Electronic Supplementary Material (ESI) for Chemical Communications.

Supplementary Information

Tuning Macrocycles to Design 'Turn-on' Fluorescence Probes for Manganese (II) Sensing in Live Cells

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General Procedures and Materials

Unless otherwise mentioned all chemicals were of analytical grade, obtained from commercial sources, and used without further purification. Dry tetrahydrofuran was obtained by distillation over sodium metal for 1h. Water used for experiments was deionized using a Milli Q Integral 3 water purification unit (Millipore Corp. Billerica, MA, USA). Silica gel (230-400 mesh size, Merck & Co., Inc.) and Alumina (Brockmann grade, S D Fine-Chem Ltd.) were used for column chromatography. Solvents used for chromatography were of analytical grade and used without distillation. Eluting systems for column chromatography purifications were determined by thin layer chromatography (TLC) analysis. TLC analyses were performed on silica gel 60 F_{254} (Merck & Co., Inc.) and aluminium oxide 60 F_{254} (Merck & Co., Inc.) TLC plates and the plates were visualized under UV light, 254 nm and 365 nm. Solvents were evaporated under reduced pressure using a rotary evaporator (BÜCHI Labortechnik AG).

^{1}H NMR and ^{13}C NMR spectra were collected in either $CDCl_3$ or $(CD_3)_2SO$ (Cambridge Isotope Laboratories, Cambridge, MA) at 25 °C on either Avance Bruker 500 MHz or Varian 600 MHz spectrometers at the National NMR facility, Tata Institute of Fundamental Research, Mumbai, India. All chemical shifts are reported in the standard notation of parts per million (ppm) using either the peaks of proton signals of residual solvents or tetramethylsilane as internal reference. The abbreviations used for the proton spectra multiplicities are: s, singlet; br, broad; d, doublet; t, triplet; m, multiplet. Low-resolution mass spectral analyses were carried out on a liquid chromatography mass spectrometer (LCMS-2020, Shimadzu Corp.) with an ESI probe (positive and negative ion modes). High-resolution mass spectral analyses were carried out at the Chemistry Department, Indian Institute of Technology, Bombay, India on a maXis^{TM} impact ESI-qTOF mass spectrometer (Bruker Corp.).

Synthesis and Characterization of M1 and M2

***N*-Phenyldiethanolamine ditosylate (1a).** Compound **1a** was synthesized according to a previously reported procedure.[1]

4,7-Dimethyl-1,10-bis(p-tolylsulfonyl)-1,4,7,10-tetraazadecane (2a). Compound **2a** was synthesized according to a previously reported procedure.[2]

1,4-dimethyl-10-phenyl-7,13-ditosyl-1,4,7,10,13-pentaazacyclopentadecane (3a). *t*-butyl-ammonium bromide (0.25 g, 0.78 mmol) was added to a mixture of lithium hydroxide in water (2.5 %, 20 mL) and toluene (20 mL) and the resultant mixture was refluxed at 80 ºC for 30 min. A suspension of compound **1a** (1.66 g, 3.39 mmol) and compound **2a** (1.64 g, 3.40 mmol) in toluene (20 mL) was added to the reaction mixture and the mixture was refluxed for 12 h. The reaction mixture was cooled to room temperature and the organic phase was washed with water, dried over sodium sulphate, and evaporated to dryness. Purification by column chromatography (silica gel, 4:1 ethyl acetate / hexanes) afforded compound **3a** as a white solid (0.62 g, 29 %).

^{1}H NMR (500 MHz, $CDCl_3$, 298 K): δ (ppm) 7.70 (4H, d, *J*= 8 Hz,), 7.33-7.29 (6H, m), 6.94 (2H, d, *J*= 7.5 Hz), 6.73 (1H, t, *J*= 7.5 Hz), 3.76 (4H, t, *J*= 8 Hz), 3.29 (4H, br), 3.15 (4H, t, *J*= 8.5 Hz), 2.65 (4H, br), 2.53 (4H, s), 2.42 (6H, s), 2.23 (6H, s)

^{13}C NMR (125 MHz, $CDCl_3$, 298 K): δ (ppm) 147.3, 143.4, 135.6, 129.7, 129.6, 127.2, 115.9, 111.4, 60.0, 56.2, 50.4, 47.9, 47.0, 41.5, 21.4

HRMS (m/z): calculated for [MH^+] $C_{32}H_{46}N_5O_4S_2$ 628.2986, found 628.2989.

Dimethyl 2,2'-(10,13-dimethyl-4-phenyl-1,4,7,10,13-pentaazacyclopentadecane-1,7-diyl) di-acetate (4a). Naphthalene (0.30 g, 0.02 mmol) and sodium metal (0.70 g) were added to dry tetrahydrofuran under argon atmosphere. The mixture was sonicated for 10 min to yield a green colored solution of sodium naphthalenide. The solution was then cooled to -78 ºC using an isopropanol / dry ice bath. Compound **3a** (0.13 g, 0.21 mmol) was dissolved in dry tetrahydrofuran and added to the cooled sodium naphthalenide solution. The resultant mixture was stirred at -78 ºC for 15 min. The reaction mixture was then added in small portions to water (50 mL) while carefully avoiding contact of any un-dissolved pieces of sodium with water. The resultant mixture was dried under vacuum and triturated with methanol to remove salts. The methanol extract was dried under vacuum and the product obtained was taken forward to the next step without further purification.

A solution of methyl 2-bromoacetate (0.06 g, 0.39 mmol) dissolved in dry dimethylformamide (30 mL) was added drop-wise to a solution of the detosylated macrocyle and potassium carbonate (0.24 g, 1.71 mmol) in dry dimethylformamide (50 mL) over a period of 2 h. The

reaction mixture was stirred for 6 h at room temperature. Methyl 2-bromoacetate was further added to the reaction mixture in two additional aliquots (0.03 g, 0.21 mmol dissolved in 20 mL dimethylformamide; 0.015 g, 0.11 mmol dissolved in 15 mL dimethylformamide). Each aliquot was added to the reaction mixture dropwise for 1 h following which the reaction mixture was stirred for 4 h. The progress of the reaction was monitored by LRESI-MS (calculated for [MH^+] 464, found 464) and TLC (alumina, 98:2 dichloromethane / methanol). Upon completion the reaction mixture was added to water and the product was extracted with dichloromethane. The organic phase was washed with water, dried over sodium sulphate, and evaporated under reduced pressure. Purification by column chromatography (alumina, 98:2 dichloromethane / methanol) afforded compound **4a** as yellow oil (0.045 g, 45 %).

^{1}H NMR (500 MHz, $CDCl_3$, 298 K): δ (ppm) 7.18 (2H, t, *J*= 7.5 Hz), 6.64-6.59 (3H, m), 3.68 (6H, s), 3.50 (4H, t, *J*= 7 Hz), 3.44 (4H, s), 2.88 (4H, t, *J*= 7.5 Hz), 2.84 (4H, t, *J*= 6.5 Hz), 2.63-2.55 (8H, m), 2.27 (6H, s)

^{13}C NMR (150 MHz, $CDCl_3$, 298 K): δ (ppm) 171.8, 147.9, 129.3, 115.6, 111.3, 56.5, 55.6, 55.1, 52.0, 51.9, 51.4, 49.9, 42.8

HRMS (m/z): calculated for [MH^+] $C_{24}H_{42}N_5O_4$ 464.3231, found 464.3233.

Dimethyl 2,2'-(4-(4-formylphenyl)-10,13-dimethyl-1,4,7,10,13-pentaazacyclopentadecane-1,7-diyl)diacetate (5a). Phosphorus oxychloride (0.09 mL, 0.9 mmol) was added to anhydrous dimethylformamide (15 mL) degassed under argon atmosphere at 0 °C and the resultant solution was stirred for 30 min while gradually warming to room temperature. Compound **4a** (0.07 g, 0.15 mmol) was dissolved in anhydrous dimethylformamide and added to the reaction mixture. The mixture was stirred for 6 h at 80 ºC under argon atmosphere. The dark yellow reaction mixture was quenched with saturated potassium carbonate solution and the pH of the mixture was adjusted to 8. The resultant mixture was stirred for 10 min and extracted with dichloromethane. The organic phase was dried over sodium sulphate and evaporated under reduced pressure. Purification by column chromatography (alumina, 98:2 dichloromethane / methanol) afforded compound **5a** as yellow oil (0.024 g, 33%).

^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ (ppm) 9.73 (s, 1H), 7.72 (2H, d, *J*= 9 Hz), 6.68 (2H, d, *J*=

8.4 Hz), 3.71 (6H, s), 3.66 (4H, t, *J*= 6.6 Hz), 3.46 (4H, s), 2.94 (4H, t, *J*= 7.2 Hz), 2.88 (4H, br), 2.62 (8H, br), 2.31 (6H, s)

^{13}C NMR (150 MHz, $CDCl_3$, 298 K): δ (ppm) 190.3, 172.1, 152.9, 132.6, 125.2, 110.9, 57.1, 56.5, 56.1, 52.5, 52.1, 51.9, 50.5, 43.4

HRMS (m/z): calculated for [MH^+] $C_{25}H_{43}N_5O_5$ 492.3180, found 492.3183.

10-(4-(4,13-bis(2-methoxy-2-oxoethyl)-7,10-dimethyl-1,4,7,10,13-pentaazacyclopentadecan-1-yl)phenyl)-5,5-difluoro-1,3,7,9-tetramethyl-4-bora-3a,4a-diaza-s-indacene (M1). Compound **5a** (0.012 g, 0.024 mmol) and 2,4-dimethylpyrrole (12.3 μL, 0.12 mmol) were dissolved in anhydrous dichloromethane (15 ml) under argon atmosphere. A few drops of trifluoroacetic acid were added to the reaction mixture and the resultant red solution was stirred under argon for 5 h at room temperature in the dark. Formation of the dipyrromethane adduct was confirmed by LRESI-MS; calculated for [MH^+] and [MNa^+] 664 and 686 respectively, found 664 and 686 respectively. *p*-Chloranil (0.009 g, 0.036 mmol) dissolved in dichloromethane (1 mL) was added to the reaction mixture and the mixture was stirred for 1 h. The formation of dipyrromethene was confirmed by LRESI-MS; calculated for [MH^+] and [MNa^+] 662 and 684 respectively, found 662 and 684 respectively. Di-isopropylethylamine (0.74 mL, 4.2 mmol) was then added dropwise to the reaction mixture over a period of 10 min, and the resultant solution was stirred for an additional 10 min. Boron trifluoride diethyl etherate (0.37 mL, 3 mmol) was added drop wise over a period of 5 min. The reaction mixture was allowed to stir for 12 h. The product was extracted in dichloromethane and washed multiple times with water. The organic phase was dried over sodium sulphate, evaporated under reduced pressure, and purified twice by column chromatography (alumina, 98:2 dichloromethane / methanol) to afford compound **M1** as an orange solid (5 mg, 29.4%).

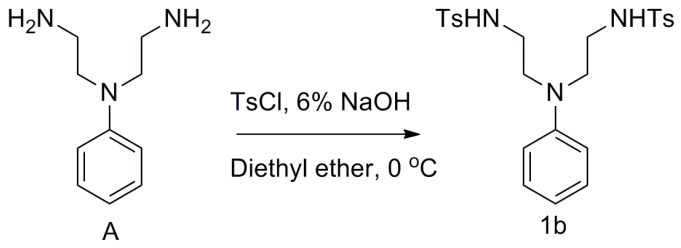
^{1}HNMR (600 MHz, $CDCl_3$, 298 K): δ (ppm) 6.99 (2H, d, *J*= 8.4 Hz), 6.68 (2H, d, *J*= 9 Hz), 5.96 (2H, s), 3.68 (6H, s), 3.56 (4H, t, *J*= 6.6 Hz), 3.45 (4H, s), 2.93 (4H, t, *J*= 7.2 Hz), 2.87 (4H, br), 2.63 (8H, br), 2.54 (6H, s), 2.30 (6H, s), 1.48 (6H, s)

^{13}CNMR (150 MHz, $CDCl_3$, 298 K): δ (ppm) 172.1, 155.1, 148.7, 143.5, 139.6, 132.5, 129.4, 123.8, 121.2, 112, 56.8, 55.9, 52.2, 52.0, 51.9, 50.6, 43.0, 15.1, 14.9

HRMS (m/z): calculated for [MH^+] $C_{37}H_{54}N_7O_4BF_2$ 710.4377 found 710.4377.

***N'*-(2-aminoethyl)-*N'*-phenylethane-1,2-diamine (A).** Compound **A** was synthesized in three steps as reported in a previous procedure .[1]

Scheme S1. Synthesis of macrocycle precursor 1b.



