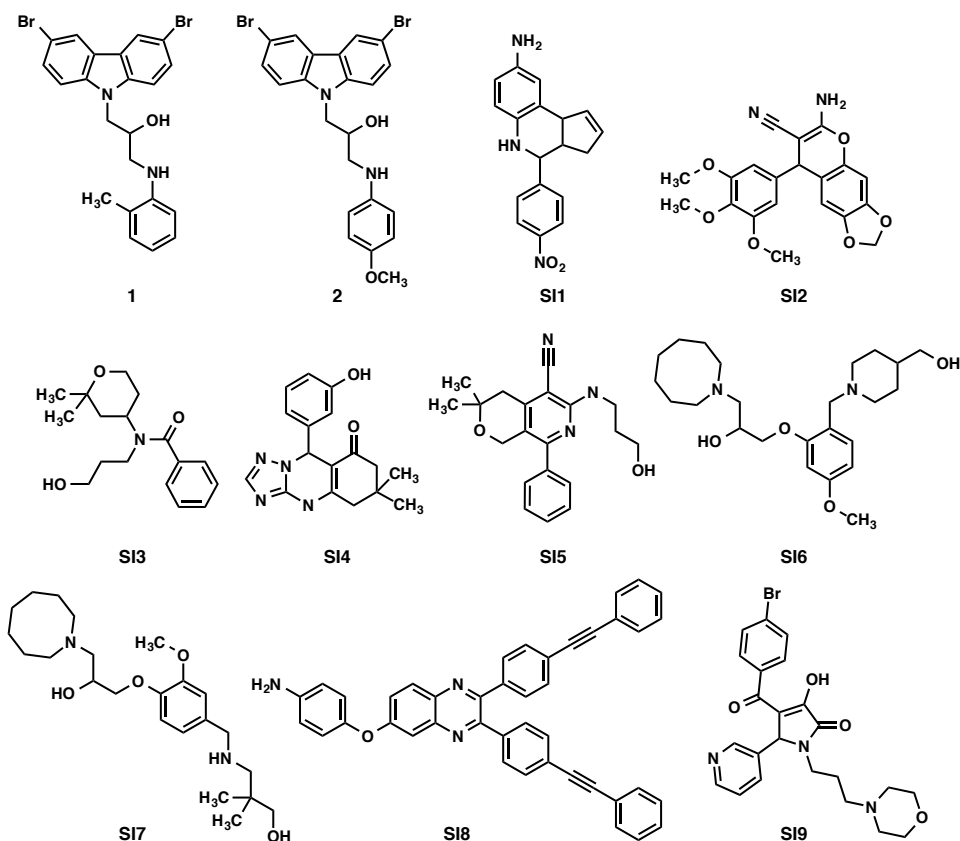


## **Discovery of Inhibitors of MicroRNA-21 Processing Using Small Molecule Microarrays**

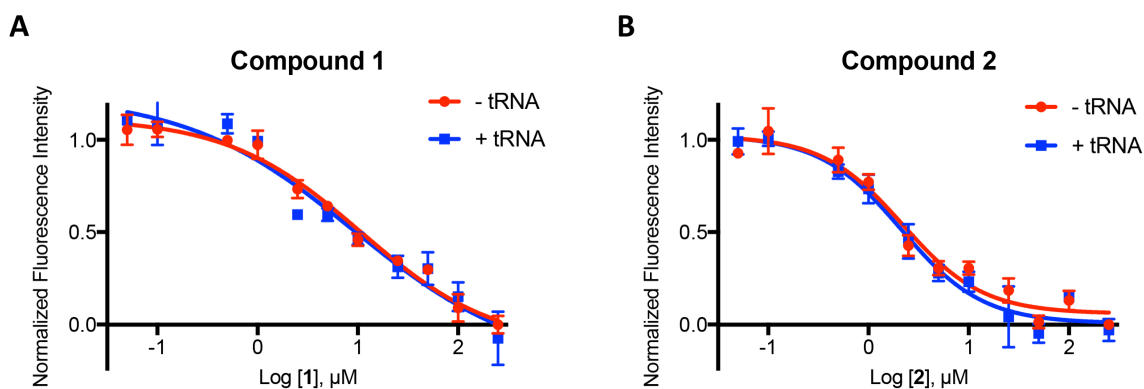
Colleen M. Connelly, Robert E. Boer, Michelle H. Moon, Peter Gareiss, and John S. Schneekloth, Jr.\*

### **Supporting Information**

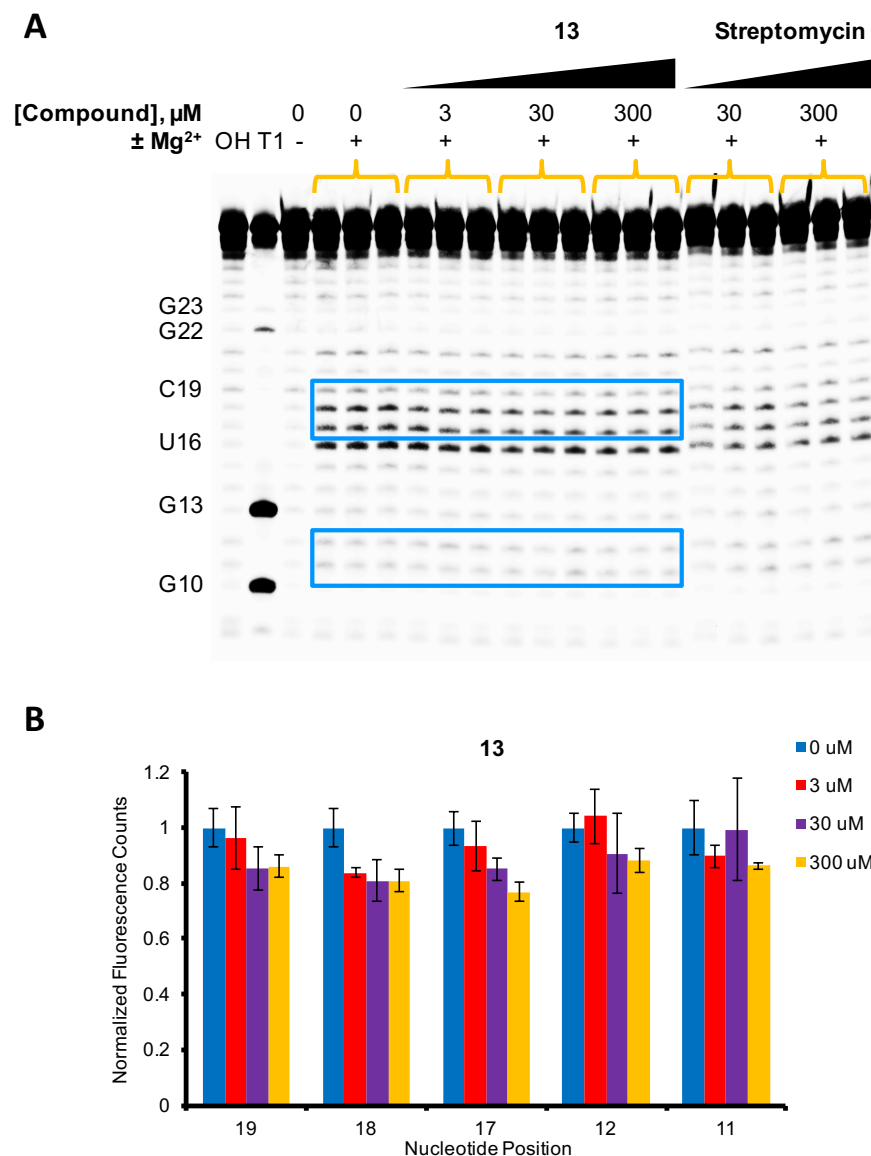
- A. Supporting Figures S1-S3
- B. Table S1
- C. General Chemistry Methods
- D. Experimental Procedures
- E.  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data



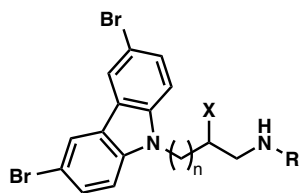
**Figure S1.** Structures of 11 hit compounds that were identified to selectively bind the miR-21 hairpin over the TAR hairpin by SMM. Compounds were selected for further analysis and purchased from commercial vendors.



**Figure S2.** FIA affinity titrations of compounds 1 and 2 in the presence of 10x excess yeast tRNA. Compounds were incubated with labeled miR-21-hp, yeast tRNA, and were allowed to equilibrate for 30 minutes before analyzing as described in the Methods section.



**Figure S3.** Analysis of small molecule binding to miR-21-hp RNA by  $\text{Mg}^{2+}$  induced in-line cleavage. (A) In-gel fluorescence of 5'-Cy5-labeled miR-21-hp RNA after treatment with **13** (3, 30 or 300  $\mu\text{M}$ ), streptomycin (30 or 300  $\mu\text{M}$ ) or a DMSO control in triplicate in the absence (-) or presence (+) of 1 mM  $\text{MgCl}_2$ . OH and T1 are a partial alkaline hydrolysis ladder and ribonuclease T1 digestion, respectively. Blue boxes designate nucleotide positions where the cleavage efficiency was significantly altered by treatment with **13**. (B) Quantification of fluorescent band intensity from triplicate experiments treated with **13** normalized to the average band intensity of DMSO control treated samples. The error bars indicate the standard deviation determined from the three independent experiments.



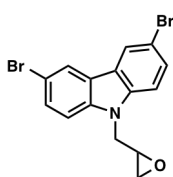
Compound	R	n	X	$\Delta T_m$ (°C)	FIA $K_d$ ( $\mu\text{M}$ )	2-AP $K_d$ ( $\mu\text{M}$ )
S110		2	OH	$-0.4 \pm 0.6$	$2.7 \pm 0.9$	$3.1 \pm 0.5$
S111		2	OH	$-3.7 \pm 0.5$	$2.3 \pm 0.5$	$2.0 \pm 0.5$
S112		1	F	$-0.6 \pm 0.2$	$2.6 \pm 1.3$	$9.4 \pm 3.7$

**Table S1:**  $\Delta T_m$  and apparent  $K_D$  values determined by 2-aminopurine and fluorescence intensity assays for linker region derivatives.

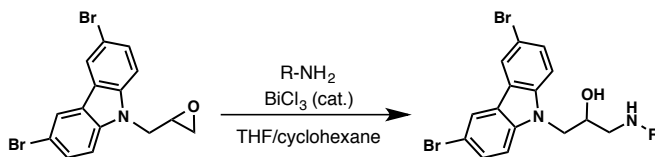
**General Chemistry Methods:** Unless otherwise noted, all chemical reagents were obtained from commercial suppliers and used without further purification. Anhydrous  $\text{CH}_2\text{Cl}_2$ , DMF, and THF were obtained from GlassContour Solvent Systems and were dried over alumina under an argon atmosphere. Solvents were removed using a Buchi rotary evaporator under reduced pressure. Flash column chromatography was performed using a Teledyne ISCO CombiFlash Rf automated chromatography system. HPLC purifications were performed using a Waters HPLC with a 2545 model pump and a Phenomenex Luna 10 micron C18 column (75 x 30 mm) using an acetonitrile/water gradient with 0.1% TFA. NMR spectra were recorded on Varian and Bruker spectrometers (at 400 or 500 MHz for  $^1\text{H}$  spectra or at 100 or 125 MHz for  $^{13}\text{C}$  spectra, respectively) and are reported relative to deuterated solvent signals. Data for  $^1\text{H}$  NMR spectra are reported as follows: chemical shift ( $\delta$  ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, app = apparent), coupling constants (Hz), and integration. Data for  $^{13}\text{C}$  NMR spectra are reported in terms of chemical shift.

High resolution mass spectrometry data were acquired on an Agilent 6520 Accurate-Mass Q-TOF LC/MS System, (Agilent Technologies, Inc.) equipped with a dual electro-spray source, operated in the positive-ion mode. Separation was performed on Zorbax 300SB-C18 Poroshell column (2.1 mm x 150 mm; particle size 5  $\mu\text{m}$ ). The analytes were eluted using a water/acetonitrile gradient with 0.1% formic acid. Data were acquired at high resolution (1,700  $m/z$ ), 4 GHz. To maintain mass accuracy during the run time, an internal mass calibration sample was infused continuously during the LC/MS runs. Data acquisition and analysis were performed using MassHunter Workstation Data Software, LCMS Data Acquisition (version B.06.01) and Qualitative Analysis (version B.07.00).

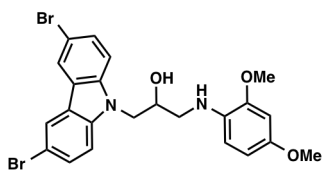
### Experimental Procedures:



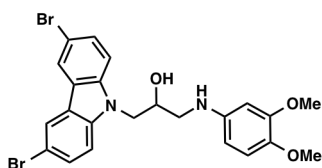
**Epoxide SI13:** To a solution of 3,6-dibromocarbazole (1.00 g, 3.08 mmol) in  $\text{CH}_3\text{CN}$  (50 mL) was added  $\text{K}_2\text{CO}_3$  (0.830 g, 6.16 mmol). Epichlorohydrin (12.4 mL, 158 mmol) was added and the reaction was heated to reflux and stirred for 4 h. The reaction was allowed to cool to rt and  $\text{H}_2\text{O}$  (150 mL) and  $\text{CH}_2\text{Cl}_2$  (200 mL) were added. The organic layer was separated and washed with 0.2 M HCl (100 mL). The organic layer was dried ( $\text{MgSO}_4$ ), filtered, and concentrated to produce a yellow solid which was subsequently rinsed with cold EtOAc (50 mL) to provide the alkylated carbazole **SI13** as an off-white solid which was used without further purification. The spectral data matched reported values.<sup>1,2</sup>



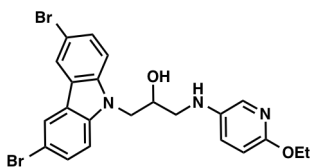
**Representative procedure for epoxide opening reactions:**<sup>2</sup> A screw-capped vial was charged with epoxide (0.131 mmol), THF (1.00 mL), and cyclohexane (2.00 mL). The requisite aniline (0.144 mmol) and BiCl<sub>3</sub> (0.0328 mmol) were added and the reaction vial was sealed. The reaction was heated to 80 °C and stirred for 16 h. After cooling to rt, the reaction mixture was diluted with EtOAc (5 mL) and H<sub>2</sub>O (5 mL). The aqueous layer was extracted with EtOAc (3 x 10 mL) and the combined organic layers were washed with brine (20 mL), dried (MgSO<sub>4</sub>), filtered, and concentrated *in vacuo*. The resulting residue was purified by RP-HPLC (H<sub>2</sub>O/CH<sub>3</sub>CN with 0.1% TFA) to provide **3-9**.



**Compound 3:** Purified by HPLC (10-95% CH<sub>3</sub>CN in H<sub>2</sub>O with 0.1% TFA, *t<sub>R</sub>* = 6.9 min) to yield **3** (37 mg, 53 %) as a light purple solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.46 (d, *J* = 1.8 Hz, 2H), 7.60 (d, *J* = 8.8 Hz, 2H), 7.57 (dd, *J* = 8.8, 1.8 Hz, 2H), 6.50 (d, *J* = 2.5 Hz, 1H), 6.39 (d, *J* = 8.6 Hz, 1H), 6.33 (dd, *J* = 8.5, 2.6 Hz, 1H), 5.29 (d, *J* = 5.3 Hz, 1H), 4.53 (t, *J* = 3.8 Hz, 1H), 4.48 (dd, *J* = 15.0, 4.5 Hz, 1H), 4.35 (dd, *J* = 14.9, 7.4 Hz, 1H), 4.06-4.13 (m, 1H), 3.78 (s, 3H), 3.66 (s, 3H), 3.15-3.20 (m, 1H), 2.96-3.01 (m, 1H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 151.3, 147.6, 139.7 (2C), 132.2, 128.7 (2C), 123.3 (2C), 123.0 (2C), 112.1 (2C), 111.3 (2C), 110.1, 104.1, 99.2, 68.1, 55.5, 55.4, 47.8, 47.5. HRMS *m/z*: calcd. for C<sub>23</sub>H<sub>23</sub>Br<sub>2</sub>N<sub>2</sub>O<sub>3</sub> [M+H]<sup>+</sup> 533.0070, found 533.0075.

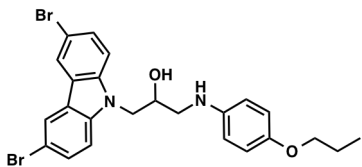


**Compound 4:** Purified by HPLC (10-95% CH<sub>3</sub>CN in H<sub>2</sub>O with 0.1% TFA, *t<sub>R</sub>* = 6.8 min) to yield **4** (45 mg, 64 %) as a light purple solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.46 (d, *J* = 1.9 Hz, 2H), 7.62 (d, *J* = 8.8 Hz, 2H), 7.56 (dd, *J* = 8.8, 1.9 Hz, 2H), 6.69 (d, *J* = 8.5 Hz, 1H), 6.29 (d, *J* = 2.6 Hz, 1H), 6.05 (dd, *J* = 8.6, 2.5 Hz, 1H), 5.28 (t, *J* = 6.2 Hz, 1H), 5.18 (d, *J* = 5.2 Hz, 1H), 4.49 (dd, *J* = 14.9, 4.0 Hz, 1H), 4.35 (dd, *J* = 14.9, 7.7 Hz, 1H), 4.04-4.10 (m, 1H), 3.64 (s, 3H), 3.61 (s, 3H), 3.15-3.23 (m, 1H), 3.07-3.12 (m, 1H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 149.9, 143.9, 140.4, 139.7 (2C), 128.6 (2C), 123.2 (2C), 123.0 (2C), 114.5, 112.3 (2C), 111.3 (2C), 102.9, 98.9, 68.2, 56.6, 55.2, 48.1, 47.4. HRMS *m/z*: calcd. for C<sub>23</sub>H<sub>23</sub>Br<sub>2</sub>N<sub>2</sub>O<sub>3</sub> [M+H]<sup>+</sup> 533.0070, found 533.0079.



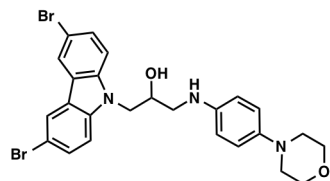
**Compound 5:** Purified by HPLC (10-95% CH<sub>3</sub>CN in H<sub>2</sub>O with 0.1% TFA, *t<sub>R</sub>* = 7.0 min) to yield **5** (5 mg, 7 %) as a dark purple oil. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.45 (d, *J* = 2.0 Hz, 2H), 7.62 (d, *J* = 8.8 Hz, 2H), 7.56 (dd, *J* = 8.7, 2.0 Hz, 2H), 7.49 (d, *J* = 2.9 Hz, 1H), 7.10 (dd, *J* = 8.8, 3.0 Hz, 1H), 6.57 (d, *J* = 8.7 Hz, 1H), 5.38 (t, *J* = 6.1 Hz, 1H), 5.25 (d, *J* = 2.7 Hz, 1H), 4.49 (dd, *J* = 14.9, 3.9 Hz, 1H), 4.36 (dd, *J* = 14.9, 7.7 Hz, 1H), 4.14 (q, *J* =

7.0 Hz, 2H), 4.03-4.10 (m, 1H), 3.15 (dt,  $J = 11.9, 5.7$  Hz, 1H), 3.02 (dt,  $J = 12.2, 5.5$  Hz, 1H), 1.25 (t,  $J = 7.0$  Hz, 3H).  $^{13}\text{C}$  NMR (125 MHz, DMSO)  $\delta$  156.0, 140.3, 140.2 (2C), 129.7, 129.1 (2C), 125.8, 123.7 (2C), 123.4 (2C), 112.7 (2C), 111.7 (2C), 110.8, 68.6, 61.0, 48.3, 47.8, 15.2. HRMS  $m/z$ : calcd. for  $\text{C}_{22}\text{H}_{22}\text{Br}_2\text{N}_3\text{O}_2$   $[\text{M}+\text{H}]^+$  518.0073, found 518.0070.



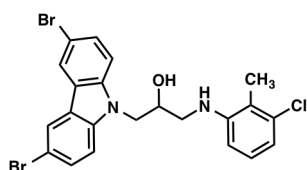
**Compound 6:** Purified by HPLC (10-95%  $\text{CH}_3\text{CN}$  in  $\text{H}_2\text{O}$  with 0.1% TFA,  $t_{\text{R}} = 7.4$  min) to yield **6** (20 mg, 29 %) as a light brown solid.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.44 (d,  $J = 1.9$  Hz, 2H), 7.59 (d,  $J = 8.8$  Hz, 2H), 7.53 (dd,  $J = 8.7, 2.0$  Hz, 2H), 6.69 (d,  $J = 8.9$  Hz, 2H), 6.53 (d,  $J = 8.8$  Hz, 2H), 4.49 (dd,  $J = 14.9, 3.9$  Hz, 1H),

4.34 (dd,  $J = 14.9, 7.7$  Hz, 1H), 4.03-4.1 (m, 1H), 3.77 (t,  $J = 6.5$  Hz, 2H), 3.11 (dd,  $J = 12.7, 5.5$  Hz, 1H), 3.02 (dd,  $J = 12.6, 6.2$  Hz, 1H), 1.65 (h,  $J = 7.1$  Hz, 2H), 0.94 (t,  $J = 7.4$  Hz, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  150.3, 143.0, 139.8 (2C), 128.6 (2C), 123.2 (2C), 123.0 (2C), 115.5 (2C), 113.5 (2C), 112.3 (2C), 111.3 (2C), 69.5, 68.3, 48.3, 47.5, 22.3, 10.5. HRMS  $m/z$ : calcd. for  $\text{C}_{24}\text{H}_{25}\text{Br}_2\text{N}_2\text{O}_2$   $[\text{M}+\text{H}]^+$  531.0277, found 531.0278.



