Supporting Information for:

Library Synthesis, Screening and Discovery of Modified Zinc(II)-bis(dipicolylamine) Probe for Enhanced Molecular Imaging of Cell Death

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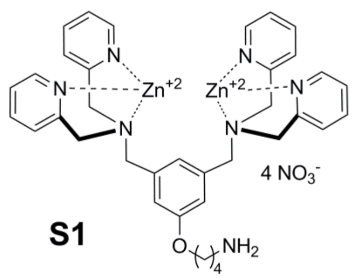
Contents

1. Experimental Details and Supplementary Figures.

Method of Measuring Zn^{2+} Concentration in RED Assay. The azo dye NO_2-PAPS is a colorimetric indicator for Zn^{2+}.[S1] Upon exposure to Zn-BDPA scaffolds the absorbance maxima of NO_2-PAPS shifts from 492 nm to 565 nm. Addition of Zn-BDPA **S1** (Chart S1) to a solution of NO_2-PAPS (75 μM) in TES buffer (10 mM TES, 145 mM NaCl, pH 7.4) produces a ratiometric change in the absorbance spectrum (Figure S1), and the linear response (Figure S1, B) demonstrates that NO_2-PAPS can be used to measure the concentration of a Zn-BDPA complex.

Chart S1. Chemical Structure of Zn-BDPA **S1**.



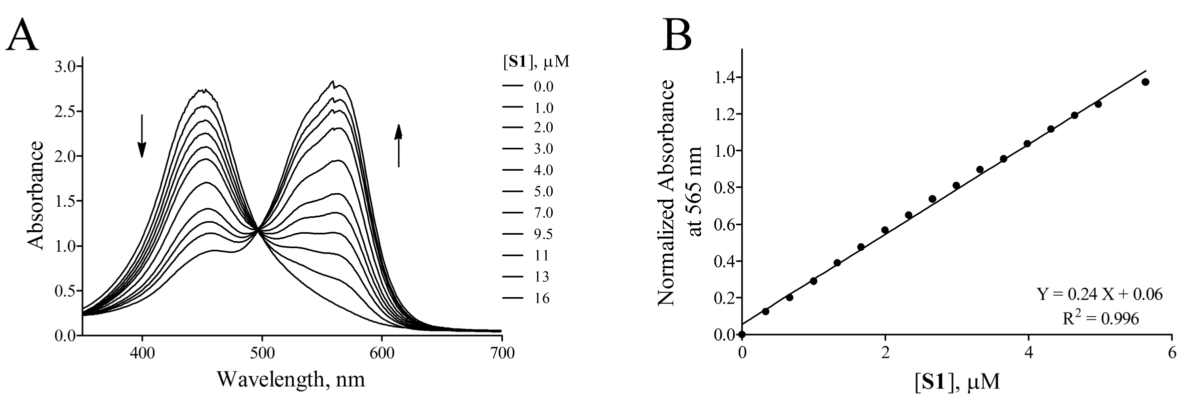


Figure S1. Absorbance profile for NO_2-PAPS (75 μM) in TES buffer (10 mM TES, 145 mM NaCl, pH 7.4) upon titration with compound **S1** (A). Beer's law plot for the addition of **S2** to NO_2-PAPS (B).

Proof that Ratio of Zn^{2+} Concentrations Reflects Zn-BDPA Binding in RED Assay. To validate the assumption that the concentration of Zn^{2+} is proportional to the concentration of Zn-DPA scaffold in the two compartments of the Rapid Equilibrium Dialysis (RED) apparatus, a series RED experiments were conducted using fluorescent probe **12** and PS-rich vesicles. Equilibrated samples were analyzed using the NO_2-PAPS assay to measure Zn^{2+} concentration, and the deep-red fluorescence of **12** was used to independently measure its concentration. Increasing the amount of PS-rich vesicles in the 'source' compartment of the RED apparatus shifts the dialysis equilibrium ratio for **12**, and the data in Figure S2 shows that the ratio of Zn^{2+} concentration in the two compartments reflects the ratio of Zn-BDPA concentration.

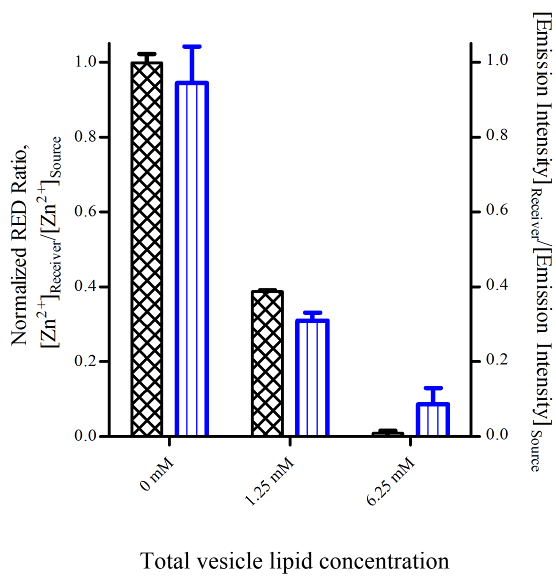


Figure S2. Validation that Zn^{2+} concentration reflects Zn-BDPA scaffold concentration in a RED assay. PS-rich vesicles (10:65:25 POPS:POPC:Cholesterol) and fluorescent probe **12** were added to the 'source' compartment, and the RED apparatus was allowed to shake for 16 hours at 180 rpm and 37 °C. The colorimetric NO_2-PAPS method was used to measure Zn^{2+} concentration (black criss-cross pattern) and the deep-red fluorescence of **12** (blue vertical lines, λ_{excite} = 600 nm, λ_{emit} = 658 nm) was used to measure its concentration in each compartment.

Measuring Membrane Affinity Using the RED Assay. Quantitative RED assays were conducted as proof-of-principle studies for measuring dissociation constants of Zn-BDPA compounds with PS-rich (POPC:Cholesterol:POPS, 65:25:10) or PC (POPC:Cholesterol, 75:25) vesicles. If the concentration of Zn-BDPA scaffold is held constant between RED compartments while the concentration of vesicles in the 'source' compartment is varied, a saturated binding isotherm can be obtained (Figure S3). Control experiments were conducted to ensure that unbiased RED equilibration occurred in the absence of vesicles.

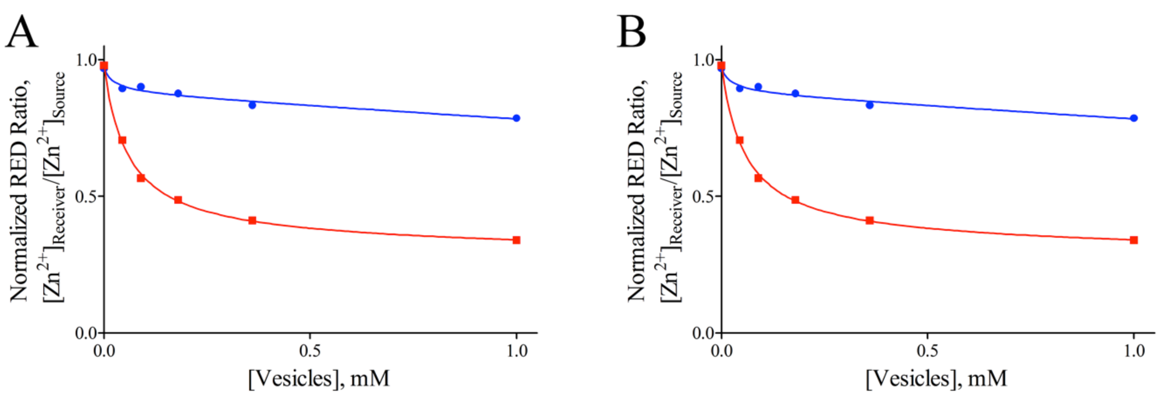


Figure S3. Binding isotherms (generated using the RED:NO_2-PAPS dialysis assay) for association of compound **11c** (A) and compound **11k** (B) to PS-rich vesicles (POPS:Cholesterol:POPC, 10:25:65, red squares) or PC vesicles (Cholesterol:POPC, 25:75, blue circles). The RED apparatus was allowed to shake for 16 hours at 180 rpm and 37 °C.

FRET Displacement Assay and Association Constant Determination. A FRET ensemble of energy acceptor **12** bound to the surface of PS-rich vesicles containing energy donor **14** was prepared by mixing **12** (10 μM) and PS-rich vesicles (20 μM total lipid; POPS:POPC:**14**, 50:49:1) in 2.0 mL of HEPES buffer (5 mM HEPES, 137 mM NaCl, 3.2 mM KCl, 1.0 mM $Zn(NO_3)_2{\bullet}6H_2O$, pH 7.4) at 25 °C. Aliquots of lead candidates **11k** or **11o** were titrated into separate samples of the FRET ensemble while stirring. After a waiting period of approximately 60 seconds for equilibration to occur, the fluorescence emission spectrum was acquired (λ_{ex} = 480 nm, λ_{em} = 500-750 nm). Plots of fluorescence intensity (I_{567nm}/I_{663nm}) as a function of lead candidate concentration were generated and fit using a computer to a competitive binding model that determined 1:1 association constants.[S2] Specifically a plot of S_t/P versus Q (equations **1-3**) produces a linear graph that has a slope equal to K_1/K_2. For equations **1-3** the parameters were defined as follows: Substrate = Zn-BDPA library candidate **11k** or **11o**, Indicator = probe **12** (10 μM), Ligand concentration was 10 μM, the total lipid concentration in the cuvette. Parameter K_1 is the association constant between PS-rich vesicles and fluorescent probe **12** while parameter K_2 is the association constant between library candidates and PS-rich vesicles. Since K_1 is easily measured using a standard FRET titration assay K_2 can be readily obtained. FRET displacement experiments were conducted using library candidates **11k** (Figure S4) and **11o** (Figure S5).

$$\frac{S_t}{P} = \frac{K_1}{K_2} Q + 1$$ **Equation 1**

Where S_t = Total substrate concentration

K_I = Ligand – Indicator binding constant

$$P = L_t - \frac{1}{Q * K_1} - \frac{I_t}{Q + 1}$$ **Equation 2**

Where L_t = Total ligand concentration

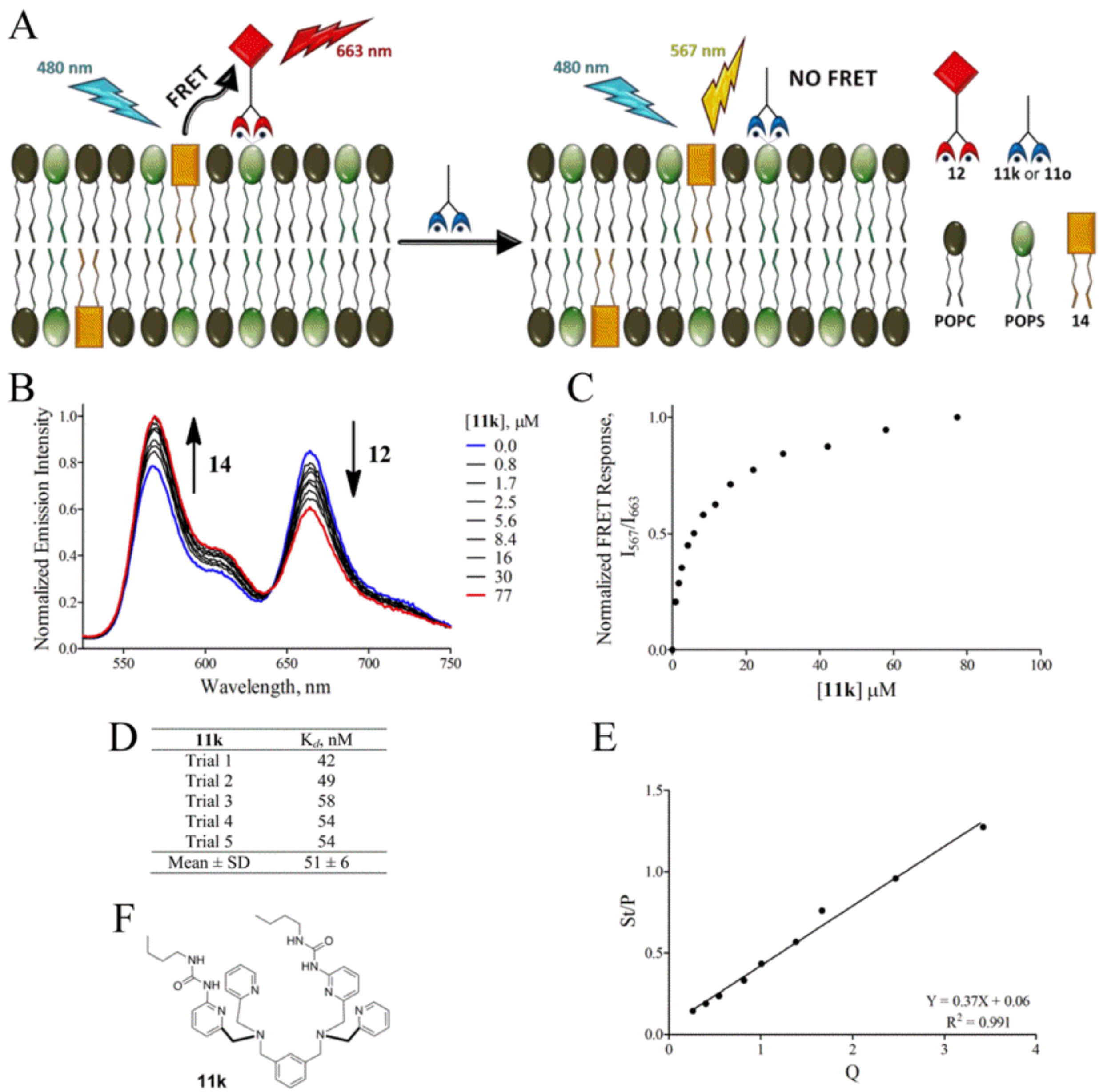
I_t = Total indicator concentration

$$Q = \frac{F_n - F_I}{F_{lim} - F_n}$$ **Equation 3**

Where F_I = Initial fluorescence intensity

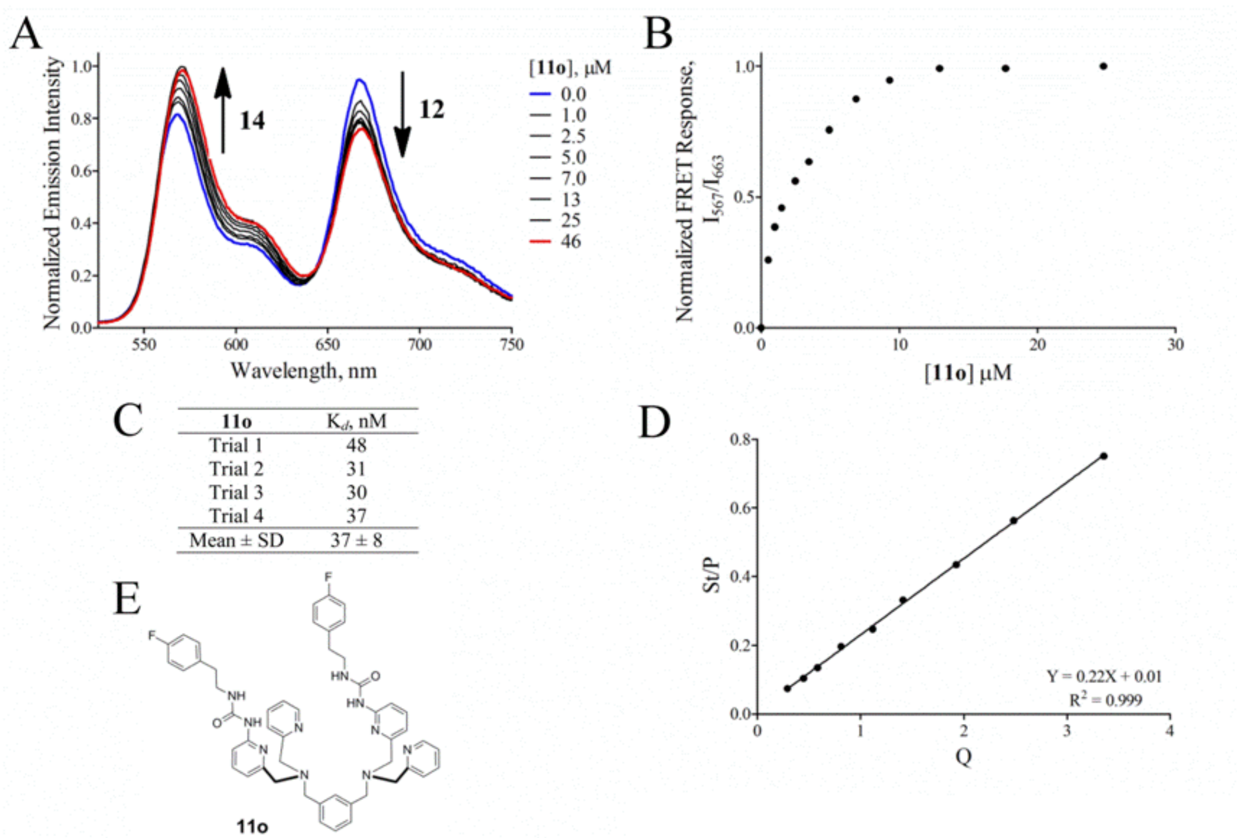
F_{lim} = Quenched fluorescence intensity

F_n = Fluorescence intensity at titration point n



11k	K_d, nM
Trial 1	42
Trial 2	49
Trial 3	58
Trial 4	54
Trial 5	54
Mean ± SD	51 ± 6

Figure S4. FRET displacement data for Zn-BDPA library candidate **11k** and PS-rich vesicles (POPS:POPC:**14**, 50:49:1). Cartoon depiction of the FRET displacement titration experiment showing that energy transfer between the FRET donor **14** and the FRET acceptor **12** is lost when a Zn-DPA library candidate (either **11k** or **11o**) has displaced **12** from the vesicle surface (A) (copy of Figure 2A). Emission profile (B), normalized FRET response (C), table of FRET displacement results (D), and fitted data (E) for the FRET displacement induced by **11k** binding to PS-rich vesicles. Chemical structure of Zn-BDPA library candidate **11k** (F).



11o	K_d, nM
Trial 1	48
Trial 2	31
Trial 3	30
Trial 4	37
Mean ± SD	37 ± 8

Figure S5. FRET displacement data for Zn-BDPA library candidate **11o** and PS-rich vesicles (POPS:POPC:**14**, 50:49:1). Emission profile (A) (copy of Figure 2C), normalized FRET response (B), table of FRET displacement results (C), and fitted data (D) for the FRET displacement induced by **11o** binding to PS-rich vesicles. Chemical structure of Zn-BDPA library candidate **11o** (E).

FRET Titration Assay and Association Constant Determination. Fluorescent probes **12** and **13** were titrated separately into aqueous samples containing either PS-rich (10 μM total lipid; POPS:POPC:**14**, 50:49:1) or PC (10 μM total lipid; POPC:**14**, 99:1) vesicles in 3.0 mL HEPES buffer (5 mM HEPES, 137 mM NaCl, 3.2 mM KCl, 1.0 mM $Zn(NO_3)_2$•$6H_2O$, pH 7.4) at 25 °C while stirring. After a waiting period of approximately 60 seconds for equilibration to occur, the fluorescence emission spectrum was acquired (λ_{ex} = 480 nm, λ_{em} = 500-750 nm). Plots of fluorescence intensity (λ_{em} = 567 nm) as a function of Zn-BDPA concentration were generated and the association constants were determined using a nonlinear least-squares fitting procedure adapted for fluorescence spectroscopy (Equation 4).[S3] The parameters for Equation 4 were defined as follows: The Host concentration was 10 μM, the total lipid concentration in the cuvette, Guest = Zn-BDPA probe **12** or **13**. FRET titration experiments were conducted using fluorescent probes **12** and **13** (Figure S6).

$$F = F_O + \frac{F_{lim} - F_O}{2C_O}\left\{C_O + C_G + \frac{1}{K} - \left[\left(C_O + C_G + \frac{1}{K}\right)^2 - 4C_OC_G\right]^{\frac{1}{2}}\right\}$$ **Equation 4**

Where F = Fluorescence intensity

F_o = Initial Fluorescence intensity

F_{lim} = Quenched fluorescence intensity (end of titration experiment)

C_O = Total host concentration

C_G = Total guest concentration

K = Association Constant

The association constants reported in this study formally correspond to 1:1 association of a Zn-BDPA compound and a phospholipid within a vesicle membrane. In the case of PS-rich vesicles the reported value is an apparent association constant since it is the weighted average of 1:1 association with either a PS or PC phospholipid in the membrane.

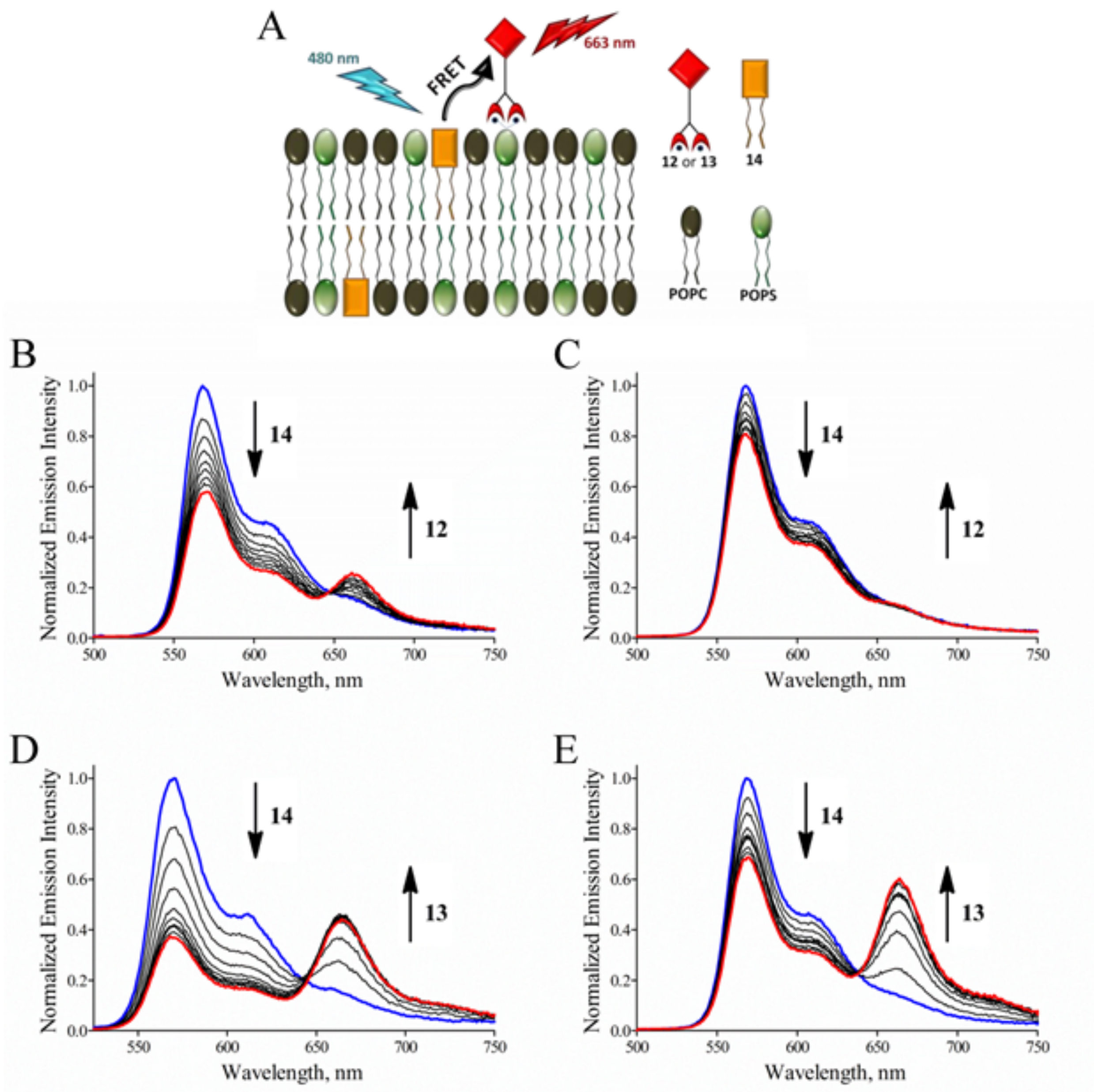


Figure S6. Schematic cartoon of FRET titration assay (A) (copy of Figure 3A). FRET profiles for fluorescent probes **12** and **13** binding to PS-rich (10 µM total lipid; POPS:POPC:**14**, 50:49:1) and PC (10 µM total lipid; POPC:**14**, 99:1) vesicles; **12** and PS-rich vesicles (B), **12** and PC vesicles (C), **13** and PS-rich vesicles (D) (copy of Figure 2D), **13** and PC vesicles (E).

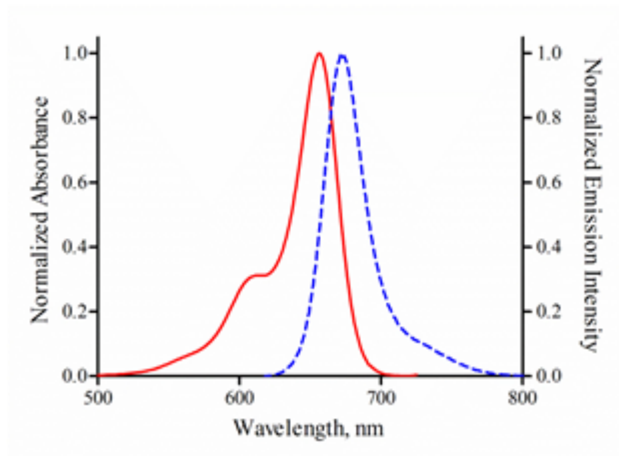


Figure S7. Absorbance (red solid line) and emission (blue dashed line) profile of **13** (2 μM) in 100 % DMSO (λ_{ex} = 610 nm).

Table S1. Photophysical Properties of **12** and **13**.[a]

Compound	Molar Absorptivity, $M^{-1}cm^{-1}$	Quantum Yield[b]	Absorbance λ_{max}, nm	Emission λ_{max}, nm
12	1.1 x 10^5	0.13	656	673
13	1.1 x 10^5	0.13	656	672

[a] 2 μM samples in DSMO at 25 °C. [b] Quantum yield measured relative to commercially purchased **12** (PSVue®643, Molecular Targeting Technologies Inc., West Chester, PA).

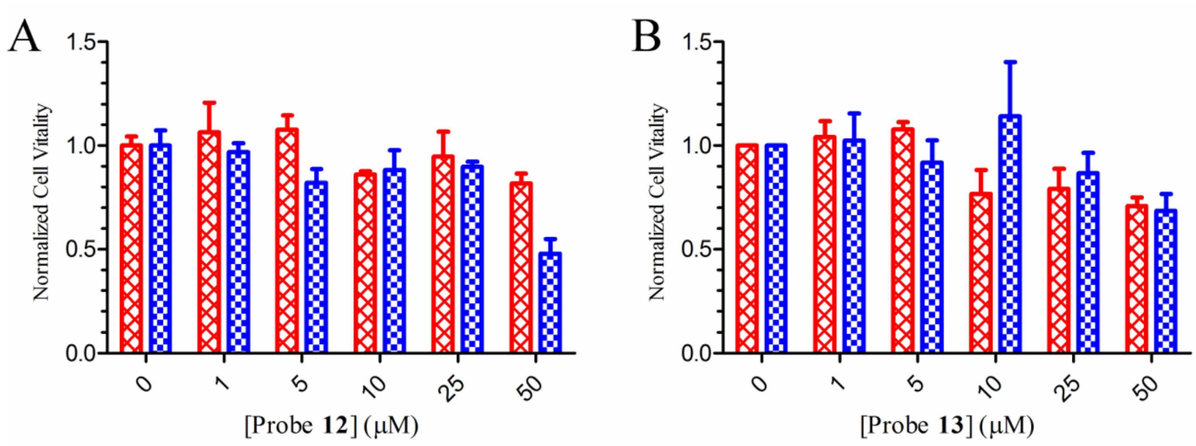


Figure S8. Cell viability of CHO-K1 cells (Blue crisscross) and MDA-MB-231 cells (red checkered) treated with either **12** (A) or **13** (B) for 18 hours at 37 °C.

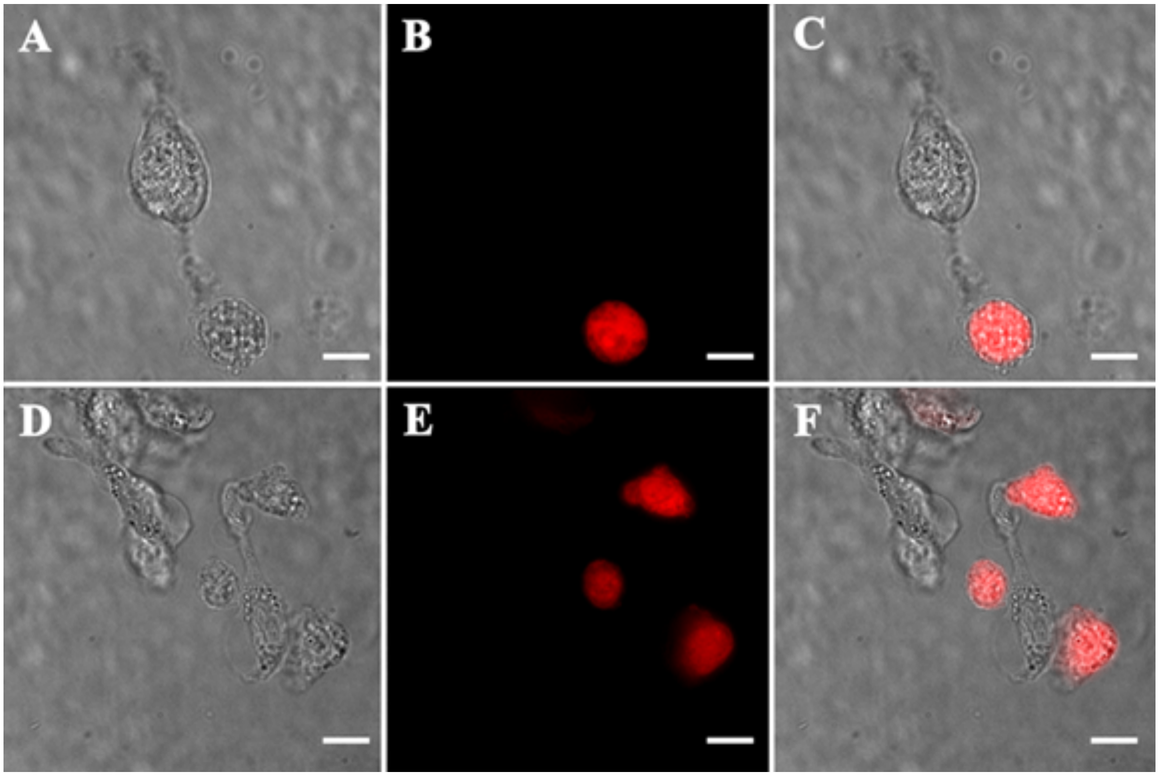


Figure S9. Fluorescence micrographs showing selective targeting of probe **13** for rounded dead/dying cells over elongated healthy cells. MDA-MB-231 cells (top, A-C) and CHO-K1 cells (bottom D-F) stained with **13**. Cells were treated with etoposide (15 μM) for 12 hours, incubated with probe **13** (10 μM) for 30 minutes at 37 °C, then washed with HEPES buffer. Micrographs C and F are merges of the brightfield images (A, D) and the deep-red Cy-5 fluorescence images (B, E). Scale bar = 25 μm.

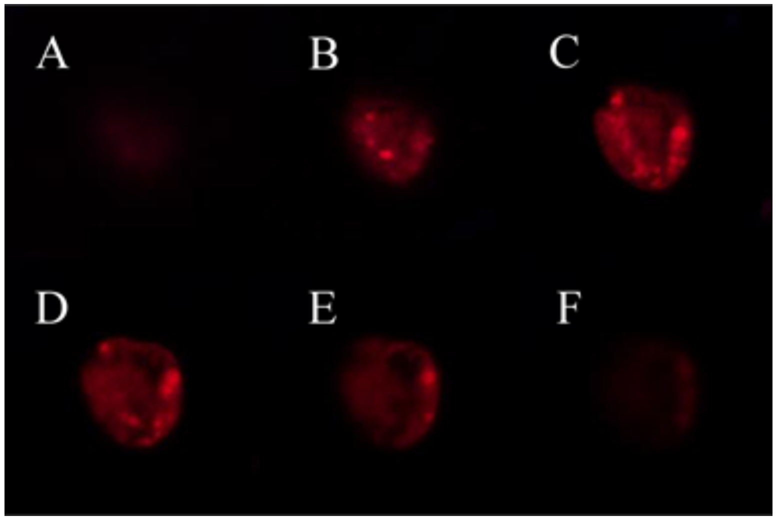


Figure S10. Confocal micrographs of MDA-MB-231 cell treated with etoposide (15 µM) for 12 hours to induce cell death, then stained with probe **13** (10 µM) for 30 minutes prior to deep-red fluorescence imaging. Images are 4 µm slices (60 x magnification) taken through the cell separated by 20 µm (A – F). The micrographs indicate probe distribution throughout the cytosol.

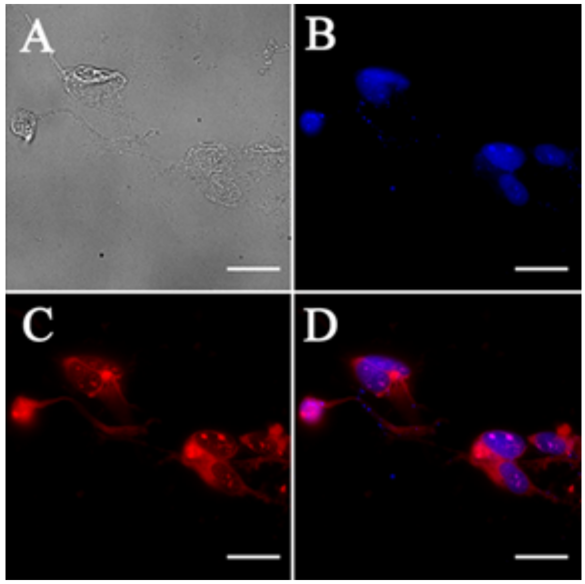


Figure S11. Fluorescence microscopy of dead MDA-MB-231 cells stained with blue-emitting nucleic acid stain SYTOX® Blue (B) and red-emitting **13** (C). A bright field (A) and merge (D) are also shown. The images show that probe **13** stains the cytosol of dead/dying cells but not the cell nucleus. Scale bar = 25 μm.

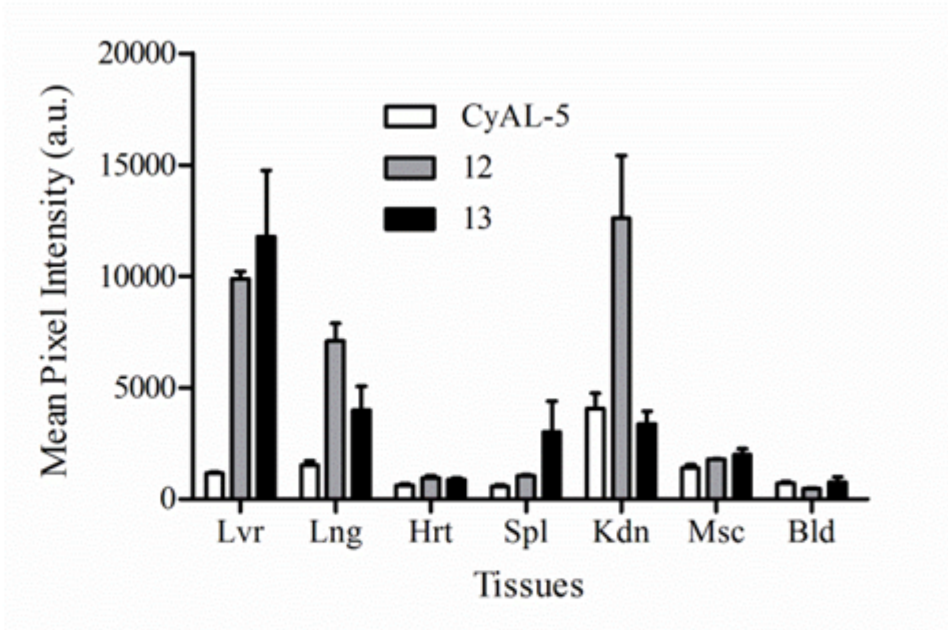


Figure S12. Biodistribution of deep-red CyAL-5 dye, probe **12**, or probe **13** in organs taken from healthy rats sacrificed at 24 hours after intravenous injection of the probe (3.0 mg/kg). Error bars are standard error of the mean (N = 4).