***N,N'*-[(phenylimino)diethane-2,1-diyl]bis(4-methylbenzenesulfonamide) (1b).** Compound **A** (1.18 g, 6 mmol) and sodium hydroxide (1.52 g, 38 mmol) were dissolved in water (25 mL) at 0 °C. Diethyl ether (25 mL) was added to the resultant mixture and the mixture was stirred vigorously. *p*-toluenesulfonyl chloride (3 g, 15.7 mmol) was added and the mixture was stirred at 0 °C for 2 h and then at room temperature for 10 h. The organic phase was washed with water, dried over sodium sulphate, and evaporated under reduced pressure. The sticky solid obtained was dissolved in minimum volume of chloroform and methanol was added to precipitate compound **1b** as a white solid (0.72 g, 25 %).

^{1}H NMR (500 MHz, $(CD_3)_2SO$, 298 K): δ (ppm) 7.56 (4H, d, *J*= 7.5 Hz), 7.14 (4H, d, *J*= 8 Hz), 6.96 (2H, t, *J*= 8 Hz), 6.42 (2H, t, *J*= 6.5 Hz), 6.39 (2H, d, *J*= 9 Hz), 3.13 (4H, t, *J*= 7 Hz), 2.67 (4H, t, *J*= 7 Hz), 2.29 (6H, s)

^{13}C NMR (125 MHz, $(CD_3)_2SO$, 298 K): δ (ppm) 147.6, 143.7, 138.5, 130.6, 130.1, 127.5, 116.8, 112.2, 51.1, 21.9

HRMS (m/z): calculated for [MH^+] $C_{44}H_{54}N_5O_8S_4$ 488.1672 found 488.1680.

Ethane-1,2-diylbis((4-methylphenyl)sulfonyliminoethane-2,1-diyl)bis(4-methyl-benzenesulfonate) (2b). Compound **2b** was synthesized according to a modified procedure than reported earlier for the same molecule.[3] The procedure followed was taken from Lee *et. al*.[4]

^{1}H NMR (600 MHz, $CDCl_3$, 298 K): δ (ppm) 7.77 (4H, d, *J*= 7.8 Hz), 7.71 (4H, d, *J*= 8.4 Hz), 7.34 (8H, d, *J*= 7.8 Hz), 4.13 (4H, t, *J*= 5.1 Hz), 3.36 (4H, t, *J*= 6 Hz), 3.30 (4 H, s), 2.44 (12H, s)

^{13}C NMR (150 MHz, $CDCl_3$, 298 K): δ (ppm) 145.5, 144.3, 132.6, 130.3, 130.0, 128.3, 127.7, 69.3, 50.1, 49.7, 21.9, 21.8

LRMS (ESI) m/z calculated for [MH^+] 765 [MNa^+]787 found [MH^+] 765 [MNa^+]787

1-phenyl-4,7,10,13-tetratosyl-1,4,7,10,13-pentaazacyclopentadecane (3b). *t*-butylammonium bromide (0.13 g, 0.39 mmol) was dissolved in lithium hydroxide in water (2.5 %, 40 mL). Toluene (10 mL) was added to the resultant solution and the mixture was heated to 90 °C. Compound **1b** (0.75 g, 1.56 mmol) and compound **2b** (1.19 g, 1.56 mmol) were dissolved in hot toluene (40 mL) and added to the reaction mixture. The resultant mixture was heated at 100 °C for 12 h. The organic phase was washed with water, dried over sodium sulphate, and evaporated under reduced pressure. Purification by column chromatography (silica gel, 2:3 ethyl acetate / hexanes) afforded compound **3b** as a white solid (0.66 g, 47 %).

^{1}H NMR (500 MHz, $CDCl_3$, 298 K): δ (ppm) 7.73 (4H, d, *J*= 8 Hz), 7.69 (4H, d, *J*= 8 Hz), 7.36 (4H, d, *J*= 8 Hz), 7.30-7.25 (5H, m), 6.84 - 6.81 (4H, m), 3.49 (4H, t, *J*= 8 Hz), 3.30 (8 H, s), 3.29 (4H, s), 3.25 (4H, t, *J*= 7.5 Hz), 2.47 (6H, s), 2.44 (6H, s)

^{13}C NMR (125 MHz, $CDCl_3$, 298 K): δ (ppm) 147.0, 144.4, 144.2, 135.7, 135.4, 130.3, 130.2, 128.3, 127.7, 127.5, 117.7, 111.9, 50.9, 50.4, 50.1, 49.6, 47.8, 21.9

HRMS (m/z): calculated for [MH^+] $C_{44}H_{54}N_5O_8S_4$ 908.2850 found 908.2849.

Tetramethyl-2,2',2'',2'''-(13-phenyl-1,4,7,10,13-pentaazacyclopentadecane-1,4,7,10-tetra-yl)tetraacetate (4b). Naphthalene (0.30 g, 2.3 mmol) and sodium metal (3 g) were added to dry

tetrahydrofuran under argon atmosphere. The mixture was sonicated for 10 min to yield a green colored solution of sodium naphthalenide. The solution was then cooled to -78 ºC using an isopropanol / dry ice bath. Compound **3b** (1.4 g, 1.54 mmol) dissolved in dry THF was added to the dark green mixture. The mixture was stirred for 5 min under argon at -78 ºC. The reaction mixture was then added in small portions to water (50 mL) while carefully avoiding contact of any un-dissolved pieces of sodium with water. A few drops of concentrated hydrochloric acid were added to the quenched reaction mixture to bring the pH up to 7. The resultant mixture was dried under vacuum and triturated with methanol to remove salts. The methanol extract was dried under vacuum and the product obtained was taken forward to the next step without further purification.

The detosylated macrocycle and methyl 2-bromoacetate (1.18 g, 7.73 mmol) were dissolved in dimethylformamide. Potassium carbonate (0.43 g, 3.10 mmol) was added to the reaction mixture and the resultant mixture was stirred for 24 h at room temperature. The reaction mixture was dissolved in water and extracted with chloroform. The organic phase was dried over sodium sulphate, evaporated under reduced pressure, and purified by column chromatography (silica gel, 2.5% methanol / chloroform) to afford compound **4b** as a brown solid (0.19 g , 20.8 %).

^{1}H NMR (600 MHz, $CDCl_3$, 298 K) δ (ppm): 7.17 (2H, t, *J*= 8.1 Hz), 6.63 – 6.60 (3H, m), 3.68 (6H, s), 3.66 (6H, s), 3.51 (4H, t, *J*= 6.9 Hz), 3.48 (4H, s), 3.42 (4H, s), 2.85 (4H, t, *J*= 7.2 Hz), 2.82 (4H, s), 2.81(8H, s)

^{13}C NMR (150 MHz, $CDCl_3$, 298 K) δ (ppm): 172.3, 172.2, 148.2, 129.6, 115.7, 111.4, 56.7, 55.4, 52.7, 52.7, 52.5, 52.4, 51.8, 51.7, 51.6, 49.8

HRMS (m/z): calculated for [MH^+] $C_{28}H_{45}N_5O_8$ 580.3341 found 580.3349

Tetramethyl-2,2',2'',2'''-(13-(4-formylphenyl)-1,4,7,10,13-pentaazacyclopentadecane-1,4,7,10-tetrayl)tetraacetate (5b). Phosphorus oxychloride (0.3 mL, 3.29 mmol) was added to anhydrous dimethylformamide degassed under argon atmosphere (10 mL) at 0 °C and the resultant solution was stirred for 30 min while gradually warming to room temperature. Compound **4b** (0.19 g, 0.32 mmol) was dissolved in anhydrous dimethylformamide and added to the reaction mixture. The mixture was stirred for 6 h at 80 ºC under argon atmosphere. The dark yellow reaction mixture was quenched with saturated potassium carbonate solution and the pH of

the mixture was adjusted to 8. The resultant mixture was stirred for 10 min and extracted with dichloromethane. The organic phase was dried over sodium sulphate and evaporated under reduced pressure. Purification by column chromatography (silica gel, 2.5% methanol / chloroform) afforded compound **5b** as a brown solid (0.61 g, 31 %).

^{1}H NMR (600 MHz, $CDCl_3$, 298 K) δ (ppm): 9.69 (1H, s), 7.68 (2H, d, *J*= 9 Hz), 6.63 (2H, d, *J*= 9 Hz), 3.68 (6H, s), 3.66 (4H, t, *J*= 6 Hz), 3.63 (6H, s), 3.49 (4H, s), 3.38 (4H, s), 2.87 (4H, t, *J*= 6.6 Hz), 2.82 (4H, s), 2.80 (8H, s)

^{13}C NMR (150 MHz, $CDCl_3$, 298 K) δ (ppm): 190.4, 171.9, 152.8, 132.7, 125.2, 110.8, 56.7, 55.3, 53.0, 52.9, 52.6, 52.4, 51.8, 51.7, 50.3

HRMS (m/z): calculated for [MH^+] $C_{29}H_{46}N_5O_9$ 608.3296 found 608.3293

10-(4-(4,7,10,13-Tetrakis(2-methoxy-2-oxoethyl)-1,4,7,10,13-pentaazacyclopentadecan-1-yl) phenyl)-5,5-difluoro-1,3,7,9-tetramethyl-4-bora-3a,4a-diaza-s-indacene (M2). Compound **5b** (0.01 g, 0.02 mmol) and 2, 4 dimethylpyrrole (10.1 µL, 0.10 mmol) were added to anhydrous dichloromethane (12 mL) under argon atmosphere. Two to three drops of trifluoroacetic acid were added to the reaction mixture. The resultant pink solution was stirred for 12 h under argon atmosphere at room temperature. Formation of the dipyrromethane adduct was confirmed by LRESI-MS; calculated for [MH^+] 780 and [MNa^+] 802, found [MH^+] 780 and [MNa^+] 802. *p*-chloranil (0.01 g, 0.05 mmol) dissolved in anhydrous dichloromethane (4 mL) was added and the reaction mixture stirred for 1 h. Dipyrromethene formation was confirmed by LRESI-MS; calculated for [MH^+] 778 and [MNa^+] 800, found [MH^+] 778 and [MNa^+] 800. Di-isopropylethylamine (0.65 mL, 3.7 mmol) was added to the resultant red mixture and the reaction mixture was stirred for 5 min. Boron trifluoride diethyl etherate (0.32 mL, 2.6 mmol) was added drop-wise and the reaction mixture was stirred for 12 h. The product was extracted in dichloromethane and washed multiple times with water. The organic phase was dried over sodium sulphate, evaporated under reduced pressure, and purified twice by column chromatography (silica gel, 1.5% methanol / dichloromethane) to afford M2 as an orange solid (2.5 mg, 14 % yield).

^{1}H NMR (600 MHz, $CDCl_3$, 298 K) δ (ppm): 7.00 (2H, d, *J*= 8.4 Hz), 6.69 (2H, d, *J*= 8.4 Hz), 5.96 (2H, s), 3.70 (6H, s), 3.68 (6H, s), 3.60 (4H, t, *J*= 6.3 Hz), 3.55 (4H, br), 3.44 (4H, br), 2.94-2.88 (15 H, m), 2.54 (6H, s), 1.48 (6H, s)

^{13}C NMR (150 MHz, $CDCl_3$, 298 K) δ (ppm): 171.9, 155.0, 148.6, 143.5, 132.5, 129.4, 121.2, 111.9, 56.5, 55.1, 53.8, 52.8, 52.4, 52.2, 51.9, 50.1, 15.0, 14.9

HRMS (m/z): [M^+] calculated for $C_{41}H_{59}N_7O_8BF_2$ 826.4486 found 826.4487