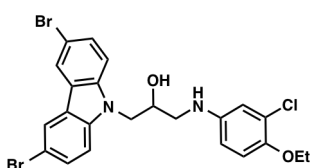
**Compound 7:** Purified by HPLC (10-95%  $\text{CH}_3\text{CN}$  in  $\text{H}_2\text{O}$  with 0.1% TFA,  $t_{\text{R}} = 6.9$  min) to yield **7** (9 mg, 12 %) as a white solid.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.46 (d,  $J = 1.8$  Hz, 2H), 7.59 (d,  $J = 8.8$  Hz, 2H), 7.55 (dd,  $J = 8.8$  Hz, 1.9 Hz, 2H), 6.75 (d,  $J = 8.9$  Hz, 2H), 6.54 (d,  $J = 8.8$  Hz, 2H), 5.20 (t,  $J = 6.2$  Hz, 1H), 5.15 (d,  $J = 5.2$  Hz, 1H), 4.49

(dd,  $J = 14.9, 3.8$  Hz, 1H), 4.34 (dd,  $J = 14.9, 7.8$  Hz, 1H), 4.02-4.08 (m, 1H), 3.80-3.60 (m, 4H), 3.13 (dt,  $J = 12.3, 6.0, 6.0$  Hz, 1H), 3.03 (dt,  $J = 13.2, 5.7, 5.7$  Hz, 1H), 2.93-2.83 (m, 4H).  $^{13}\text{C}$  NMR (125 MHz, DMSO)  $\delta$  143.3, 143.0, 140.2 (2C), 129.0 (2C), 123.7 (2C), 123.4 (2C), 118.0 (2C), 113.7 (2C), 112.7 (2C), 111.6 (2C), 68.7, 66.8 (2C), 50.9 (2C), 48.5, 47.9. HRMS  $m/z$ : calcd. for  $\text{C}_{25}\text{H}_{26}\text{Br}_2\text{N}_3\text{O}_2$   $[\text{M}+\text{H}]^+$  558.0386, found 558.0378.



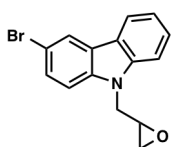
**Compound 8:** Purified by HPLC (10-95%  $\text{CH}_3\text{CN}$  in  $\text{H}_2\text{O}$  with 0.1% TFA,  $t_{\text{R}} = 9.0$  min) to yield **8** (12 mg, 16 %) as a pale yellow solid.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.46 (d,  $J = 1.9$  Hz, 2H), 7.60 (d,  $J = 8.8$  Hz, 2H), 7.56 (dd,  $J = 8.8, 2.0$  Hz, 2H), 6.96 (t,  $J = 8.1$  Hz, 1H), 6.65 (d,  $J = 7.8$  Hz, 1H), 6.48 (d,  $J = 8.2$  Hz, 1H), 5.32 (d,  $J = 5.2$  Hz, 1H), 5.15 (t,  $J =$

5.8 Hz, 1H), 4.51 (dd,  $J = 15.0, 3.9$  Hz, 1H), 4.38 (dd,  $J = 14.9, 7.4$  Hz, 1H), 4.09-4.16 (m, 1H), 3.27 (dt,  $J = 12.1, 6.1, 6.1$  Hz, 1H), 3.13 (dt,  $J = 12.6, 6.9, 5.0$  Hz, 1H), 2.17 (s, 3H).  $^{13}\text{C}$  NMR (125 MHz,  $\text{DMSO}-d_6$ )  $\delta$  147.8, 139.7 (2C), 133.4, 128.6 (2C), 127.3, 123.2 (2C), 123.0 (2C), 119.12, 116.5, 112.2 (2C), 111.2 (2C), 108.2, 67.9, 54.9, 47.6, 47.4, 13.7. HRMS  $m/z$ : calcd. for  $\text{C}_{22}\text{H}_{20}\text{Br}_2\text{ClN}_2\text{O}_2$   $[\text{M}+\text{H}]^+$  520.9625, found 520.9620.

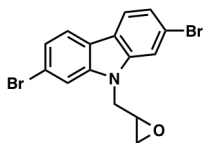


**Compound 9:** Purified by HPLC (40-95%  $\text{CH}_3\text{CN}$  in  $\text{H}_2\text{O}$  with 0.1% TFA,  $t_{\text{R}} = 7.4$  min) to yield **9** (9.2 mg, 9 %) as a pale yellow solid.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  8.46 (d,  $J = 1.9$  Hz, 2H), 7.61 (d,  $J = 8.8$  Hz, 2H), 7.56 (dd,  $J = 8.8, 1.9$  Hz, 2H), 6.89 (d,  $J = 8.8$  Hz, 1H), 6.67 (d,  $J = 2.7$  Hz, 1H),

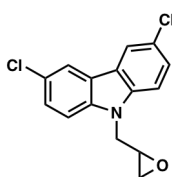
6.52 (dd,  $J = 8.9, 2.8$  Hz, 1H), 5.56 (t,  $J = 6.0$  Hz, 1H), 5.17 (d,  $J = 5.2$  Hz, 1H), 4.48 (dd,  $J = 15.0, 3.9$  Hz, 1H), 4.34 (dd,  $J = 14.9, 7.8$  Hz, 1H), 4.01-4.08 (m, 1H), 3.94 (q,  $J = 6.9$  Hz, 2H), 3.14 (ddd,  $J = 12.2, 5.9, 5.9$  Hz, 1H), 3.03 (ddd,  $J = 12.5, 5.8, 5.8$  Hz, 1H), 1.28 (t,  $J = 7.0$  Hz, 3H).  $^{13}\text{C}$  NMR (125 MHz, DMSO)  $\delta$  144.8, 144.0, 139.7 (2C), 128.6 (2C), 123.2 (2C), 123.0 (2C), 122.6, 116.5, 113.5, 112.2 (2C), 111.8, 111.2 (2C), 68.1, 65.2, 47.7, 47.3, 14.8. HRMS  $m/z$ : calcd. for  $\text{C}_{23}\text{H}_{22}\text{Br}_2\text{ClN}_2\text{O}_2$   $[\text{M}+\text{H}]^+$  550.9731, found 550.9723.



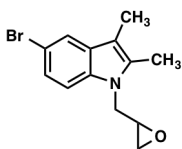
**Epoxide SI14:** To a solution of 3-bromocarbazole (0.200 g, 0.813 mmol) in DMF (1.00 mL) was added KOH (0.055 g, 2.03 mmol). The reaction was stirred for 5 min, at which point neat epichlorohydrin (0.160 mL, 2.03 mmol) was added dropwise. The reaction was stirred at rt for 16h. The reaction was treated with  $\text{H}_2\text{O}$  (5 mL) and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with  $\text{H}_2\text{O}$  (2x) and brine, dried ( $\text{MgSO}_4$ ), filtered, and concentrated. The resulting pale yellow oil was used without further purification. Spectral data matched reported values.<sup>2</sup>



**Epoxide SI15:** To a solution of 2,7-dibromocarbazole (1.00 g, 3.08 mmol) in  $\text{CH}_3\text{CN}$  (50 mL) was added  $\text{K}_2\text{CO}_3$  (0.830 g, 6.16 mmol). Epichlorohydrin (12.4 mL, 158 mmol) was added and the reaction was heated to reflux and stirred for 4 h. The reaction was allowed to cool to rt and  $\text{H}_2\text{O}$  (150 mL) and  $\text{CH}_2\text{Cl}_2$  (200 mL) were added. The organic layer was separated and washed with 0.2 M HCl (100 mL). The organic layer was dried ( $\text{MgSO}_4$ ), filtered, and concentrated to produce a yellow solid which was subsequently rinsed with cold EtOAc (50 mL) to provide the alkylated carbazole **SI15** as an off-white solid which was used without further purification.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.09 (d,  $J = 8.3$  Hz, 2H), 7.94 (d,  $J = 1.7$  Hz, 2H), 7.36 (dd,  $J = 8.3, 1.7$  Hz, 2H), 4.89 (dd,  $J = 15.7, 2.6$  Hz, 1H), 4.37 (dd,  $J = 15.8, 6.1$  Hz, 1H), 3.31-3.37 (m, 1H), 2.77 (dd,  $J = 5.0, 4.0$  Hz, 1H), 2.55 (dd,  $J = 5.0, 2.6$  Hz, 1H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  141.5 (2C), 122.4 (2C), 122.0 (2C), 120.7 (2C), 119.1 (2C), 112.9 (2C), 50.4, 44.5, 44.3.

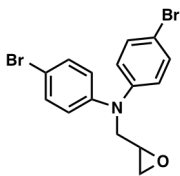


**Epoxide SI16:** Prepared according to procedure used for **SI-3**. The resulting white solid was used without further purification. Spectral data matched reported values.<sup>2</sup>

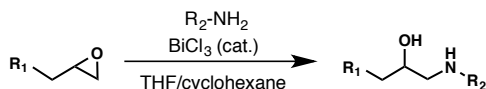


**Epoxide SI17:** Prepared according to procedure used for **SI-2**. The resulting orange oil was used without further purification. Spectral data matched reported values.<sup>2</sup>

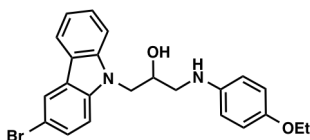




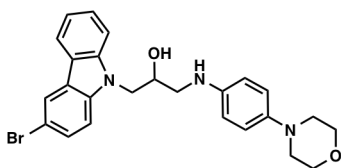
**Epoxide SI18:** To a solution of bis(4-bromophenyl)amine (0.500 g, 1.53 mmol) in DMF (1.00 mL) was added KOH (0.103 g, 1.83 mmol). The reaction stirred at rt for 30 min, at which point neat epichlorohydrin (0.300 mL, 3.82 mmol) was added dropwise. The reaction was stirred at rt for 16 h. The reaction was diluted with EtOAc (2 mL) and H<sub>2</sub>O (2 mL) and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with water (15 mL) and brine (15 mL), dried (MgSO<sub>4</sub>), filtered, and concentrated *in vacuo*. The resulting light brown solid **SI18** was used without further purification. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.37 (d, *J* = 8.8 Hz, 2H), 6.92 (d, *J* = 8.8 Hz, 2H), 3.99 (dd, *J* = 16.0, 3.1 Hz, 1H), 3.73 (dd, *J* = 16.0, 5.2 Hz, 1H), 3.19 (ddt, *J* = 5.5, 4.0, 2.9 Hz, 1H), 2.79 (dd, *J* = 4.8, 4.0 Hz, 1H), 2.55 (dd, *J* = 4.8, 2.7 Hz, 1H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 146.7 (2C), 132.6 (2C), 122.8 (2C), 114.8 (2C), 54.0, 50.2, 45.8. HRMS *m/z*: calcd. for C<sub>15</sub>H<sub>14</sub>Br<sub>2</sub>NO [M+H]<sup>+</sup> 381.9437, found 381.9434.



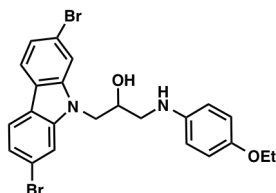
**Representative procedure for epoxide opening reactions:**<sup>2</sup> A screw-capped vial was charged with epoxide (1 eq), THF (1.00 mL), and cyclohexane (2.00 mL). The requisite aniline (1.1 eq) and BiCl<sub>3</sub> (0.25 eq) were added and the reaction vial was sealed. The reaction was heated to 80 °C and stirred for 16 h. After cooling to rt, the reaction mixture was diluted with EtOAc (5 mL) and H<sub>2</sub>O (5 mL). The aqueous layer was extracted with EtOAc (3 x 10 mL) and the combined organic layers were washed with brine (20 mL), dried (MgSO<sub>4</sub>), filtered, and concentrated *in vacuo*. The resulting residue was purified by RP-HPLC (H<sub>2</sub>O/CH<sub>3</sub>CN with 0.1% TFA) to provide **18-27**.



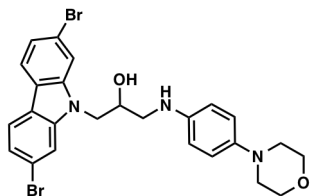
**Compound 18:** Purified by HPLC (10-95% CH<sub>3</sub>CN in H<sub>2</sub>O with 0.1% TFA, *t<sub>R</sub>* = 6.6 min) to yield **18** (7.7 mg, 11 %) as a light brown solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.38 (d, *J* = 1.9 Hz, 1H), 8.19 (d, *J* = 7.8 Hz, 1H), 7.60 (d, *J* = 8.2 Hz, 1H), 7.58 (d, *J* = 8.6 Hz, 1H), 7.52 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.44 (ddd, *J* = 8.3, 7.1, 1.2 Hz, 1H), 7.20 (ddd, *J* = 7.9, 7.1, 0.9 Hz, 1H), 6.69 (d, *J* = 8.9 Hz, 2H), 6.53 (d, *J* = 8.9 Hz, 2H), 5.22 (t, *J* = 6.1 Hz, 1H), 5.15 (d, *J* = 5.3 Hz, 1H), 4.49 (dd, *J* = 14.8, 4.1 Hz, 1H), 4.34 (dd, *J* = 14.8, 7.6 Hz, 1H), 4.05-4.12 (m, 1H), 3.88 (q, *J* = 6.9 Hz, 2H), 3.12 (ddd, *J* = 12.3, 6.8, 5.6 Hz, 1H), 3.03 (ddd, *J* = 12.4, 5.9, 5.9 Hz, 1H), 1.26 (t, *J* = 6.9 Hz, 3H). <sup>13</sup>C NMR (125 MHz, DMSO-*d*<sub>6</sub>) δ 150.5, 143.4, 141.4, 139.8, 128.2, 126.8, 124.4, 123.1, 121.5, 121.1, 119.6, 115.8 (2C), 113.8 (2C), 112.3, 111.2, 110.5, 68.7, 63.7, 48.7, 47.8, 15.3. HRMS *m/z*: calcd. for C<sub>23</sub>H<sub>24</sub>BrN<sub>2</sub>O<sub>2</sub> [M+H]<sup>+</sup> 439.1016, found 431.1014.



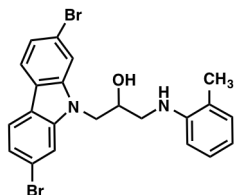
**Compound 19:** Purified by HPLC (10-95% CH<sub>3</sub>CN in H<sub>2</sub>O with 0.1% TFA,  $t_R = 6.4$  min) to yield **19** (8.0 mg, 10 %) as a brown oil. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.38 (d,  $J = 1.9$  Hz, 1H), 8.19 (d,  $J = 7.7$  Hz, 1H), 7.60 (d,  $J = 8.1$  Hz, 1H), 7.57 (d,  $J = 8.3$  Hz, 1H), 7.52 (dd,  $J = 8.7, 2.0$  Hz, 1H), 7.43 (ddd,  $J = 8.3, 7.0, 1.2$  Hz, 1H), 7.20 (t,  $J = 7.4$  Hz, 1H), 6.74 (d,  $J = 8.3$  Hz, 2H), 6.54 (d,  $J = 8.2$  Hz, 2H), 5.21 (t<sub>3</sub>,  $J = 6.3$  Hz, 1H), 5.15 (d,  $J = 5.2$  Hz, 1H), 4.49 (dd,  $J = 14.9, 4.0$  Hz, 1H), 4.34 (dd,  $J = 14.9, 7.7$  Hz, 1H), 4.05-4.12 (m, 1H), 3.63-3.78 (m, 4H), 3.08-3.15 (m, 1H), 3.02-3.08 (m, 1H), 2.86-2.92 (m, 4H). HRMS  $m/z$ : calcd. for C<sub>25</sub>H<sub>27</sub>BrN<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup> 480.1281, found 480.1271.



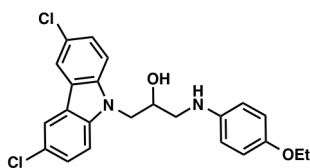
**Compound 20:** Purified by HPLC (10-95% CH<sub>3</sub>CN in H<sub>2</sub>O with 0.1% TFA,  $t_R = 7.0$  min) to yield **20** (43 mg, 64%) as a light yellow solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.09 (d,  $J = 8.3$  Hz, 2H), 7.86 (d,  $J = 1.7$  Hz, 2H), 7.33 (dd,  $J = 8.3, 1.7$  Hz, 2H), 6.71 (d,  $J = 8.9$  Hz, 1H), 6.59 (d,  $J = 8.9$  Hz, 1H), 5.28 (d,  $J = 8.4$  Hz, 1H), 5.26-5.29 (m, 1H), 4.49 (dd,  $J = 14.9, 3.4$  Hz, 1H), 4.34 (dd,  $J = 14.9, 8.1$  Hz, 1H), 4.03-4.10 (m, 1H), 3.88 (q,  $J = 6.9$  Hz, 1H), 3.14-3.19 (m, 1H), 3.04-3.10 (m, 1H), 1.26 (t,  $J = 7.0$  Hz, 3H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  150.1, 142.9, 141.8 (2C), 122.1 (2C), 122.0 (2C), 120.8 (2C), 119.0 (2C), 115.5 (2C), 113.5 (2C), 113.1 (2C), 68.3, 63.4, 48.1, 47.4, 14.9. HRMS  $m/z$ : calcd. for C<sub>23</sub>H<sub>23</sub>Br<sub>2</sub>N<sub>2</sub>O<sub>2</sub> [M+H]<sup>+</sup> 517.0121, found 517.0112.



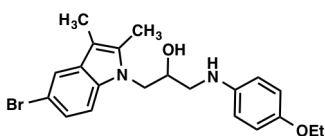
**Compound 21:** Purified by HPLC (10-95% CH<sub>3</sub>CN in H<sub>2</sub>O with 0.1% TFA,  $t_R = 6.7$  min) to yield **21** (23 mg, 32 %) as a light brown solid. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.10 (d,  $J = 8.3$  Hz, 2H), 7.86 (d,  $J = 1.7$  Hz, 2H), 7.33 (dd,  $J = 8.3, 1.7$  Hz, 2H), 6.76 (d,  $J = 8.9$  Hz, 2H), 6.59 (d,  $J = 8.9$  Hz, 2H), 5.28 (t,  $J = 5.8$  Hz, 1H), 5.21 (d,  $J = 5.4$  Hz, 1H), 4.49 (dd,  $J = 15.0, 3.4$  Hz, 1H), 4.34 (dd,  $J = 15.0, 8.2$  Hz, 1H), 4.02-4.08 (m, 1H), 3.63-3.76 (m, 4H), 3.14-3.20 (m, 1H), 3.13-3.03 (m, 1H), 2.82-2.96 (m, 4H). <sup>13</sup>C NMR (126 MHz, DMSO)  $\delta$  142.8, 142.6, 141.8 (2C), 122.1 (2C), 122.0 (2C), 120.8 (2C), 119.0 (2C), 117.7 (2C), 113.3 (2C), 113.1 (2C), 68.3, 66.4 (2C), 50.6 (2C), 48.0, 47.4. HRMS  $m/z$ : calcd. for C<sub>25</sub>H<sub>26</sub>Br<sub>2</sub>N<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup> 558.0386, found 558.0378.



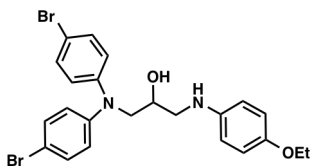
**Compound 22:** Purified by HPLC (30-95% CH<sub>3</sub>CN in H<sub>2</sub>O with 0.1% TFA,  $t_R = 7.8$  min) to yield **22** (2.8 mg, 6 %) as a brown solid. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.12 (d,  $J = 8.3$  Hz, 2H), 7.87 (d,  $J = 1.7$  Hz, 2H), 7.35 (dd,  $J = 8.3, 1.7$  Hz, 2H), 6.97-7.01 (m, 2H), 6.51-6.56 (m, 2H), 5.27 (d,  $J = 5.4$  Hz, 1H), 4.86 (t,  $J = 5.9$  Hz, 1H), 4.53 (dd,  $J = 15.1, 3.4$  Hz, 1H), 4.38 (dd,  $J = 15.0, 7.9$  Hz, 1H), 4.12 (p,  $J = 8.6, 7.4$  Hz, 3.29-3.34 (m, 1H), 3.14 (ddd,  $J = 12.4, 6.7, 4.8$  Hz, 1H), 2.14 (s, 1H). <sup>13</sup>C NMR (125 MHz, DMSO)  $\delta$  146.6, 142.2 (2C), 130.3, 127.2, 122.5 (2C), 122.4 (2C), 122.3, 121.2 (2C), 119.4 (2C), 116.5, 113.5 (2C), 109.8, 68.7, 47.9, 47.6, 18.1. HRMS  $m/z$ : calcd. for C<sub>22</sub>H<sub>21</sub>Br<sub>2</sub>N<sub>2</sub>O [M+H]<sup>+</sup> 487.0015, found 487.0006.



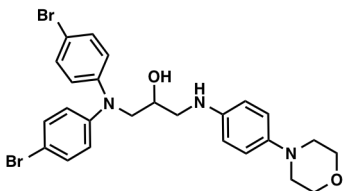
**Compound 23:** Purified by HPLC (10-95% CH<sub>3</sub>CN in H<sub>2</sub>O with 0.1% TFA, *t<sub>R</sub>* = 7.0 min) to yield **23** (12.0 mg, 16 %) as a dark orange oil. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 8.31 (d, *J* = 2.1 Hz, 2H), 7.64 (d, *J* = 8.8 Hz, 2H), 7.44 (dd, *J* = 8.7, 2.2 Hz, 2H), 6.69 (d, *J* = 8.8 Hz, 2H), 6.54 (d, *J* = 8.9 Hz, 2H), 5.23 (t, *J* = 5.2 Hz, 1H), 5.19 (d, *J* = 5.2 Hz, 1H), 4.50 (dd, *J* = 15.0, 3.8 Hz, 1H), 4.35 (dd, *J* = 14.9, 7.8 Hz, 1H), 4.07 (dt, *J* = 8.8, 4.5 Hz, 1H), 3.88 (q, *J* = 6.9 Hz, 2H), 3.12 (ddd, *J* = 10.7, 5.0 Hz, 1H), 3.03 (ddd, *J* = 12.2, 7.5, 4.0 Hz, 1H), 1.26 (t, *J* = 6.9 Hz, 3H). <sup>13</sup>C NMR (126 MHz, DMSO) δ 150.04, 142.9, 139.6 (2C), 126.0 (2C), 123.4 (2C), 122.4 (2C), 120.2 (2C), 115.4 (2C), 113.4 (2C), 111.8 (2C), 68.3, 63.3, 48.2, 47.5, 14.9. HRMS *m/z*: calcd. for C<sub>23</sub>H<sub>23</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub> [M+H]<sup>+</sup> 429.1131, found 429.1128.