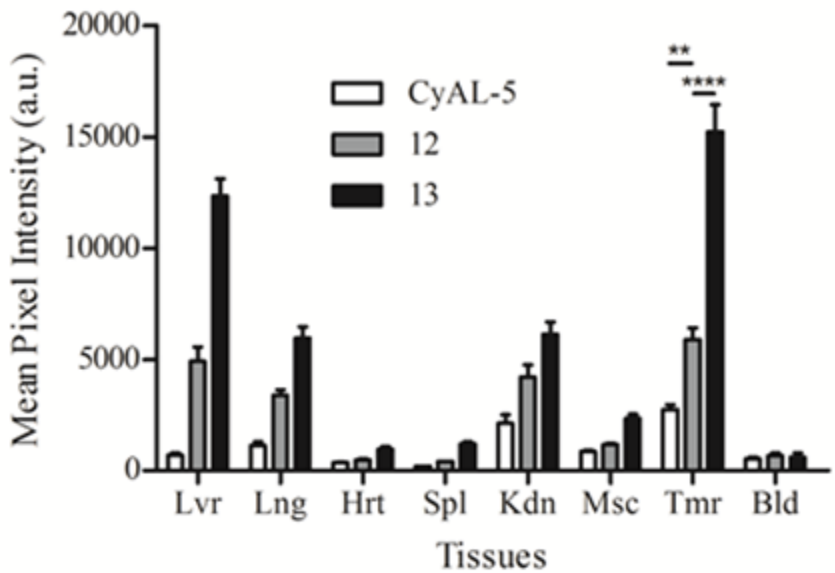
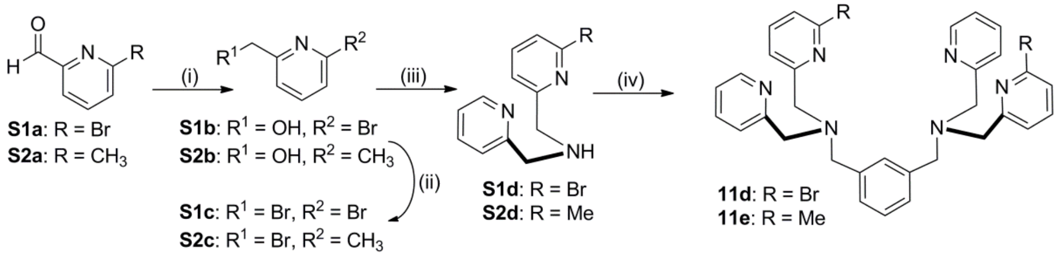


Figure S13. Biodistribution of deep-red CyAL-5 dye, probe **12**, or probe **13** in organs taken from rats bearing a subcutaneous prostate tumor at 24 hours after intravenous injection of the probe (3.0 mg/kg). Error bars are standard error of the mean (N = 4). $^{**}P = 0.004$; $^{****}P < 0.0001$.

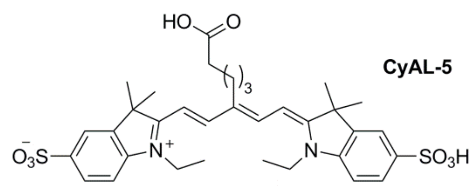
Synthesis – Reaction conditions. The synthesis of library candidates **11d** and **11e** are shown below in Scheme S1. The synthetic route for both compounds follow the same chemical transformations; a benzylic aldehyde is reduced to a secondary alcohol which is then brominated via an Appel reaction. Treatment of alkyl bromides **S1c** and **S2c** with 2-picolylamine leads to the desired modified DPA building blocks **S1d** and **S2d** which are appended to the desired *m*-xylene scaffold.

Scheme S1. Synthesis of compounds **11d-e** (Chart 2, Entries 13 & 14)[a]



[a]Reagents and conditions: (i) $NaBH_4$, $CaCl_2$, EtOH; (ii) PPh_3, CBr_4, K_2CO_3, DCM, 0 °C → rt; (iii) 2-Picolylamine, Na_2CO_3, MeCN; (iv) *m*-Xylene dibromide, $CHCl_3$, DIPEA.

Chart S2. Chemical structure of CyAL-5



2. COMPOUND CHARACTERIZATION.

6c. To a solution of **6b** (20.0 mg, 62.0 μmol) in $CHCl_3$ (250 μL) was added butylisocyanate (70.5 μL, 630 μmol). The reaction was allowed to stir under argon for 12 hours. Solvent was removed and the crude material was purified using silica gel column chromatography with 0-2 % MeOH:$CHCl_3$ as the eluent to yield the desired product as a white powder (26.3 mg, 81 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.94 (t, J = 7 Hz, 6H), 1.42 (sextet, J = 7 Hz, 4H), 1.58 (p, J = 7 Hz, 4H), 3.39 (q, J = 7 Hz, 4H), 3.67 (s, 4H), 3.68 (s, 2H), 6.71 (d, J = 8 Hz, 2H), 7.08 (d, J = 7 Hz, 2H), 7.25 (t, J = 8 Hz, 1H), 7.32 (t, J = 7 Hz, 2H), 7.38 (d, J = 8 Hz, 2H), 7.54 (t, J = 8 Hz, 2H), 9.03 (br s, NH, 2H), 9.62 (br s, NH, 2H) ppm; ^{13}C NMR (600 MHz, $CDCl_3$) δ 13.8, 20.3, 32.1, 39.5, 58.6, 59.8, 110.3, 115.2, 127.2, 1284, 128.6, 138.5, 138.6, 153.1, 156.0, 156.4 ppm; HRMS (ESI, MeCN): m/z = 518.3232 ($[M+H]^+$).

6d. To a solution of **6b** (7.0 mg, 21.9 μmol) in $CHCl_3$ (2 mL) was added DIPEA (20 μL, 120 μmol) and butyric anhydride (11 μL, 67 μmol). The reaction was allowed to stir under argon for 12 hours. The reaction was quenched upon addition of H_2O (1 mL) and the crude product was extracted using chloroform. The organic layer was dried over $MgSO_4$ and solvent was removed. The crude material was purified using silica gel column chromatography with 0-2 % MeOH:CHCl3 as the eluent to yield the desired product as a clear oil (9.3 mg, 92 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.00 (t, J = 7 Hz, 6H), 1.76 (sextet, J = 7 Hz, 4H), 2.36 (t, J = 7 Hz, 4H), 3.68 (s, 6H), 7.22-7.28 (m, 3H), 7.32 (t, J = 7 Hz, 2H), 7.38 (d, J = 7 Hz, 2H), 7.67 (t, J = 7 Hz, 2H), 7.91 (br s, NH, 2H), 8.07 (d, J = 7 Hz, 2H) ppm; ^{13}C NMR (600 MHz, $CDCl_3$) δ 13.7, 18.8, 39.7, 58.4, 59.3, 112.0, 118.5, 127.2, 128.4, 128.9, 138.9, 150.6, 171.6 ppm; HRMS (ESI, MeCN): m/z = 460.2716 ($[M+H]^+$).

10c. To a solution of **10b** (15.0 mg, 49.3 μmol) in $CHCl_3$ (100 μL) was added butylisocyanate (50 μL, 440 μmol). The reaction was allowed to stir at room temperature for 12 hours. The crude material was purified using silica gel column chromatography with 0-3 % MeOH:$CHCl_3$ as the eluent to yield the desired product as a clear oil (12.0 mg, 60 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.94 (t, J = 7 Hz, 3H), 1.42 (s, J = 7 Hz, 2H), 1.58 (p, J = 7 Hz, 2H), 3.39 (q, J = 7 Hz, 2H), 3.68 (s, 4H), 3.80 (s, 2H), 6.62 (d, J = 8 Hz, 1H), 7.10 (d, J = 8 Hz, 1H), 7.14 (d of d of d, J = 1, 6, 8 Hz, 1H), 7.24 (t, J = 7 Hz, 1H), 7.32 (t, J = 7 Hz, 2H), 7.40 (d, J = 7 Hz, 2H), 7.54 (t, J = 7 Hz, 1H), 7.5 (d, J = 8 Hz, 1H), 7.65 (d of t, J = 2, 8 Hz, 1H), 8.30 (s, 1H), 8.50-8.51 (m, 1H), 9.59 (s, 1H) ppm; ^{13}C NMR (600 MHz, $CDCl_3$) δ 13.8, 20.3, 32.1, 39.5, 58.6, 59.7, 60.1, 110.0, 115.4, 122.0, 122.7, 127.1, 128.3, 128.7, 136.4, 138.6, 138.7, 148.9, 152.9, 156.1, 156.3, 159.7 ppm; HRMS (ESI, MeCN): m/z = 404.2431 ($[M+H]^+$).

11c. To a solution of α,α'-Dibromo-m-xylene (102.7 mg, 390 μmol) in $CHCl_3$ (2 mL) was added DIPEA (0.17 mL, 980 μmol), and 2,2'-Dipicolylamine (0.15 mL, 830 μmol). The reaction mixture was allowed to stir at room temperature for 4 hours. The resulting solution was washed once with water and was dried over $MgSO_4$. The crude residue was purified by column chromatography with 0-15 % MeOH:EtOAc as the eluent to yield the desired product as a brown oil (60 mg, 31 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.67 (s, 4H), 3.81 (s, 8H), 7.11 (m, 4H), 7.29 (m, 4H), 7.48 (s, 1H), 7.60 (m, 7H), 8.51 (d, J = 5 Hz, 4H) ppm; ^{13}C NMR (500 MHz, $CDCl_3$) δ 58.5, 59.9, 122.0, 122.8, 127.7, 128.3, 129.3, 136.5, 138.8, 148.9, 159.4 ppm; HRMS (ESI, MeCN): m/z = 501.2757 ($[M+H]^+$).

11d. To a solution of α,α'-Dibromo-m-xylene (17.8 mg, 67.4 μmol) in $CHCl_3$ (4 mL) was added DIPEA (50 μL, 290 μmol), and **20d** (41.3 mg, 150 μmol). The reaction mixture was allowed to stir at room temperature overnight. The resulting solution was washed once with water and the organic layer was dried over $MgSO_4$. The crude residue was purified by column chromatography with 0-20 % MeOH:$CHCl_3$ as the eluent to yield the desired product as a yellow oil (41.5 mg, 93 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.71 (s, 4H), 3.81 (s, 4H), 3.82 (s, 4H), 7.16-7.18 (m, 2H), 7.27-7.28 (m, 3H), 7.33 (d, J = 8 Hz, 2H), 7.47 (s, 1H), 7.50 (t, J = 8 Hz, 2H), 7.58 (t, J = 8 Hz, 4H), 7.66 (dt, J = 2, 8 Hz, 2H), 8.53-8.55 (m, 2H) ppm; ^{13}C NMR (500 MHz, $CDCl_3$) δ 58.7, 59.5, 60.3, 121.6, 122.3, 123.0, 126.4, 127.9, 128.6, 129.5, 136.7, 139.0, 141.4, 149.3, 159.7, 161.9 ppm; HRMS (ESI, MeCN): m/z = 657.0976 ($[M+H]^+$).

11e. To a solution of α,α'-Dibromo-m-xylene (11.4 mg, 43.2 μmol) in 3mL $CHCl_3$ was added DIPEA (50 μL, 290 μmol), and **21d** (20.0 mg, 93.7 μmol). The reaction mixture was allowed to stir at room temperature overnight. The resulting solution was washed once with water and the organic layer was dried over $MgSO_4$. The crude residue was purified by column chromatography with 0-10 % MeOH:$CHCl_3$ as the eluent to yield the desired product as a yellow oil (7.7 mg, 34 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.51 (s, 6H), 3.67 (s, 4H), 3.77 (s, 4H), 3.80 (s, 4H), 6.98 (d, J = 8 Hz, 2H), 7.11-7.14 (m, 2H), 7.26-7.30 (m, 3H), 7.44 (d, J = 8 Hz, 2H), 7.48 (s, 1H), 7.51 (t, J = 8 Hz, 2H), 7.59-7.63 (m, 4H), 8.51 (dt, J = 1, 5 Hz, 2H) ppm; ^{13}C NMR (500 MHz, $CDCl_3$) δ 24.7, 58.8, 60.3, 60.4, 119.6, 121.6, 122.1, 122.9, 127.8, 128.5, 129.4, 136.6, 136.9, 139.4, 149.2, 157.7, 159.5, 160.2 ppm; HRMS (ESI, MeCN): m/z = 529.3079 ($[M+H]^+$).

11f. To a solution of **11b** (15.1 mg, 28.5 μmol) in $CHCl_3$ (2 mL) was added acetic anhydride (40 μL, 430 μmol). The reaction mixture was allowed stir overnight. Solvent was removed under reduced pressure and the crude residue was purified by column chromatography with 0-10 % MeOH:$CHCl_3$ as the eluent to yield the desired product as a brown oil (10 mg, 57 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.87 (s, 6H), 3.64 (s, 4H), 3.75 (s, 4H), 3.82 (s, 4H), 7.18-7.27 (m, 7H), 7.48, (d, J = 8 Hz, 2H), 7.58 (td, J = 2, 8 Hz, 2H), 7.71 (t, J = 8 Hz, 2H), 7.80 (s, 1H), 8.15 (d, J = 8 Hz, 2H), 8.50-8.52 (m, 2H), 9.30 (br s, NH, 2H) ppm; ^{13}C NMR (500 MHz, $CDCl_3$) δ 24.4, 58.1, 59.4, 60.4, 112.8, 119.5, 122.5, 123.4, 128.2, 130.3, 136.7, 139.4, 149.1, 151.7, 169.3 ppm; HRMS (ESI, MeCN): m/z = 615.3178 ($[M+H]^+$).

11g. To a solution of **11b** (19.0 mg, 35.8 μmol) in $CHCl_3$ (2.0 mL) at 0 °C was added freshly distilled trifluoroacetic anhydride (50 μL, 350 μmol). The reaction mixture was allowed to warm to room temperature while stirring overnight. The resulting solution was washed with water and the organic layer was dried over $MgSO_4$. The crude residue was purified by column chromatography with 0-10 % MeOH:$CHCl_3$ as the eluent to yield the desired product as a brown oil (13.4 mg, 52 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.67 (s, 4H), 3.71 (s, 4H), 3.81 (s, 4H), 7.14-7.16 (m, 2H), 7.23-7.29 (m, 3H), 7.39 (d, J = 8 Hz, 2H), 7.47 (s, 1H), 7.51 (d, J = 8 Hz, 2H), 7.61 (td, J = 2, 8 Hz, 2H), 7.74 (t, J = 8 Hz, 2H), 7.99 (br s, 2H), 8.50-8.52 (m, 2H), 9.35 (br s, NH, 2H) ppm; ^{13}C NMR (500 MHz, $CDCl_3$) δ 58.5, 59.1, 60.3, 113.3, 120.8, 122.4, 123.1, 128.2, 128.6, 129.8, 136.7, 138.4, 139.6, 149.2, 159.4 ppm; HRMS (ESI, MeCN): m/z = 723.2616 ($[M+H]^+$).

11h. To a solution of **11b** (15.0 mg, 28.3 μmol) in $CHCl_3$ (2.0 mL) was added benzoic anhydride (63.3 mg, 280 μmol). The reaction mixture was allowed to stir overnight. Solvent was removed under reduced pressure and the crude residue was purified by column chromatography with 0-2 % MeOH:$CHCl_3$ as the eluent to yield the desired product as an off-white solid (18.0 mg, 86 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.65 (s, 4H), 3.69 (s, 4H), 3.82 (s, 4H), 7.12-7.17 (m, 2H), 7.27 (s, 2H), 7.31 (d, J = 8 Hz, 2H), 7.38 (t, J = 7 Hz, 4H), 7.47-7.52 (m, 2H), 7.53-7.63 (m, 5H), 7.73 (t, J = 8 Hz, 2H), 7.81 (d, J = 8 Hz, 4H), 8.11 (d, J = 7 Hz, 1H), 8.31 (d, J = 8 Hz, 2H), 8.51-8.53 (m, 2H), 9.28 (br s, NH, 2H) ppm; ^{13}C NMR (500 MHz, $CDCl_3$) δ 58.6, 59.5, 60.3, 112.7, 119.3, 122.3, 123.0, 127.4, 128.1, 128.4, 128.5, 128.8, 129.6, 130.2, 132.2, 133.1, 134.6, 136.8, 139.3, 149.0, 151.5, 158.3, 159.7, 166.4 ppm; HRMS (ESI, MeCN): m/z = 739.3526 ($[M+H]^+$).

11k. To a solution of **11b** (25.0 mg, 47.1 μmol) in $CHCl_3$ (3.0 mL) was added butyl isocyanate (27 μL, 240 μmol). The reaction mixture was allowed stir for 36 hours and solvent was removed under reduced pressure. The crude residue was purified by alumina column chromatography with 0-2 % MeOH:$CHCl_3$ as the eluent to yield the desired product as a brown oil (21.6 mg, 63 % yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.83 (t, J = 8 Hz, 6H), 1.31 (sextet, J = 8 Hz, 4H), 1.47 (p, J = 8 Hz, 4H), 3.28 (q, J = 8 Hz, 4H), 3.57 (s, 4H), 3.59 (s, 4H), 3.70 (s, 4H), 6.59 (d, J = 8 Hz, 2H), 7.00 (d, J = 8 Hz, 2H), 7.02-7.05 (m, 2H), 7.19-7.21 (m, 2H), 7.31 (s, 1H), 7.41 (t, J = 8 Hz, 2H), 7.44 (d, J = 8 Hz, 2H), 7.52 (dt, J = 2, 8 Hz, 2H), 8.40-8.41 (m, 2H), 8.66 (s, 2H), 9.47 (s, 2H) ppm; ^{13}C NMR (600 MHz, $CDCl_3$) δ 13.8, 20.2, 25.7, 32.1, 39.5, 58.6, 59.8, 60.1, 110.1, 115.2, 122.0, 122.6, 127.5, 128.4, 129.0, 136.4, 138.5, 138.9, 149.0, 153.0, 156.2, 156.3, 159.6 ppm; HRMS (ESI, MeCN): m/z = 729.4350 ($[M+H]^+$).

11m. To a solution of **11b** (14.9 mg, 28.1 μmol) in $CHCl_3$ (3.0 mL) was added *p*-tolyl isocyanate (20 μL, 160 μmol). The reaction mixture was allowed stir overnight and solvent was removed under reduced pressure. The crude residue was purified by alumina column chromatography with 0-2 % MeOH:$CHCl_3$ as the eluent to yield the desired product as a brown oil (16.1 mg, 73 % yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.31 (s, 6H), 3.66 (s, 4H), 3.71 (s, 4H), 3.81 (s, 4H), 6.72 (s, 2H), 7.09-7.11 (m, 8H), 7.21-7.24 (m, 1H), 7.28 (d, J = 8 Hz, 2H), 7.41-7.43 (m, 5H), 7.49-7.56 (m, 6H), 8.48-8.49 (m, 2H), 8.74 (s, 2H), 11.94 (s, 2H) ppm; ^{13}C NMR (600 MHz, $CDCl_3$) δ 20.9, 58.7, 59.6, 60.2, 110.4, 115.9, 120.1, 122.1, 122.9, 127.8, 128.4, 129.4, 132.7, 136.0, 136.5, 138.5, 139.0, 149.0, 152.4, 153.6, 156.3, 159.3 ppm; HRMS (ESI, MeCN): m/z = 797.4057 ($[M+H]^+$).

11n. To a solution of **11b** (30.0 mg, 56.5 μmol) in $CHCl_3$ (4.0 mL) was added 3,5-Bis(trifluoromethyl)phenyl isocyanate (49 μL, 280 μmol). The reaction mixture was allowed stir for overnight and solvent was removed under reduced pressure. The crude material was purified using preparatory TLC methods (alumina gel preparatory TLC plate, with 5 % MeOH:$CHCl_3$ as the mobile phase. The purified material was dissolved in $CHCl_3$, washed with water, and dried over Na_2SO_4 to yield the desired product as a white solid (15.2 mg, 26 % yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.65 (s, 4H), 3.69 (s, 4H), 3.82 (s, 4H), 6.80 (s, 2H), 7.04 (d, J = 8 Hz, 2H), 7.09-7.11 (m, 2H), 7.17-7.20 (m, 1H), 7.23 (d, J = 8 Hz, 1H), 7.42 (d, J = 8 Hz, 2H), 7.46 (s, 2H), 7.52-7.56 (m, 4H), 7.99 (s, 4H), 8.43-8.44 (m, 2H) ppm; ^{13}C NMR (600 MHz, $CDCl_3$) δ 58.4, 59.2, 59.6, 110.5, 116.1, 116.7, 119.1, 120.4, 122.2, 122.9, 124.0, 125.9, 128.1, 128.4, 129.6, 131.4 (q, J = 133 Hz), 136.7, 137.6, 139.2, 140.1, 148.8, 151.8, 153.2, 156.3, 158.7 ppm; HRMS (ESI, MeCN): m/z = 1041.3249 ([M+H]$^+$).

11o. To a solution of **11b** (20.0 mg, 37.7 μmol) in $CHCl_3$ (500 μL) was added 4-fluorophenethyl isocyanate (27.4 μL, 190 μmol). The reaction was allowed to stir at room temperature for 12 hours. Solvent was removed and the crude material was purified using silica gel column chromatography with 0-10 % MeOH:$CHCl_3$ as the eluent to yield the desired product as a clear oil (8.7 mg, 27 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.85 (t, J = 7 Hz, 4H), 3.44 (s, 4H), 3.61-3.65 (m, 8H), 3.74 (s, 4H), 6.60 (s, 2H), 6.91-6.95 (m, 4H), 7.01-7.17 (m, 8H), 7.27-7.32 (m, 3H), 7.42 (s, 1H), 7.49 (t, J = 7 Hz, 2H), 7.52 (d, J = 7 Hz, 2H), 7.60 (t of d, J = 8 Hz, J = 1 Hz, 2H), 8.12 (br s, 2H), 8.50-8.52 (m, 2H), 9.47 (br s, 2H) ppm; ^{13}C NMR (500 MHz, $CDCl_3$) δ 35.7, 41.2, 58.9, 59.6, 60.4, 110.0, 115.5, 115.7, 122.3, 122.9, 127.8, 128.7, 129.2, 130.4, 135.3, 136.7, 139.3, 149.3, 152.7, 156.1, 158.7, 160.8, 162.7 ppm; HRMS (ESI, MeCN): m/z = 861.4184 ([M+H]$^+$).

11p. To a solution of **11b** (40.0 mg, 75.4 μmol) in $CHCl_3$ (250 μL) was added 6-Isocyanato-1,4-benzodioxane (51.5 μL, 380 μmol). The reaction was allowed to stir at room temperature for 12 hours. Solvent was removed and the crude material was purified using silica gel column chromatography with 0-10 % MeOH:$CHCl_3$ as the eluent to yield the desired product as a clear oil (14.8 mg, 22 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.67 (s, 4H), 3.71 (s, 4H), 3.80 (s, 4H), 4.20-4.24 (m, 8H), 6.72 (s, 2H), 6.78 (d, J = 9 Hz, 2H), 6.93 (dd, J = 2, 8 Hz, 2H), 7.10 (t, J = 7 Hz, 4H), 7.20-7.23 (m, 3H), 7.27 (d, J = 8 Hz, 2H), 7.41 (s, 1H), 7.49-7.56 (m, 6H), 8.48 (d, J = 4 Hz, 2H), 8.83 (br s, 2H), 11.9 (br s, 2H) ppm; ^{13}C NMR (600 MHz, $CDCl_3$) δ 58.7, 59.6, 60.2, 64.3, 64.5, 109.7, 110.4, 113.8, 115.8, 117.1, 122.1, 122.9, 127.8, 128.4, 132.4, 136.5, 138.6, 139.0, 139.7, 143.4, 148.9, 152.4, 153.6, 156.2, 159.3 ppm; HRMS (ESI, MeCN): m/z = 885.3846 ([M+H]$^+$).

11q. To a solution of **11b** (40.0 mg, 75.4 μmol) in $CHCl_3$ (250 μL) was added 4-phenoxyphenyl isocyanate (68 μL, 380 μmol). The reaction was allowed to stir at room temperature for 12 hours. Solvent was removed and the crude material was purified using silica gel column chromatography with 0-10 % MeOH:$CHCl_3$ as the eluent to yield the desired product as a clear oil (2.6 mg, 4 %). ^{1}H NMR (600 MHz, CD_3OD) δ 3.54 (s, 4H), 3.62 (s, 4H), 3.67 (s, 4H), 6.75 (d, J = 8 Hz, 2H), 6.77-6.79 (m, 4H), 6.82-6.84 (m, 4H), 6.95-6.97 (m, 5H), 7.03-7.05 (m, 1H), 7.08-7.10 (m, 4H), 7.20-7.23 (m, 4H), 7.28 (s, 1H), 7.31-7.33 (m, 4H), 7.44 (d, J = 8 Hz, 2H), 7.48-7.53 (m, 3H), 8.25-8.26 (m, 2H) ppm; HRMS (ESI, MeCN): m/z = 954.4256 ($[M+H]^+$).

11r. To a solution of **11b** (40.0 mg, 75.4 μmol) in $CHCl_3$ (250 μL) was added allyl isocyanate (31 μL, 360 μmol). The reaction was allowed to stir at room temperature for 12 hours. Solvent was removed and the crude material was purified using silica gel column chromatography with 0-10 % MeOH:$CHCl_3$ as the eluent to yield the desired product as a clear oil (20.1 mg, 38 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.65 (s, 4H), 3.66 (s, 4H), 3.78 (s, 4H), 4.02-4.03 (m, 4H), 5.11 (dq, J = 2, 10 Hz, 2H), 5.25 (dq, J = 2, 17 Hz, 2H), 5.95 (dp, J = 5, 17 Hz, 2H), 6.69 (d, J = 8 Hz, 2H), 7.05 (d, J = 7 Hz, 2H), 7.10-7.13 (m, 2H), 7.24-7.31 (m, 3H), 7.36 (s, 1H), 7.48-7.53 (m, 4H), 7.60 (td, J = 2, 8 Hz, 2H), 8.48-8.49 (m, 4H), 8.84 (br s, NH, 2H), 9.72 (br s, NH, 2H) ppm; ^{13}C NMR (600 MHz, $CDCl_3$) δ 42.2, 58.7, 59.6, 60.1, 110.2, 115.2, 115.5, 115.9, 122.0, 122.6, 127.5, 128.4, 129.1, 135.1, 136.5, 138.6, 138.9, 148.9, 152.9, 156.2, 159.6 ppm; HRMS (ESI, MeCN): m/z 697.3712 ($[M+H]^+$).

11s. To a solution of **11b** (40.0 mg, 75.4 μmol) in $CHCl_3$ (250 μL) was added 2-(2-thienyl)ethyl isocyanate (50 μL, 380 μmol). The reaction was allowed to stir at room temperature for 12 hours. Solvent was removed and the crude material was purified using silica gel column chromatography with 0-10 % MeOH:$CHCl_3$ as the eluent to yield the desired product as a clear oil (37.1 mg, 59 %). ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.09 (t, J = 6 Hz, 4H), 3.48 (s, 4H), 3.63 (s, 4H), 3.69 (q, J = 6 Hz, 4H), 3.75 (s, 4H), 6.66 (d, J = 8 Hz, 2H), 6.82 (dd, J = 2, 4 Hz, 2H), 6.84 (dd, J = 3, 6 Hz, 2H), 7.06 (dd, J = 1, 5 Hz, 2H), 7.09 (d, J = 8 Hz, 2H), 7.12-7.14 (m, 2H), 7.27-7.29 (m, 3H), 7.40 (s, 1H), 7.48 (t, J = 8 Hz, 2H), 7.52 (d, J = 8 Hz, 2H), 7.60 (td, J = 2, 8 Hz, 2H), 8.49-8.51 (m, 2H), 8.72 (br s, NH, 2H), 9.69 (br s, NH, 2H) ppm; ^{13}C NMR (600 MHz, $CDCl_3$) δ 30.6, 41.1, 58.6, 59.4, 60.1, 105.0, 115.0, 122.0, 122.5, 123.7, 125.1, 127.0, 127.5, 128.4, 128.9, 136.5, 138.6, 139.0, 141.9, 149.0, 152.7, 156.2, 156.5, 159.7 ppm; HRMS (ESI, MeCN): m/z = 837.3481 ($[M+H]^+$).

apo-12. To a solution of **CyAL-5** (37 mg, 52 μmol) in anhydrous DMF (550 μL) was added triethylamine (110 μL, 780 μmol) and *N,N'*-Disuccinimidyl carbonate (54 mg, 210 μmol). The reaction mixture was allowed to stir under argon at room temperature for 24 hours. To the reaction mixture was added a solution of **S1** (155 mg, 260 μmol, 5 eq) dissolved in DMF (250 μL). The reaction mixture was allowed to stir under argon at room temperature for 48 hours. Solvent was removed under reduced pressure and the crude material was purified using preparatory TLC methods (silica gel preparatory TLC plate, 80:20:2 $CHCl_3$:MeOH:NH_4OH as the mobile phase, redeveloped 3 times to achieve good separation) to yield the desired product as a blue solid (61.6 mg, 93 % Yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.25 (t, J = 6 Hz, 6H), 1.43-1.48 (m, 2H), 1.50-1.54 (m, 2H), 1.64-1.69 (m, 4H), 1.70 (s, 12H), 2.15 (t, J = 6 Hz, 2H), 2.62 (t, J = 6 Hz, 4H), 3.07-3.10 (m, 2H), 3.57 (s, 4H), 3.69 (s, 8H), 3.91 (t, J = 6 Hz, 2H), 4.17-4.21 (m, 4H), 6.18 (d, J = 12 Hz, 2H), 6.78 (s, 2H), 7.06 (s, 1H), 7.22-7.24 (m, 4H), 7.30 (d, J = 12 Hz, 2H), 7.54-7.58 (m, 4H), 7.63 (d, J = 12 Hz, 2H), 7.70-7.74 (m, 4H), 7.80-7.83 (m, 4H), 8.16 (d, J = 12 Hz, 2H), 8.45-8.49 (m, 4H); HRMS (ESI, MeCN): m/z = 1240.9654 ($[M-H]^-$).

S1b. To a solution of 6-bromopicolinaldehyde (500 mg, 2.7 mmol) and $CaCl_2$ (600 mg, 5.4 mmol) in EtOH (25 mL) at 0 °C was added $NaBH_4$ (310 mg, 8.2 mmol). After stirring for 3 hours at 0 °C H_2O was added to the reaction mixture. The reaction mixture was extracted three times with chloroform and the organic layer was dried over $MgSO_4$. The crude residue was purified by silica gel column chromatography with 0-10 % MeOH in $CHCl_3$ as the eluent to yield the desired product as a colorless oil (200 mg, 40 % yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.23 (s, OH, 1H), 4.67 (s, 2H), 7.26-7.30 (m, 2H), 7.48 (dd, J = 7, 8 Hz, 1H) ppm; ^{13}C NMR (300 MHz, $CDCl_3$) δ 64.3, 119.6, 126.6, 139.3, 141.2, 162.0 ppm; HRMS (ESI, MeCN): m/z = 187.9722 ($[M+H]^+$), m/z = 209.9553 ($[M+Na]^+$).

S1c. To a solution of **S1b** (200 mg, 1.1 mmol), CBr_4 (700 mg, 2.1 mmol), and K_2CO_3, (430 mg, 3.1 mmol) in anhydrous dichloromethane (10 mL) at 0°C was added by dropwise addition a solution of PPh_3 (550 mg, 2.1 mmol) in anhydrous dichloromethane (5.5 mL). The reaction mixture was allowed to warm to room temperature while stirring overnight. The reaction mixture was filtered to remove any insoluble material. The crude residue was purified by silica gel column chromatography with 0-5 % MeOH in $CHCl_3$ as the eluent to yield the desired product as an off-white solid (139 mg, 52 % yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.50 (s, 2H), 7.40-7.43 (m, 2H), 7.56 (t, J = 8 Hz, 1H) ppm; ^{13}C NMR (300 MHz, $CDCl_3$) δ 32.7, 122.7, 127.7, 139.6, 141.7, 158.3 ppm; HRMS (ESI, MeCN): m/z = 249.8877 ($[M+H]^+$).

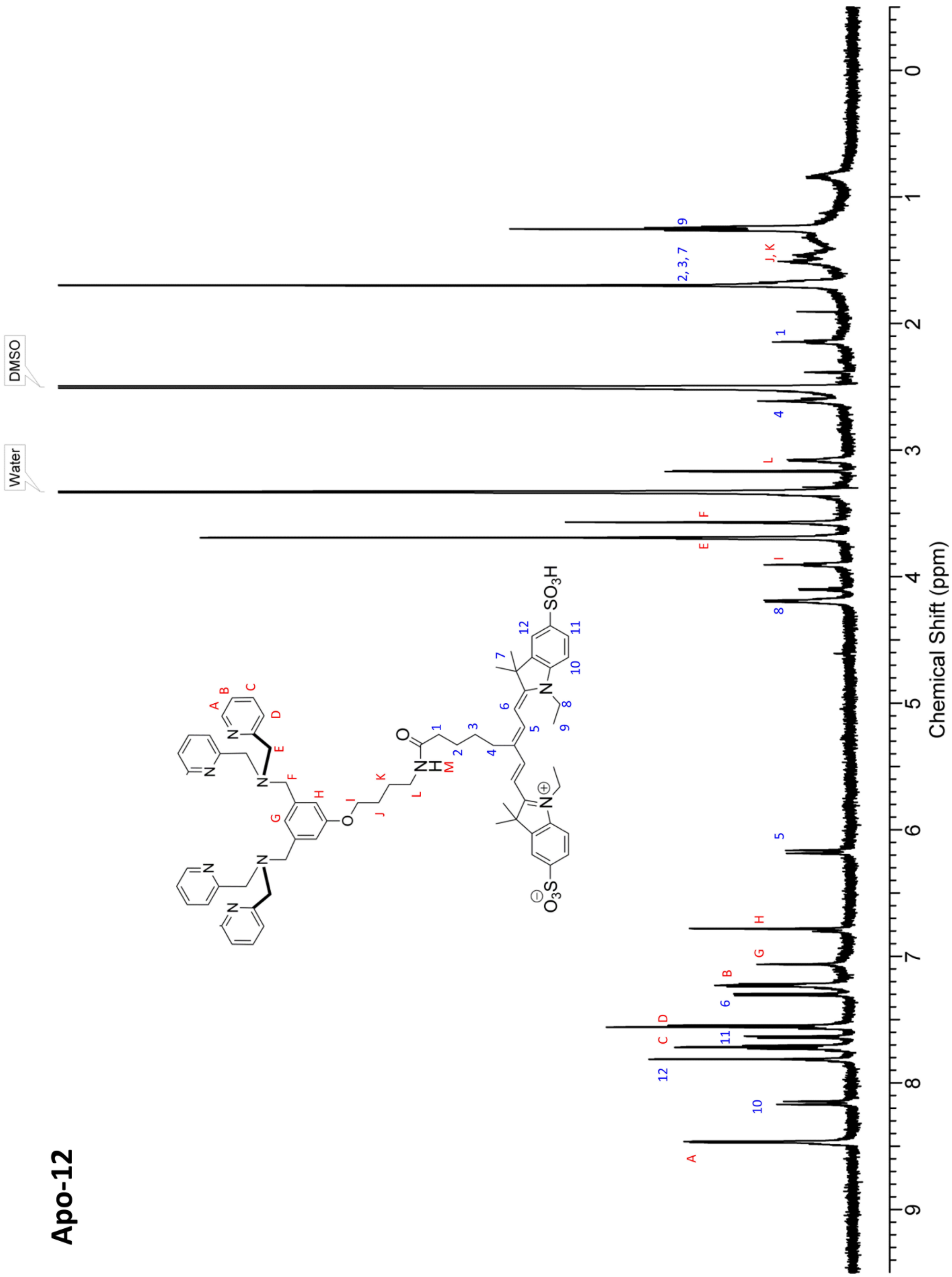
S1d. To a solution of 2-picolylamine (0.22 mL, 2.2 mmol) and Na_2CO_3 (250 mg, 2.4 mmol) in MeCN (8 mL) at 0 °C was added by dropwise addition a solution of **S1c** (138.6 mg, 0.55 mmol) in MeCN (12 mL). The reaction mixture was allowed to warm to room temperature overnight while stirring. The reaction mixture was filtered to remove any insoluble material. The residue was dissolved in EtOAc (25 mL) and wash with water (5 x 25 mL). The organic layer was dried over $MgSO_4$ and solvent was evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography with 0-10 % MeOH in $CHCl_3$ as the eluent to yield the desired product as a yellow oil (41.3 mg, 27 %). ^1H NMR (500 MHz, $CDCl_3$) δ 2.48 (s, 1H), 3.96 (s, 2H), 3.97 (s, 2H), 7.17 (ddd, J = 1, 5, 10 Hz, 1H), 7.34-7.38 (m, 3H), 7.51 (t, J = 8 Hz, 1H), 7.657 (td, J = 2, 7 Hz, 1H), 8.56-8.58 (m, 1H) ppm; ^{13}C NMR (500 MHz, $CDCl_3$) δ 54.4, 54.9, 121.2, 122.3, 122.6, 126.5, 136.7, 139.1, 141.9, 149.6, 159.7, 161.9 ppm; HRMS (ESI, MeCN): m/z = 278.0312 ($[M+H]^+$.