LC-MS Traces for M1 and M2

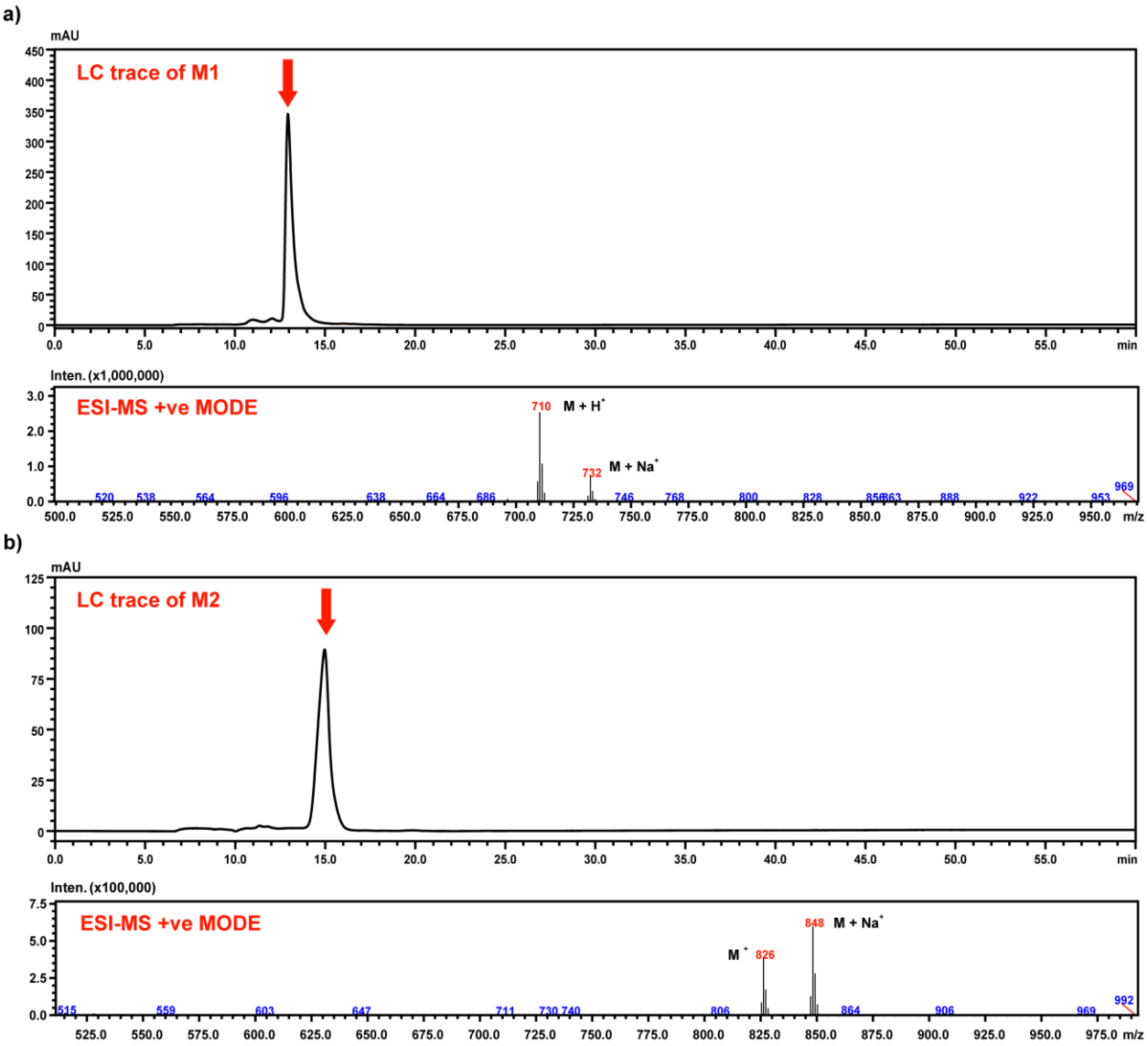


Figure S1. LC-ESI traces for (a) M1 and (b) M2 confirming sample purity (LC depicting absorption intensity at 498 nm). A gradient of 5% Solvent A (1 mM NH_4Ac in 1 : 9 water : methanol) to 100% Solvent B (1 mM NH_4Ac in Acetonitrile) in 38 min followed by 100% solvent B, was run through a C8 column (5µm, 150 x 4.6 mm, Kromasil®) for the analysis. The major elution peak is pointed with a red arrow and the corresponding ESI-MS trace in the +ve mode is shown with the major peaks highlighted in red.

Fluorescence and Absorbance Measurements

All spectroscopic measurements were performed in acetonitrile at room temperature. UV-Visible spectrophotometric measurements for the sensors were performed on a double beam Perkin-Elmer (Lambda 750-UV/Vis/NIR) spectrophotometer in a quartz cuvette having a path length of 1 cm. The absorption spectra of M1 and M2 are shown in Figure S3. Fluorescence spectra were recorded on either FluoroLog®-3 (Horiba Jobin Yvon Inc.) or Fluoromax®-3 (Horiba Jobin Yvon Inc.) spectrofluorometer using quartz cuvettes with either 10 mm x 4 mm (Hellma® Analytics) or 10 mm x 2 mm (Starna Cells, Inc.) inner dimensions. Fluorescence spectra were obtained by excitation at 480 nm with slit width 2 nm for both excitation and emission for M1 and M2. $MnCl_2.4H_2O$ was used as the source of Mn^{2+}. Fluorescence quantum yields were determined with reference to fluorescein in 0.1 N NaOH ($\Phi = 0.95$).[5] Excitation was provided at 480 nm and collected emission was integrated from 490 to 550 nm for M1 and M2. Slit widths for both excitation and emission were 1 nm. All fluorescence measurements for quantum yield calculations were performed in 10 mm x 4 mm quartz cuvettes.

The apparent dissociation constants (K_d) were determined from a plot of observed fluorescence intensity (F) over fluorescence intensity of the dye in the absence of any metal ion (F_0) at 508 nm versus $[Mn^{2+}]$.

The data were fitted to the following equation for M1[6]:

$$\frac{F}{F_0} = 1+(F_{max}-F_0)\times \frac{\left([L]_t+[Mn^{2+}]_t+K_d\right) - \sqrt{([L]_t+[Mn^{2+}]_t+K_d)^2 - 4[L]_t[Mn^{2+}]_t}}{2\times[L]_t\times F_0} \quad \ldots\ldots\ldots\ldots (S1)$$

where, F_{max} is the fluorescence for the Mn^{2+} bound sensor complex. $[L]_t$ is the total ligand concentration and $[Mn^{2+}]_t$ is the total Mn^{2+} concentration.

The data for M2 was fitted to the following equation:

$$\frac{F}{F_o} = \frac{F_{max}[Mn^{2+}]_t^{\,2} + F_o K}{\left(K + [Mn^{2+}]_t^{\,2}\right) \times F_o} \ldots\ldots\ldots (S2)$$

The apparent K_d has been reported as $\sqrt{K}$.

The Hill coefficients were determined from the slopes of the linear least squares fits of $\log[(F - F_0) / (F_{max} - F)]$ vs. $\log[Mn^{2+}]_{free}$ plots, where $[Mn^{2+}]_{free}$ is the free Mn^{2+} concentration calculated by using the following equation:

$$[Mn^{2+}]_{free} = [Mn^{2+}]_t - [L]_t \frac{F - F_0}{F_{max} - F_0} \ldots\ldots\ldots (S3)$$

Other metal ions were used to test the selectivity of M1 and M2 towards Mn^{2+}. Mn^{2+} was delivered in the form of $MnCl_2.4H_2O$ from a stock solution of 5 mM $MnCl_2.4H_2O$ in 10 % water in acetonitrile. Fe^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Na^+, K^+, Cd^{2+}, and Al^{3+} were delivered in the form of their chlorides or nitrates as $FeCl_2$, $NiCl_2$, $CuCl_2$, $ZnCl_2$, NaCl, KCl, $Cd(NO_3)_2$ and $Al(NO_3)_3.9H_2O$ from 5 mM stock solutions prepared in 10 % water in acetonitrile. Ca^{2+} and Mg^{2+} were delivered in the form of their chlorides as $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$ from 5 mM stock solutions prepared in 11.5 % water in acetonitrile. The stock solutions of Fe^{2+} were prepared in degassed water and acetonitrile. Cu^+ was supplied as tetrakis(acetonitrile)copper(I) hexafluorophosphate and Fe^{3+} was supplied as $FeCl_3$ from a 5 mM stock solution in acetonitrile. All stock solutions were diluted in acetonitrile when required, to prepare 0.5 mM secondary stocks.

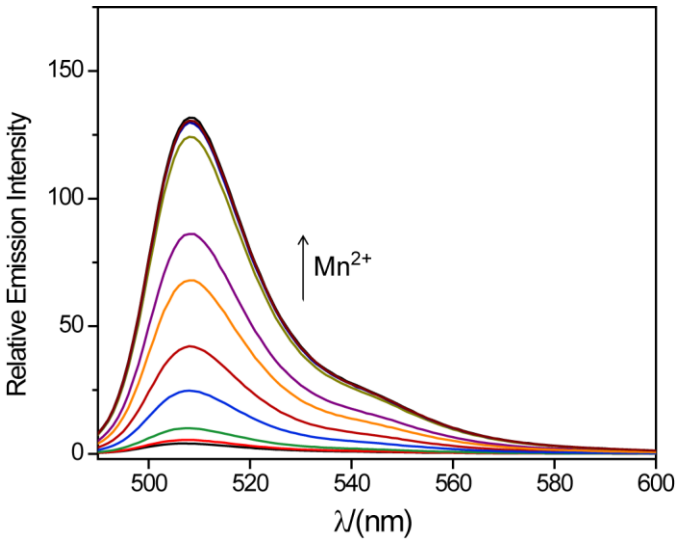


Figure S2. Fluorescence response of M2 (2.5 μM) to Mn^{2+} (0 - 48 μM) in acetonitrile. λ_{ex} = 480 nm.

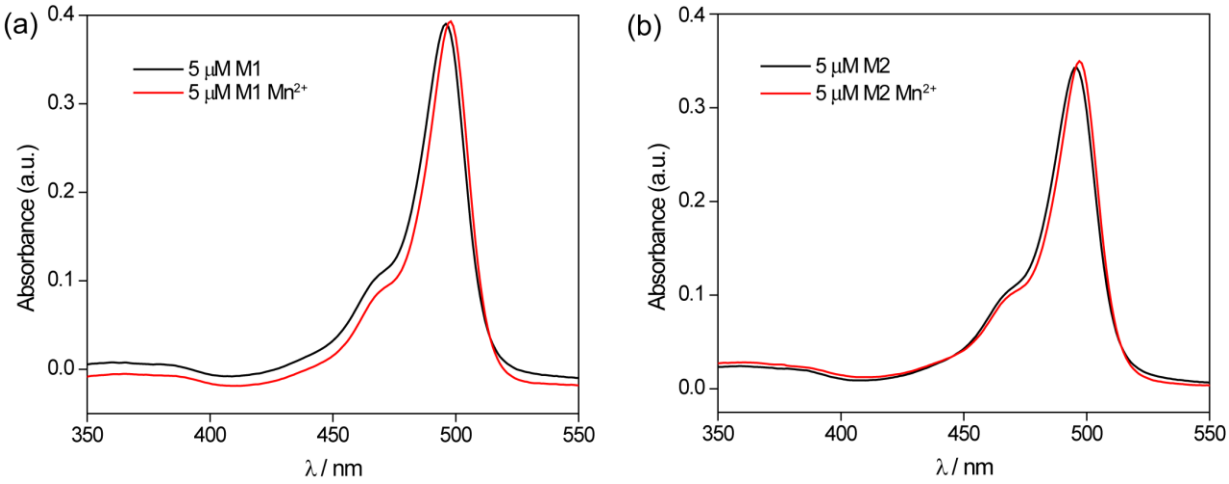


Figure S3. Absorption spectra in acetonitrile a) M1 (5 μM) and b) M2 (5 μM). Black curves represent the absorption of M1 and M2 in the absence of Mn^{2+}. Red traces show the absorption of M1 and M2 in the presence of Mn^{2+} (13 μM and 59 μM for M1 and M2 respectively).

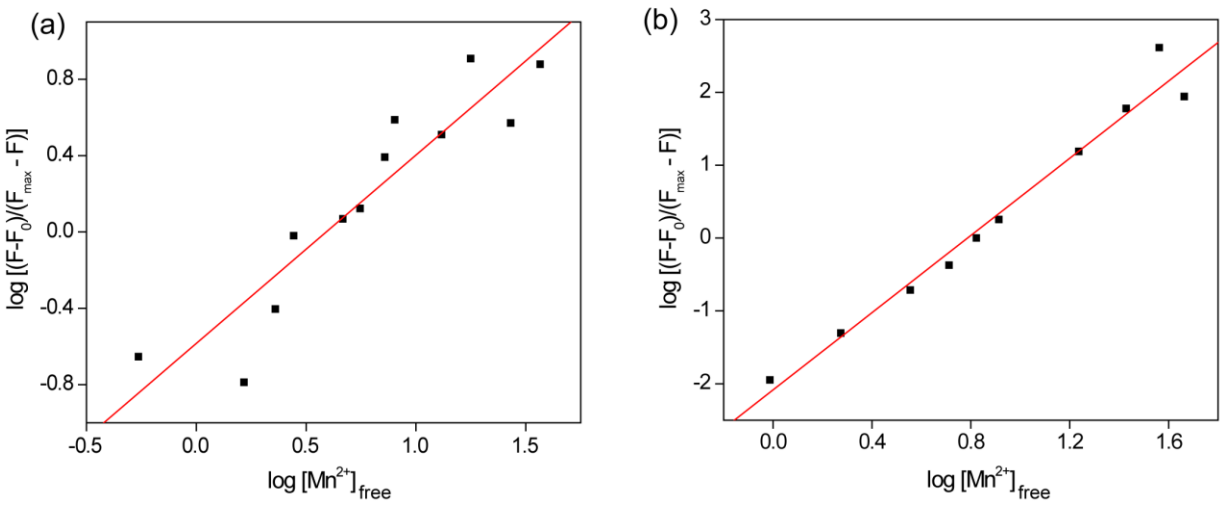


Figure S4. Hill plots for a) M1 (2.5 μM) and b) M2 (2.5 μM). [Mn^{2+}] = (0 - 48 μM). Least-squares fitting (red line) of the plots afforded slopes of 0.98 ± 0.13 for M1 and 2.65 ± 0.16 for M2. λ_{ex} = 480 nm; λ_{em} = 508 nm.

