Supporting Information for:

Synthesis of a fluorogenic cyclooctyne activated by Cu-free click chemistry

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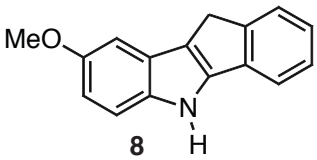
General Synthetic Procedures.

All chemical reagents were purchased from Sigma-Aldrich, Acros and TCI chemicals and used without purification unless noted otherwise. Anhydrous DMF and MeOH were purchased from Aldrich or Acros in sealed bottles; all other solvents were purified as described by Pangborn *et al.*[1] In all cases, magnesium sulfate was used as a drying agent and solvent was removed by reduced pressure with a Buchi Rotovapor R-114 equipped with a Welch self-cleaning dry vacuum. Products were further dried by reduced pressure with an Edwards RV5 high vacuum. Thin layer chromatography was performed EMD Silica Gel 60 F_{254} plates. Unless otherwise specified, R_f values are reported in the solvent system the reaction was monitored in. Flash chromatography was performed using Silicycle SiliaFlash® P60 230-400 mesh silica.

All ^1H and ^{13}C NMR spectra are reported in ppm and referenced to solvent peaks. Spectra were obtain on Bruker AVQ-400®, AVB-400®, DRX-500®, AV-500®, or AV-600® instruments. High resolution electrospray ionization (ESI) mass spectra were obtained from the UC Berkeley Mass Spectrometry Facility.

Synthesis of Compounds



8

To a slurry of 4-methoxyphenylhydrazine•HCl (7.63 g, 43.7 mmol) and 1-indanone (5.79 g, 43.7 mmol) in ethanol (175 mL) was added acetic acid (0.15 mL). The solution was fitted with a reflux condenser and heated to 85 ˚C with stirring for 14 hours. The solution became clear and red/orange after ~10 minutes, but at ~1 h precipitate was observed to be forming. After the 14 hours was complete the solution was cooled in an ice bath and subsequently vacuum filtered and dried under vacuum. The light tan solid (9.10 g, 38.7 mmol, 88%) was pure by NMR and TLC and was consistent with literature precedent.[2] It was used without further purification. ^1H NMR (600 MHz, $CDCl_3$) δ 7.64 (d, *J* = 7.6 Hz, 1H), 7.52 (d, *J* = 7.4 Hz, 1H), 7.34 (t, *J* = 7.5 Hz, 1H), 7.22 (dd, *J* = 7.9, 5.5 Hz, 2H), 7.02 (s, 1H), 6.78 (dd, *J* = 8.7, 2.1 Hz, 1H), 4.01 (s, 3H), 3.65 (s, 2H). HRMS (ESI) calcd for $C_{16}H_{14}ON$ $[M+H]^+$: 236.1070); found: 236.1070.

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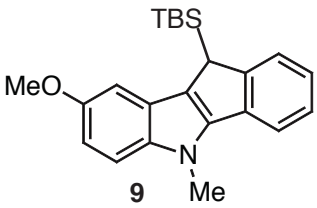
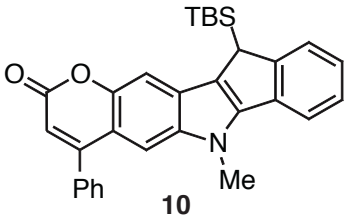
[1] Pangborn, A.B.; Giardello, M.A.; Grubbs, R.H.; Rosen, R.K.; Timmers, F.J. *Organometallics* **1996**, *15*, 1518.

[2] Brown, D. W.; Graupner, P. R.; Sainsbury, M.; Shertzer, H. G. *Tetrahedron* **1991**, *47*, 4383-4408.



9

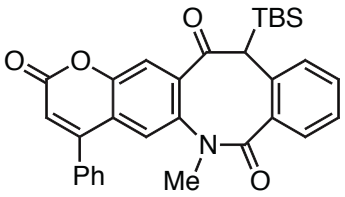
To a solution of **8** (2.70 g, 11.5 mmol) in THF (100 mL) was added *n*-BuLi (14.3 mmol, 1.6 M in hexanes, 23 mmol) over 40 min via syringe pump at 0 ˚C. After an additional 30 minutes TBSCl (1.76 g, 11.7 mmol) in THF (5 mL) was added over 5 minutes to the red solution. The solution was allowed to warm up to room temperature and stir for 30 minutes at room temperature (at which point the red color dissipated) and subsequently cooled back to 0 ˚C for 30 minutes. MeI (1.5 mL, excess) was added to the solution and it was allowed to warm to room temperature and stir overnight. The following morning the solution was concentrated to an orange oil which was purified by silica gel column chromatography (20:1 to 10:1 hexanes:ethyl acetate, product $R_f \sim 0.45$ in 10:1) to give the desired compound, **9**, as an off-white solid (2.23 g, 6.14 mmol, 53%). The desired compound is not completely stable to silica gel column chromatography (decomposition is noted on 2D TLC plates). Note, the non-silylated compound is located just below the desired compound. Additionally, if care is not taken to ensure red color is completely quenched by the TBSCl, methylation of the benzylic position can occur. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 (d, *J* = 7.6 Hz, 1H), 7.56 (d, *J* = 7.6 Hz, 1H), 7.30 (t, *J* = 7.5 Hz, 1H), 7.21-7.16 (m, 3H), 6.90 (dd, *J* = 8.8, 2.4 Hz, 1H), 3.91 (s, 3H), 3.86 (s, 3H), 3.56 (s, 1H), 0.48 (s, 9H), 0.33 (s, 3H), 0.31 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.63, 150.68, 143.71, 137.41, 133.80, 125.20, 125.12, 124.00, 123.49, 123.42, 117.62, 110.37, 110.05, 102.74, 55.83, 35.67, 31.02, 27.00, 18.34, -3.54, -3.63. HRMS (ESI) calcd for $C_{23}H_{30}ONSi$ $[M+H]^+$: 364.2091; found: 364.2092



10

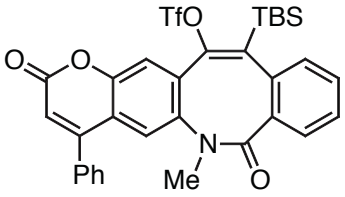
To a solution of **9** (680. mg, 1.87 mmol) in CH_2Cl_2 (20 mL) was added ethylbenzoylacetate (0.64 mL, 3.8 mmol) followed by TsOH (0.66 mL, 7.5 mmol) at room temperature. Upon addition of the acid the solution became dark brown. After 5 days the reaction was diluted with CH_2Cl_2 (30 mL) and neutralized with $NaHCO_3$ (20 mL, sat. aq.). The organic layer was separated and dried over $MgSO_4$ and to the solution was added silica gel. The slurry was concentrated in vacuo and loaded onto the top of a silica gel column (loaded with 6:1 hexanes : ethyl acetate). A gradient column 6:1 to 4:1 hexanes : ethyl acetate was used to isolate starting material **9** (255 mg, 38% recovered, $R_f \sim 0.7$ in 2:1 H:EA), and the desired product, **10**, as a yellow solid (385 mg, 43%, 69% based on recovered sm, $R_f \sim 0.3$ in 2:1 H:EA, highly fluorescent yellow on TLC with 350nm lamp). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.70 (d, *J* = 7.6 Hz, 1H), 7.60-7.49 (m, 7H), 7.33 (d, *J* = 7.4 Hz, 1H), 7.31 (s,

