Brightly Luminescent and Kinetically Inert Lanthanide Bioprobes Based on Linear and Pre-organized Chelators.

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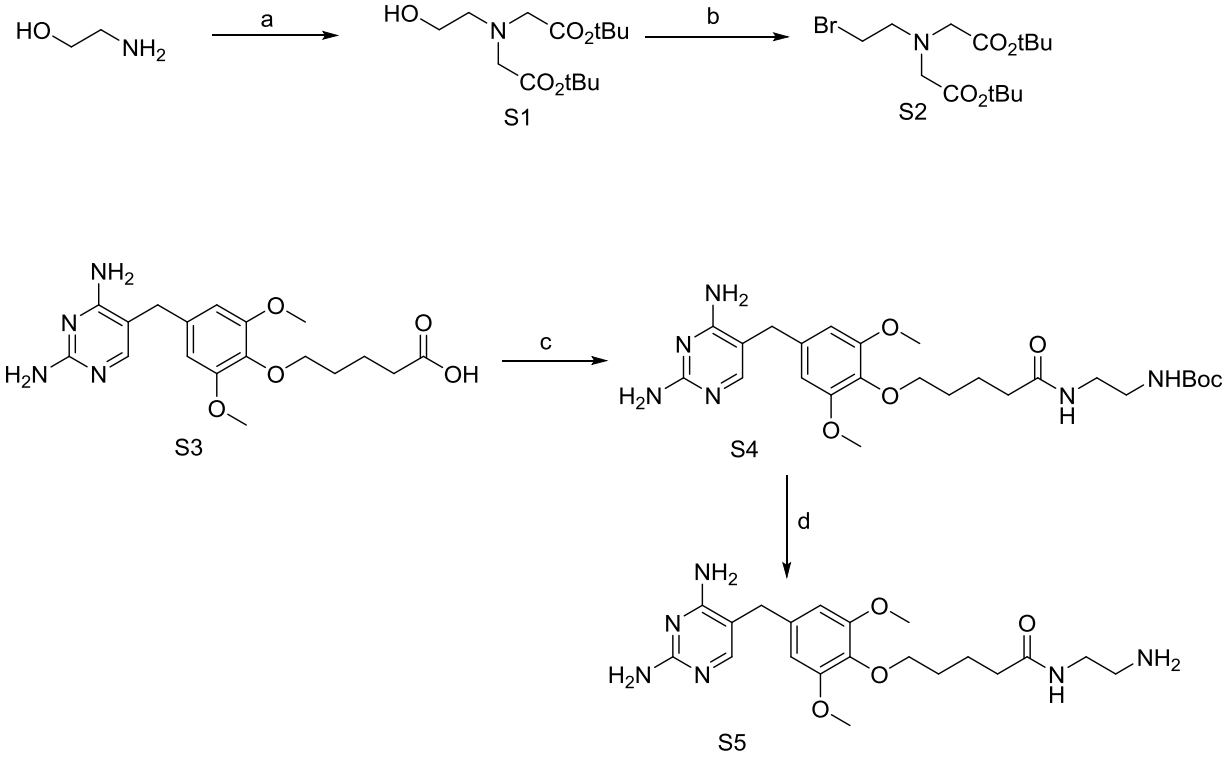
I. Syntheses of Luminescent Lanthanide Complexes.

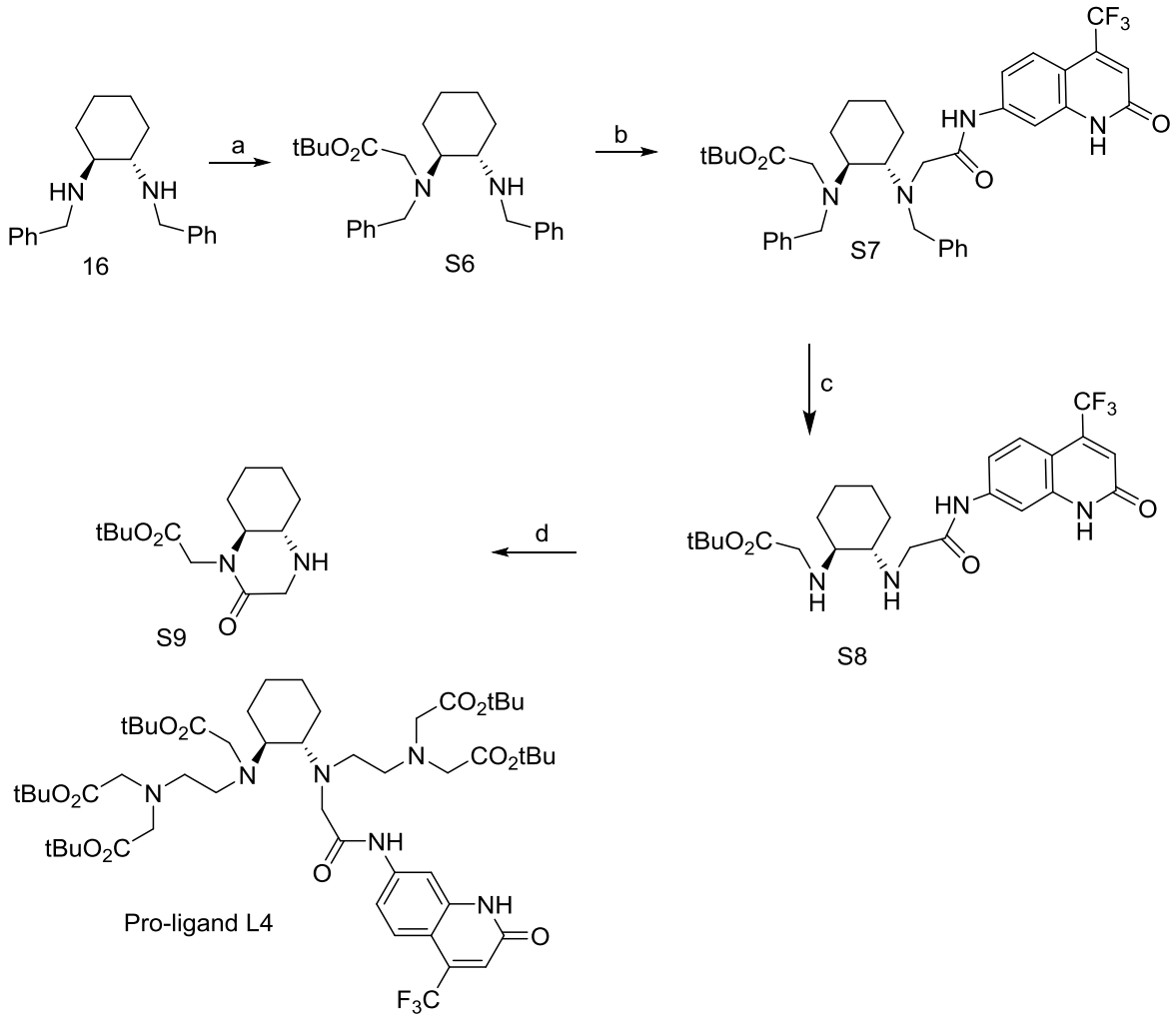
A. Supporting Schemes.

Scheme S1.



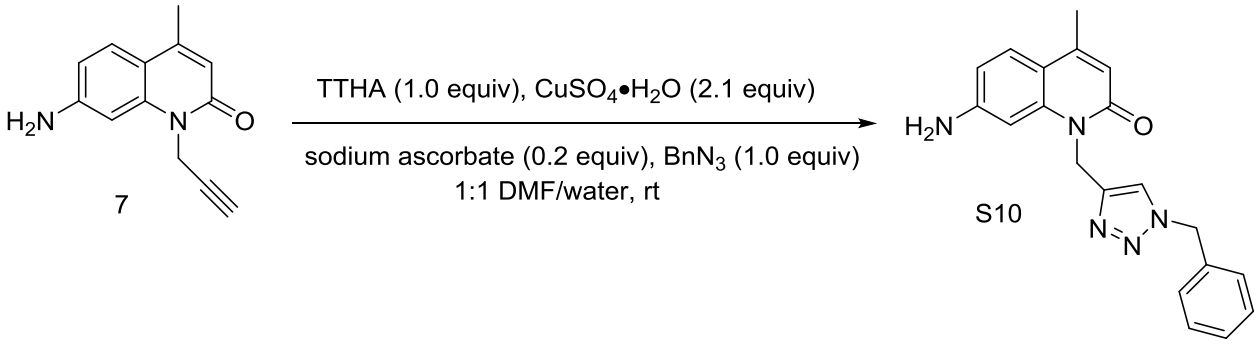
Reagents and conditions: (a) t-butyl bromoacetate (2.25 equiv), Na_2CO_3 (2.5 equiv), DMF, rt. (b) NBS (1.2 equiv), PPh_3 (1.5 equiv), DCM, 0 °C- rt. (c) EDCI (1.1 equiv), DIEA (4.0 equiv), tert-butyl (2-aminoethyl)carbamate (1.0 equiv), DMF, rt. (d) TFA/DCM, rt

Scheme S2.



Reagents and conditions: (a) t-butyl bromoacetate (0.25 equiv), Na_2CO_3 (1.0 equiv), MeCN, rt. (b) 4 (1.1 equiv), Na_2CO_3 (2.0 equiv), MeCN, 60 °C. (c) Pd/C (10 wt %), H_2, MeOH, rt. (d) S2 (2.1 equiv), Na_2CO_3 (3.0 equiv), MeCN, 60 °C.

Scheme S3.


TTHA (1.0 equiv), CuSO4•H2O (2.1 equiv)
sodium ascorbate (0.2 equiv), BnN3 (1.0 equiv)
1:1 DMF/water, rt
H2N
N
O
7
S10

B. Synthetic Methods.

1. General Information

All reactions were performed in flame-dried round-bottomed or modified Schlenk flasks fitted with rubber septa under a positive pressure of nitrogen, unless otherwise noted. Air- and moisture-sensitive liquids and solutions were transferred via syringe or stainless steel cannula. All chemicals were purchased from Sigma-Aldrich, Inc. except *trans*-1,2-diaminocyclohexane (TCI America, Inc.). Where necessary, solvents were deoxygenated by sparging with nitrogen for at least 1 hour unless otherwise noted. All other reagents were used directly from the supplier without further purification unless otherwise noted. Column chromatography was carried out employing Silicycle Silica-P flash silica gel (40-63 μm). Organic solutions were concentrated by rotary evaporation at ~25 mbar in a water bath heated to 40 °C unless otherwise noted. Thin layer chromatography plates were visualized by exposure to ultraviolet light and/or exposure to iodine, ninhydrine or a basic solution of potassium permanganate followed by heating on a hot plate. ^{1}H NMR and ^{13} C NMR spectra were recorded at the UIC Research Resources Center (RRC) in the deuterated solvents stated. ^{1} H and ^{13} C chemical shifts were referenced to internal solvent resonances and reported relative to $SiMe_4$. Low-resolution and high-resolution electrospray (ESI) mass spectra were obtained at the UIC RRC.

2. Experimental Procedures.

tert-butyl 2,2'-(2-hydroxyethylazanediyl)diacetate (S1). In a round bottom flask, sodium carbonate (5.32 g, 50.2 mmol, 2.5 equiv) and dry DMF (50 mL) were mixed and 2-Ethanolamine (1.20 mL, 20.0 mmol) was added in one portion, and the flask was placed in an ice bath. To this suspension, tert-butylbromoacetate (6.64 mL, 45.0 mmol, 2.25 equiv) was added over 1 h. The contents were stirred for 24 h at room temperature. The product was extracted with EtOAc and washed with water (100 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude mixture purified via flash column chromatography over silica gel to obtain the product as colorless, viscous oil. Yield 99%. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 3.47 (t, J = 4.7 Hz, 2H), 3.36 (s, 4H), 2.95 (t, J = 5.0 Hz, 2H), 1.34 (s, 18H). ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 170.7, 81.3, 56.9, 42.5, 28.4. ESIMS (m/z) 290 $[M+H]^+$, 312 $[M+Na]^+$, 288 $[M-H]^-$

tert-butyl 2,2'-(2-bromoethylazanediyl)diacetate (**S2**). **S1** (5.78 g, 20.0 mmol) and triphenylphosphine (7.86 g, 30.0 mmol, 1.5 equiv) were dissolved in dry DCM (100 mL) in an ice bath. N-Bromosuccinimide (4.27 g, 24.0 mmol, 1.2 equiv) was added in small portions over 40 minutes. The contents were allowed to warm to the room temperature and stirred for 3 h. The solvent was removed on a rotavap to obtain brown-red viscous oil that was washed with diethyl ether and was filtered through a small silica column to obtain colorless viscous oil. The crude mixture was purified using flash chromatography over silica gel to obtain product as colorless, viscous oil. Yield 98%. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 3.48 (s, 4H), 3.43 (t, J = 7.2 Hz, 2H), 3.12 (t, J = 7.40 Hz, 2H), 1.46 (s, 18H). ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 170.5, 81.3, 56.4, 30.3, 28.1. ESIMS (m/z) 352 $[M+H]^+$, 374 $[M+Na]^+$, 350 $[M-H]^-$

5-(4-((2,4-diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)pentanoic acid (S3). Prepared according to a literature protocol.[1]

tert-butyl (2-(5-(4-((2,4-diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)pentanamido)ethyl)carbamate (**S4**). To a well stirred solution of **S3** (286 mg, 1.0 mmol) in DMF (5 mL) was added N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (191 mg, 1.0 mmol, 1.1 equiv.) and DIEA (697 uL, 4.0 mmol, 4.0 equiv) and the solution stirred for 15 min. To the resulting solution was added tert-butyl (2-aminoethyl)carbamate (218 mg, 1.0 mmol, 1.0 equiv) and the mixture was stirred for 24 h at room temperature. The DMF was evaporated in vacuum and the residue was dissolved in ethyl acetate and washed with brine solution. The combined organic phases were dried over $MgSO_4$; the solvent was removed under reduced pressure and purified by column chromatography. Yield 90%. ^{1}H NMR (500 MHz, CD_3CN): δ ppm 7.34 (s, 1H), 6.79 (s, 2H), 6.30 (s, 2H), 3.86 (s, 2H), 3.76 (s, 6H), 3.07-3.18 (m, 4H), 2.19-2.22 (m, 2H), 1.62-1.75 (m, 4H), 1.39 (s, 9H). ESIMS (m/z) 519 $[M+H]^+$

N-(2-aminoethyl)-5-(4-((2,4-diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)pentanamide (**S5**). To **S4** (25 mg, 0.05 mmol) was added deprotection mixture (TFA/CH_2Cl_2, 1:1, 5.0 mL). Solvent was removed by evaporation under reduced pressure after stirring for 24 h. TLC shows complete conversion of the substrate to **S5**. HRMS $[C_{20}H_{30}N_6O_4 -H]^-$ calc. 417.1606; meas. 417.1604.

tert-butyl 2-(benzyl((1S,2S)-2-(benzylamino)cyclohexyl)amino)acetate (S6). To a solution **16** (1.764 mg, 6.0 mmol, 3.0 equiv.) and sodium carbonate (212 mg, 2.0 mmol, 1.0 equiv) in dry acetonitrile was added dropwise a solution of tert-butyl bromoacetate (147 uL, 1.0 mmol). The reaction mixture was stirred at 18 h at room temperature under N_2. Sodium carbonate was filtered and the filtrate concentrated in vacuo to dryness. EtOAc and water were added and aqueous layer were extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a waxy, colorless solid. Product was purified by silica column chromatography to afford **S6** as yellow oil. Yield 86%. ^{1}H NMR (400 MHz, CD_3OD): δ ppm 7.23-7.40 (m, 10H), 3.70-3.80 (m, 2H), 3.54-3.61 (m, 2H), 3.18 (d, J = 12.8 Hz, 1H), 3.03 (d, J = 12.8 Hz, 1H), 2.40-2.42 (m, 2H), 2.14-2.16 (m, 2H), 1.95-1.97 (m, 1H), 1.60-1.78 (m, 2H), 1.37 (s, 9H), 1.07-1.21 (m, 4H). ^{13}C NMR (100 MHz, CD_3OD): δ ppm 172.2, 139.5, 139.4, 129.2, 128.8, 128.5, 128.4, 127.3, 80.9, 64.7, 57.8, 50.8, 30.6, 29.8, 27.4, 25.6, 24.5, 24.0. ESIMS (m/z) 409 $[M+H]^+$, 431 $[M+Na]^+$, 407 $[M-H]^-$

tert-butyl 2-(benzyl((1S,2S)-2-(benzyl(2-oxo-2-(2-oxo-4-(trifluoromethyl)-1,2-dihydroquinolin-7-ylamino)ethyl)amino)cyclohexyl)amino)acetate (S7). To compound **S6** (408 mg, 1.0 mmol) and **8** (334 mg, 1.1 mmol, 1.1 equiv) in 10 mL DMF was added sodium carbonate (212 mg, 2.0 mmol, 2.0 equiv), and the reaction mixture was heated to 60 °C for 24 h under N_2. After cooling to room temperature, the reaction mixture was diluted with EtOAc, washed with H_2O and brine, aqueous layer were extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a yellow powder. Product was purified by silica column chromatography to afford **S7** as yellow oil. Yield 60%. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 10.41 (s, 1H), 8.89 (s, 1H), 6.99-7.75 (m, 14H), 3.65-3.75 (m, 2H), 3.39-3.50 (m, 2H), 2.88-2.99 (m, 2H), 2.51-2.62 (m, 2H), 2.05-2.18 (m, 2H), 1.71-1.78 (m, 2H), 1.55 (s, 9H), 1.03-1.26 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 173.7, 172.1, 161.9, 141.1, 141.0,

137.9, 129.9, 129.5, 128.4, 128.1, 127.4, 127.3, 124.8, 123.8, 121.7, 119.4, 116.55, 110.5, 106.9, 81.0, 60.3, 58.8, 58.4, 56.7, 55.9, 53.9, 51.4, 28.2, 25.4, 23.8, 21.0, 14.2. ESIMS (m/z) 677 $[M+H]^+$, 699 $[M+Na]^+$.

tert-butyl 2-((1S,2S)-2-(2-oxo-2-(2-oxo-4-(trifluoromethyl)-1,2-dihydroquinolin-7-ylamino)ethylamino)cyclohexylamino)acetate (S8). To a solution of **S7** (330 mg, 0.5 mmol) in methanol (5 mL) was added Pd/C (44 mg, 10 wt %). Hydrogen gas was bubbled through the solution for 5 min, and then the reaction mixture was stirred under hydrogen for 24 h. The Pd/C was filtered out over Celite, rinsing well with methanol, and the filtrate was evaporated to dryness in vacuo. The crude mixture was used for the next synthetic step without further purification. ESIMS (m/z) 497 $[M+H]^+$, 519 $[M+Na]^+$

tert-butyl 2-((4aS,8aS)-2-oxooctahydroquinoxalin-1(2H)-yl)acetate (S9). To a solution of **S8** (248 mg, 0.5 mmol) and **S2** (369 mg, 1.05 mmol, 2.1 equiv) in DMF was added sodium carbonate (159 mg, 1.5 mmol, 3.0 equiv). The solution was heated for 24 h at 60 °C under N_2. Sodium carbonate was removed by filtration. The filtrate was washed with H_2O and aqueous phase extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. Product was purified by silica column chromatography to afford **S9** as colorless powder. Yield 55%. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 4.20 (d, *J* = 15.0 Hz, 1H), 3.95 (d, *J* = 15.0 Hz, 1H), 3.61-3.62 (m, 2H), 3.08-3.12 (m, 1H), 2.54-2.58 (m, 2H), 1.72-1.93 (m, 4H), 1.41 (s, 9H), 1.22-1.27 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 169.1, 168.5, 81.7, 62.4, 58.5, 50.1, 43.9, 31.7, 29.3, 28.0, 24.3. ESIMS (m/z) 269 $[M+H]^+$, 291 $[M+Na]^+$, 267 $[M-H]^-$

7-amino-1-((1-benzyl-2,3-dihydro-1H-1,2,3-triazol-4-yl)methyl)-4-methylquinolin-2(1H)-one (S10). To **7** (212 mg, 1.0 mmol) and TTHA (494 mg, 1.0 mmol, 1.0 equiv) in the solution of 1:1 DMF/water (10.0 mL) was added copper sulfate pentahydrate (319 mg, 2.1 mmol, 2.1 equiv) and the resulting solution stirred for 30 min at room temperature which after that (azidomethyl)benzene (133 mg, 1.0 mmol, 1.0 equiv) and sodium ascorbate (40 mg, 0.2 mmol, 0.2 equiv) added and the reaction mixture stirred at room temperature for overnight. The solution was concentrated in vacuo and the precipitate dissolved in 1:1 THF/water (10 mL) and sodium sulfide (390 mg, 5.0 mmol, 5.0 equiv). The brown solid was filtered out using a fine fritted filter and the colorless filtrate was concentrated in vacuo to dryness. The resulting powder purified by silica column chromatography to afford **S10** as a light brown powder. Yield 90%. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 7.87 (s, 1H), 7.25-7.43 (m, 6H), 6.65 (d, *J* = 2.0 Hz, 1H), 6.50 (dd, *J* = 9.0, 2.0 Hz), 6.12 (s, 1H), 5.85 (s, 2H), 5.51 (s, 2H), 5.31 (s, 2H), 2.30 (s, 3H). ESIMS (m/z) 348 $[M+H]^+$

7-amino-4-(trifluoromethyl)quinolin-2(1H)-one (2). 1,3- phenylenediamine (1.08 g, 10.0 mmol) and ethyl 4,4,4-trifluoro-3-oxobutanoate (1.46 mL, 10.0 mmol, 1.0 equiv) were refluxed in DMF for 24 h. The product was extracted with EtOAc and washed with water (100 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude mixture was purified using flash chromatography over silica gel to obtain product as a yellow powder. Yield 95%. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 11.80 (s, 1H), 7.33 (d, *J* = 8.5 Hz, 1H), 6.55 (d, *J* = 8.5 Hz, 1H), 6.45 (s, 1H), 6.41 (s, 1H), 6.16 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.3, 152.5, 142.6, 137.0, 125.5, 124.4, 113.9, 112.2, 103.9, 97.1. ESIMS (m/z) 229 $[M+H]^+$, 251 $[M+Na]^+$, 227 $[M-H]^-$

7-amino-4-methylquinolin-2(1H)-one (3). 1,3-Phenylenediamine (1.08 g, 10.0 mmol) and ethyl 3-oxobutanoate (1.26 mL, 10.0 mmol, 1.0 equiv) were refluxed in DMF for 24 h. The product was extracted with EtOAc and washed with water (100 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude mixture purified via flash column chromatography over silica gel to obtain the product as a yellow powder. Yield 75%. ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ ppm 11.14 (s, 1H), 7.31 (d, *J* = 8.5 Hz, 1H), 6.44 (d, *J* = 8.5 Hz, 1H), 6.35 (s, 1H), 5.93 (s, 1H), 5.72 (s, 2H), 2.26 (s, 3H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ ppm 162.8, 151.5, 148.3, 141.2, 126.0, 115.1, 110.8, 97.2, 18.9. ESIMS (m/z) 175 $[M+H]^+$, 197 $[M+Na]^+$, 173 $[M-H]^-$

