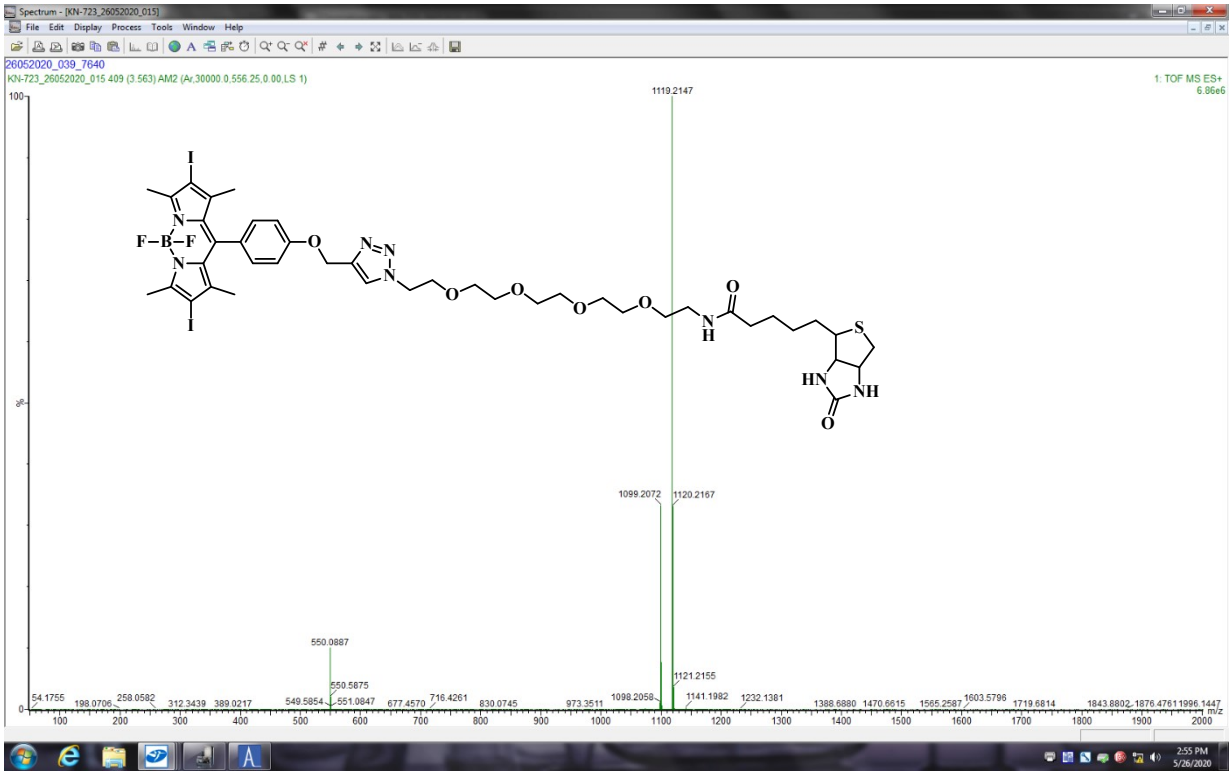



**Figure S11:** HRMS Spectrum of **KDP2**

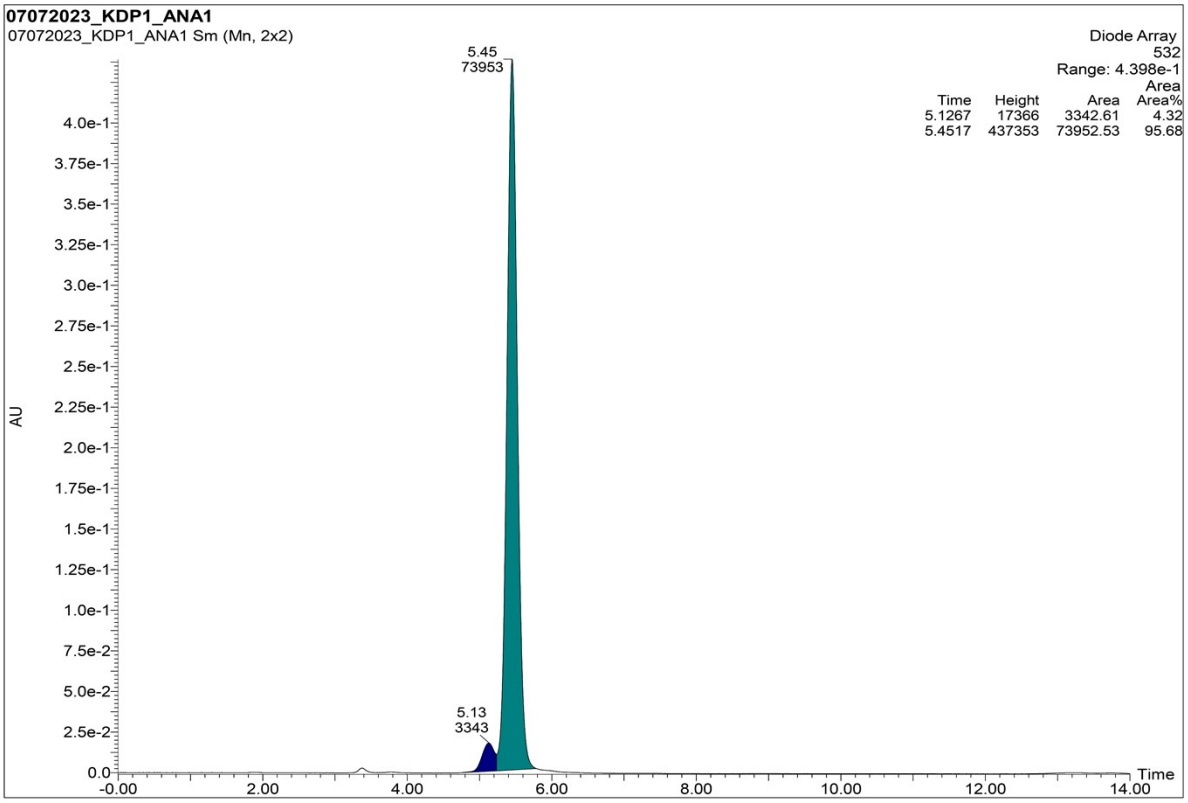



**Figure S12:** HPLC Chromatogram of compound **KDP1 (monitored at 532 nm)**

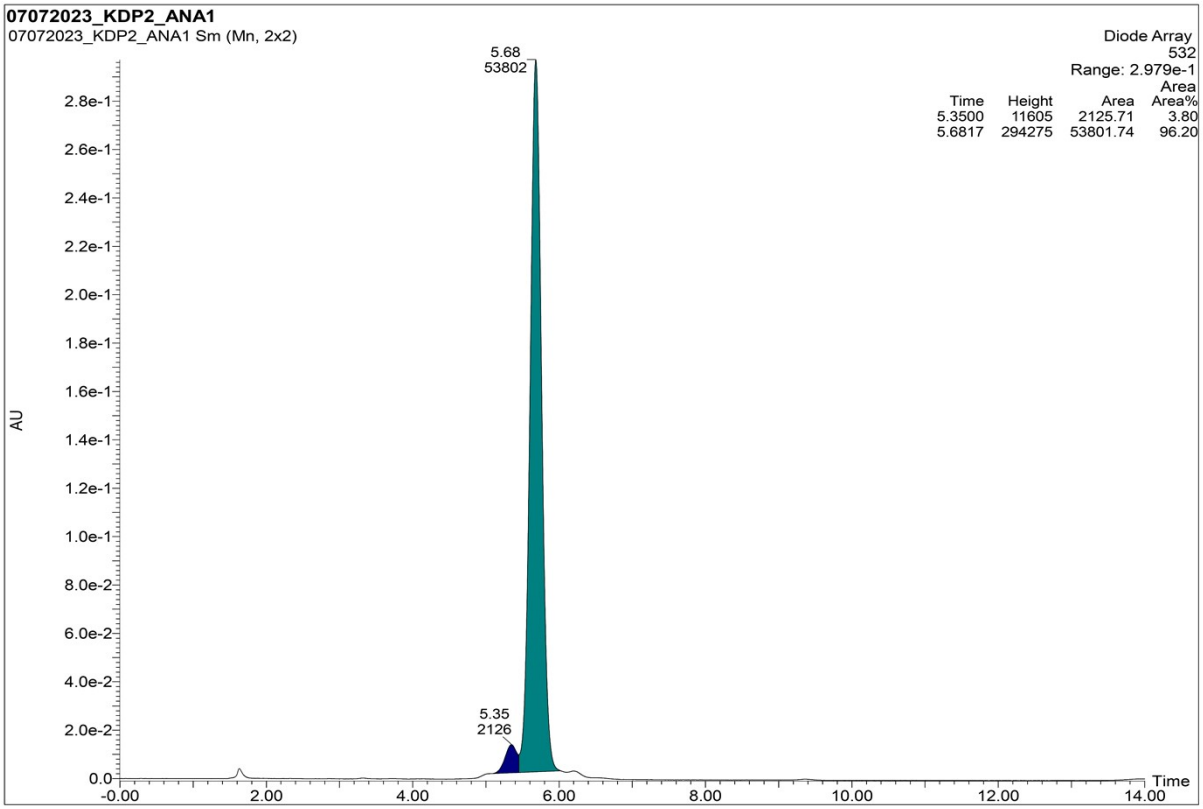



**Figure S13:** HPLC Chromatogram of compound **KDP2 (monitored at 532 nm)**

## References:


1 C. Zhang, J. Zhao, S. Wu, Z. Wang, W. Wu, J. Ma, S. Guo and L. Huang, *J Am Chem Soc*, 2013, **135**, 10566–10578.

2 D. Dutta, R. R. Nair, S. Mangalath, S. Asha Nair, J. Joseph, P. Gogoi and D. Ramaiah, *ACS Omega*, 2023, **8**, 26180-26190.