1H), 7.26 (dd, *J* = 7.5, 1.0 Hz, 1H), 6.27 (s, 1H), 3.89 (s, 3H), 3.56 (s, 1H), 0.42 (s, 9H), 0.28 (s, 3H), 0.25 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.14, 156.61, 151.67, 148.38, 148.05, 139.35, 136.46, 132.55, 129.47, 128.90, 128.59, 126.18, 125.49, 125.22, 123.34, 118.72, 113.43, 112.23, 106.76, 106.32, 35.89, 31.43, 26.88, 18.32, -3.63, -3.75. HRMS (ESI) calcd for $C_{31}H_{32}O_2NSi$ $[M+1]^+$: 478.2197; found: 478.2198



11

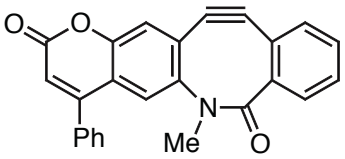
To a 0 ˚C solution of **10** (330. mg, 0.69 mmol) in CH_2Cl_2 (30 mL) and $NaHCO_3$ (7 mL, sat. aq.) was added *m*CPBA (400 mg, 75%, 1.7 mmol) portionwise over 3 minutes. The solution was stirred at 0 ˚C for 30 minutes and then warmed to room temperature for an additional 30 minutes of stirring. The yellow solution lost some of its yellow color as the reaction progressed. The reaction was diluted with CH_2Cl_2 (20 mL) and worked up with 1 N NaOH (25 mL). The organic layer was dried over $MgSO_4$ and concentrated to yield an off white solid that was pure enough for the next step (300. mg, 85%). This compound is stable to silica gel column chromatography and can be purified using hexanes and ethyl acetate ($R_f \sim 0.65$ in 2:1 hexanes : ethyl acetate) on the silica gel column if so desired. The yield of the following step was not affected by using the unpurified compound provided it was pure by NMR. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57-7.51 (m, 3H), 7.33-7.30 (m, 3H), 7.23-7.18 (m, 1H), 7.16-7.13 (m, 3H), 6.91 (d, *J* = 7.5 Hz, 1H), 6.36 (s, 1H), 3.95 (s, 1H), 3.27 (s, 3H), 1.00 (s, 9H), 0.20 (s, 3H), -0.27 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 204.78, 170.53, 159.70, 154.27, 153.43, 142.97, 136.46, 136.39, 136.25, 134.35, 130.57, 130.54, 130.38, 129.39, 128.92, 128.38, 128.32, 127.07, 124.09, 120.77, 116.76, 115.33, 54.03, 37.48, 27.41, 26.93, 18.29, -5.46, -5.87. HRMS (ESI) calcd for $C_{31}H_{32}O_4NSi$ $[m+1]^+$: 510.2095; found: 510.2096.



12

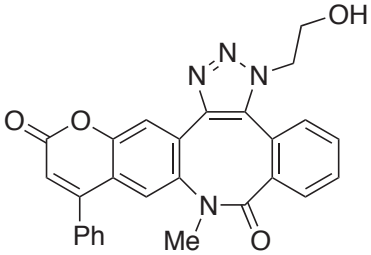
To a -78 ˚C solution of **11** (300. mg, 0.60 mmol) in THF (20 mL) was added KHMDS (0.5 M in toluene, 1.32 mL, 0.66 mmol) dropwise. After stirring for 30 minutes at -78 ˚C Tf_2O (0.11 mL, 0.63 mmol) was added dropwise to the red/orange solution. The color went away upon addition and after an additional 30 minutes of stirring at low temperature the reaction was diluted with Et_2O (40 mL) and quenched with $NaHCO_3$ (sat. aq., 10 mL) as it warmed to room temperature. The organic layer was separated from the aqueous and the aqueous layer was further extracted with a 1:1 mixture of hexanes:ethyl acetate (40 mL). The organic layers were combined and

dried over $MgSO_4$, filtered and concentrated in vacuo. The compound was purified using silica gel column chromatography with a gradient of 4:1 → 3:1 hexanes:ethyl acetate to yield an off-white solid (R_f ~ 0.55 in 2:1 hexanes:ethyl acetate) which was a 9:1 mixture of desired product to starting ketone (178 mg total, therefore 165 mg desired product by NMR ratio, 0.26 mmol, 43%). These two compounds were seemingly inseparable by TLC and silica gel chromatography. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.55-7.51 (m, 3H), 7.35-7.33 (m, 2H), 7.24-7.17 (m, 4H), 7.16-7.12 (m, 2H), 6.90 (d, *J* = 7.9 Hz, 1H), 6.37 (s, 1H), 3.37 (s, 3H), 1.00 (s, 9H), 0.31 (s, 3H), 0.12 (s, 3H). ^{19}F NMR (565 MHz, $CDCl_3$) δ -74.55. ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.25, 159.43, 154.08, 151.84, 148.07, 140.52, 138.39, 135.86, 135.39, 134.51, 134.17, 130.39, 129.34, 128.88, 128.33, 127.73, 127.36, 123.73, 121.17, 117.17, 60.44, 37.60, 27.36, 18.70, -3.48, -3.61.



3

To a 0 ˚C solution of **12** (120. mg, 0.19 mmol) in THF (10. mL) was added TBAF (1.0 M in THF) dropwise. The solution went dark red upon addition of the fluoride source. After 10 minutes the solution was warmed to room temperature and stirred for an additional 30 minutes at room temperature. The solution was diluted with ethyl acetate (30 mL) and washed with NH_4Cl (sat. aq., 15 mL). The organic layer was separated, dried over $MgSO_4$, filtered and concentrated in vacuo. The crude yellow oil was purified by silica gel column chromatography with a gradient of 3:1 → 2:1 hexanes : ethyl acetate to yield **3** as an off-white solid (20.4 mg, 0.054 mmol, 28%, R_f = 0.50 in 1:1 hexanes : ethyl acetate, just above the non-silylated ketone). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.59-7.54 (m, 4H), 7.50-7.46 (m, 4H), 7.43-7.39 (m, 2H), 6.43 (s, 1H), 2.70 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.65, 160.12, 155.12, 153.46, 151.82, 149.53, 134.76, 130.14, 129.84, 129.78, 129.27, 128.58, 127.28, 127.00, 126.11, 125.83, 121.50, 119.86, 116.34, 116.04, 113.29, 107.07, 39.01. HRMS (ESI) calcd for $C_{25}H_{16}O_3N$ $[m+1]^+$: 378.1125; found: 378.1128.