2-chloro-N-(2-oxo-4-(trifluoromethyl)-1,2-dihydroquinolin-7-yl)acetamide (4). To **2** (456 mg, 2.0 mmol) in DMF (0.20 M) was added NEt_3 (836 uL, 6.0 mmol, 3.0 equiv) and the solution was cooled to 0 °C before chloroacetyl chloride (175 uL, 2.2 mmol, 1.1 equiv) diluted in DMF was added dropwise to the reaction solution. After 6 h, the reaction mixture was diluted with EtOAc and washed with H_2O. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude mixture purified via flash column chromatography over silica gel to obtain the product as a yellow powder. Yield 76%. ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ ppm 12.29 (s, 1H), 10.80 (s, 1H), 7.95 (s, 1H), 7.66 (d, *J* = 9.0 Hz, 1H), 7.40 (d, *J* = 9.0 Hz, 1H), 6.82 (s, 1H), 3.32 (s, 2H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ ppm 165.8, 160.8, 141.7, 141.2, 136.5, 125.5, 120.2, 115.2, 109.6, 105.6, 44.0. ESIMS (m/z) 305 $[M+H]^+$, 327 $[M+Na]^+$, 303 $[M-H]^-$

2-chloro-N-(4-methyl-2-oxo-1,2-dihydroquinolin-7-yl)acetamide (5). To **3** (348 mg, 2.0 mmol) in DMF (0.20 M) was added NEt_3 (836 uL, 6.0 mmol, 3.0 equiv) and the solution was cooled to 0 °C before chloroacetyl chloride (175 uL, 2.2 mmol, 1.1 equiv) diluted in DMF was added dropwise to the reaction solution. After 6 h, the reaction mixture was diluted with EtOAc and washed with H_2O and brine to give a grey precipitate in the organic layer. After filtration, the solid was dried under high vacuum to give product as a white solid (76%). ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ ppm 11.60 (s, 1H), 10.67 (s, 1H), 7.77 (s, 1H), 7.66 (d, *J* = 9.0 Hz, 1H), 7.35 (d, *J* = 9.0 Hz, 1H), 6.29 (s, 1H), 3.36 (s, 2H), 2.39 (s, 3H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ ppm 165.4, 162.4, 148.0, 140.6, 139.9, 125.9, 119.7, 116.4, 114.0, 105.2, 44.0, 18.8. ESIMS (m/z) 251 $[M+H]^+$, 273 $[M+Na]^+$, 249 $[M-H]^-$

7-amino-1-(prop-2-yn-1-yl)-4-(trifluoromethyl)quinolin-2(1H)-one (**6**). To **2** (456 mg, 2.0 mmol) in DMF (0.2 M) was added NaH (48 mg, 2.0 mmol, 1.0 equiv) and stirred at room temperature for 15 min. Propargyl bromide (178 uL, 2.0 mmol, 1.0 equiv) was added dropwise and stirred at rt. After 12 h, 100 mL water added and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a yellow solid. The crude product was purified by silica gel column chromatography. Yield 80%. ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ ppm 7.46-7.47 (d, *J* = 8.5 Hz, 1H), 6.67-6.70 (m, 2H), 6.60 (s, 1H), 6.40 (s, 2H), 4.96 (s, 2H), 3.34 (s, 1H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ ppm 159.87, 153.24, 141.89, 126.68, 112.49, 111.92, 104.49, 97.77, 78.96, 75.15, 31.96. HRMS $[C_{13}H_9N_2OF_3 +H]^+$ calc. 267.0745; meas. 267.0743.

7-amino-4-methyl-1-(prop-2-ynyl)quinolin-2(1H)-one (7). To **3** (348 mg, 2.0 mmol) in DMF (0.2 M) was added NaH (48 mg, 2.0 mmol, 1.0 equiv) and stirred at room temperature for 15 min. Propargyl bromide (178 uL, 2.0 mmol, 1.0 equiv) was added dropwise and stirred at rt. After 12

h, 100 mL water added and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a yellow solid. The crude product was purified by silica gel column chromatography. Yield 80%. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 7.42 (d, J = 9.0 Hz, 1H), 6.54-6.59 (m, 2H), 6.09 (s, 1H), 5.95 (s, 2H), 4.89 (s, 2H), 3.19 (s, 1H), 2.28 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.0, 152.1, 148.0, 140.6, 127.1, 113.9, 111.5, 110.5, 97.5, 79.9, 74.4, 31.0, 18.9. ESIMS (m/z) 213 $[M+H]^+$, 235 $[M+Na]^+$, 211 $[M-H]^-$

2-chloro-N-(2-oxo-1-(prop-2-yn-1-yl)-4-(trifluoromethyl)-1,2-dihydroquinolin-7-yl)acetamide (8). Prepared in similar way as **4**, except **6** used instead of **2.** The crude mixture was used for the next synthetic step without further purification. ESIMS (m/z) 343 $[M+H]^+$

chloro-N-(4-methyl-2-oxo-1-(prop-2-ynyl)-1,2-dihydroquinolin-7-yl)acetamide (9). Prepared in similar way as **5**, except **7** used instead of **3**. Yield 80%. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 11.20 (s, 1H), 8.06 (s, 1H), 7.75 (d, J = 11.0 Hz, 1H), 7.58 (dd, J = 11.0, 1.5 Hz, 1H), 6.44 (s, 1H), 4.95 (s, 2H), 4.42 (s, 2H), 3.27-3.28 (m, 1H), 2.40 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 166.1, 161.0, 148.2, 141.9, 139.5, 127.2, 118.9, 117.7, 114.5, 105.2, 79.6, 75.4, 44.5, 31.7, 19.3. ESIMS (m/z) 289 $[M+H]^+$, 311 $[M+Na]^+$, 287 $[M-H]^-$

2-(7-amino-2-oxo-4-(trifluoromethyl)-1,2-dihydroquinolin-3-yl)acetic acid (10). Prepared according to a literature protocol.[2]

2-(7-amino-2-oxo-4-(trifluoromethyl)-1,2-dihydroquinolin-3-yl)-N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)acetamide (**11**). To a well stirred solution of **10** (286 mg, 1.0 mmol) in DMF (5 mL) was added N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (191 mg, 1.0 mmol, 1.1 equiv.) and DIEA (697 uL, 4.0 mmol, 4.0 equiv) and the solution stirred for 15 min. To the resulting solution was added 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-amine (218 mg, 1.0 mmol, 1.0 equiv) and the mixture was stirred for 24 h at room temperature. The DMF was evaporated in vacuum, the residue was dissolved in ethyl acetate and washed with brine solution. The combined organic phases were dried over $MgSO_4$; the solvent was removed under reduced pressure and purified by column chromatography. Yield 90%. ^{1}H NMR (500 MHz, CD_3OD): δ ppm 7.54 (dd, J = 9.0, 2.5 Hz, 1H), 6.63 (dd, J = 9.0, 2.5 Hz, 1H), 6.49 (d, J = 2.0 Hz, 1H), 3.87 (s, 2H), 3.58-3.64 (m, 12H), 3.35-3.36 (m, 4H). HRMS $[C_{20}H_{25}F_3N_6O_5+H]^+$ calcd. 487.1917; meas. 487.1917.

N-(3-(14-azido-2-oxo-6,9,12-trioxa-3-azatetradecyl)-2-oxo-4-(trifluoromethyl)-1,2-dihydroquinolin-7-yl)-2-chloroacetamide (**12**). To **11** (1125 mg, 2.0 mmol) in DMF (0.20 M) was added NEt_3 (836 uL, 6.0 mmol, 3.0 equiv) and the solution was cooled to 0 °C before chloroacetyl chloride (175 uL, 2.2 mmol, 1.1 equiv) diluted in DMF was added dropwise to the reaction solution. After 6 h, the reaction mixture was diluted with EtOAc and washed with H_2O. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude mixture purified via flash column chromatography over silica gel to obtain the product as a yellow powder. Yield 78%. ^{1}H NMR (500 MHz, CD_3OD): δ ppm 7.97-8.08 (m, 2H), 7.77-7.79 (m, 2H), 7.24-7.26 (m, 2H), 4.22 (s, 2H), 3.95 (s, 2H), 3.54-3.64 (m, 12H), 3.37-3.39 (m, 4H). HRMS $[C_{23}H_{26}N_6O_6F_3Cl+H]^+$ calc: 563.1633; meas: 563.1637.

N,N′-(Benzyl)ethylenediamine (15). To a solution of ethylenediamine (1.33 mL, 20.0 mmol) in dry methanol (100 mL) was added benzaldehyde (4.06 mL, 40.0 mmol, 2.0 equiv). The solution was refluxed for 10 h and then cooled via an ice bath. Addition of $NaBH_4$ (3.25 g, 86.0 mmol, 4.3 equiv) was performed slowly and in small portions to prevent boiling, and the reaction mixture was stirred for 4 h until completion. The solvent was evaporated in vacuo, and then saturated $NaHCO_3$ (50 mL), water (50 mL) and EtOAc (200 mL) were added. The aqueous layer was extracted twice with EtOAc (100 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a waxy, yellow solid. The crude mixture purified via flash column chromatography over silica gel to obtain the product as yellow oil. Yield 75%. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.36 (d, *J* = 10.0 Hz, 8H), 7.27-7.32 (m, 2H), 3.81 (s, 4H), 2.79 (s, 4H), 1.63 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 140.6, 128.4, 128.2, 126.9, 54.0, 48.9. ESIMS (m/z) 241 $[M+H]^+$, 263 $[M+Na]^+$, 239 $[M-H]^-$

di-tert-butyl 6,9-dibenzyl-3,12-bis(2-tert-butoxy-2-oxoethyl)-3,6,9,12-tetraazatetradecane-1,14-dioate (17). To a solution of **15** (2.40 g, 10.0 mmol) and **S2** (7.39 g, 21.0 mmol, 2.1 equiv) in MeCN was added sodium carbonate (3.17 g, 30.0 mmol, 3.0 equiv). The solution was heated for 24 h at 60 °C under N_2. Sodium carbonate was removed by filtration and the filtrate was washed with H_2O and aqueous phase extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude mixture purified via flash column chromatography over silica gel to obtain the product as yellow oil. Yield 89%. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.18-7.24 (m, 10H), 3.55 (s, 4H), 3.37 (s, 8H), 2.76-2.79 (m, 4H), 2.54-2.57 (m, 8H), 1.41 (s, 36H). ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 170.6, 139.6, 128.8, 128.0, 126.7, 80.7, 59.2, 56.1, 53.0, 52.1, 28.1. ESIMS (m/z) 783 $[M+H]^+$, 805 $[M+Na]^+$, 781 $[M-H]^-$

di-tert-butyl 3,12-bis(2-tert-butoxy-2-oxoethyl)-3,6,9,12-tetraazatetradecane-1,14-dioate (19). To a solution of **17** (1.56 g, 2.0 mmol) in methanol (7 mL) was added Pd/C (84 mg, 10 wt %). Hydrogen gas was bubbled through the solution for 5 min, and then the reaction mixture was stirred under hydrogen for 18 h. The Pd/C was filtered out over Celite, rinsing well with methanol, and the filtrate was evaporated to dryness in vacuo. The crude product was purified by silica gel column chromatography to afford the product as a waxy yellow solid. Yield 95%. ^{1}H NMR (500 MHz, CD_3OD): δ ppm 3.55-3.63 (m, 10H), 3.13-3.35 (m, 10H), 1.49 (s, 36H). ^{13}C NMR (126 MHz, CD_3OD): δ ppm 172.0, 81.6, 56.4, 50.8, 46.1, 43.3, 27.3. ESIMS (m/z) 603 $[M+H]^+$, 625 $[M+Na]^+$, 601 $[M-H]^-$

di-tert-butyl 3,6,12-tris(2-tert-butoxy-2-oxoethyl)-3,6,9,12-tetraazatetradecane-1,14-dioate (21). To a solution **19** (3.6 g, 6.0 mmol) and sodium carbonate (317 mg, 3.0 mmol, 0.5 equiv) in MeCN was added dropwise a solution of tert-butyl bromoacetate (221 uL, 1.5 mmol, 0.25 equiv). The reaction mixture was stirred for 18 h at room temperature under N_2. Sodium carbonate was filtered and the filtrate concentrated in vacuo to dryness. EtOAc and water were added and aqueous layer extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a waxy, yellow solid. The crude product was purified by silica gel column chromatography to afford the product as yellow oil. Yield 90%. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 3.76-3.79 (m, 2H), 2.82-3.18 (m, 14H), 2.49-2.53 (m, 6H), 1.13 (m, 45H). ESIMS (m/z) 717 $[M+H]^+$, 739 $[M+Na]^+$, 715 $[M-H]^-$

di-tert-butyl 3,6,12-tris(2-(tert-butoxy)-2-oxoethyl)-9-(2-((4-methyl-2-oxo-1,2-dihydroquinolin-7-yl)amino)-2-oxoethyl)-3,6,9,12-tetraazatetradecanedioate (pro-ligand L1). To compound **21** (143 mg, 0.2 mmol) and **5** (55 mg, 0.22 mmol, 1.1 equiv) in 10 mL DMF was added sodium carbonate (42 mg, 0.4 mmol, 2.0 equiv), and the reaction mixture was heated to 60 °C for 24 h under N_2. After cooling to room temperature, the reaction mixture was diluted with EtOAc, washed with H_2O and brine, aqueous layer were extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a waxy, yellow solid. Product was purified by silica column chromatography to afford pro-ligand **L1** as yellow oil. Yield 60%. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.78-7.82 (m, 1H), 7.70 (d, *J* = 10.0 Hz, 1H), 7.56 (d, *J* = 10.0 Hz, 1H), 6.41 (s, 1H), 3.34-3.47 (m, 12H), 2.73-2.84 (m, 12H), 2.44 (s, 3H), 1.40-1.45 (m, 45H). ESIMS (m/z) 931 $[M+H]^+$, 953 $[M+Na]^+$, 929 $[M-H]^-$. **L1** was prepared according to deprotection procedure. HRMS $[C_{28}H_{38}N_6O_{12}+H]^-$ calc. 651.2626; meas. 651.2609.

3,6,12-tris(carboxymethyl)-9-(2-oxo-2-((2-oxo-4-(trifluoromethyl)-1,2-dihydroquinolin-7-yl)amino)ethyl)-3,6,9,12-tetraazatetradecanedioic acid (pro-ligand L2). Prepared in a similar way as pro-ligand **L1**, except **4** was used instead of **5**. Yield 55%. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 8.19 (m, 1H), 7.63-7.70 (m, 2H), 6.90 (s, 1H), 3.24-3.47 (m, 12H), 2.74-2.88 (m, 12H), 1.40-1.44 (m, 45H). ESIMS (m/z) 985 $[M+H]^+$, 1007 $[M+Na]^+$, 983 $[M-H]^-$. **L2** was prepared according to deprotection procedure. HRMS $[C_{28}H_{35}F_3N_6O_{12}+H]^+$ calc. 705.2243; meas. 705.2359.

(1S,2S)-N1,N2-dibenzylcyclohexane-1,2-diamine (16). To a solution of (1S,2S)-cyclohexane-1,2-diamine (1.14 g, 10.0 mmol) in dry methanol was added benzaldehyde (2.03 mL, 20.0 mmol, 2.0 equiv). The solution was refluxed for 4 h and then cooled via an ice bath. Addition of $NaBH_4$ (1.62 g, 43.0 mmol, 4.3 equiv) was performed slowly and in small portions to prevent boiling, and the reaction mixture was stirred for 12 h until completion. The solvent was evaporated in vacuo, and then saturated $NaHCO_3$ (50 mL), water (50 mL) and EtOAc (200 mL) were added and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a waxy, yellow solid. Product was purified by silica column chromatography to afford **16** as yellow oil. Yield 90%. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.25-7.37 (m, 10H), 3.92 (d, *J* = 13.3 Hz, 2H), 3.69 (d, *J* = 13.3 Hz, 2H), 2.17-2.32 (m, 6H), 1.76-1.77 (m, 2H), 1.07-1.29 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 141.1, 128.4, 128.1, 126.8, 60.9, 50.9, 31.5, 25.1. ESIMS (m/z) 295 $[M+H]^+$, 317 $[M+Na]^+$, 293 $[M-H]^-$

tert-butyl 2,2',2'',2'''-(2,2'-(1S,2S)-cyclohexane-1,2-diylbis(benzylazanediyl)bis(ethane-2,1-diyl))bis(azanetriyl)tetraacetate (18). To a solution of **16** (294 mg, 1.0 mmol) and **S2** (739 mg, 2.1 mmol, 2.1 equiv) in DMF was added sodium carbonate (318 mg, 3.0 mmol, 3.0 equiv). The solution was heated for 24 h at 60 °C under N_2. Sodium carbonate was removed by filtration. The filtrate was washed with H_2O and aqueous phase extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. Product was purified by silica column chromatography to afford **18** as yellow oil. Yield 88%. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.07-7.28 (m, 10H), 3.73 (d, *J* = 13.5 Hz, 2H), 3.37 (d, *J* = 13.5 Hz, 2H), 3.26 (s, 8H), 2.53-2.73 (m, 8H), 1.91-1.95 (m, 4H), 1.63-1.64 (m, 2H), 1.35 (s, 36H), 1.02-1.11 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 170.5, 141.1, 128.8, 127.8, 126.3, 80.4, 61.0, 60.2, 55.9, 54.8, 53.8, 48.4, 28.0, 26.6, 26.0, 20.9, 14.1. ESIMS (m/z) 837 $[M+H]^+$, 859 $[M+Na]^+$, 835 $[M-H]^-$

tert-butyl 2,2',2'',2'''-(2,2'-(1S,2S)-cyclohexane-1,2-diylbis(azanediyl)bis(ethane-2,1-diyl))bis(azanetriyl)tetraacetate (20). To a solution of **18** (837 mg, 1.0 mmol) in methanol (7 mL) was added Pd/C (88 mg, 10 wt %). Hydrogen gas was bubbled through the solution for 5 min, and then the reaction mixture was stirred under hydrogen for 24 h. The Pd/C was filtered out over Celite, rinsing well with methanol, and the filtrate was evaporated to dryness in vacuo. The crude product was purified by silica gel column chromatography to afford the product **20** as a waxy yellow solid. Yield 90%. ^{1}H NMR (500 MHz, CD_3OD): δ ppm 3.19-3.70 (m, 16H), 2.35-2.37 (m, 2H), 1.87-1.91 (m, 4H), 1.49 (m, 40H). ^{13}C NMR (126 MHz, CD_3OD): δ ppm 172.1, 81.8, 56.7, 56.4, 51.5, 44.2, 27.3, 25.4, 21.4. ESIMS (m/z) 657 $[M+H]^+$, 679 $[M+Na]^+$, 655 $[M-H]^-$

tert-butyl 2,2'-(2-((1S,2S)-2-((2-(bis(2-tert-butoxy-2-oxoethyl)amino)ethyl)(2-tert-butoxy-2-oxoethyl)amino)cyclohexylamino)ethylazanediyl)diacetate (22). To a solution **20** (984 mg, 1.5 mmol) and sodium carbonate (79 mg, 0.75 mmol, 0.5 equiv) in dry acetonitrile was added dropwise a solution of tertbutyl bromoacetate (55 uL, 0.37 mmol, 0.25 equiv). The reaction mixture was stirred at 18 h at room temperature under N_2. Sodium carbonate was filtered and the filtrate concentrated in vacuo to dryness. EtOAc and water were added and aqueous layer were extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a waxy, yellow solid. Product was purified by silica column chromatography to afford **22** as yellow oil. Yield 85%. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 2.37-3.75 (m, 18H), 1.45-1.82 (m, 6H), 0.89-1.14 (m, 40H). ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 170.5, 170.4, 170.3, 170.1, 82.4, 80.9, 80.7, 80.3, 59.8, 59.5, 56.1, 55.8, 52.9, 50.5, 43.1, 41.6, 27.8, 20.6, 13.8. ESIMS (m/z) 771 $[M+H]^+$, 793 $[M+Na]^+$, 769 $[M-H]^-$

di-tert-butyl 2,2'-((2-(((1S,2S)-2-((2-(bis(2-(tert-butoxy)-2-oxoethyl)amino)ethyl)(2-((4-methyl-2-oxo-1,2-dihydroquinolin-7-yl)amino)-2-oxoethyl)amino)cyclohexyl)(2-(tert-butoxy)-2-oxoethyl)amino)ethyl)azanediyl)diacetate (pro-ligand L3). Prepared in a similar way as **L1**, except **22** was used instead of **21**. Yield 52%. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.52-7.94 (m, 3H), 6.38 (m, 1H), 2.15-3.44 (m, 25H), 1.23-1.43 (m, 53H). ESIMS (m/z) 985 $[M+H]^+$, 1007 $[M+Na]^+$, 983 $[M-H]^-$. **L3** was prepared according to deprotection procedure. HRMS $[C_{36}H_{52}N_6O_{12}-H]^-$ calc. 759.3565; meas. 759.3841.

di-tert-butyl 2,2'-((2-(((1S,2S)-2-((2-(bis(2-(tert-butoxy)-2-oxoethyl)amino)ethyl)(2-(tert-butoxy)-2-oxoethyl)amino)cyclohexyl)(2-oxo-2-((2-oxo-4-(trifluoromethyl)-1,2-dihydroquinolin-7-yl)amino)ethyl)amino)ethyl)azanediyl)diacetate (pro-ligand L4). Prepared in a similar way as **L2**, except **22** was used instead of **21**. Yield 52%. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.44-7.63 (m, 3H), 6.87 (s, 1H), 2.03-3.49 (m, 22H), 1.33-1.44 (m, 53H). ESIMS (m/z) 985 $[M+H]^+$, 1007 ESIMS (m/z) 1039 $[M+H]^+$, 1061 $[M+Na]^+$, 1037 $[M-H]^-$. **L4** was prepared according to deprotection procedure. HRMS $[C_{36}H_{52}N_6O_{12}+H]^+$ calc. 759.2813; meas. 759.2814.