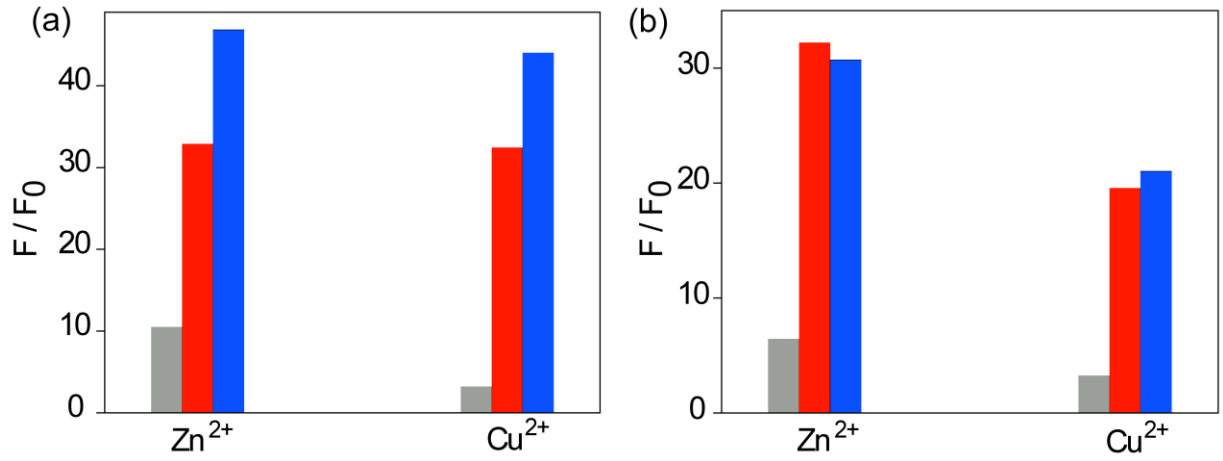


Figure S5. Observed fluorescence (F) over initial fluorescence in absence of metal ion (F_0) at 508 nm, in acetonitrile for (a) M1 (2.5 μM) and (b) M2 (2.5 μM). Gray bars represent F/F_0 values for the probes upon addition of either Zn^{2+} or Cu^{2+} ([M^{2+}] = 10 μM). Red and blue bars represent F/F_0 values upon subsequent addition of Mn^{2+} (final concentration 10 μM) and Mn^{2+} (final concentration 20 μM), respectively. λ_{ex} = 480 nm.

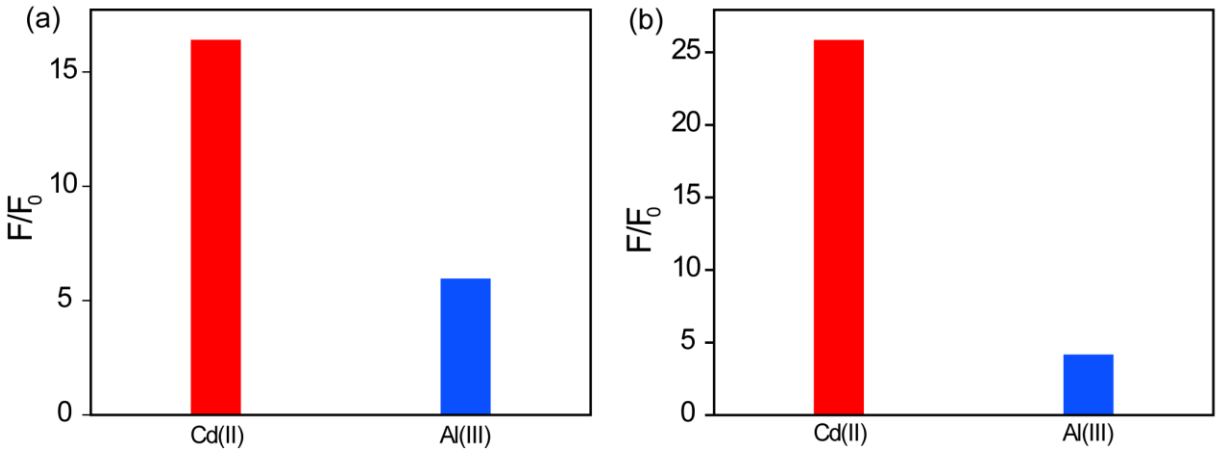


Figure S6. Observed fluorescence (F) over initial fluorescence in absence of metal ion (F_0) at 508 nm, in acetonitrile for (a) M1 (2.5 μM) and (b) M2 (2.5 μM). Red bars represent F/F_0 values for the probes upon addition of Cd^{2+} (1 μM). Blue bars represent F/F_0 values upon addition of Al^{3+} (20 μM). λ_{ex} = 480 nm.

Cell Studies and Confocal Imaging

HEK 293T cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich®) supplemented with Fetal Bovine Serum (10%, Gibco®), Penicillin (50 units/ml, Gibco®) and Streptomycin (50 μg/ml, Gibco®) in T25 culture plates at 37 °C under humidified air containing 5% CO_2. A day before the imaging, the cells were plated on home-made glass coverslip bottomed petriplates (35 mm diameter, Tarsons) coated with polyornithine (0.1 mg/mL) and fibronectin (100 μg/mL).

Fluorescence images of the cells were recorded on a confocal microscope (LSM 510, Carl Zeiss, Germany) using 40x oil immersion objectives. Z-series were obtained with a spacing of 1.00 μM. 488 nm laser (Argon source) was used for M1 excitation. Modified Thomson's buffer (TB) consisting of sodium HEPES (20 mM), NaCl (146 mM), KCl (5.4 mM), $MgSO_4$ (0.8 mM), KH_2PO_4 (0.4 mM), Na_2HPO_4 (0.3 mM) and glucose (5.5 mM); pH adjusted to 7.4 was used during the confocal studies.

A stock solution of M1 (3.5 mM) was prepared in DMSO. M1 staining was done directly on the microscopic stage. The cells were washed with TB and incubated with M1 (5 μM in TB) for 15 min. After staining, the cells were washed three times with TB and imaged. For Mn treatment,

cells were incubated with $MnCl_2$ (25 μM in DMEM) for 1 h at 37 °C under humidified air containing 5% CO_2. Then the cells were washed with TB multiple times to remove excess $MnCl_2$, stained with M1 as mentioned above and imaged. Both control and Mn-loaded M1 stained cells were treated with Tetrakis-(2-pyridylmethyl)ethylenediamine (TPEN, 2 mM in TB) for 20 min and imaged.

For the lipid co-localization studies the Mn treated cells were stained with M1 for 15 min, washed, and then stained with nile red (100 nM) for 5 min. The cells were washed again with TB and imaged. 543 nm laser (Helium Neon source) was used for nile red excitation.

References

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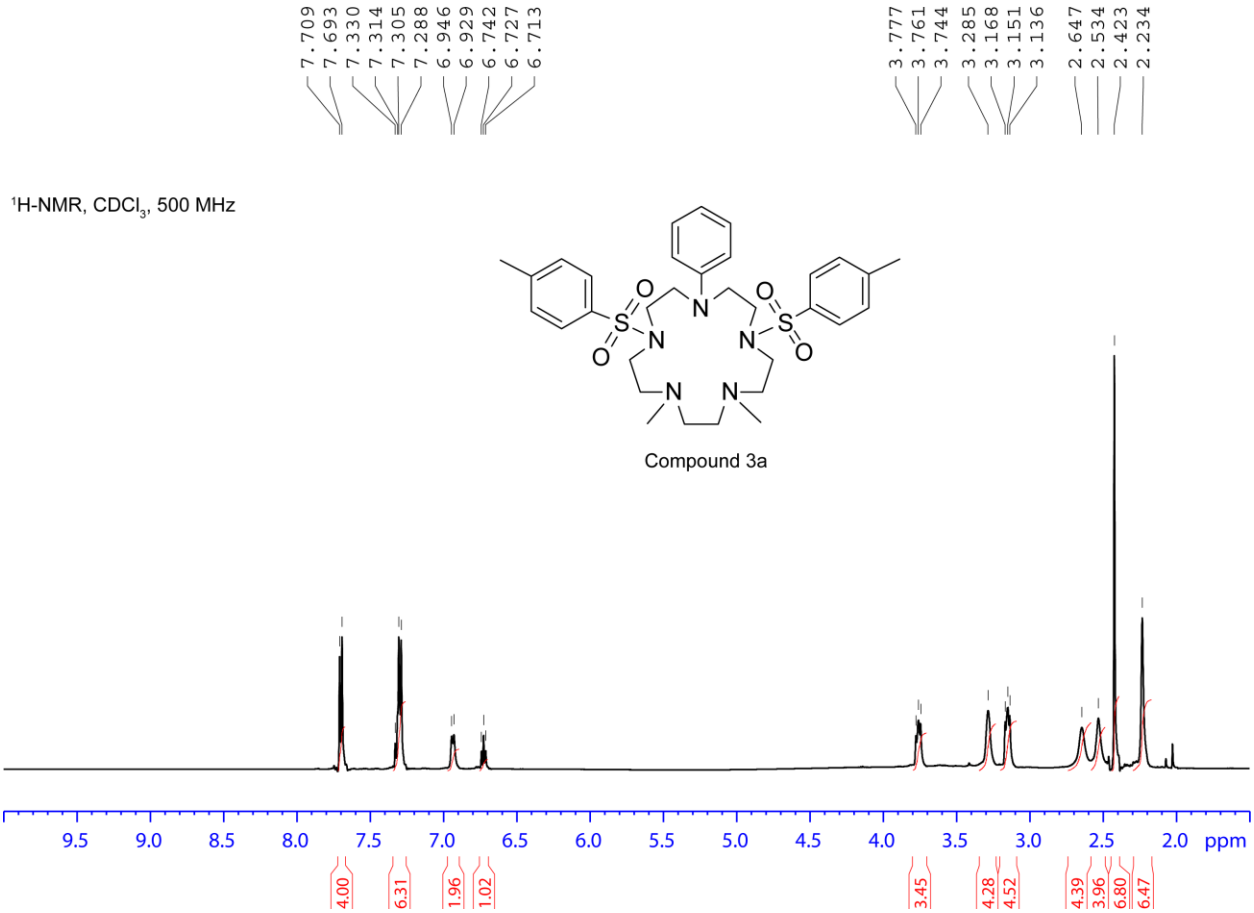
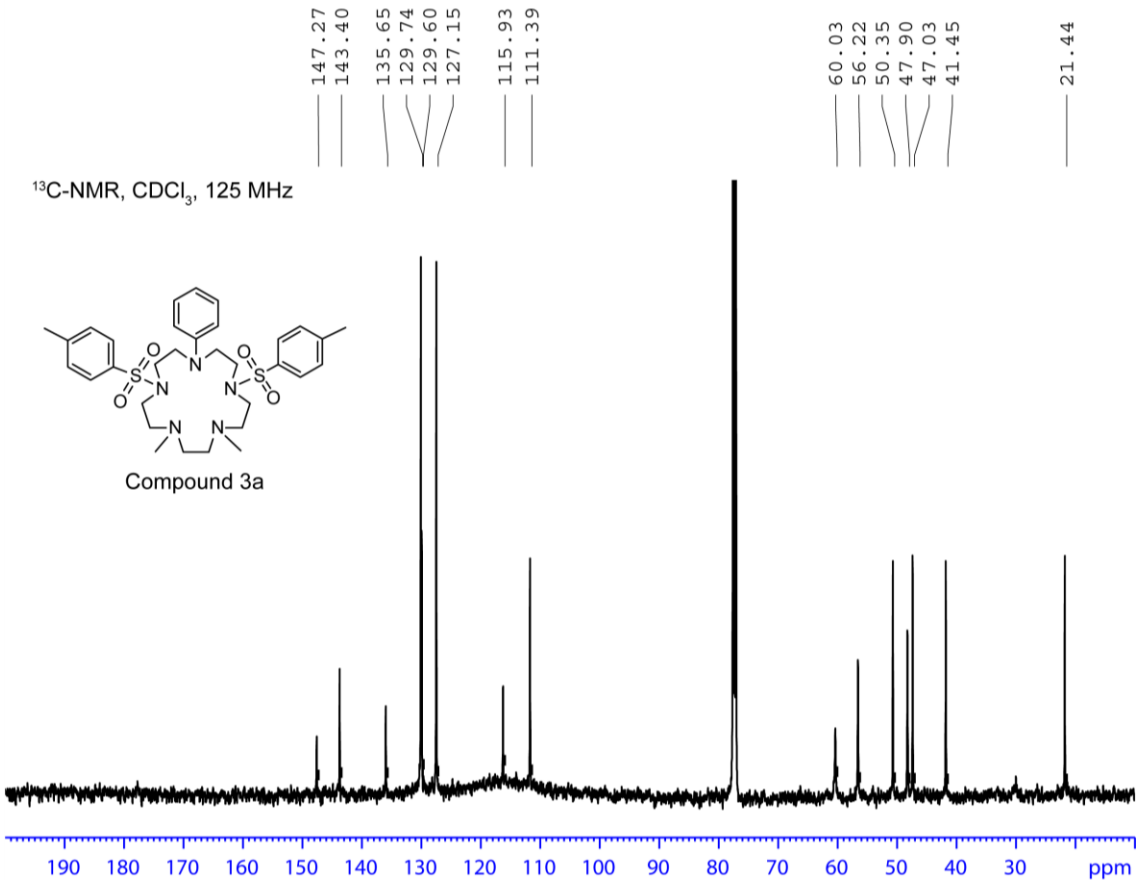
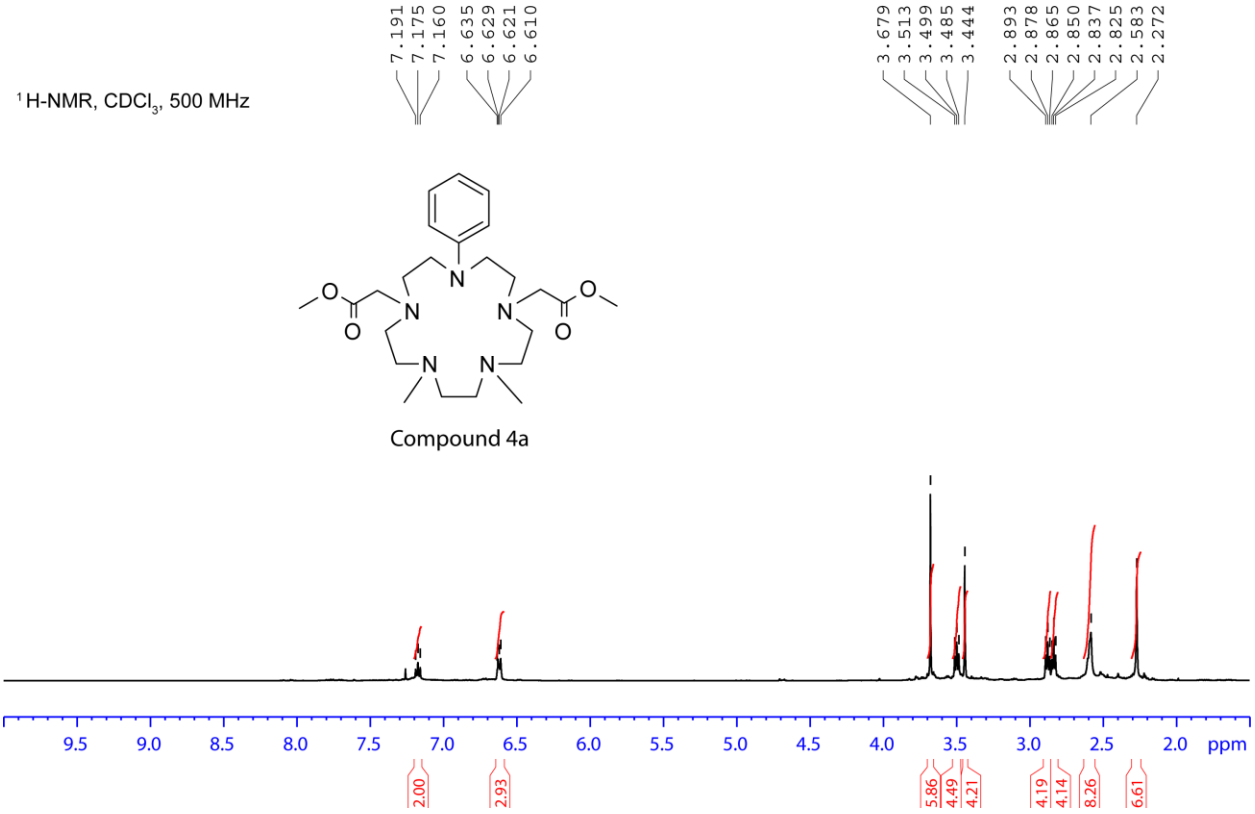
[1]H-NMR and [13]C-NMR Spectra

