Supplementary Information

Click Reaction-Mediated Functionalization of Near-Infrared Pyrrolopyrrole Cyanine Dyes for Biological Imaging Applications

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General Experimental Methods

All chemicals and reagents were purchased from commercial sources and were used without further purification. Millipore grade water was used for the study. NMR data were recorded on either a 400 MHz Variant spectrometer or a Varian Unity-600 at ambient temperature and referenced to tertramethylsilane (TMS). The absorption spectra were collected on a Beckman Coulter DU640 spectrophotometer (Fullerton, CA, USA). The emission spectra were record on a Fluorolog III fluorometer (Horiba Jobin Yvon, Edison, NJ, USA) and the lifetime was calculated with the integrated software. The molar extinction coefficient was determined by Beer's Law between the concentration range of 0.2 – 0.6 μM. The relative fluorescence quantum yield was

determined using 1,1',3,3,3',3'-Hexamethylindotricarbocyanine iodide (HITCI) as a reference standard, which has a of 0.28 in EtOH. The quantum yield was calculated with the following equation:

$$\Phi_{sample} = \Phi_{standard} \times (L_{sample} / L_{standard}) \times (Abs_{standard} / Abs_{sample}) \times (n_{sample} / n_{standard})^2$$

Syntheses and Characterizations

2-methyl-6-((4-pentyn-1-yloxy)methyl)quinoline (1)

To a solution of (2-methyl-6-quinolinyl) methanol (1.25g, 7.2 mmol) in anhydrous DMF (ca. 100 mL) at 0 ^{o}C was added sodium hydride (0.60 g, 25.0 mmol). The reaction mixture was stirred at r.t. for 2 h and was added 5-chloro-1-pentyne (3 g, 29.4 mmol). The reaction mixture was stirred at r.t. overnight. After reaction, water (100 mL) was added, and the mixture was extracted with dichloromethane. The solvents were removed under reduced pressure. Purification by silica gel flash column chromatography (ethyl acetate: hexanes = 1:5) afforded **1** as a light yellow oil (1.30 g, 75%). ^{1}H NMR (400 MHz $CDCl_3$) δ 7.97 (1H, d, *J* = 8.7 Hz), 7.94 (1H, d, *J* = 8.3 Hz), 7.67 (1H, s), 7.59 (1H, dd, *J* = 8.7 Hz, 1.8 Hz), 7.21 (1H, d, *J* = 2.7 Hz), 4.62 (2H, s), 3.58 (2H, t, *J* = 5.7 Hz), 2.69 (3H, s), 2.30 (2H, m), 1.89-1.76 (3H, m).

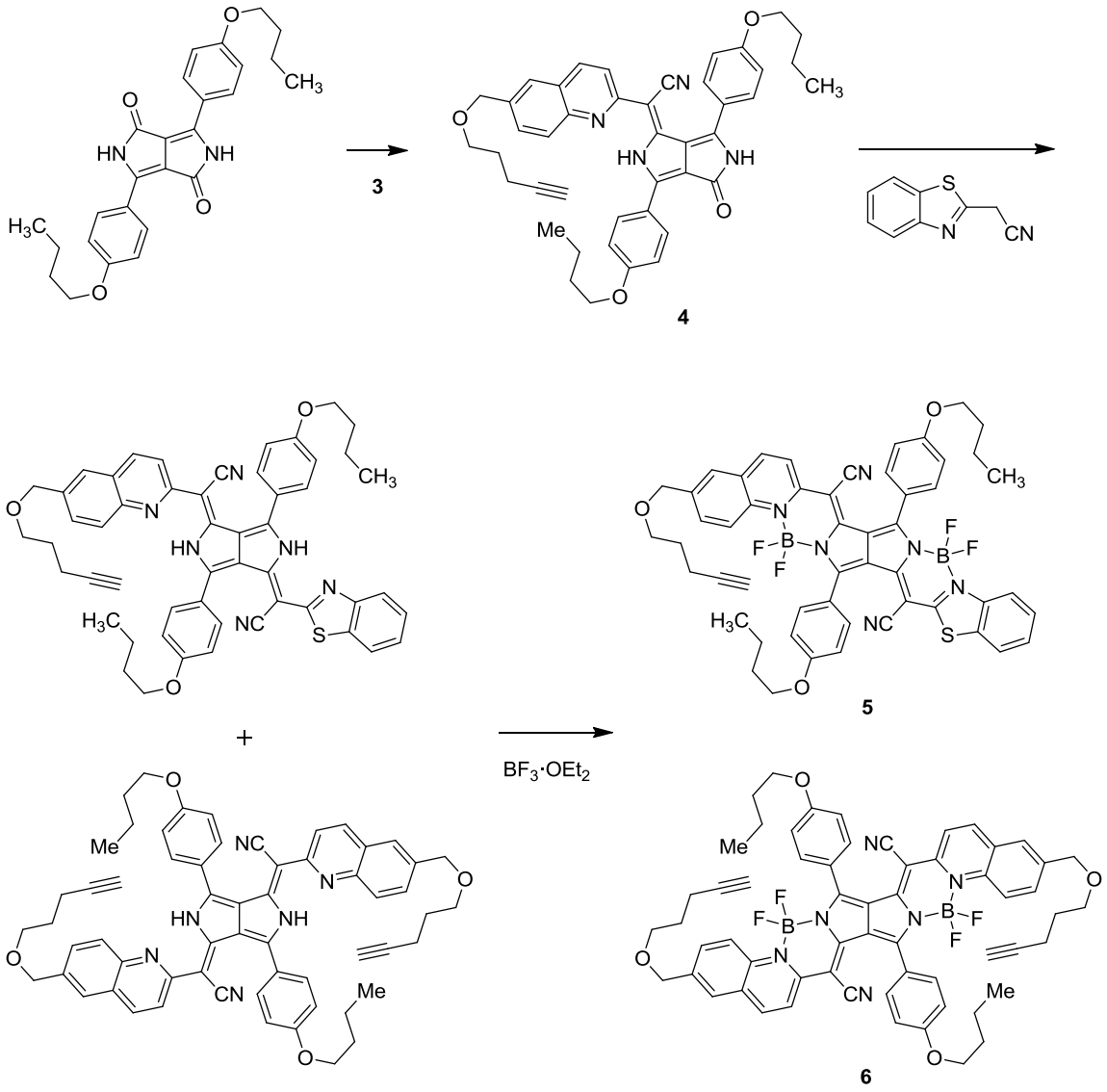
2-(chloromethyl)-6-((4-pentyn-1-yloxy)methyl)quinoline (2)