S2b. To a solution of 6-bromopicolinaldehyde (84 mg, 700 μmol) and $CaCl_2$ (160 mg, 1.4 mmol) in EtOH (10 mL) at 0 °C was added $NaBH_4$ (190 mg, 4.9 mmol). After stirring for 3 hours at 0 °C H_2O was added to the reaction mixture. The reaction mixture was extracted three times with chloroform and the organic layer was dried over $MgSO_4$. The crude residue was purified by silica gel column chromatography with 1:1 Hexanes:EtOAc as the eluent to yield the desired product as a colorless oil (54.8 mg, 64 % Yield). ^1H NMR (500 MHz, $CDCl_3$) δ 2.49 (s, 3H), 4.69 (s, 2H), 4.83 (s, OH, 1H), 7.00 (d, J = 8 Hz, 1H), 7.09 (d, J = 8 Hz, 1H), 7.53 (t, J = 8 Hz, 1H) ppm; ^{13}C NMR (500 MHz, $CDCl_3$) δ 24.1, 64.2, 117.8, 121.9, 137.1, 157.4, 159.0 ppm; HRMS (ESI, MeCN): m/z = 124.0760 ($[M+H]^+$).

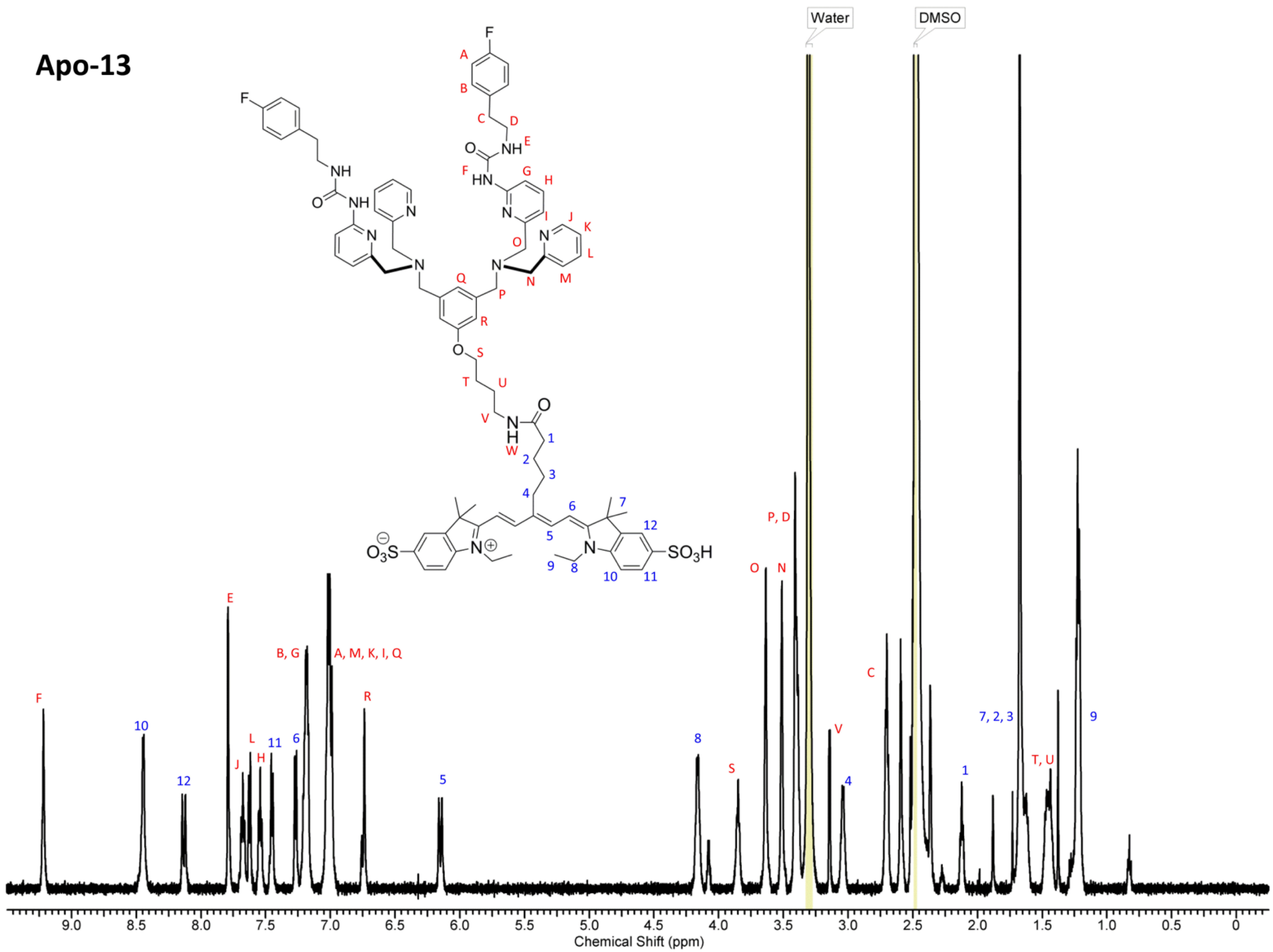
S2c. To a solution of **S2b** (600 mg, 4.9 mmol), CBr_4 (3.2 g, 9.7 mmol), and K_2CO_3, (2.0 g, 14 mmol) in anhydrous dichloromethane (40 mL) at 0°C was added by dropwise addition a solution of PPh_3 (2.5 g, 9.7 mmol) in anhydrous dichloromethane (25 mL). The reaction mixture was allowed to warm to room temperature while stirring overnight. The reaction mixture was filtered to remove any insoluble material. The crude residue was purified by silica gel column chromatography with 25 % EtOAc in hexanes as the eluent to yield the desired product as a red solid (479.5 mg, 53 % yield). ^1H NMR (300 MHz, $CDCl_3$) δ 2.55 (s, 3H), 4.51 (s, 2H), 7.06 (d, J = 8 Hz, 1H), 7.23 (d, J = 8 Hz, 1H), 7.56 (t, J = 8 Hz, 1H) ppm; ^{13}C NMR (300 MHz, $CDCl_3$) δ 24.6, 34.4, 120.7, 122.9, 137.5, 156.3, 158.6 ppm; HRMS (ESI, MeCN): m/z = 185.9908($[M+H]^+$).

S2d. To a solution of 2-picolylamine (1.0 mL, 9.7 mmol) and Na_2CO_3 (120 mg, 1.1 mmol) in MeCN (2 mL) at 0 °C was added by dropwise addition a solution of **S2c** (50 mg, 270 μmol) in MeCN (15 mL). The reaction mixture was allowed to warm to room temperature overnight while stirring. The reaction mixture was filtered to remove any insoluble material. The residue was dissolved in EtOAc (25 mL) and wash with water (5 x 25 mL). The organic layer was dried over $MgSO_4$ and solvent was evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography with 0-10 % MeOH in EtOAC as the eluent to yield the desired product as a dark orange oil (14.4 mg, 25 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.54 (s, 3H), 3.02 (br s, NH, 1H), 3.95 (s, 2H), 3.99 (s, 2H), 7.02 (d, J = 8 Hz, 1H), 7.15-7.17 (m, 2H), 7.36 (d, J = 8 Hz, 1H), 7.53 (t, J = 8 Hz, 1H), 7.64 (d of t, J = 2, 8 Hz, 1H), 8.56 (d of q, J = 1, 5 Hz, 1H) ppm; ^{13}C NMR (500 MHz, $CDCl_3$) δ 24.7, 54.9, 55.0, 119.3, 121.8, 122.2, 122.6, 136.7, 137.0, 149.5, 158.2, 158.9, 159.8 ppm; HRMS (ESI, MeCN): m/z = 214.1368 ($[M+H]^+$.

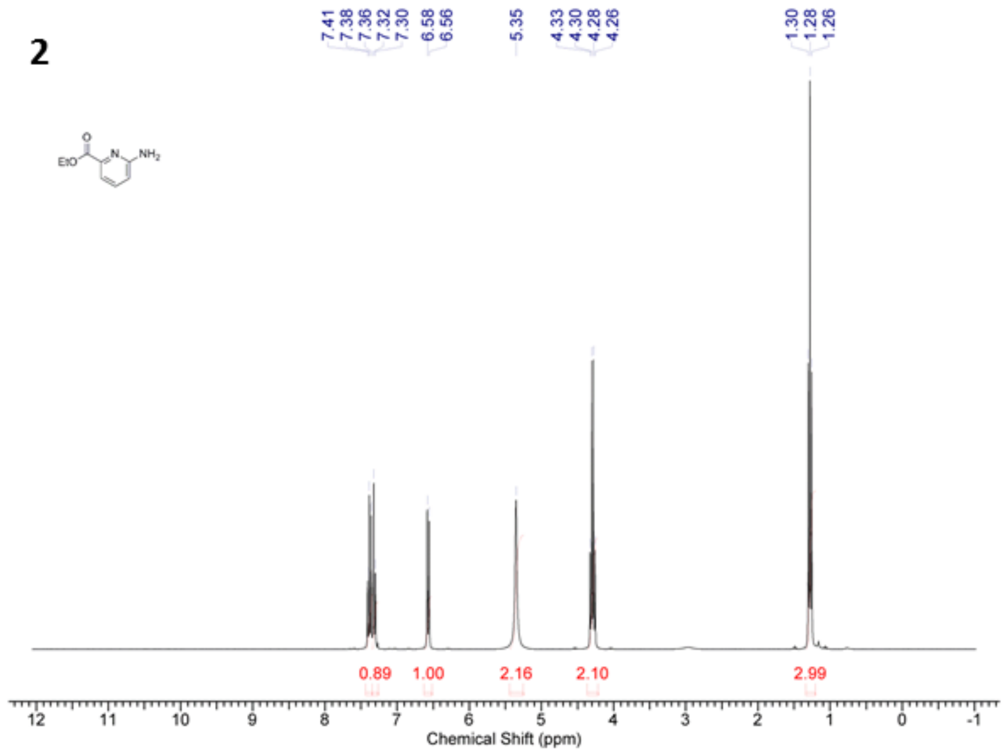
3. **Assigned NMR Spectra of apo-12 and apo-13.**

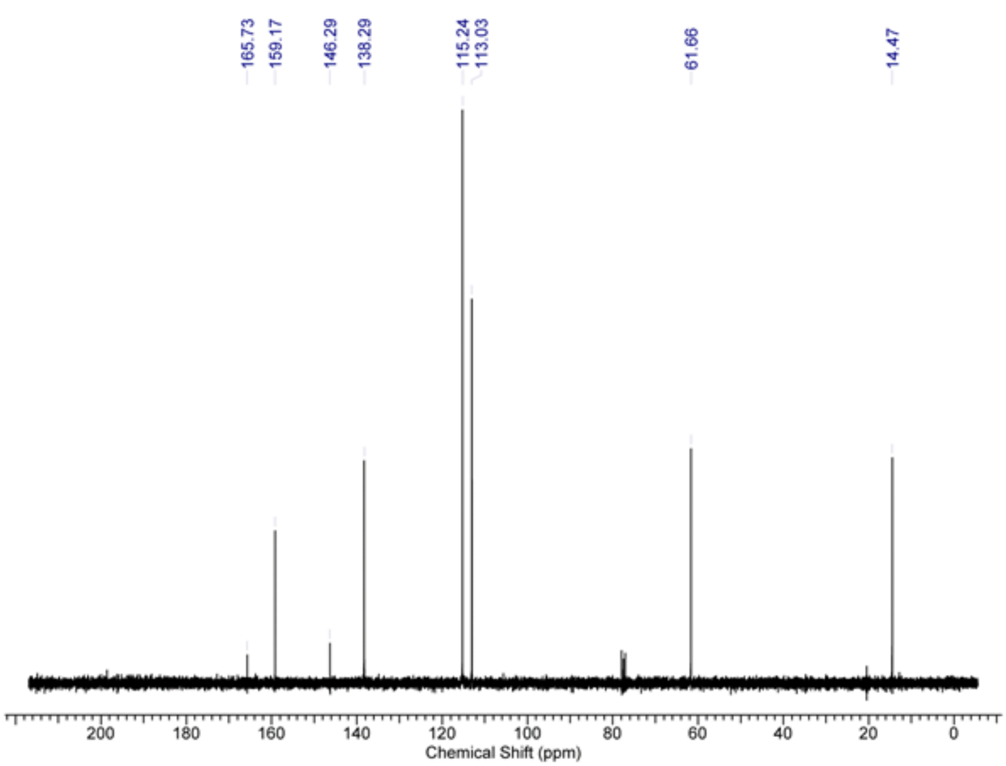


Apo-13

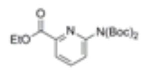

Water
DMSO
F
10
12
E
J
L
H
11
6
B, G
A, M, K, I, Q
R
5
8
S
O
N
P, D
V
4
C
1
7, 2, 3
T, U
9
Chemical Shift (ppm)

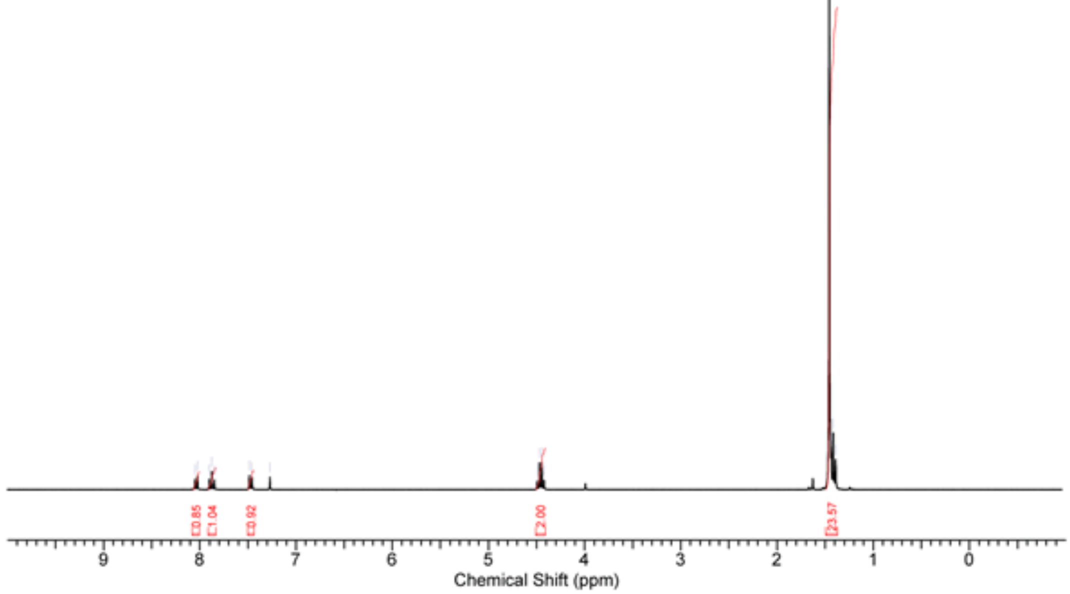
4. **^{1}H and ^{13}C NMR data.**

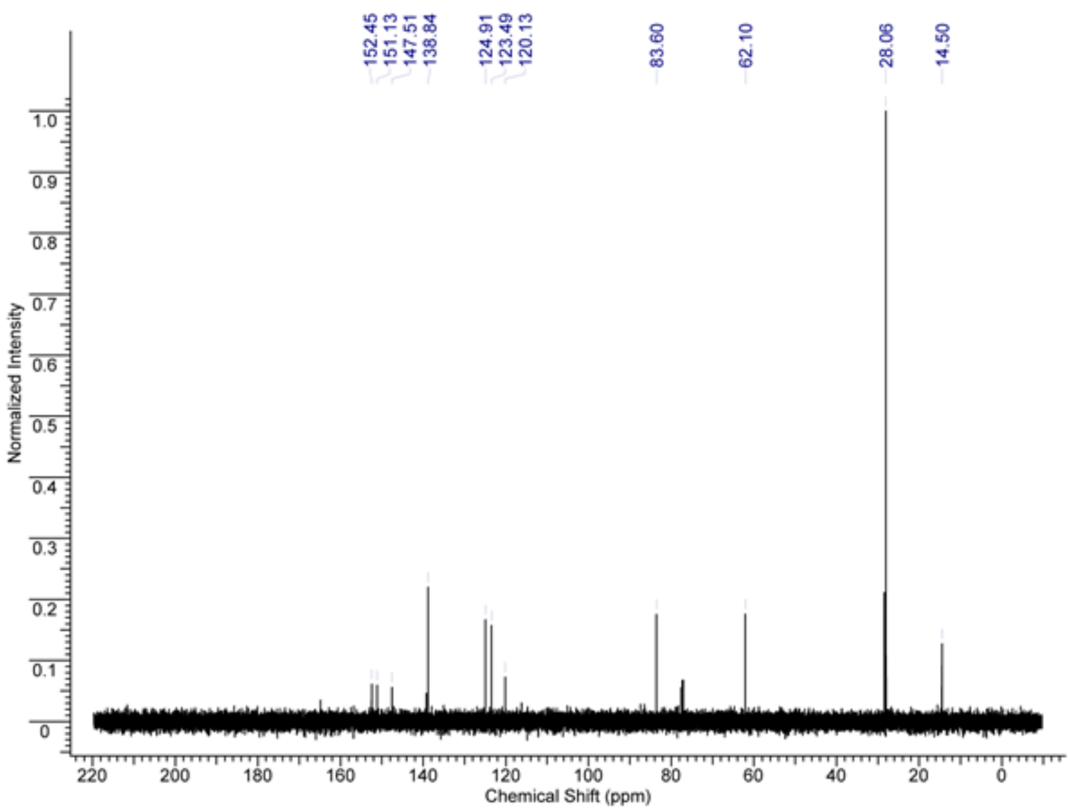




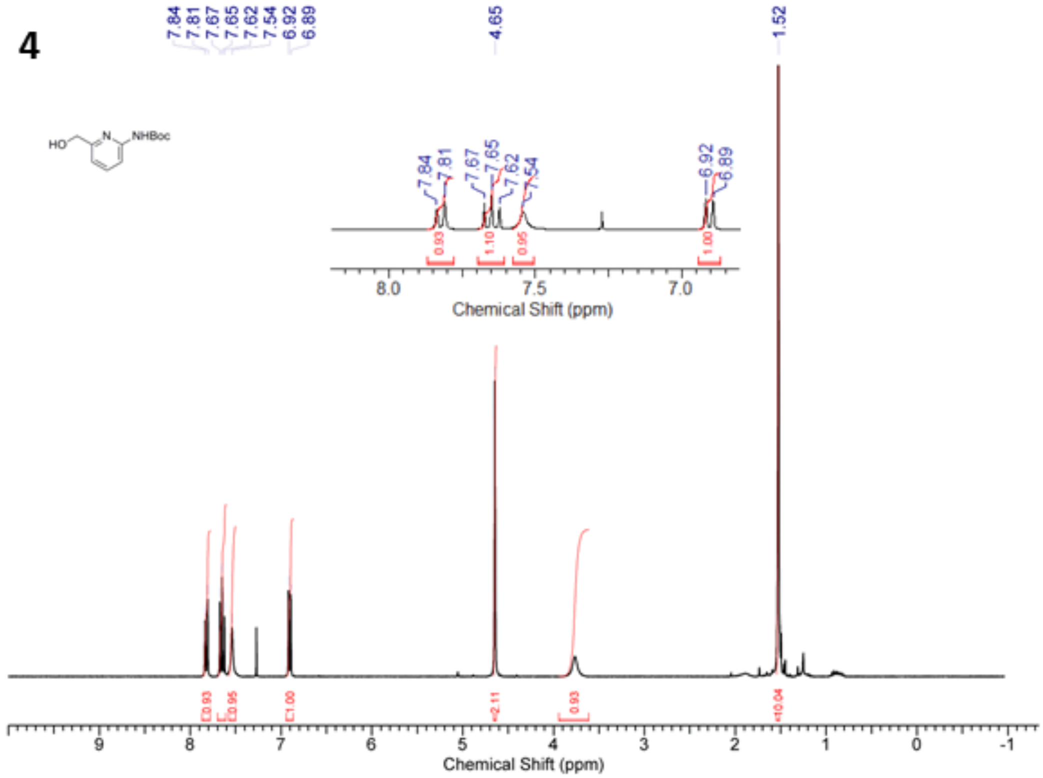
3

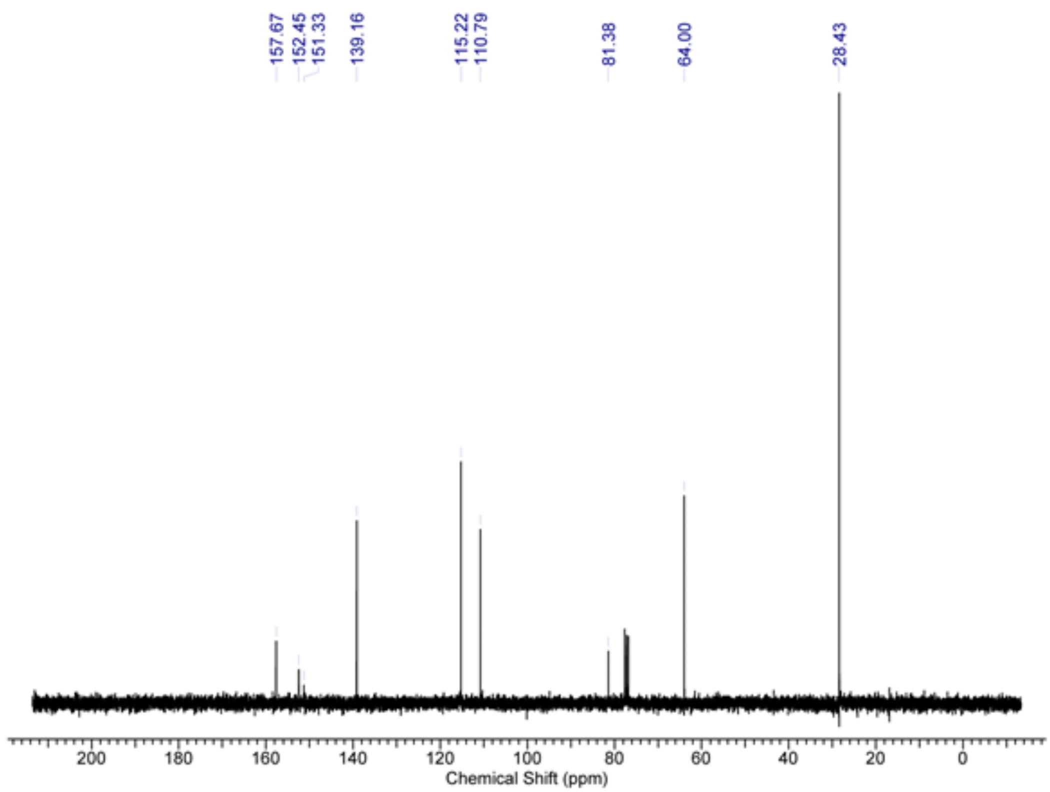

O
EtO
N
N(Boc)2


8.05
8.02
7.90
7.88
7.85
7.48
7.46
7.27
4.49
4.47
4.45
4.42
1.46
1.44
1.41
1.39
0.85
1.04
0.92
2.00
23.57
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

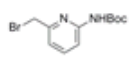

152.45
151.13
147.51
138.84
124.91
123.49
120.13
83.60
62.10
28.06
14.50
Normalized Intensity
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
220
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

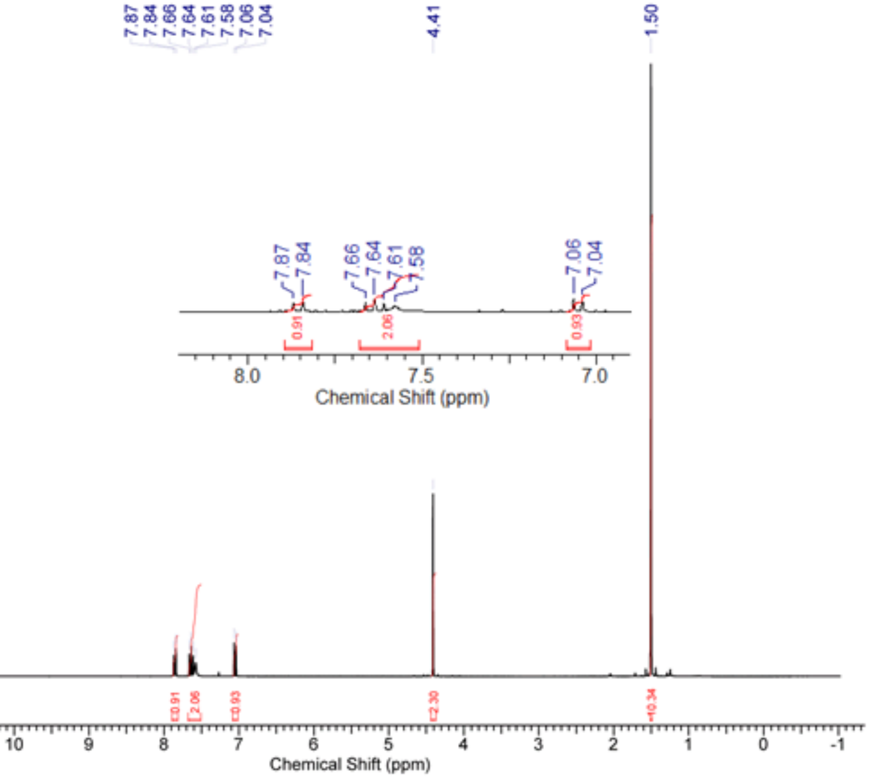
4

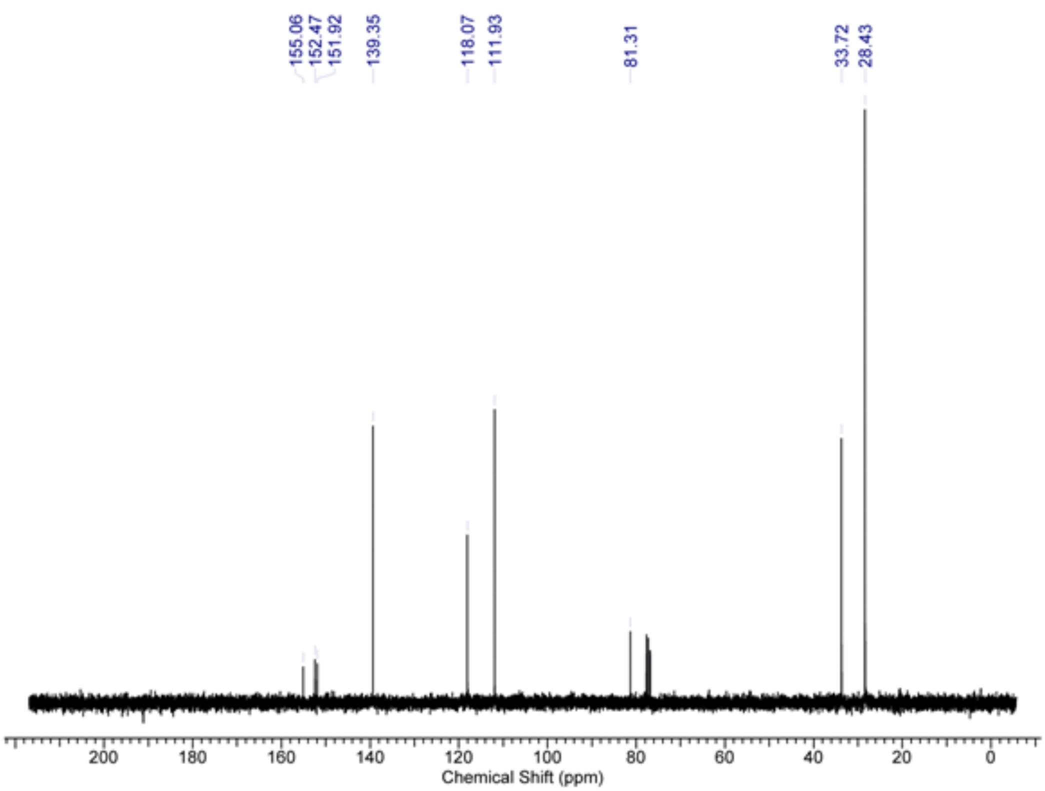

7.84
7.81
7.67
7.65
7.62
7.54
6.92
6.89
4.65
1.52
HO
N
NHBoc
0.93
1.10
0.95
1.00
8.0
7.5
7.0
Chemical Shift (ppm)
0.93
0.95
1.00
2.11
0.93
10.04
9
8
7
6
5
4
3
2
1
0
-1
Chemical Shift (ppm)


157.67
152.45
151.33
139.16
115.22
110.79
81.38
64.00
28.43
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

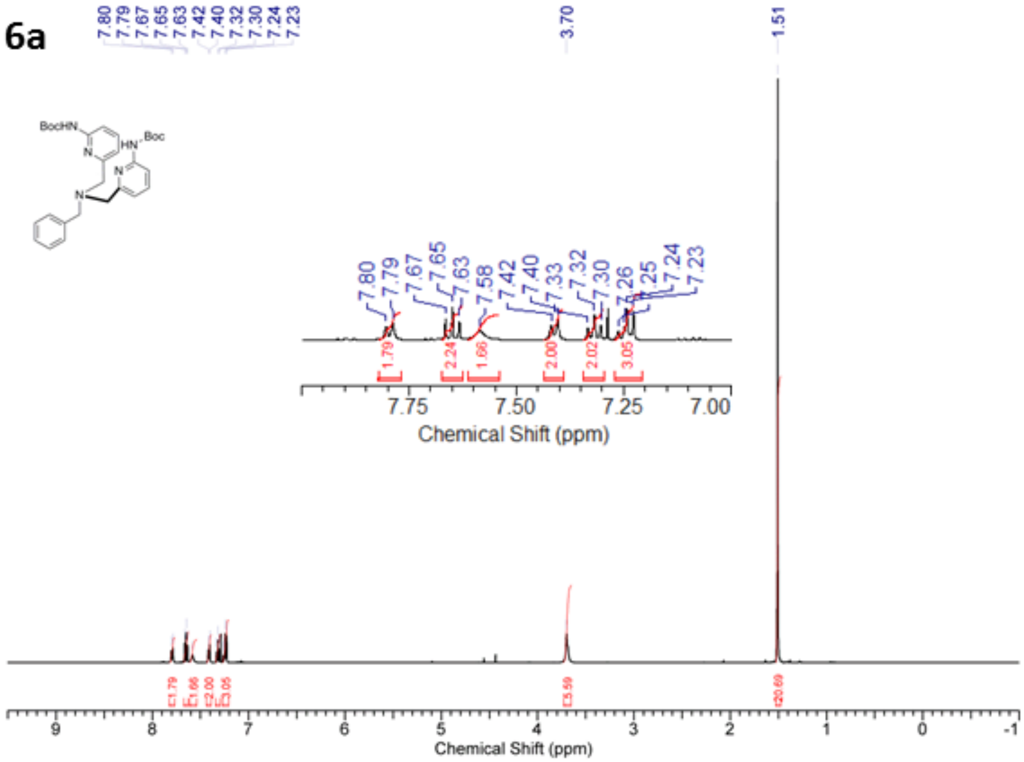
5

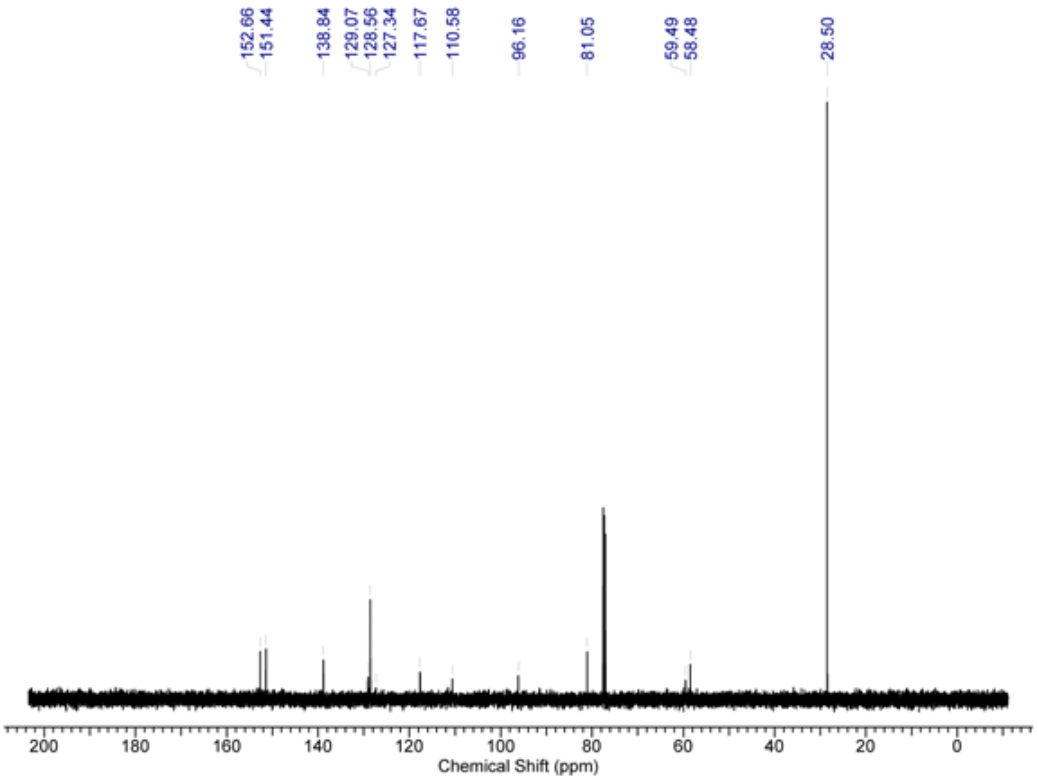

Br
N
NHBoc


7.87
7.84
7.66
7.64
7.61
7.58
7.06
7.04
4.41
1.50
0.91
2.06
0.93
2.30
10.34
8.0
7.5
7.0
Chemical Shift (ppm)
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
Chemical Shift (ppm)