**Compound 24:** Purified by HPLC (10-95% CH<sub>3</sub>CN in H<sub>2</sub>O with 0.1% TFA, *t<sub>R</sub>* = 6.5 min) to yield **24** (9.9 mg, 13 %) as a dark orange oil. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 7.53 (d, *J* = 1.9 Hz, 1H), 7.28 (d, *J* = 8.6 Hz, 1H), 7.07 (dd, *J* = 8.6, 2.0 Hz, 1H), 6.69 (d, *J* = 8.9 Hz, 2H), 6.53 (d, *J* = 8.9 Hz, 2H), 5.17 (t, *J* = 6.1 Hz, 1H), 5.11 (d, *J* = 5.2 Hz, 1H), 4.23 (dd, *J* = 14.8, 3.9 Hz, 1H), 4.02 (dd, *J* = 14.8, 8.0 Hz, 1H), 3.88 (q, *J* = 7.0 Hz, 3H), 3.85-3.90 (m, 1H), 2.94-3.06 (m, 2H), 2.32 (s, 3H), 2.14 (s, 3H), 1.26 (t, *J* = 7.0 Hz, 3H). <sup>13</sup>C NMR (125 MHz, DMSO) δ 150.4, 143.3, 135.6, 135.2, 130.4, 122.6, 120.1, 115.8 (2C), 113.8 (2C), 111.9, 111.3, 105.5, 69.2, 63.7, 48.6, 47.8, 15.3, 10.7, 9.0. HRMS *m/z*: calcd. for C<sub>21</sub>H<sub>26</sub>BrN<sub>2</sub>O<sub>2</sub> [M+H]<sup>+</sup> 417.1172, found 417.1167.

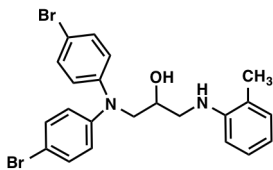


**Compound 25:** Purified by HPLC (10-95% CH<sub>3</sub>CN in H<sub>2</sub>O with 0.1% TFA, *t<sub>R</sub>* = 7.0 min) to yield **25** (11.3 mg, 17 %) as a dark orange oil. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.36 (d, *J* = 8.7 Hz, 4H), 6.93 (d, *J* = 8.7 Hz, 4H), 6.77 (d, *J* = 8.8 Hz, 2H), 6.57 (d, *J* = 8.7 Hz, 2H), 4.11-4.15 (m, 1H), 3.96 (q, *J* = 7.0 Hz, 2H), 3.86 (dd, *J* = 15.1, 4.5 Hz, 1H), 3.76 (dd, *J* = 15.1, 7.9 Hz, 1H), 3.25 (dd, *J* = 12.8, 3.6 Hz, 1H), 3.07 (dd, *J* = 12.7, 7.4 Hz, 1H), 1.37 (t, *J* = 7.0 Hz, 3H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 152.2, 147.1 (2C), 142.0 (2C), 132.6 (4C), 123.1 (4C), 115.9 (2C), 115.0 (2C), 114.9, 67.9, 64.2, 56.6, 49.0, 15.1. HRMS *m/z*: calcd. for C<sub>23</sub>H<sub>25</sub>Br<sub>2</sub>N<sub>2</sub>O<sub>2</sub> [M+H]<sup>+</sup> 519.0277, found 519.0276.

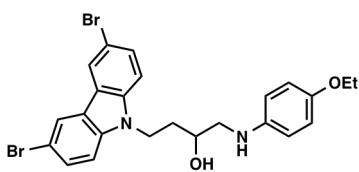


**Compound 26:** Purified by HPLC (10-95% CH<sub>3</sub>CN in H<sub>2</sub>O with 0.1% TFA, *t<sub>R</sub>* = 6.8 min) to yield **26** (3.0 mg, 3 %) as a brown solid. <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 7.37 (d, *J* = 8.9 Hz, 4H), 7.00 (d, *J* = 8.9 Hz, 4H), 6.73 (d, *J* = 8.8 Hz, 2H), 6.51 (d, *J* = 8.8 Hz, 2H), 5.12 (d, *J* = 5.0 Hz, 1H), 5.08 (t, *J* = 6.1 Hz, 1H), 3.92 (dd, *J* = 14.9, 4.1 Hz, 1H), 3.85-3.88 (m, 1H), 3.67-3.72 (m, 4H), 3.54 (dd, *J* = 14.8, 7.5 Hz, 1H), 3.00-3.06 (m, 1H), 2.93-2.98 (m, 1H), 2.87-2.92 (m, 4H). <sup>13</sup>C NMR (125 MHz, DMSO) δ 147.2

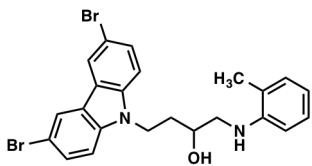
(2C), 143.3, 142.9, 132.3 (4C), 123.3 (4C), 118.0 (2C), 113.6 (2C), 113.2 (2C), 66.8 (2C), 66.6, 56.7, 51.0 (2C), 48.5. HRMS  $m/z$ : calcd. for  $C_{25}H_{28}Br_2N_3O_2$   $[M+H]^+$  560.0543, found 560.0535.



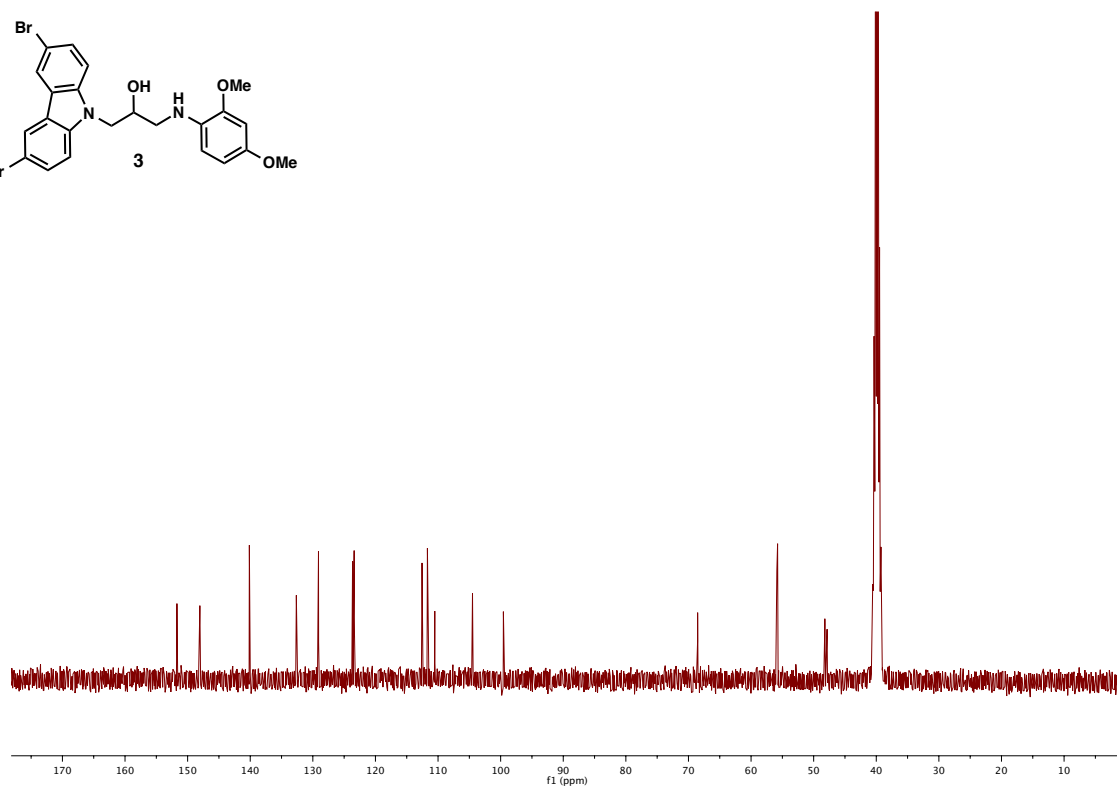
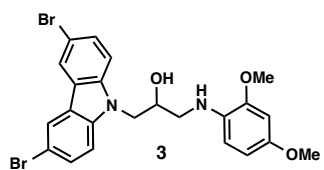
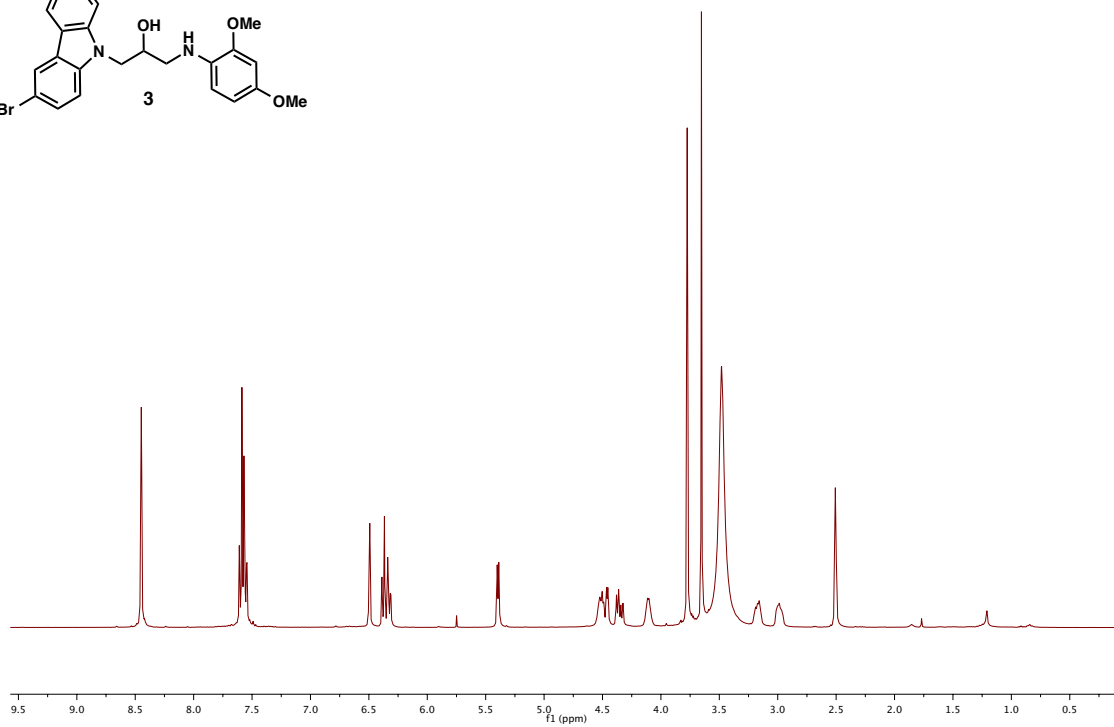
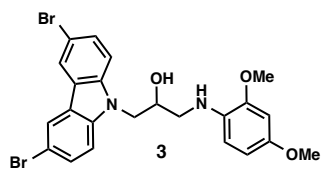
**Compound 27:** Purified by HPLC (10-95%  $CH_3CN$  in  $H_2O$  with 0.1% TFA,  $t_R = 8.1$  min) to yield **27** (3.5 mg, 5 %) as a X.  $^1H$  NMR (500 MHz,  $DMSO-d_6$ )  $\delta$  7.37 (d,  $J = 8.8$  Hz, 4H), 7.01 (d,  $J = 8.9$  Hz, 4H), 6.95-6.98 (m, 2H), 6.47-6.52 (m, 2H), 5.26 (d,  $J = 5.0$  Hz, 1H), 4.71 (t,  $J = 5.9$  Hz, 1H), 3.93-3.97 (m, 2H), 3.59 (dd,  $J = 16.1, 8.6$  Hz, 1H), 3.17 (ddd,  $J = 12.0, 6.8, 5.1$  Hz, 1H), 3.04 (ddd,  $J = 12.4, 6.5, 5.0$  Hz, 1H), 2.08 (s, 3H).  $^{13}C$  NMR (125 MHz,  $DMSO$ )  $\delta$  146.8 (2C), 146.2, 131.9 (4C), 129.8, 126.7, 122.9 (4C), 121.7, 115.9, 112.8 (2C), 109.3, 66.1, 56.3, 47.4, 17.5. HRMS  $m/z$ : calcd. for  $C_{22}H_{23}Br_2N_2O$   $[M+H]^+$  489.0172, found 489.0163.

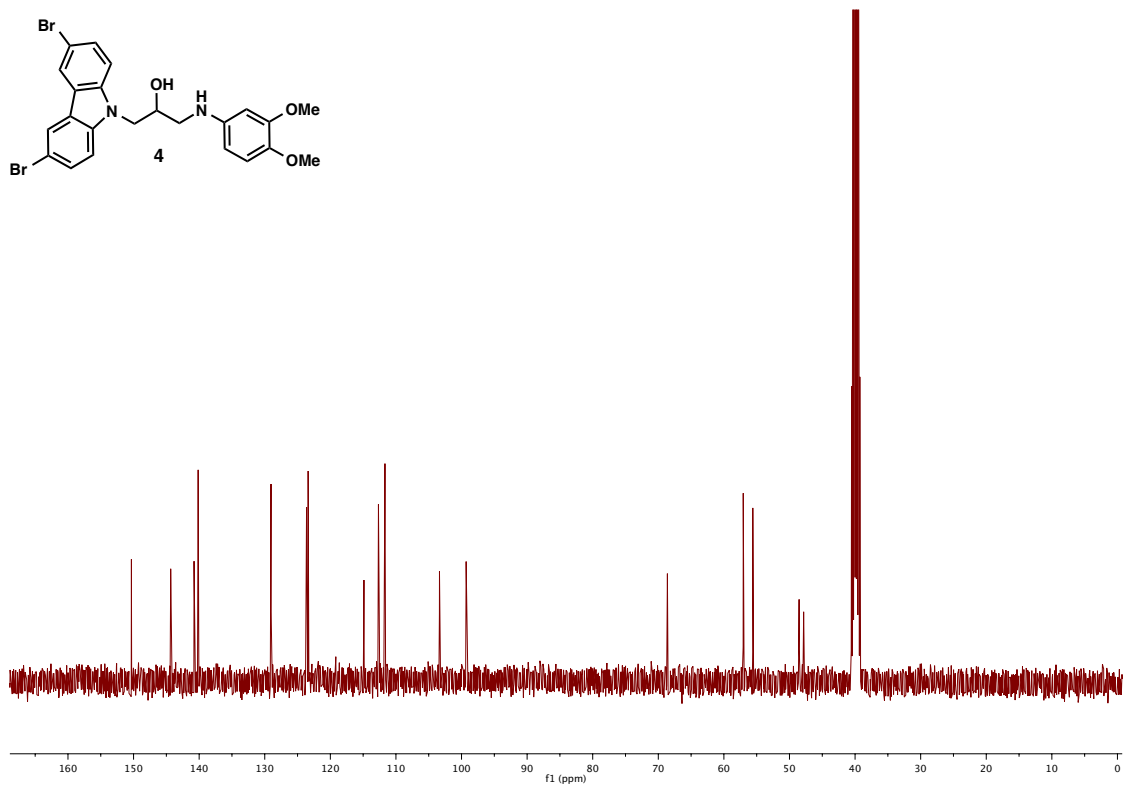
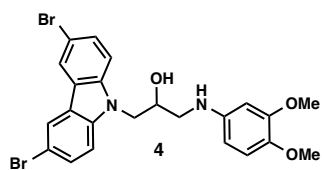
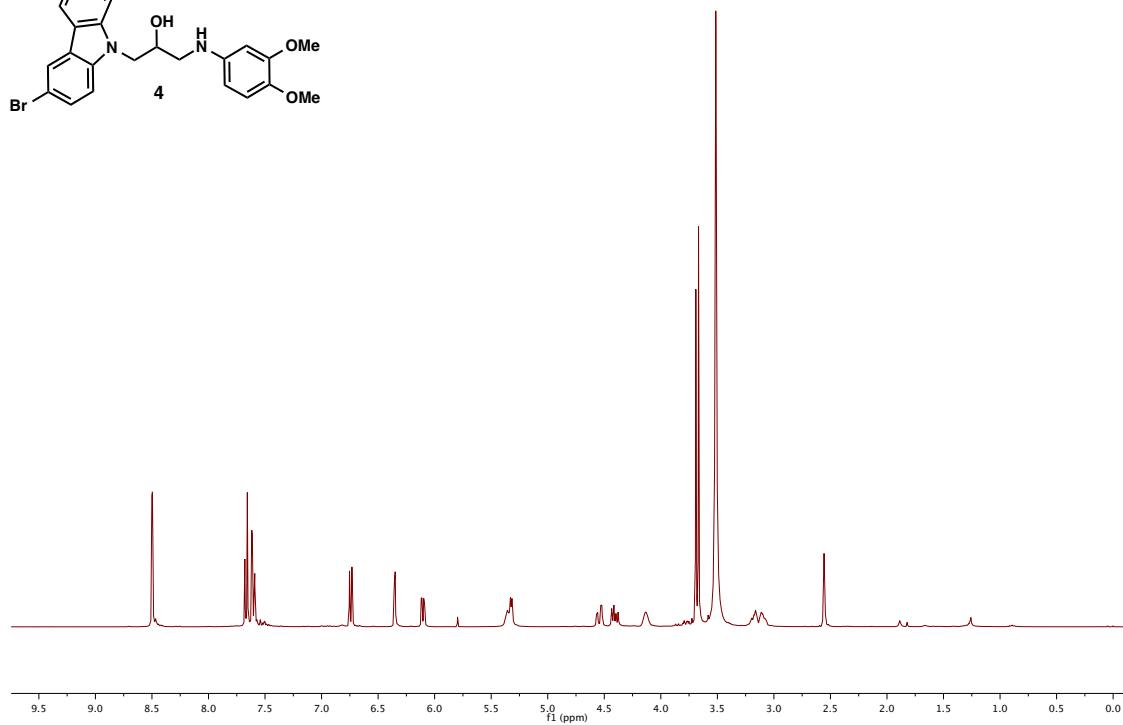
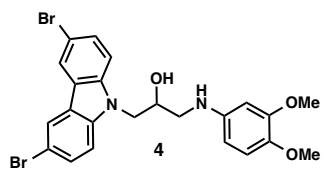