14

Upon mixing coumBARAC (**3**, 2 mg, 5 μmol) with 2-azidoethanol (50 μL, 1 M solution in H_2O) in MeOH / H_2O (1 mL, 1:1) two new, more polar spots were observed by TLC. After 15 minutes the solvent was removed in vacuo and product was purified by silica gel column chromatograph two give the triazole products (1.1 mg, higher R_f spot, 0.5 in pure ethyl acetate, and 1.3 mg of the lower R_f spot). These

ratios nearly matched the 43:57 ratio observed by NMR integration of the crude reaction mixture. *Less polar triazole*: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.62-7.56 (m, 3H), 7.52 (s, 1H), 7.51-7.47 (m, 2H), 7.43 (dd, *J* = 10.6, 4.4 Hz, 1H), 7.39 (d, *J* = 8.1 Hz, 2H), 7.37 (s, 1H), 6.43 (s, 1H), 4.71 (ddd, *J* = 14.1, 5.1, 3.0 Hz, 1H), 4.53 (ddd, *J* = 14.1, 7.8, 3.5 Hz, 1H), 4.19-4.13 (m, 1H), 4.13-4.08 (m, 1H), 3.14 (s, 3H), 2.76 (t, *J* = 5.5 Hz, 1H). *More polar triazole:* ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.62-7.55 (m, 5H), 7.47-7.44 (m, 3H), 7.42-7.39 (m, 2H), 7.34-7.29 (m, 1H), 6.41 (s, 3H), 4.64 (ddd, *J* = 14.2, 7.7, 3.4 Hz, 1H), 4.46 (ddd, *J* = 14.1, 5.5, 3.1 Hz, 1H), 4.32-4.23 (m, 2H), 3.18 (s, 3H), 2.58-2.53 (br t, 1H). HRMS (ESI) calcd for $C_{27}H_{21}O_4N_4$ $[m+1]^+$: 465.1557; found: 465.1560.

Spectroscopic Materials and Methods. All spectroscopic measurements of coumBARAC and the triazole products were performed in a mixture of 1% DMSO in PBS buffer, pH 7.4. Fluorescence spectra were recorded on a Photon Technology International Quanta Master 4 L-format scanning spectrofluorometer equipped with an LPS-220B 75-W xenon lamp and power supply, A-1010B lamp housing with an integrated igniter, switchable 814 photon counting/analog photomultiplier detection unit, and MD5020 motor driver. Samples for emission measurements were contained in a 1-cm x 0.1-cm quartz cuvette. Absorption spectra were recorded using an Agilent 8453 spectrophotometer.

Quantum yield was determined using a reference sample of quinine sulfate (QS, 1 mM solution in 0.1 M H_2SO_4 from Invitrogen) diluted into 0.5 M H_2SO_4. For coumBARAC, triazole products and QS, the absorbance spectra were measured within an absorbance range of 0.01 to 0.1. The quantum yield was calculated as an average of 5 points according to the equation:

$\Phi_{sample} = \Phi_{QS} (A_{QS} / A_{sample})(F_{sample} / F_{QS})(\eta_{sample}/\eta_{standard})^2$;

where Φ is the quantum yield, Φ_{QS} = 0.55 in 0.5 M H_2SO_4, "*A*" is the absorbance at the excitation frequency, "*F*" is the area under the emission curve , and η is the refractive index of the solvent.[3] The refractive indexes of the solutions were assumed to be equal.