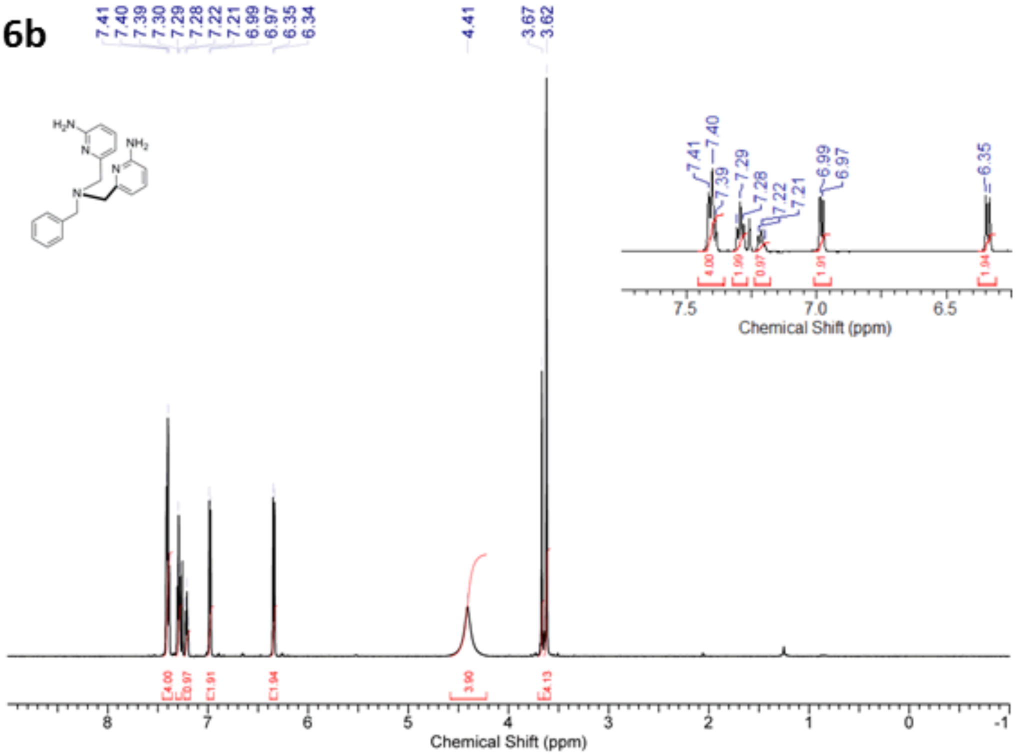

155.06
152.47
151.92
139.35
118.07
111.93
81.31
33.72
28.43
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

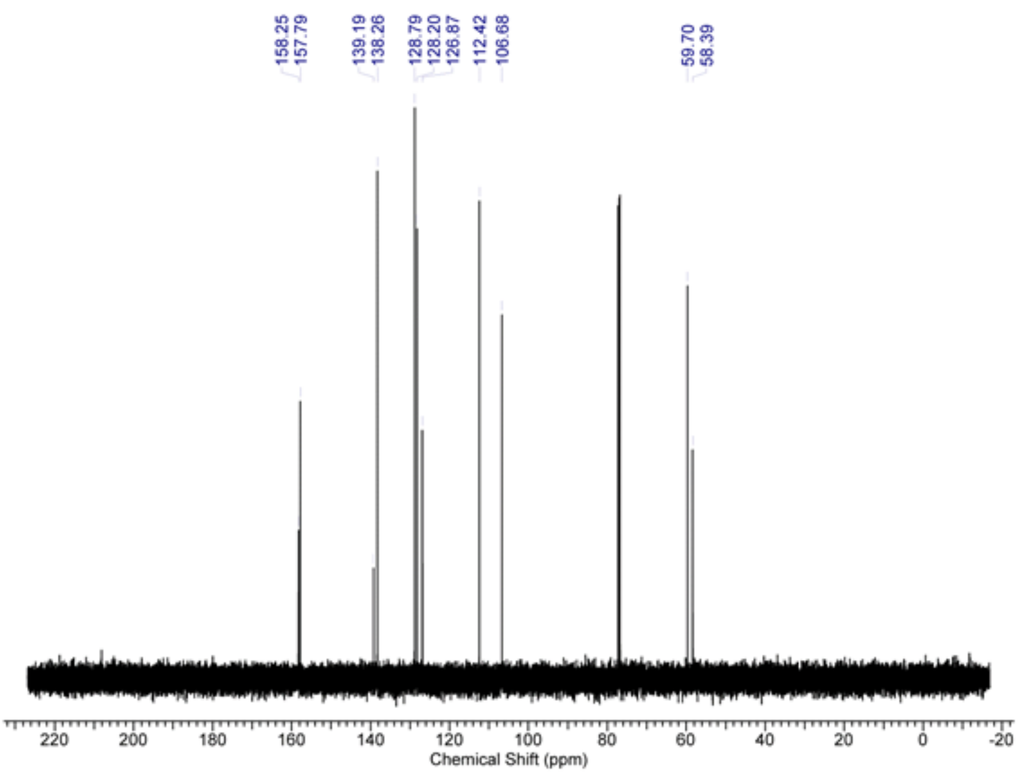
6a


7.80
7.79
7.67
7.65
7.63
7.42
7.40
7.32
7.30
7.24
7.23
3.70
1.51
BocHN
HN
Boc
N
7.58
7.33
7.26
7.25
1.79
2.24
1.66
2.00
2.02
3.05
5.59
20.69
Chemical Shift (ppm)

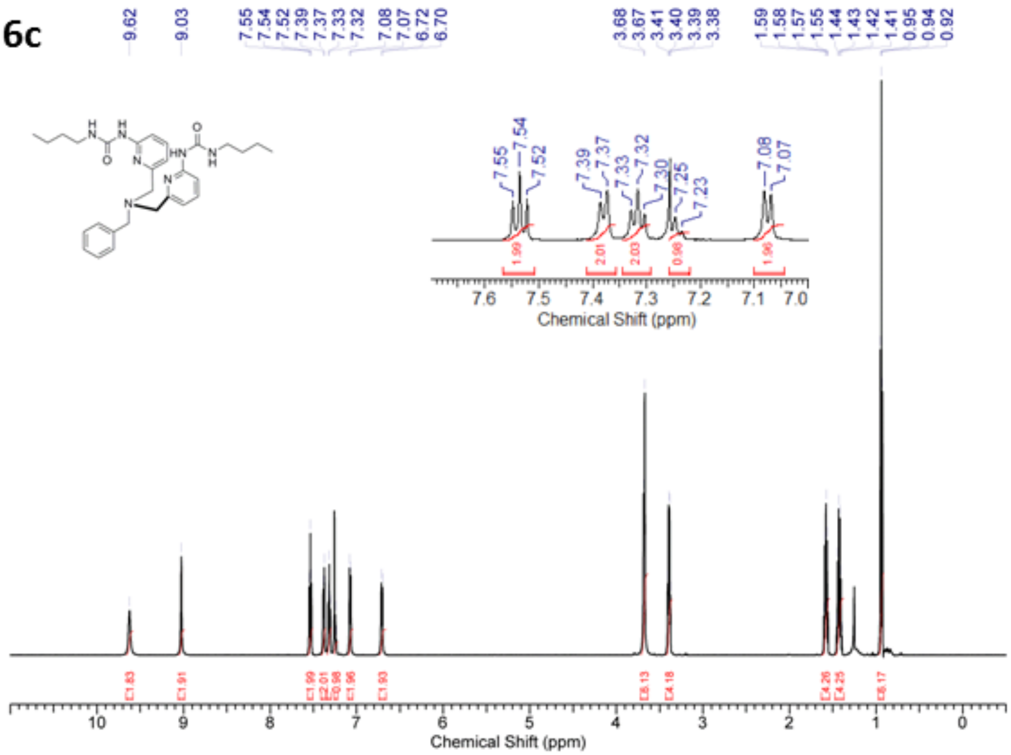

152.66
151.44
138.84
129.07
128.56
127.34
117.67
110.58
96.16
81.05
59.49
58.48
28.50
Chemical Shift (ppm)

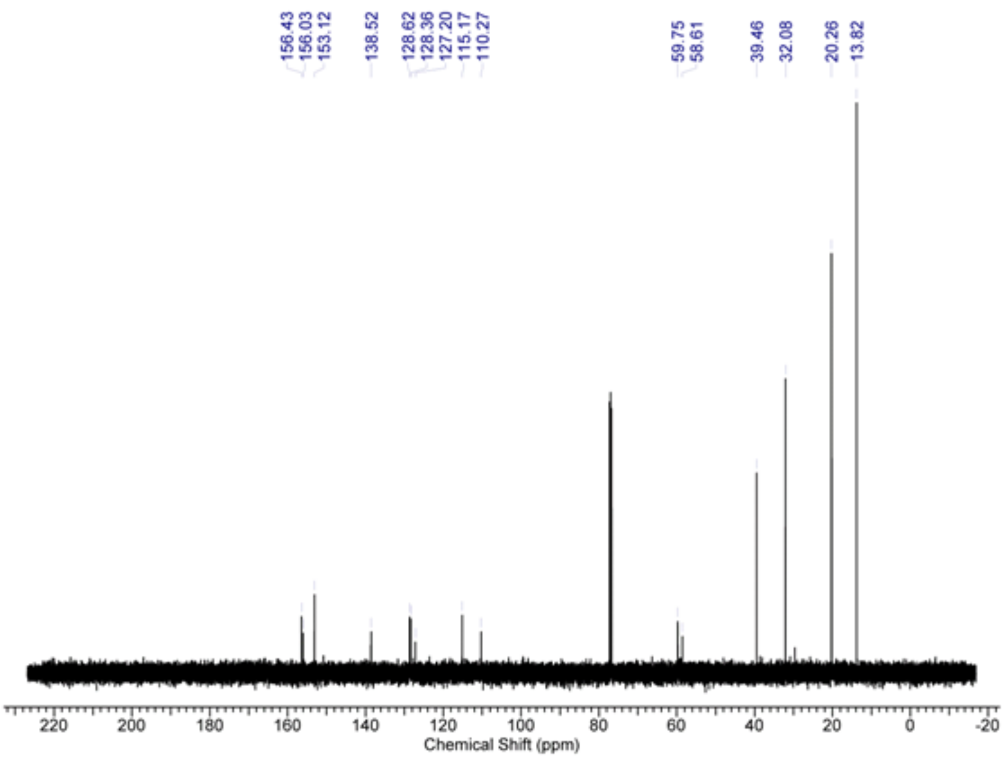
6b


7.41
7.40
7.39
7.30
7.29
7.28
7.22
7.21
6.99
6.97
6.35
6.34
4.41
3.67
3.62
H2N
NH2
N
4.00
0.97
1.91
1.94
3.90
4.13
1.99
Chemical Shift (ppm)

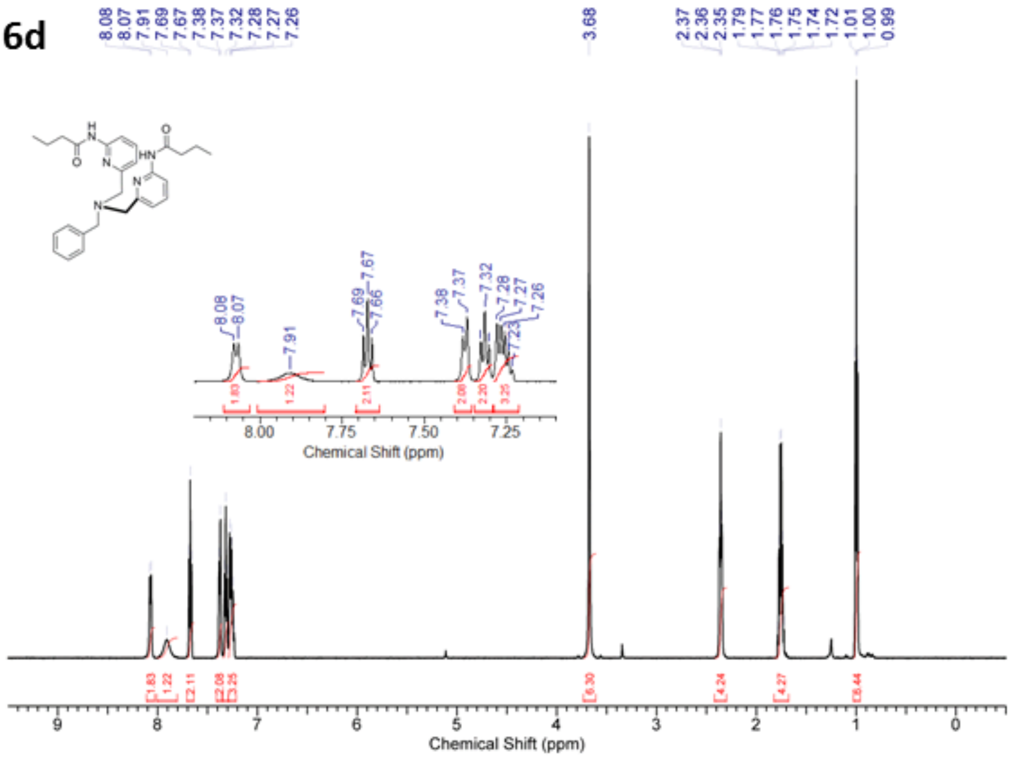

158.25
157.79
139.19
138.26
128.79
128.20
126.87
112.42
106.68
59.70
58.39
Chemical Shift (ppm)

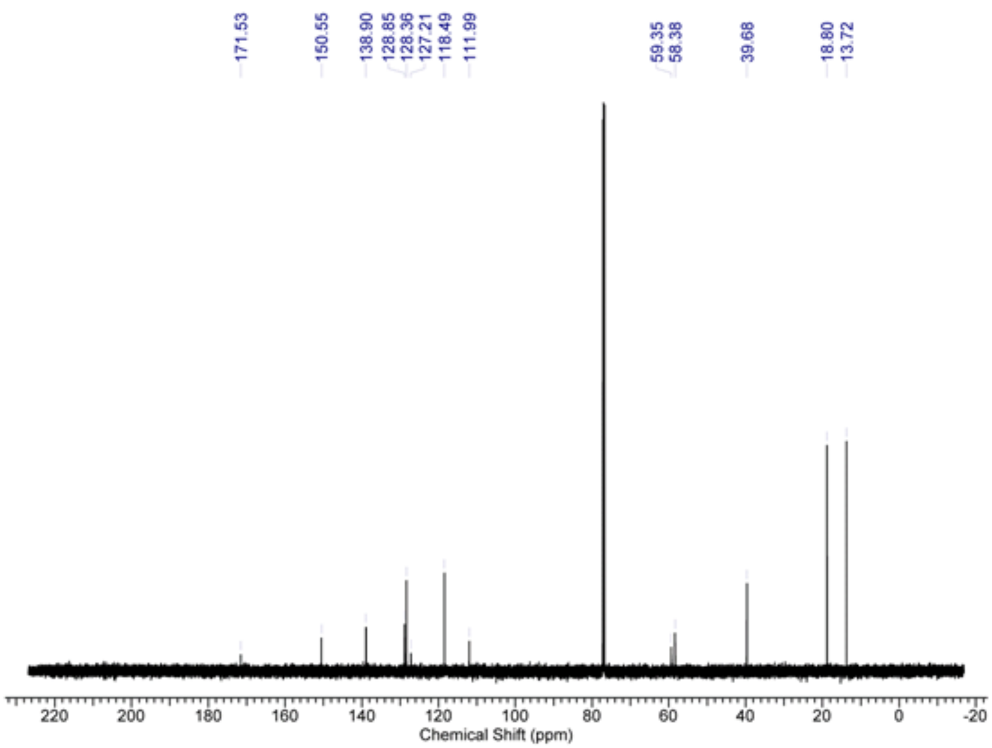
6c


9.62
9.03
7.55
7.54
7.52
7.39
7.37
7.33
7.32
7.08
7.07
6.72
6.70
3.68
3.67
3.41
3.40
3.39
3.38
1.59
1.58
1.57
1.55
1.44
1.43
1.42
1.41
0.95
0.94
0.92
7.30
7.25
7.23
1.99
2.01
2.03
0.98
1.96
Chemical Shift (ppm)
1.83
1.91
1.99
2.01
0.98
1.96
1.93
8.13
4.18
4.26
4.25
6.17
Chemical Shift (ppm)

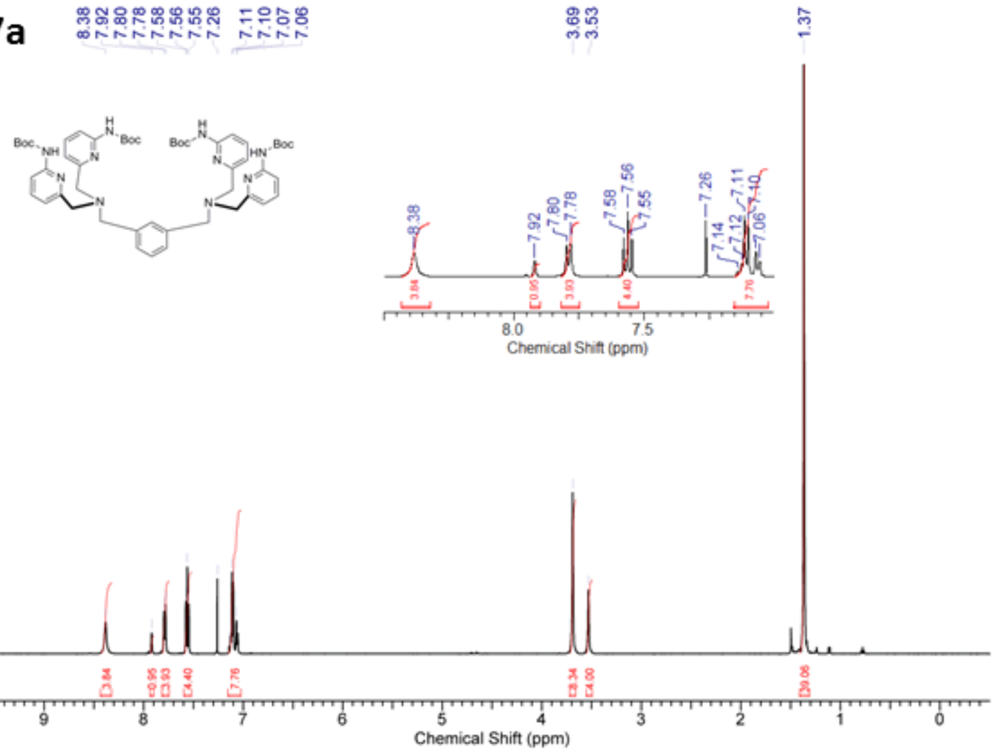

156.43
156.03
153.12
138.52
128.62
128.36
127.20
115.17
110.27
59.75
58.61
39.46
32.08
20.26
13.82
Chemical Shift (ppm)

6d

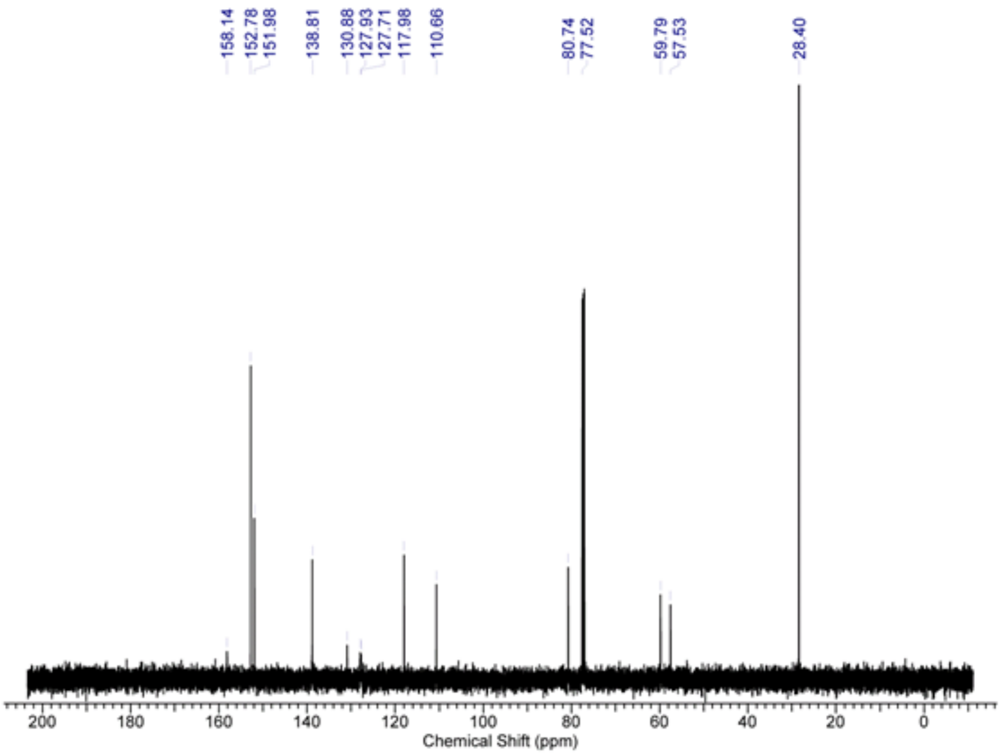




7a

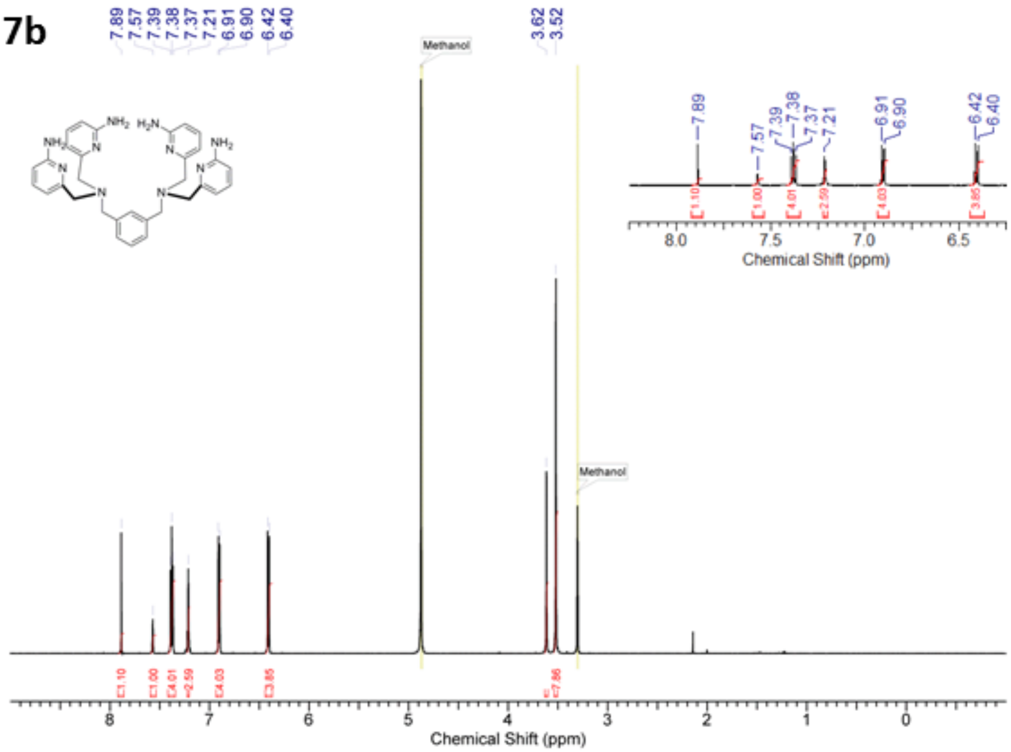


8.38
7.92
7.80
7.78
7.58
7.56
7.55
7.26
7.11
7.10
7.07
7.06
3.69
3.53
1.37
Boc
NH
N
HN
Chemical Shift (ppm)

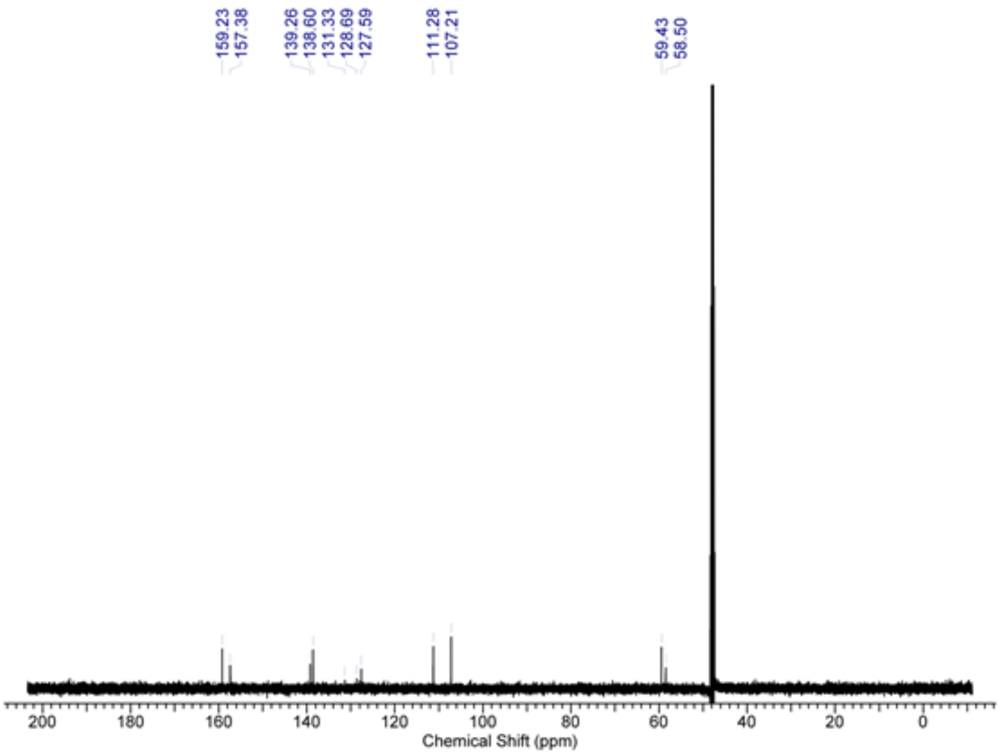


158.14
152.78
151.98
138.81
130.88
127.93
127.71
117.98
110.66
80.74
77.52
59.79
57.53
28.40
Chemical Shift (ppm)

7b

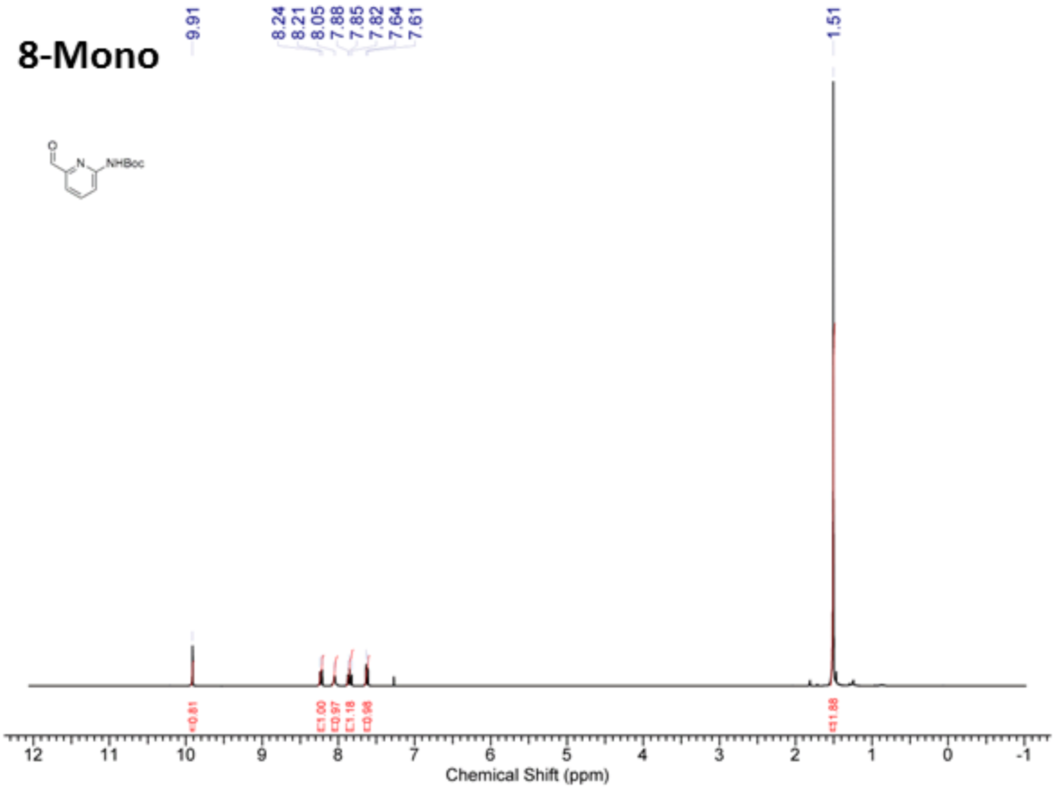


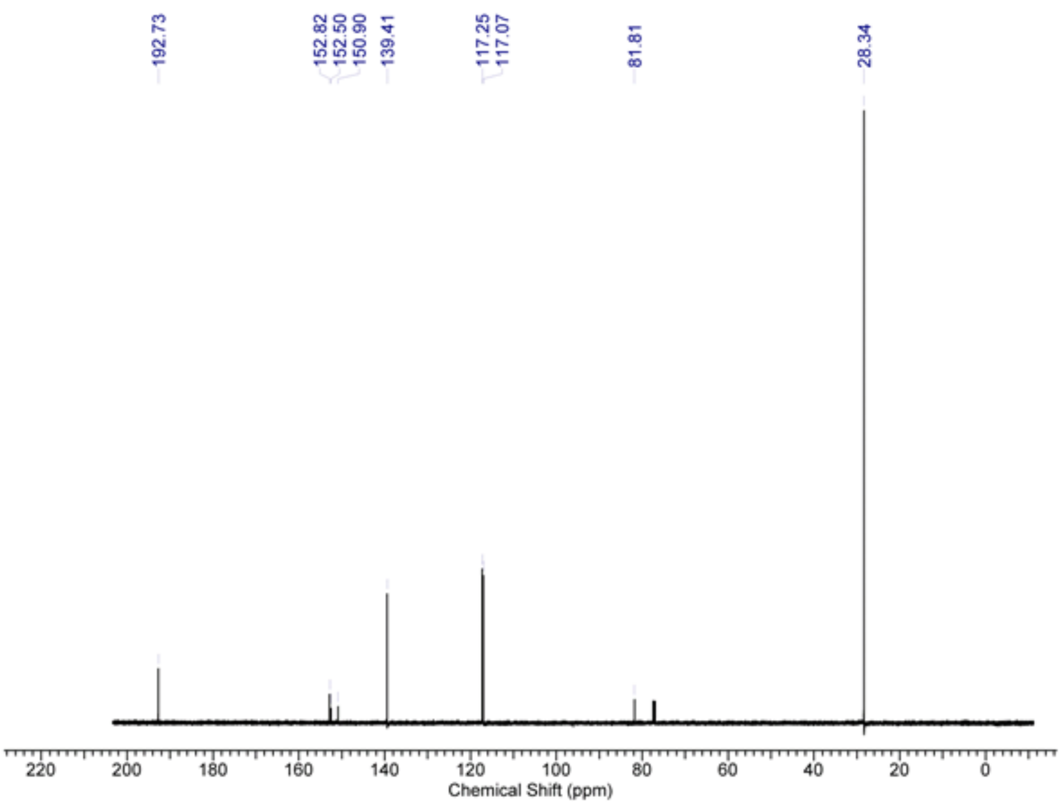
7.89
7.57
7.39
7.38
7.37
7.21
6.91
6.90
6.42
6.40
3.62
3.52
Methanol
Methanol
NH2
NH2
H2N
NH2
N
1.10
1.00
4.01
2.59
4.03
3.85
7.86
Chemical Shift (ppm)

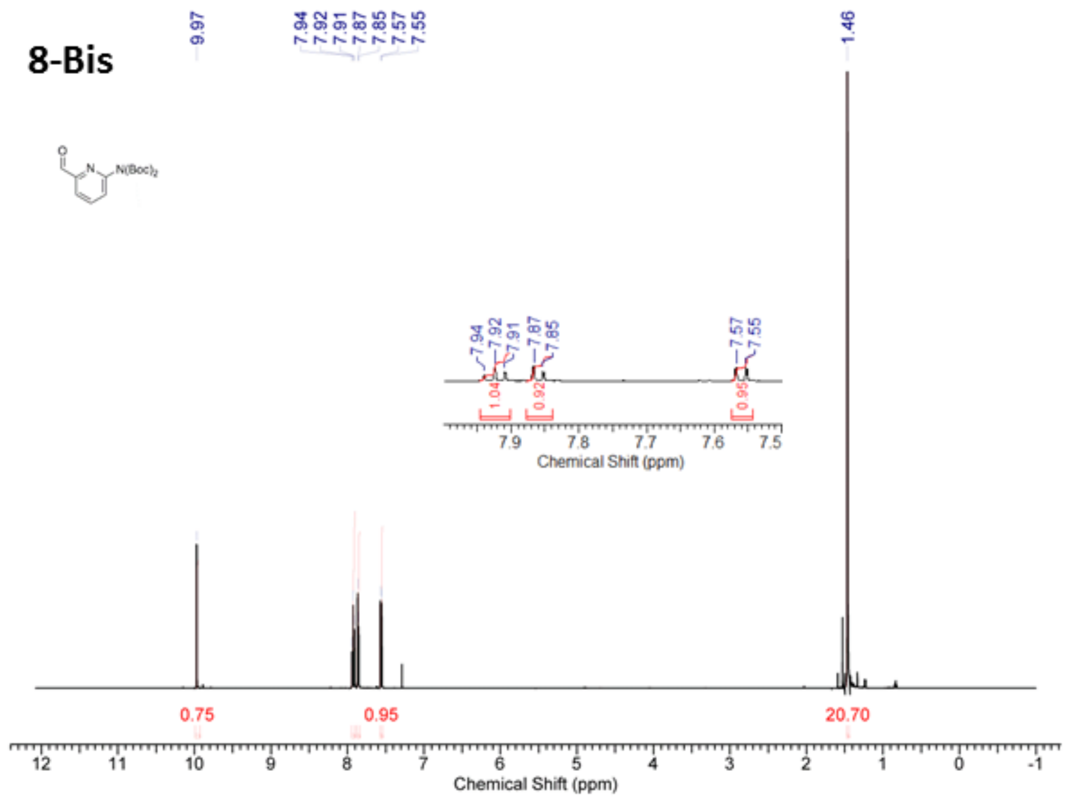


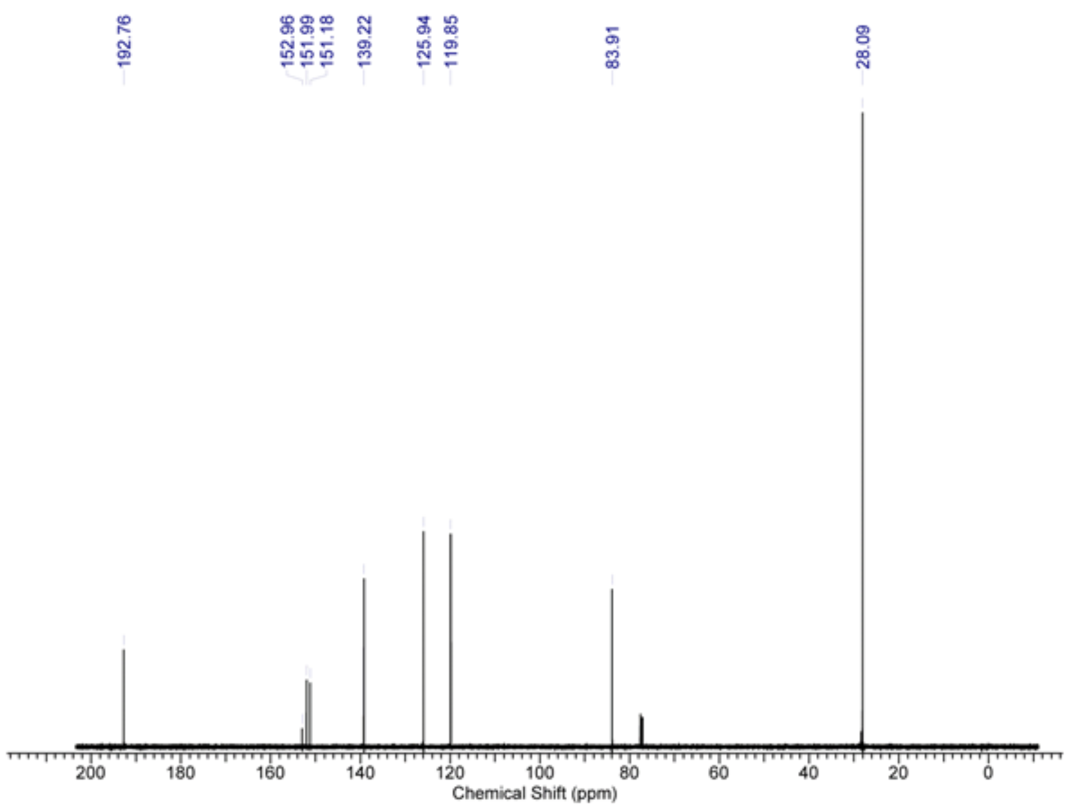
159.23
157.38
139.26
138.60
131.33
128.69
127.59
111.28
107.21
59.43
58.50
Chemical Shift (ppm)