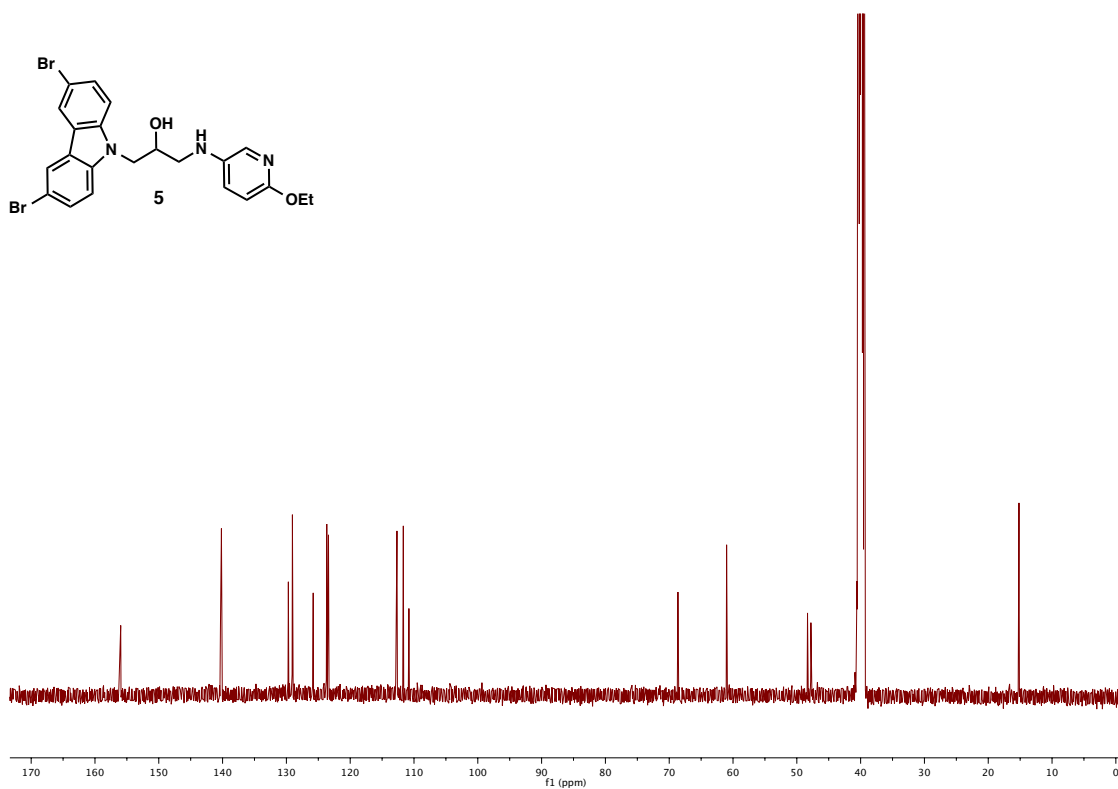
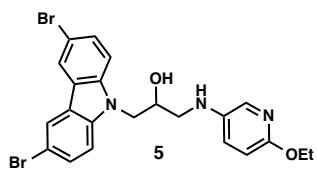
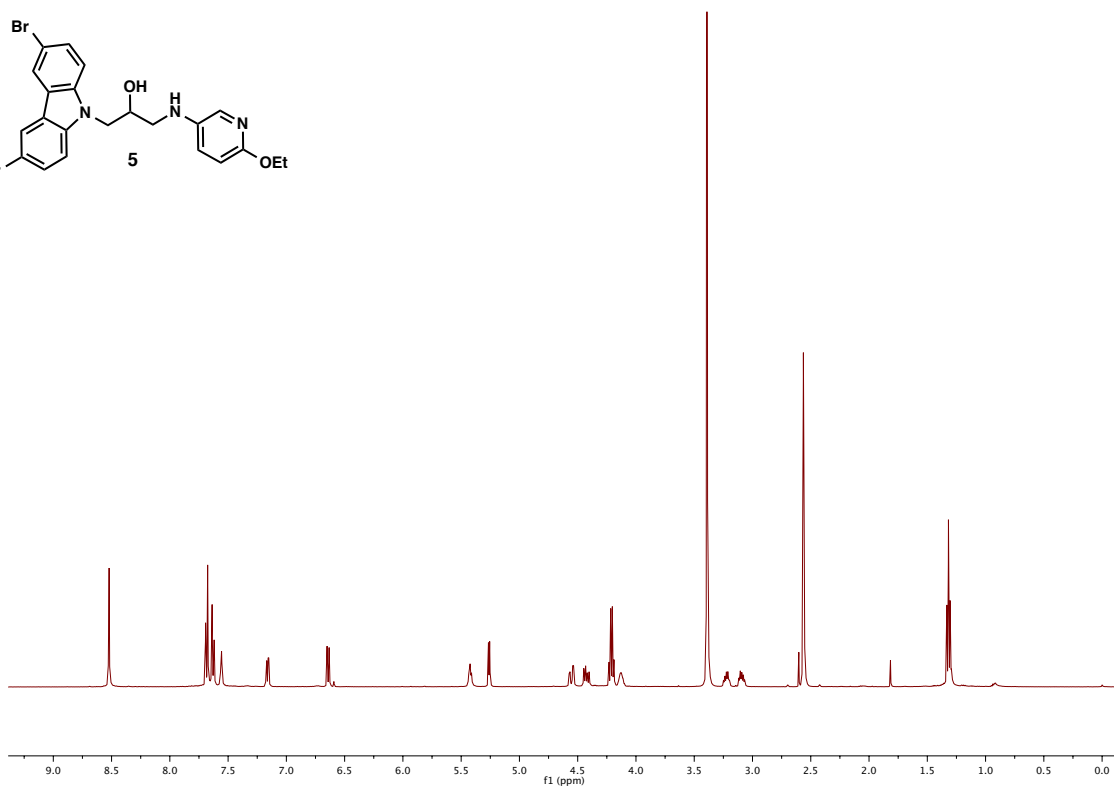
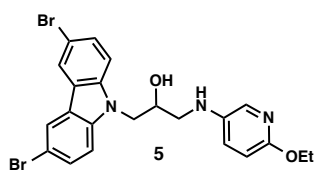
**Compound SI10:** Purified by HPLC (40-95%  $CH_3CN$  in  $H_2O$  with 0.1% TFA,  $t_R = 5.9$  min) to yield **SI10** (14.2 mg, 21 %) as a white solid.  $^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta$  8.14 (d,  $J = 2.2$  Hz, 2H), 7.56 (dd,  $J = 8.7, 2.0$  Hz, 2H), 7.38 (d,  $J = 8.5$  Hz, 2H), 6.73 (d,  $J = 9.0$  Hz, 2H), 6.53 (d,  $J = 8.9$  Hz, 2H), 4.55 (ddd,  $J = 15.3, 9.1, 6.3$  Hz, 1H), 4.43 (ddd,  $J = 14.9, 6.8, 4.1$  Hz, 1H), 3.94 (q,  $J = 7.0$  Hz, 1H), 3.61-3.66 (m, 1H), 3.07 (dd,  $J = 13.1, 3.1$  Hz, 1H), 2.95 (dd,  $J = 13.0, 8.6$  Hz, 1H), 2.03-2.10 (m, 1H), 1.88-1.95 (m, 1H), 1.36 (t,  $J = 6.9$  Hz, 2H).  $^{13}C$  NMR (125 MHz,  $CDCl_3$ )  $\delta$  152.3, 142.0, 139.5 (2C), 129.3 (2C), 123.7 (2C), 123.4 (2C), 115.9 (2C), 115.1 (2C), 112.3 (2C), 110.7 (2C), 67.5, 64.2, 52.0, 39.8, 33.6, 15.1. HRMS  $m/z$ : calcd. for  $C_{24}H_{25}Br_2N_2O_2$   $[M+H]^+$  531.0277, found 531.0267.

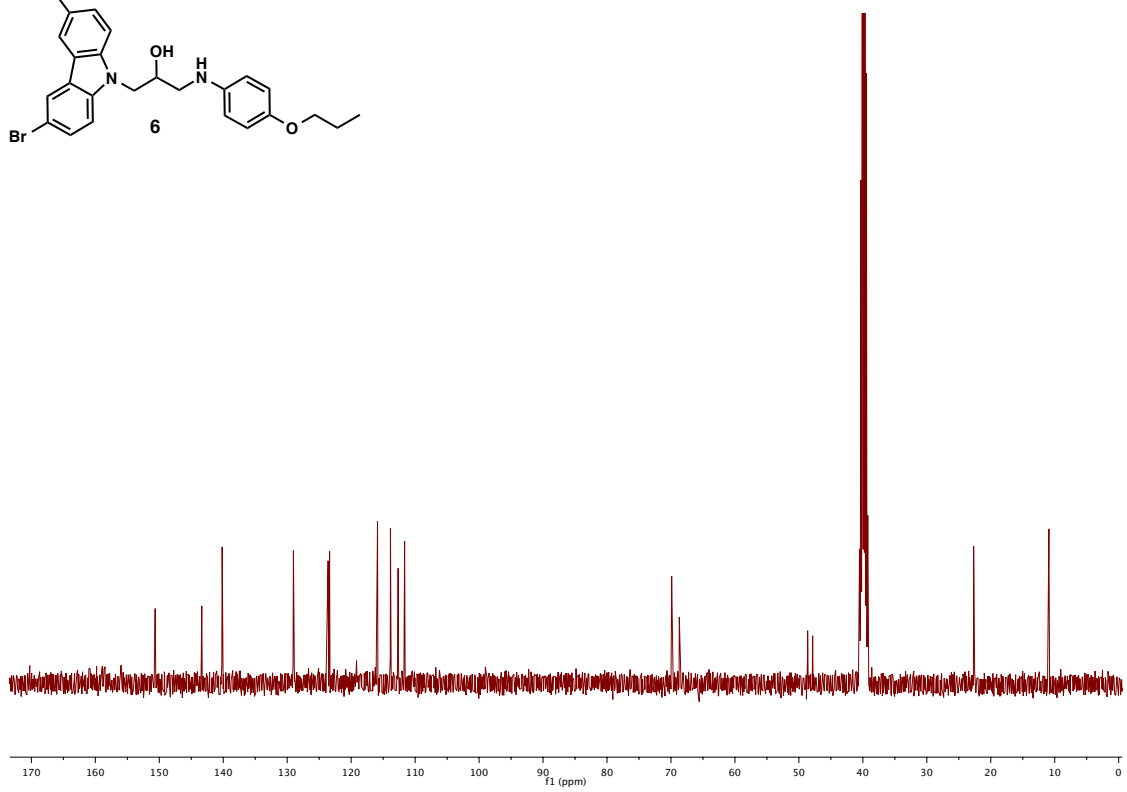
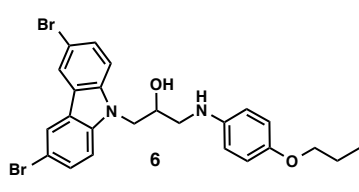
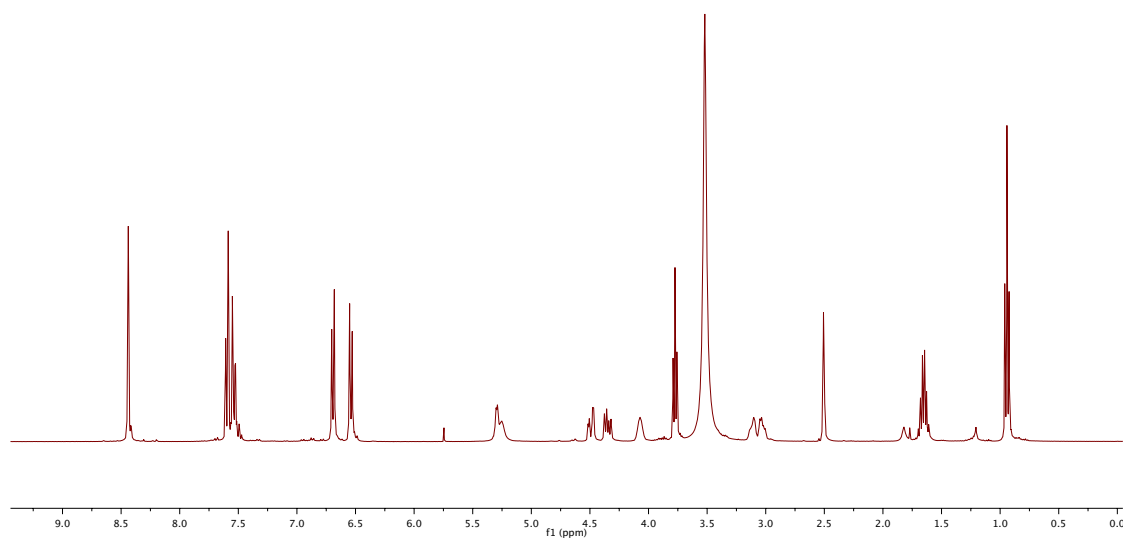
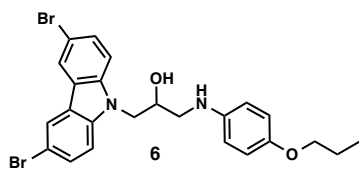


**Compound SI11:** Purified by HPLC (40-95%  $CH_3CN$  in  $H_2O$  with 0.1% TFA,  $t_R = 7.0$  min) to yield **SI11** (3.6 mg, 6 %) as a yellow oil.  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  8.15 (d,  $J = 1.9$  Hz, 2H), 7.57 (dd,  $J = 8.7, 1.9$  Hz, 2H), 7.38 (d,  $J = 8.7$  Hz, 2H), 7.04-7.09 (m, 2H), 6.69 (t,  $J = 7.3$ , 1H), 6.53 (d,  $J = 7.9$  Hz, 1H), 4.57 (ddd,  $J = 15.2, 9.0, 6.3$  Hz, 1H), 4.45 (ddd,  $J = 15.0, 7.1, 4.4$  Hz, 1H), 3.70-3.77 (m, 1H), 3.19 (dd,  $J = 13.2, 3.4$  Hz, 1H), 3.08 (dd,  $J = 13.2, 8.4$  Hz, 1H), 2.12 (s, 3H), 2.09-2.18 (m, 1H), 1.91-2.00 (m, 1H).  $^{13}C$  NMR (126 MHz,  $CDCl_3$ )  $\delta$  145.8, 139.5 (2C), 130.5, 129.3 (2C), 127.3, 123.7 (2C), 123.5 (2C), 122.9, 118.2, 112.3 (2C), 110.7 (2C), 110.4, 67.%, 50.7, 39.8, 33.7, 17.7. HRMS  $m/z$ : calcd. for  $C_{23}H_{23}Br_2N_2O$   $[M+H]^+$  501.0172, found 501.0165.

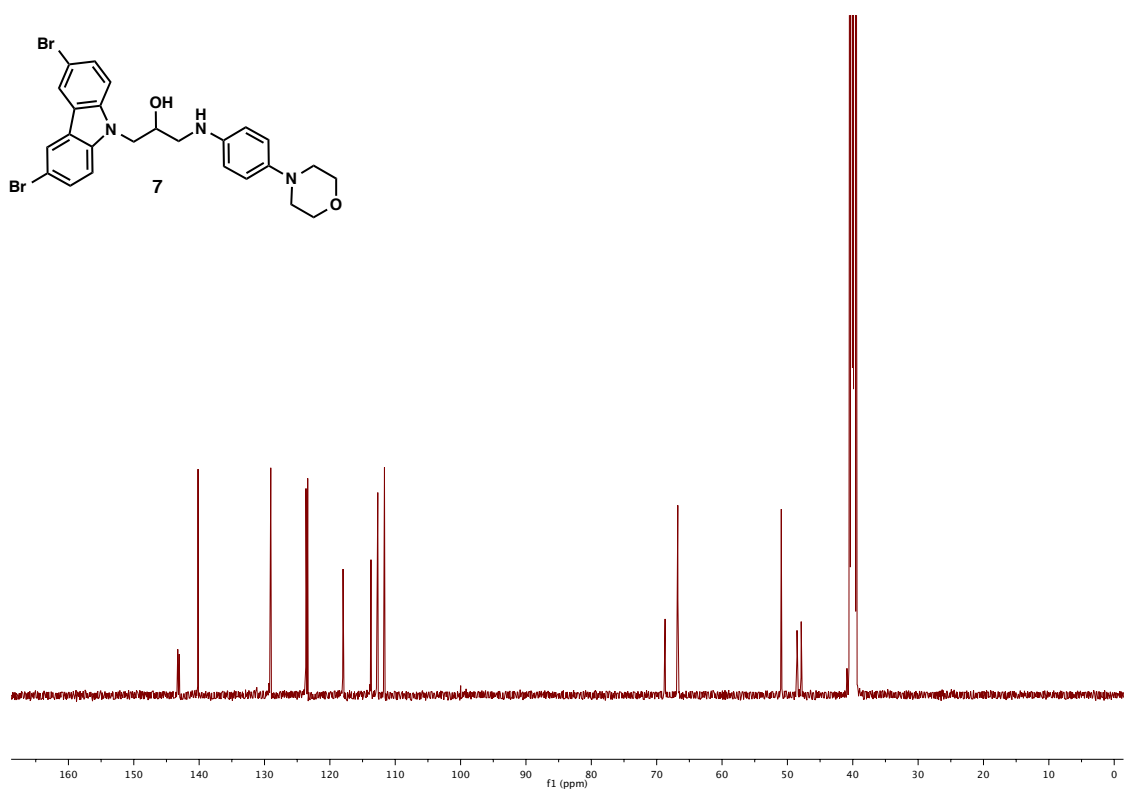
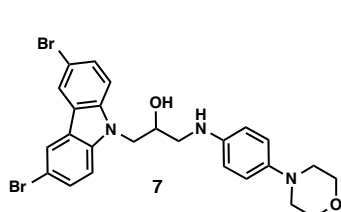
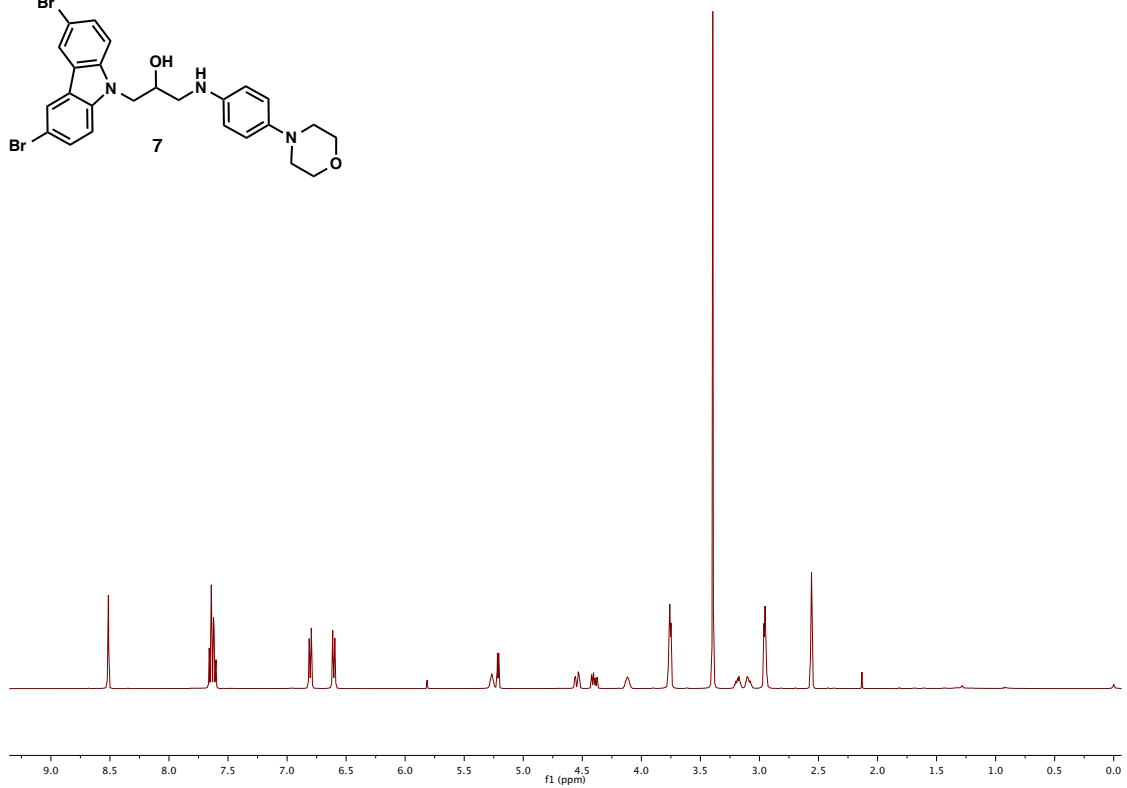
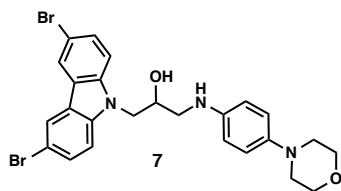


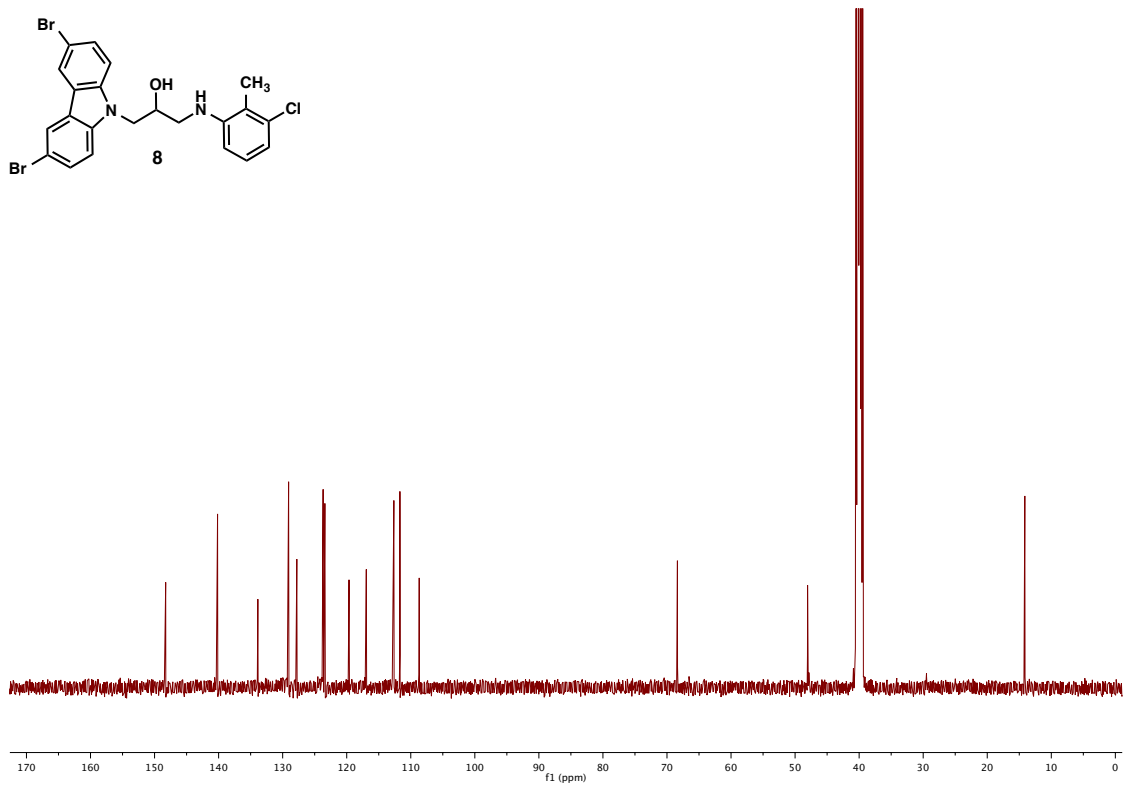
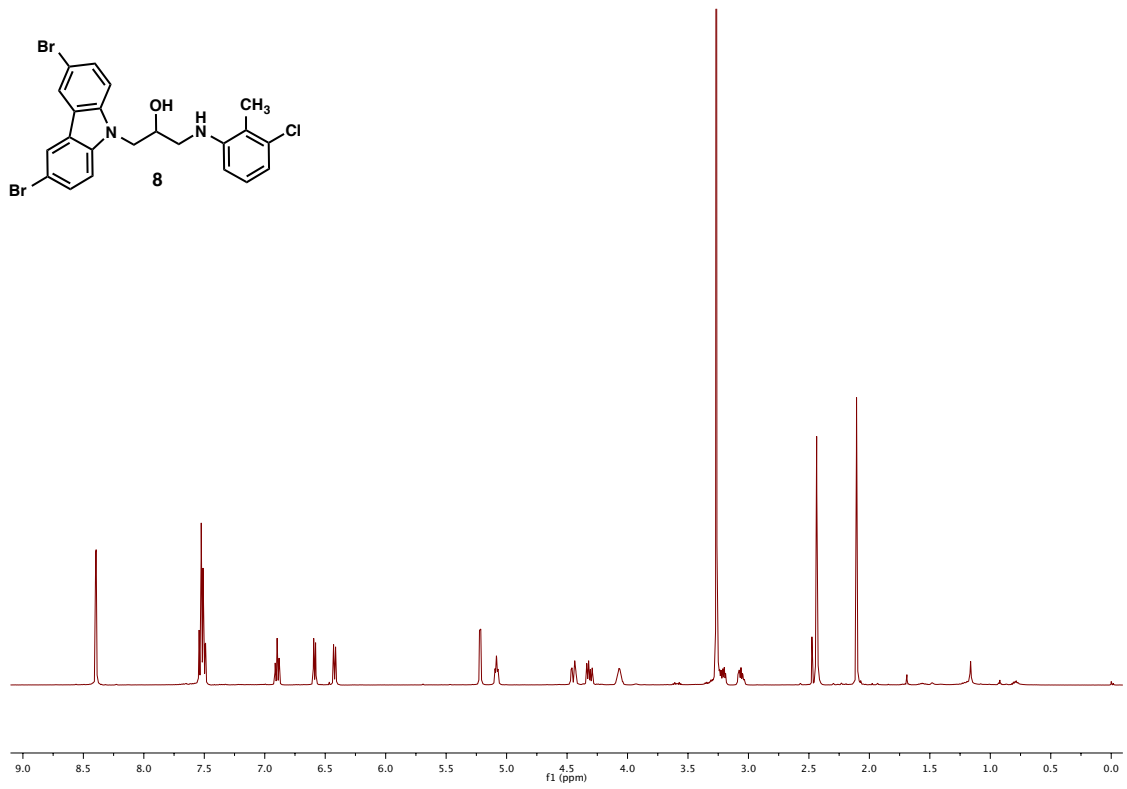