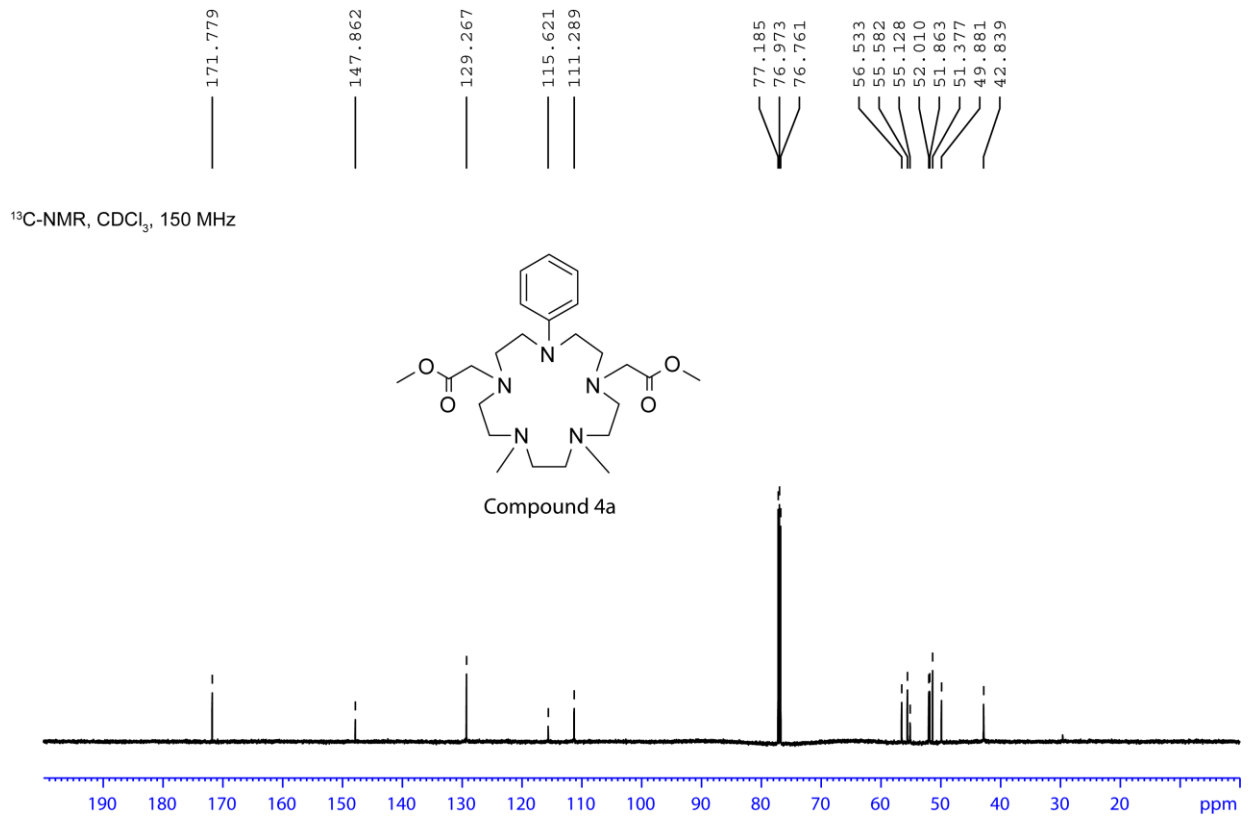



147.27
143.40
135.65
129.74
129.60
127.15
115.93
111.39
60.03
56.22
50.35
47.90
47.03
41.45
21.44
13C-NMR, CDCl3, 125 MHz
Compound 3a
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 ppm