8-Mono

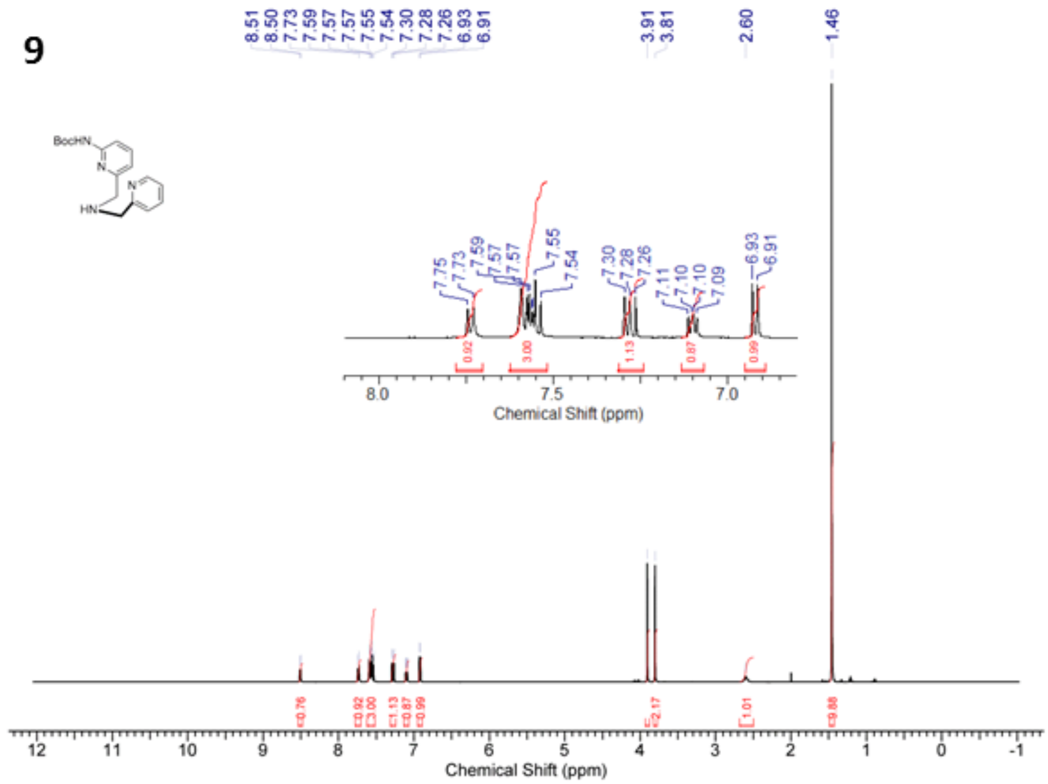

O
N
NHBoc
9.91
8.24
8.21
8.05
7.88
7.85
7.82
7.64
7.61
1.51
0.81
1.00
0.97
1.18
0.98
11.88
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
Chemical Shift (ppm)

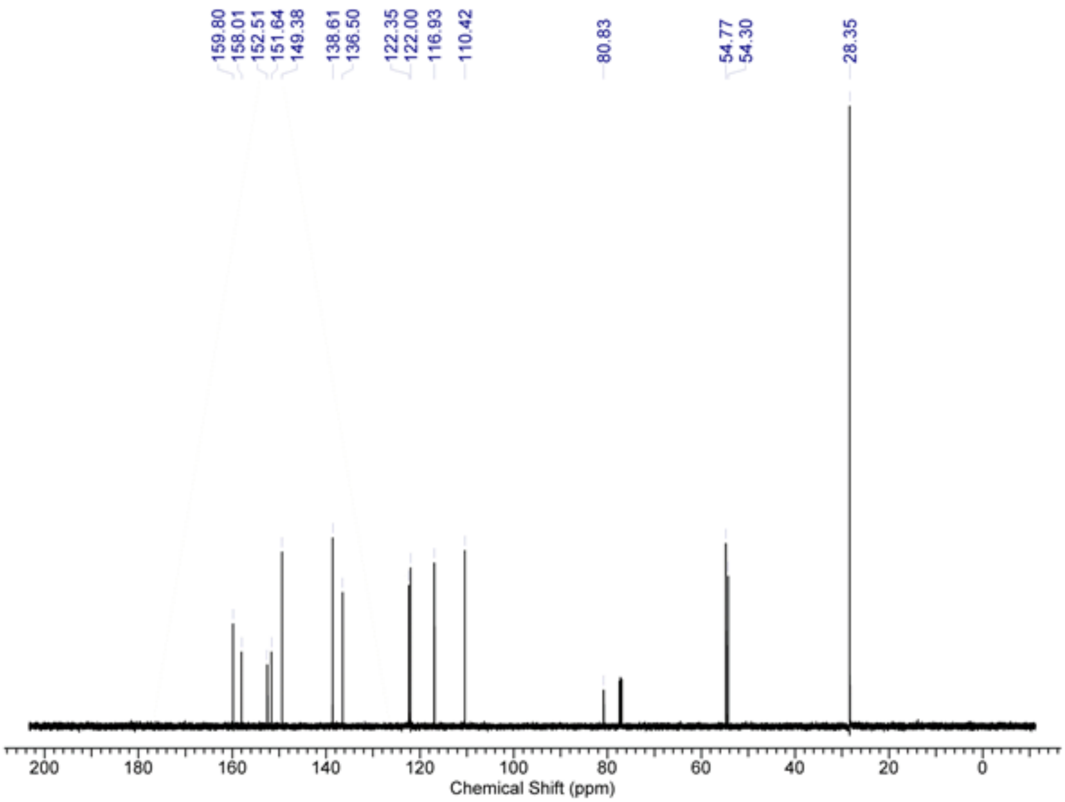

192.73
152.82
152.50
150.90
139.41
117.25
117.07
81.81
28.34
220
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)


8-Bis
N(Boc)2
9.97
7.94 7.92 7.91 7.87 7.85 7.57 7.55
1.46
1.04
0.92
0.95
0.75
0.95
20.70
Chemical Shift (ppm)

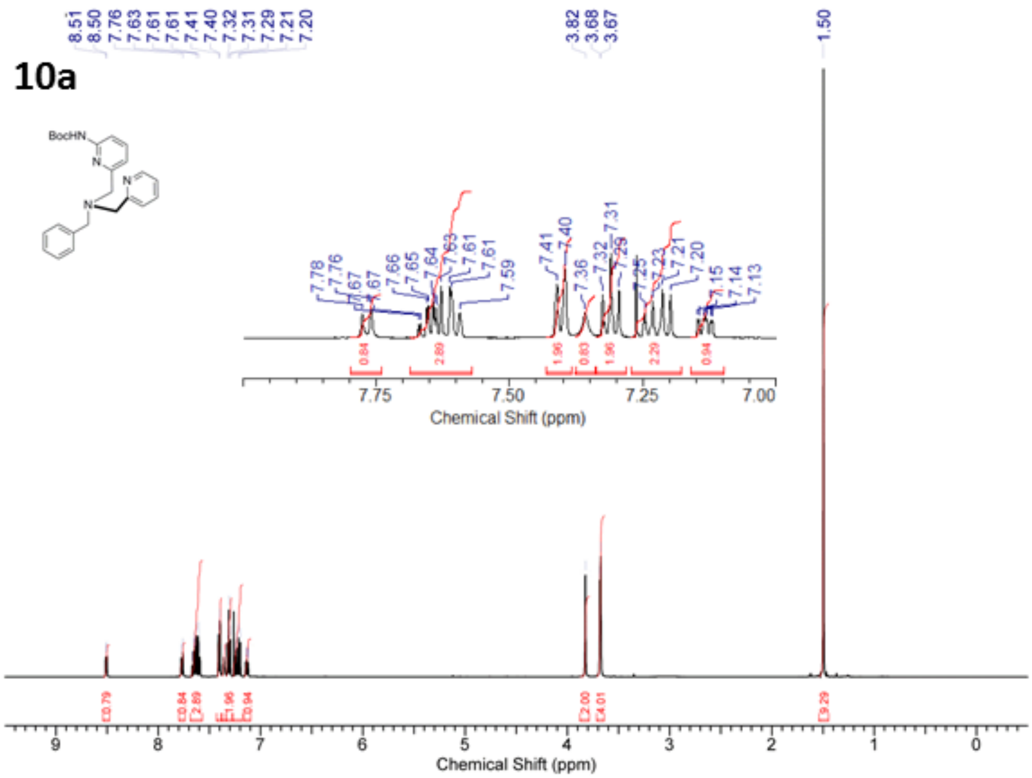

192.76
152.96 151.99 151.18
139.22
125.94 119.85
83.91
28.09
Chemical Shift (ppm)

9

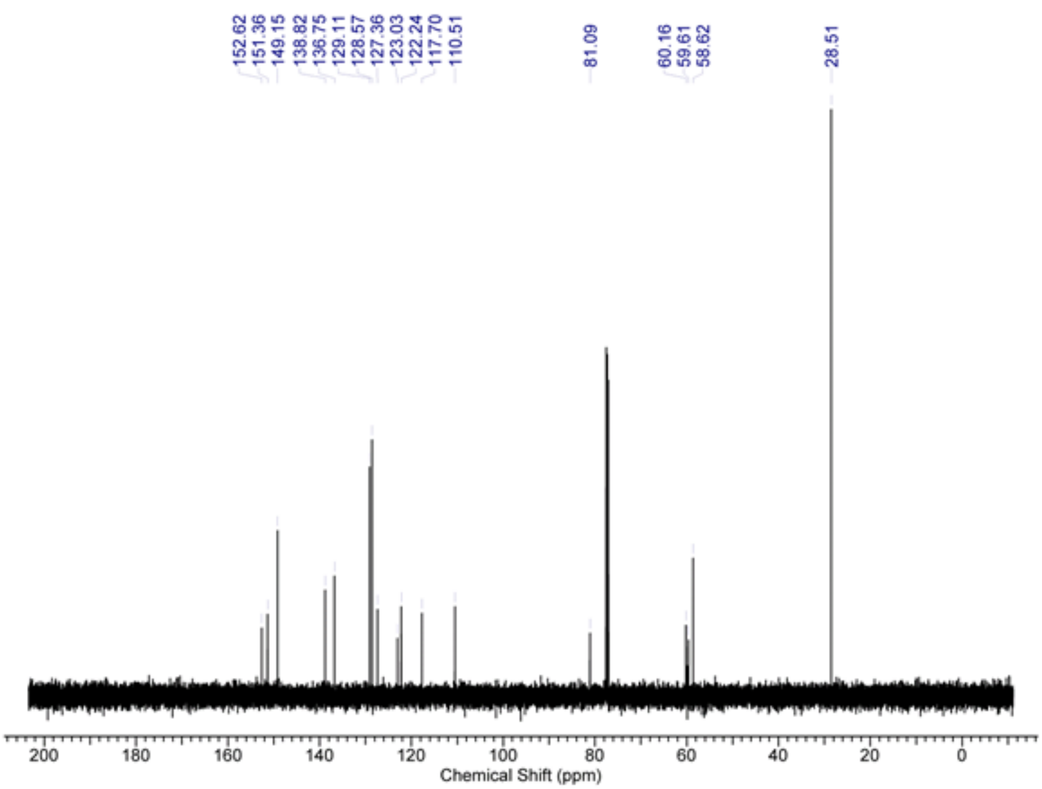

BocHN
N
N
HN
8.51
8.50
7.73
7.59
7.57
7.57
7.55
7.54
7.30
7.28
7.26
6.93
6.91
3.91
3.81
2.60
1.46
7.75
7.73
7.59
7.57
7.57
7.55
7.54
7.30
7.28
7.26
7.11
7.10
7.10
7.09
6.93
6.91
0.92
3.00
1.13
0.87
0.99
8.0
7.5
7.0
Chemical Shift (ppm)
0.76
0.92
3.00
1.13
0.87
0.99
2.17
1.01
9.88
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
Chemical Shift (ppm)


159.80
158.01
152.51
151.64
149.38
138.61
136.50
122.35
122.00
116.93
110.42
80.83
54.77
54.30
28.35
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

10a

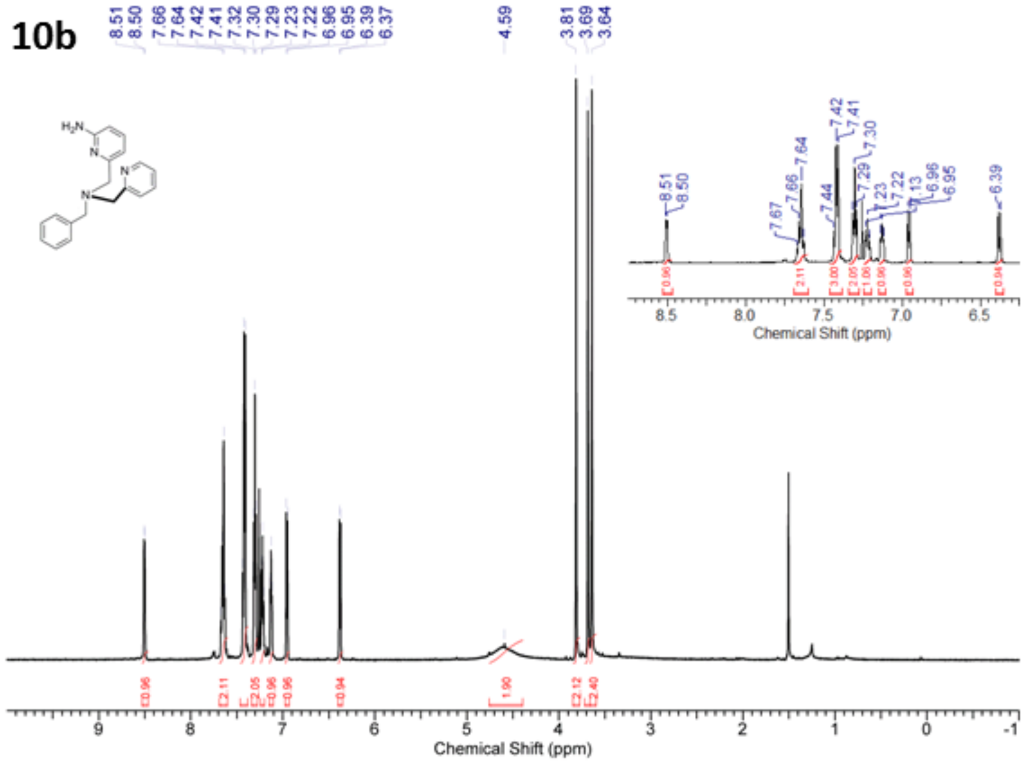


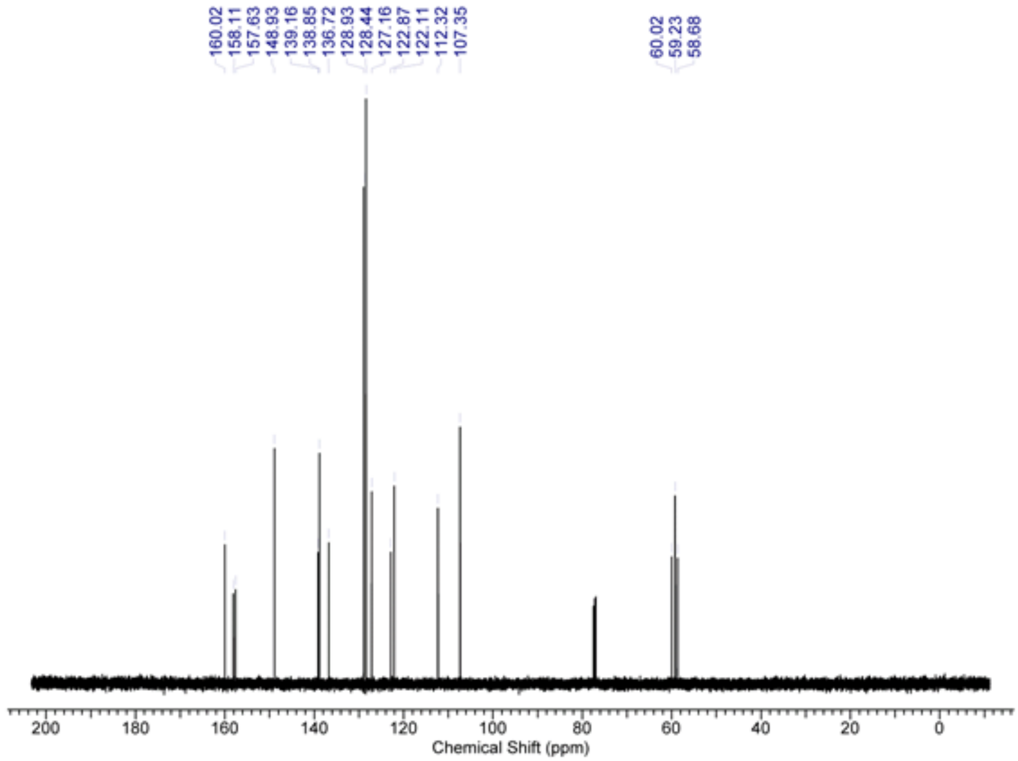
BocHN
N
N
N
Chemical Shift (ppm)



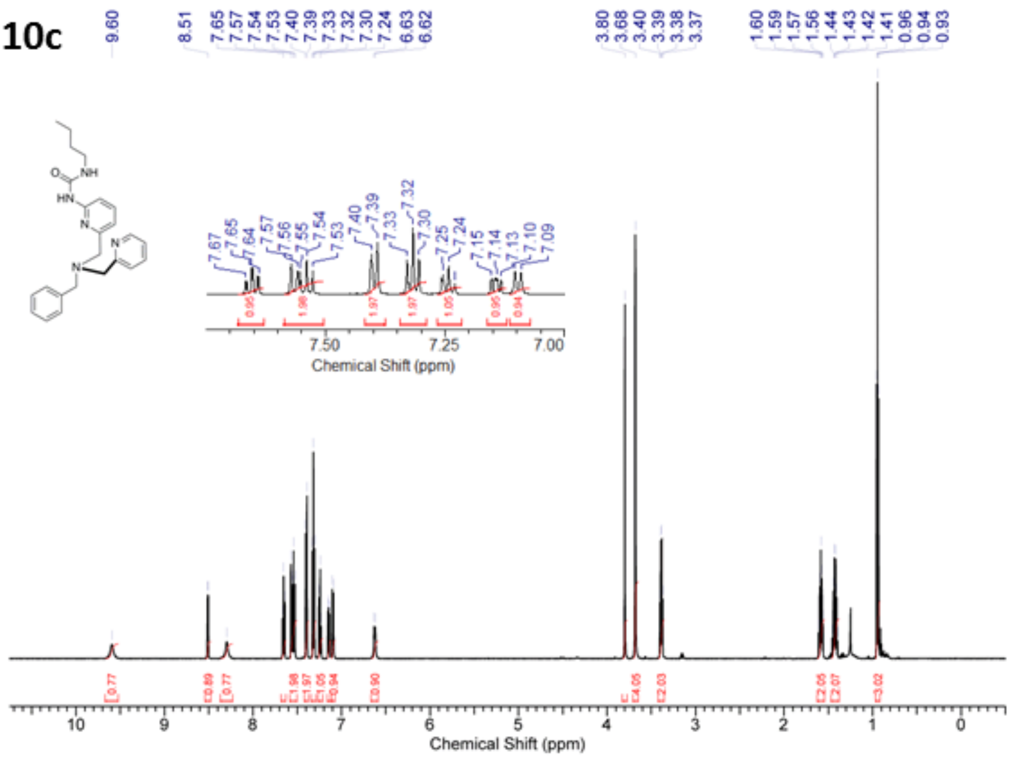
152.62
151.36
149.15
138.82
136.75
129.11
128.57
127.36
123.03
122.24
117.70
110.51
81.09
60.16
59.61
58.62
28.51
Chemical Shift (ppm)

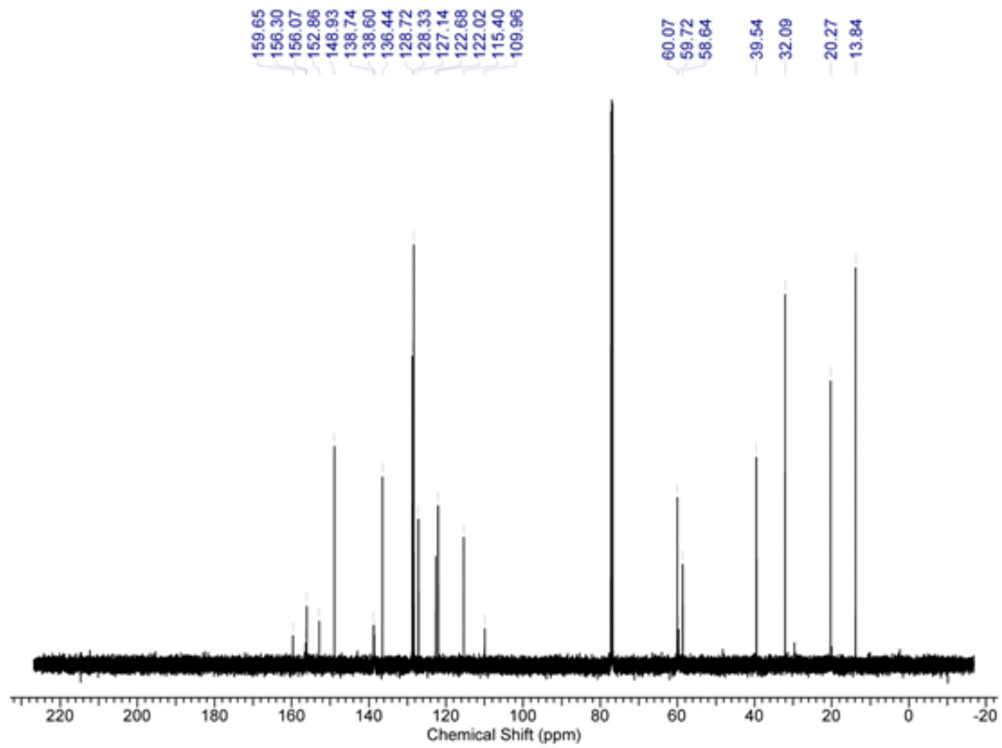
10b



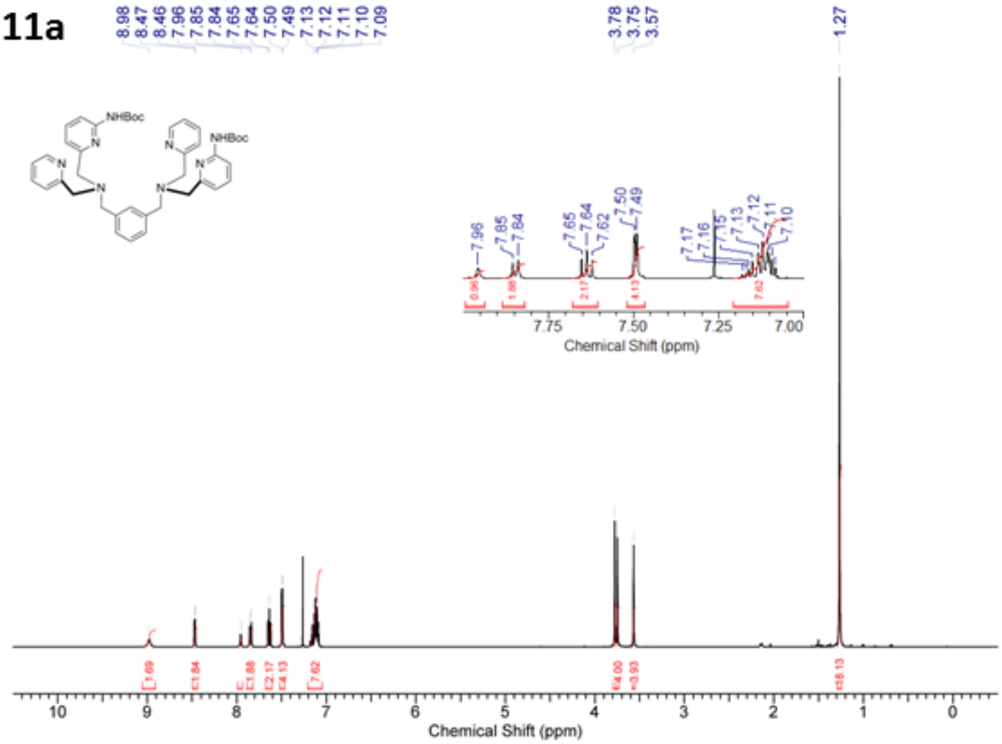


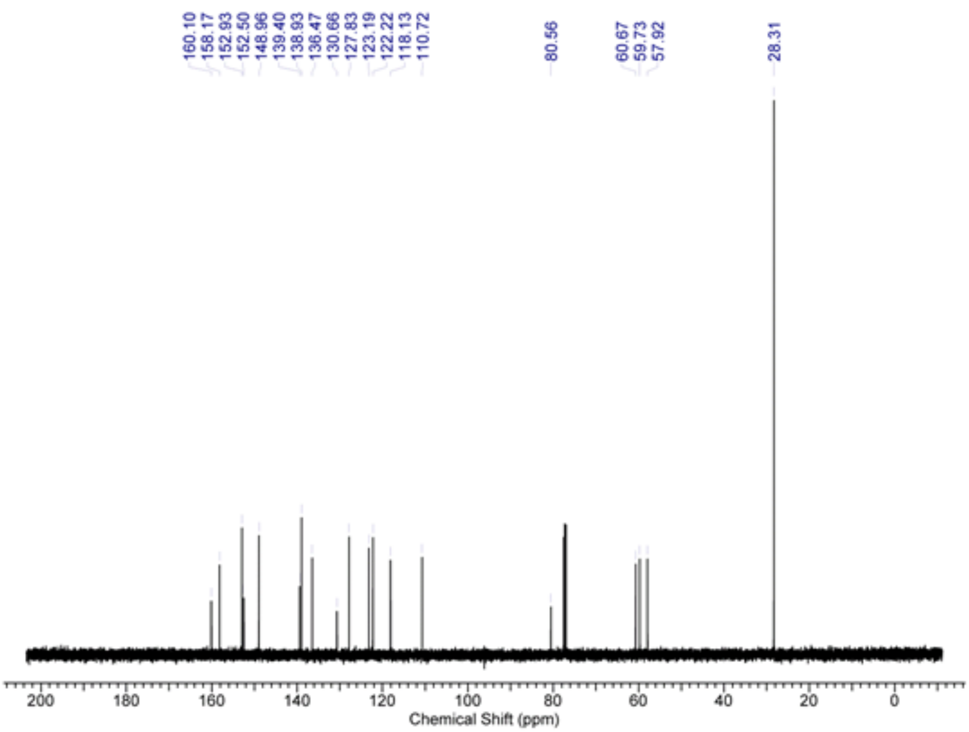
10c



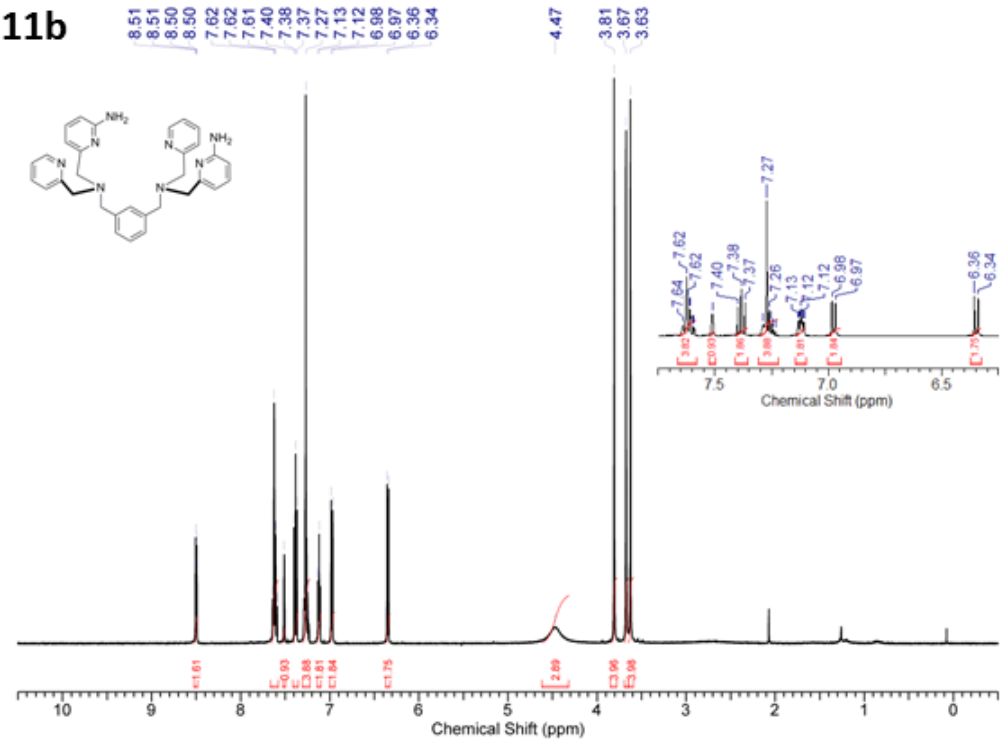


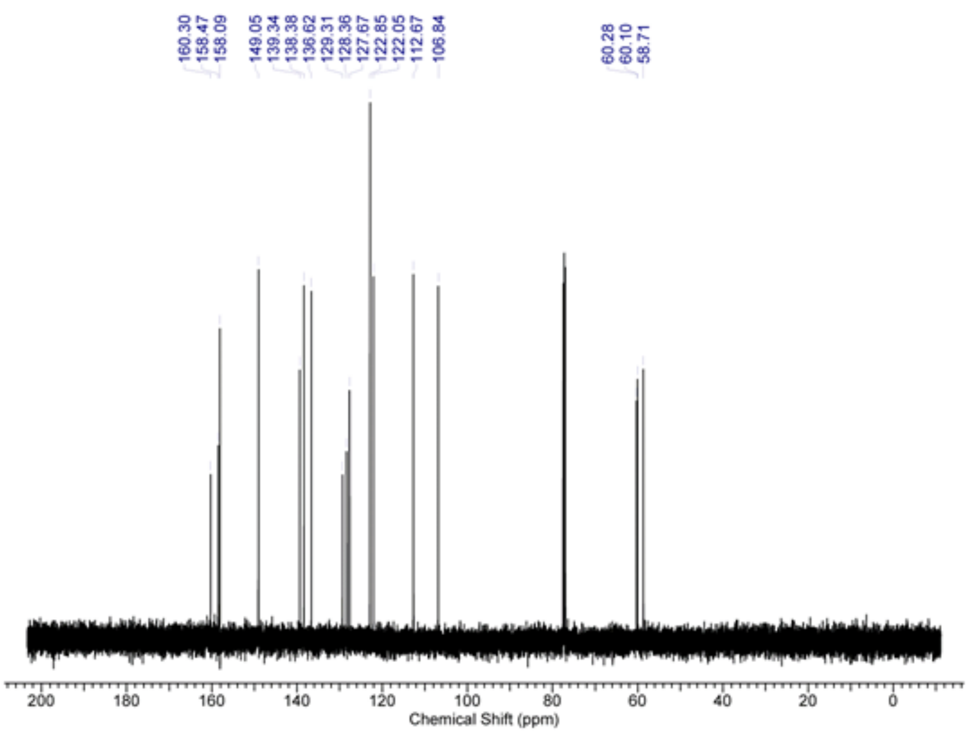
11a


NHBoc
NHBoc
N
8.98
8.47
8.46
7.96
7.85
7.84
7.65
7.64
7.50
7.49
7.13
7.12
7.11
7.10
7.09
3.78
3.75
3.57
1.27
Chemical Shift (ppm)

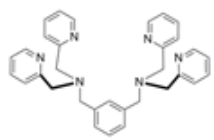

160.10
158.17
152.93
152.50
148.96
139.40
138.93
136.47
130.66
127.83
123.19
122.22
118.13
110.72
80.56
60.67
59.73
57.92
28.31
Chemical Shift (ppm)

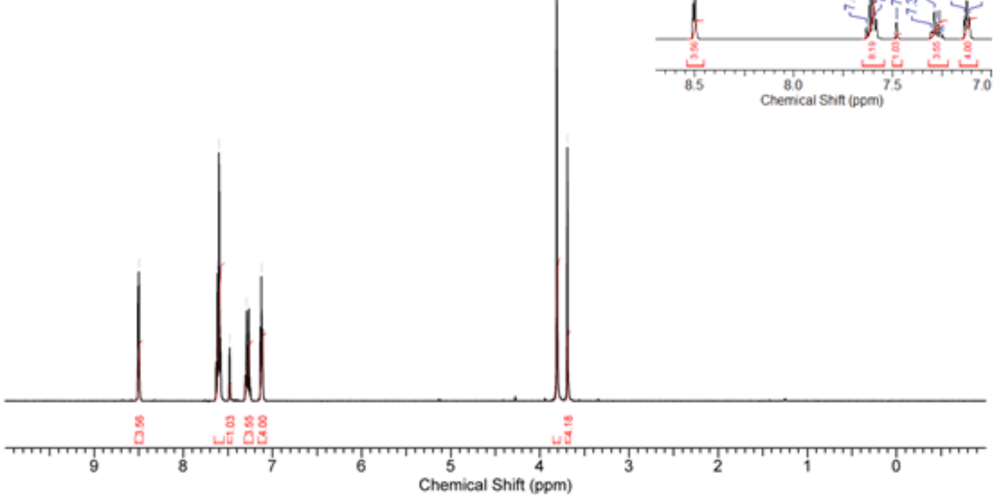
11b

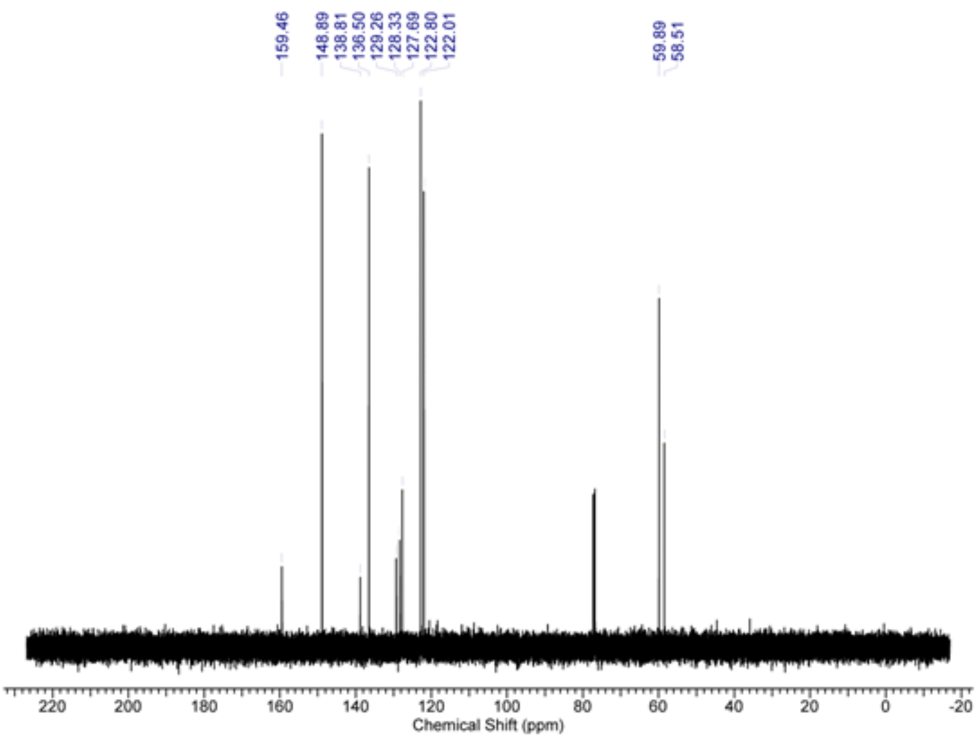

8.51
8.51
8.50
8.50
7.62
7.62
7.61
7.40
7.38
7.37
7.27
7.13
7.12
6.98
6.97
6.36
6.34
4.47
3.81
3.67
3.63
NH2
NH2
N
N
N
N
N
N
Chemical Shift (ppm)
Chemical Shift (ppm)