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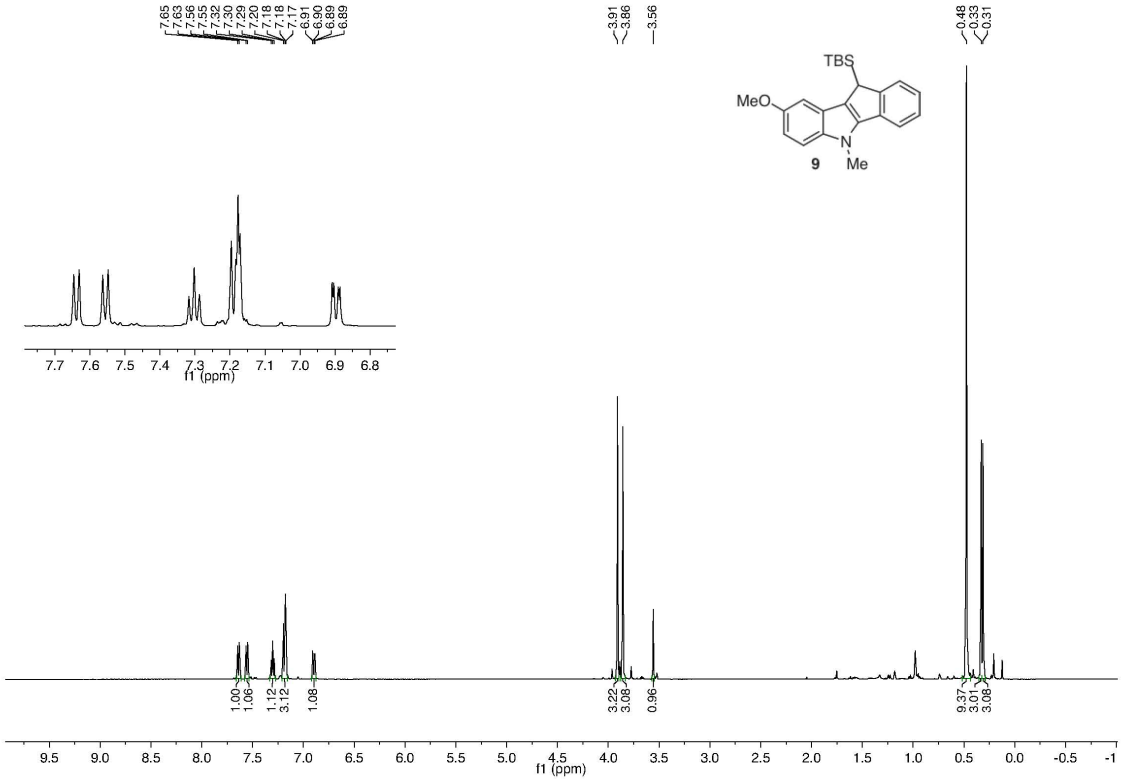
[3] Fery-Forgues, S.; Lavabre, D. *J. Chem. Ed.* **1999**, *76*, 1260-1264.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6.89