6-(2-((3-(14-azido-2-oxo-6,9,12-trioxa-3-azatetradecyl)-2-oxo-4-(trifluoromethyl)-1,2-dihydroquinolin-7-yl)amino)-2-oxoethyl)-3,9,12-tris(carboxymethyl)-3,6,9,12-tetraazatetradecanedioic acid (L5). The corresponding pro-ligand was prepared in a similar way as **L2**, except **12** was used instead of **4.** After deprotection step**,** the product was purified by reverse-phase HPLC. (A, 0.1% trifluoroacetic acid in water; B, CH_3CN; 30-100% B; 30 min,

5mL/min). Product fractions were pooled and lyophilized. HRMS $[C_{37}H_{51}F_3N_{12}O_{16}+H]^+$ calc. 949.3436; meas. 949.3434.

di-tert-butyl 2,2'-((2-(((1S,2S)-2-((2-(bis(2-(tert-butoxy)-2-oxoethyl)amino)ethyl)(2-((4-methyl-2-oxo-1-(prop-2-yn-1-yl)-1,2-dihydroquinolin-7-yl)amino)-2-oxoethyl)amino)cyclohexyl)(2-(tert-butoxy)-2-oxoethyl)amino)ethyl)azanediyl)diacetate (pro-ligand L6) Prepared in a similar way as **L3**, except **9** was used instead of **5**. Yield 52%. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 8.13 (s, 1H), 7.81-7.83 (m, 1H), 7.55-7.57 (m, 1H), 6.49 (s, 1H), 5.09 (s, 2H), 4.25 (s, 1H), 3.57-3.58 (m, 2H), 3.44 (s, 6H), 3.30-3.31 (m, 4H), 2.70-2.80 (m, 7H), 2.49-2.50 (m, 4H), 1.47-1.51 (m, 53H). ESIMS (m/z) 1023 $[M+H]^+$, 1045 $[M+Na]^+$, 1021 $[M-H]^-$. **L6** was prepared according to deprotection procedure. HRMS $[C_{55}H_{86}N_6O_{12}+H]^+$ calc. 1023.6382; meas. 1023.6380.

di-tert-butyl 3,6,12-tris(2-(tert-butoxy)-2-oxoethyl)-9-(quinolin-2-ylmethyl)-3,6,9,12-tetraazatetradecanedioate (pro-ligand 23). Prepared in a similar way as **L1**, except 2-(chloromethyl)quinoline was used instead of **5**. Yield 75%. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 8.10 (d, *J* = 10.5 Hz, 1H), 7.99 (d, *J* = 10.5 Hz, 1H), 7.75 (d, *J* = 10.0 Hz, 1H), 7.60-7.66 (m, 2H), 7.47-7.48 (m, 1H), 3.98-3.49 (m, 2H), 3.39 (s, 5H), 3.46-3.51 (m, 2H), 3.39 (s, 4H), 3.32-3.35 (m, 3H), 2.72-2.88 (m, 10 H), 1.34-1.43 (m, 45H). ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 171.0, 169.3, 166.7, 160.9, 147.9, 136.8, 129.6, 129.3, 127.9, 127.7, 126.4, 121.5, 81.9, 81.2, 61.9, 58.7, 56.9, 56.4, 53.2, 52.3, 52.0, 49.7, 47.5, 44.7, 28.5. ESIMS (m/z) 858 $[M+H]^+$, 880 $[M+Na]^+$, 856 $[M-H]^-$. **23** was prepared according to deprotection procedure. HRMS $[C_{26}H_{35}N_5O_{10}+H]^+$ calc. 578.2462; meas. 578.2460.

3,6-bis(carboxymethyl)-12-(2-((2-(5-(4-((2,4-diaminopyrimidin-5-yl)methyl)-2,6-dimethoxyphenoxy)pentanamido)ethyl)amino)-2-oxoethyl)-9-(2-((4-methyl-2-oxo-1,2-dihydroquinolin-7-yl)amino)-2-oxoethyl)-3,6,9,12-tetraazatetradecanedioic acid (24). A suspension of **L1** (32 mg, 0.05 mmol), in Ac_2O (19 uL, 0.2 mmol, 4.0 equiv) and pyridine (1.0 mL) was stirred for 24 h at 65 °C. The solvent dried under vacuum and the precipitate washed with Et_2O. To the well stirred solution of the mixture in DMF (1.0 mL) and DIEA (87 uL, 0.5 mmol, 10.0 equiv) was added **S5** (11 mg, 25 umol, 0.5 equiv) in DMF (1.0 mL) dropwise and stirred at room temperature 12 h. A few drops of water were added to quench the reaction. The product was purified by reverse-phase HPLC. (A, 0.1% trifluoroacetic acid in water; B, CH_3CN; 30-100% B; 30 min, 5mL/min). Product fractions were pooled and lyophilized. HRMS $[C_{48}H_{66}N_{12}O_{15}-H]^-$ calc. 1049.4692; meas. 1049.4691.

3,6,12-tris(carboxymethyl)-9-(2-oxo-2-((2-oxo-1-((1-(13-oxo-17-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-3,6,9-trioxa-12-azaheptadecyl)-1H-1,2,3-triazol-4-yl)methyl)-4-(trifluoromethyl)-1,2-dihydroquinolin-7-yl)amino)ethyl)-3,6,9,12-tetraazatetradecanedioic acid (25). The corresponding clickable substrate prepared in situ and in a similar way as **L2**, except **8** was used instead of **4**. The crude reaction mixture (~ 0.05 mmol, 37 mg) was suspended in 1:1 DMF/water (5.0 mL), and then (3aS,4S,6aR)-4-(1-azido-3,6,9-trioxa-12-azaheptadecan-17-yl)tetrahydro-1H-thieno[3,4-d]imidazol-2(3H)-one (biotinPEG3azide) (25 mg, 0.05 mmol, 1.0 equiv) was added followed by copper(II) sulfate (26 mg, 0.03 mmol, 2.1 equiv). After 30 min stirring, sodium ascorbate (~2 mg, 0.1 mmol, 0.2 equiv) was added, and the reaction mixture stirred at room temperature for overnight. The solution was concentrated in vacuo

and the precipitate dissolved in 1:1 THF/water (5.0 mL) and sodium sulfide (19 mg, 5.0 equiv). The solid was filtered out using a fine fritted filter and the filtrate was concentrated in vacuo to dryness. The resulting colorless solid was dissolved in water (3.0 mL) and purified by reverse-phase HPLC. (A, water; B, 0.1% trifluoroacetic acid in acetonitrile; 30-100% B; 30 min, 5mL/min). Product fractions were pooled and lyophilized. ^{1}H NMR (500 MHz, D_2O): δ ppm 7.81-8.10 (m, 2H), 7.42 (d, *J* = 8.50 Hz, 1H), 7.09 (s, 1H), 5.55 (s, 2H), 4.49-4.55 (m, 2H), 3.25-4.02 (m, 45H), 1.98-2.15 (m, 2H), 1.16-1.53 (m, 6H). HRMS $[C_{49}H_{69}N_{12}O_{17}F_3S + H]^+$ calc. 1187.4660; meas. 1187.4655.

Deprotection of tert-butyl esters. To the corresponding ester was added deprotection mixture (TFA/CH_2Cl_2/iPr_3SiH, 5:5:1.8, v/v, 11.8 mL). After stirring for 24 h, toluene (10 mL) was added, and the solvent was removed by evaporation under reduced pressure. Ethyl acetate (5 mL) was added and the heterogeneous mixture was stirred for 1 h at 60 °C. The resulting precipitate was filtered off and washed with ethyl acetate (10 mL). The precipitate dissolved in water and lyophilized to afford a pure chelator. For the probes **L5** additional HPLC purification is needed.

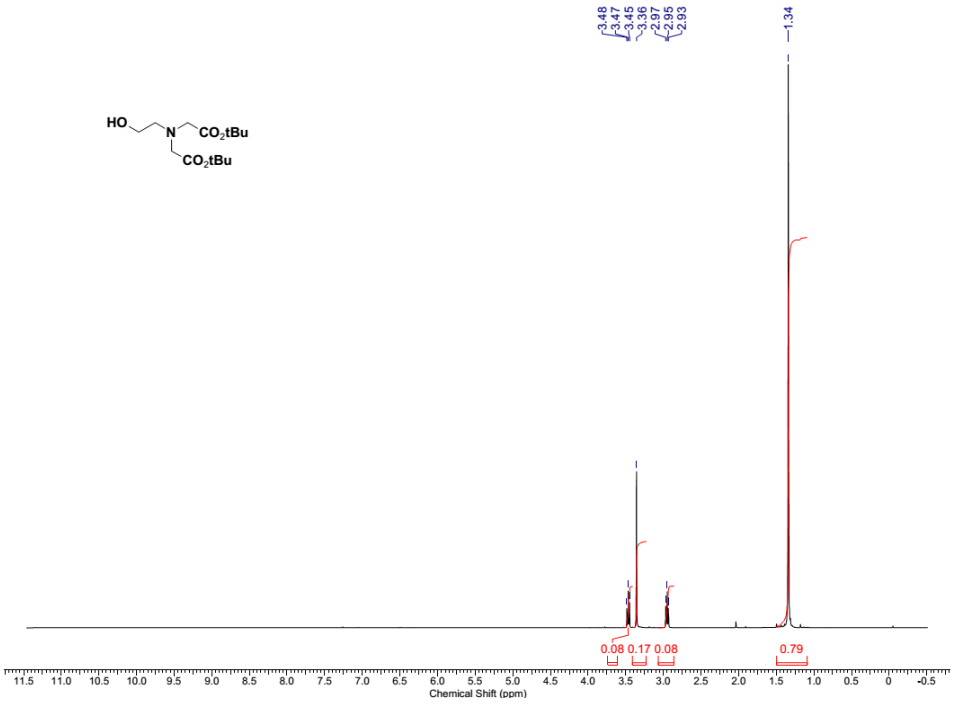
C. Supporting References.

(1) Rajapakse, H. E., Reddy, D. R., Mohandessi, S., Butlin, N. G., and Miller, L. W. (2009) Luminescent Terbium Protein Labels for Time-Resolved Microscopy and Screening. *Angew Chem Int Edit 48*, 4990-4992.

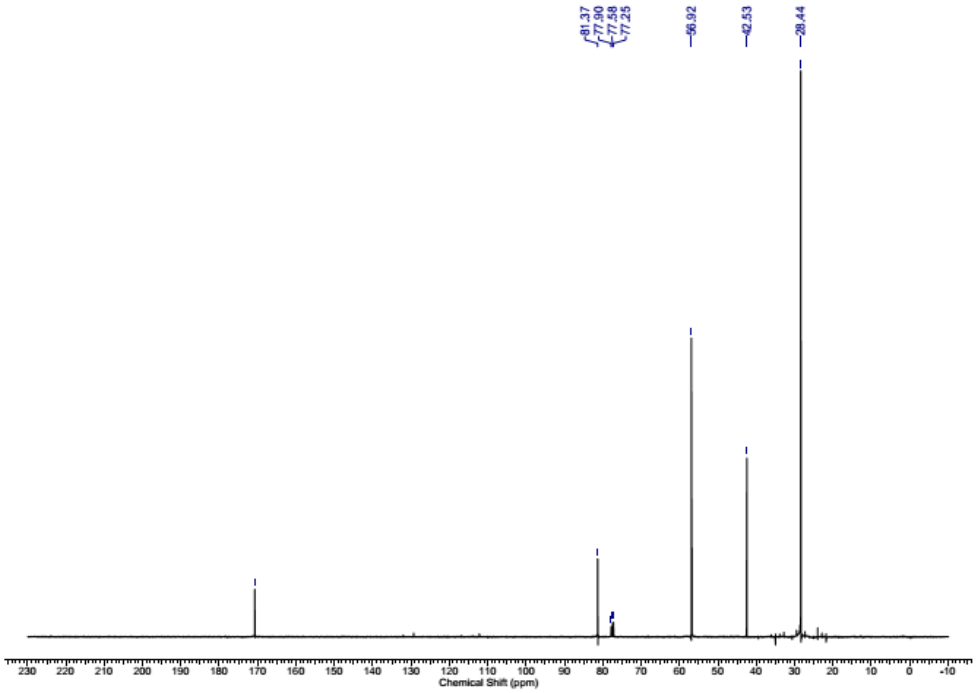
(2) Reddy, D. R., Rosa, L. E. P., and Miller, L. W. (2011) Luminescent Trimethoprim-Polyaminocarboxylate Lanthanide Complex Conjugates for Selective Protein Labeling and Time-Resolved Bioassays. *Bioconjugate Chem 22*, 1402-1409.