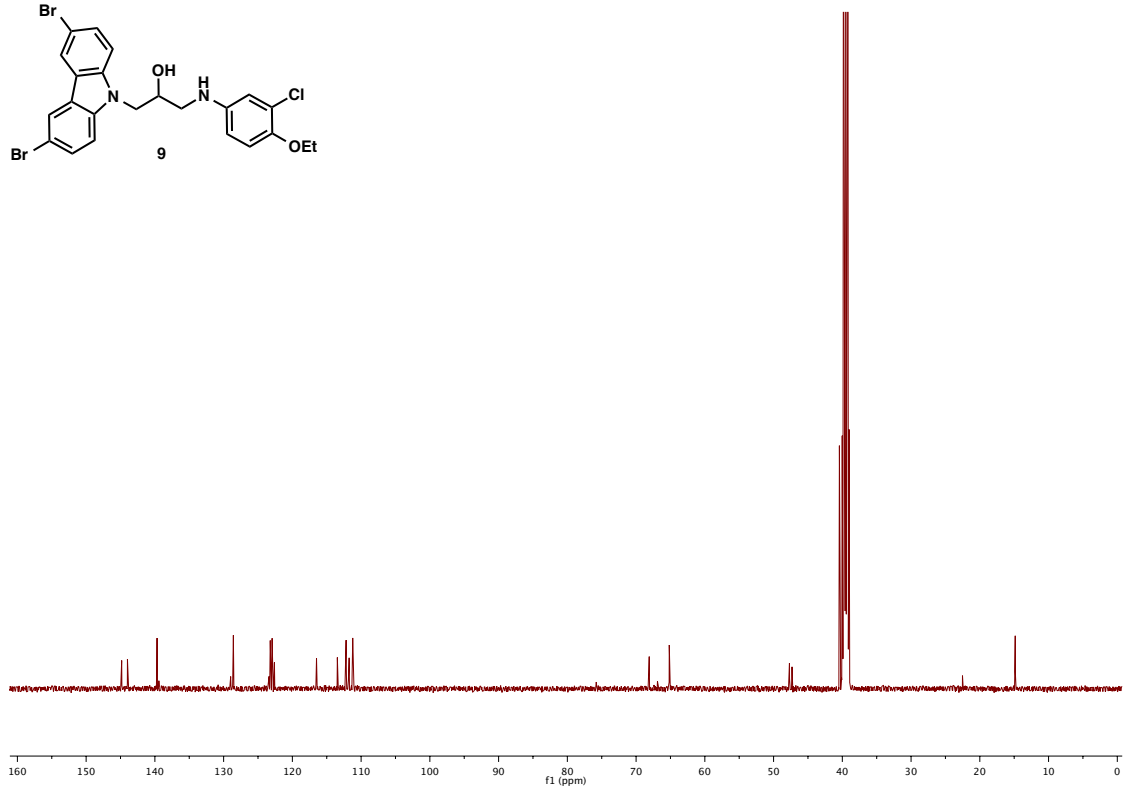
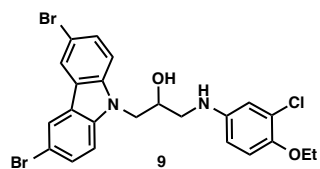
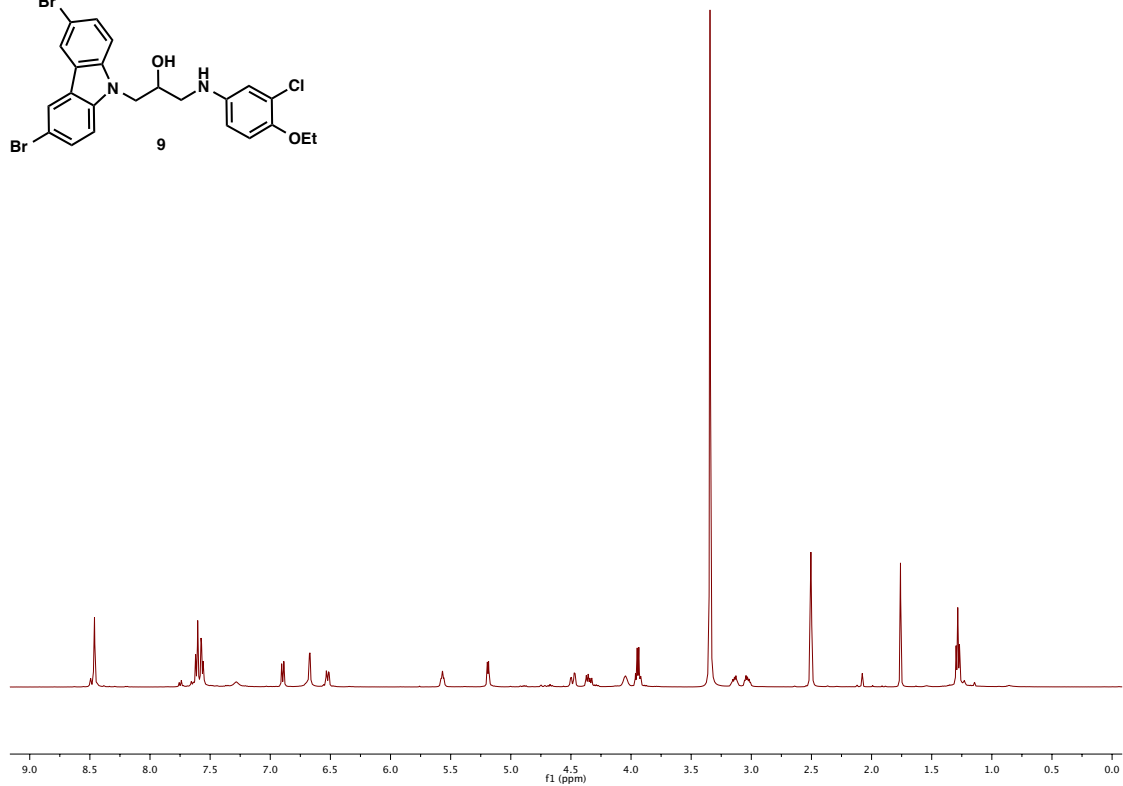
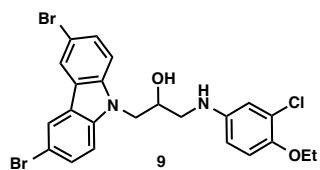


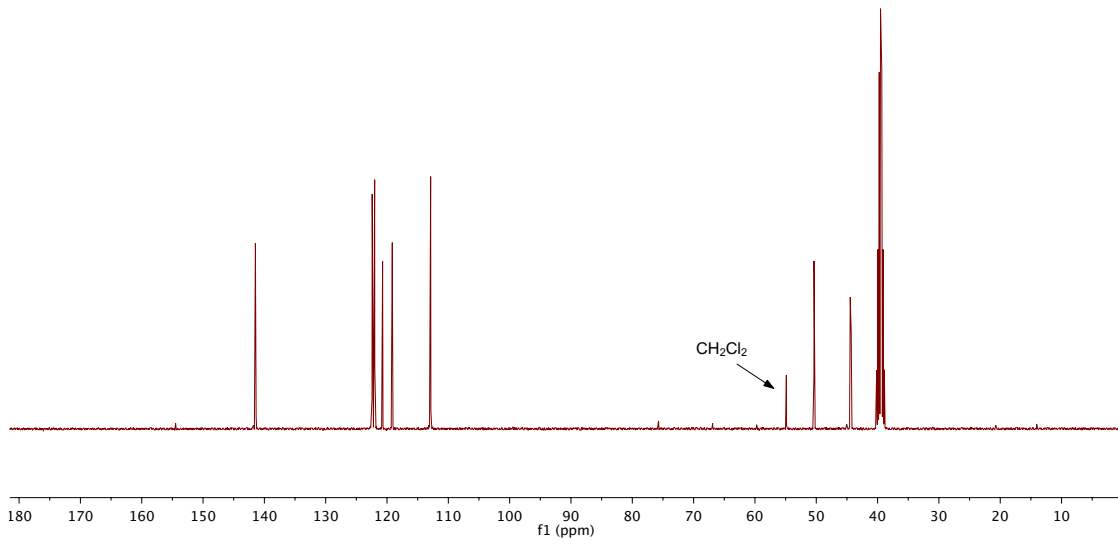
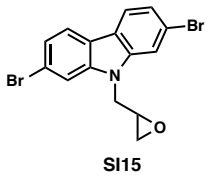
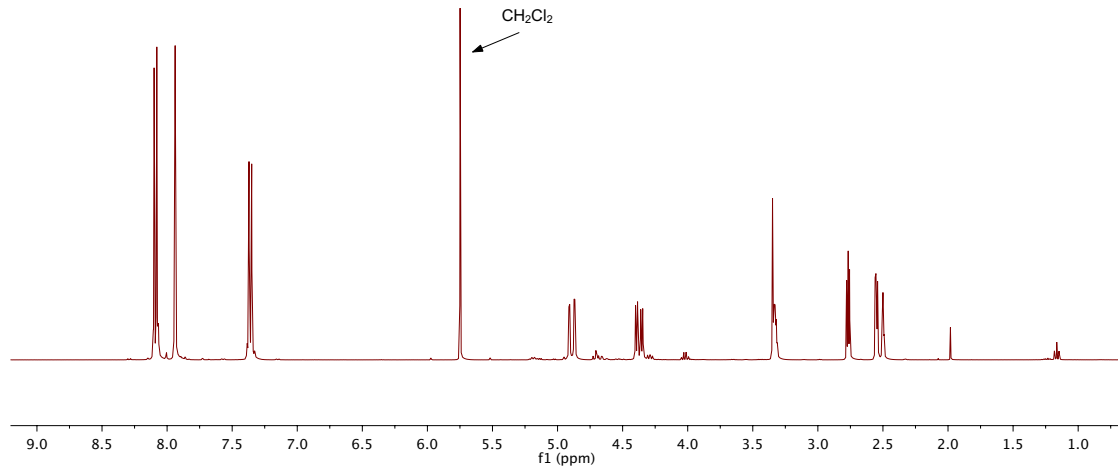
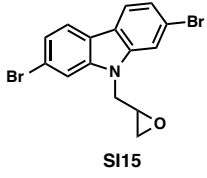


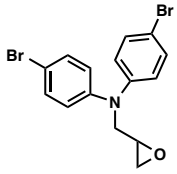




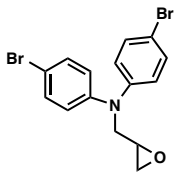
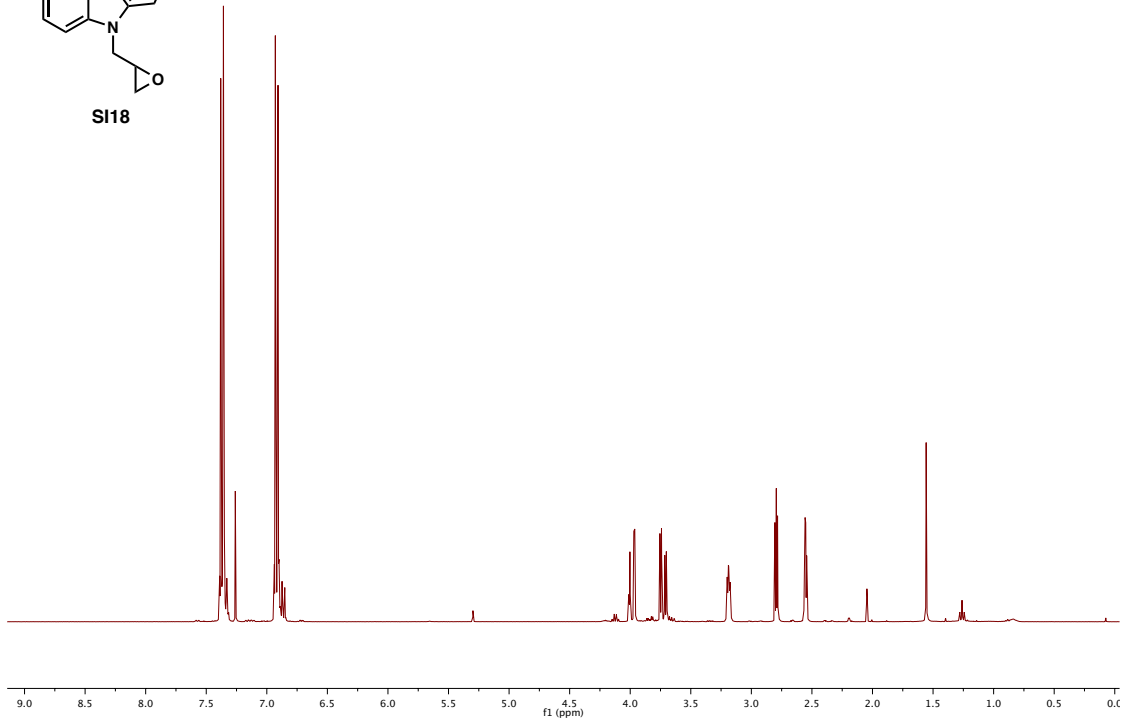




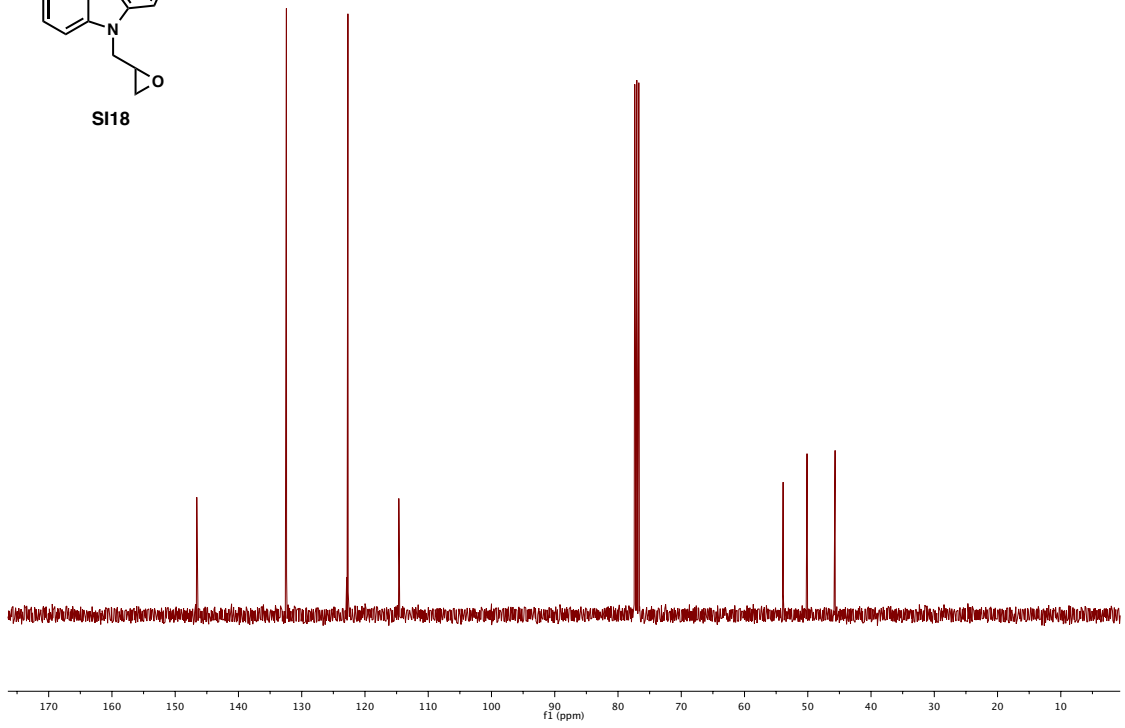


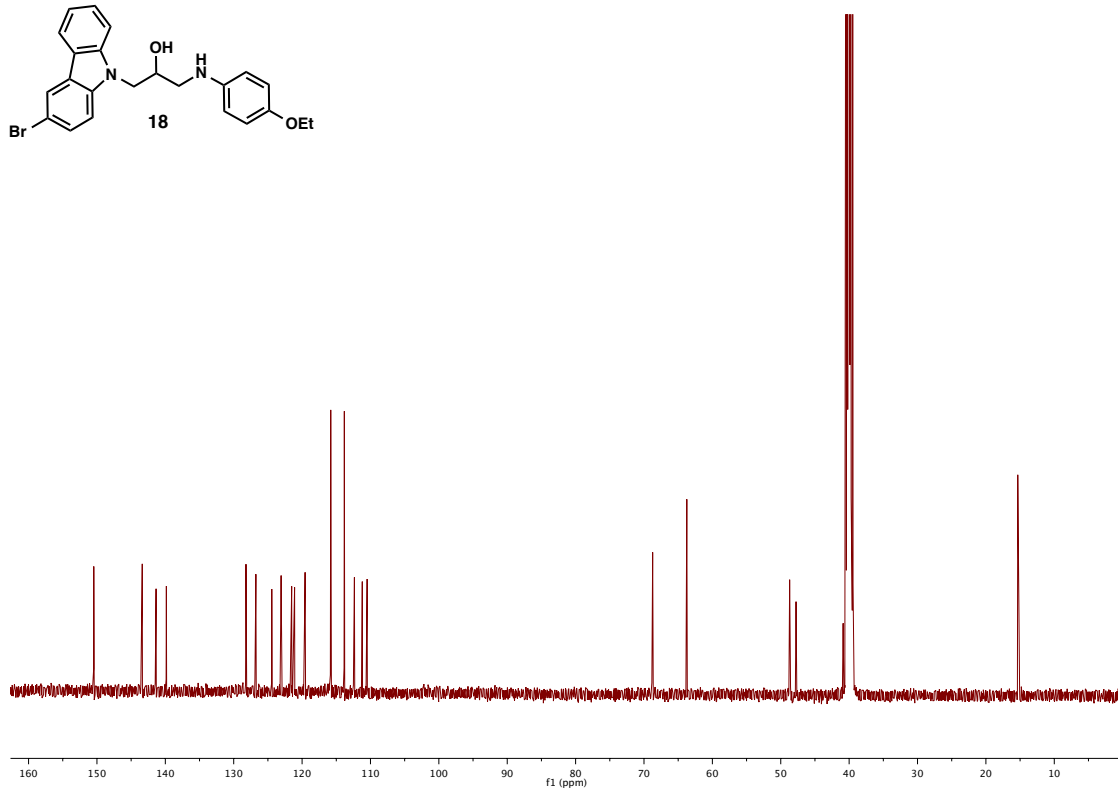
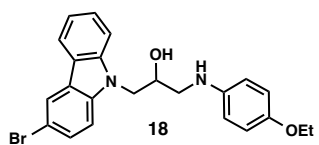
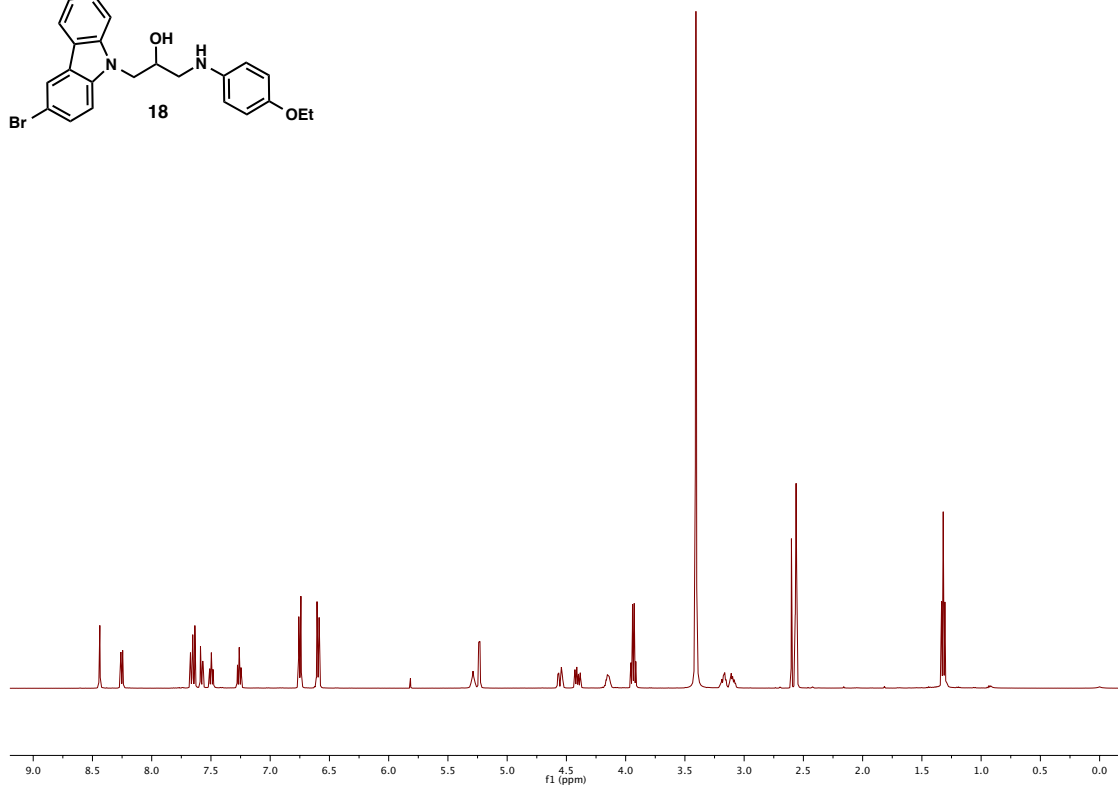
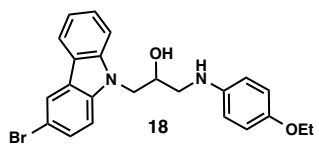