160.30
158.47
158.09
149.05
139.34
138.38
136.62
129.31
128.36
127.67
122.85
122.05
112.67
106.84
60.28
60.10
58.71
Chemical Shift (ppm)

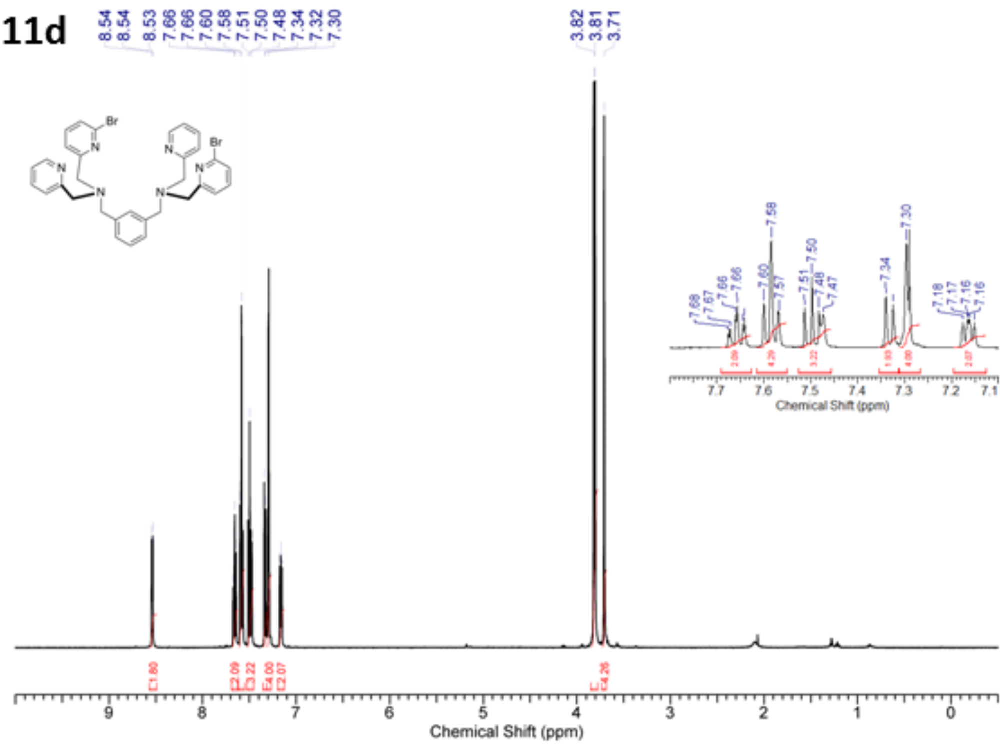
11c

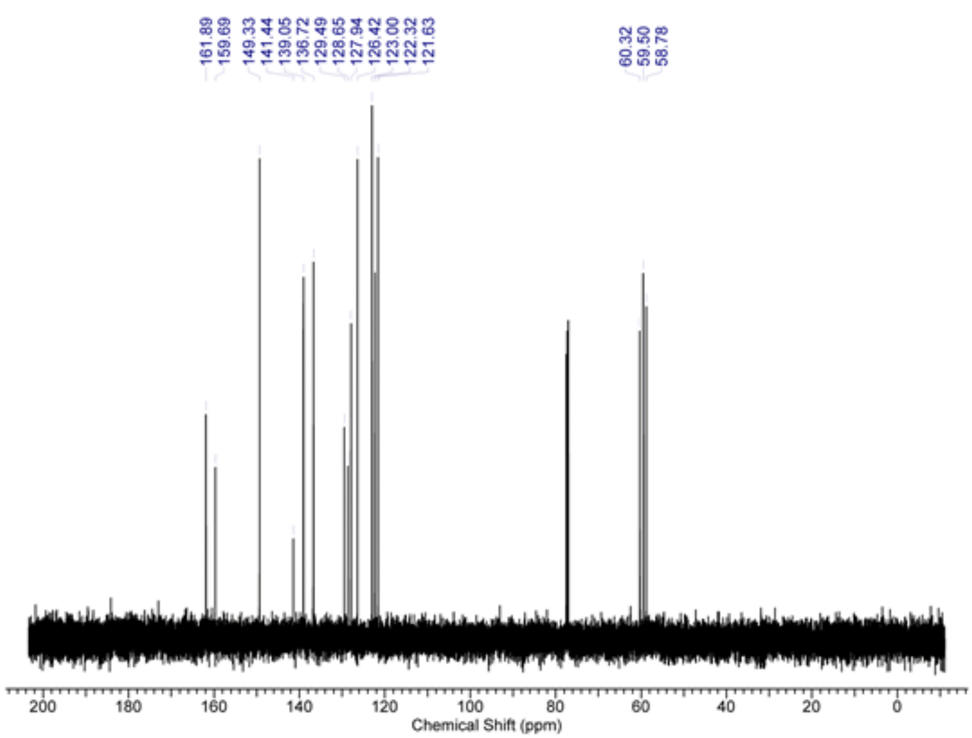



8.51
8.50
7.63
7.62
7.60
7.58
7.48
7.29
7.28
7.14
7.13
7.11
3.81
3.69
Chemical Shift (ppm)

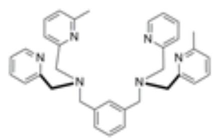

159.46
148.89
138.81
136.50
129.26
128.33
127.69
122.80
122.01
59.89
58.51
Chemical Shift (ppm)

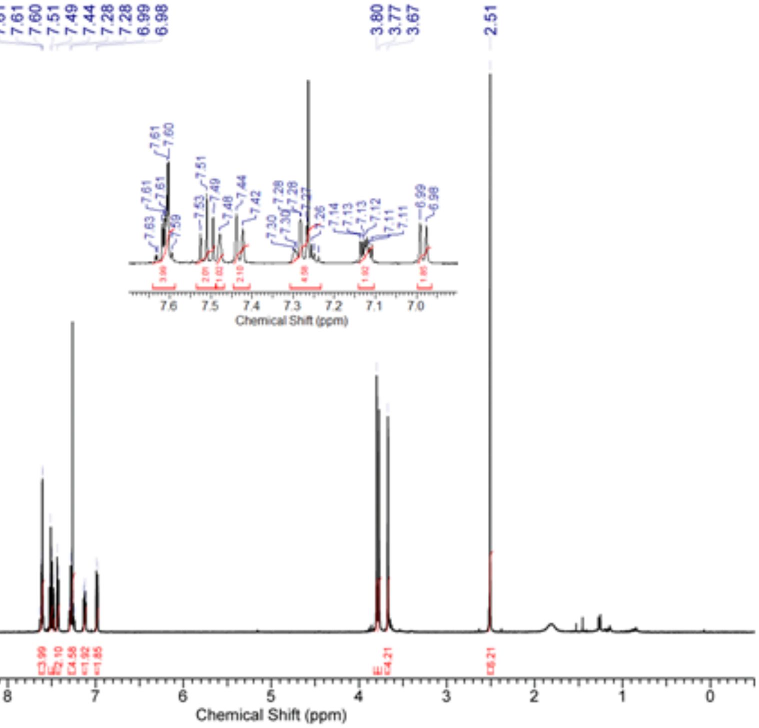
11d

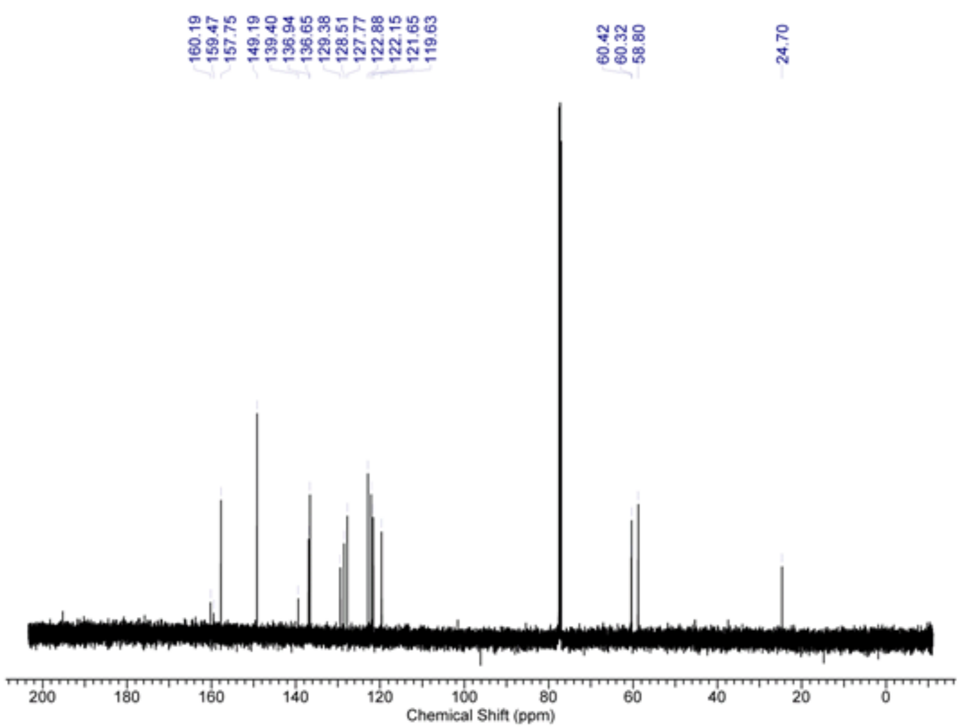




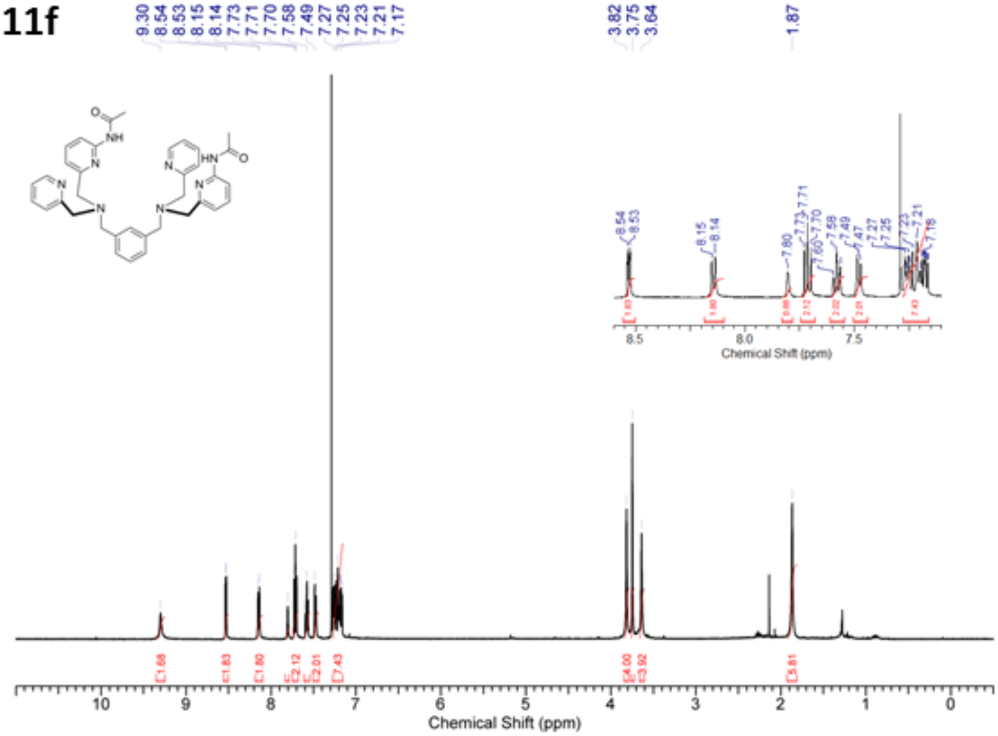
11e

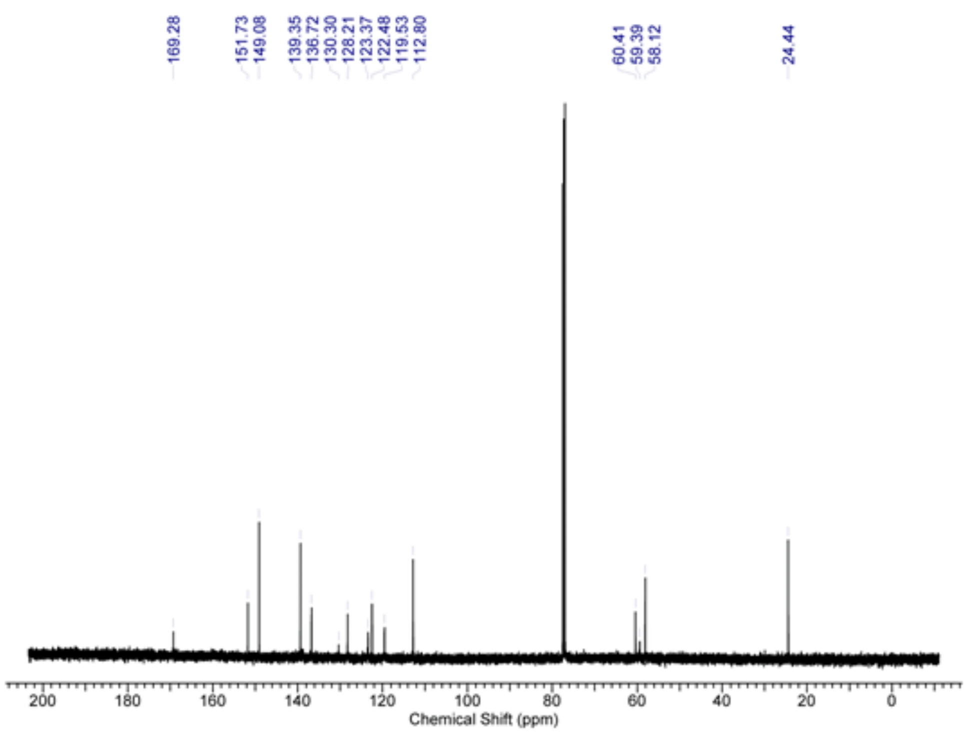




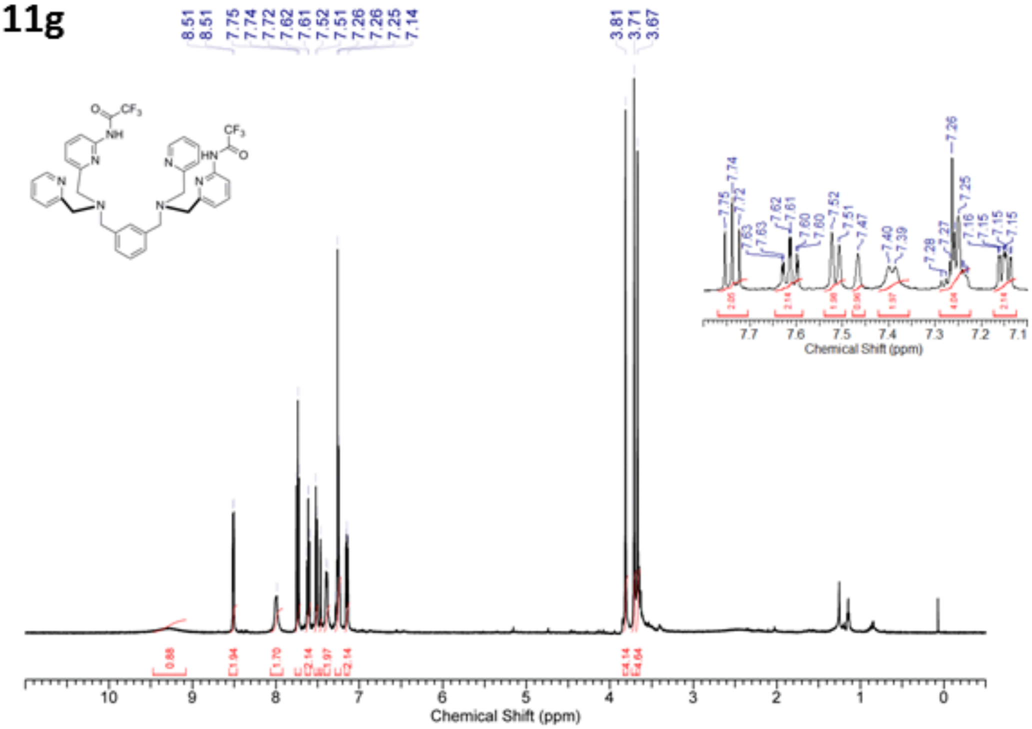


11f

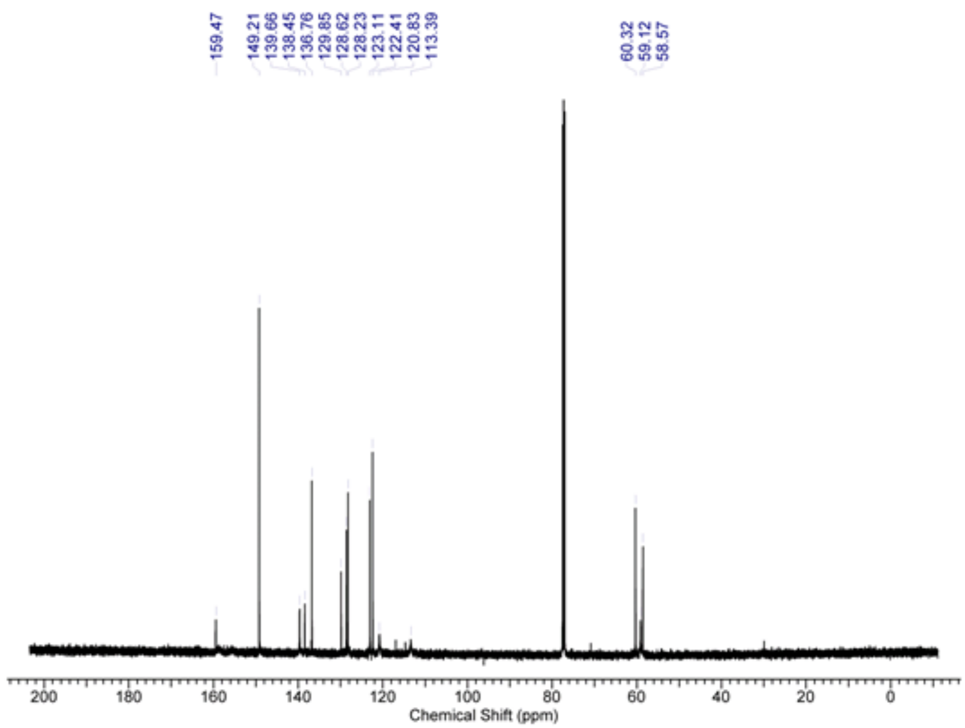

9.30 8.54 8.53 8.15 8.14 7.73 7.71 7.70 7.58 7.49 7.27 7.25 7.23 7.21 7.17
3.82 3.75 3.64
1.87
1.68 1.83 1.80 2.12 2.01 7.43 4.00 3.92 5.81
Chemical Shift (ppm)


169.28
151.73 149.08
139.35 136.72 130.30 128.21 123.37 122.48 119.53 112.80
60.41 59.39 58.12
24.44
Chemical Shift (ppm)

11g

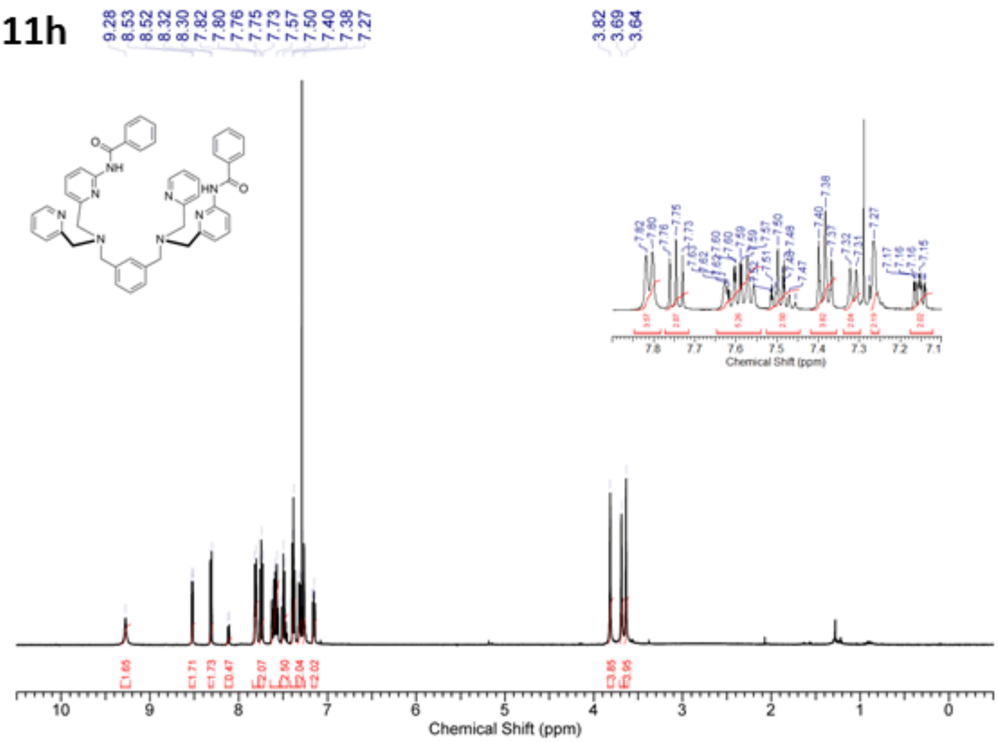


8.51 8.51 7.75 7.74 7.72 7.62 7.61 7.52 7.51 7.26 7.26 7.25 7.14
3.81 3.71 3.67
CF3
NH
HN
N
0.88 1.94 1.70 2.14 1.97 2.14 4.14 4.64
Chemical Shift (ppm)

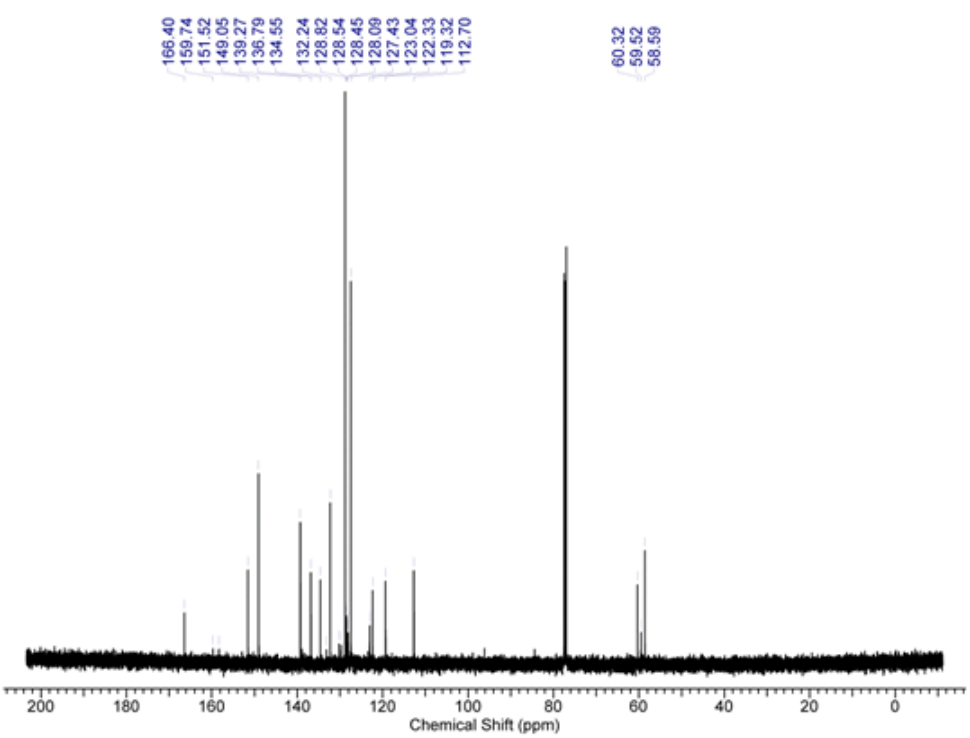


159.47
149.21 139.66 138.45 136.76 129.85 128.62 128.23 123.11 122.41 120.83 113.39
60.32 59.12 58.57
Chemical Shift (ppm)

11h

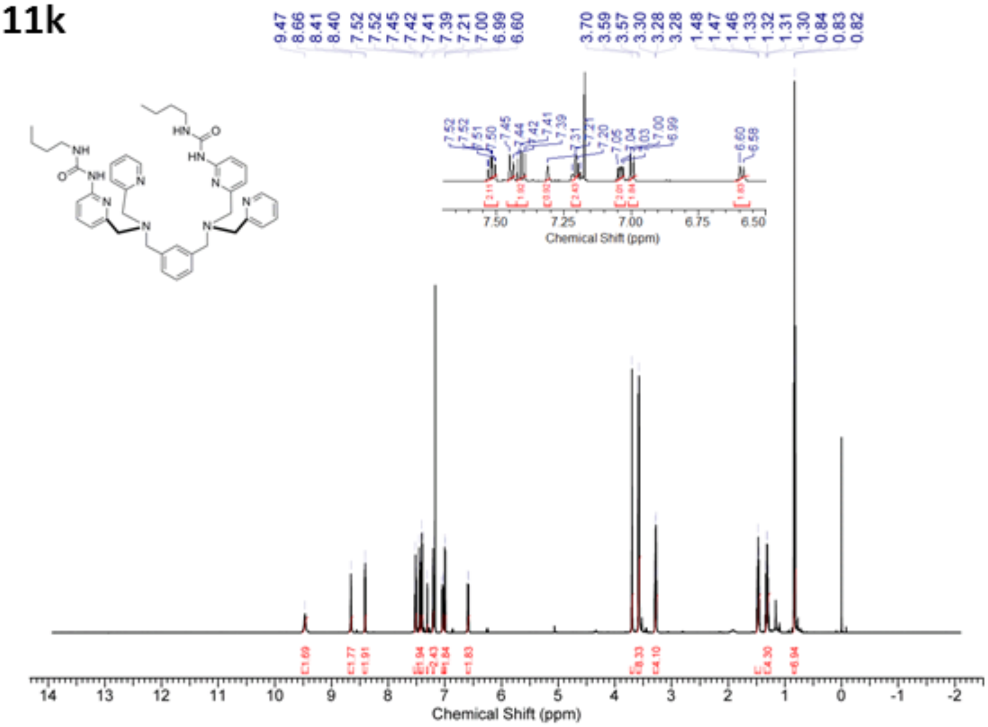


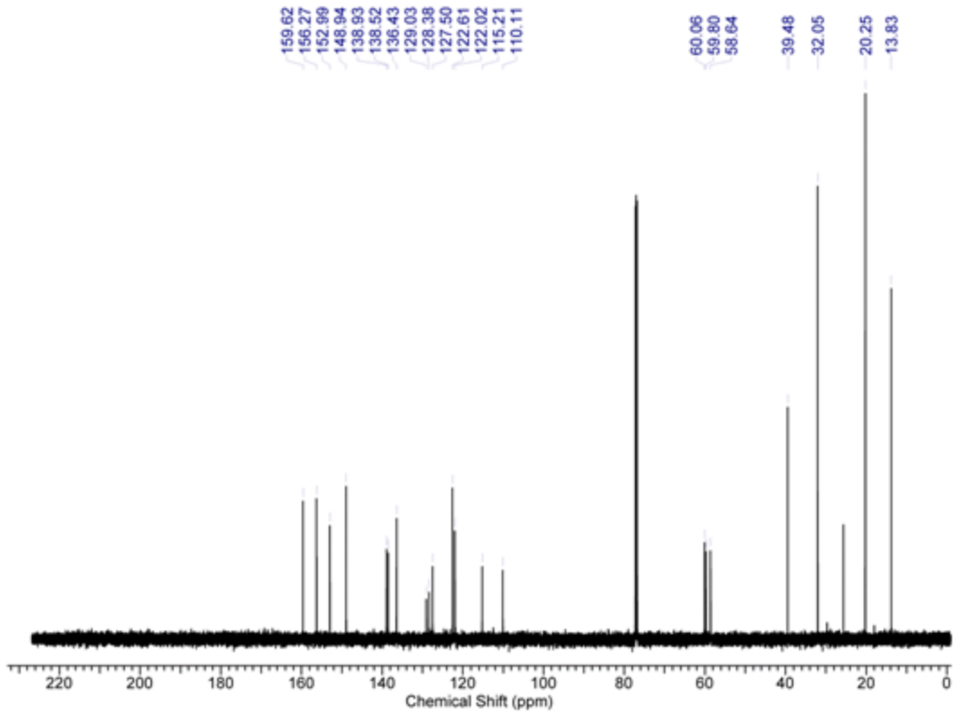
O
NH
N
HN
9.28 8.53 8.52 8.32 8.30 7.82 7.80 7.76 7.75 7.73 7.57 7.50 7.40 7.38 7.27
3.82 3.69 3.64
1.65 1.71 1.73 0.47 2.07 2.00 2.04 2.02
3.85 3.95
Chemical Shift (ppm)



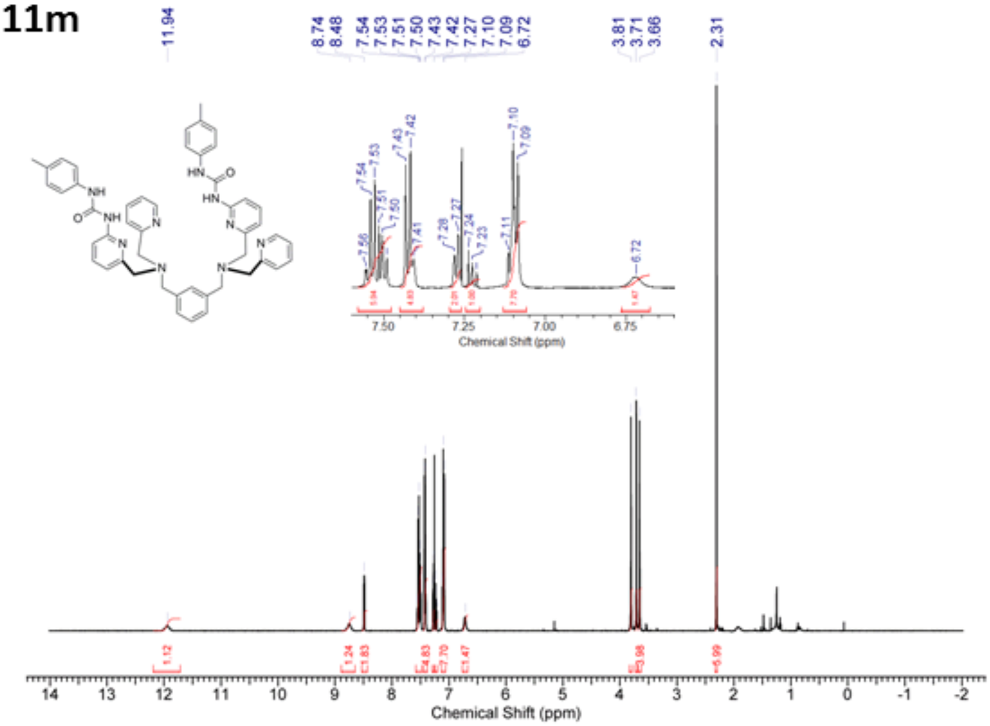
166.40 159.74 151.52 149.05 139.27 136.79 134.55 132.24 128.82 128.54 128.45 128.09 127.43 123.04 122.33 119.32 112.70
60.32 59.52 58.59
Chemical Shift (ppm)

11k

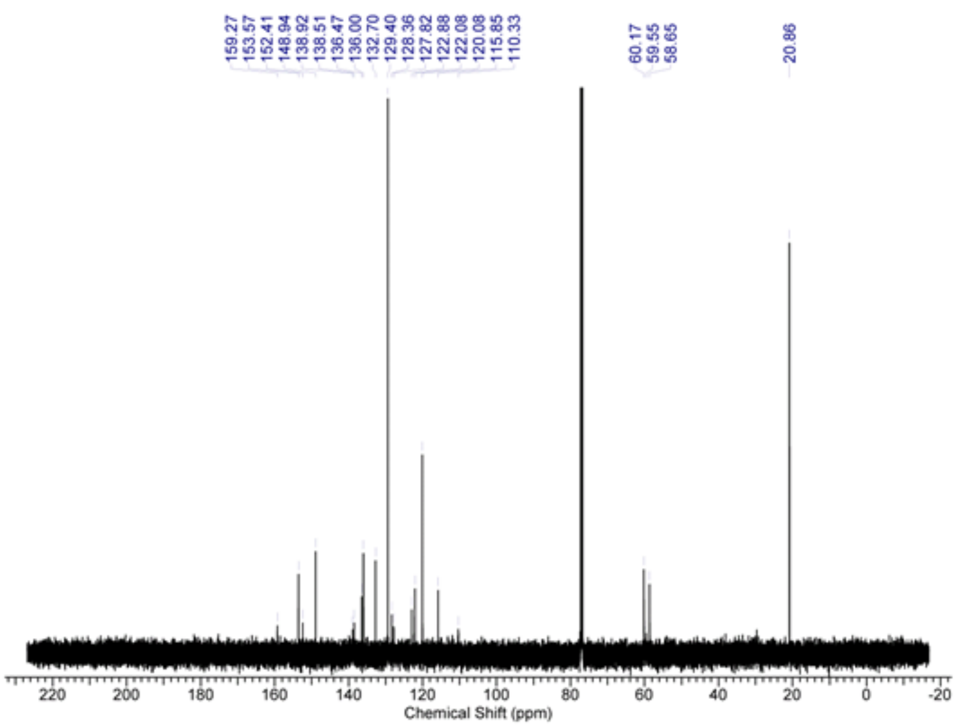




11m

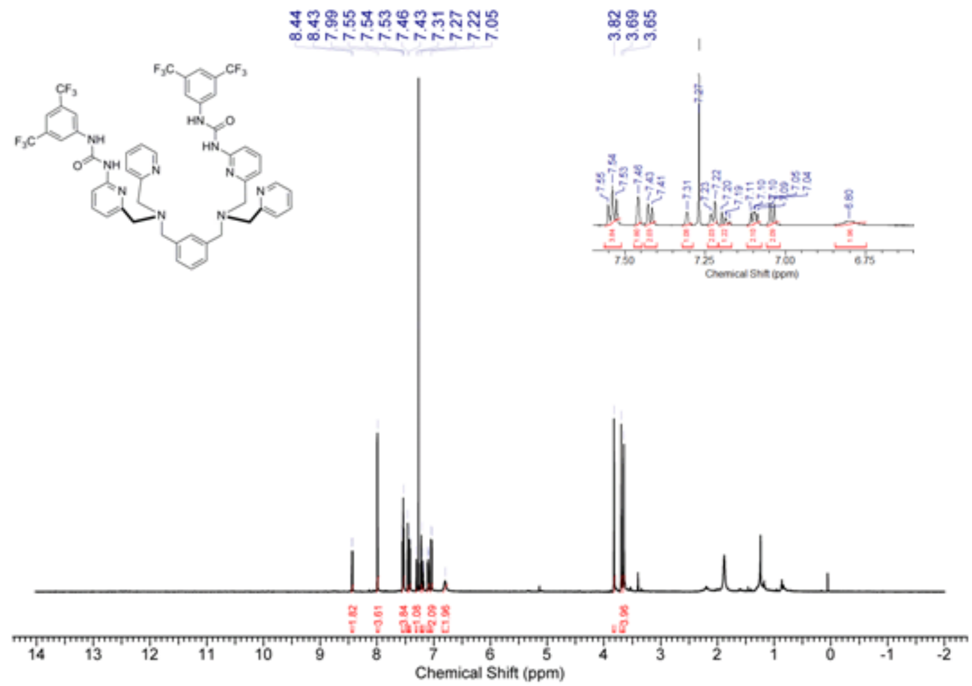


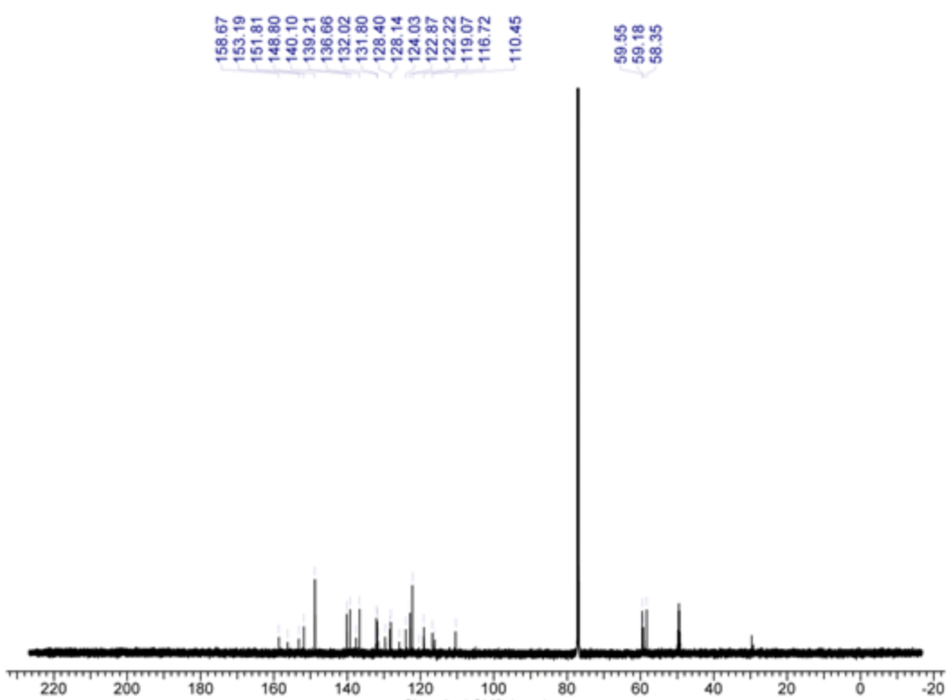
11.94
8.74
8.48
7.54
7.53
7.51
7.50
7.43
7.42
7.27
7.10
7.09
6.72
3.81
3.71
3.66
2.31
Chemical Shift (ppm)