D. NMR Spectral Data.

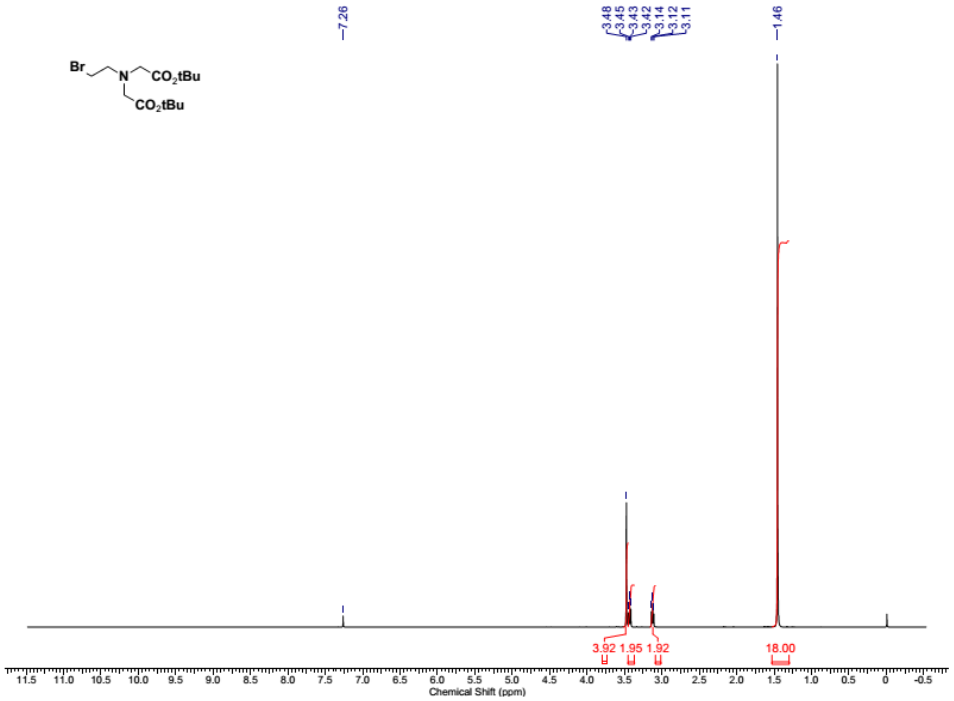
^{1}HNMR spectrum of S1



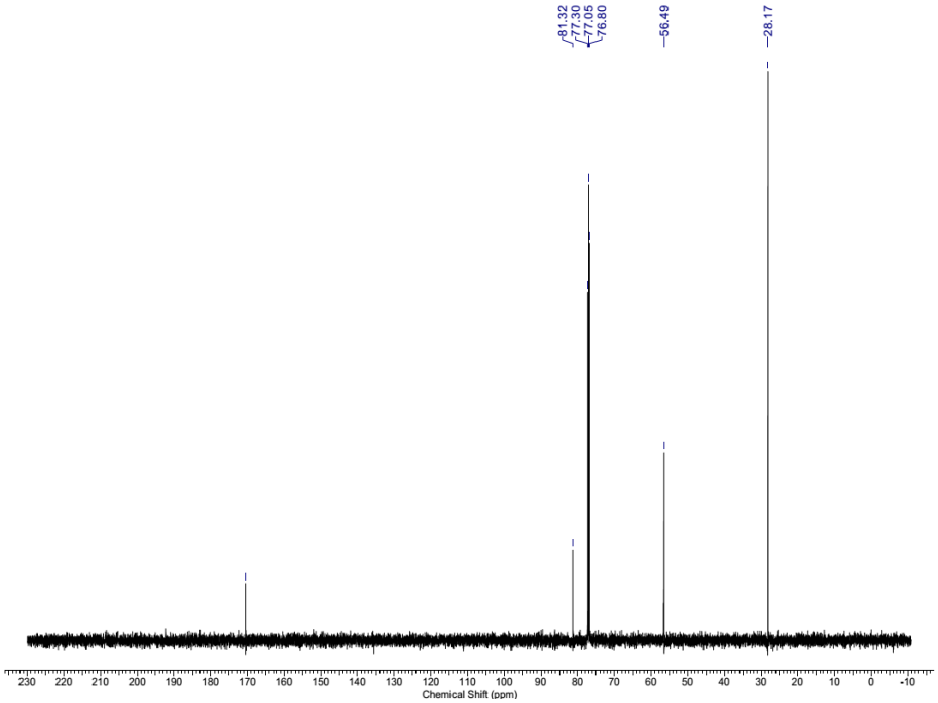
^{13}CNMR spectrum of S1



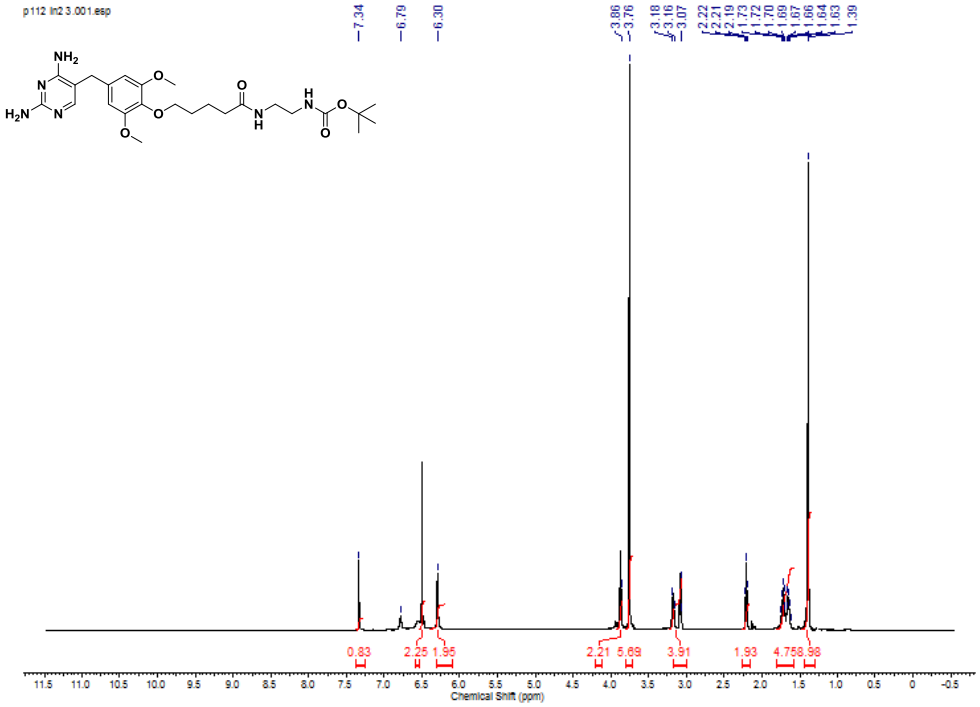
^{1}HNMR spectrum of S2



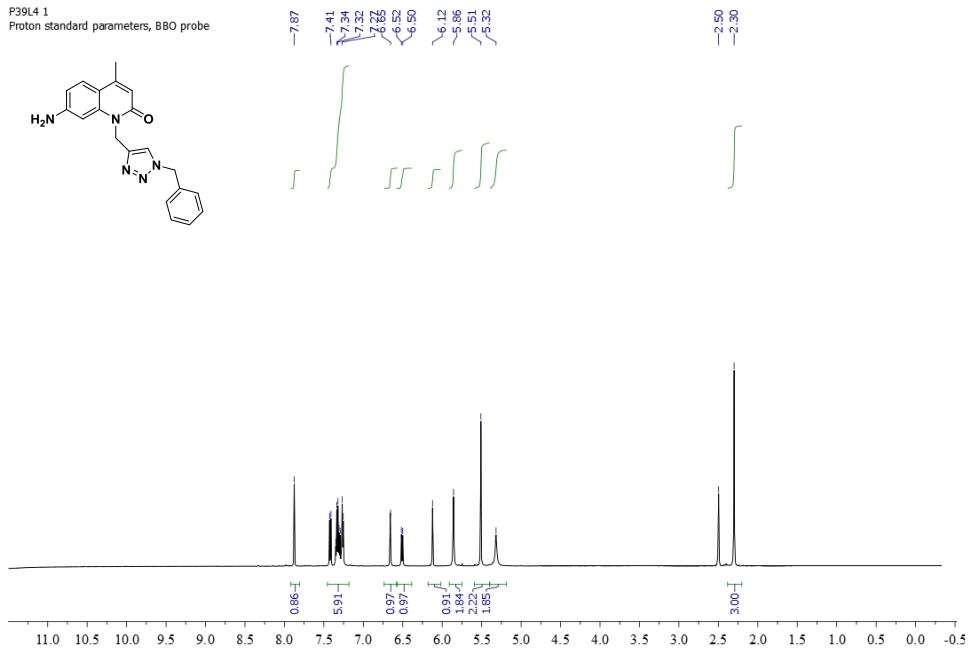
[13]CNMR spectrum of S2



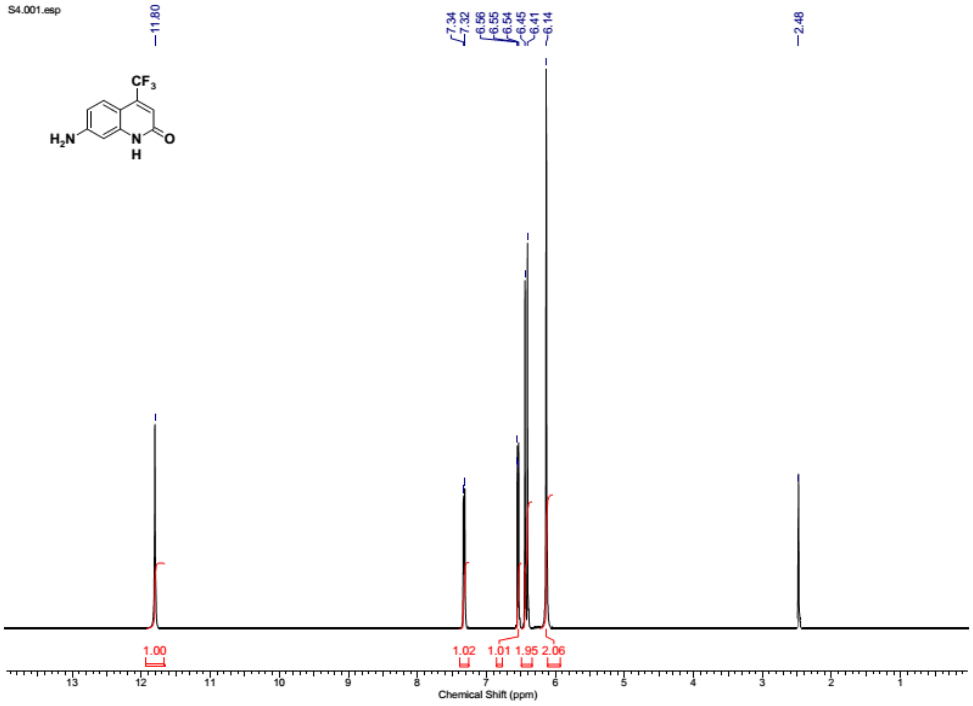
[1]HNMR spectrum of S4



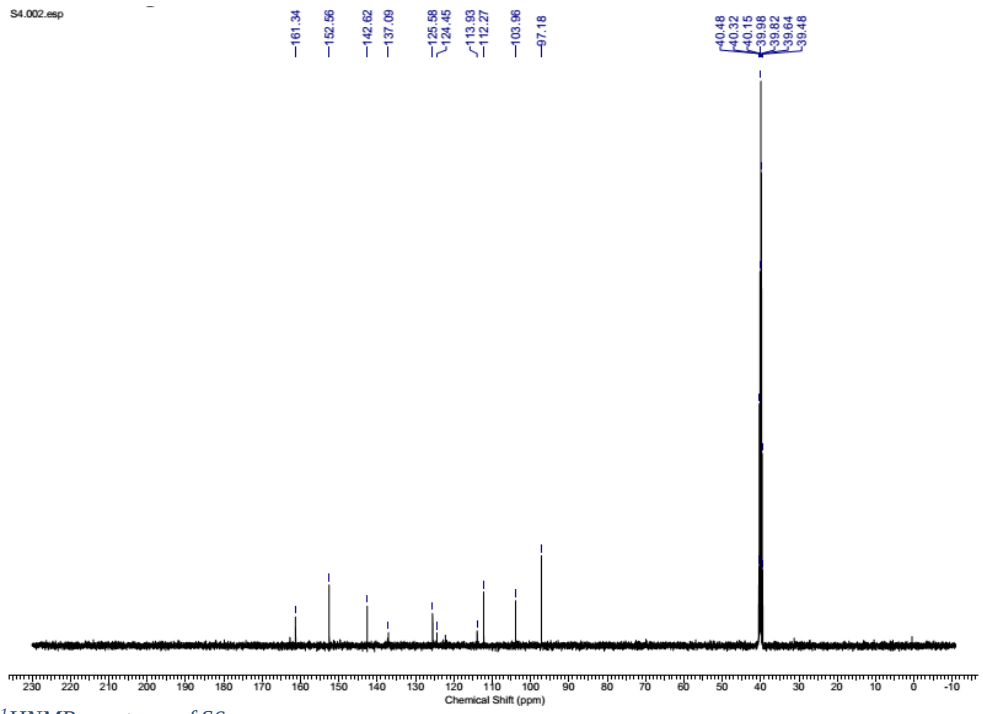
[1]HNMR spectrum of S10



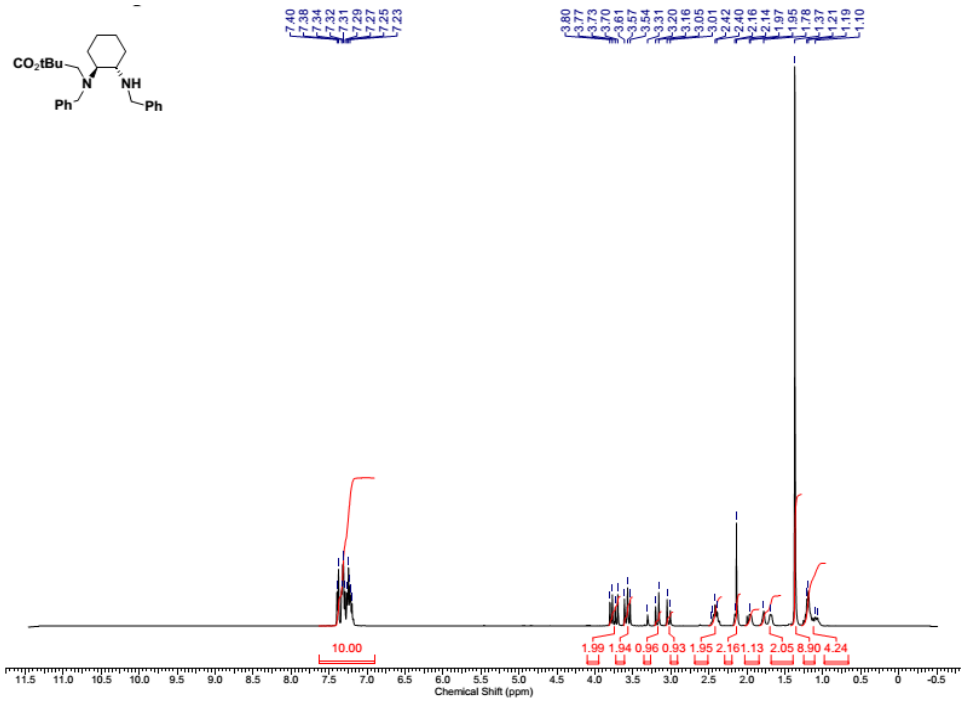
[1]HNMR spectrum of 2



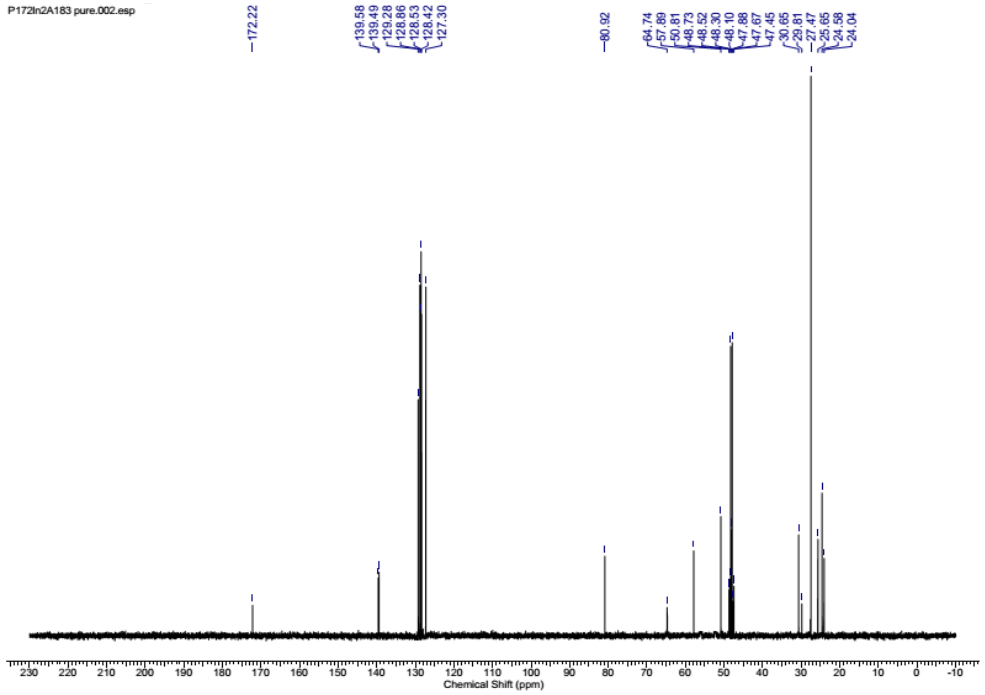
^{13}CNMR spectrum of S2



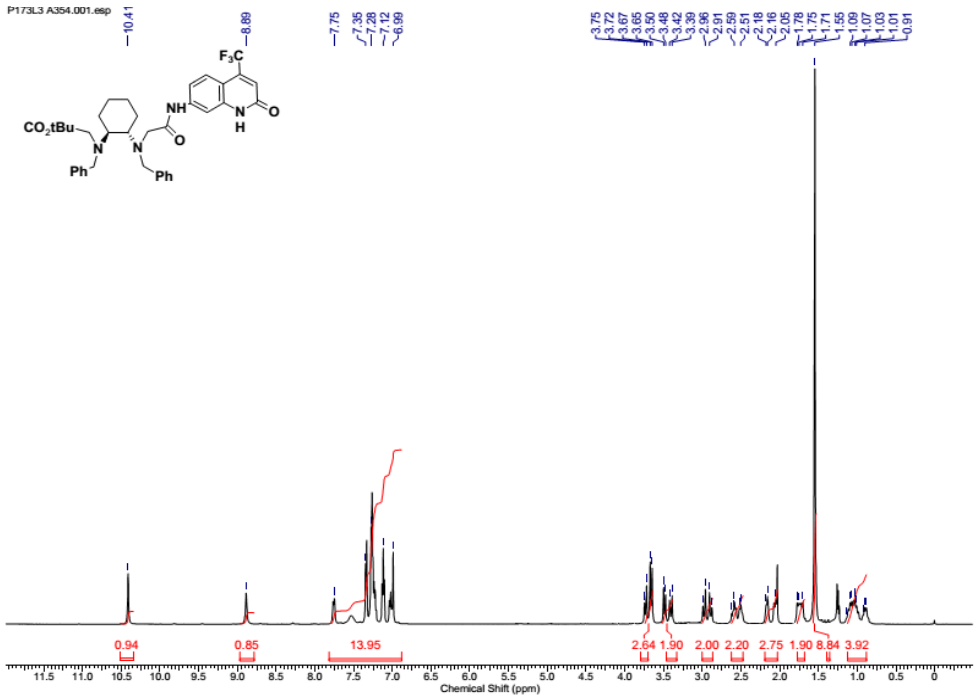
^{1}HNMR spectrum of S6



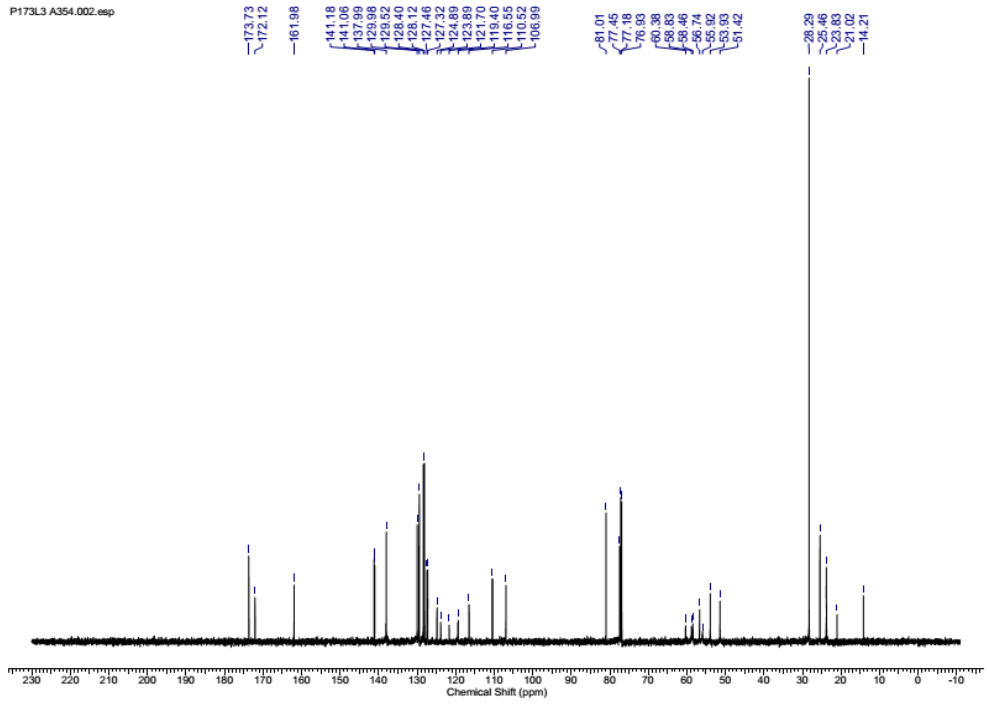
^{13}CNMR spectrum of S6



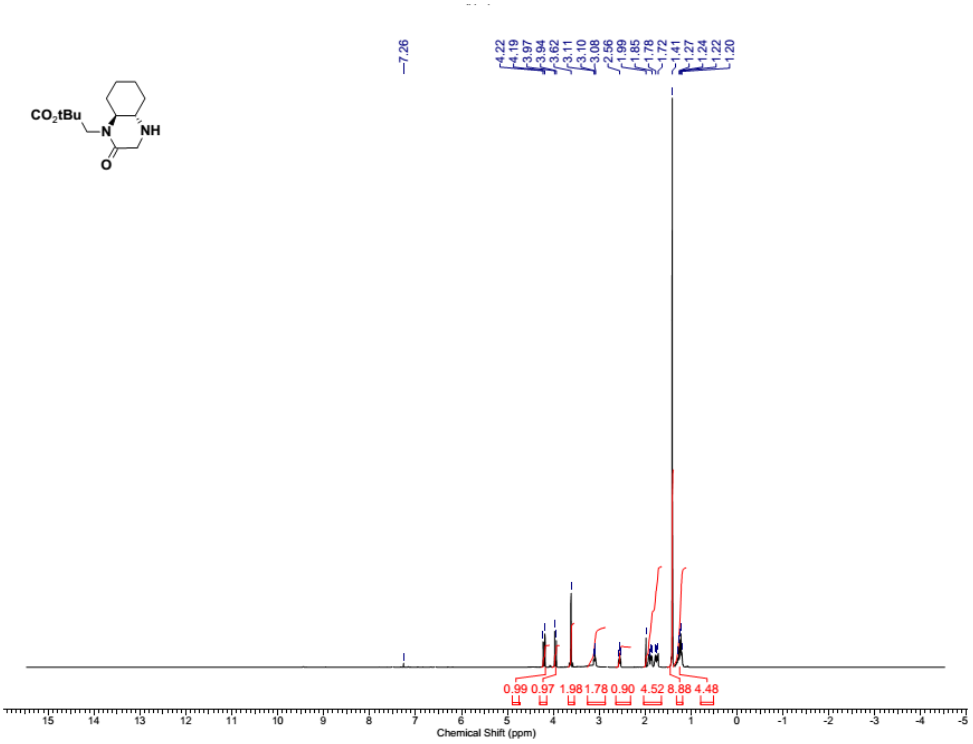
[1]HNMR spectrum of S7



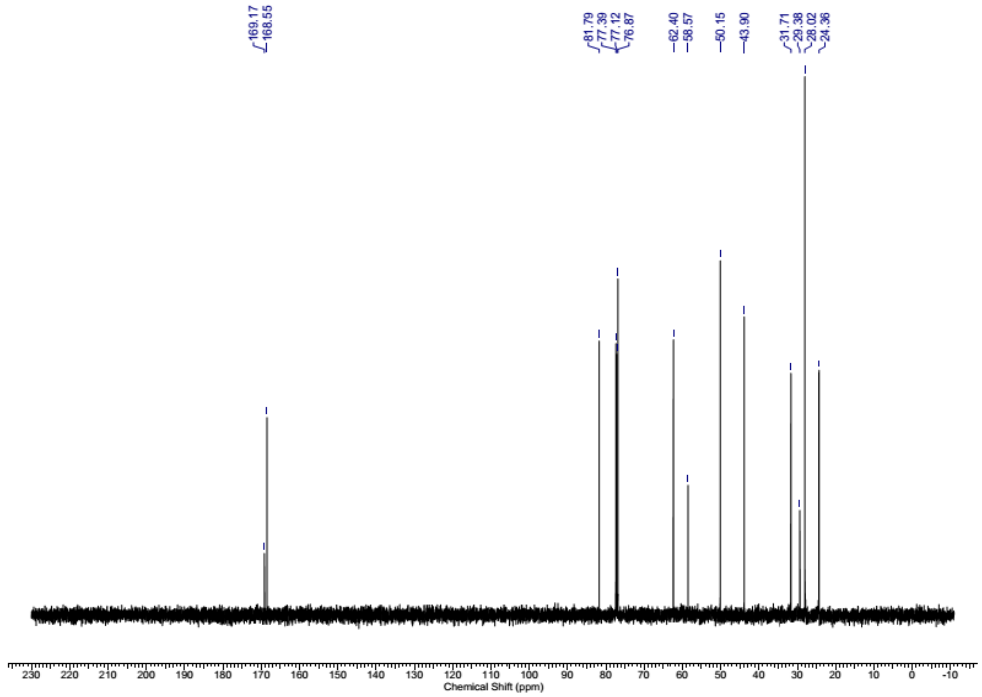
[13]CNMR spectrum of S7



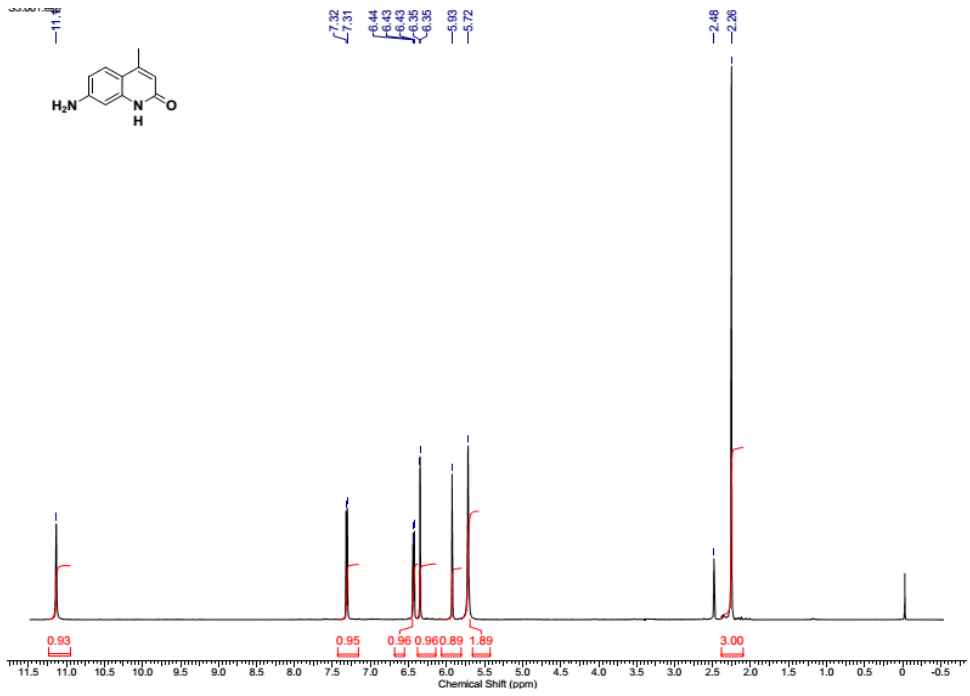
[1]HNMR spectrum of S9



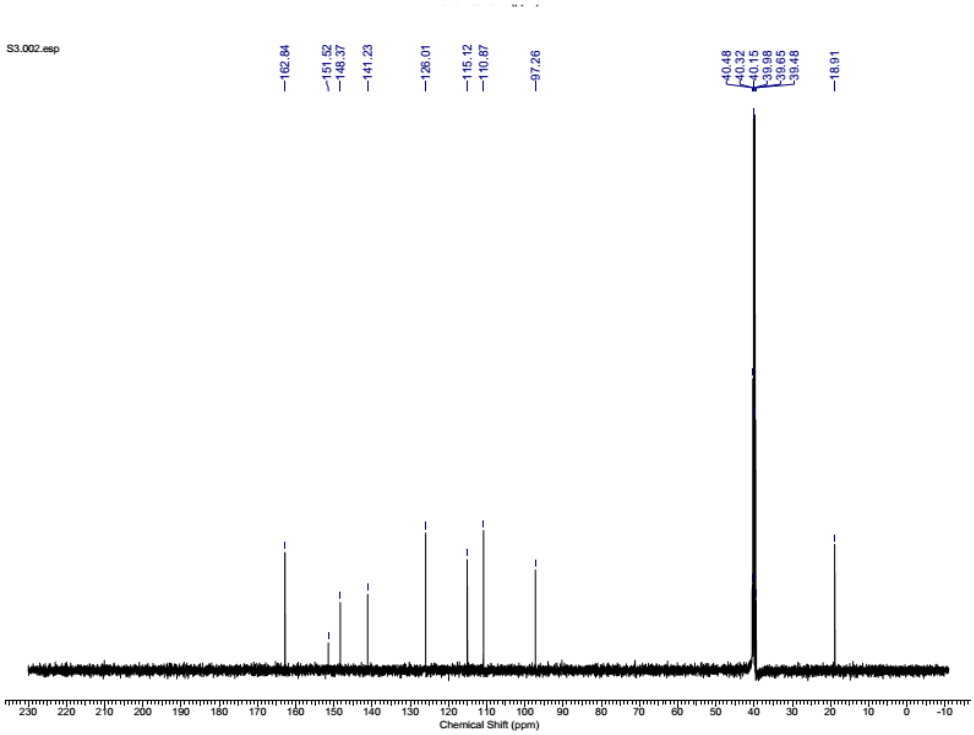
[13]CNMR spectrum of S9



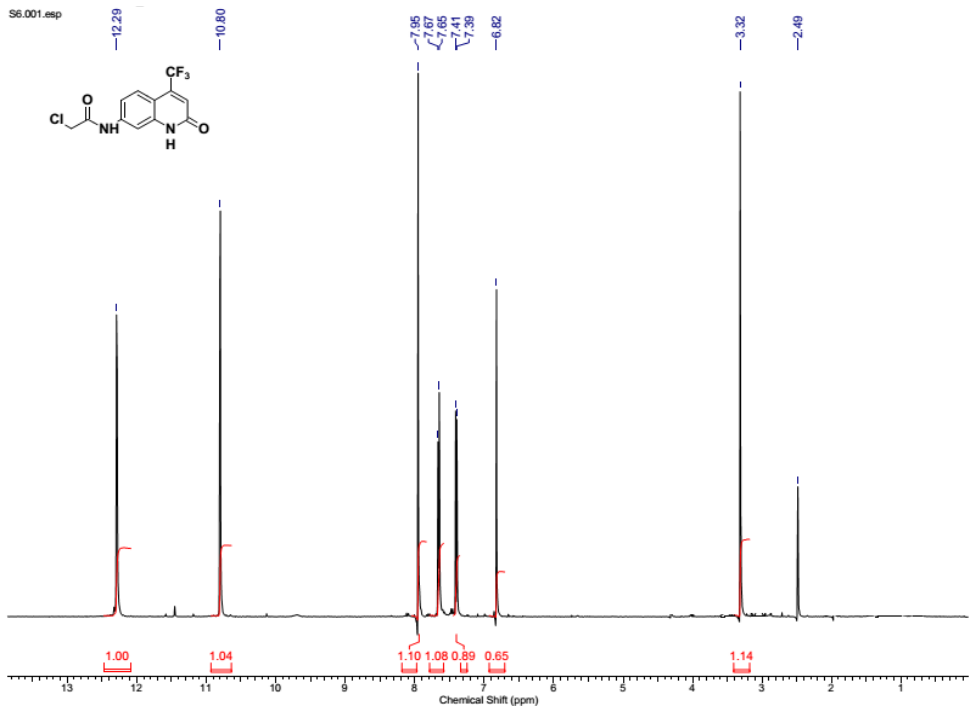
[1]HNMR spectrum of 3



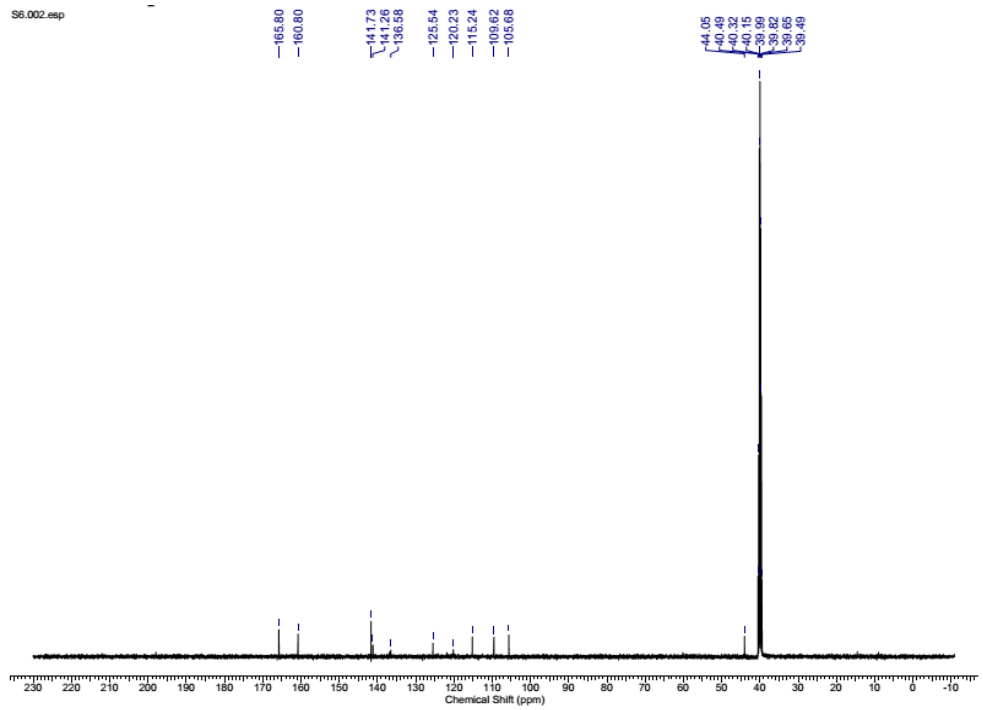