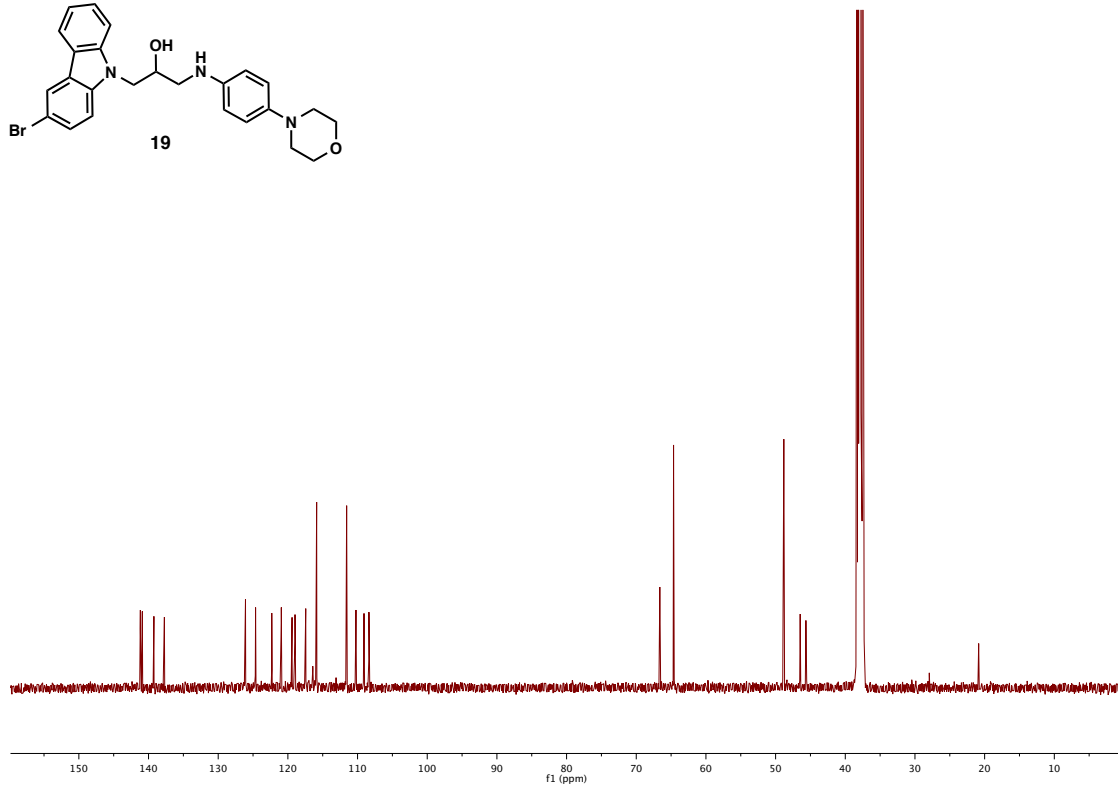
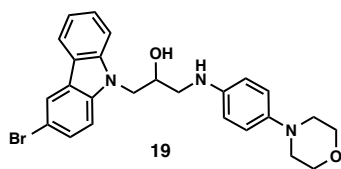
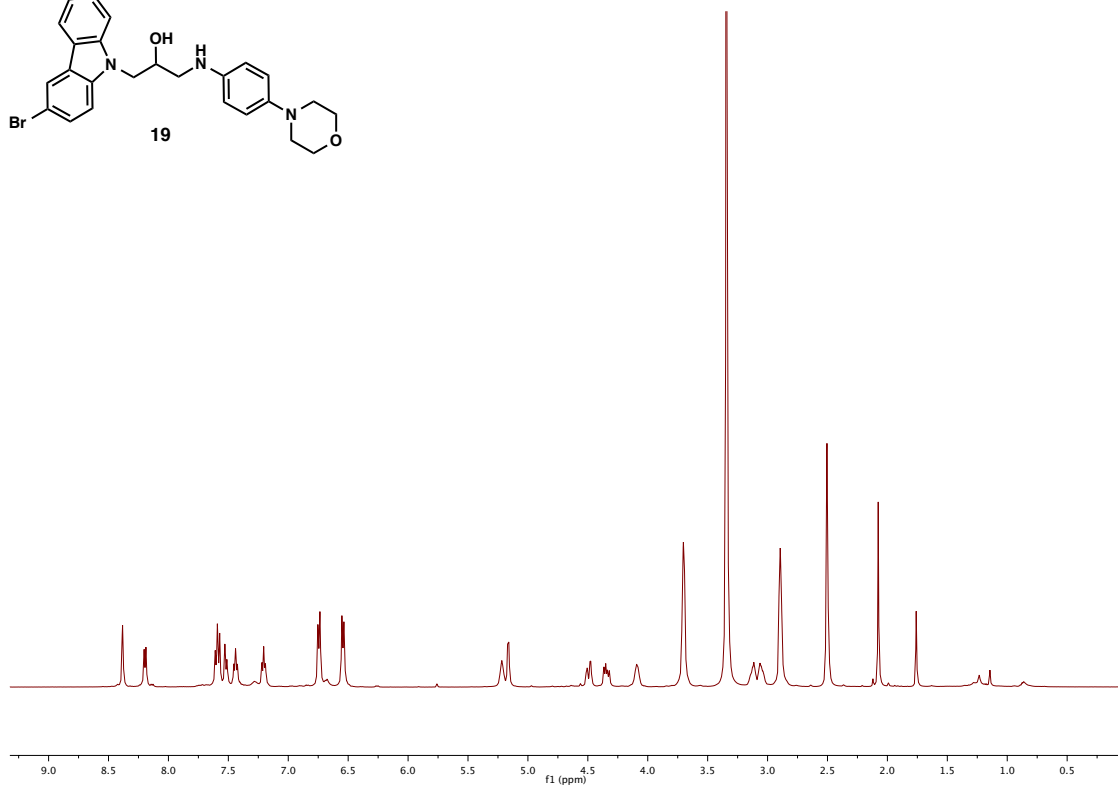
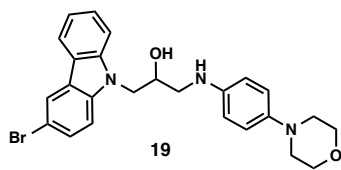
SI18

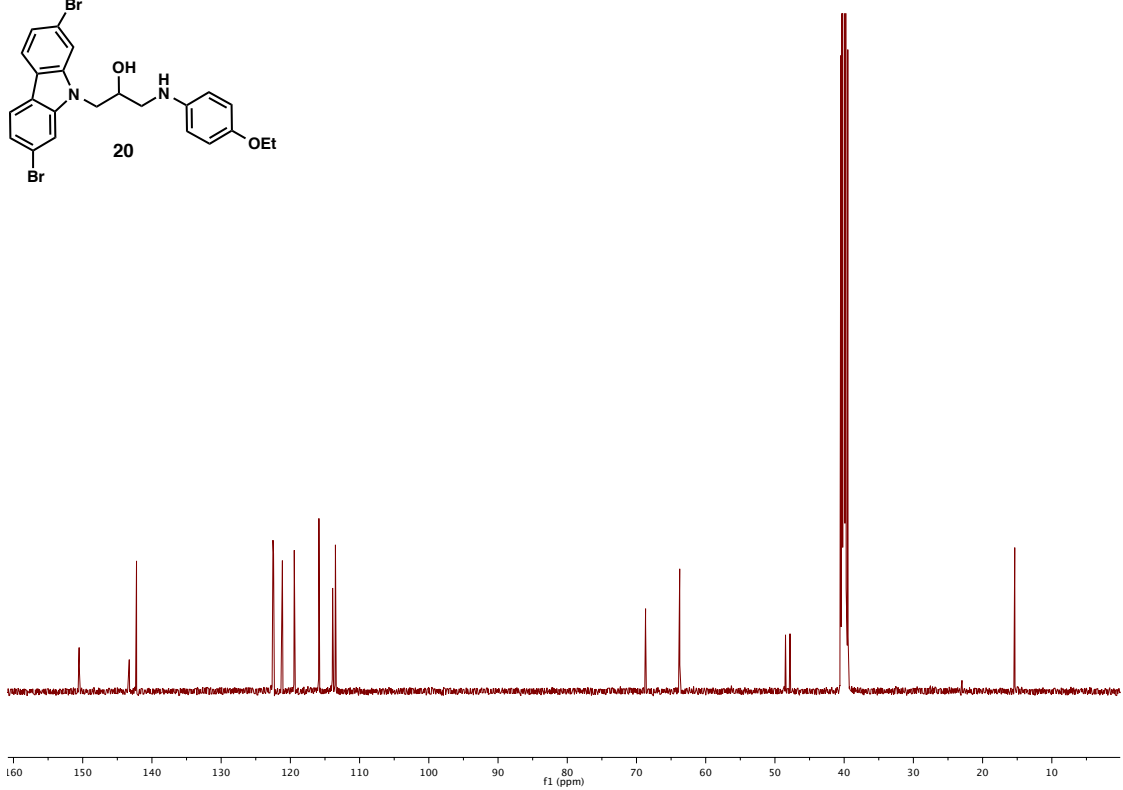
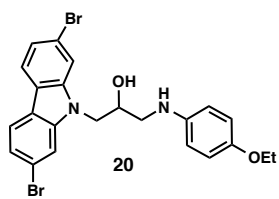
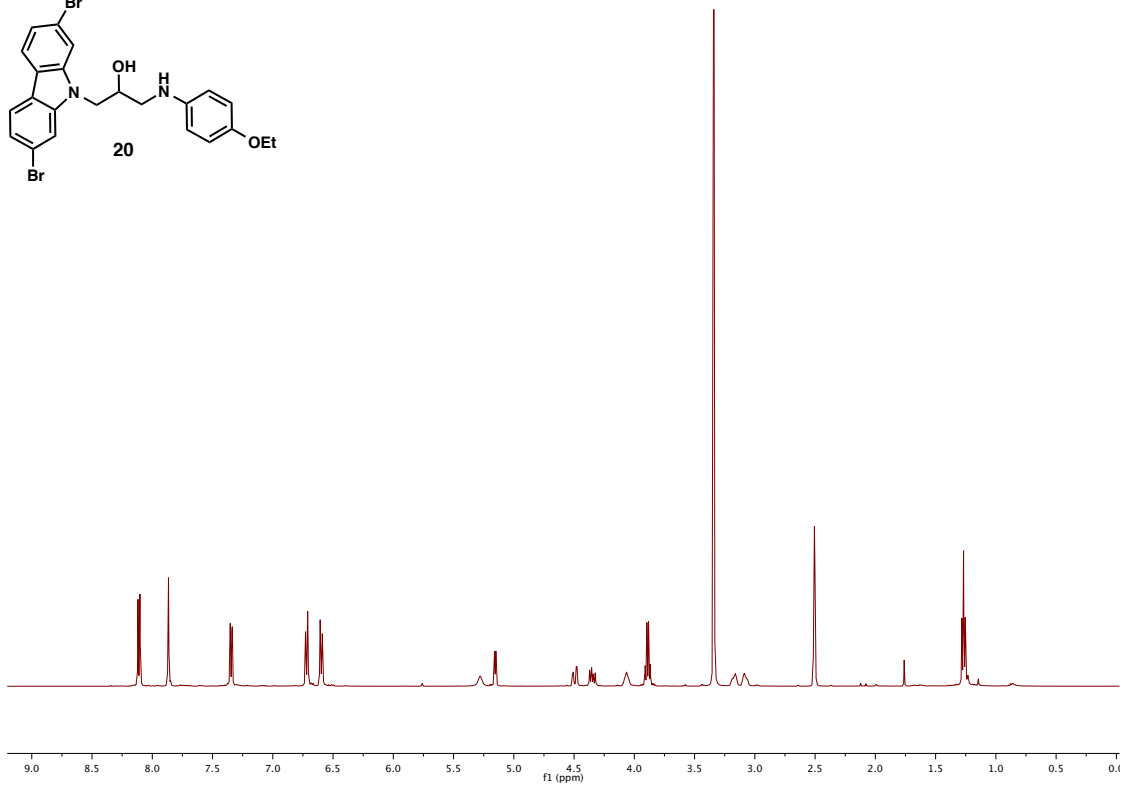
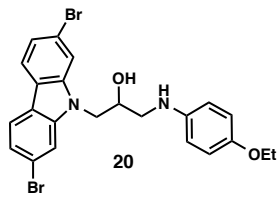


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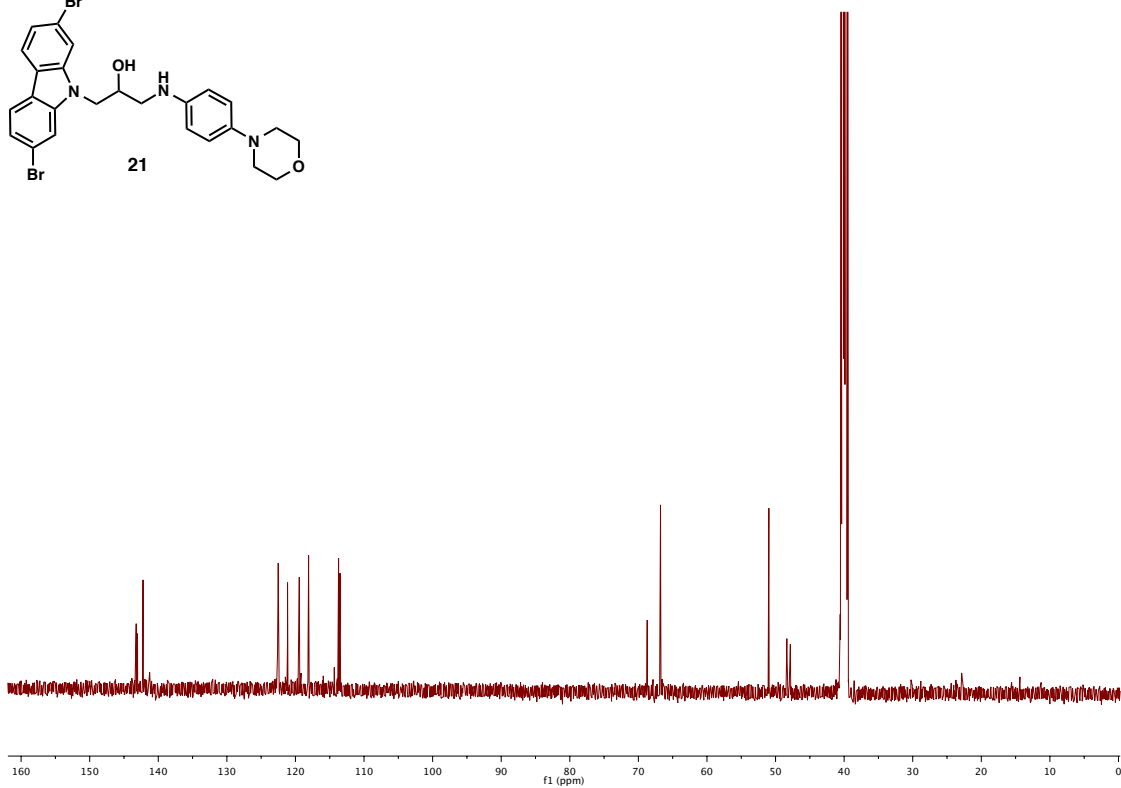
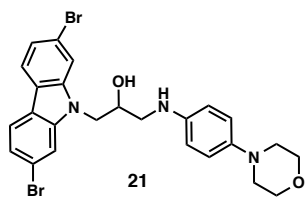
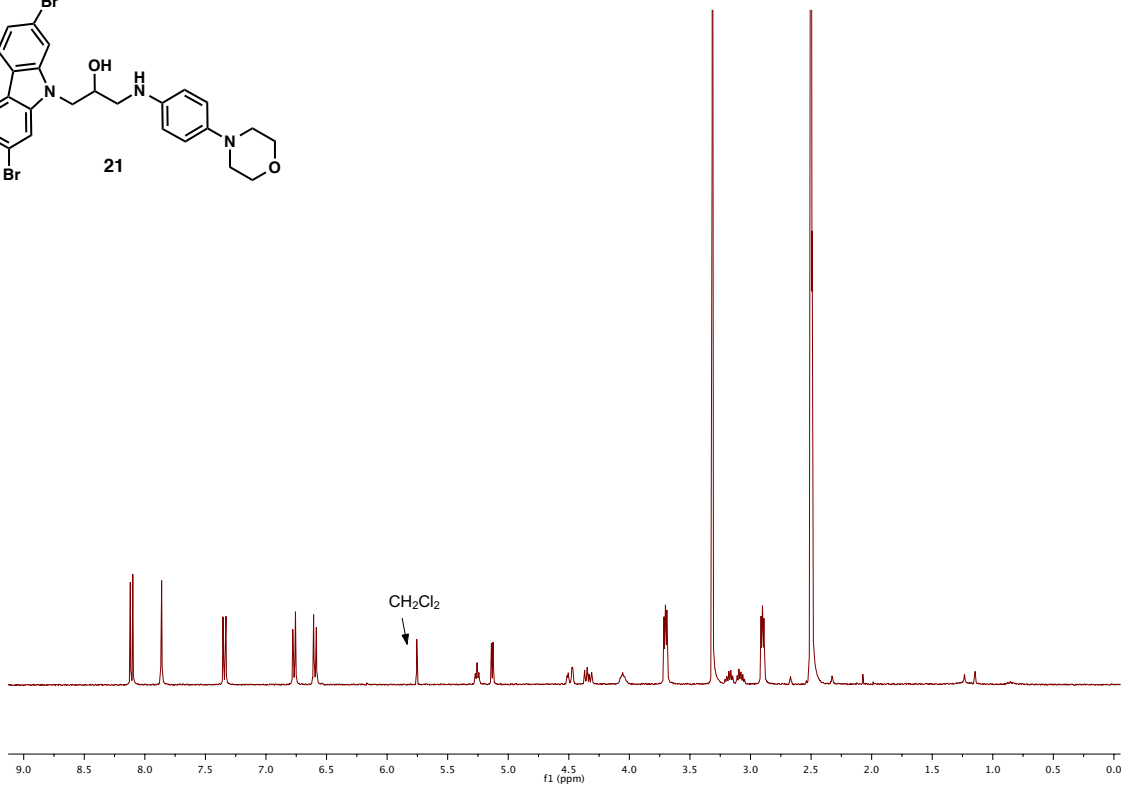
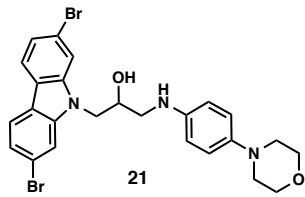