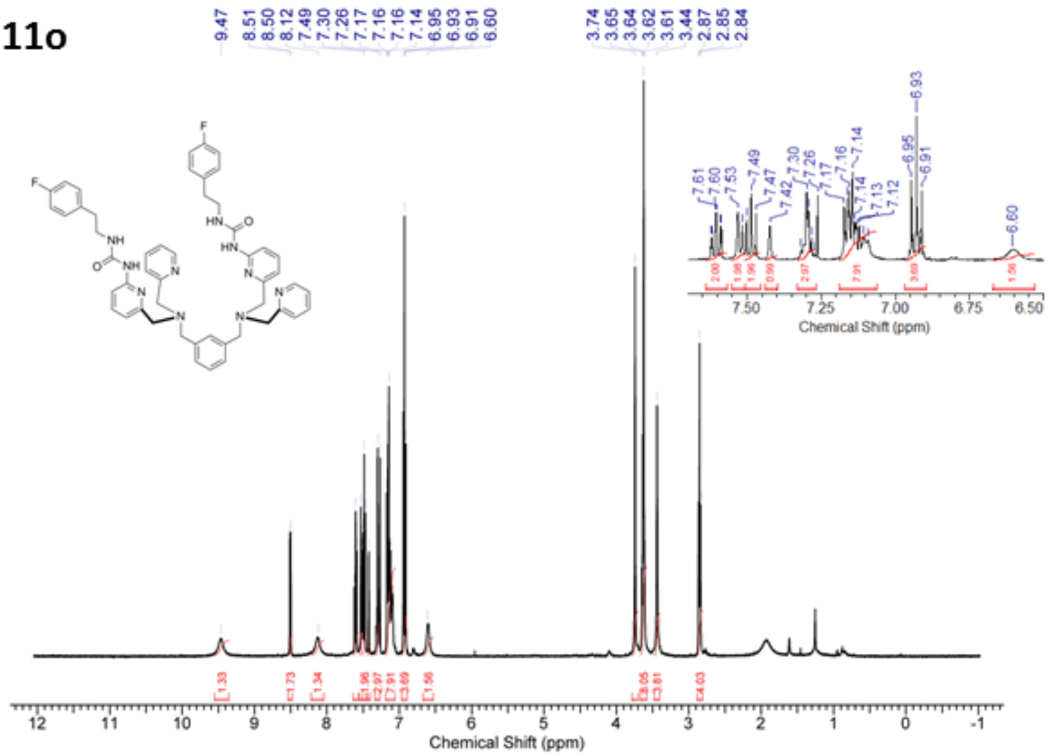
159.27
153.57
152.41
148.94
138.92
138.51
136.47
136.00
132.70
129.40
128.36
127.82
122.88
122.08
120.08
115.85
110.33
60.17
59.55
58.65
20.86
Chemical Shift (ppm)

11n

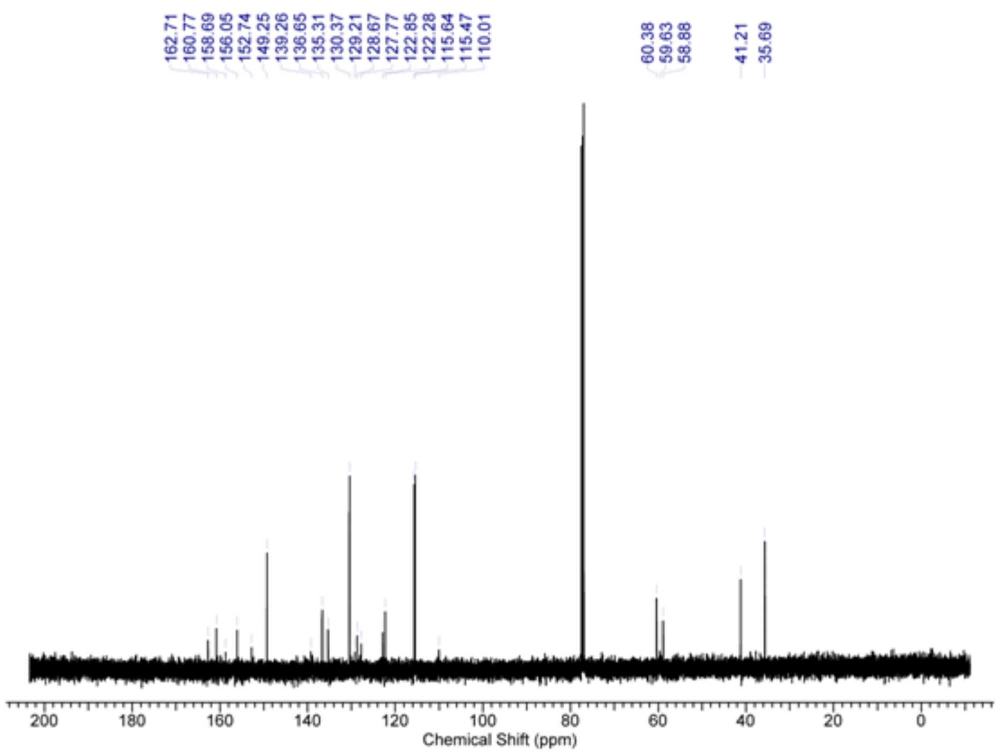




11o

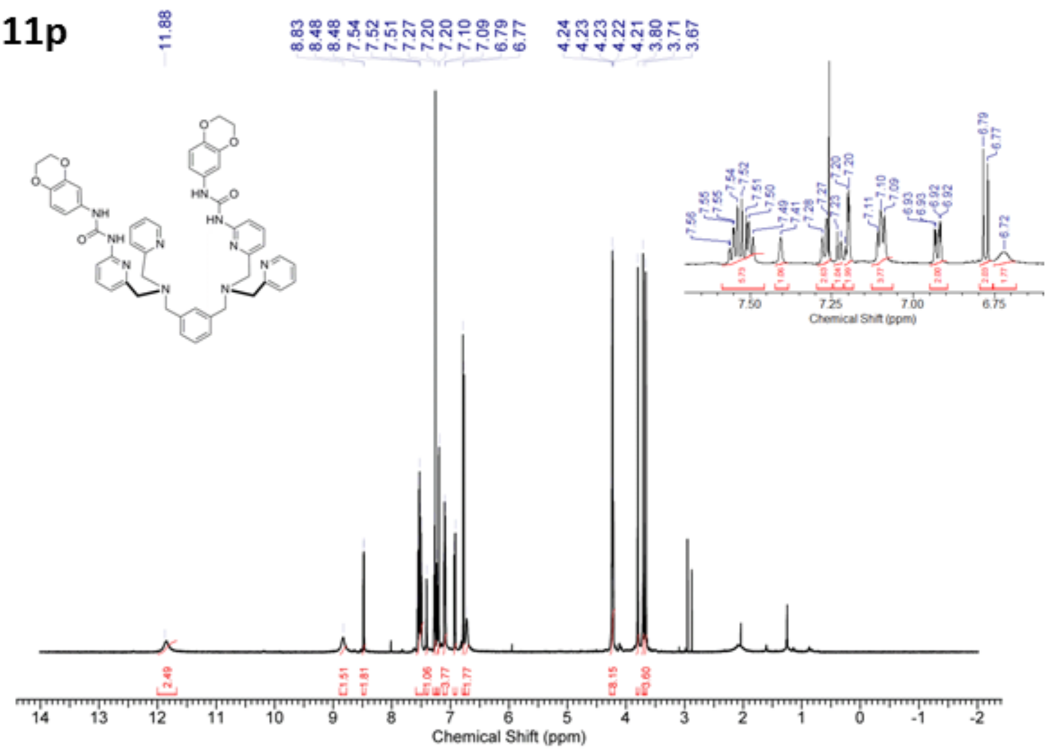


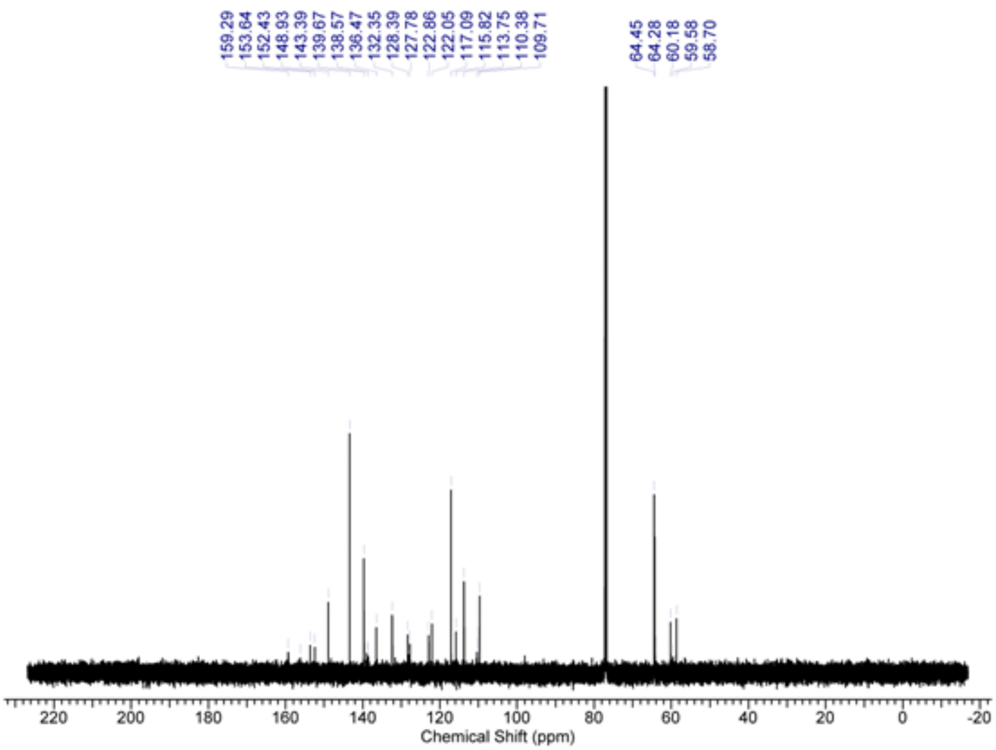
9.47
8.51
8.50
8.12
7.49
7.30
7.26
7.17
7.16
7.16
7.14
6.95
6.93
6.91
6.60
3.74
3.65
3.64
3.62
3.61
3.44
2.87
2.85
2.84
Chemical Shift (ppm)



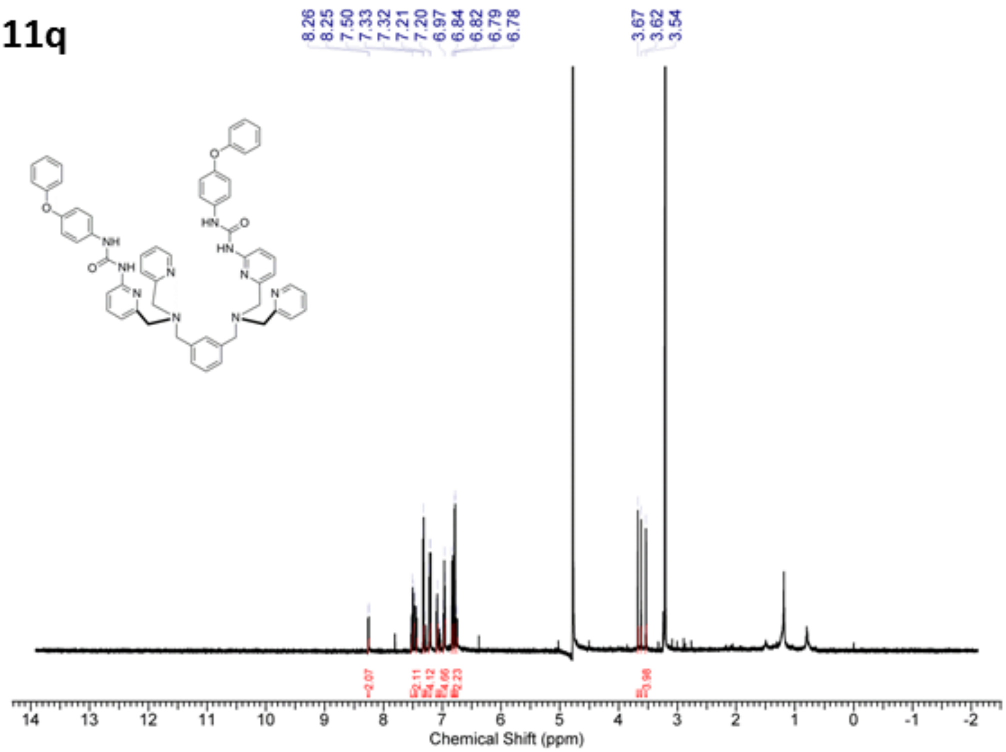
162.71
160.77
158.69
156.05
152.74
149.25
139.26
136.65
135.31
130.37
129.21
128.67
127.77
122.85
122.28
115.64
115.47
110.01
60.38
59.63
58.88
41.21
35.69
Chemical Shift (ppm)

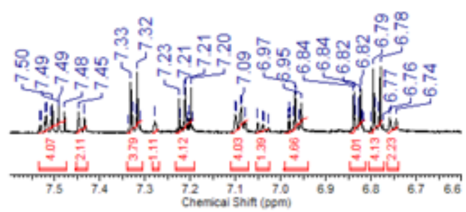
11p


11.88
8.83
8.48
8.48
7.54
7.52
7.51
7.27
7.20
7.20
7.10
7.09
6.79
6.77
4.24
4.23
4.23
4.22
4.21
3.80
3.71
3.67
Chemical Shift (ppm)

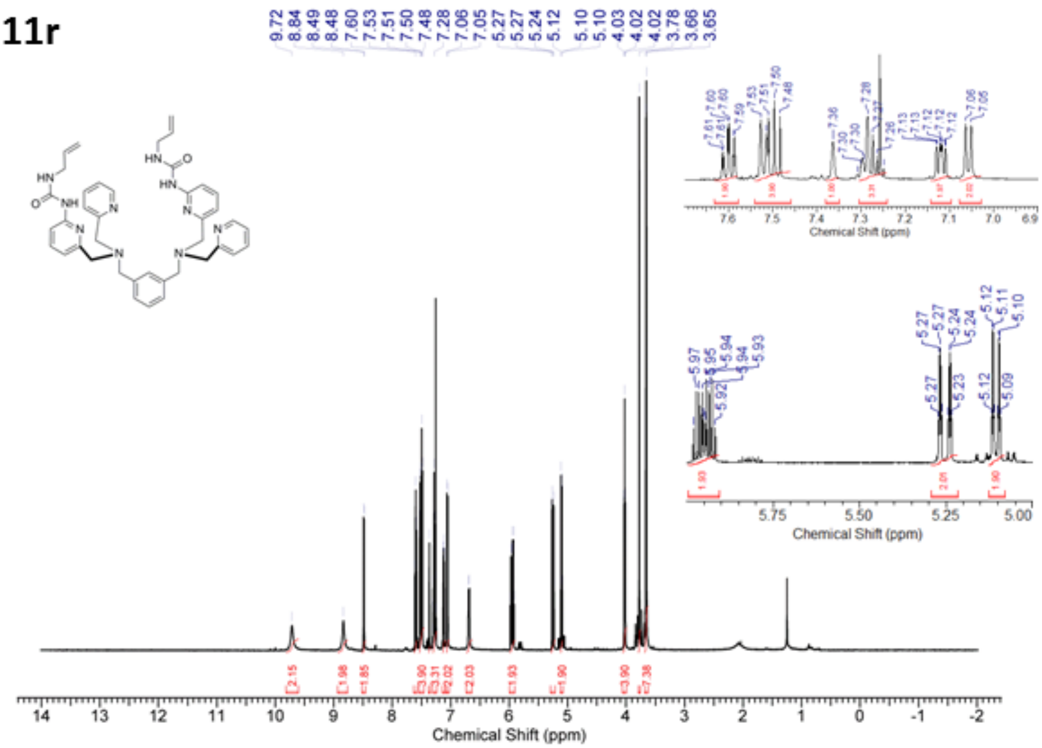

159.29
153.64
152.43
148.93
143.39
139.67
138.57
136.47
132.35
128.39
127.78
122.86
122.05
117.09
115.82
113.75
110.38
109.71
64.45
64.28
60.18
59.58
58.70
Chemical Shift (ppm)

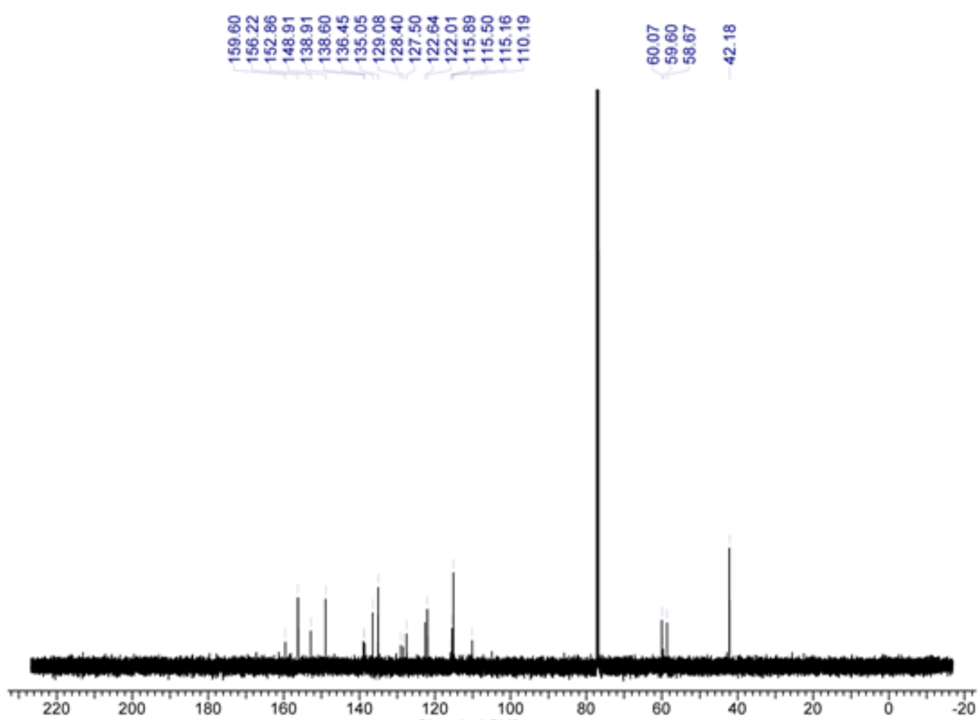
11q


8.26
8.25
7.50
7.33
7.32
7.21
7.20
6.97
6.84
6.82
6.79
6.78
3.67
3.62
3.54
Chemical Shift (ppm)

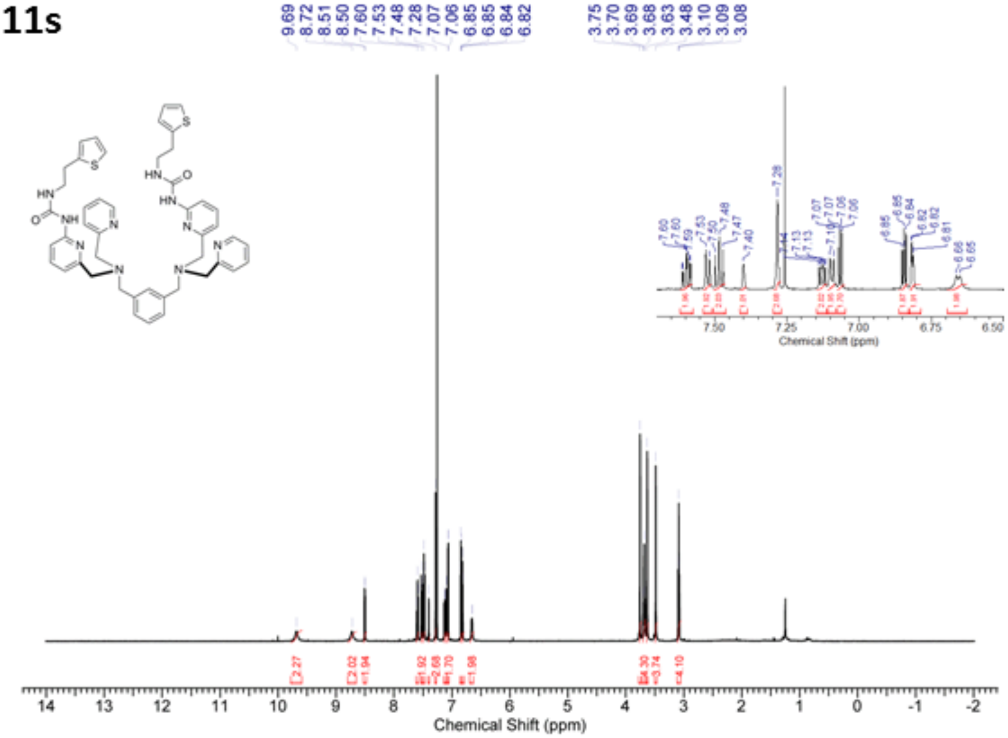

Chemical Shift (ppm)

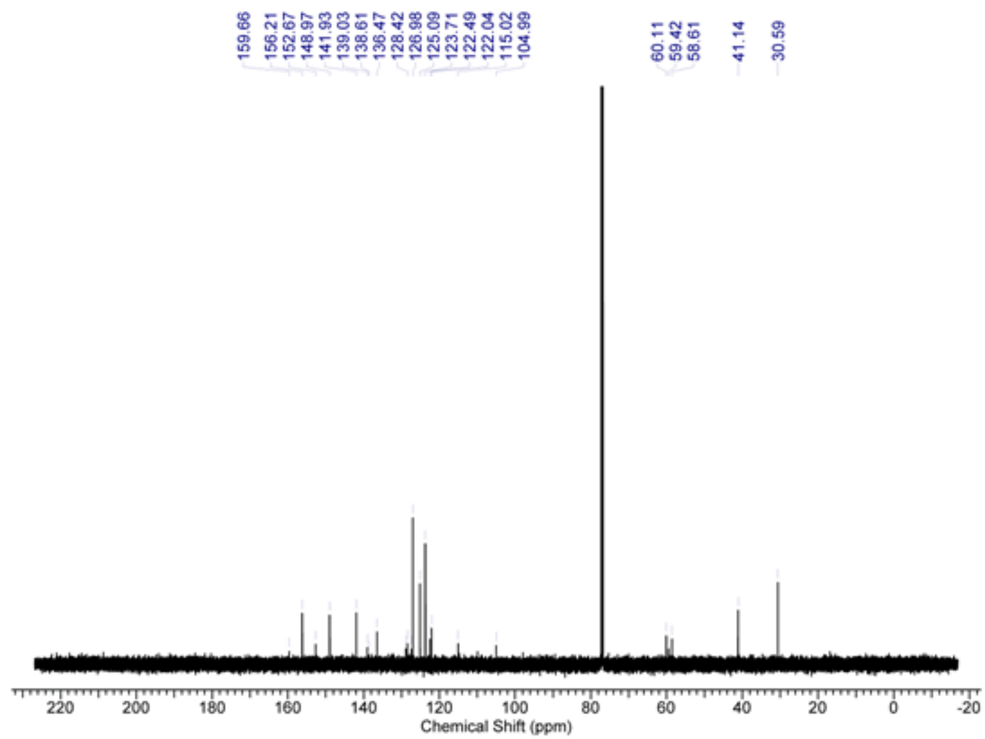
11r



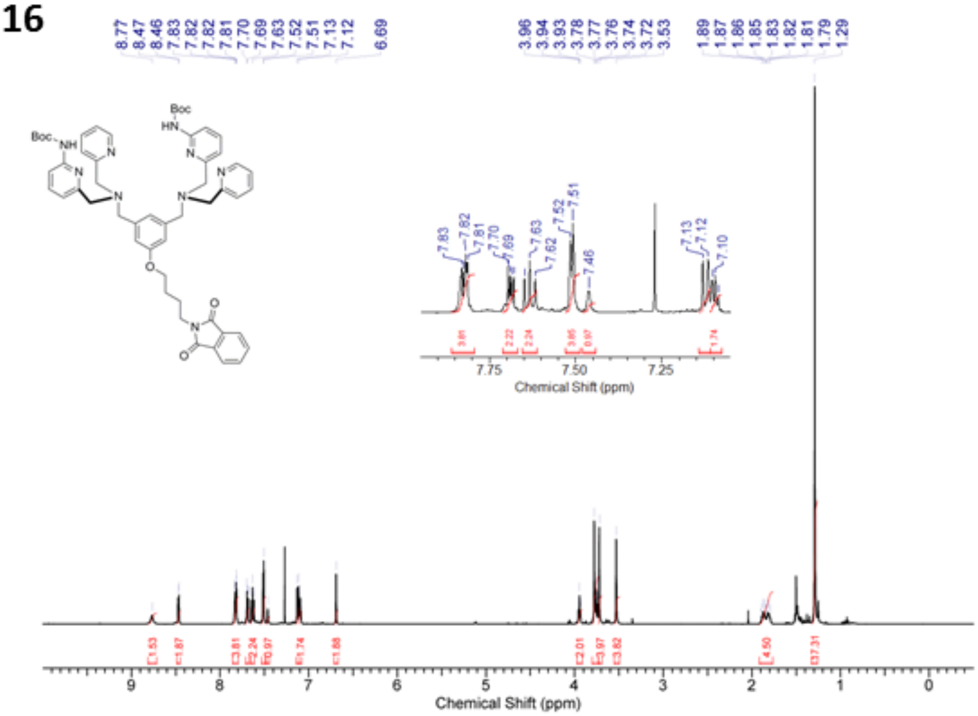


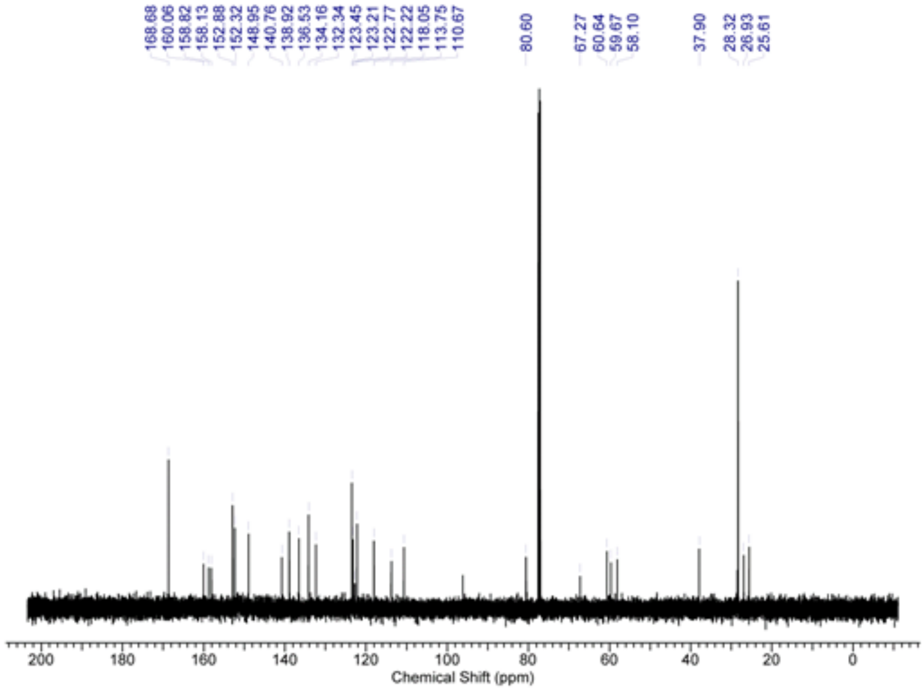
11s



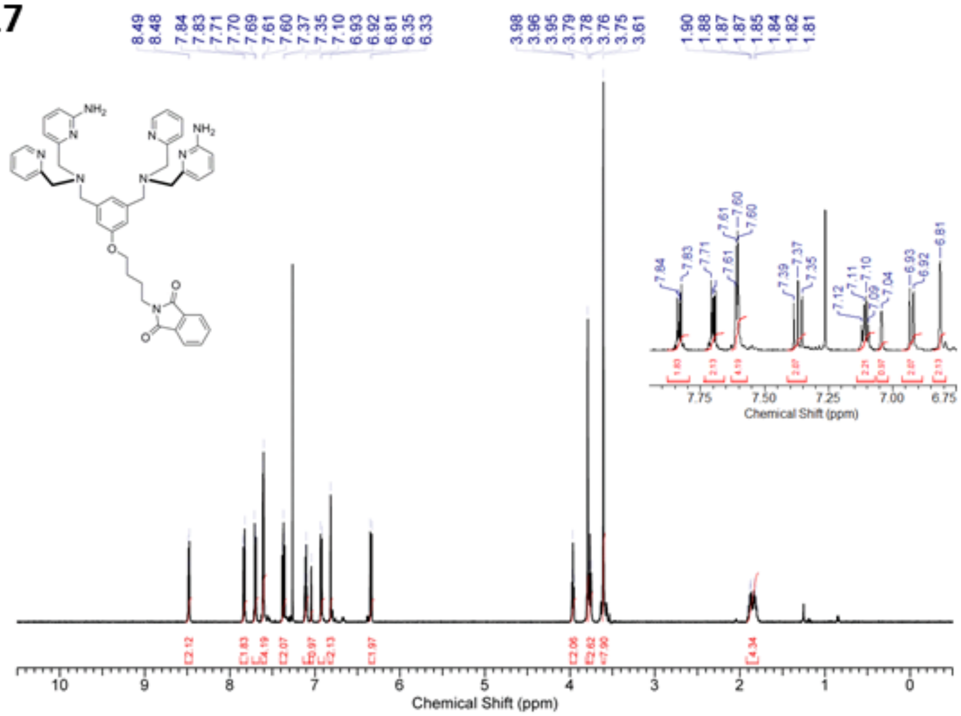


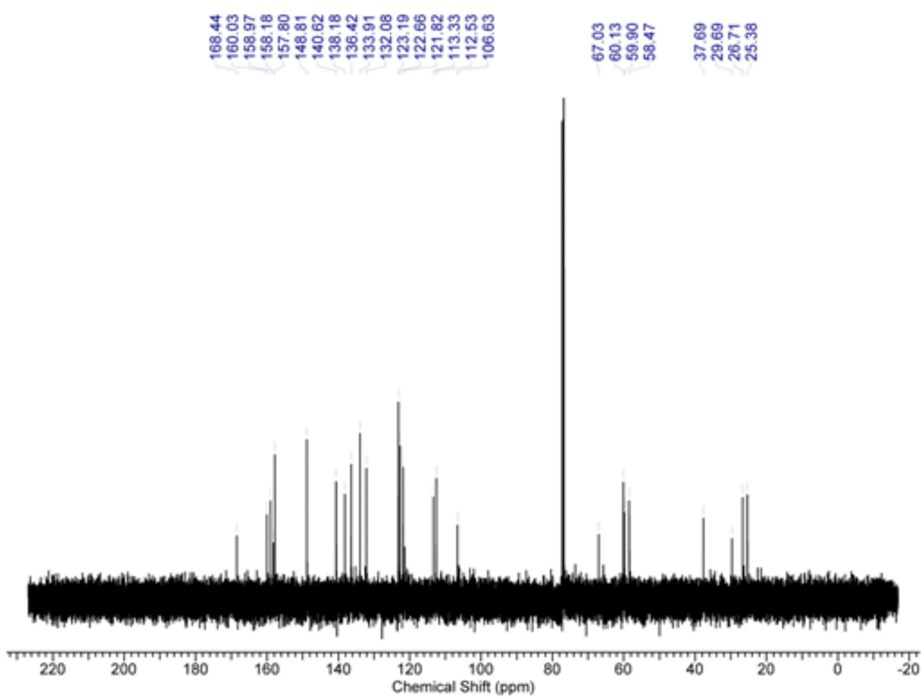
16



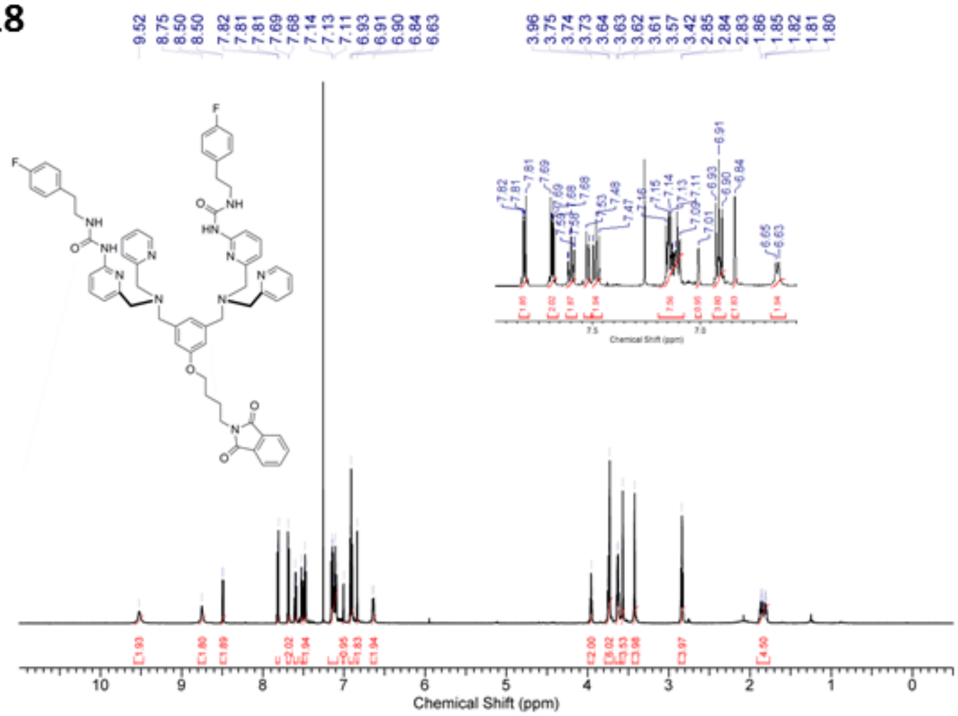


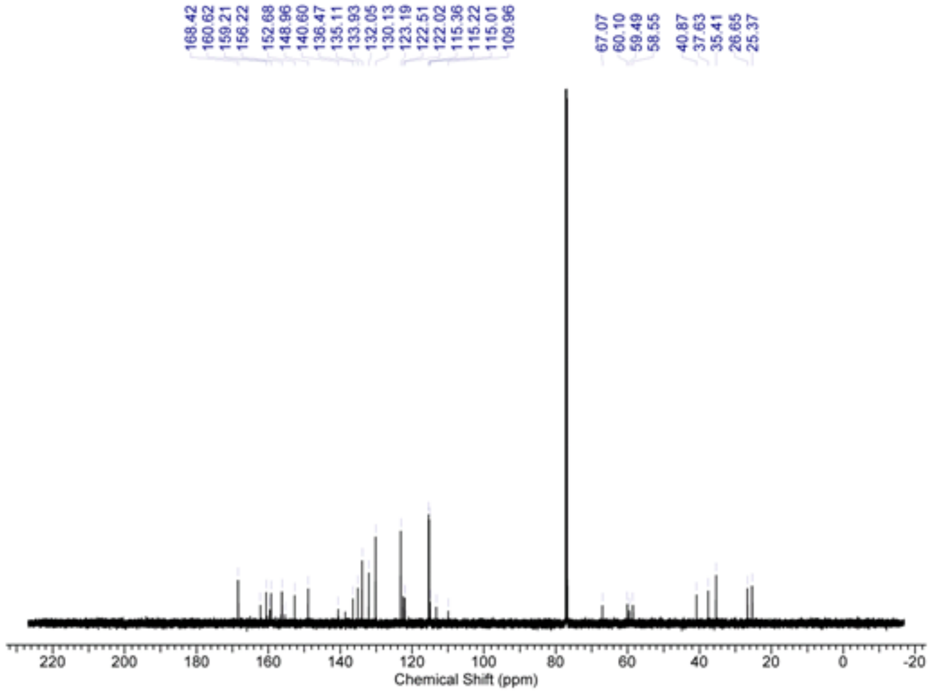
17


8.49
8.48
7.84
7.83
7.71
7.70
7.69
7.61
7.60
7.37
7.35
7.10
6.93
6.92
6.81
6.35
6.33
3.98
3.96
3.95
3.79
3.78
3.76
3.75
3.61
1.90
1.88
1.87
1.87
1.85
1.84
1.82
1.81
Chemical Shift (ppm)