To a solution of **1** (0.27 g, 1.1 mmol) in 10 mL chloroform was added trichloroisocyanuric acid (0.07 g, 0.3 mmol) in portions. The mixture was stirred at r.t. for 1 h and the cyanuric acid was filtered off and the solvent were removed under reduced pressure. Purification by silica gel flash column chromatography (ethyl acetate:hexanes = 1:10) afforded **2** as a yellow oil (0.17 g, 67%). ^{1}H NMR (400 MHz $CDCl_3$) δ 8.17 (1H, d, *J* = 8.5 Hz,), 8.06 (1H, d, *J* = 8.7 Hz), 7.77 (1H, s), 7.69 (1H, d, *J* = 8.7 Hz), 7.59 (1H, dd, *J* = 8.5, 1.6 Hz), 4.84 (2H, s), 4.68 (2H, s), 3.64 (2H, t, *J* = 6.5 Hz), 2.36 (2H, td, *J* = 7.0, 2.6 Hz), 1.96 (3H, t, *J* = 2.7 Hz). ^{13}C NMR (100 MHz $CDCl_3$) δ

156.5, 146.8, 137.5, 137.4, 129.7, 129.1, 127.2, 125.5, 120.7, 83.8, 72.4, 68.9, 68.7, 47.2, 28.6, 15.3.

2-(6-((4-pentyn-1-yloxy)methyl)quinolin-2-yl)acetonitrile (3)

To a solution of **2** (0.11 g, 0.4 mmol) in 10 mL DMF was added NaCN (0.03 g, 0.6 mmol) and a trace amount of NaI (0.006 g, 0.04 mmol). The reaction mixture was stirred at 60 $^{\circ}$C for 2 h. After removing the solvent, the remaining residue was extracted between ethyl acetate and H_2O. The organic layer was then concentrated under reduce pressure. Purification by silica gel flash column chromatography (ethyl acetate:hexanes = 1:10) afforded **3** as a yellow solid (0.05 g, 50%). ^{1}H NMR (400 MHz $CDCl_3$) δ 8.17 (1H, d, *J* = 8.4 Hz), 8.01 (1H, d, *J* = 8.7 Hz), 7.77 (1H, s), 7.70 (1H, d, *J* = 8.7 Hz), 7.50 (1H, d, *J* = 8.4Hz), 4.68 (2H, s), 4.09 (2H, s), 3.64 (2H, t, *J* = 6.1 Hz), 2.34 (2H, td, *J* = 7.0, 2.6 Hz), 1.92 (1H, t, *J* = 2.7 Hz), 1.89 – 1.81 (2H, m). ^{13}C NMR (100 MHz $CDCl_3$) δ 150.3, 147.4, 137.6, 137.6, 129.9, 129.1, 127.0, 125.5, 119.8, 116.9, 83.8, 72.4, 69.0, 68.6, 28.56, 27.3, 15.3. HRMS (ESI) calcd. for $C_{17}H_{17}N_2O$ [M + H]+ 265.1335, found 265.1343



PPCy dye was synthesized by following a reported procedure.[1] Interestingly, when molecule **4** was condensed with 2-benzothiazoleacetonitrile followed by $BF_3{\cdot}OEt_2$ reaction, both **5** and **6** were formed, with a 1.5:1 ratio using both the reported procedure[1] and the procedure used to synthesize **4**. This might be caused by the fact that the condensation reaction is a reversible reaction; 2-quinolinylacetonitrile condensation can form a larger conjugation system; therefore, **6** is the thermodynamically more stable product.

Compound 4

3,6-Bis(4-butoxyphenyl)pyrrolo[3,4-c]pyrrole-1,4(2H,5H)-dione DPP molecule (75 mg; 0.17 mmol), synthesized according to a reported method,[2] was refluxed in 2 mL $POCl_3$ for 2 h. $POCl_3$ was then removed by reduced pressure and the residue was dried under vacuum overnight. Compound **3** (45 mg; 0.17 mmol) in 10 mL anhydrous THF was then added and the mixture was refluxed until the starting material disappeared. The reaction mixture was then concentrated under reduce pressure and extracted between DCM and saturated aqueous solution of Na_2CO_3. Purification by silica gel flash column chromatography (ethyl acetate: DCM = 1:20) afforded **4** as a blue solid (50 mg, 50%). ^{1}H NMR (600 MHz $CDCl_3$) ppm ^{1}H NMR (600 MHz, cdcl3) δ 8.45 (1 H, d, *J* = 8.5 Hz), 8.21 (1 H, d, *J* = 8.4 Hz), 8.08 (1 H, d, *J* = 8.8 Hz), 8.03 (1 H, d, *J* = 8.5 Hz), 8.01 (1 H, d, *J* = 8.6 Hz,), 7.82 – 7.76 (2 H, m), 7.75 – 7.68 (3 H, m), 7.54 (2 H, dd, *J* = 8.0, 6.2 Hz), 7.16 (1 H, d, *J* = 8.4 Hz), 7.06 (1 H, d, *J* = 8.4 Hz), 4.72 (2 H, s), 4.14 (2 H, t, *J* = 6.2), 4.08 (2 H, t, *J* = 6.4), 3.69 (2 H, t, *J* = 6.0), 2.44 – 2.35 (2 H, m), 1.98 (1 H, s), 1.94 – 1.80 (6 H, m), 1.06 – 1.00 (6 H, m), 0.93 – 0.88 (6 H, m). ^{13}C NMR (150 MHz $CDCl_3$) δ 161.2, 155.3, 150.5, 146.2, 141.1, 139.5, 137.6, 132.5, 131.6, 128.1, 126.8, 126.3, 126.1, 123.4, 123.2, 122.3, 122.5, 121.9, 121.7, 120.0, 118.6, 1163, 113.4, 83.6, 79.0, 71.6, 69.0, 68.7, 67.7, 50.1, 35.9, 31.3, 29.7, 28.5, 19.3, 15.2, 13.9. . HRMS (ESI) calcd. for $C_{43}H_{43}N_4O_4$ [M + H]+ 679.3279, found 679.3300