^{1}H-NMR, $CDCl_3$, 500 MHz

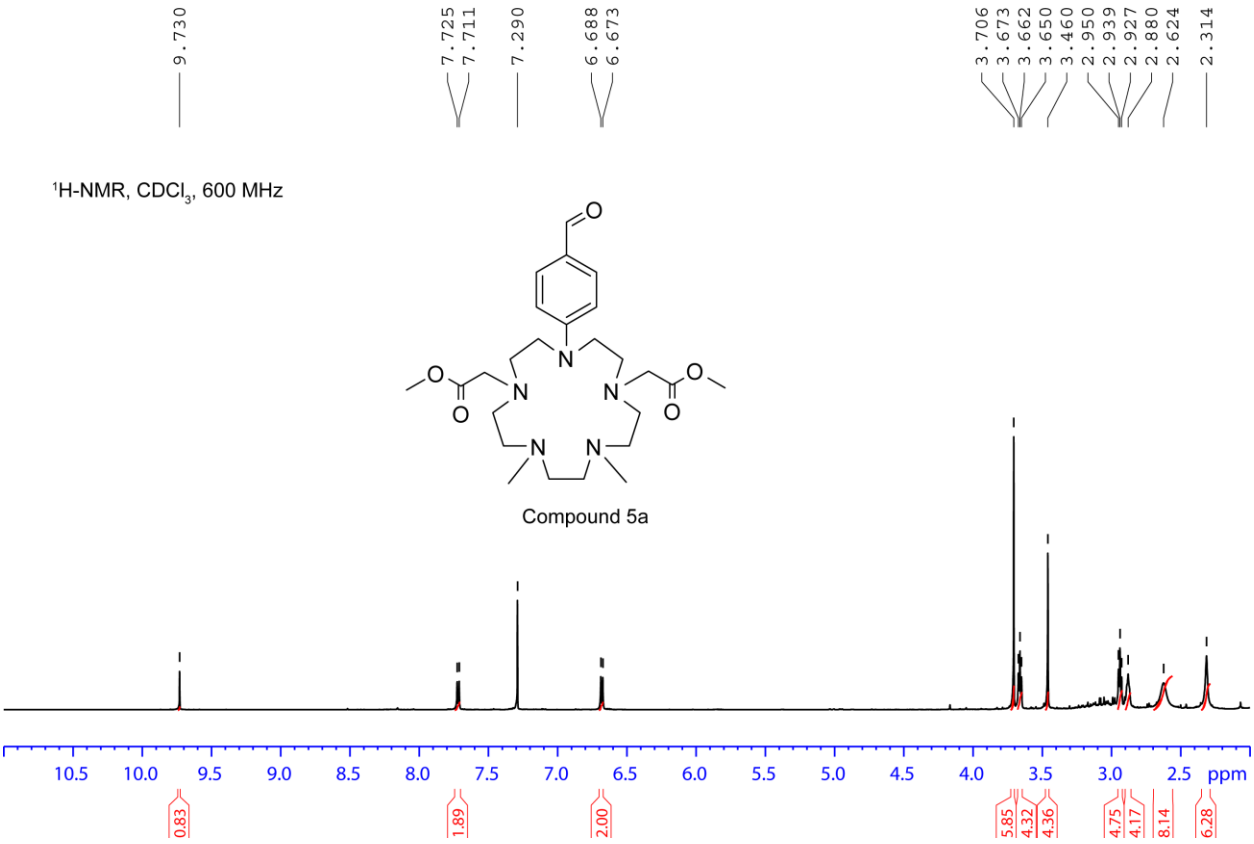


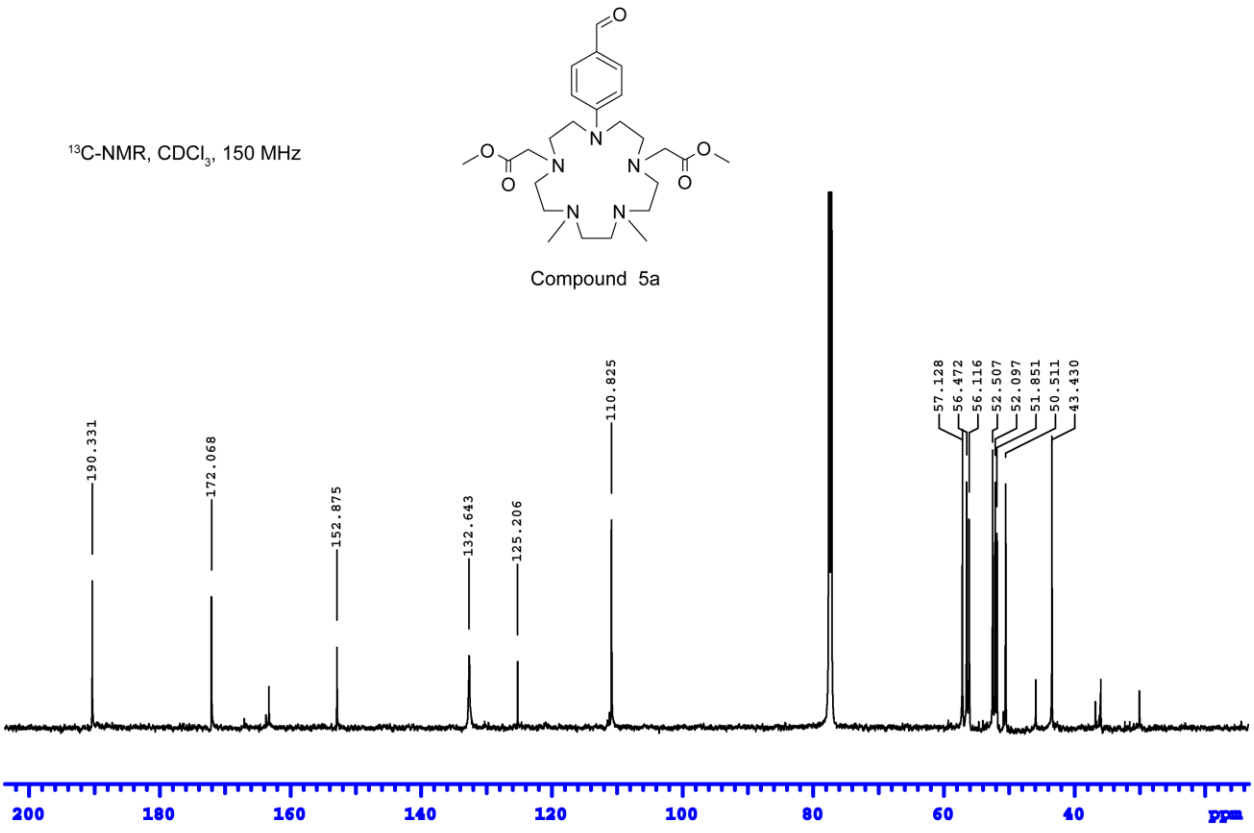
Compound 4a

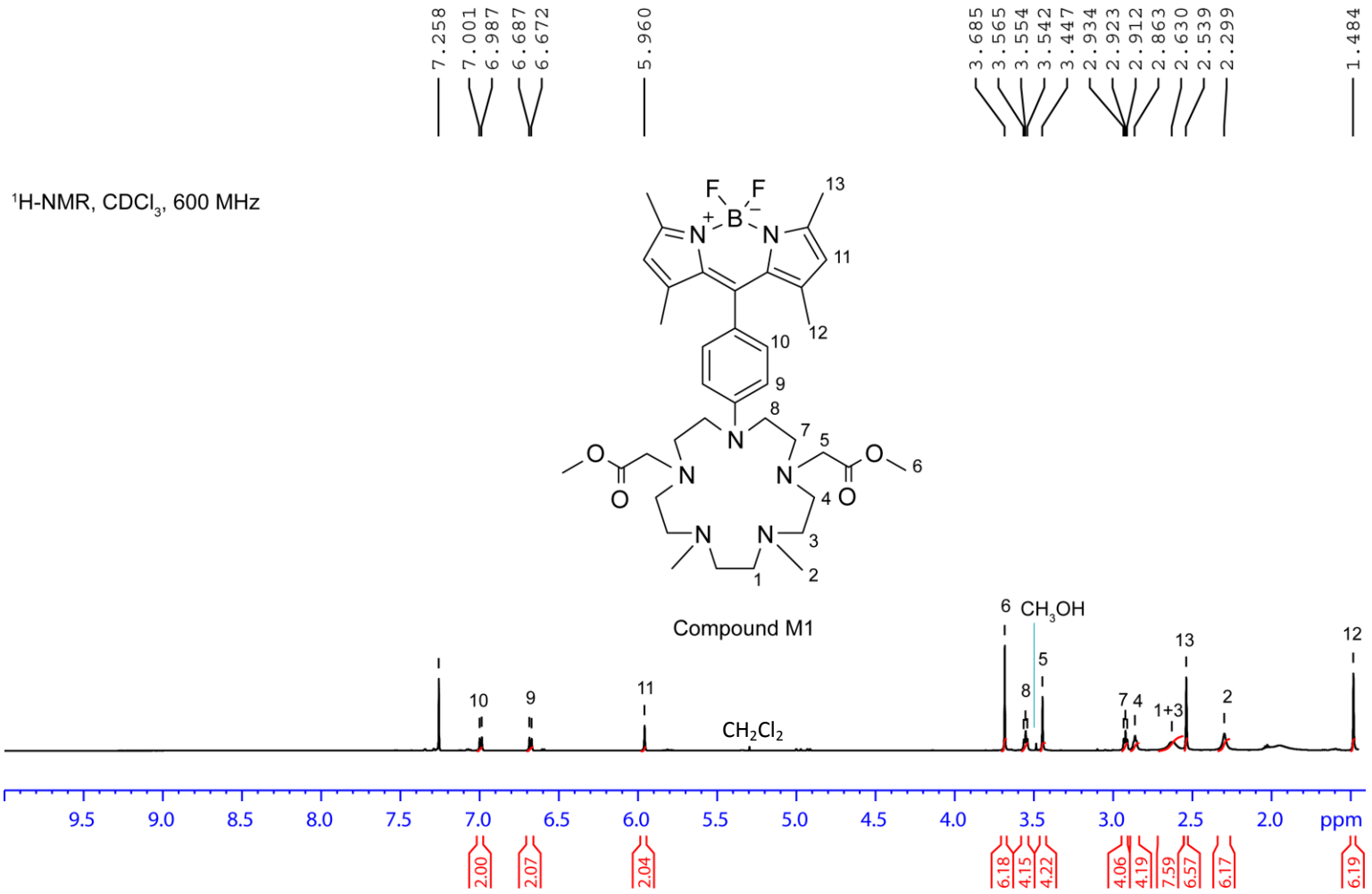


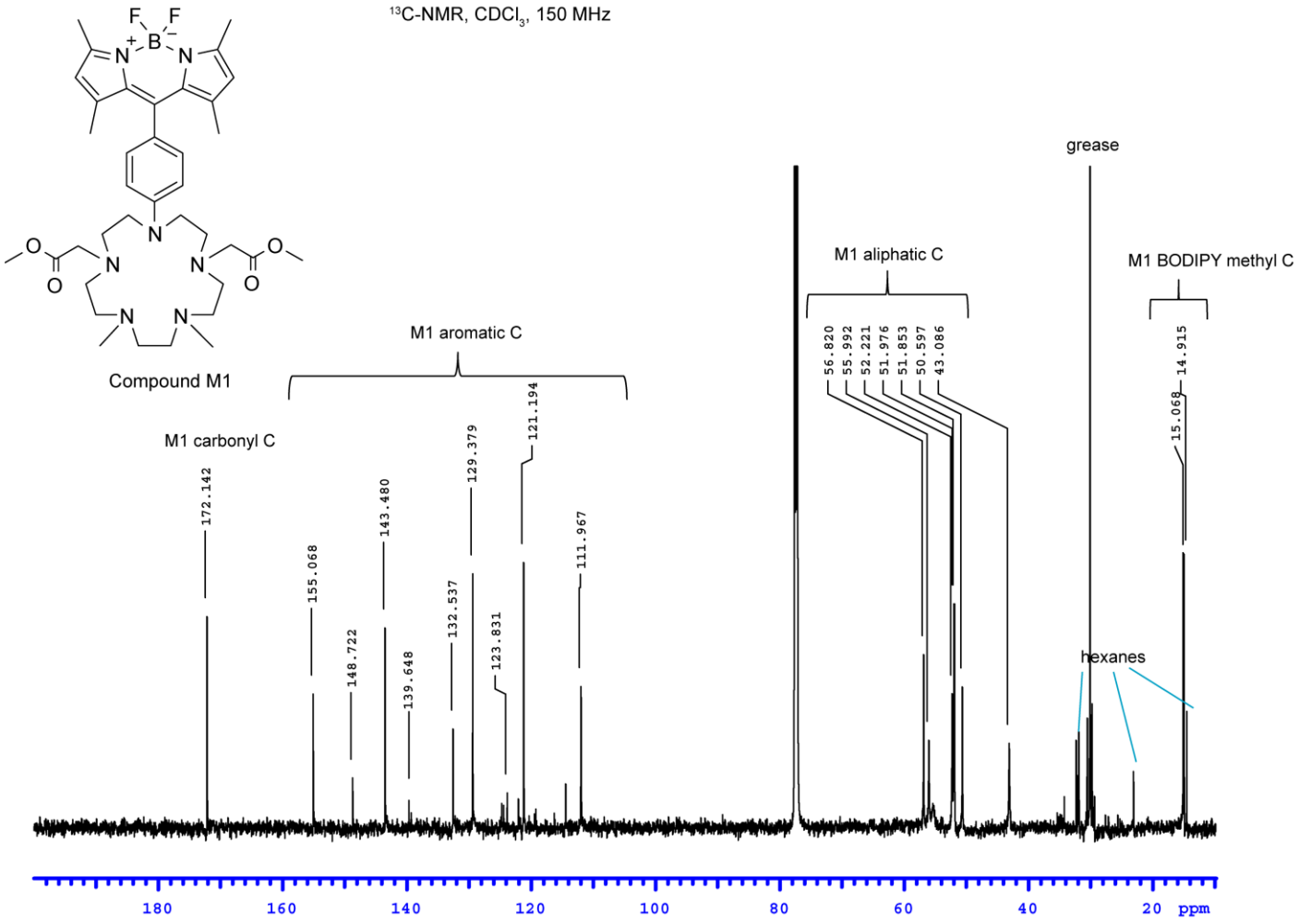
^{13}C-NMR, $CDCl_3$, 150 MHz

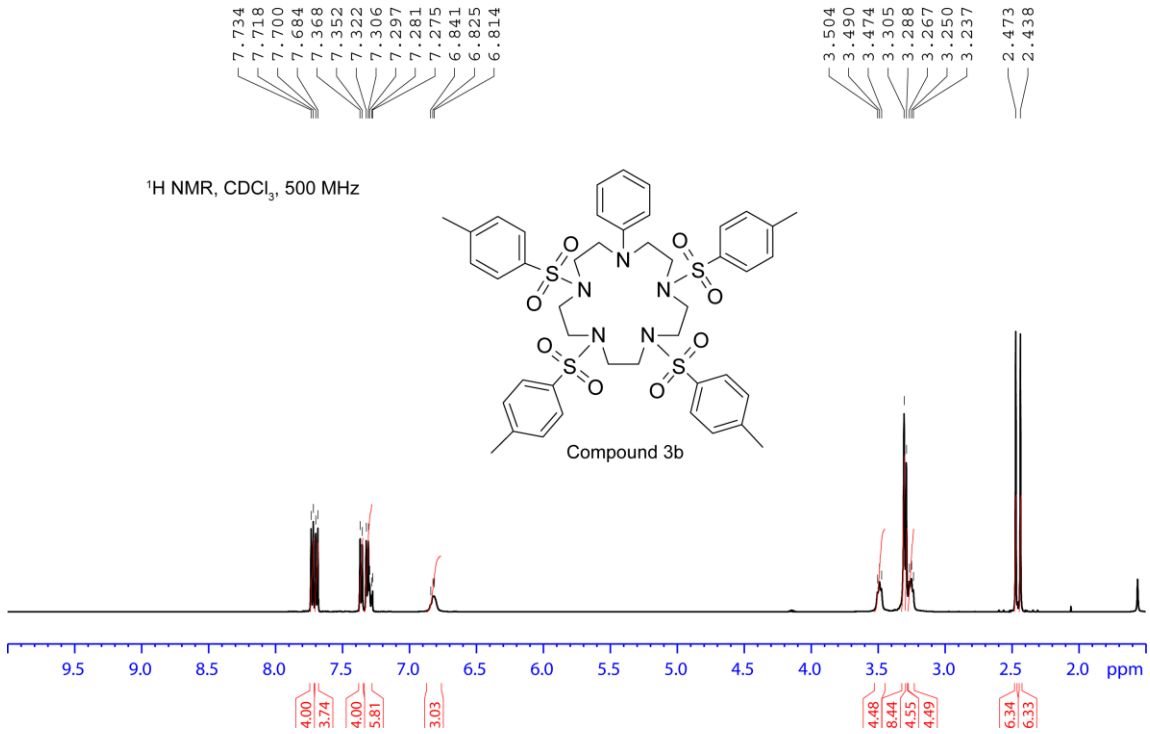
Compound 4a

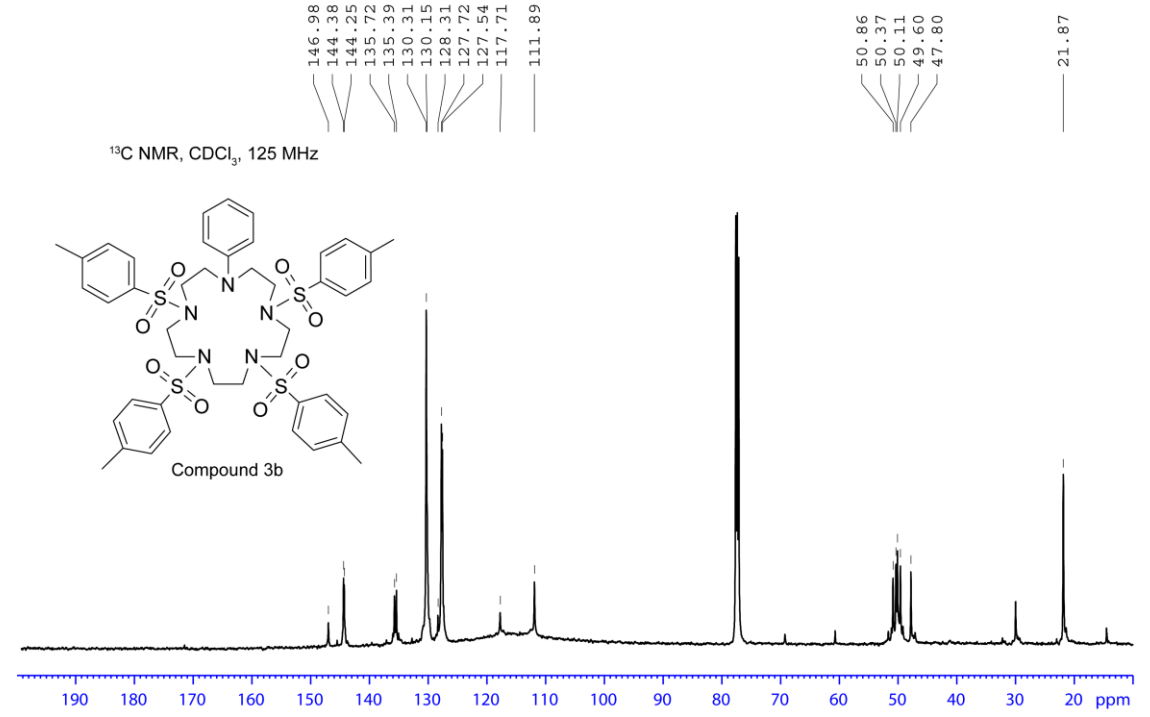

9.730
7.725
7.711
7.290
6.688
6.673
3.706
3.673
3.662
3.650
3.460
2.950
2.939
2.927
2.880
2.624
2.314
^{1}H-NMR, CDCl$_3$, 600 MHz
Compound 5a
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 ppm
0.83
1.89
2.00
5.85
4.32
4.36
4.75
4.17
8.14
6.28

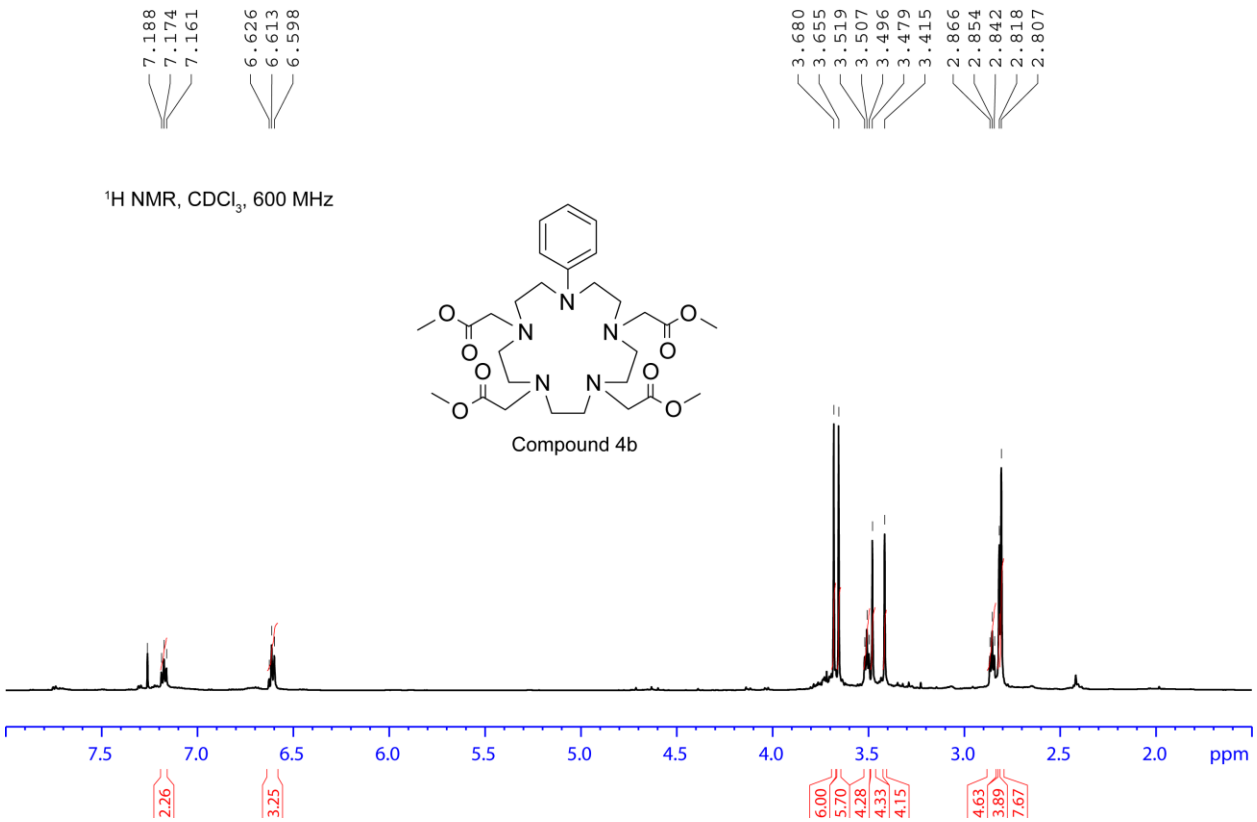

^{13}C-NMR, CDCl$_3$, 150 MHz
Compound 5a
190.331
172.068
152.875
132.643
125.206