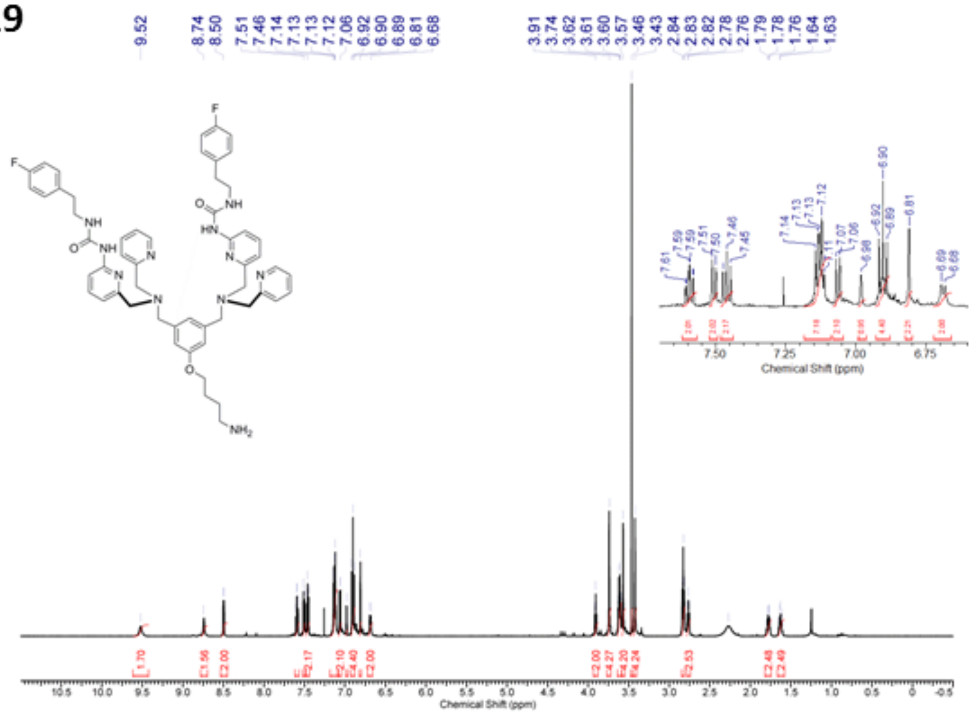

168.44
160.03
158.97
158.18
157.80
148.81
140.62
138.18
136.42
133.91
132.08
123.19
122.66
121.82
113.33
112.53
106.63
67.03
60.13
59.90
58.47
37.69
29.69
26.71
25.38
Chemical Shift (ppm)

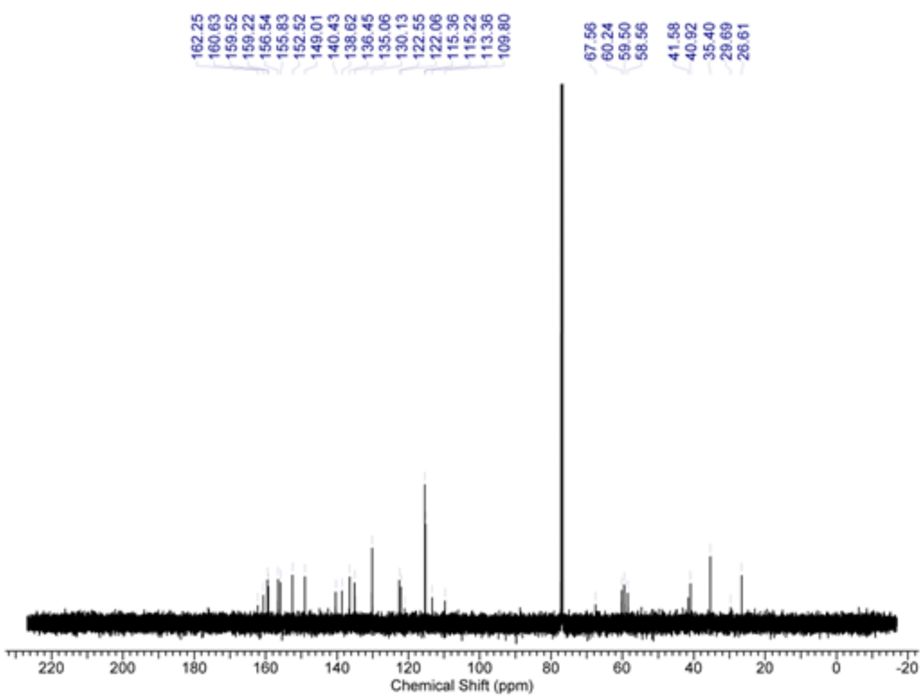
18



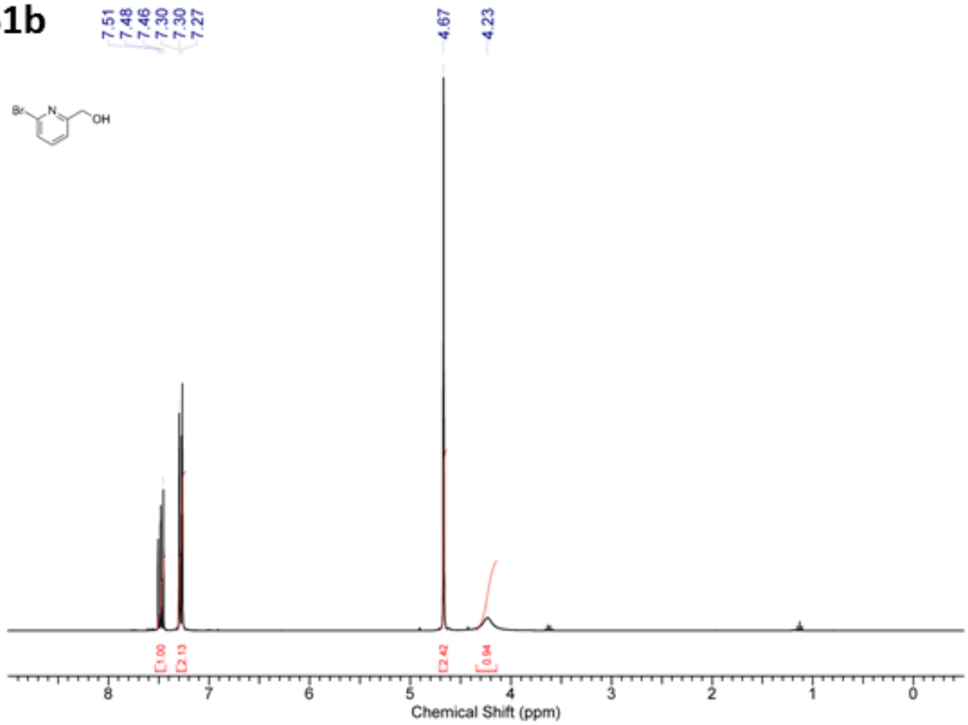


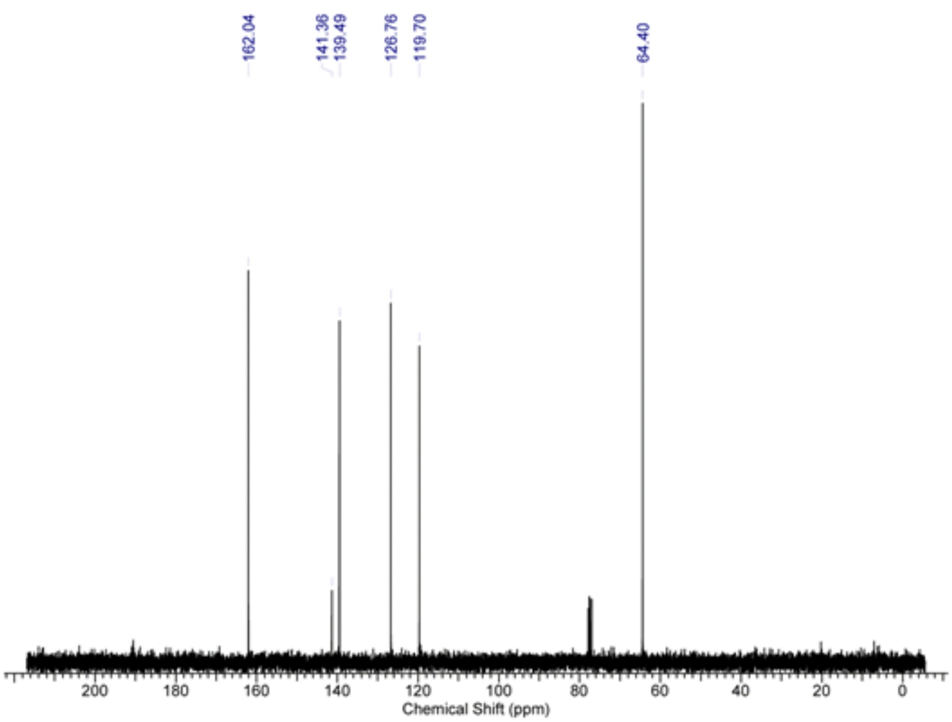
19



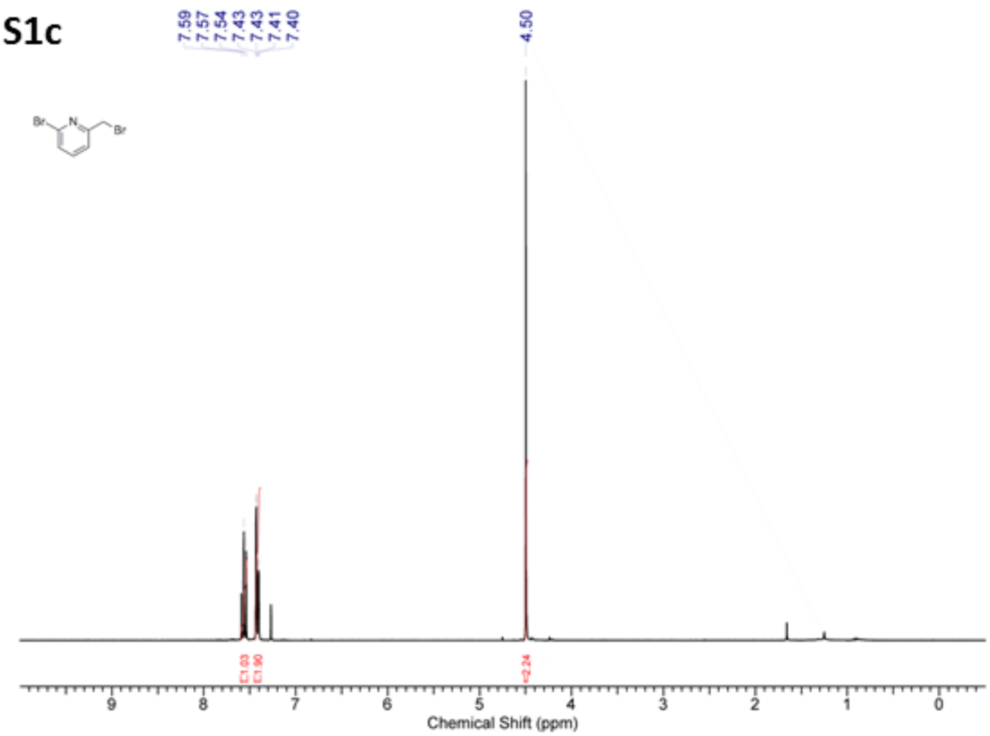


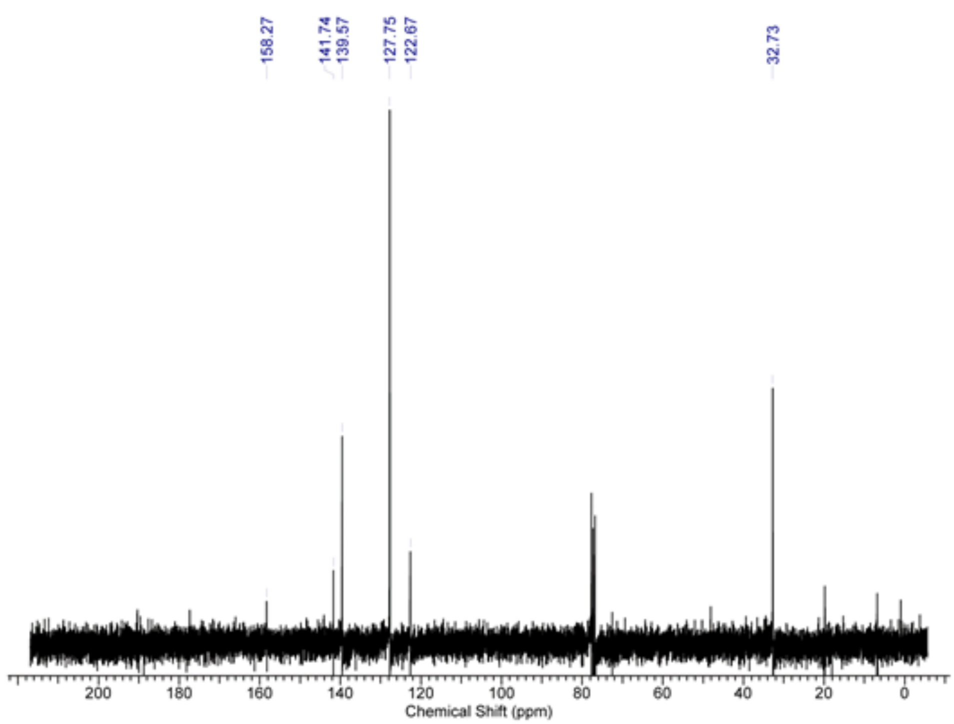
S1b


7.51
7.48
7.46
7.30
7.30
7.27
4.67
4.23
1.00
2.13
2.42
0.94
Chemical Shift (ppm)

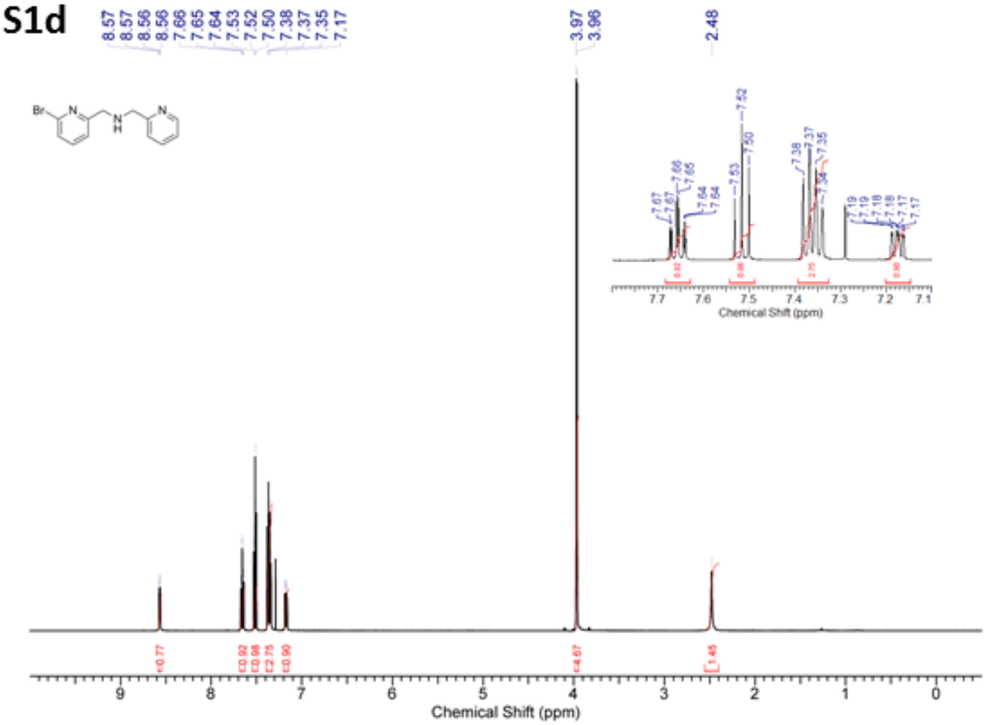

162.04
141.36
139.49
126.76
119.70
64.40
Chemical Shift (ppm)

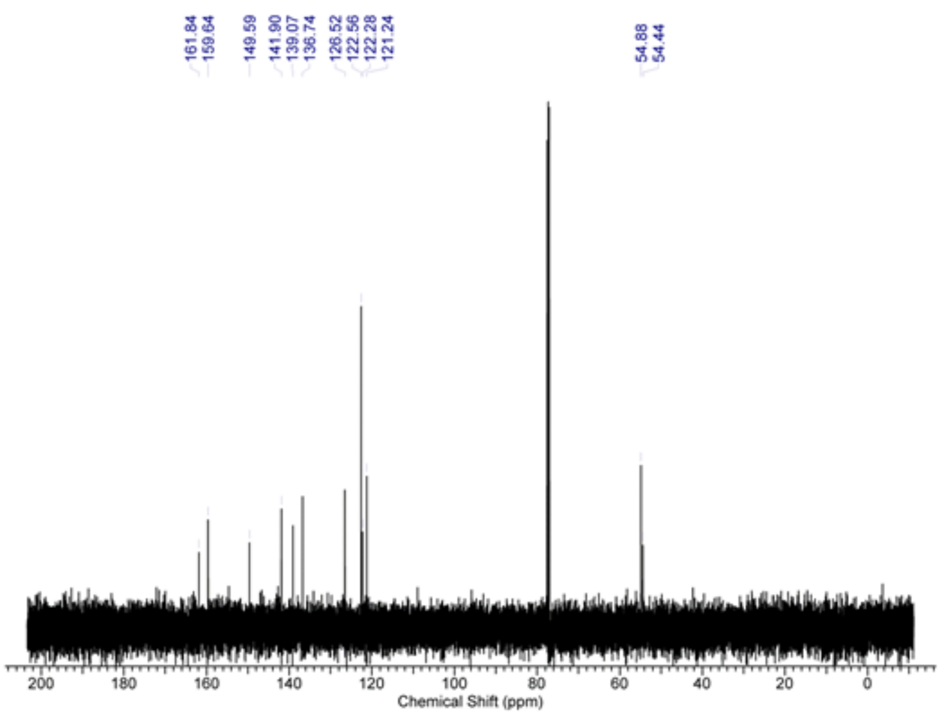
S1c


7.59
7.57
7.54
7.43
7.43
7.41
7.40
4.50
Br
N
Br
1.03
1.90
2.24
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

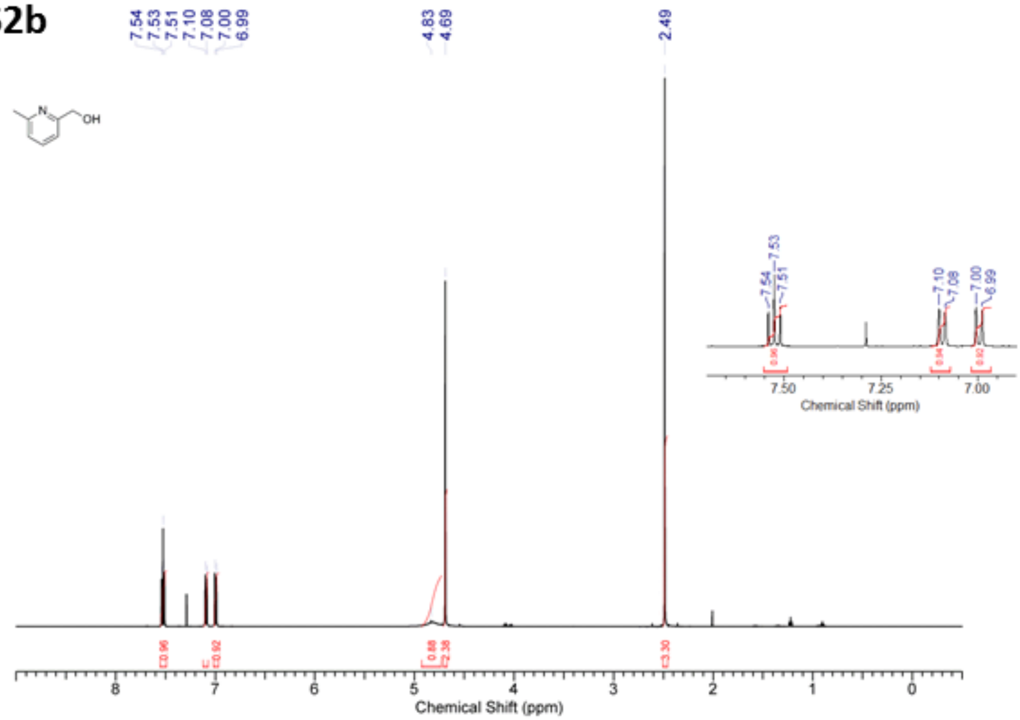

158.27
141.74
139.57
127.75
122.67
32.73
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

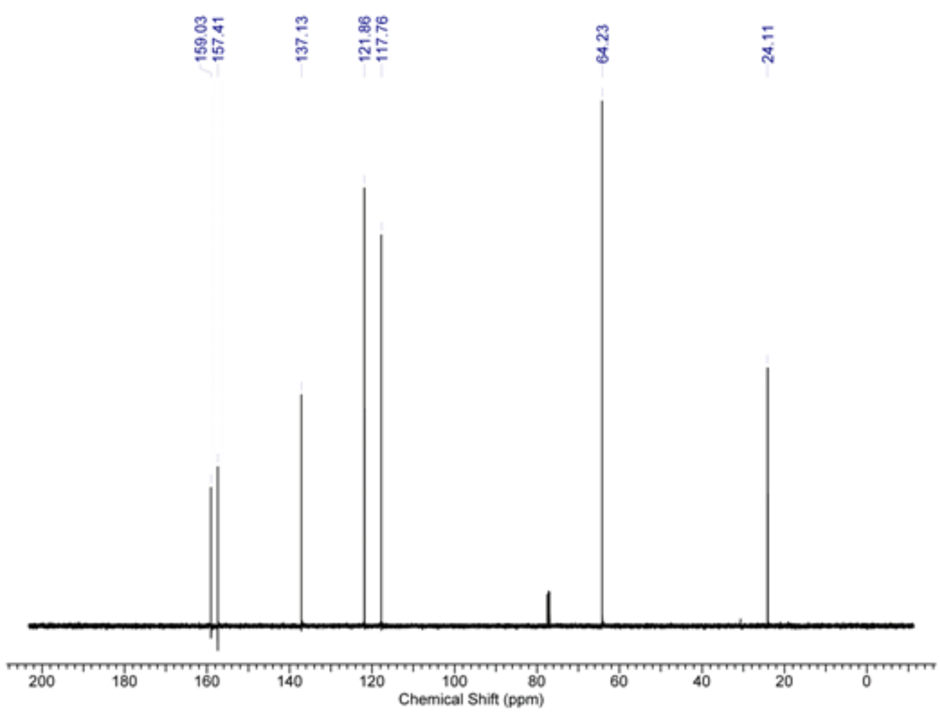
S1d


8.57
8.57
8.56
8.56
7.66
7.65
7.64
7.53
7.52
7.50
7.38
7.37
7.35
7.17
3.97
3.96
2.48
Chemical Shift (ppm)

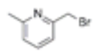

161.84
159.64
149.59
141.90
139.07
136.74
126.52
122.56
122.28
121.24
54.88
54.44
Chemical Shift (ppm)

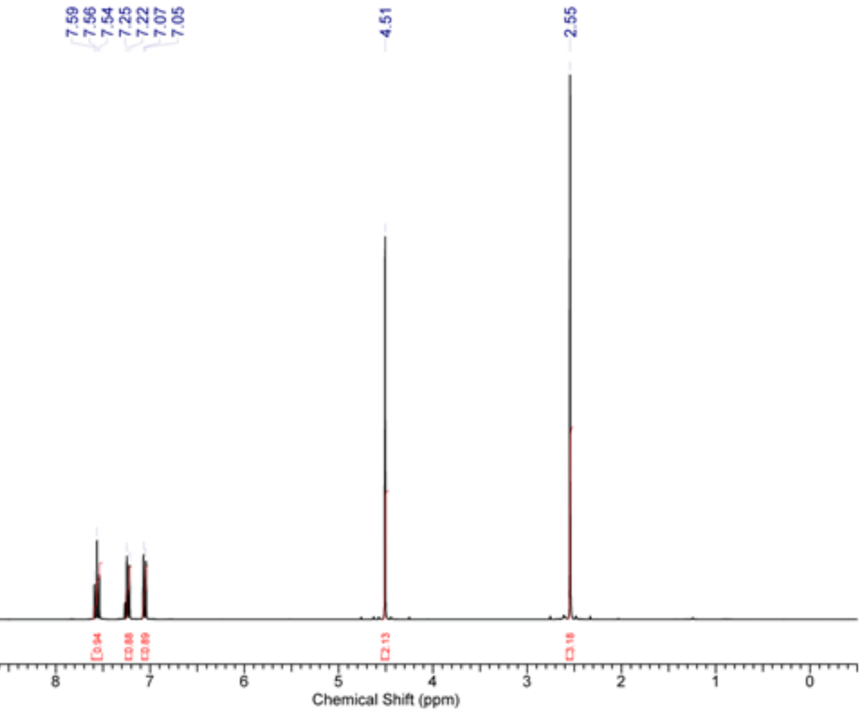
S2b

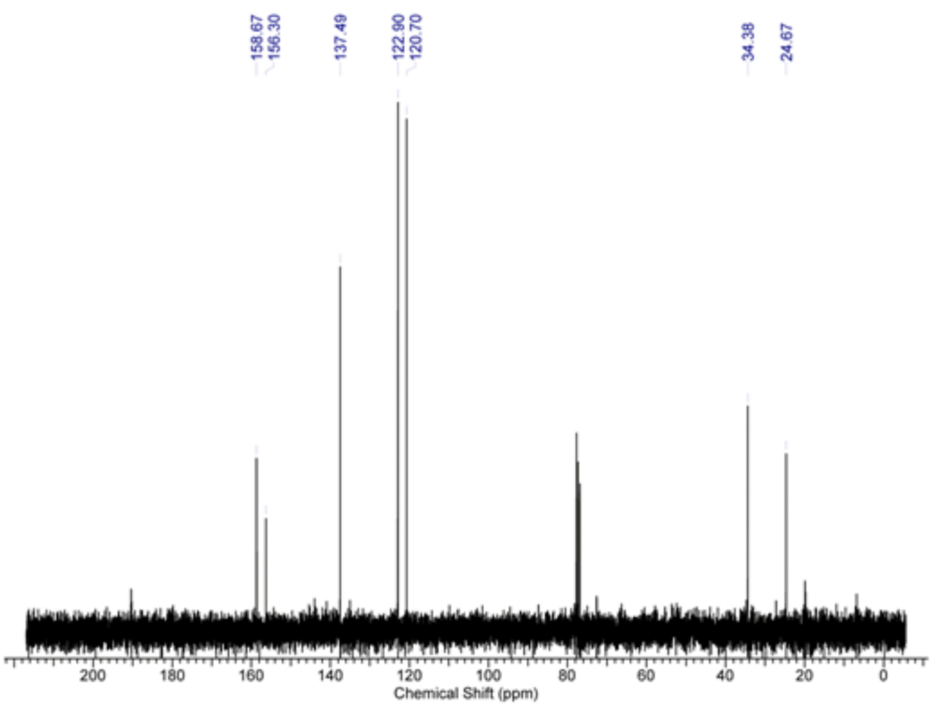

N
OH
7.54
7.53
7.51
7.10
7.08
7.00
6.99
4.83
4.69
2.49
0.96
0.92
0.88
2.38
3.30
7.50
7.25
7.00
Chemical Shift (ppm)
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)


159.03
157.41
137.13
121.86
117.76
64.23
24.11
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

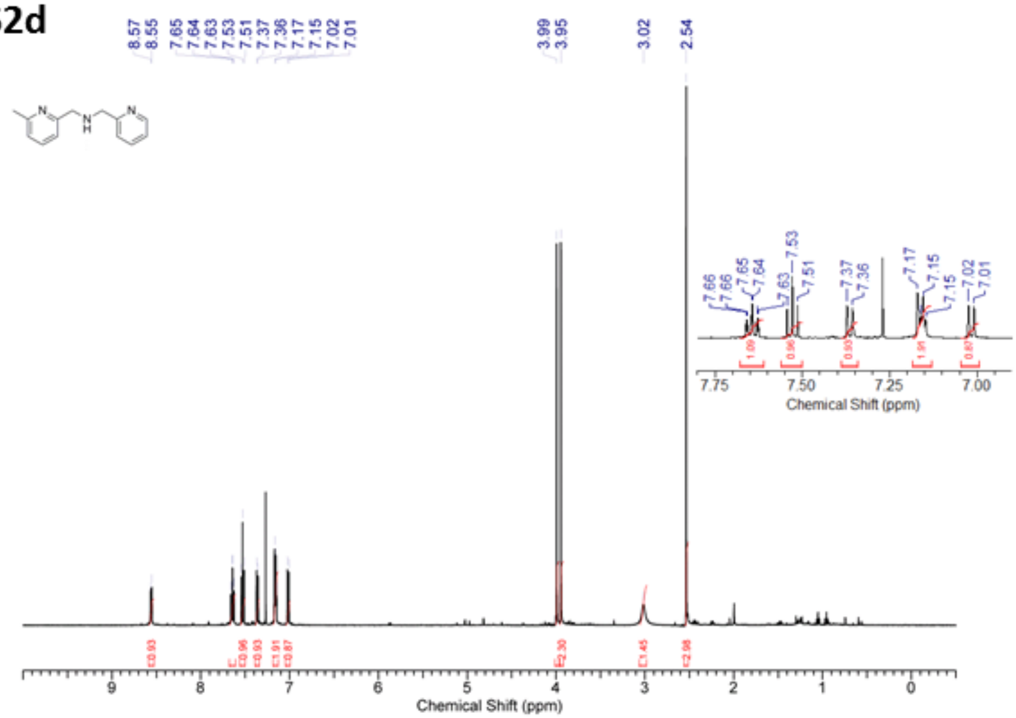
S2c

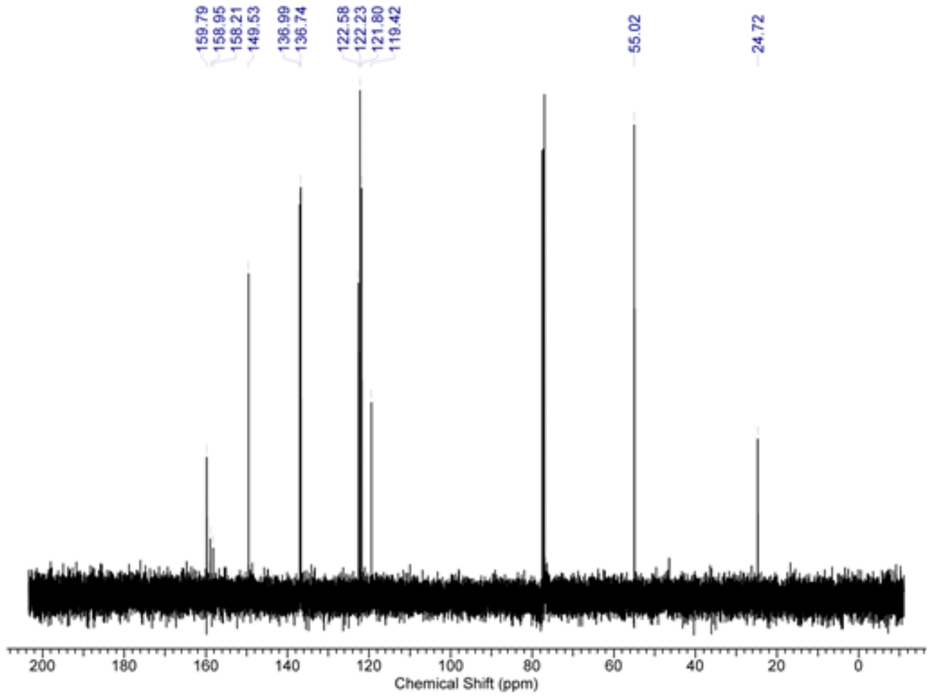



7.59
7.56
7.54
7.25
7.22
7.07
7.05
4.51
2.55
0.94
0.88
0.89
2.13
3.18
Chemical Shift (ppm)


158.67
156.30
137.49
122.90
120.70
34.38
24.67
Chemical Shift (ppm)

S2d


8.57
8.55
7.65
7.64
7.63
7.53
7.51
7.37
7.36
7.17
7.15
7.02
7.01
3.99
3.95
3.02
2.54
0.93
0.96
0.93
1.91
0.87
2.30
1.45
2.98
Chemical Shift (ppm)


159.79
158.95
158.21
149.53
136.99
136.74
122.58
122.23
121.80
119.42
55.02
24.72
Chemical Shift (ppm)

5. References.

S1. Ying, T.; Li, Z. J.; Juan, Z.; Pan, J. M. (2005) Recent advances of the derivatives of pyridylazo as reagents in analytical chemistry. *Rev. Anal. Chem. 24*, 103-147.

S2. Connors, K. A., *The measure of molecular complex stability*. John Wiley & Sons: New York, 1987.

S3. Xie, H. Z.; Yi, S.; Wu, S. K. (1999) Study on host-guest complexation of anions based on tri-podal naphthylthiourea derivatives. *J. Chem. Soc., Perkin Trans. 2*, 2751-2754.