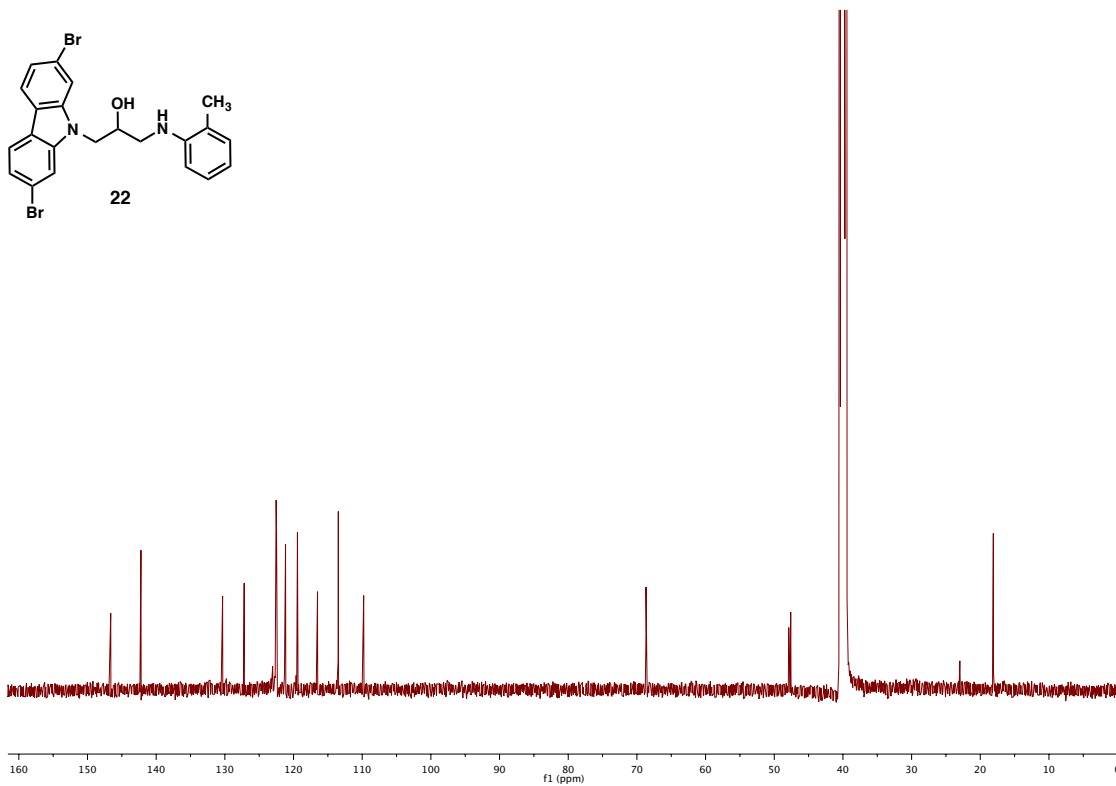
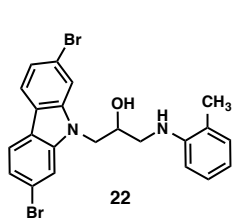
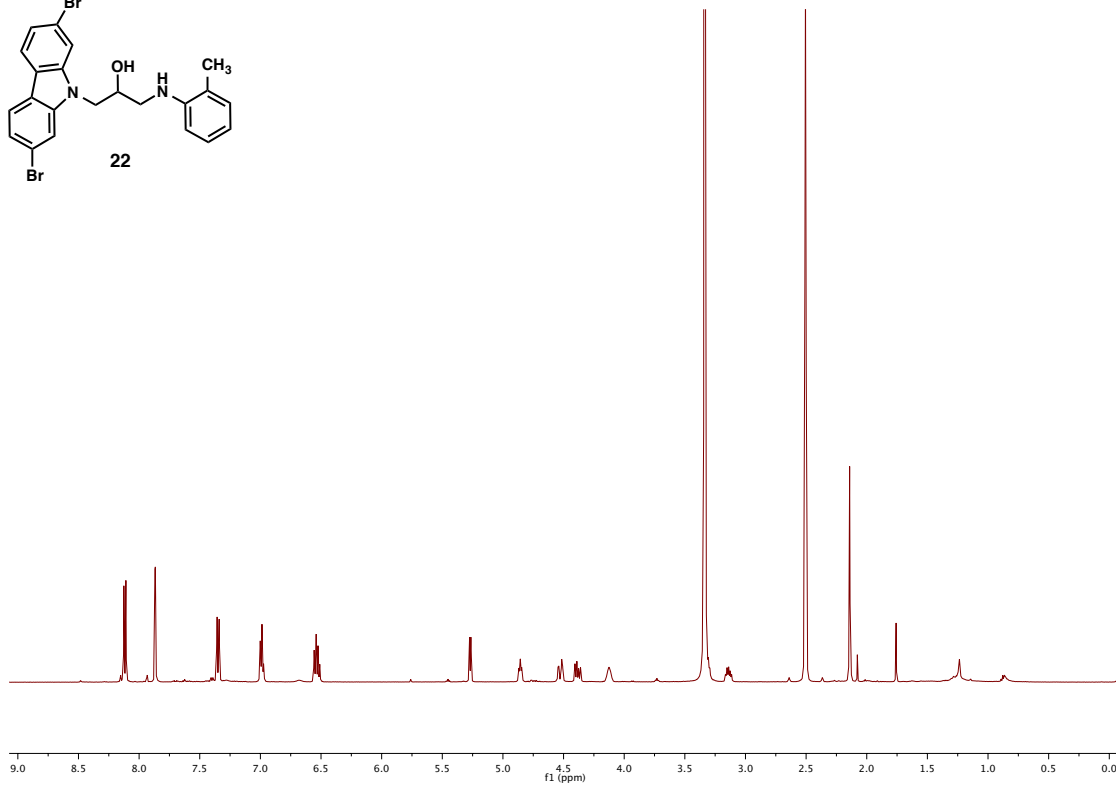
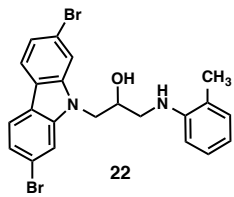


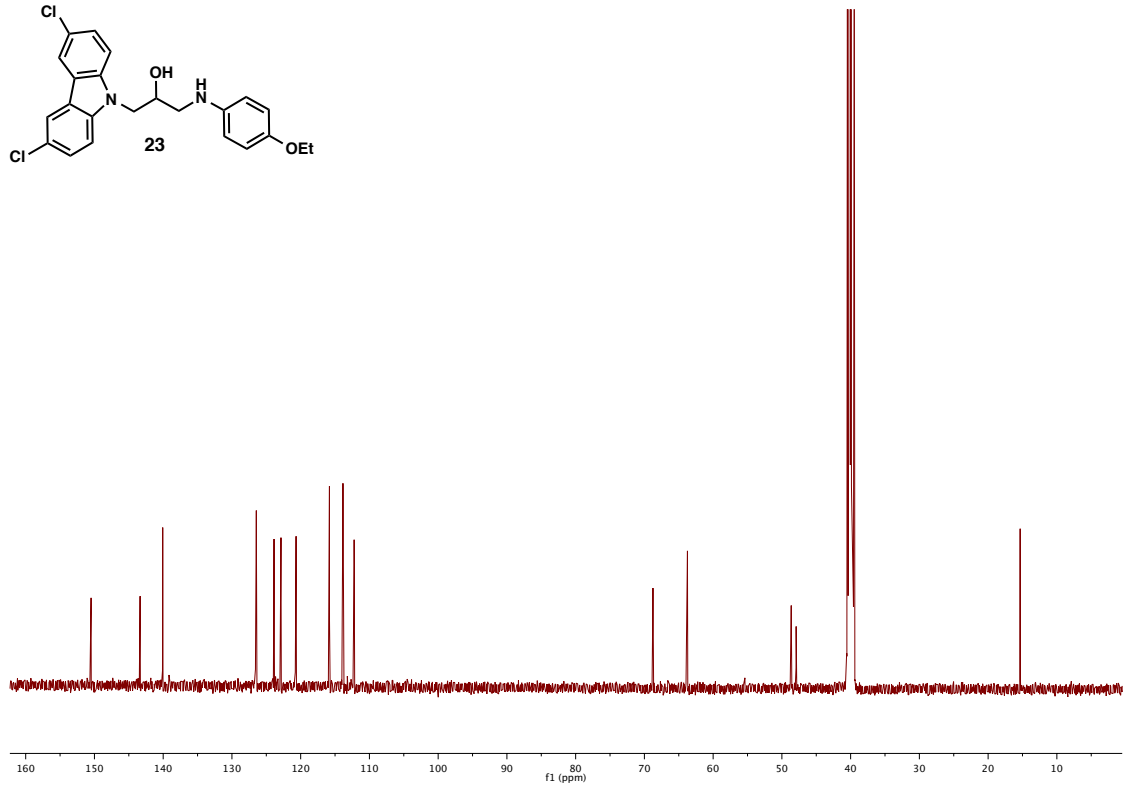
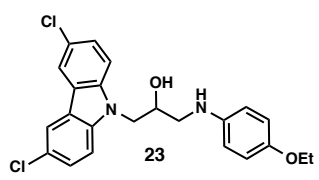
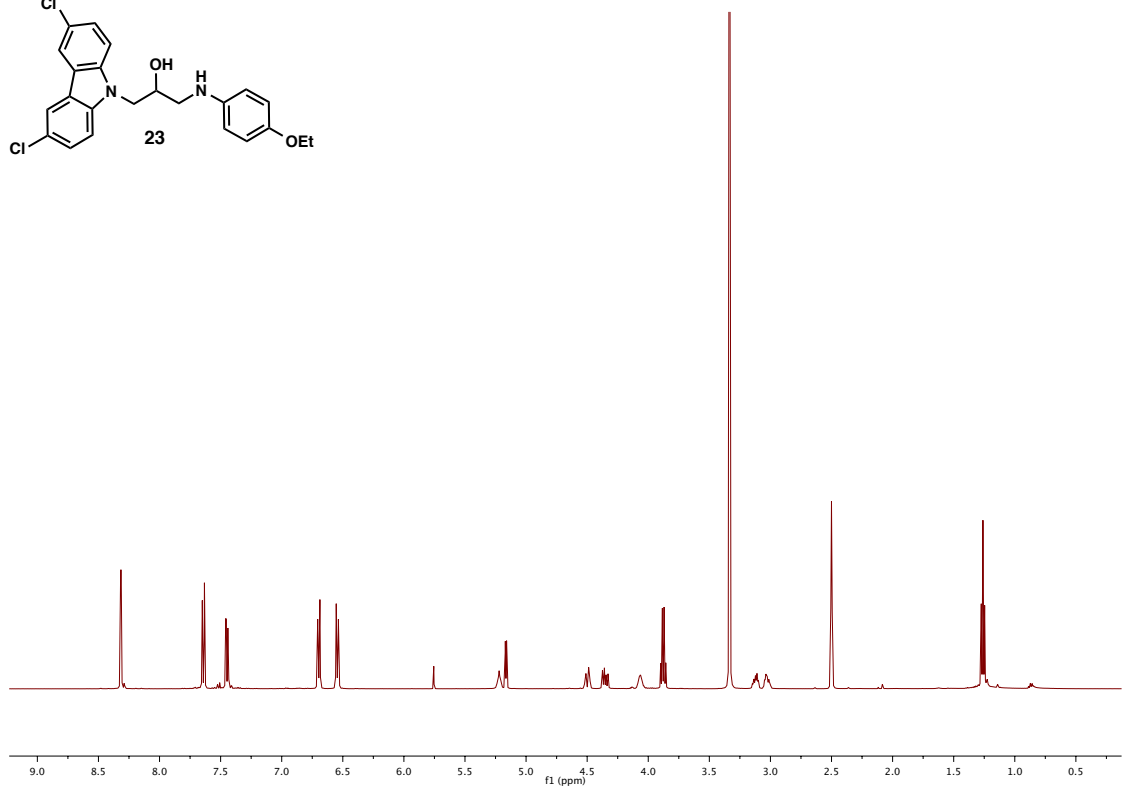
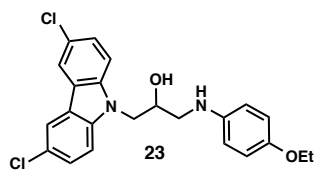


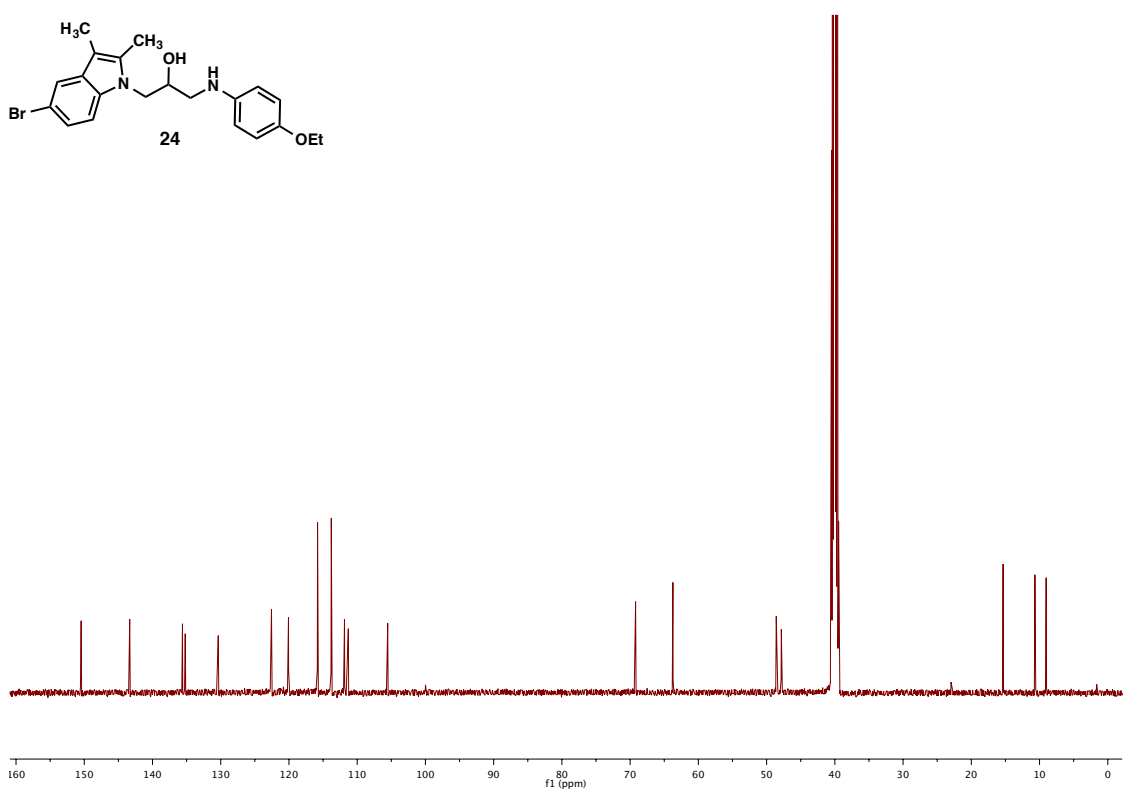
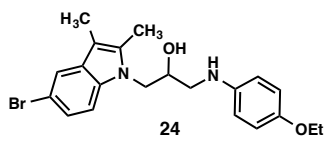
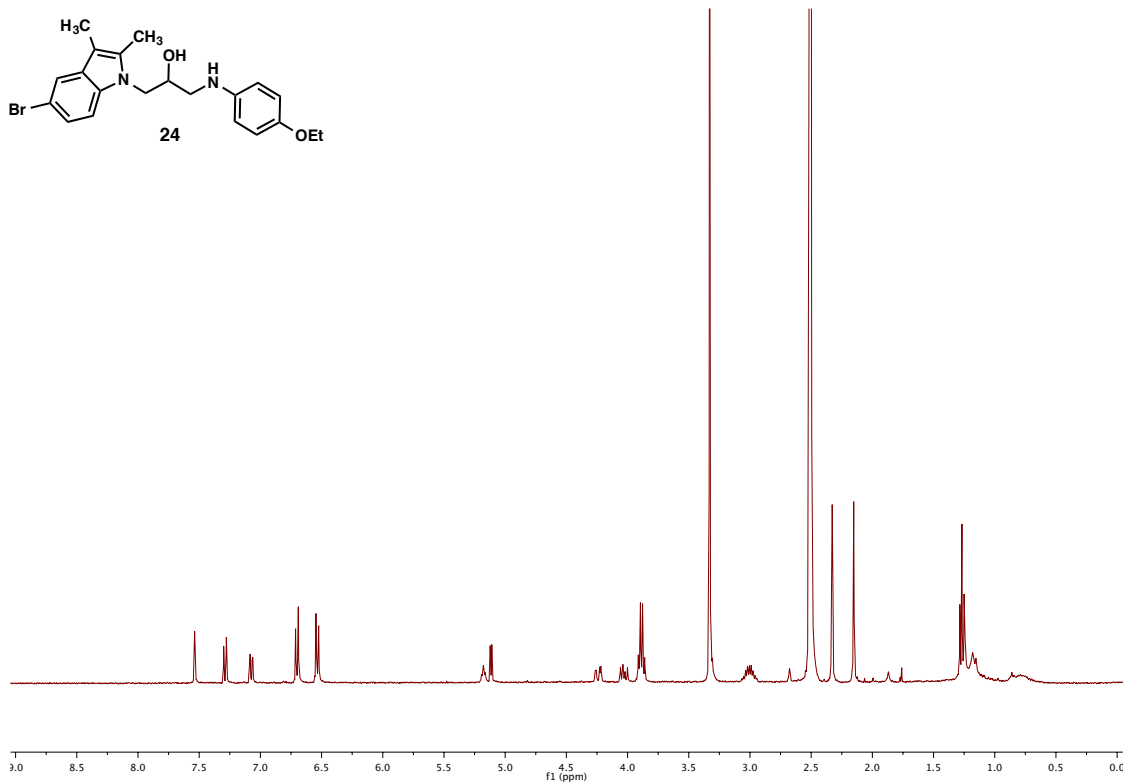
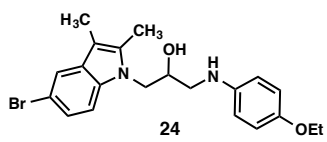


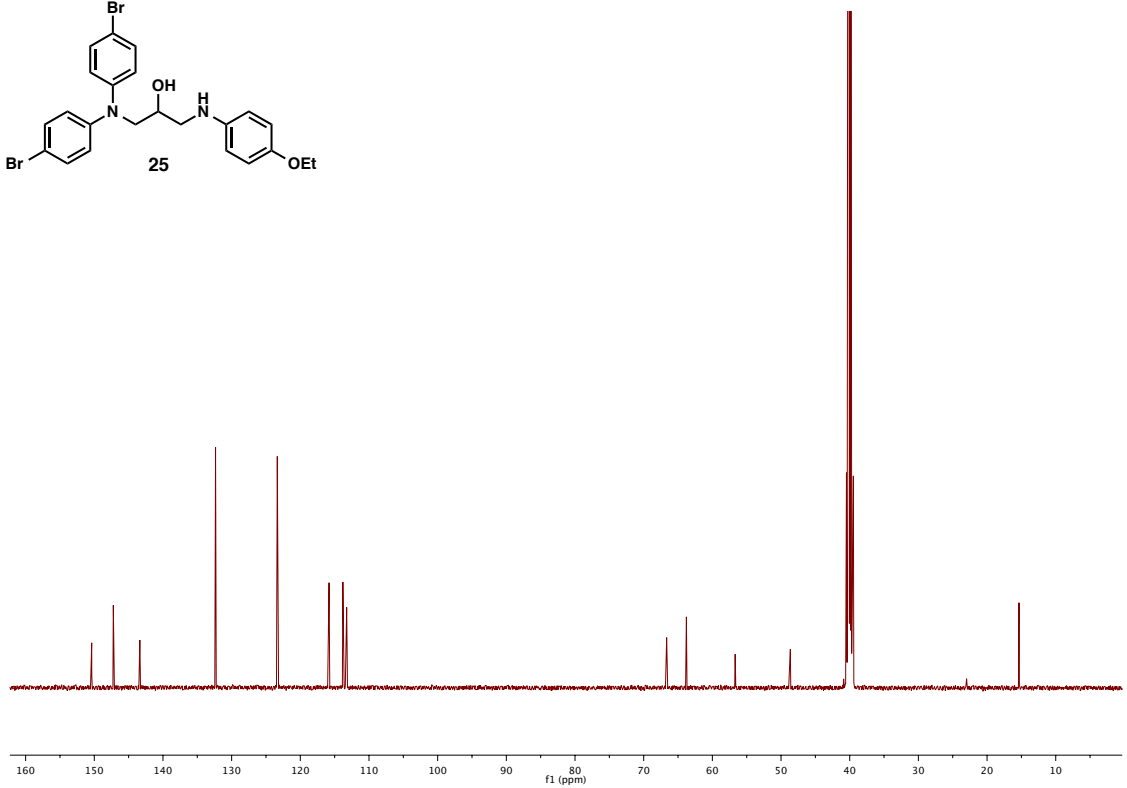
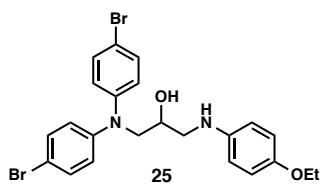
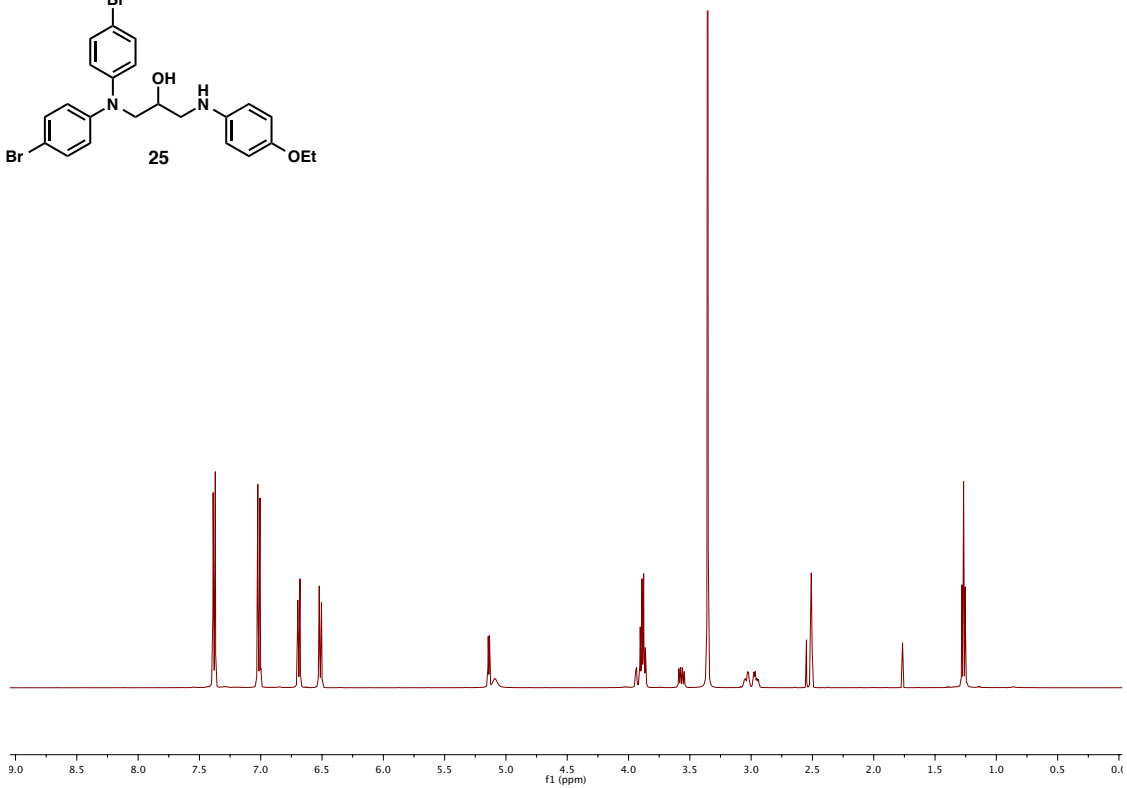
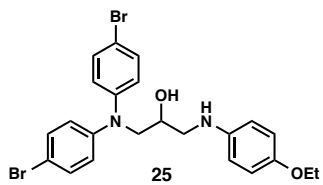