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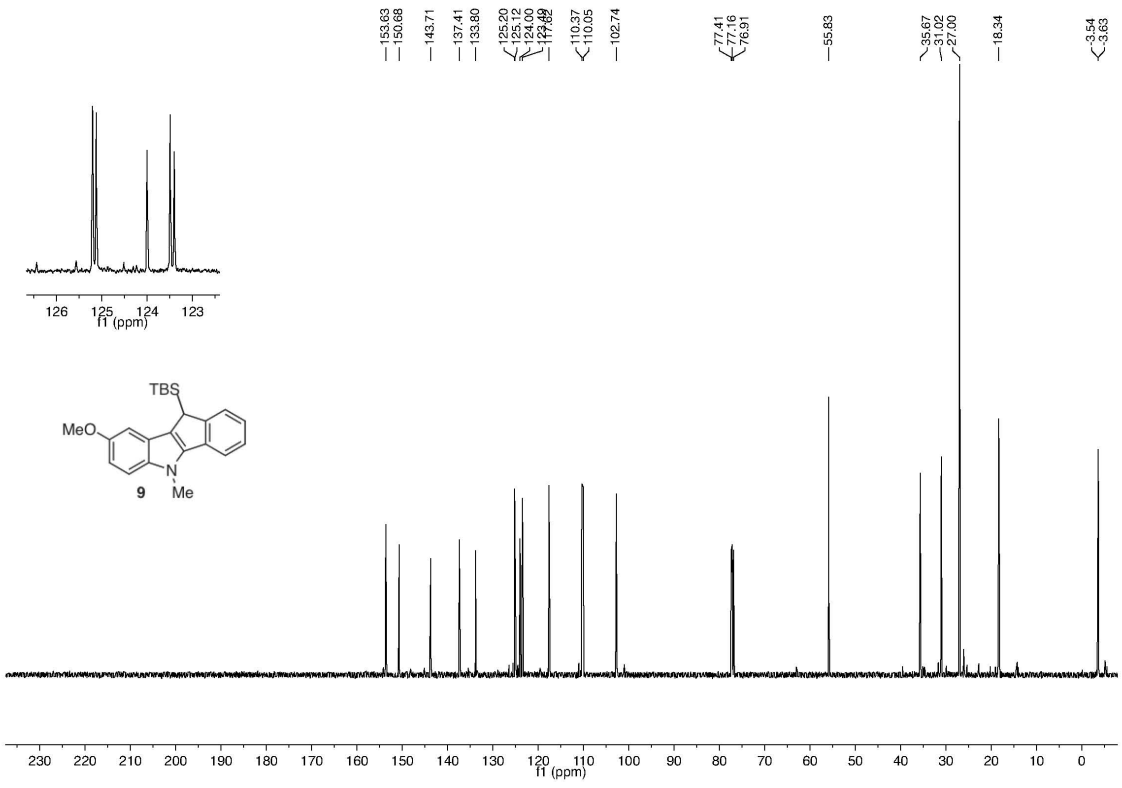
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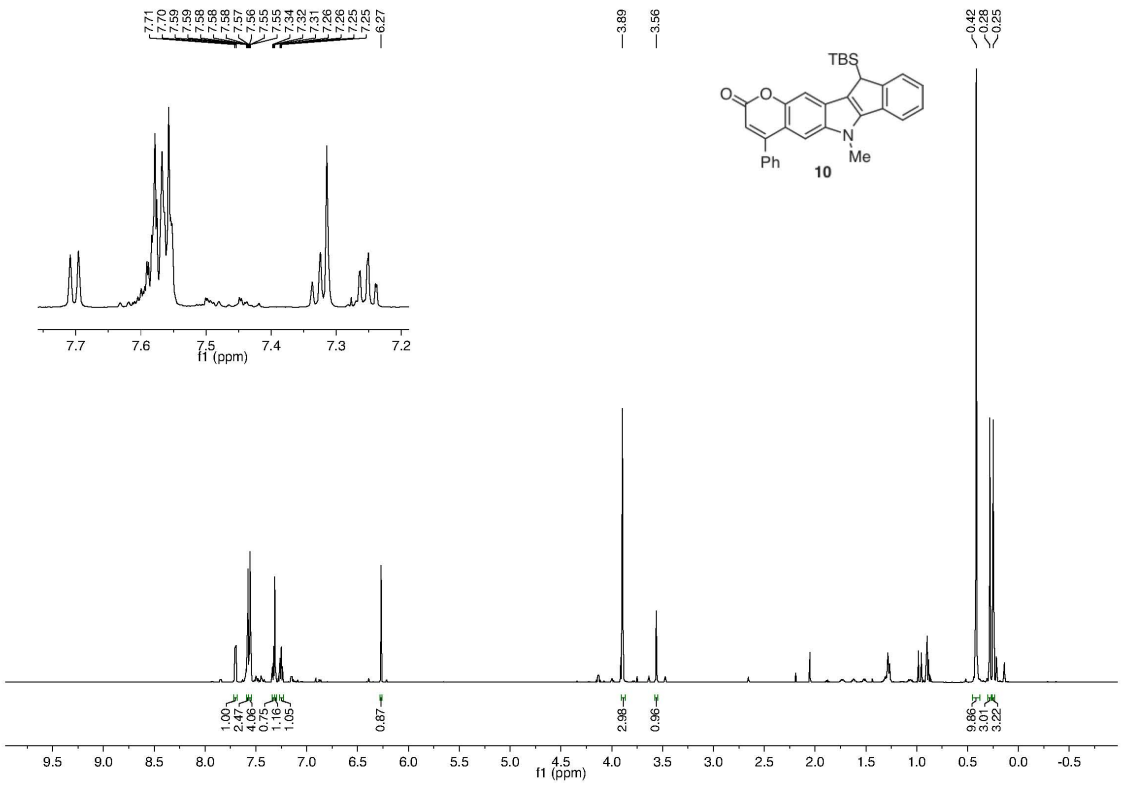
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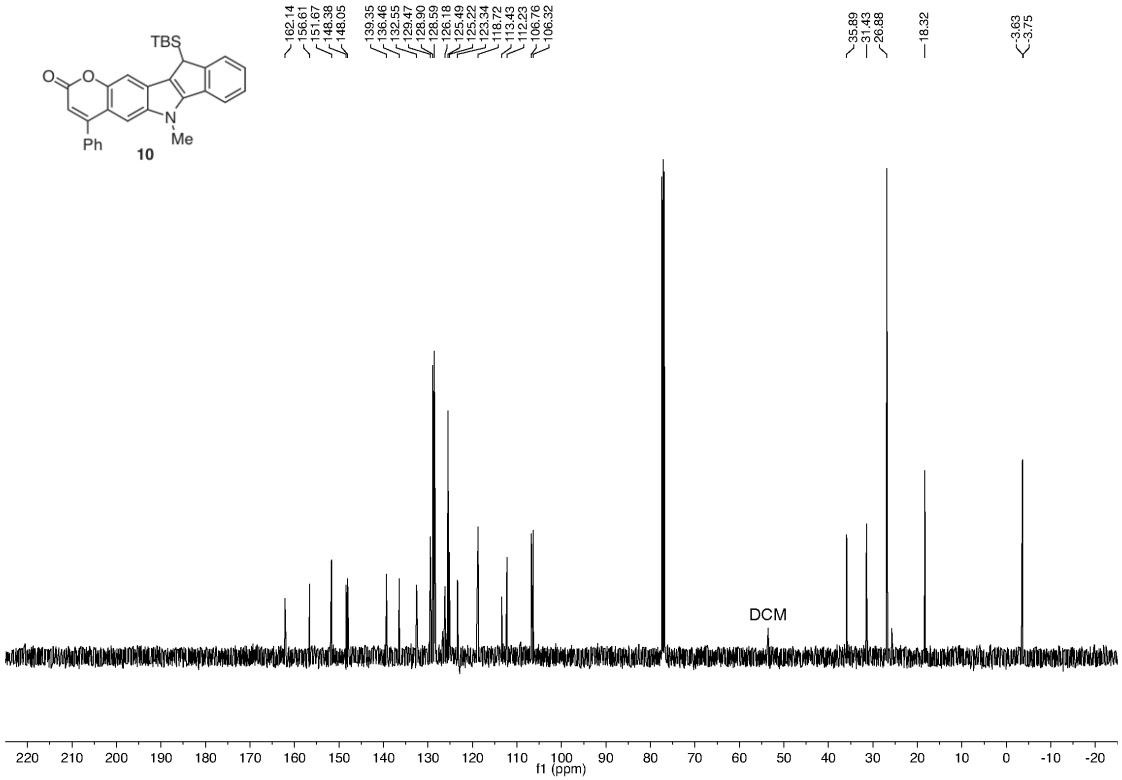