Compounds 5 and 6

Compound **4** (58 mg; 0.085 mmol) was refluxed in 2 mL $POCl_3$ for 2 h. $POCl_3$ was then removed by reduced pressure and the residue was dried under vacuum overnight. 2-Benzothiazoleacetonitrile (30 mg; 0.17 mmol) in 5 mL anhydrous THF was then added and the mixture was refluxed until the starting material disappeared. The reaction mixture was then concentrated under reduce pressure and extracted between DCM and saturated aqueous solution of Na_2CO_3. The organic layer was concentrated under reduced pressure, and the residue was used without further purification. BF_3·OEt (314 mg, 2.2 mmol) was added to the residue dissolved in 5

mL chloroform. The reaction mixture was refluxed for 30 min and was then concentrated by reduced pressure. Purification by silica gel flash column chromatography (ethyl acetate:DCM = 1:40) afforded **5** (20 mg, 29%) and **6** (38 mg, 44%) as green solids. **5**: ^{1}H NMR (400 MHz CDCl3) δ 8.48 (1 H, d, *J* = 8.9), 8.15 (1 H, d, *J* = 9.0), 7.97 (1 H, d, *J* = 8.2), 7.78 – 7.64 (7 H, m), 7.61 (2 H, d, *J* = 9.2), 7.44 (1 H, t, *J* = 7.7), 7.36 (1 H, t, *J* = 7.5), 7.07 (4 H, t, *J* = 8.6), 4.62 (2 H, s), 4.07 (4 H, dd, *J* = 9.8, 6.1), 3.61 (2 H, t, *J* = 6.1), 2.38 – 2.26 (2 H, m), 1.92 (1 H, s), 1.89 – 1.73 (6 H, m), 1.61 – 1.46 (4 H, m), 1.00 (6 H, t, *J* = 7.3). MALDI-MS: m/z calcd for $C_{52}H_{44}B_2F_4N_6O_3S$: 930.331, found 930.340. **6:** ^{1}H NMR (400 MHz CDCl3) δ 8.47 (2 H, d, *J* = 8.8), 8.08 (2 H, d, *J* = 9.1), 7.77 – 7.64 (8 H, m), 7.59 (2 H, d, *J* = 9.2), 7.07 (4 H, d, *J* = 8.6), 4.61 (4 H, s), 4.08 (4 H, t, *J* = 6.5), 3.60 (4 H, t, *J* = 6.1), 2.32 (4 H, td, *J* = 6.9, 2.3), 1.92 (1 H, s), 1.89 – 1.74 (8 H, m), 1.61 – 1.46 (4 H, m), 1.00 (6 H, t, *J* = 7.4)., ^{13}C NMR (125 MHz $CDCl_3$) δ 161.3, 155.3, 152.8, 150.5, 146.2, 141.1, 139.6, 137.6, 132.6, 131.6, 128.1, 126.8, 126.3, 126.3, 126.1, 125.8, 125.7, 123.4, 123.3, 123.2, 122.7, 122.6, 122.0, 121.7, 120.0, 118.6, 116.4, 113.4, 83.7, 79.0, 71.6, 69.0, 68.7, 67.7, 36.0, 31.4, 29.7, 28.5, 19.3, 15.2, 14.0. MALDI-MS: m/z calcd for $C_{60}H_{54}B_2F_4N_6O_4$: 1020.433, found 1020.461.

General conjugation procedure of 6

To a solution of **6** (20 μmol), $CuSO_4$ (1 μmol), (+)-sodium L-ascorbate (20 μmol), TBTA (2 μmol) in degased *tert*-butanol/H_2O/THF (1/1/1) 5 mL was added the azide compound. The reaction mixture was stirred at r.t. until **6** disappeared on TLC plate. The solvent was then removed by reduced pressure. Further purification was done by silica gel flash column chromatography.

7a

Column chromatography (MeOH:DCM = 1:5) yielded **7a** (70%) as green solid. ^{1}H NMR (400 MHz DMSO-d^{6}) δ 8.63 (2 H, d, *J* = 0.9), 7.98 (1 H, s), 7.89 – 7.62 (2 H, m), 7.07 (4 H, d, *J* = 8.6), 5.14 – 5.08 (2 H, m), 4.96 – 4.86 (2 H, m), 4.63 (4 H, s), 4.45 – 4.34 (2 H, m), 4.08 (4 H, t, *J* = 6.3), 4.04 – 3.82 (6 H, m), 3.77 – 3.68 (4 H, m), 2.73 – 2.67 (4 H, m), 1.97 – 1.85 (8 H, m), 1.54 – 1.47 (4 H, m), 0.99 (6 H, t, *J* = 7.4). ^{13}C NMR (150 MHz $CDCl_3$) δ 163.1, 152.4, 148.7, 141.9, 139.9, 136.1, 134.5, 133.0, 132.8, 130.6, 128.7, 127.7, 125.4, 124.7, 115.2, 96.8, 93.4, 78.6, 76.0, 73.2, 72.8, 70.0, 65.1, 63.4, 33.7, 31.4, 24.4, 21.1, 15.3.