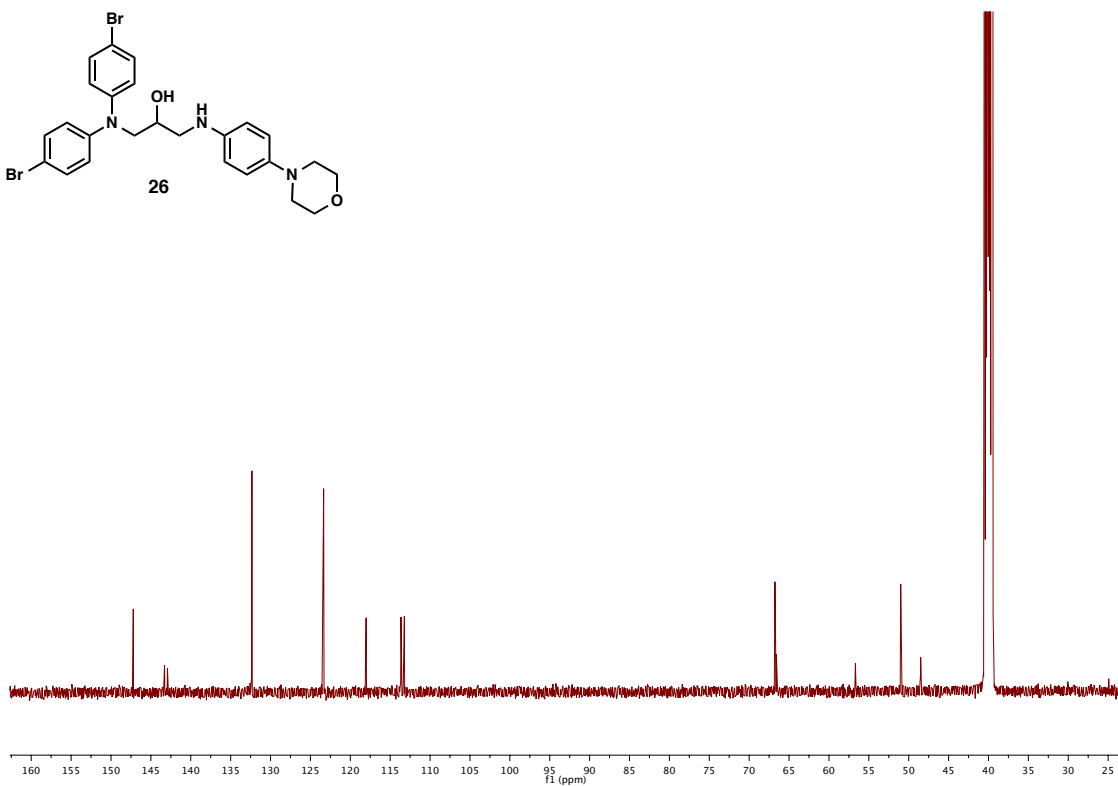
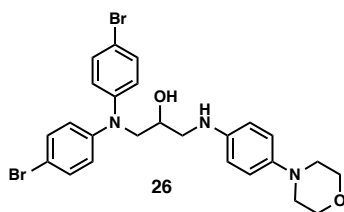
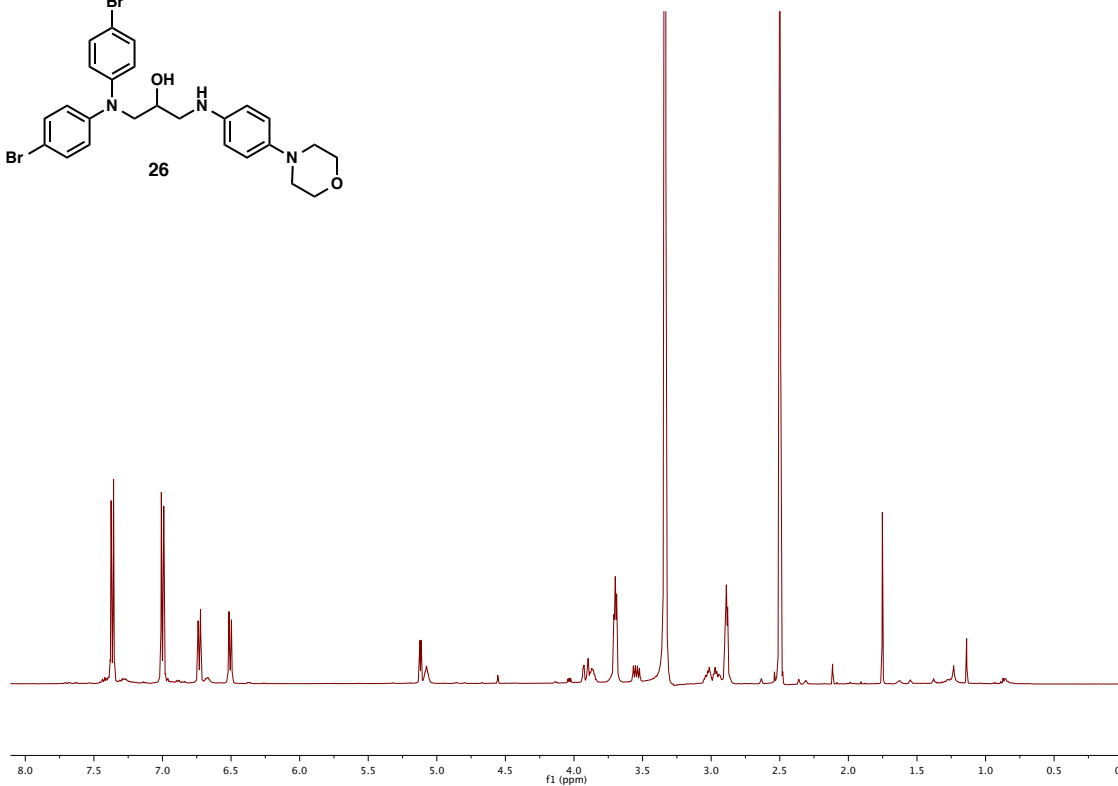
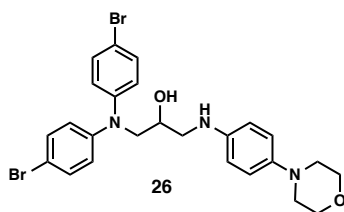


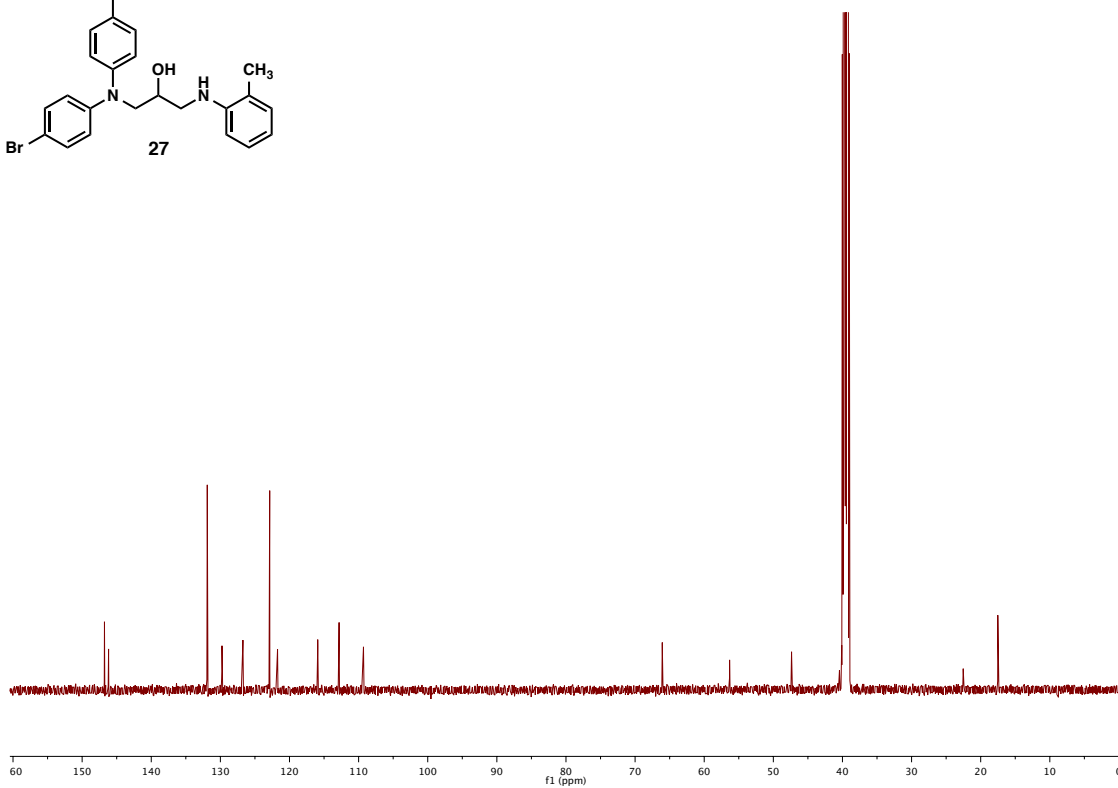
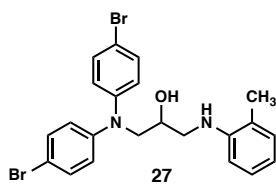
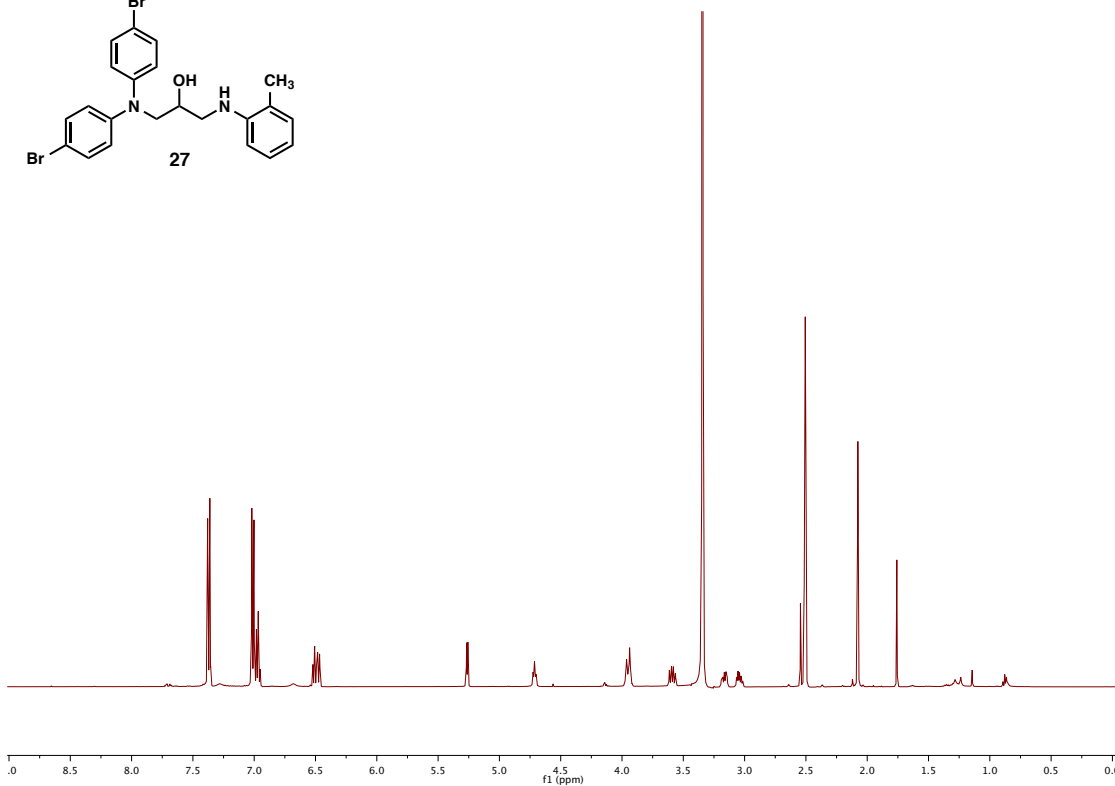
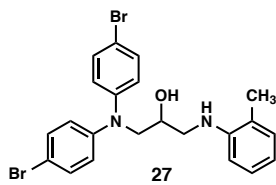


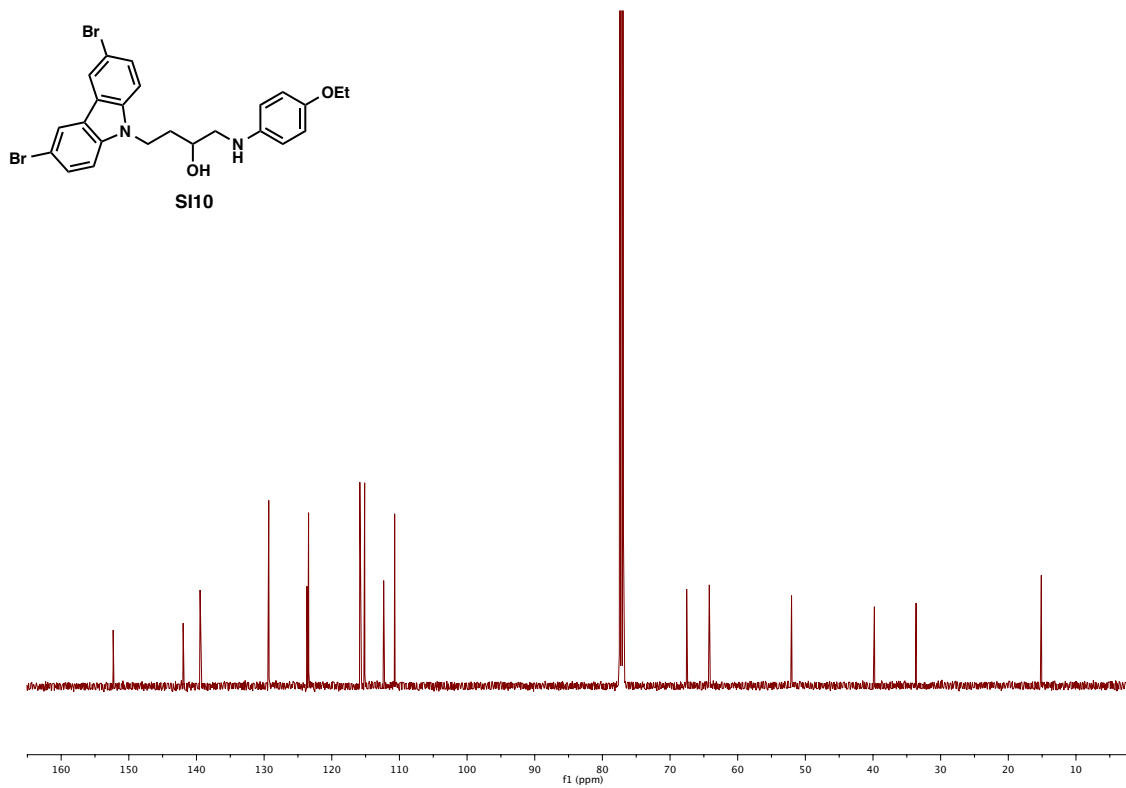
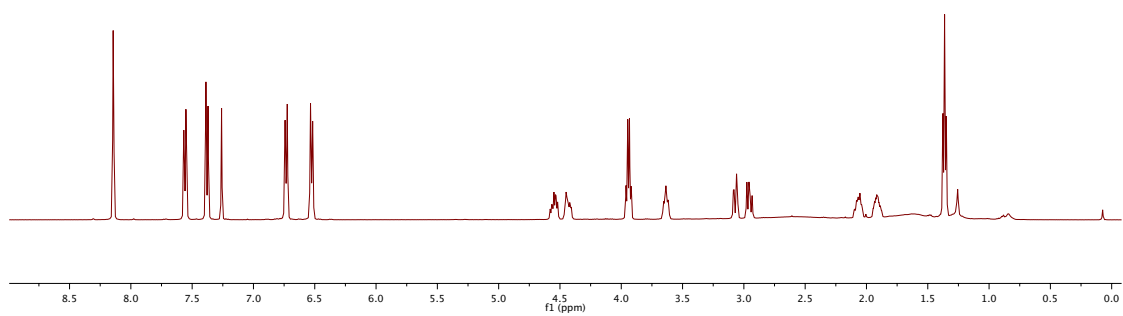
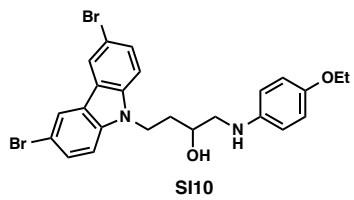




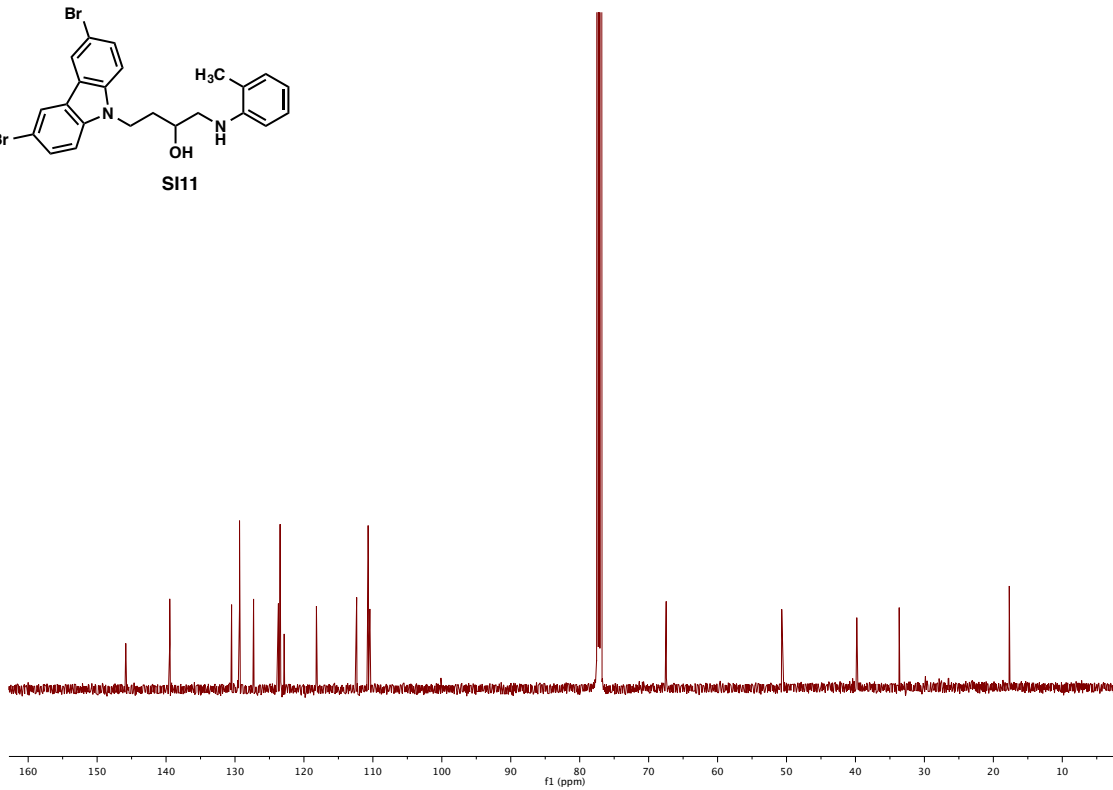
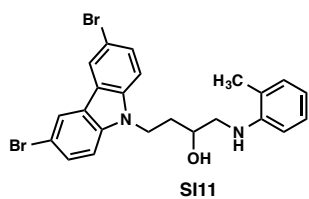
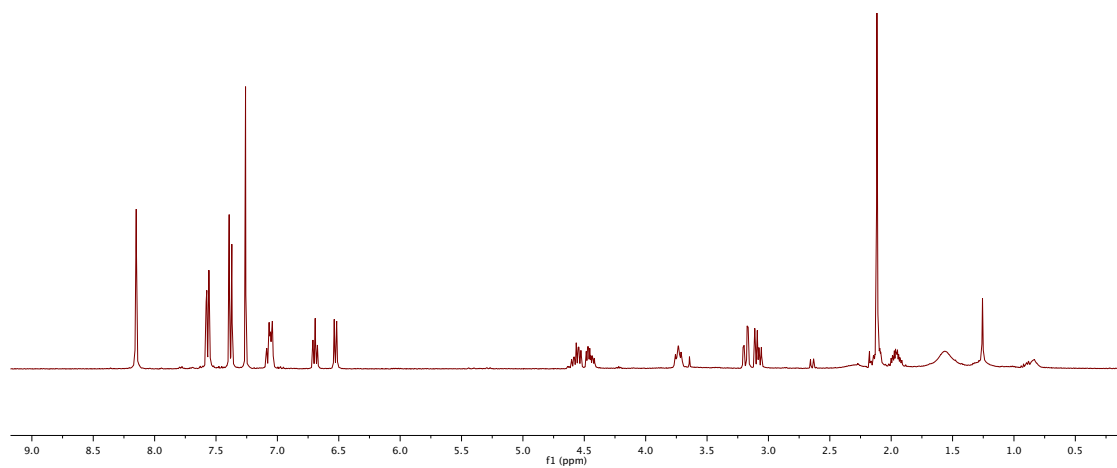
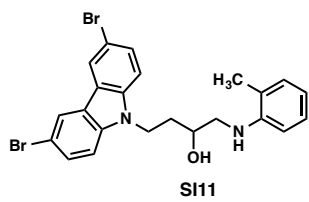












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2. MacMillan, K. S., Naidoo, J. et al. *J. Am. Chem. Soc.* **2011**, *133*, 1428.