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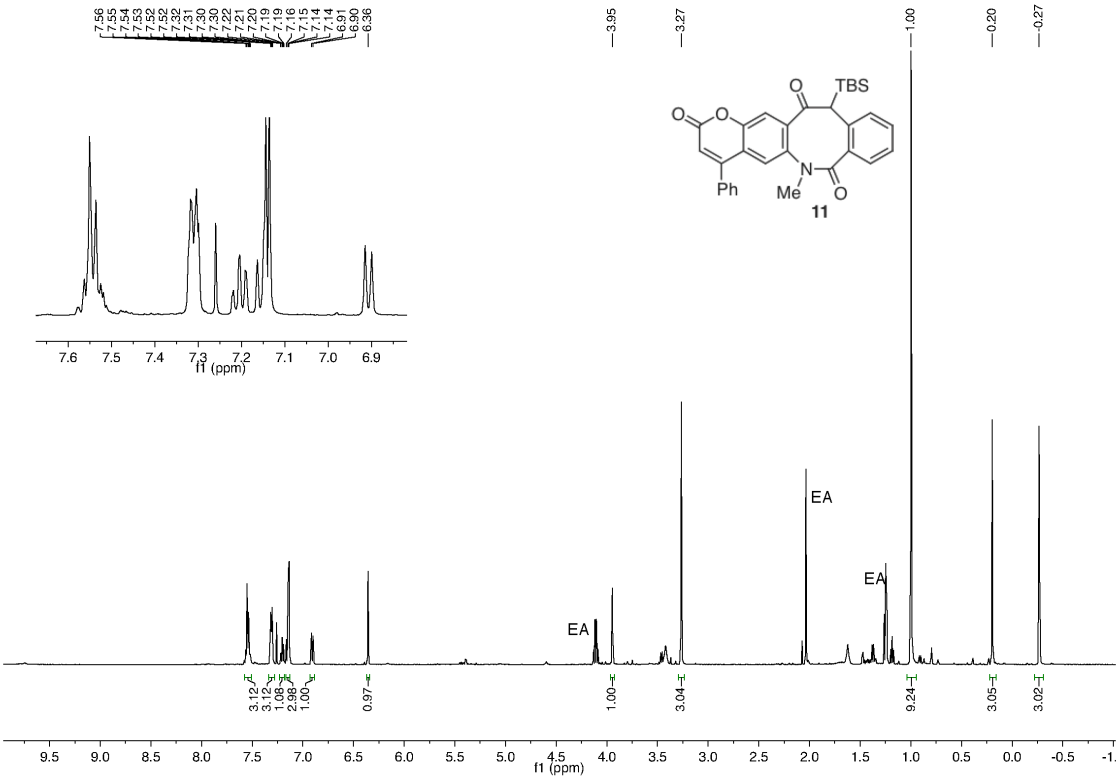
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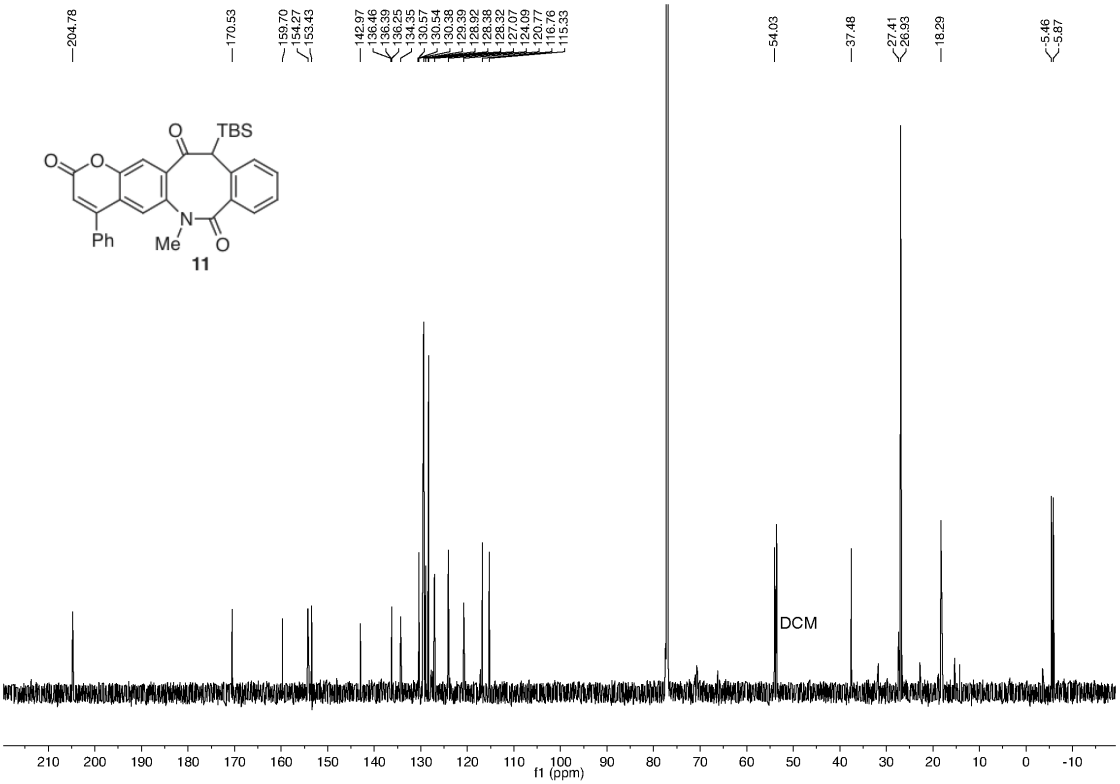
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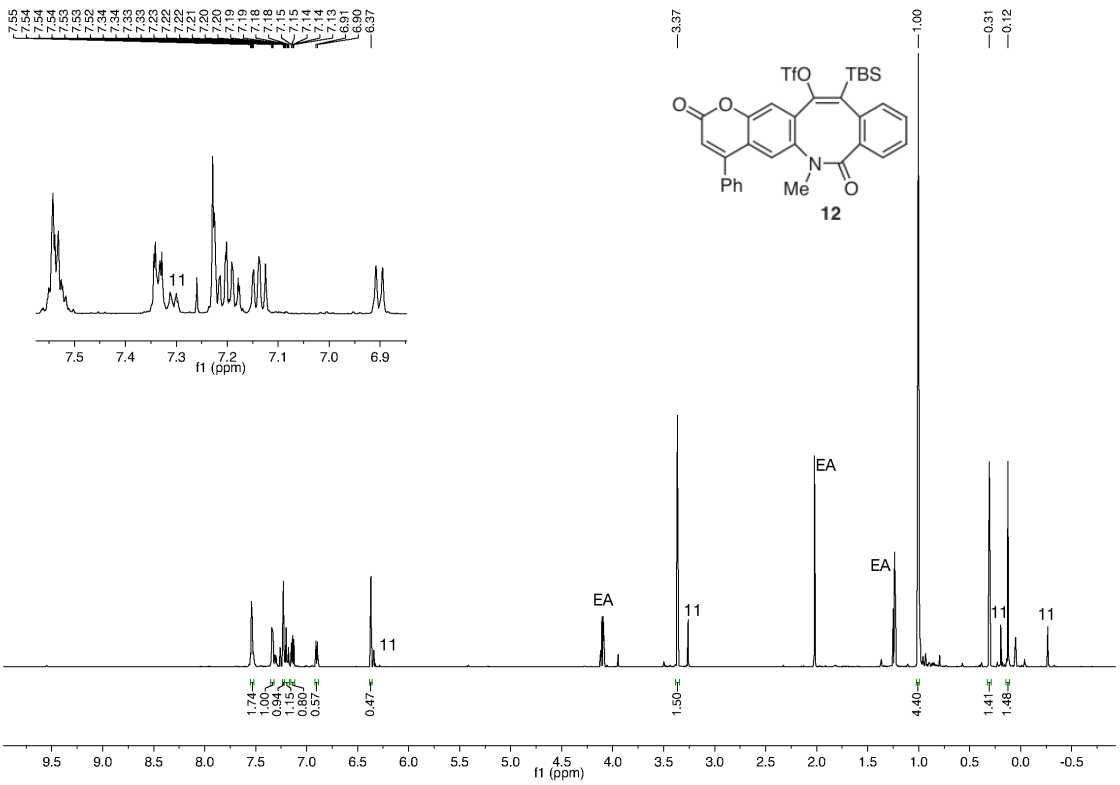