7b

Column chromatography (MeOH:DCM = 1:20) yielded **7b** (80%) as green solid. ^{1}H NMR (600 MHz CDCl3) δ 8.41 (1 H, d, *J* = 8.6), 8.05 (1 H, d, *J* = 9.1), 7.67 – 7.61 (8 H, m), 7.53 (2 H, d, *J* = 9.1), 7.32 – 7.22 (14 H, m), 7.01 (4 H, d, *J* = 8.4), 5.12 (1 H, s), 5.06 (4 H, s), 4.54 (4 H, s), 4.02 (4 H, t, *J* = 6.4), 3.48 (4 H, t, *J* = 6.0), 2.79 (4 H, t, *J* = 7.3), 1.99 – 1.93 (4 H, m), 1.79 – 1.74 (4 H, m), 1.50 – 1.44 (4 H, m), 0.95 (6 H, t, *J* = 7.3). ^{13}C NMR (150 MHz $CDCl_3$) δ 166.2, 161.2, 155.3, 150.4, 147.8, 146.1, 141.2, 139.5, 137.6, 134.5, 132.5, 131.7, 128.8, 128.7, 128.6, 128.4, 126.5, 126.4, 126.3, 123.2, 122.6, 122.6, 122.5, 122.2, 120.0, 116.3, 113.4, 71.5, 69.5, 67.9, 67.7, 50.7, 33.7, 26.7, 19.3, 14.1.

7c

Column chromatography (MeOH:DCM = 1:10) yielded **7c** (83%) as green solid. ^{1}H NMR (600 MHz CDCl3) δ 8.41 (1 H, d, *J* = 8.8), 8.06 (1 H, d, *J* = 9.1), 7.72 – 7.60 (8 H, m), 7.54 (2 H, d, *J* = 9.2), 7.40 (2 H, s), 7.32 – 7.25 (2 H, m), 7.01 (4 H, d, *J* = 8.3), 4.56 (4 H, s), 4.41 (4 H, t, *J* = 4.9), 4.03 (4 H, t, *J* = 6.4), 3.77 (4 H, t, *J* = 4.9), 3.53 – 3.44 (20 H, m), 3.29 (6 H, s), 2.76 (4 H, t, *J* = 7.5), 1.99 – 1.92 (4 H, m), 1.82 – 1.72 (4 H, m), 1.53 – 1.43 (4 H, m), 0.95 (6 H, t, *J* = 7.4). ^{13}C NMR (150 MHz $CDCl_3$) δ 161.2, 155.3, 150.5, 147.3, 146.2, 141.2, 139.5, 137.7, 132.52,

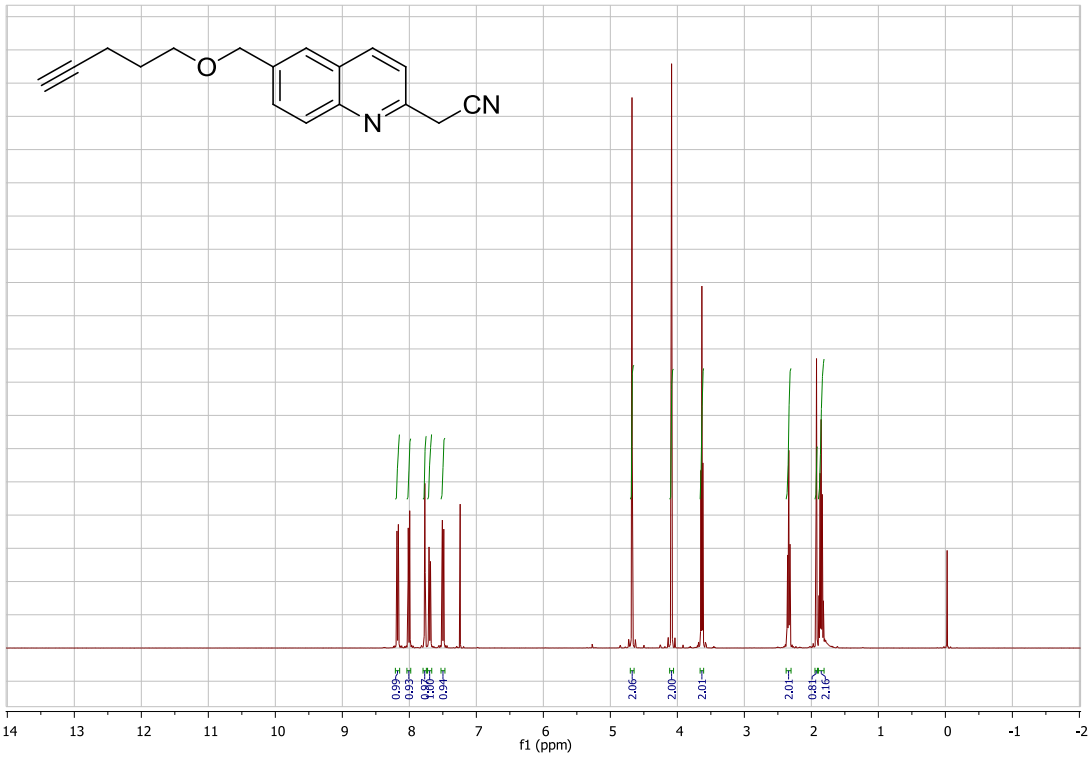
131.6, 129.1, 128.0, 126.4, 126.3, 123.2, 122.6, 122.6, 122.0, 116.3, 113.4, 71.9, 71.5, 70.5, 69.9, 69.5, 67.7, 59.0, 50.8, 50.1, 31.3, 29.7, 29.4, 22.3, 19.3, 13.9.