[13]CNMR spectrum of 3



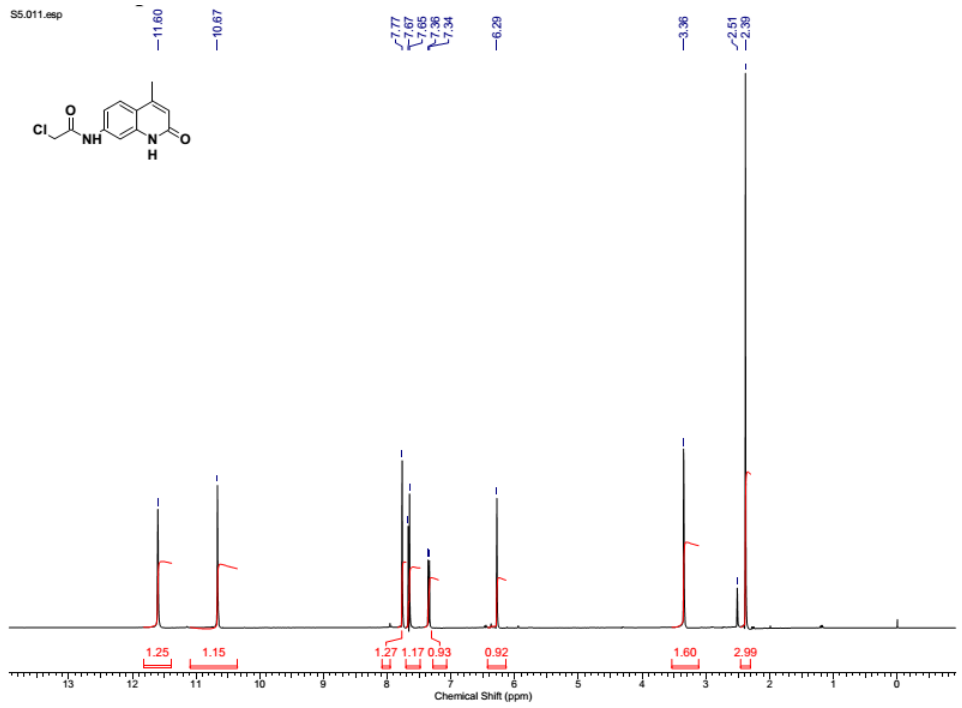
[1]HNMR spectrum of 4



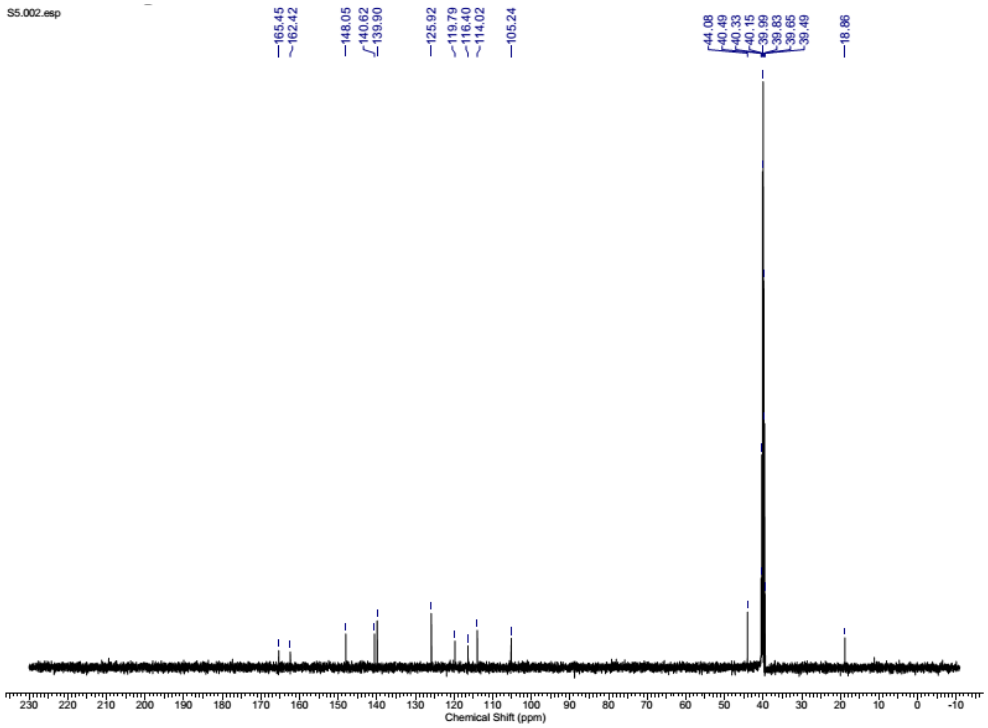
1HNMR spectrum of 4



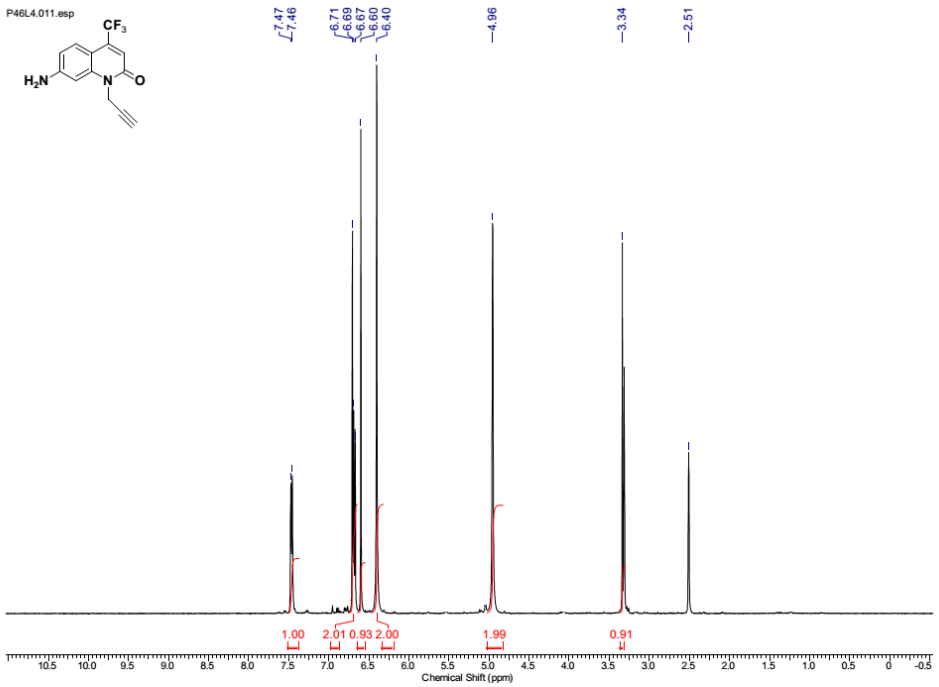
[1]HNMR spectrum of 5



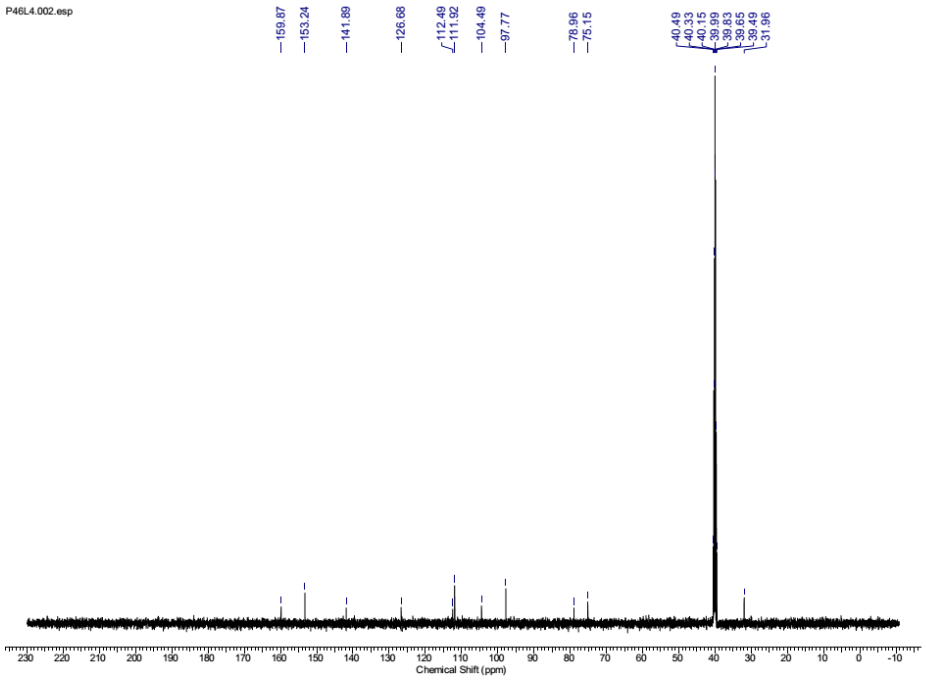
[13]CNMR spectrum of S5



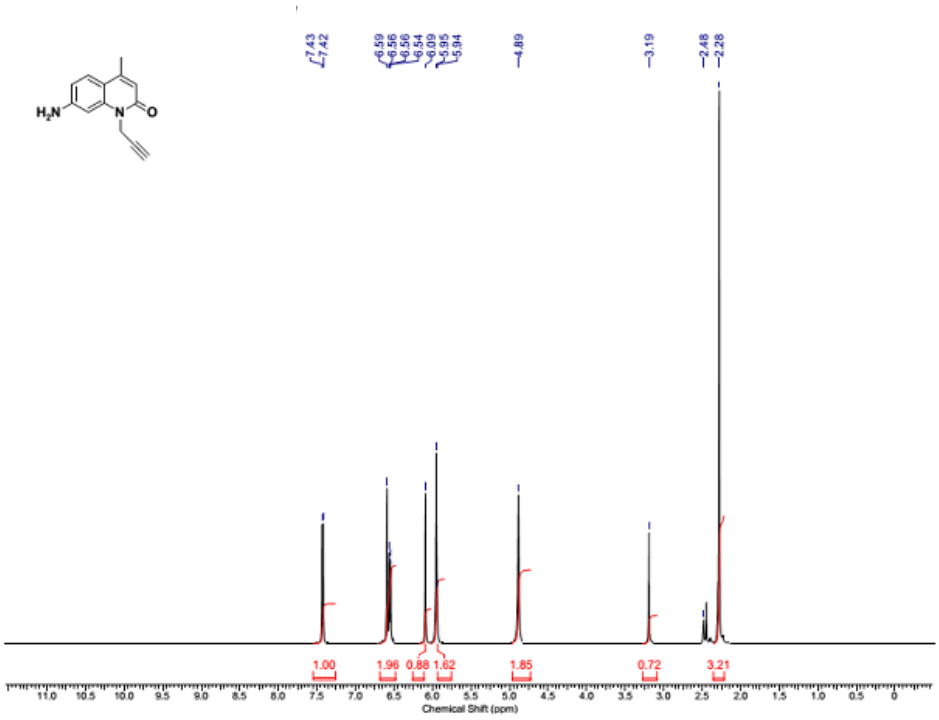
[1]HNMR spectrum of 6



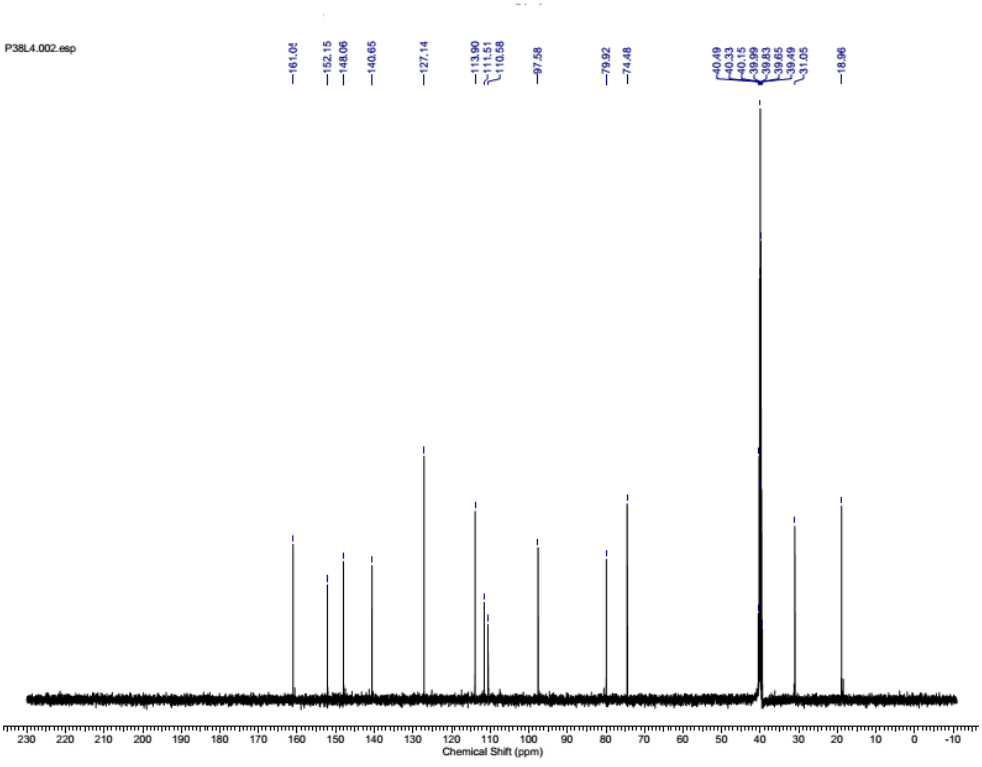
[13]CNMR spectrum of 6



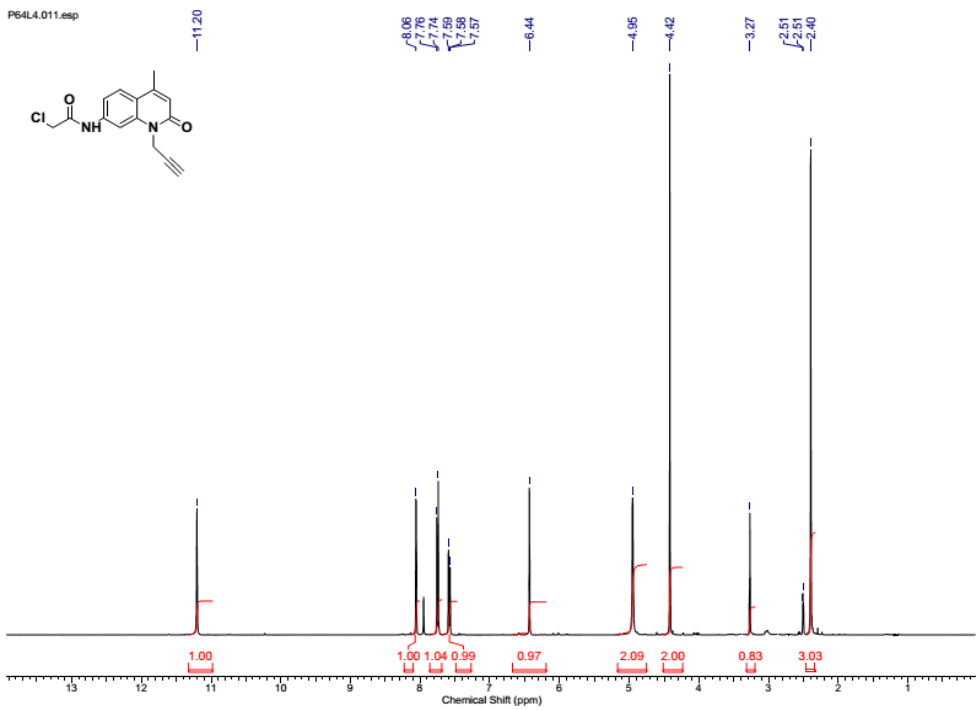
[1]HNMR spectrum of 7



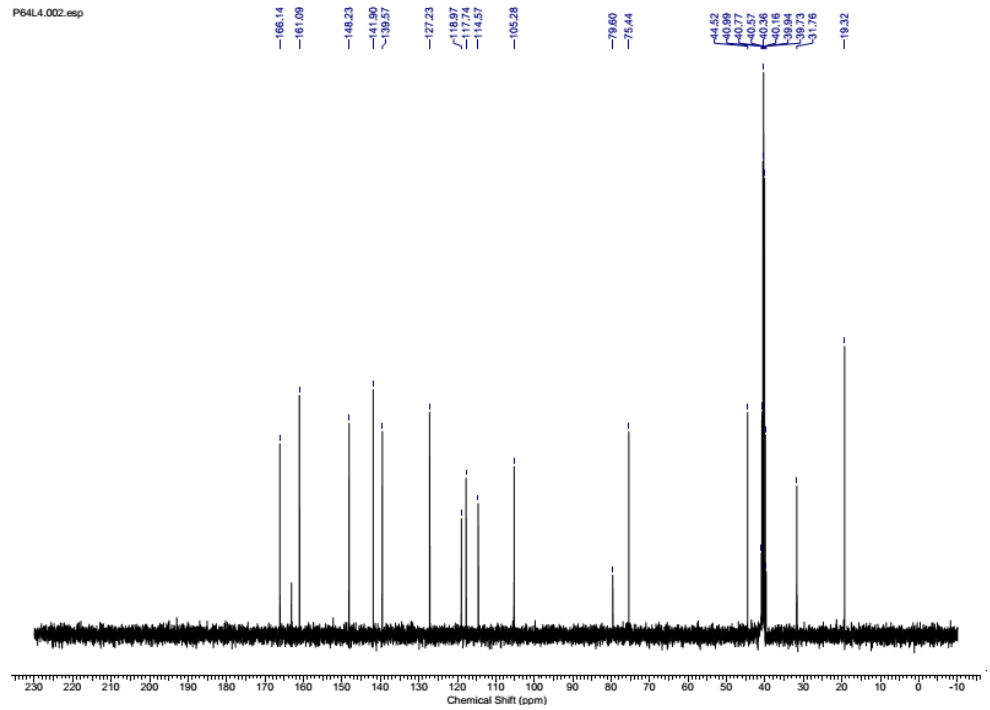
[1]HNMR spectrum of 7



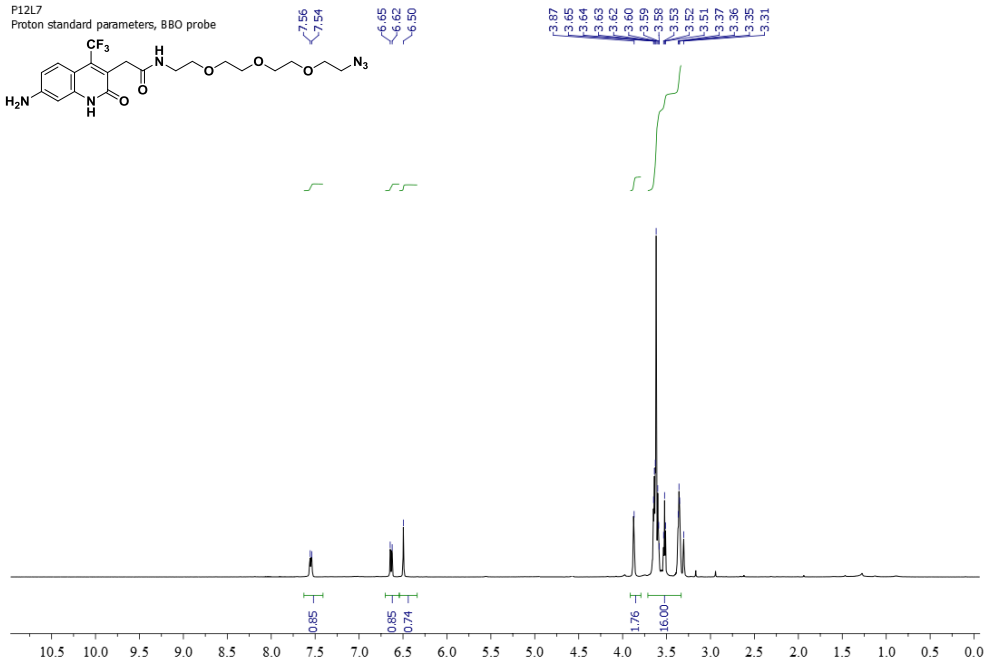
[1]HNMR spectrum of 9



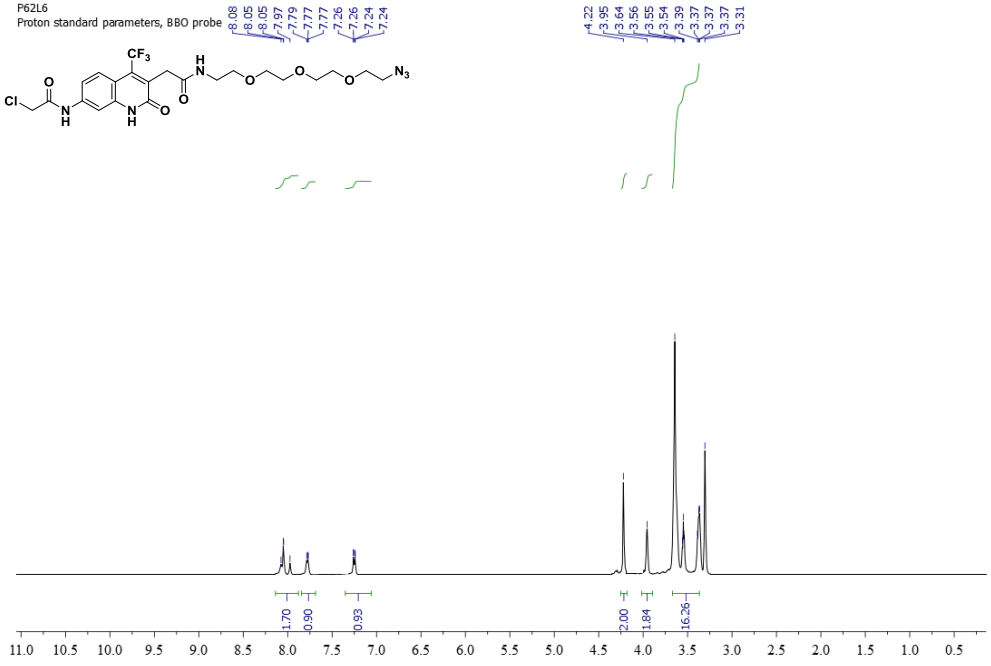
[13]CNMR spectrum of 9



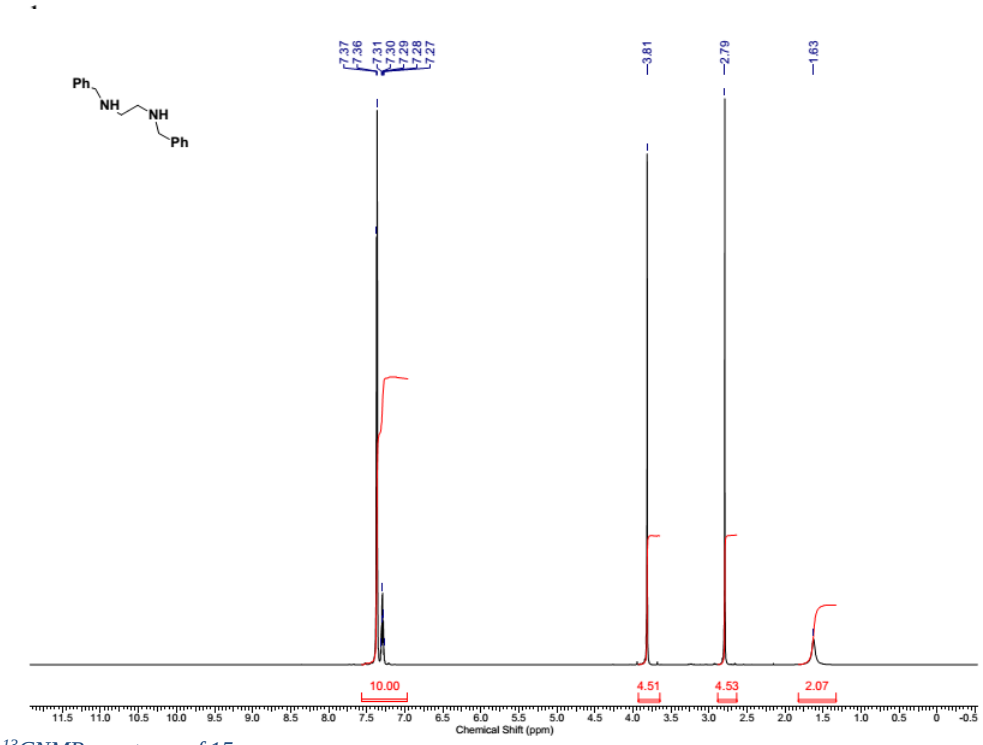
[1]HNMR spectrum of 11



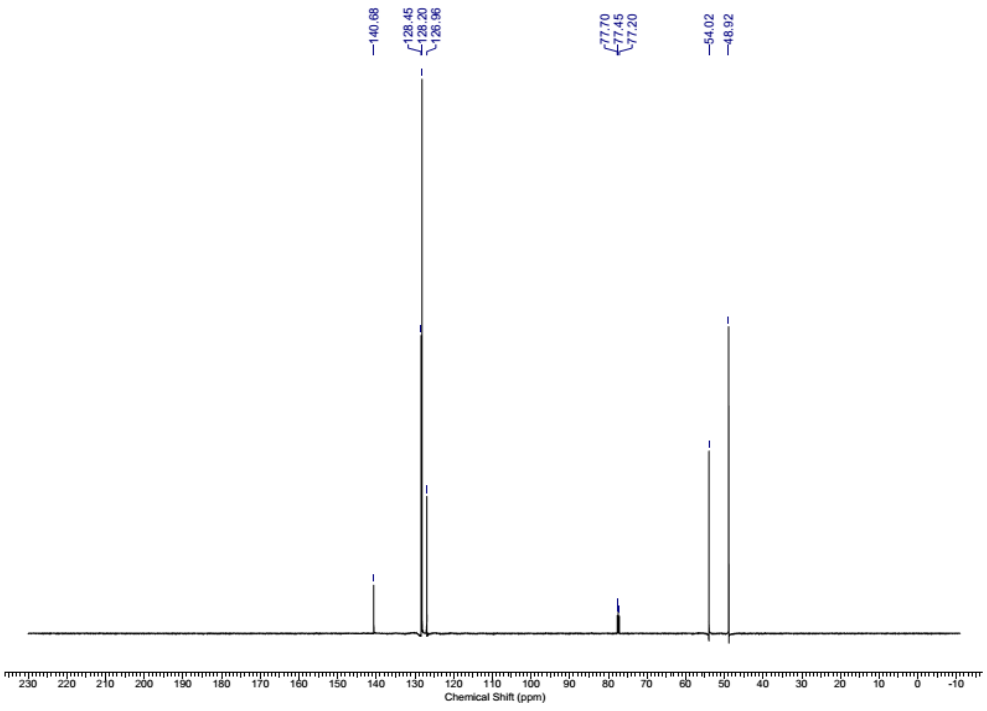
[1]H NMR spectrum of 12



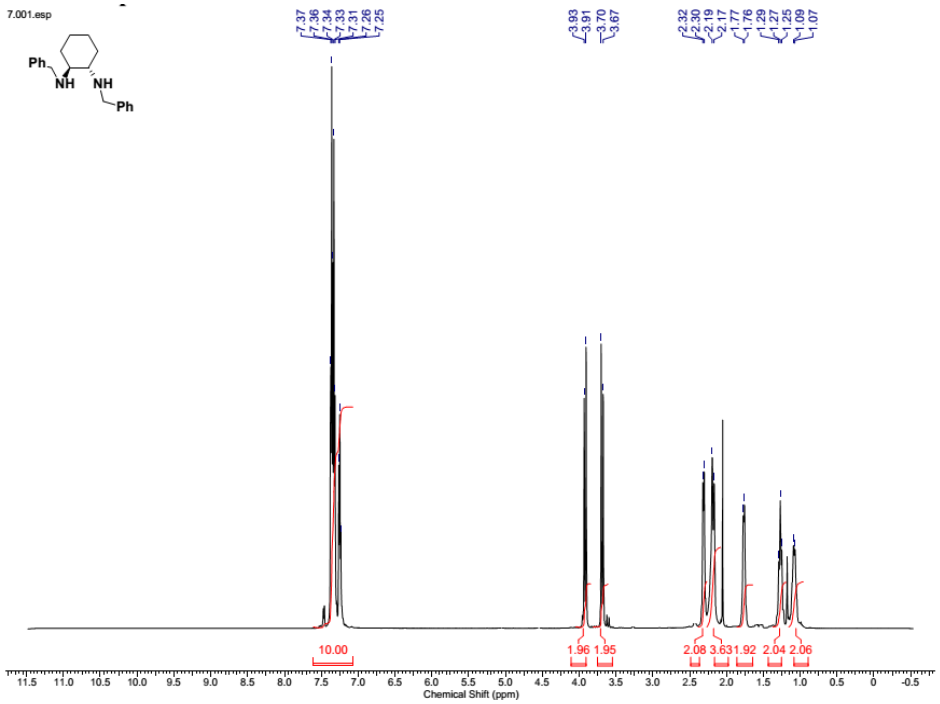
[1]HNMR spectrum of 15



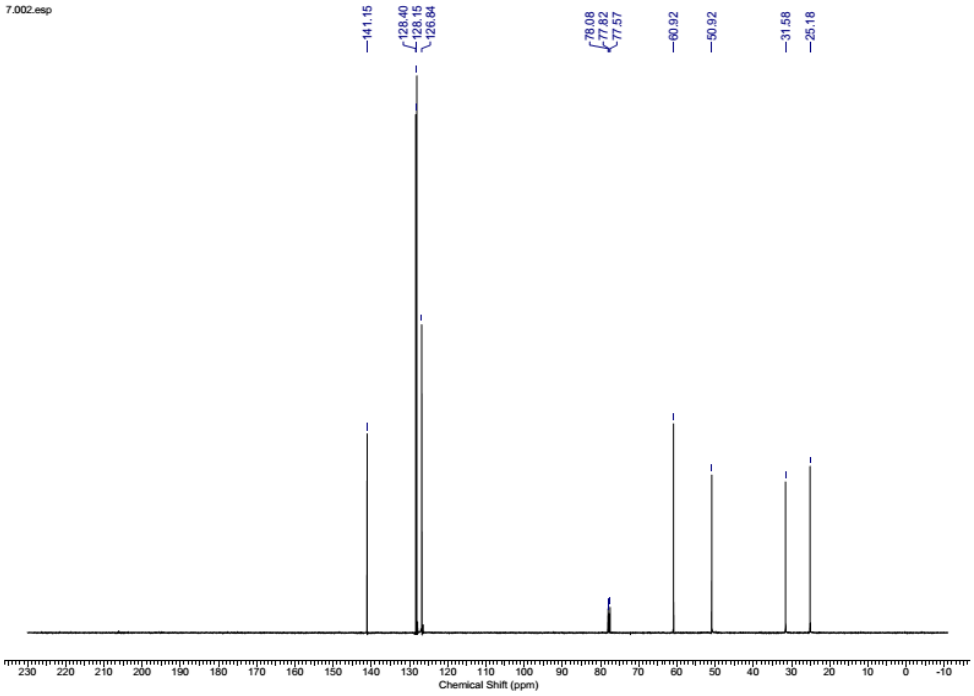
[13]CNMR spectrum of 15



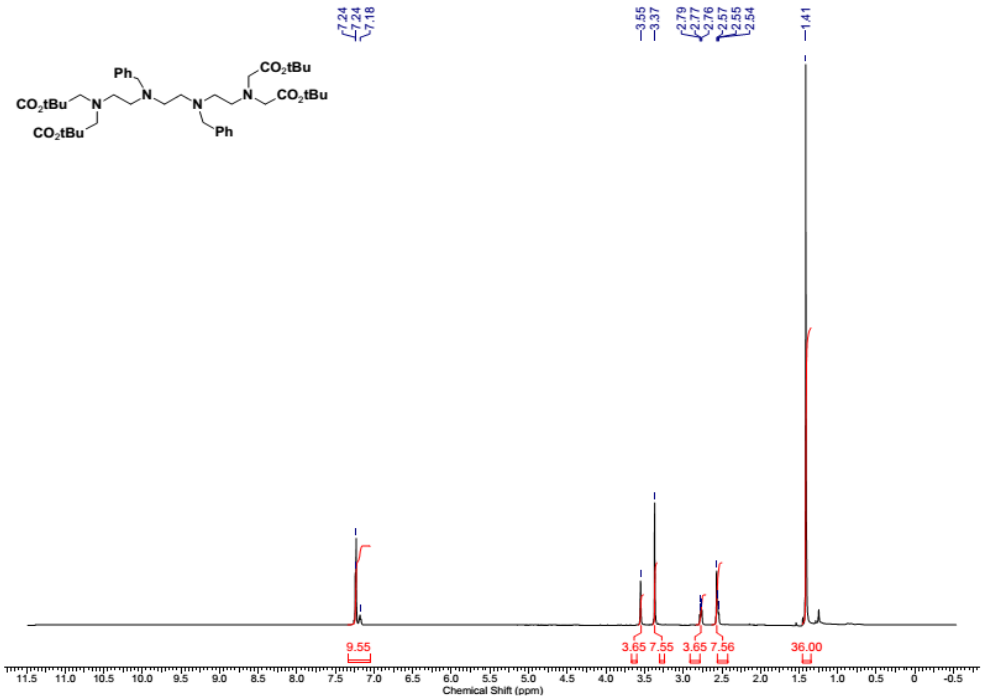
[1]HNMR spectrum of 16



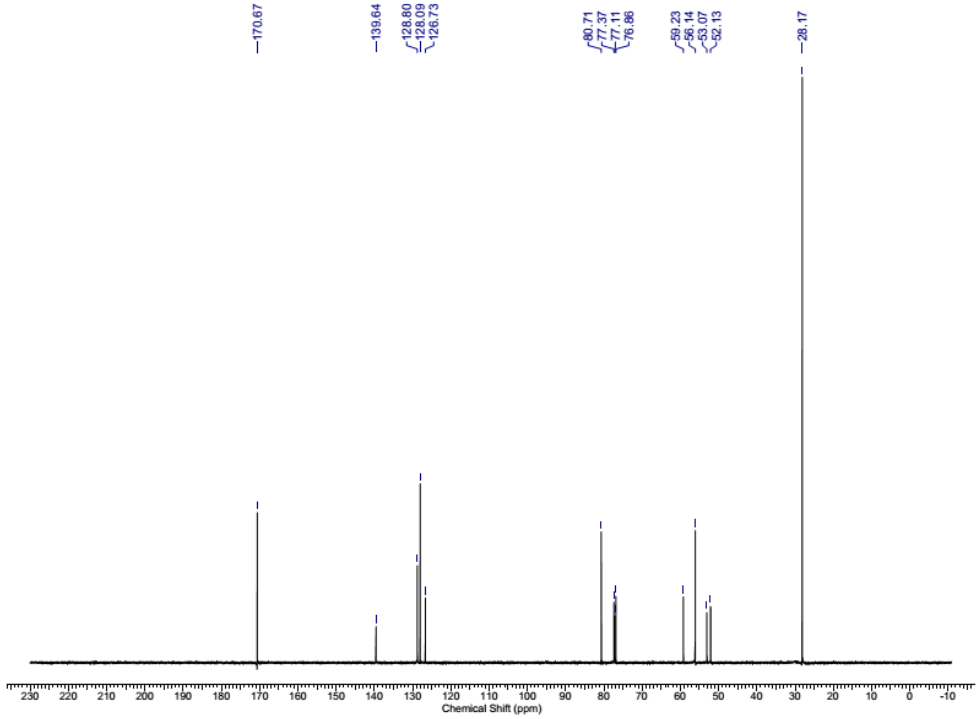