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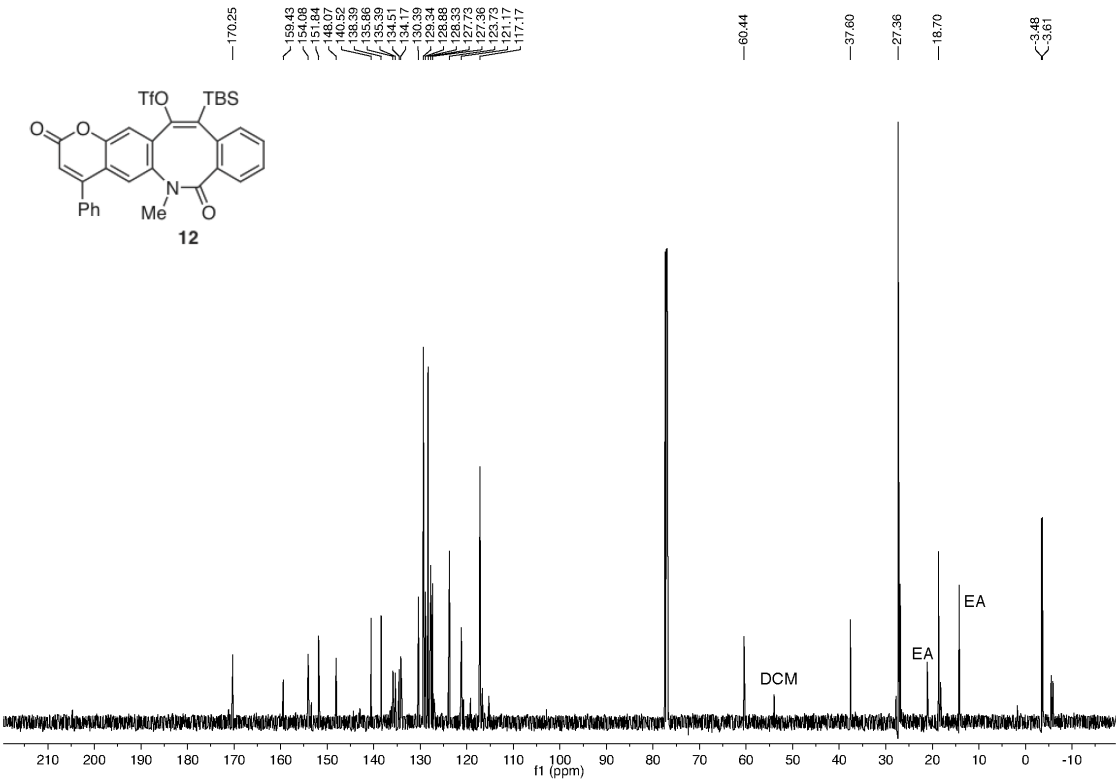
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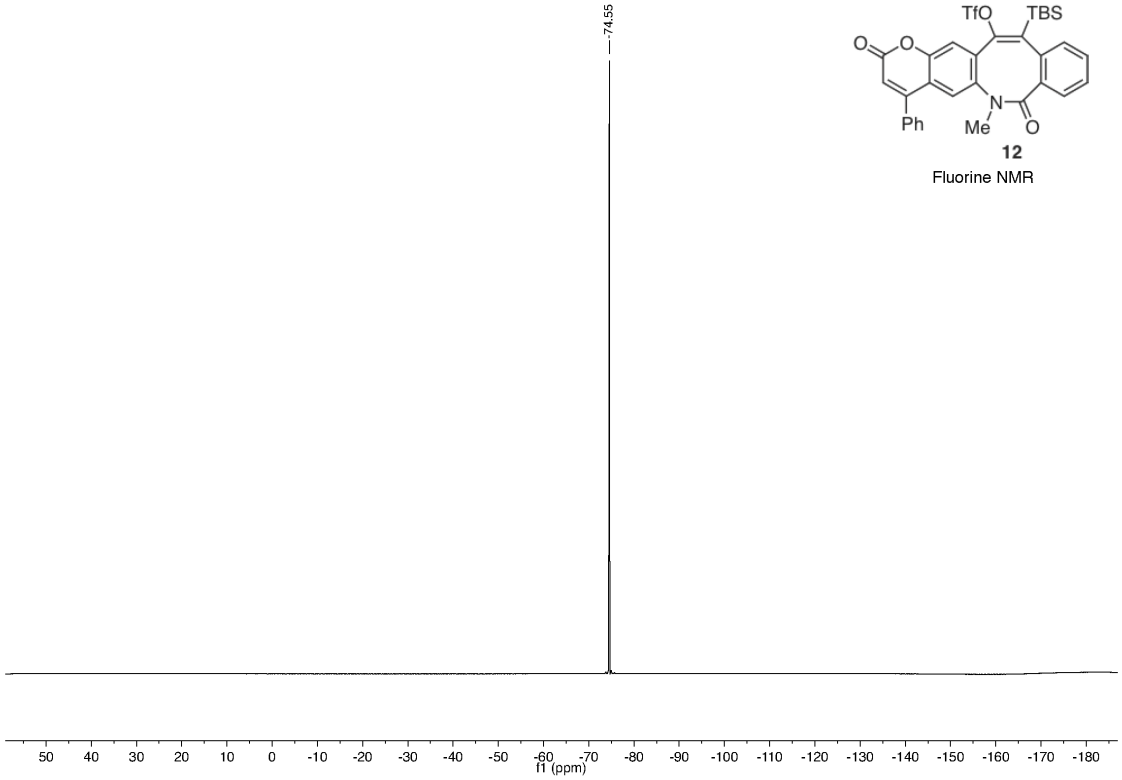
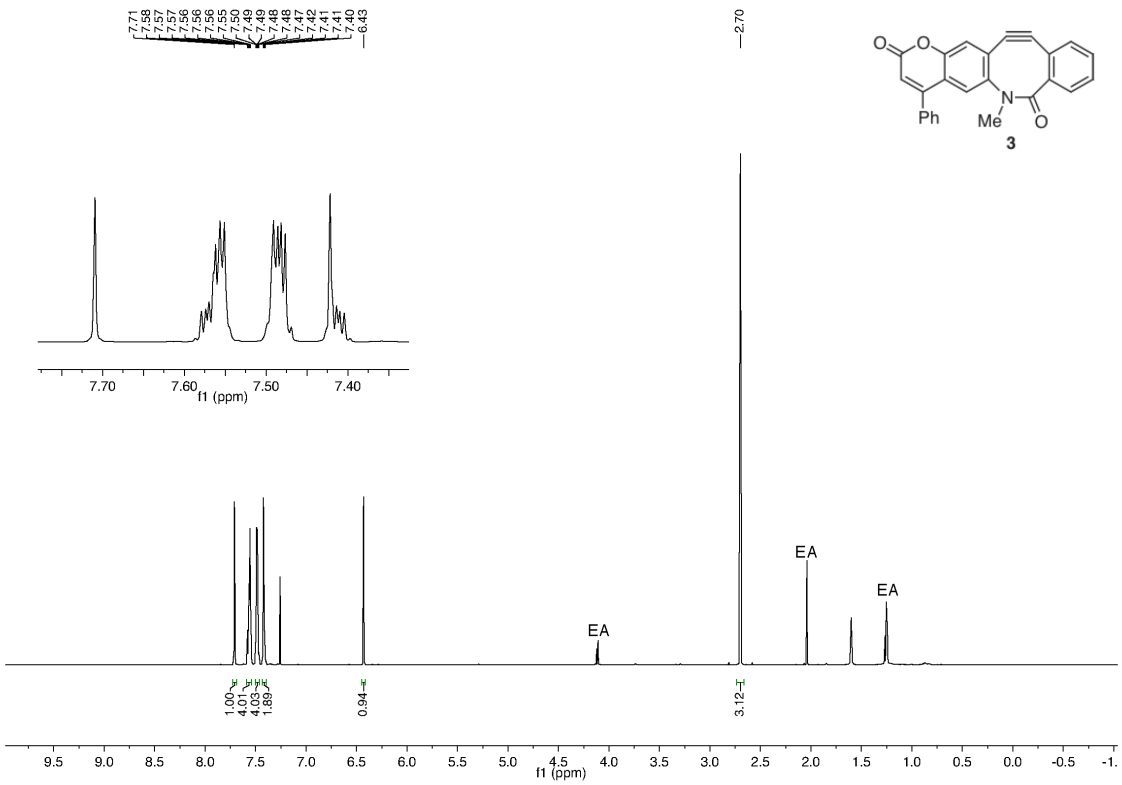