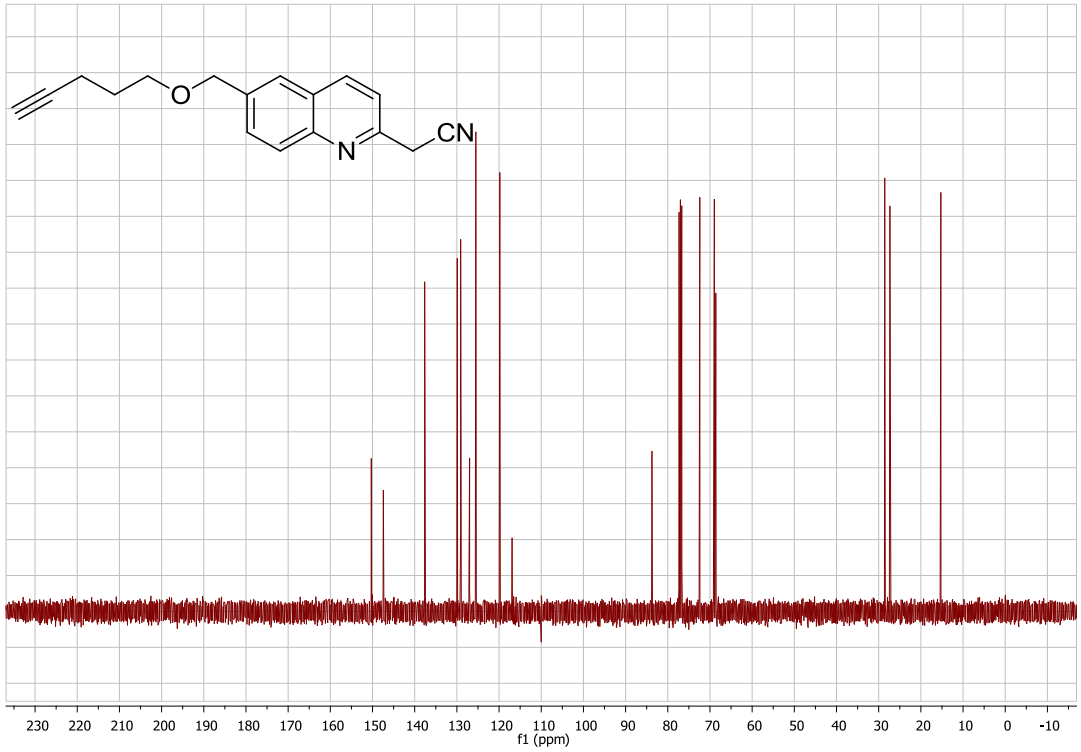
Reference

1. G. M. Fischer, C. Jungst, M. Isomaki-Krondahl, D. Gauss, H. M. Moller, E. Daltrozzo and A. Zumbusch, *Chem Commun (Camb)*, 2010, **46**, 5289-5291.
2. H. Bronstein, Z. Y. Chen, R. S. Ashraf, W. M. Zhang, J. P. Du, J. R. Durrant, P. S. Tuladhar, K. Song, S. E. Watkins, Y. Geerts, M. M. Wienk, R. A. J. Janssen, T. Anthopoulos, H. Sirringhaus, M. Heeney and I. McCulloch, *Journal of the American Chemical Society*, 2011, **133**, 3272-3275.

NMR spectra of key compounds:

2-(6-((4-pentyn-1-yloxy)methyl)quinolin-2-yl)acetonitrile (3)