[13]CNMR spectrum of 16



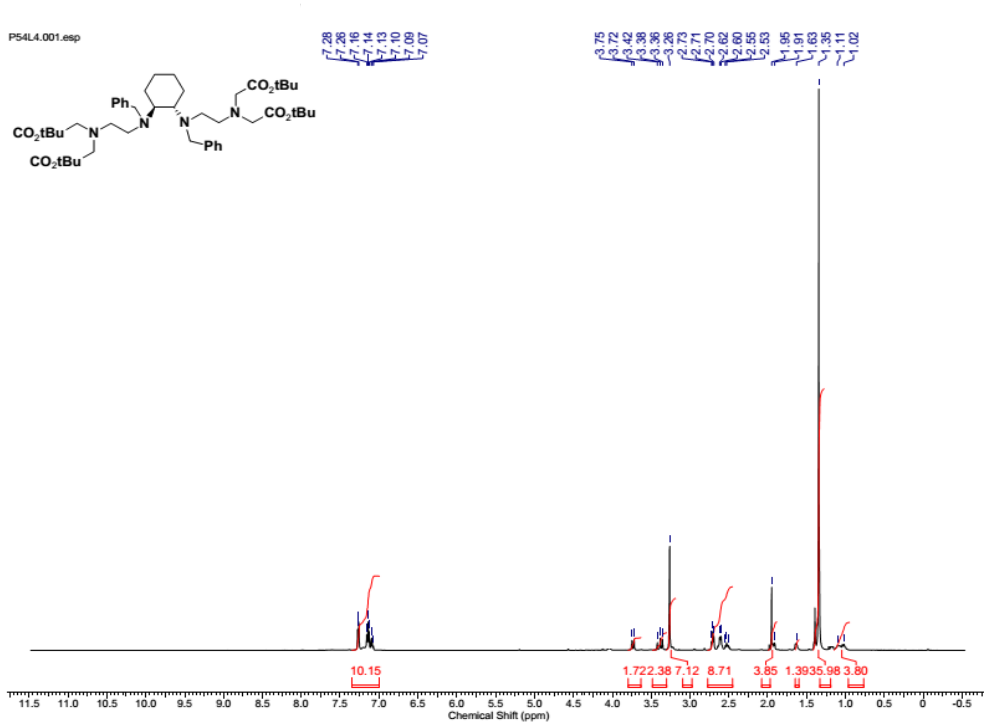
[1]HNMR spectrum of 17



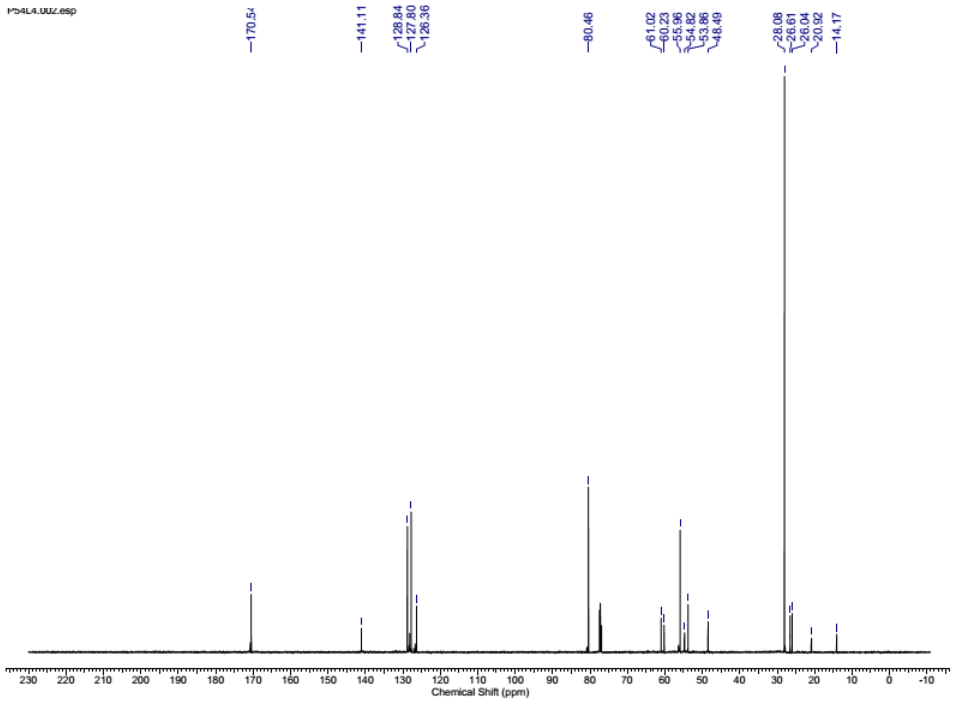
[13]CNMR spectrum of 17



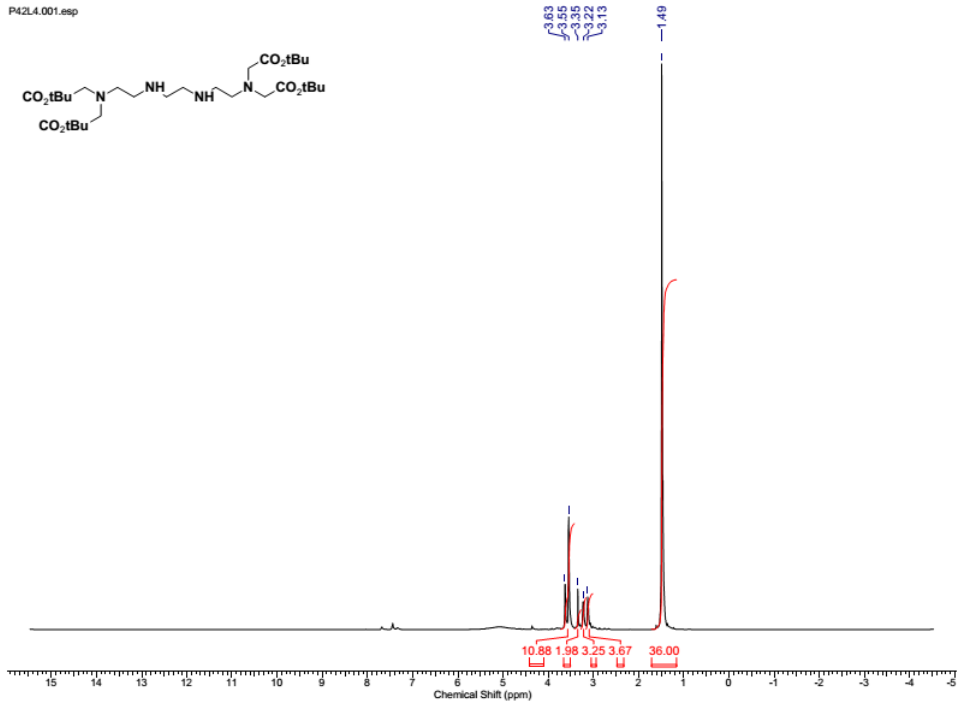
[1]HNMR spectrum of 18



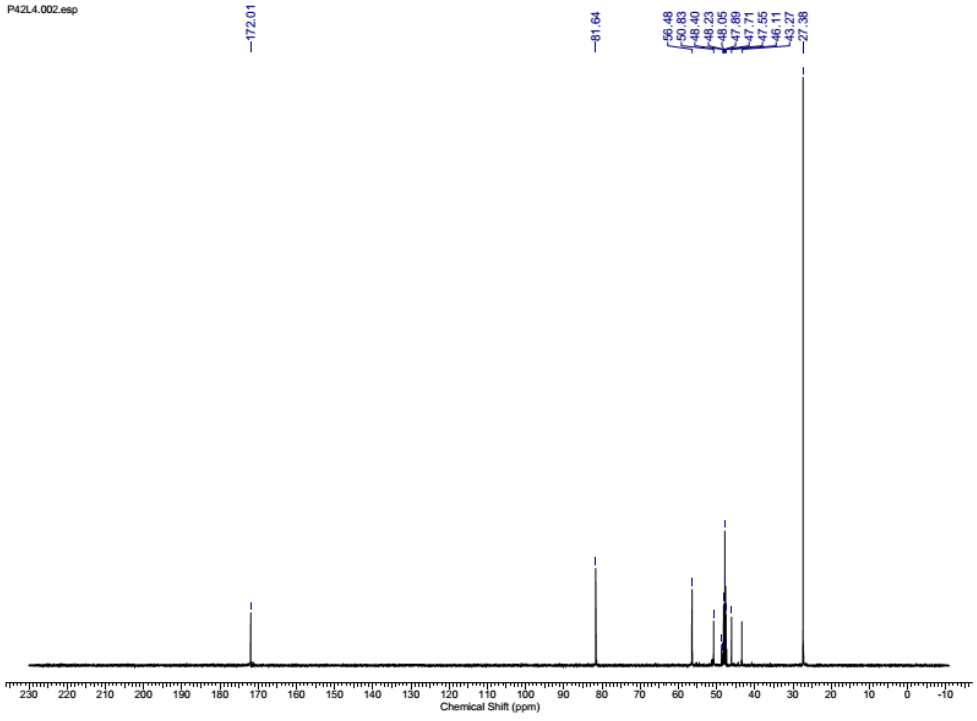
[13]CNMR spectrum of 18



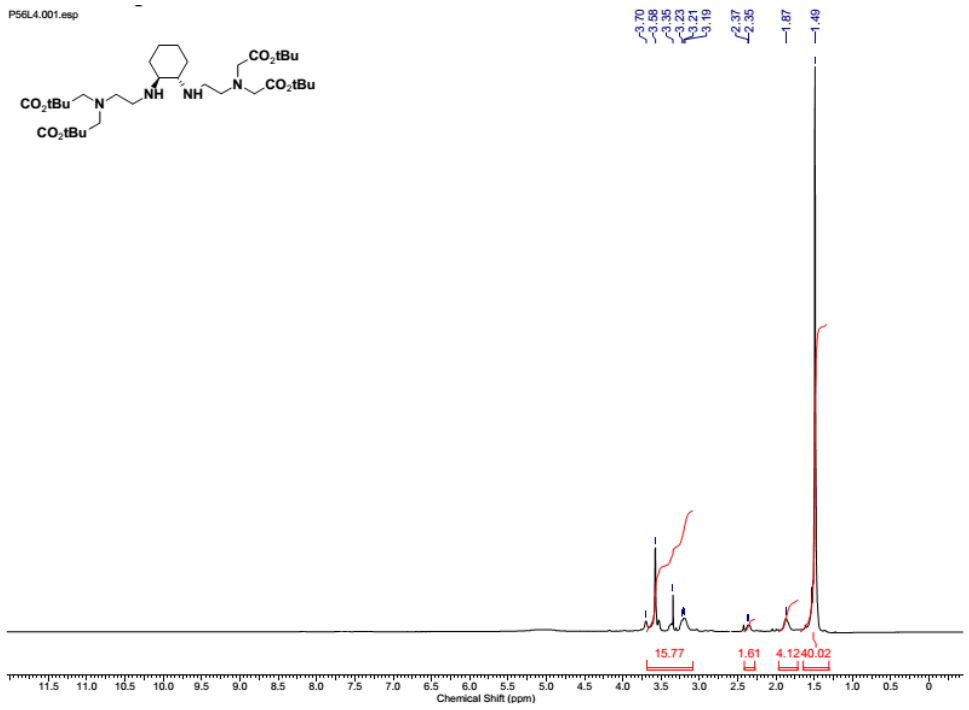
[1]HNMR spectrum of 19



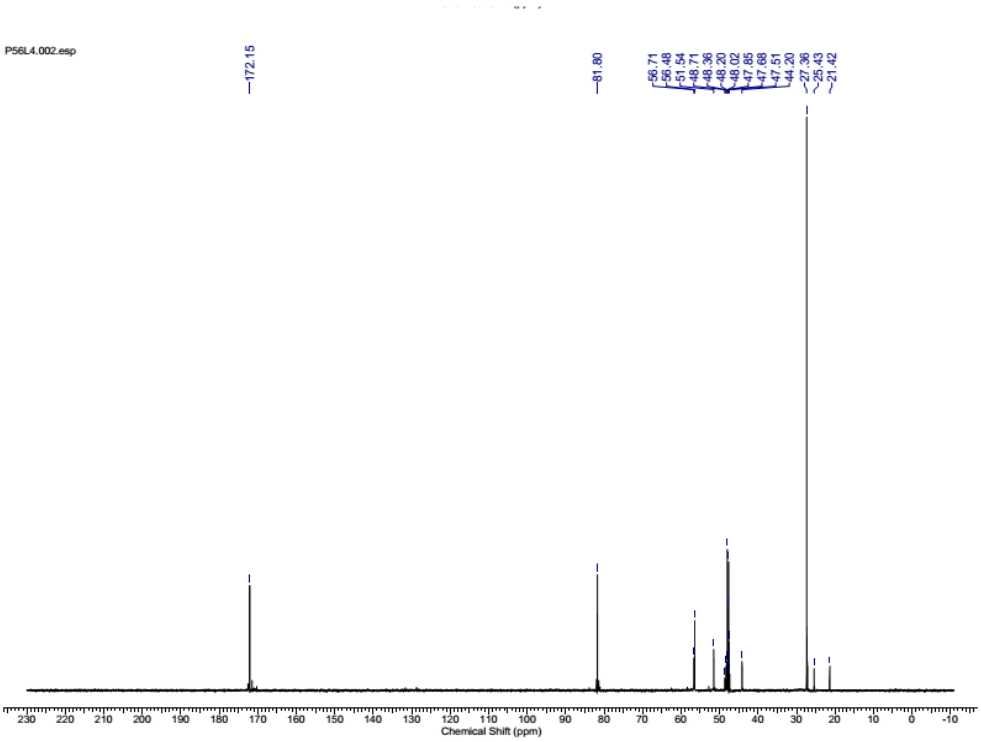
[13]CNMR spectrum of 19



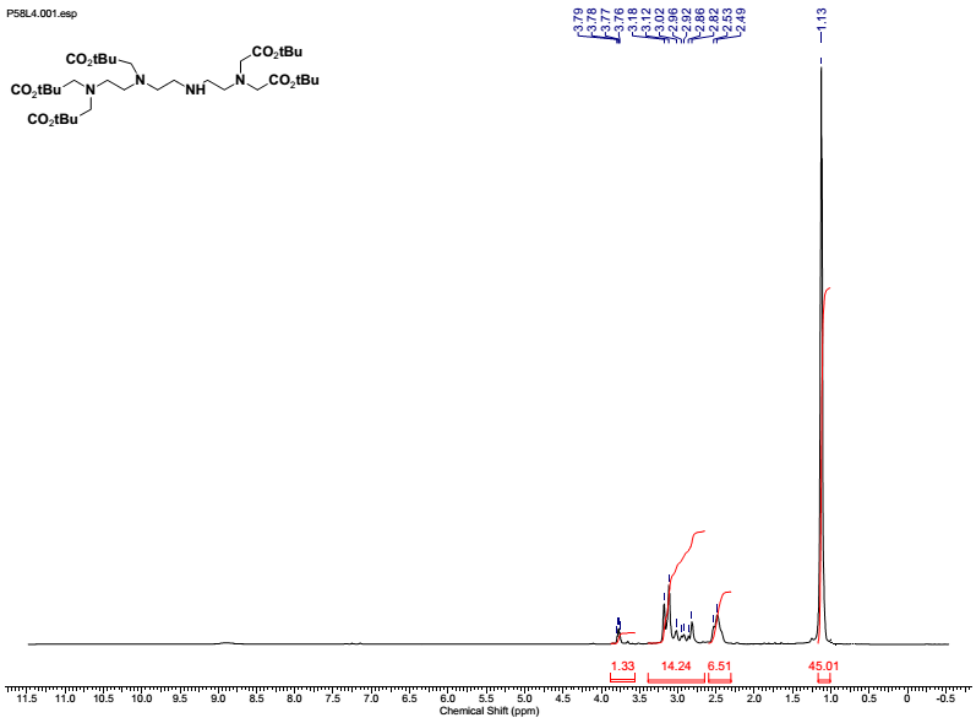
[1]HNMR spectrum of 20



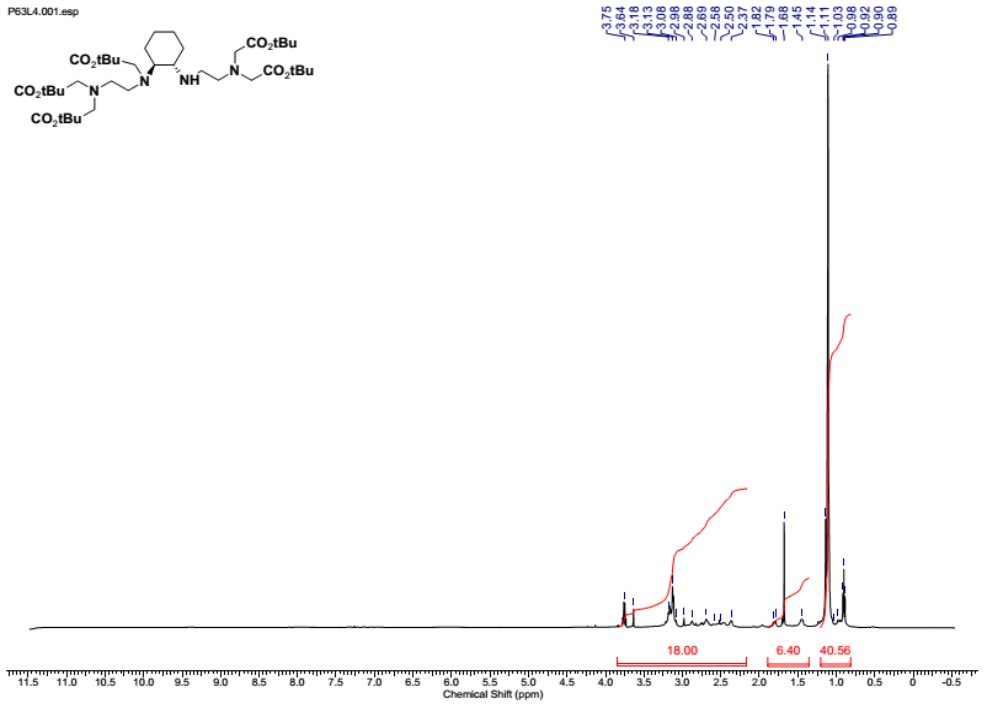
[13]CNMR spectrum of 20



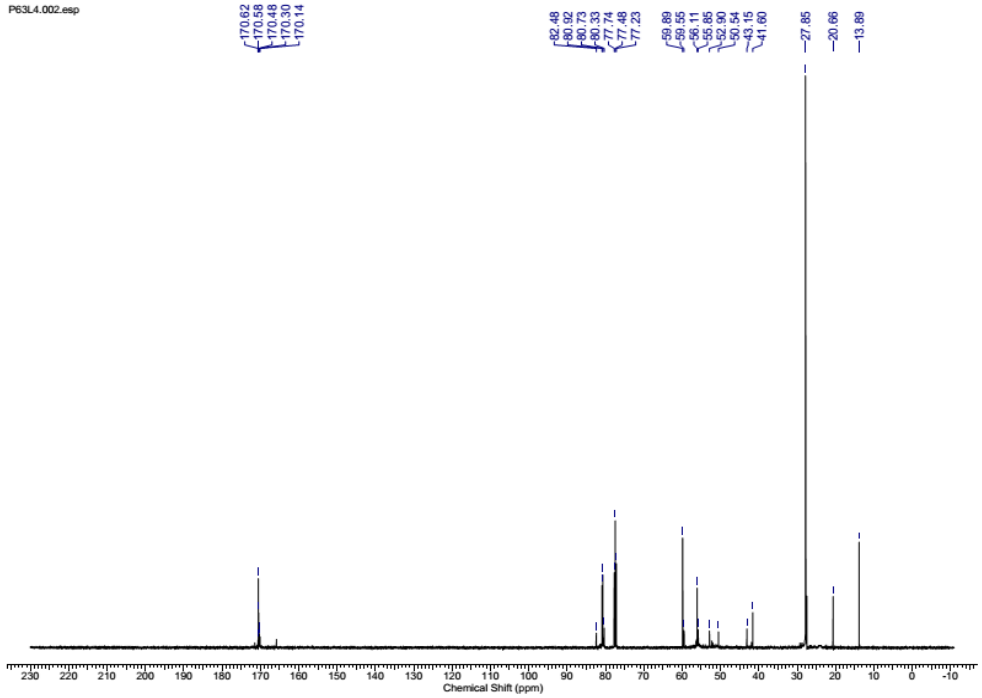
1HNMR spectrum of 21



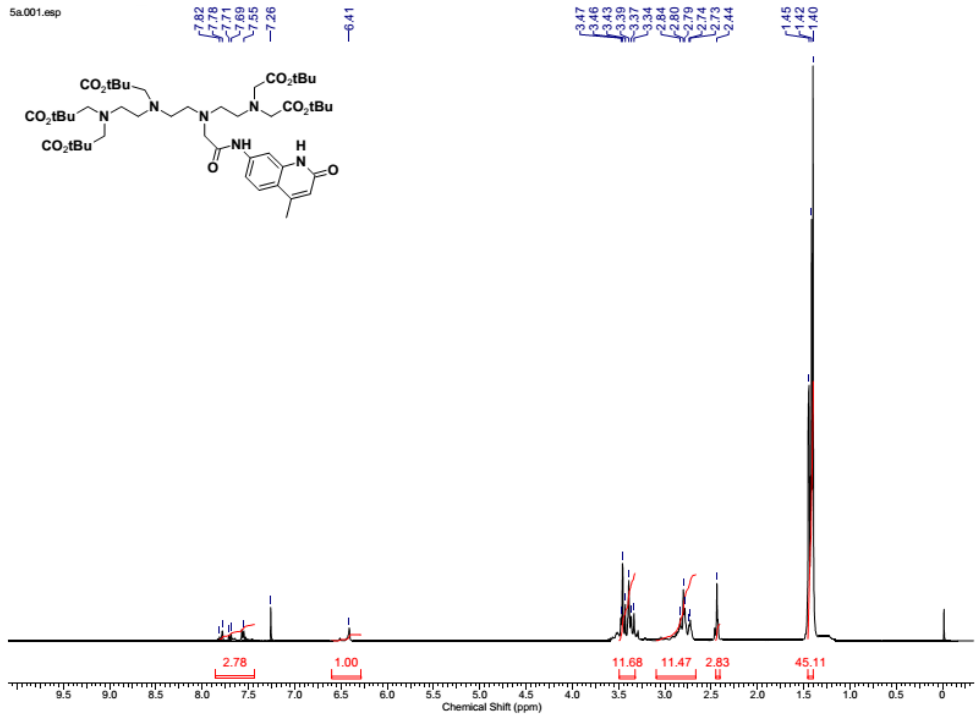
[1]HNMR spectrum of pro-ligand 22



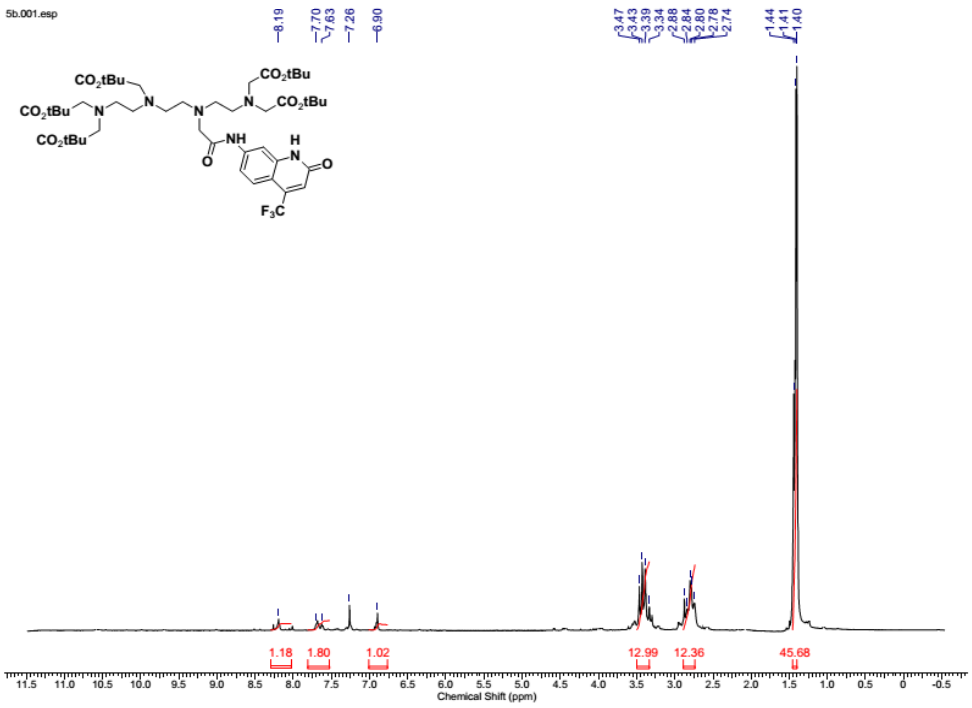
[13]CNMR spectrum of pro-ligand 22



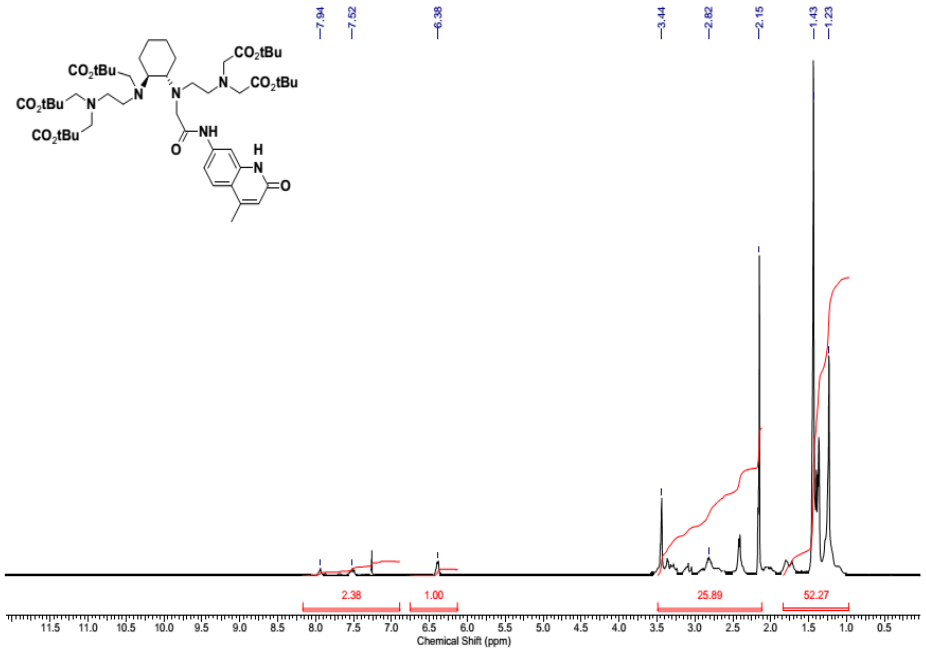
[1]HNMR spectrum of pro-ligand L1



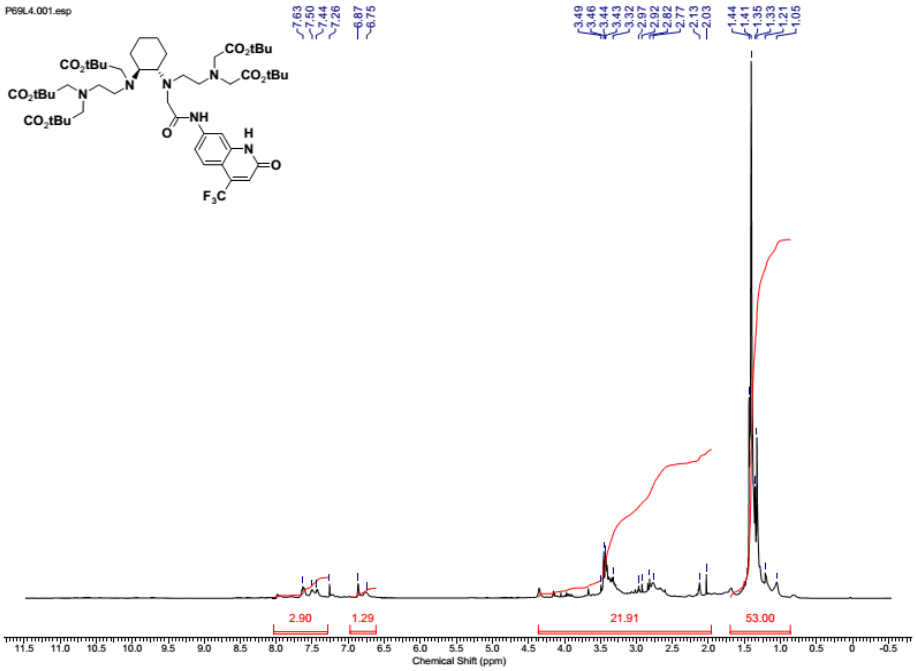
[1]HNMR spectrum of pro-ligand L2



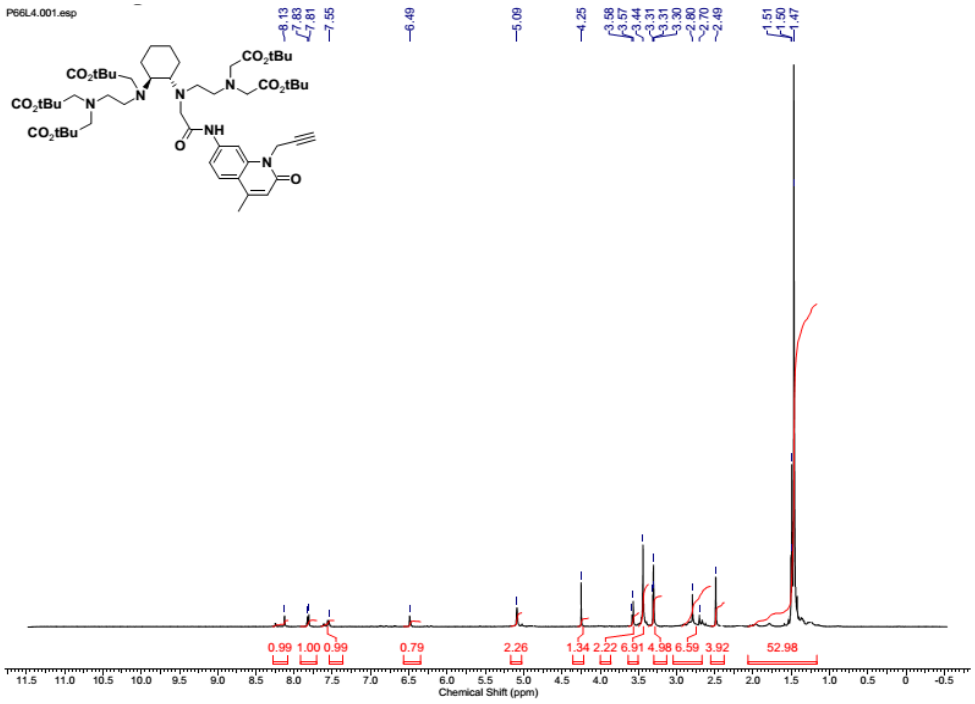
[1]HNMR spectrum of pro-ligand L3



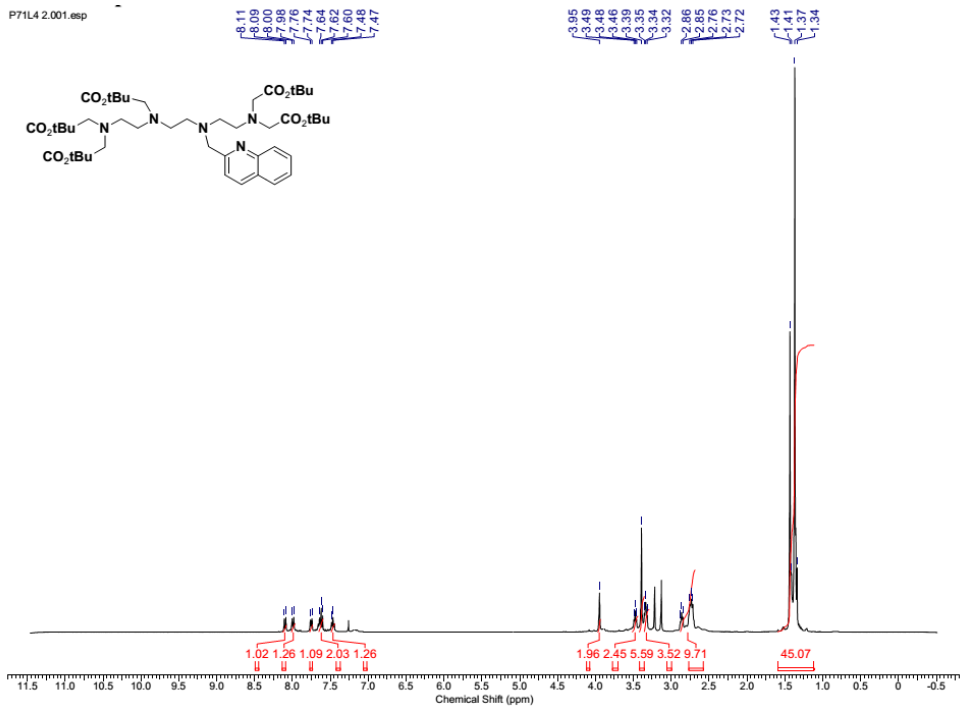