110.825
57.128
56.472
56.116
52.507
52.097
51.851
50.511
43.430
200 180 160 140 120 100 80 60 40 ppm

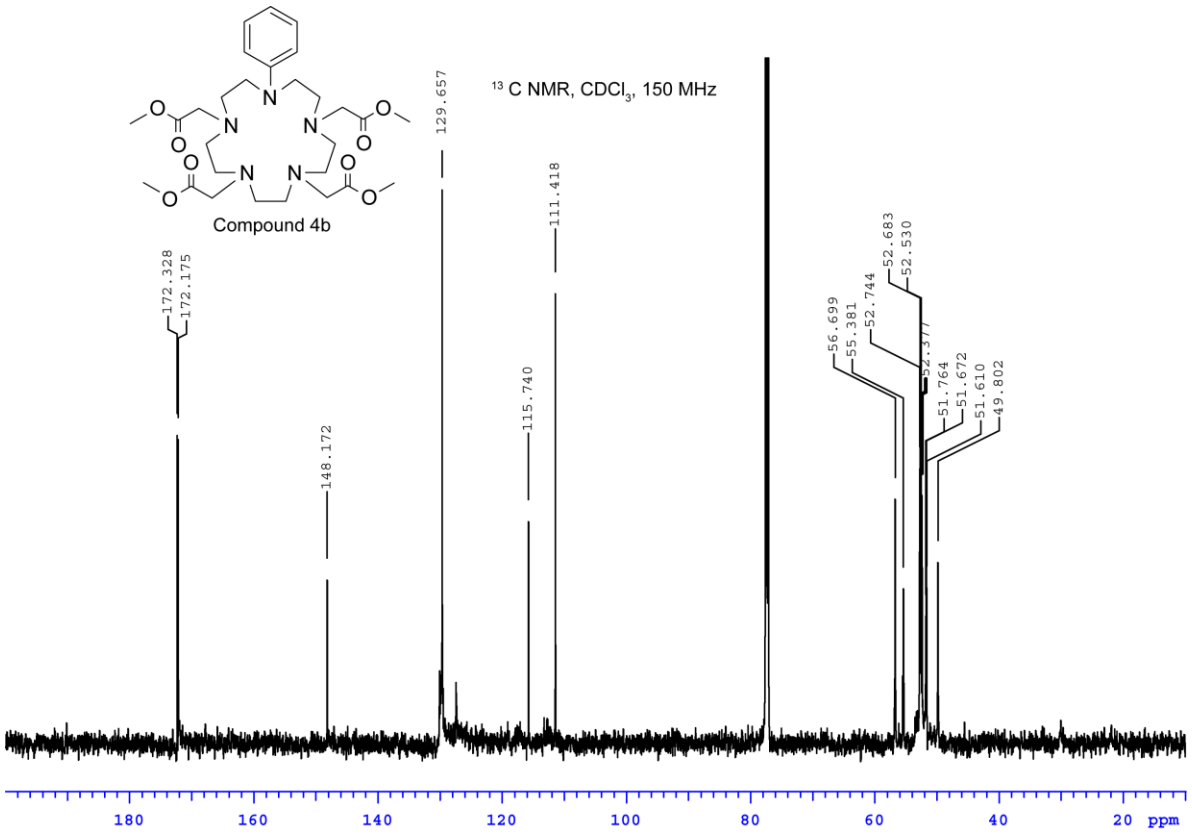

7.258
7.001
6.987
6.687
6.672
5.960
3.685
3.565
3.554
3.542
3.447
2.934
2.923
2.912
2.863
2.630
2.539
2.299
1.484
^{1}H-NMR, $CDCl_3$, 600 MHz
Compound M1
CH_2Cl_2
CH_3OH
ppm
2.00
2.07
2.04
6.18
4.15
4.22
4.06
4.19
7.59
6.57
6.17
6.19

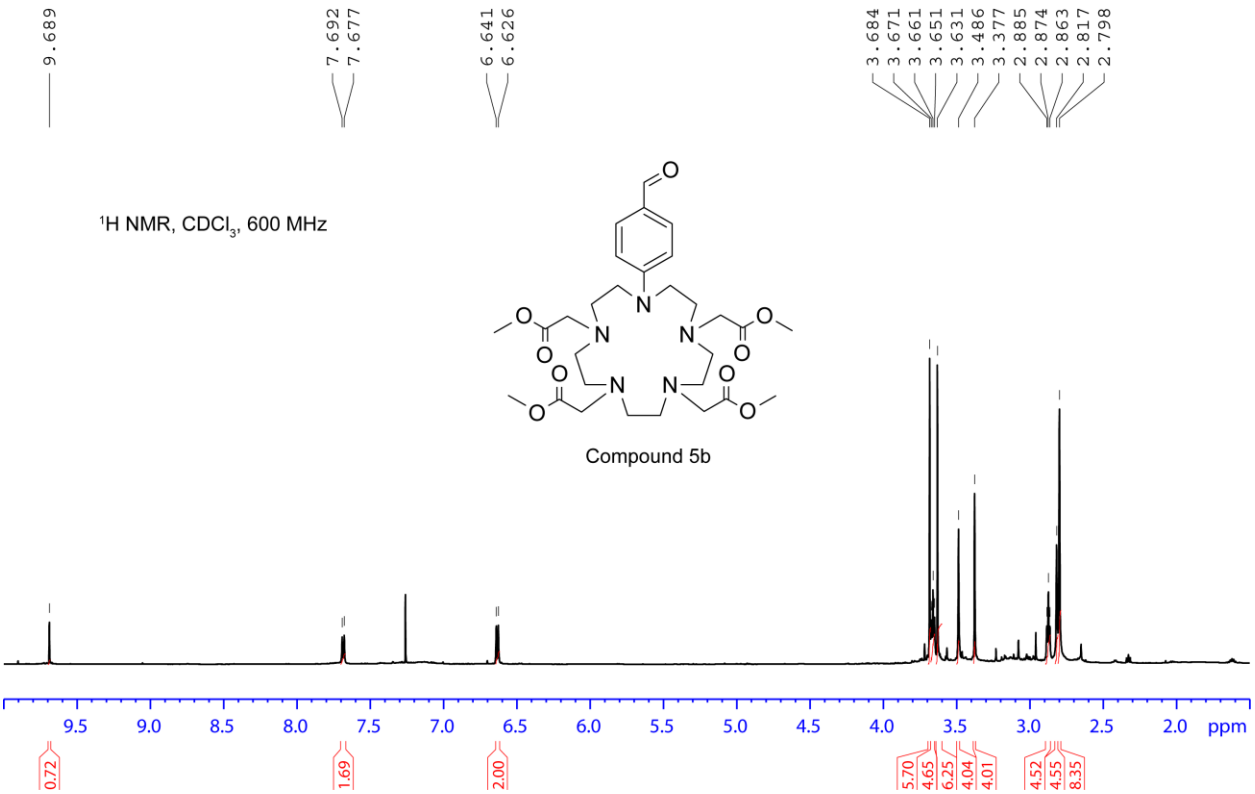

^{13}C-NMR, $CDCl_3$, 150 MHz
Compound M1
M1 carbonyl C
M1 aromatic C
M1 aliphatic C
grease
M1 BODIPY methyl C
hexanes
172.142
155.068
148.722
143.480
139.648
132.537
129.379
123.831
121.194
111.967
56.820
55.992
52.221
51.976
51.853
50.597
43.086
15.068
14.915
180
160
140
120
100
80
60
40
20
ppm