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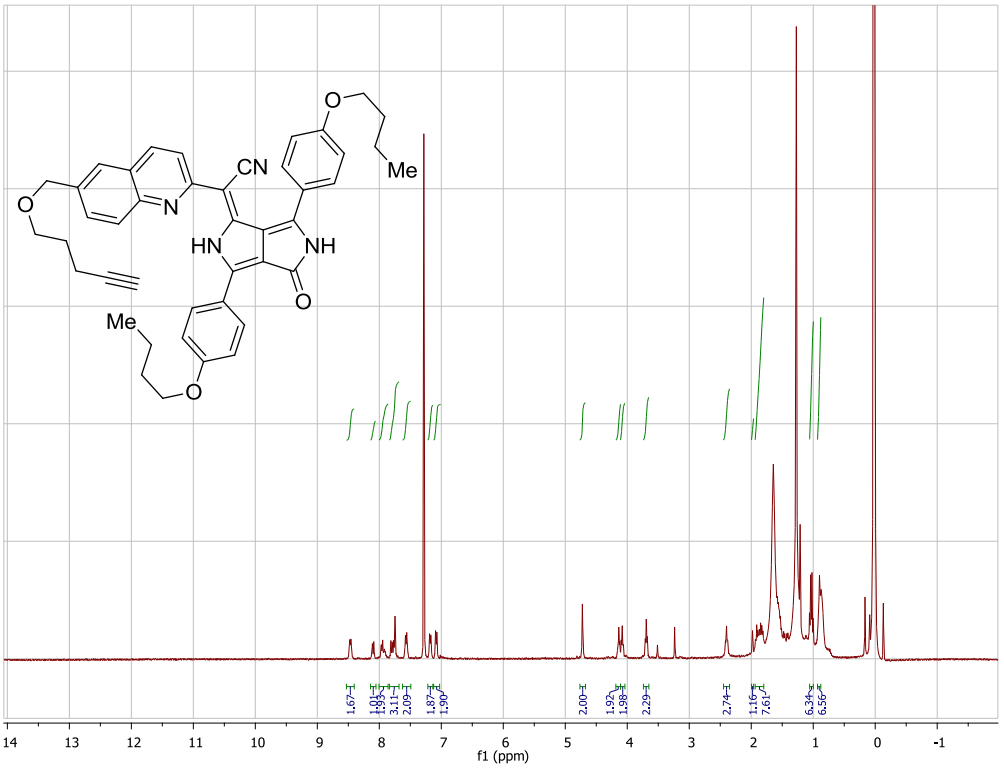
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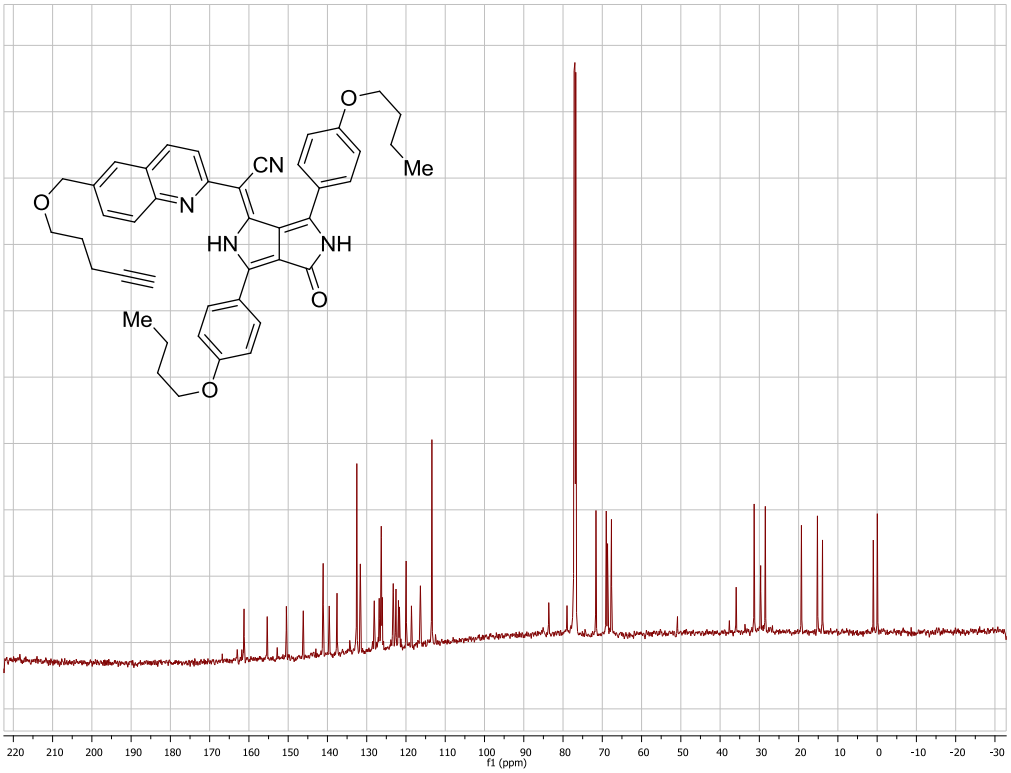
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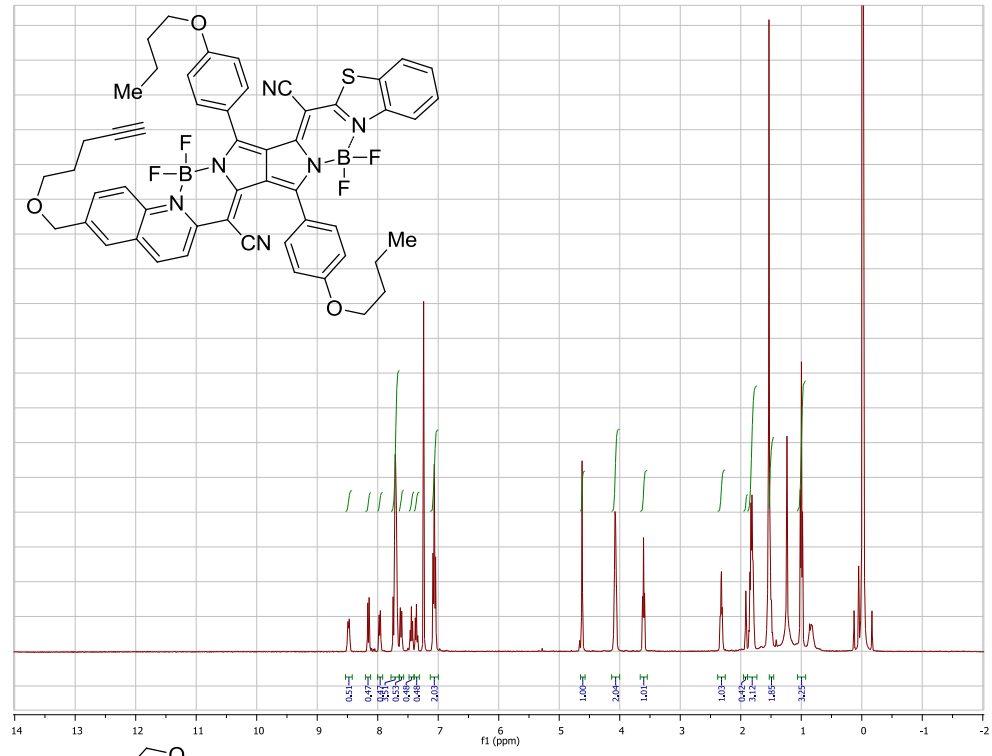