[1]HNMR spectrum of pro-ligand L4



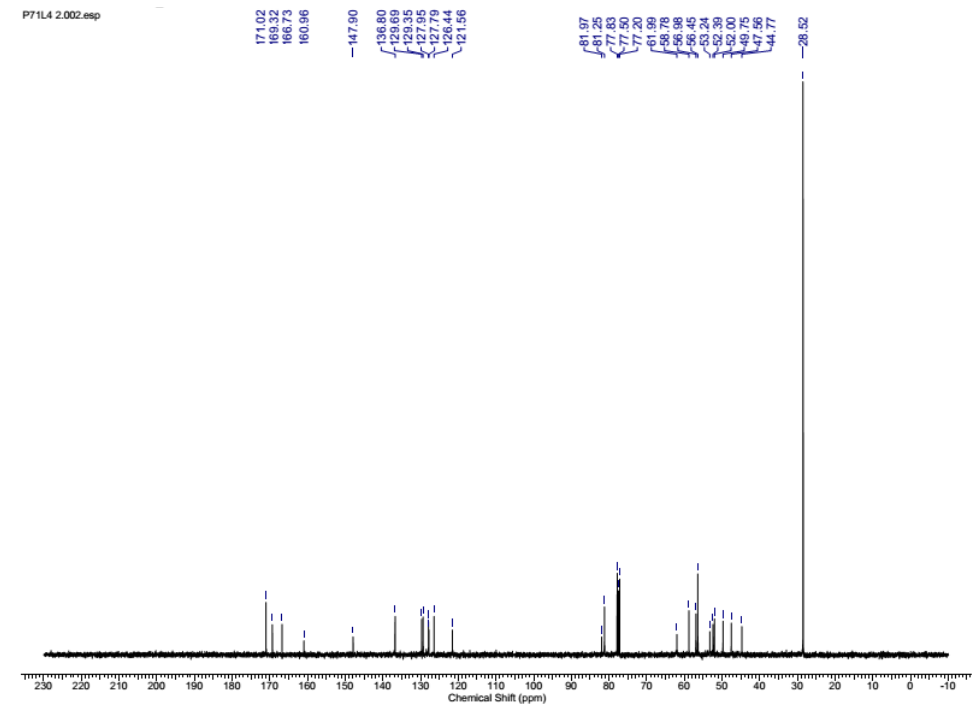
[1]HNMR spectrum of pro-ligand L6



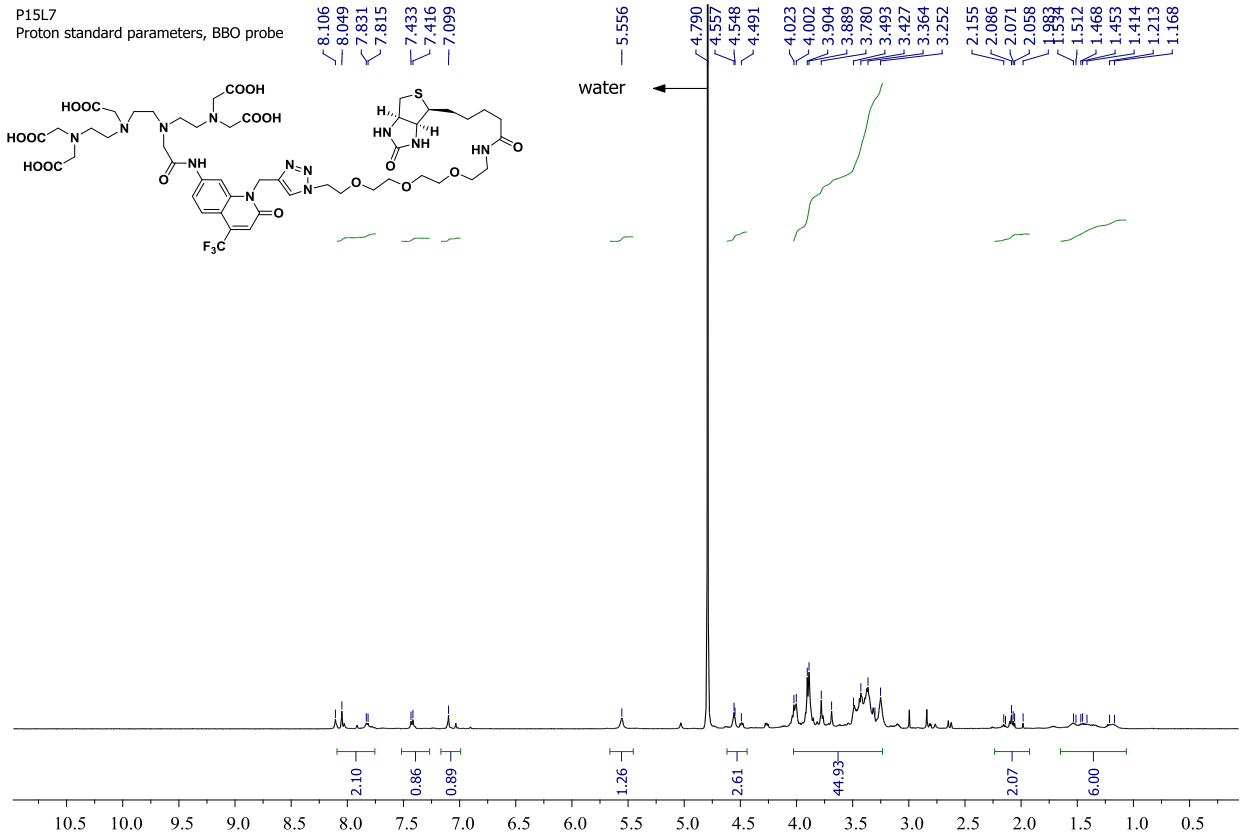
[1]HNMR spectrum of pro-ligand 23



[13]CNMR spectrum *pro-ligand 23*



[1]HNMR spectrum of 25



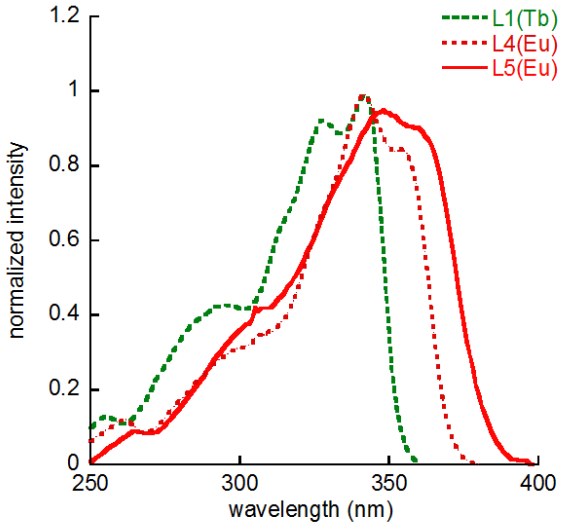


Figure S1. Normalized excitation spectra of representative lanthanide complexes in TBS buffer at pH 7.4 upon monitoring the $^5D_4 \rightarrow {}^7F_5$ emission for Tb(III) and $^5D_0 \rightarrow {}^7F_2$ for Eu(III).

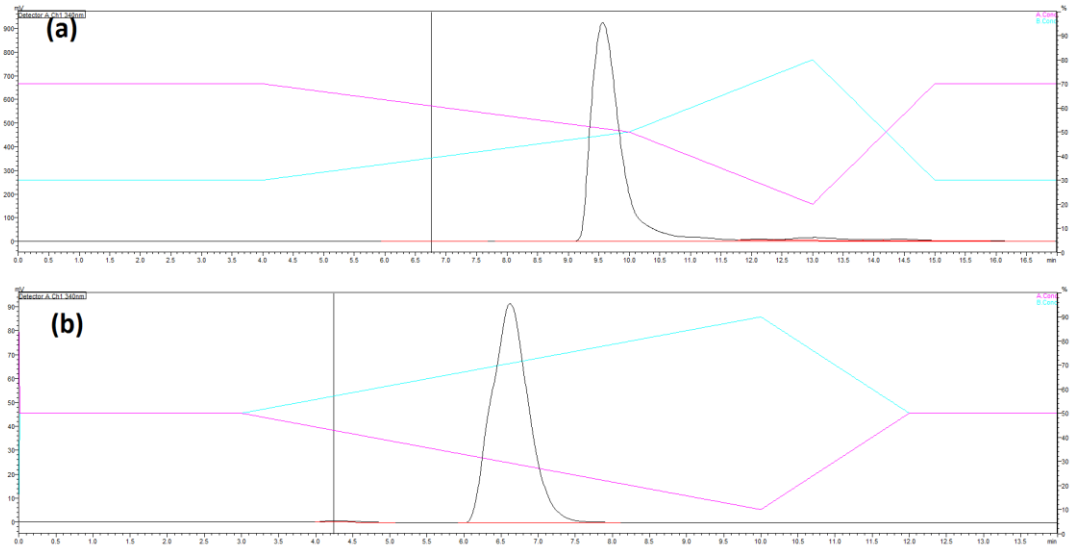


Figure S2. HPLC chromatogram of a representative sensitized lanthanide chelator **L5**. (Linear gradients of solvents A and B were used (A = de-ionized water containing 0.1 % (v/v) trifluoroacetic acid; B = HPLC grade acetonitrile containing 0.1 % (v/v) trifluoroacetic). (a) before complexation (30-50% B over 7 min followed by a steep gradient to 90% B and equilibrium back to 30% B; retention time ~ 9.1 min). (b) after incubation for 15 min at room temperature **L5(Eu)**. (45-90% B over 7 min followed equilibrium back to 45% B; retention time ~ 6.0 min).