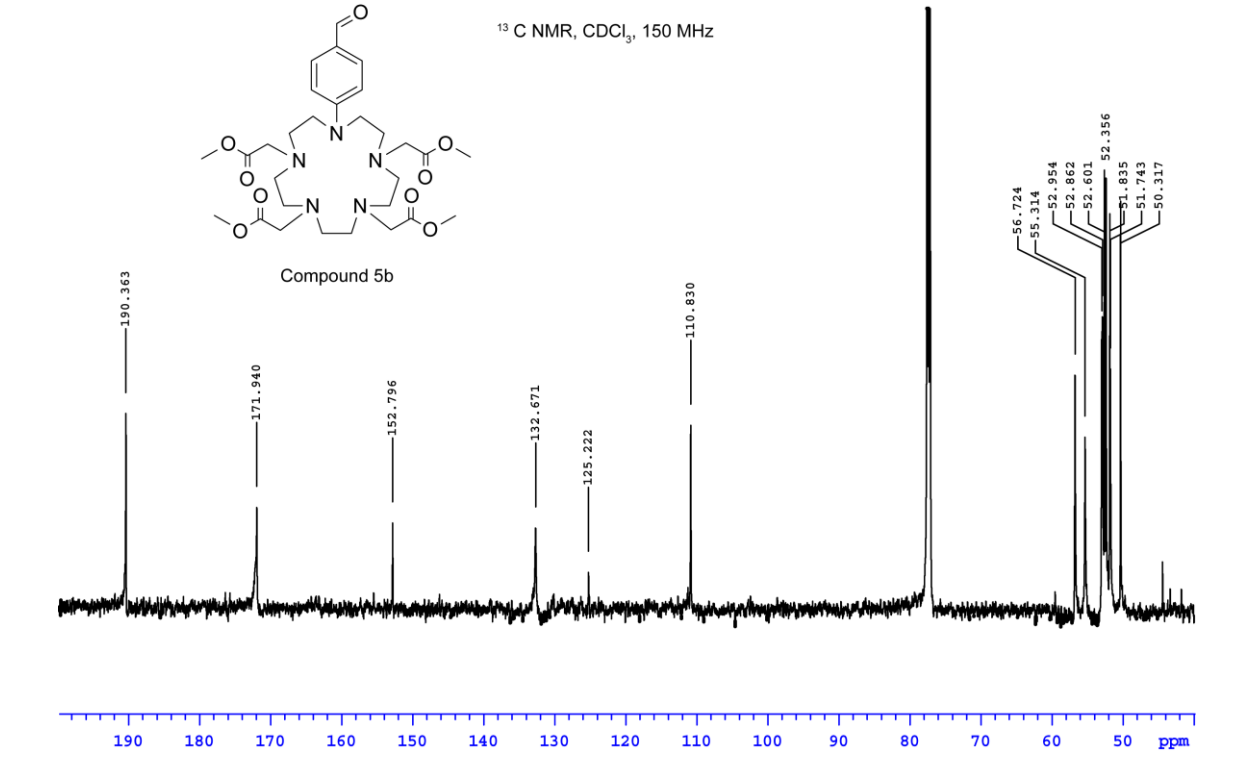

7.734
7.718
7.700
7.684
7.368
7.352
7.322
7.306
7.297
7.281
7.275
6.841
6.825
6.814
3.504
3.490
3.474
3.305
3.288
3.267
3.250
3.237
2.473
2.438
1H NMR, CDCl3, 500 MHz
Compound 3b
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
4.00
3.74
4.00
5.81
3.03
4.48
8.44
4.55
4.49
6.34
6.33

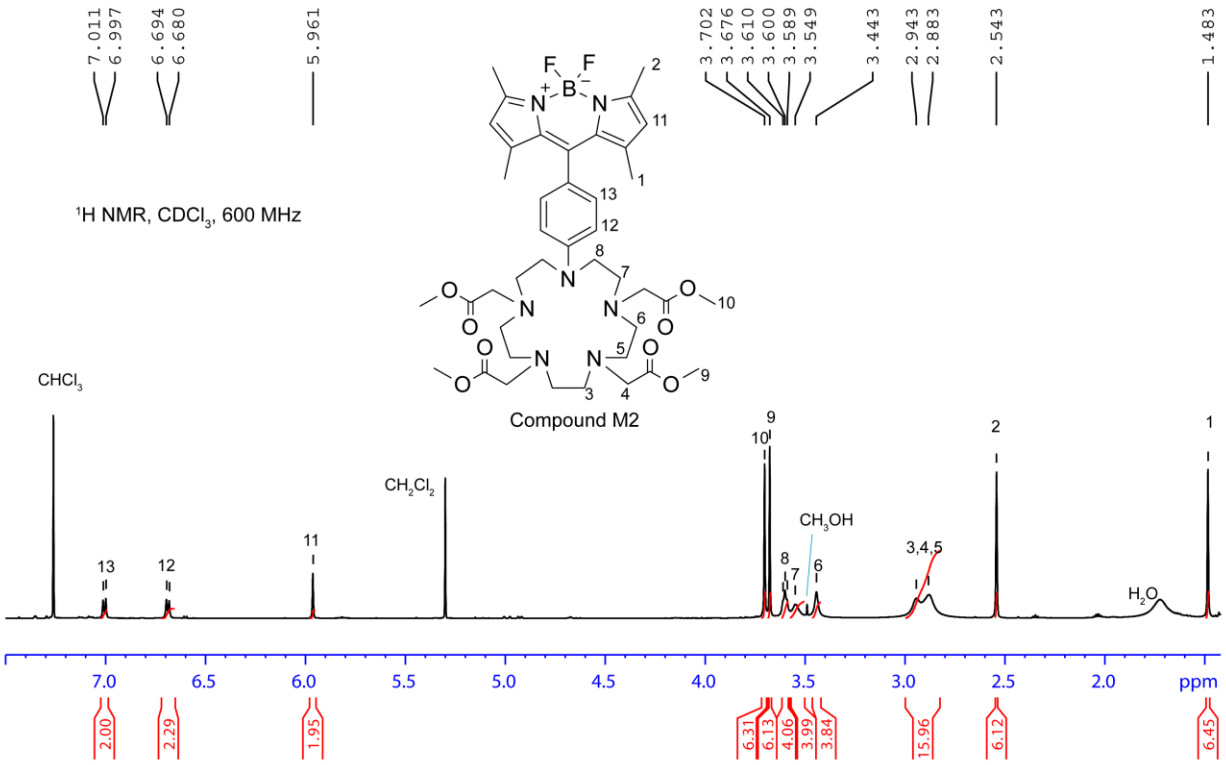

146.98
144.38
144.25
135.72
135.39
130.31
130.15
128.31
127.72
127.54
117.71
111.89
50.86
50.37
50.11
49.60
47.80
21.87
13C NMR, CDCl3, 125 MHz
Compound 3b
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

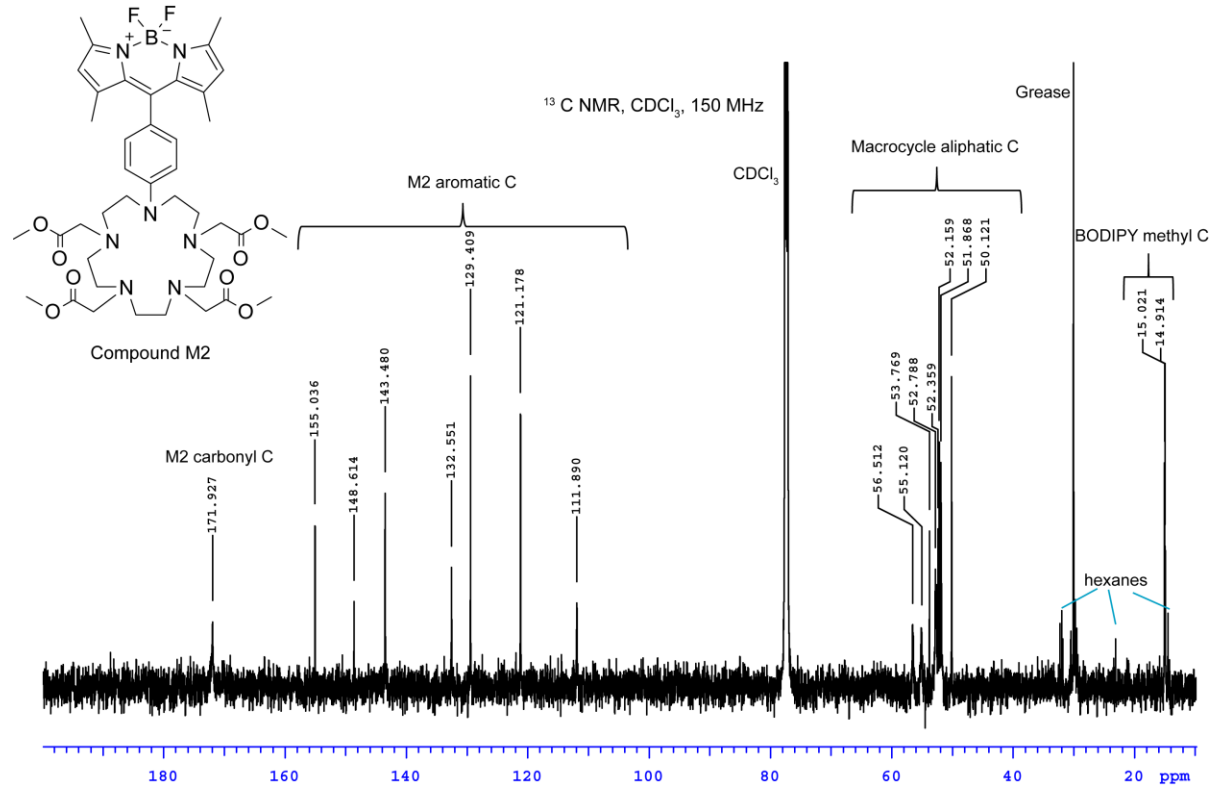

7.188
7.174
7.161
6.626
6.613
6.598
3.680
3.655
3.519
3.507
3.496
3.479
3.415
2.866
2.854
2.842
2.818
2.807
^{1}H NMR, CDCl$_3$, 600 MHz
Compound 4b
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
2.26
3.25
6.00
5.70
4.28
4.33
4.15
4.63
3.89
7.67


Compound 4b
^{13} C NMR, CDCl$_3$, 150 MHz
172.328
172.175
148.172
129.657
115.740
111.418
56.699
55.381
52.744
52.683
52.530
51.764
51.672
51.610
49.802
180
160
140
120
100
80
60
40
20
ppm


9.689
7.692
7.677
6.641
6.626
3.684
3.671
3.661
3.651
3.631
3.486
3.377
2.885
2.874
2.863
2.817
2.798
^{1}H NMR, CDCl$_3$, 600 MHz
Compound 5b
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 ppm
0.72
1.69
2.00
5.70
4.65
6.25
4.04
4.01
4.52
4.55
8.35


^{13} C NMR, CDCl$_3$, 150 MHz
Compound 5b
190.363
171.940
152.796
132.671
125.222
110.830
56.724
55.314
52.954
52.862
52.601
52.356
51.835
51.743
50.317
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 ppm


7.011
6.997
6.694
6.680
5.961
3.702
3.676
3.610
3.600
3.589
3.549
3.443
2.943
2.883
2.543
1.483
¹H NMR, CDCl₃, 600 MHz
Compound M2
CHCl₃
CH₂Cl₂
CH₃OH
H₂O
ppm
2.00
2.29
1.95
6.31
6.13
4.06
3.99
3.84
15.96
6.12
6.45


Compound M2
¹³ C NMR, CDCl₃, 150 MHz
CDCl₃
M2 aromatic C
Macrocycle aliphatic C
Grease
BODIPY methyl C
M2 carbonyl C
171.927
155.036
148.614
143.480
132.551
129.409
121.178
111.890
56.512
55.120
53.769
52.788
52.359
52.159
51.868
50.121
15.021
14.914
hexanes
ppm