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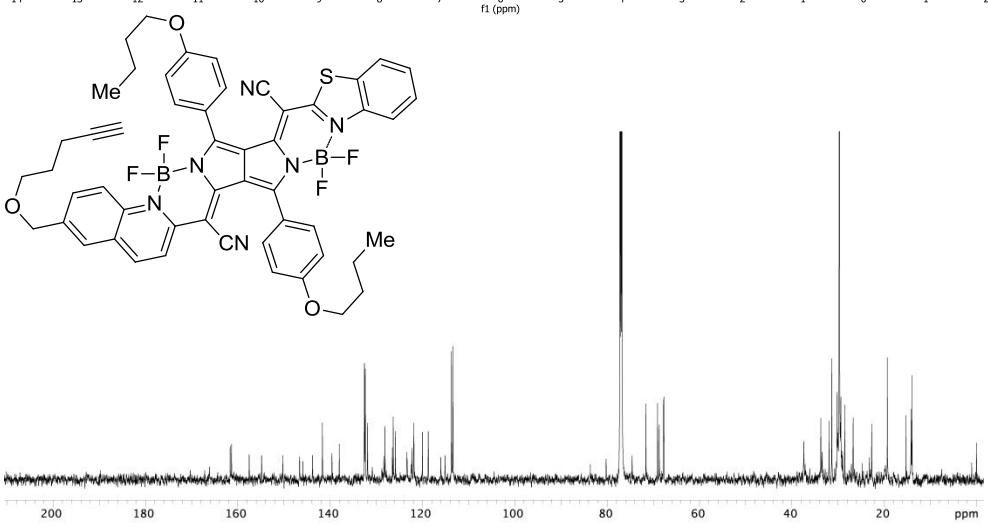
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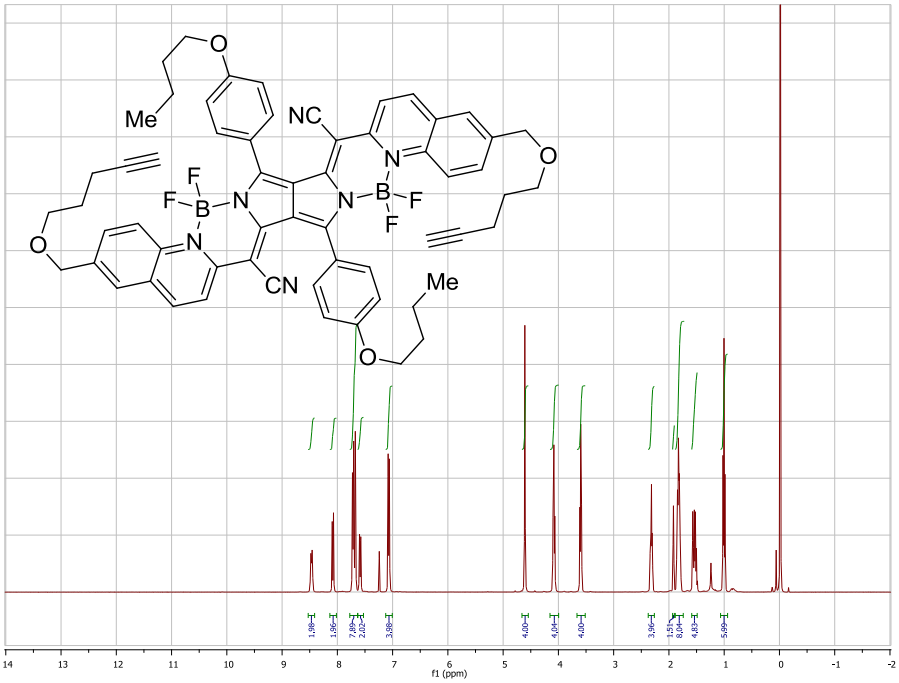
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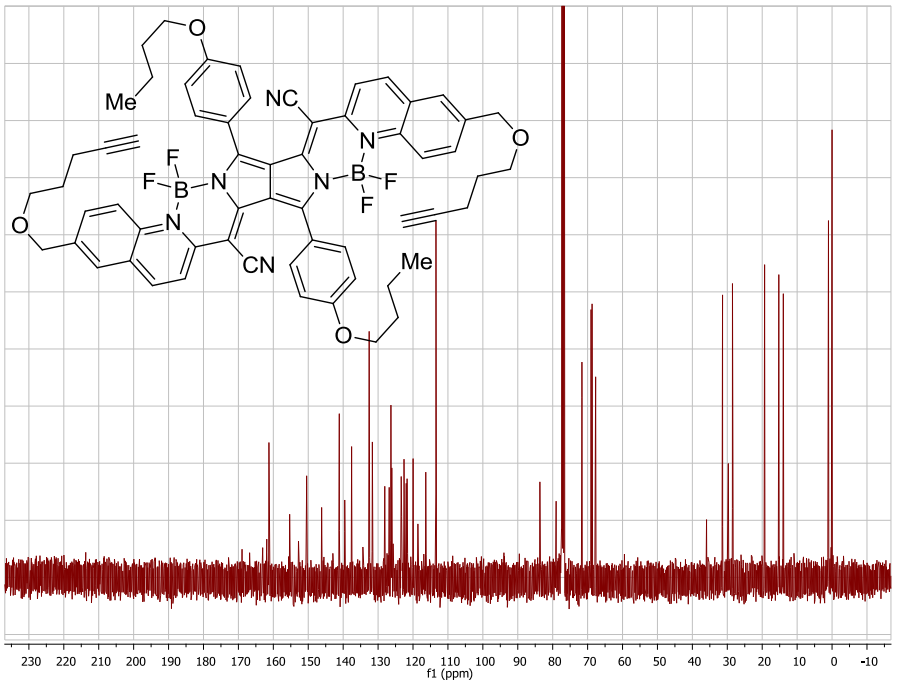