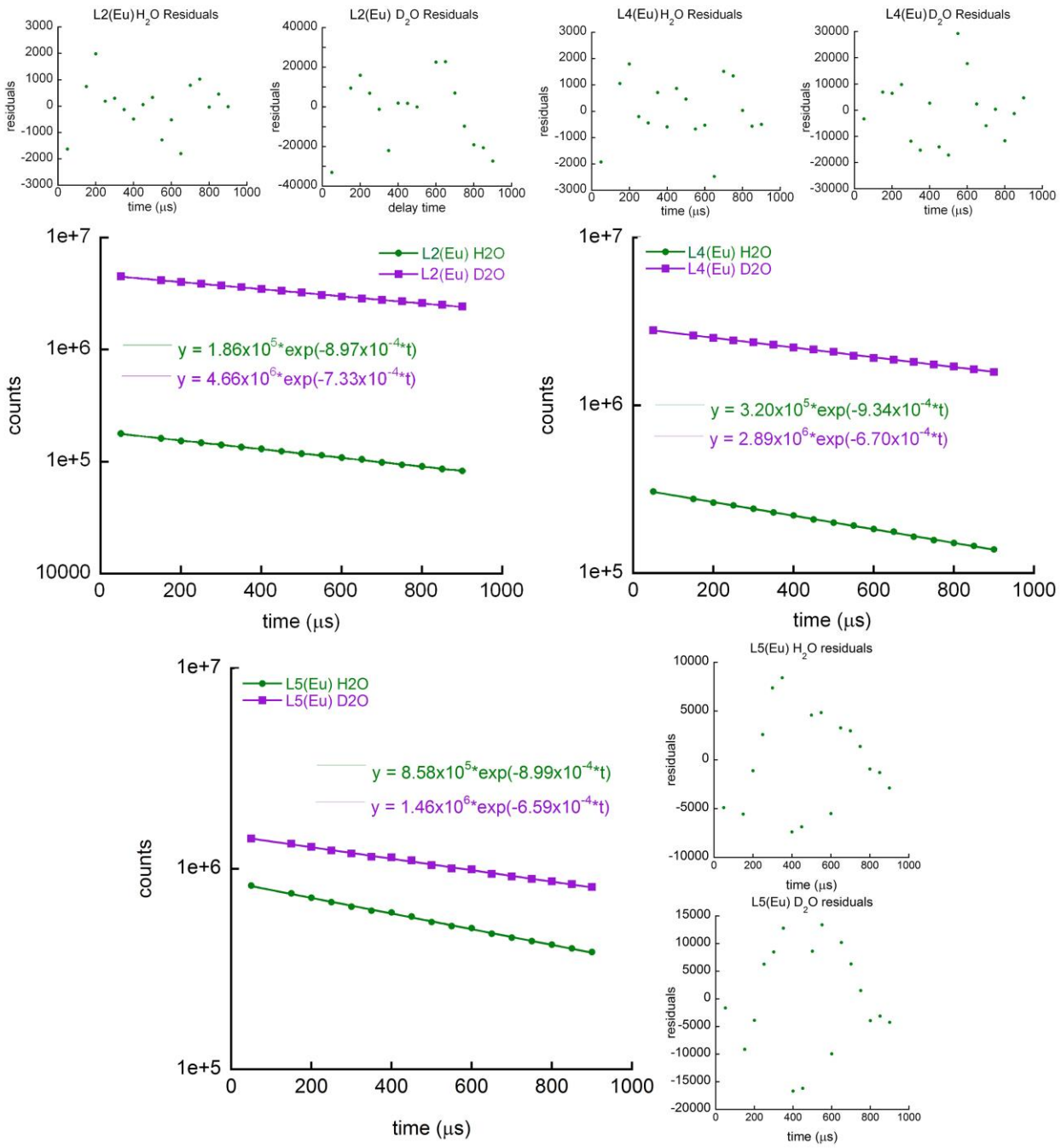


Figure S3. Time-gated luminescence intensity plotted as a function of gate delay width for Eu(III) complexes dissolved in H_2O and D_2O. Data were fit to a single exponential decay model, $I(t) = A*exp(-1/\tau*t)$, in order to estimate excited state lifetimes. Goodness of fit judged from R^2 values (> 0.99 in all cases) and lack of structure in residuals plots.

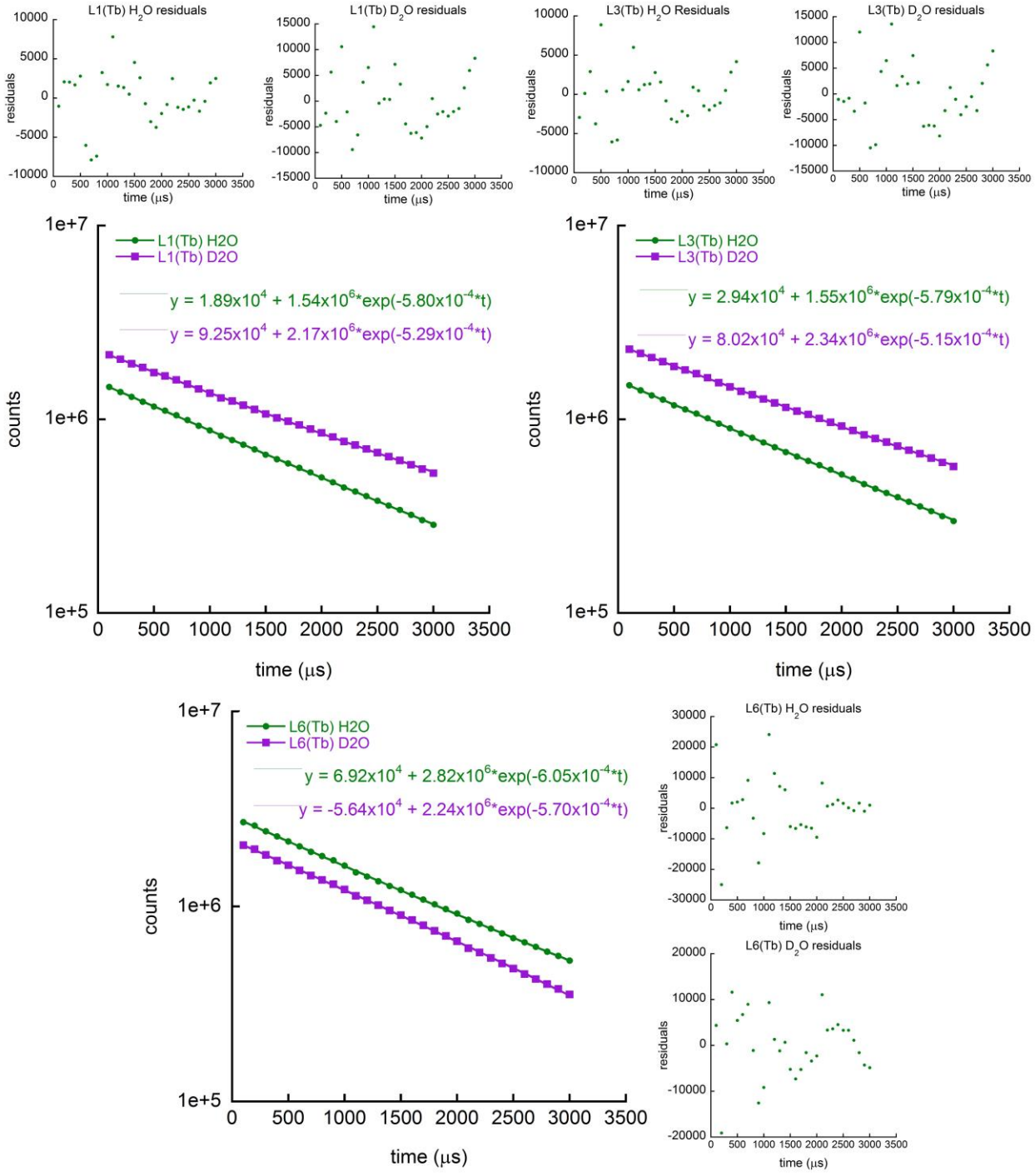
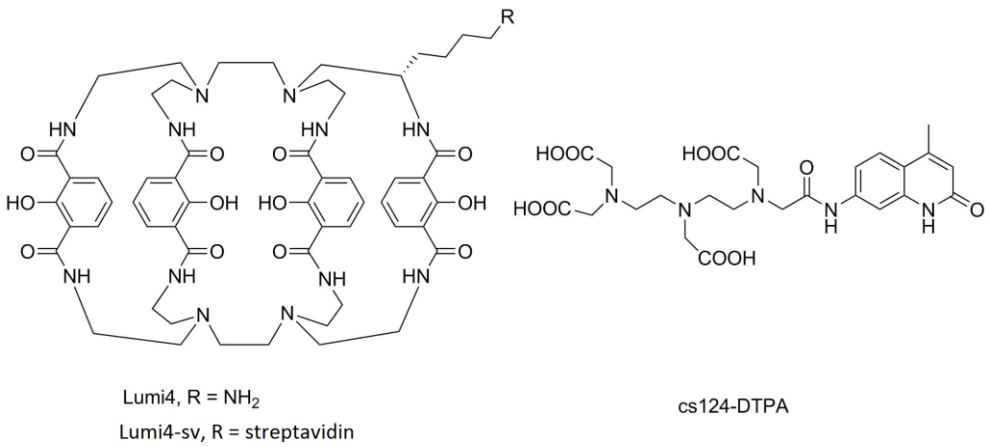


Figure S4. Time-gated luminescence intensity plotted as a function of gate delay width for Tb(III) complexes dissolved in H_2O and D_2O. Data were fit to a single exponential decay model with offset, $I(t) = A*exp(-1/\tau*t) + B$, in order to estimate excited state lifetimes. Goodness of fit judged from R^2 values (> 0.99 in all cases) and lack of structure in residuals plots.



Lumi4, R = NH_2

Lumi4-sv, R = streptavidin

cs124-DTPA

Figure S5. Structures of chelators used for comparative kinetic stability studies in solution or in live cells.