12

Fluorine NMR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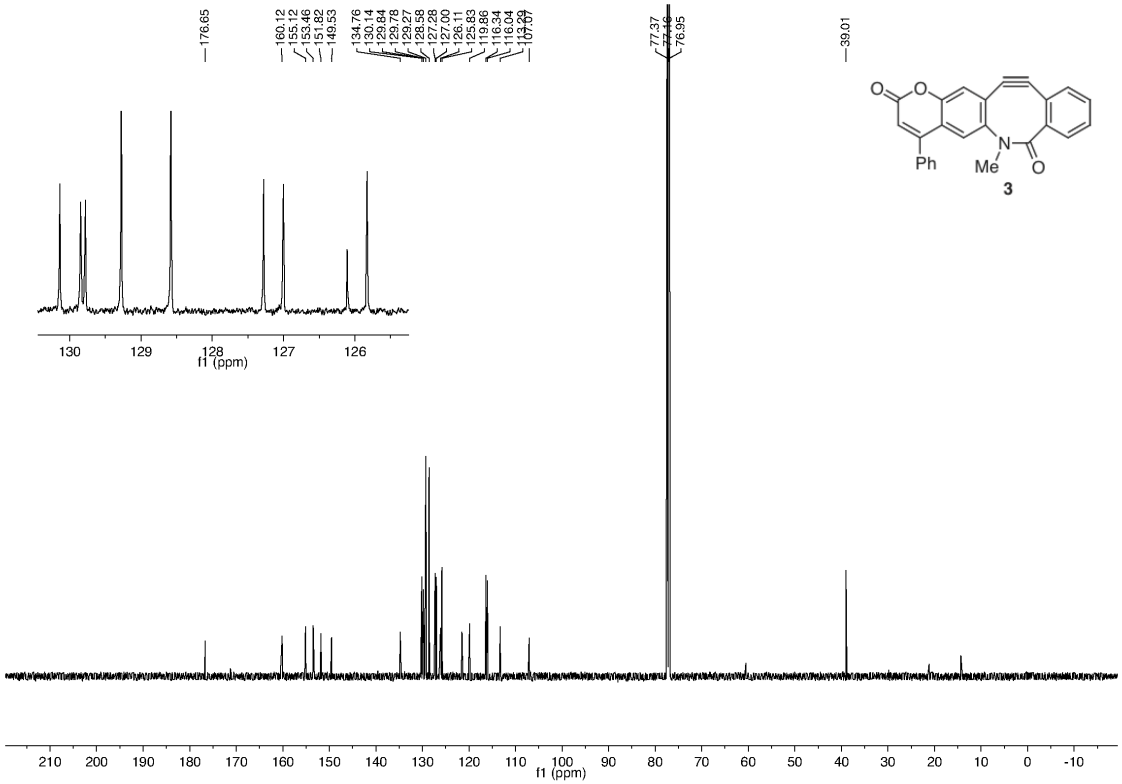
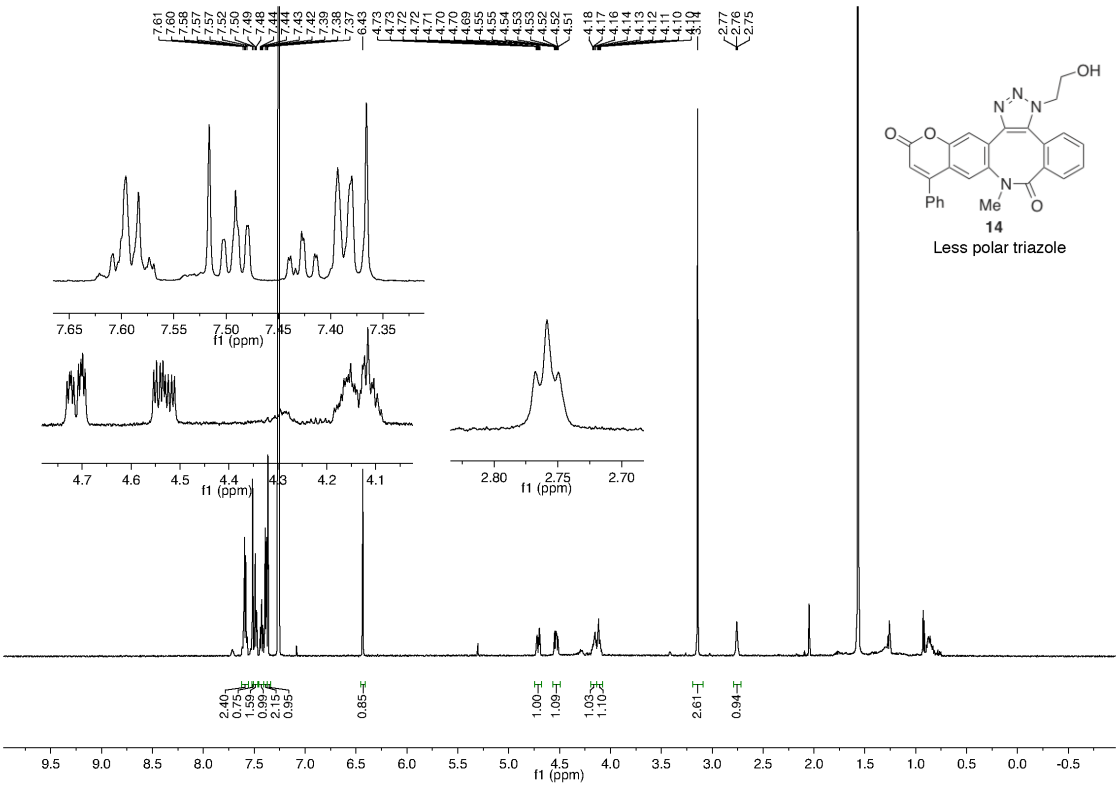
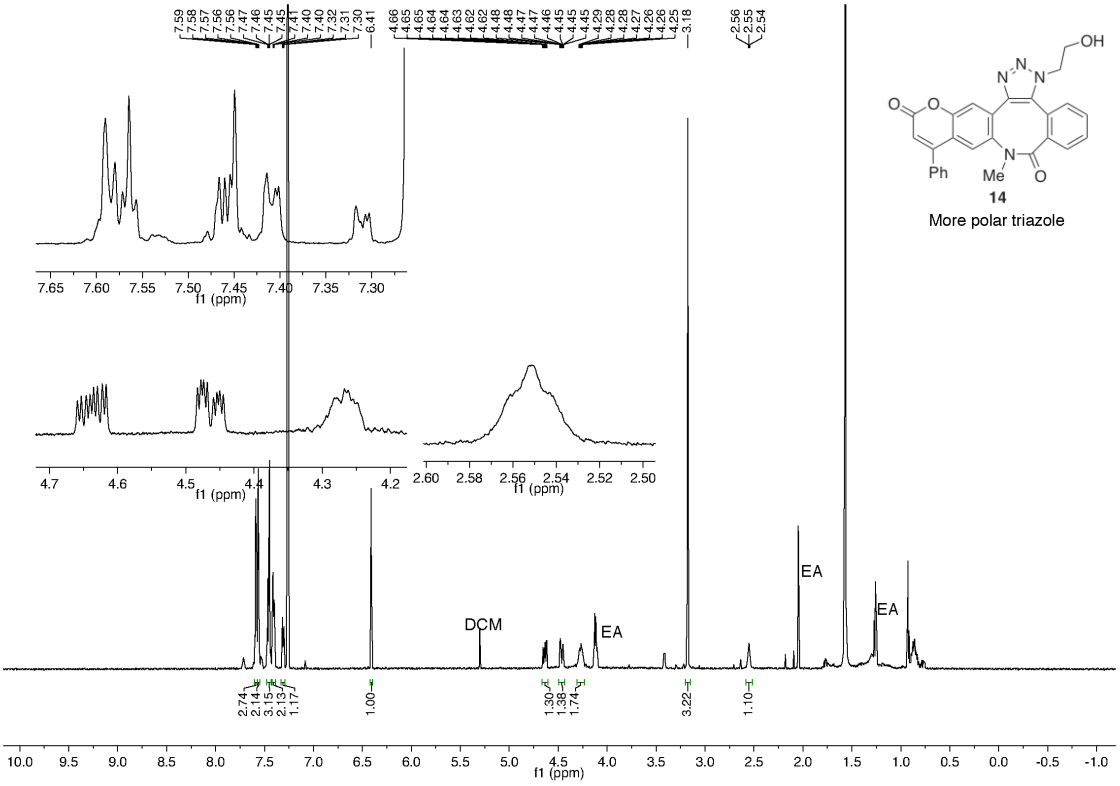
Me
3


176.65
160.12
155.12
155.46
151.82
149.53
134.76
130.14
129.84
129.78
129.27
128.58
127.28
127.00
126.11
125.83
119.86
116.34
116.04
113.29
107.07
77.37
77.16
76.95
39.01
f1 (ppm)
Ph
Me
3


OH
O
O
N
N
N
Ph
Me
O
14
Less polar triazole


OH
O
O
N
N
N
Ph
Me
O
14
More polar triazole
DCM
EA
EA
EA