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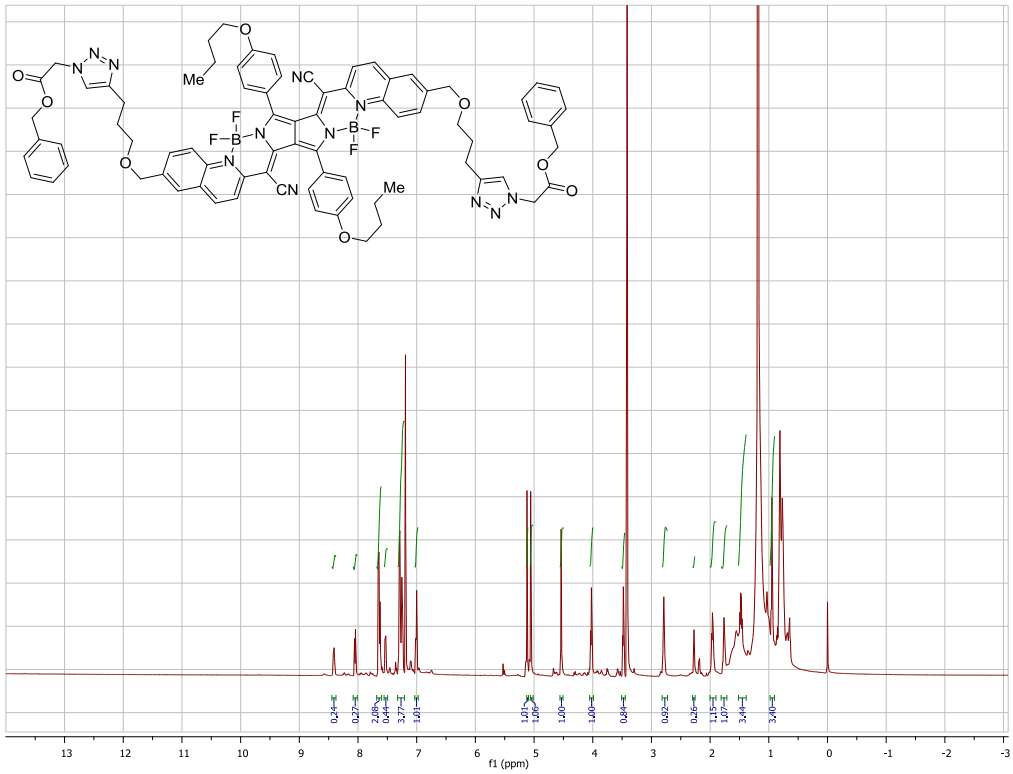
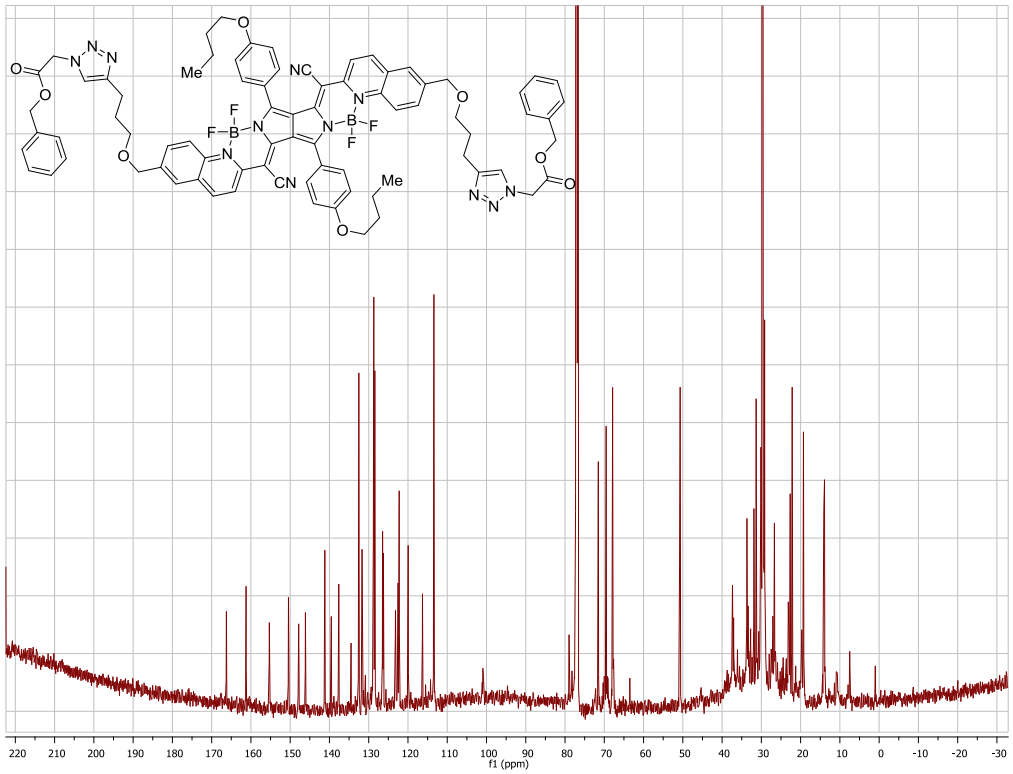
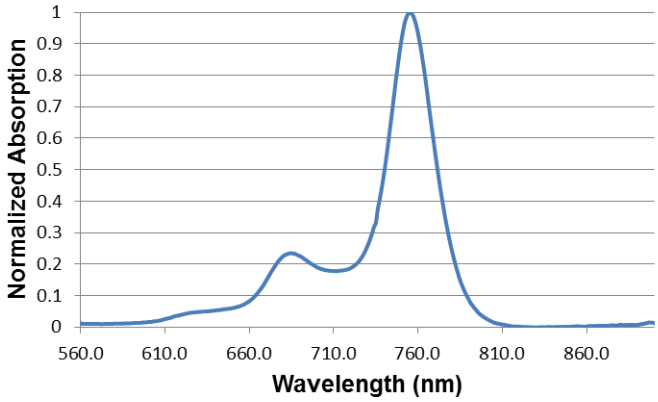
7b





UV-vis spectrum of key compounds

7a in DMSO



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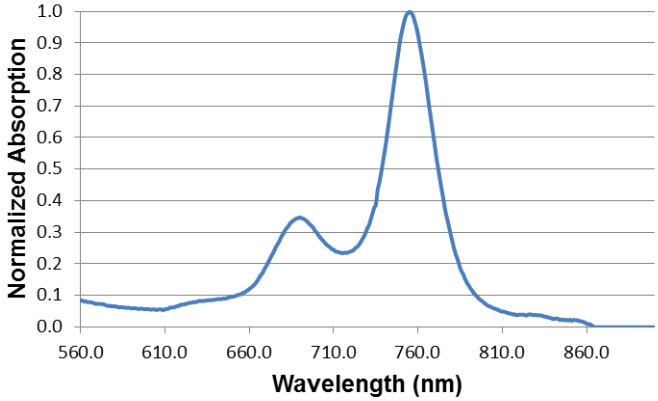

7b in 20%DMSO/80%H_2O: