Synthetic Methods: PPT1 promotes tumor growth and is the molecular target of chloroquine derivatives in cancer.

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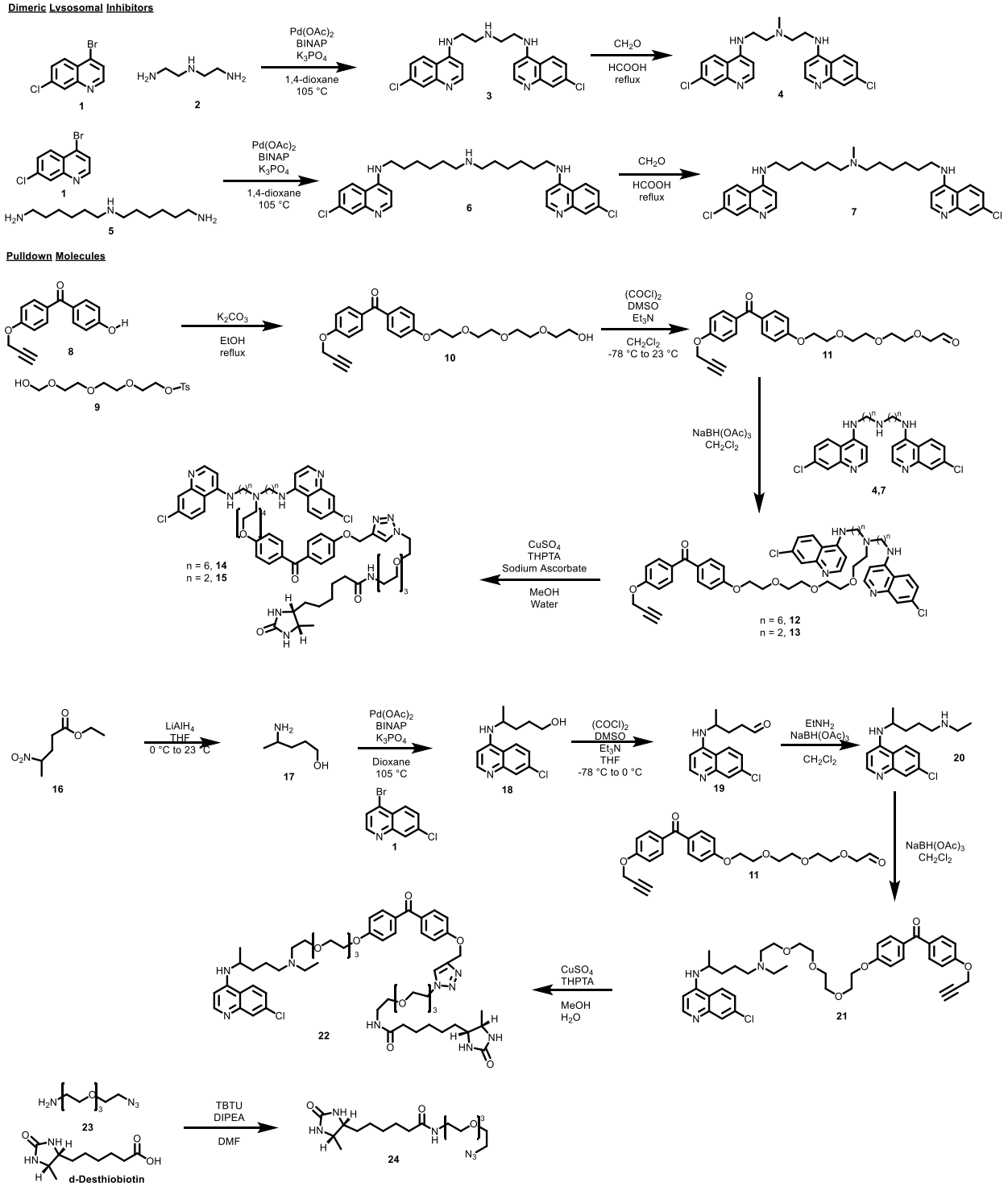
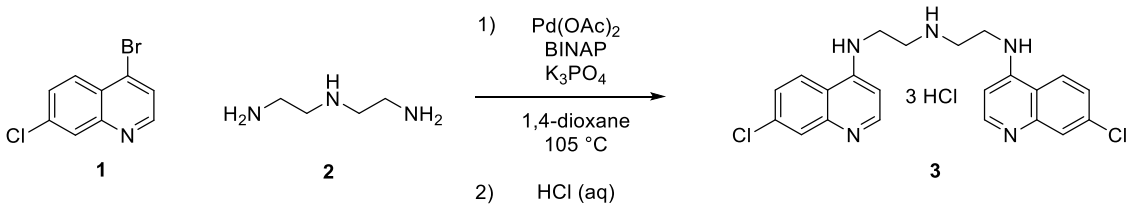
Synthetic Scheme



General Synthetic Methods

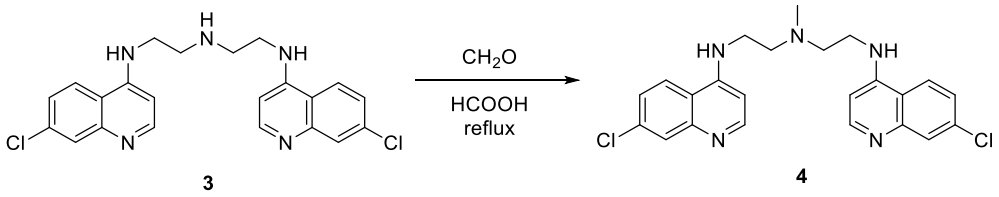
Solvents used for extraction and purification were HPLC grade from Fisher Scientific. Unless otherwise indicated, all reactions were run under an inert atmosphere of argon. Anhydrous tetrahydrofuran, ethyl ether and methylene chloride were obtained via passage through an activated alumina column. Commercial reagents were used as received. VWR pre-coated silica gel plates (250 μm, 60 F254) were used for analytical TLC. Spots were visualized using 254 nm ultraviolet light or with potassium permanganate if no chromophore was available. Chromatographic purifications were performed on Sorbent Technologies silica gel (particle size 32-63 microns). ^{1}H and ^{13}C NMR spectra were recorded at 500 MHz and 125 MHz, respectively in $CDCl_3$, CD_3OD, or DMSO-d^6, on a Bruker AM-500 or DRX-500 spectrometer. Chemical shifts are reported relative to internal chloroform (δ = 7.26 for ^{1}H, δ = 77.00 for ^{13}C), methanol (δ = 3.31 for ^{1}H, δ = 49.00 for ^{13}C), or DMSO (δ = 2.50 for ^{1}H, δ = 39.00 for ^{13}C). HPLC purification was performed utilizing a Shimadzu HPLC (Water [0.1% TFA v/v, MeCN]) with reverse phase columns from Waters (Analytical X-Select, C_{18}, 5 um pore size, column dimensions 4.6 mm x 250 mm; Preparative X-Select, C_{18}, 5 um pore size, column dimensions 19 mm x 250 mm). Infrared spectra were recorded on a NaCl plate using a Perkin-Elmer 1600 series Fourier transform spectrometer. Melting points were obtained on a Thomas Hoover capillary melting point apparatus and are uncorrected. Accurate mass measurement analyses were conducted on either a Waters GCT Premier, time-of-flight, GCMS with electron ionization (EI), or an LCT Premier XE, time-of-flight, LCMS with electrospray ionization (ESI). Samples were taken up in a suitable solvent for analysis. The signals were mass measured against an internal lock mass reference of perfluorotributylamine (PFTBA) for EI-GCMS, and leucine enkephalin for ESI-LCMS. Waters software calibrates the instruments, and reports measurements, by use of neutral atomic masses. The mass of the electron is not included. We thank Dr. Charles W. Ross III and Joo Myung Jun for their measurement and analysis of compound accurate mass.

Synthesis of Dimeric Chloroquine Inhibitors



DC220

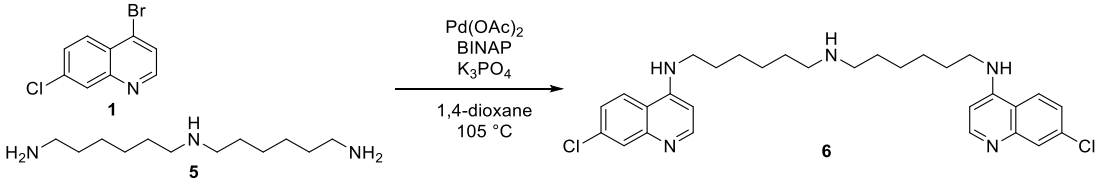
To a flame-dried round bottom flask, the triamine linker, **2**, (TCI America) (1.9 mL, 17.6 mmol, 1.0 eq.), 4-bromo-7-chloroquinoline[1] (9.00g, 37.0 mmol, 2.2 eq.), $Pd(OAc)_2$ (Strem Chemicals) (160mg, 0.71mmol, 0.04 eq.), (±)-2,2′-bis(diphenylphosphino)-1,1′-binaphthalene (BINAP) (Strem Chemicals) (880mg, 1.41 mmol, 0.08 eq.), K_3PO_4 (Acros Organics)(11.27g, 52.9 mmol, 3.0 eq.) were added at 25°C. The reagents were placed under an argon atmosphere and dissolved in 1,4-dioxane (44mL, 0.4 M). The reaction was then heated to 105 °C under a reflux condenser. The reaction was monitored by ^{1}H NMR ($CDCl_3$) where the disappearance of the primary amine linker marks consumption of the limiting reagent (peak at δ ~2.7 ppm). The reaction was then cooled to 23 °C and filtered through Celite™ using 300 mL of a 2:1 mixture of $CHCl_3$:MeOH. The resulting solution was concentrated under reduced pressure to afford a yellow solid (15.0g). The solid was triturated three times with boiling CH_2Cl_2 (100 mL) to afford a crude white solid (9.75 g) which was collected via vacuum filtration. The solid was purified by salt formation (recrystallization in 1N HCl; 700 mL), yielding a white solid (5.72g, 61%), the structure of which was confirmed by ^{1}H NMR. TLC (R_f = 0.2, 10:89:1; MeOH:CH_2Cl_2:NH_4OH). Melting point (mp) (H_2O) = >240 °C (decomposition). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.89 (s, 2H), 9.81 – 9.74 (m, 2H), 8.82 (d, J = 9.2 Hz, 2H), 8.63 (d, J = 7.1 Hz, 2H), 8.08 (d, J = 2.1 Hz, 2H), 7.77 (dd, J = 9.1, 2.1 Hz, 2H), 7.05 (d, J = 7.1 Hz, 2H), 3.97 (q, J = 5.8 Hz, 4H), 3.41 (t, J = 6.0 Hz, 4H). ^{13}C NMR (126 MHz, D_2O): δ 155.64, 142.67, 139.69, 137.26, 127.73, 123.73, 118.82, 114.77, 98.48, 44.59, 39.15. FTIR (thin film) λ (cm^{-1}): 3021.73, 2920.36, 2783.11, 1626.95, 1611.13, 1584.16, 1550.07, 1451.45, 1365.71, 1343.21, 1228.39, 1210.89. HRMS (ESI) $C_{22}H_{24}Cl_5N_5$: Calculated for [M+H-3HCl] $C_{22}H_{22}N_5Cl_2$, 426.1252; found: 426.1247.



3 4

Lys 05 (DC221) [2]

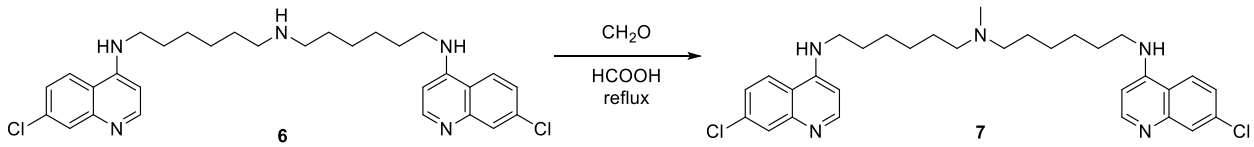
DC220-3HCl (5.00 g, 9.3 mmol, 1.0 eq) was added to a two-neck round bottom flask with a stir bar. A septum was placed in one neck, while a reflux condenser was placed over the other neck. The entire apparatus was placed under an atmosphere of argon. Formic acid (HCOOH) (Acros Organics) (31 mL) was added to the reaction vessel via a syringe, resulting in a 0.3M solution. Formaldehyde (CH_2O) (Fisher Scientific) (1.2 mL, 13.6 mmol, 1.45 eq.) was added as a solution (37% w/w aqueous) via a syringe to the reaction vessel. The reaction was heated to reflux with an oil bath preset to 105 °C. The reaction was monitored via TLC, whereby consumption of the starting material DC220 (R_f = 0.2, 10:90:1, MeOH:CH_2Cl_2:NH_4OH) and appearance of the product as a UV 254 nm active spot (purple in color, R_f = 0.45, 10:90:1, MeOH:CH_2Cl_2:NH_4OH) indicated completion of the reaction. The resulting mixture was then cooled to 23 °C and poured onto 200 mL of ice water. More ice was added to a final volume of 300 mL, and the pH of the solution brought to 12 with concentrated NH_4OH (ca. 60 mL) at which point a white solid precipitated. The solid was collected via vacuum filtration (3.5g). Further purification was affected by recrystallization from hot ethyl acetate (EtOAc), which on cooling to -20°C resulted in the formation of small off-white needles whose structure was confirmed by ^{1}H NMR. The product was collected via vacuum filtration (2.41g, 59%) and washed with cold EtOAc (-20 °C). The structure of the resulting product was confirmed by ^{1}H NMR, and found to match literature precedent.[2] ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.53 (2H, d, *J* = 5.5 Hz) ppm, 7.94 (2H, d, *J* = 2.0 Hz), 7.41 (2H, d, *J* = 9.0 Hz), 6.99 (2H, dd, *J* = 9.0, 2.0 Hz), 6.38 (2H, d, *J* = 5.5 Hz), 5.45 (2H, s(br)), 3.40 (4H, quart., *J* = 5.0 Hz), 2.89 (4H, t, *J* = 6.0 Hz), 2.46 (1H, s).



1 5 6

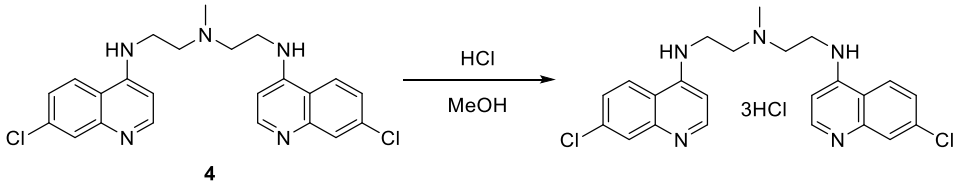
DC660

To a flame-dried round bottom flask, bishexamethylene triamine (TCI America) (3.799 g, 17.64 mmol, 1.0 eq.), 4-bromo-7-chloroquinoline[1] (9.43, 38.8 mmol, 2.2 eq.), $Pd(OAc)_2$ (199mg, 0.706mmol, 0.05 eq.), BINAP (1098 mg, 1.412 mmol, 0.1 eq.), K_3PO_4 (11.272g, 52.92 mmol, 3.0 eq.) was added. The reagents were placed under an argon atmosphere and dissolved in 1,4-dioxane (44mL) to give a 0.4M reaction concentration. The reaction was then heated in an oil bath preset to 105 °C under a reflux condenser. The reaction was monitored by ^{1}H NMR ($CDCl_3$) where the consumption of primary amine-containing linker marks complete consumption of the limiting reagent (peak at ~2.7 ppm). The reaction was then cooled to 23 °C and filtered through Celite™ using 300 mL of a 4:1 mixture of $CHCl_3$:MeOH. The solution was concentrated under reduced pressure to afford an orange-yellow solid (13.37 g), which was recrystallized from CH_2Cl_2 (heated to reflux and then cooled to -20 °C) to afford a white solid (8.516 g, 90%), the structure of which was verified by ^{1}H NMR. TLC (R_f = 0.25, 90:10:1, CH_2Cl_2, MeOH, NH_4OH). Melting point (mp) = 100 – 102 ˚C; ^{1}H NMR (500 MHz, CD_3OD): δ 1.38 – 1.55 (12H, m), 1.73 – 1.78 (4H, m), 2.55 (4H, t, *J* = 7.5 Hz), 3.36 (4H, t, *J* = 7.0 Hz), 6.50 (2H, d, *J* = 5.5 Hz), 7.38 (2H, dd, *J* = 2.0, 9.0 Hz), 7.76 (2H, d, *J* = 2.0 Hz), 8.09 (2H, d, *J* = 9.0 Hz), 8.34 (2H, d, *J* = 5.5 Hz). ^{13}C NMR (125 MHz, CD_3OD): δ 26.7 (2C), 27.9, 28.8, 42.5, 49.1, 98.2, 117.4, 122.9, 124.5, 126.2, 134.8, 148.3, 151.0 (2C). FTIR (thin film) λ (cm^{-1}): 3054, 1420, 1265, 896. HRMS (ESI) $C_{30}H_{37}Cl_2N_5$: Calculated for [M+H] $C_{30}H_{38}Cl_2N_5$, 538.2507; found: 538.2509.



DC661

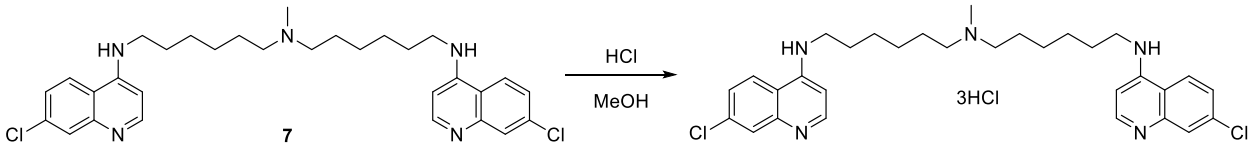
Compound **6** (7.02 g, 13.0 mmol, 1.0 eq.) was added to a round bottom flask, under a reflux condenser and placed under an atmosphere of argon. Formic acid (HCOOH, 19.3 mL) was added to the reaction vessel to give a 0.3M reaction concentration. While stirring, aqueous formaldehyde (37 % w/w) (0.95 mL, 12.75 mmol, 2.2 eq.) was added to the reaction vessel via a syringe. The reaction was then heated to reflux in an oil bath preset to 105 °C. The reaction was stirred at reflux until the starting material (DC660) was observed by TLC (90:10:1, CH_2Cl_2, MeOH, NH_4OH, R_f = 0.25) to be consumed. The formic acid was diluted with water (300 mL) resulting in a black aqueous solution. The pH of the solution was brought to 12 using concentrated NH_4OH (50 mL), and then extracted with $CHCl_3$ (3 x 100 mL). Upon extraction, a gray precipitate forms which was identified by ^{1}H NMR to be mostly product. The solid (1.1g) was collected via vacuum filtration of the remaining aqueous layer. The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford a gray-brown foam (5.4g). The foam was dissolved in CH_2Cl_2, and stirred with decolorizing carbon (2.0 g) for 45 min. The carbon was then removed by filtration through a plug of Celite™, using CH_2Cl_2 (100 mL). The gray solid was dissolved in a 4:1 mixture of $CHCl_3$:MeOH (100 mL) and stirred with decolorizing carbon (1.0 g) for 45 min. The carbon was them removed by filtration through a plug of Celite™. The filtered carbon was washed using CH_2Cl_2 (100 mL). The combined filtrates were then concentrated under reduced pressure to afford a beige-white crude solid. Purification was affected by recrystallization from a three-solvent system, where boiling CH_2Cl_2 yields a slightly cloudy solution, which was not clarified by the addition of more boiling CH_2Cl_2. To this solution methanol was added dropwise stirring until the boiling solution clarifies. The solution was then cooled to 20 °C, diethyl ether was then added until a droplet caused the solution to turn cloudy. The solution was cooled to -20°C for 24 hours to yield white circular crystals which were collected via vacuum filtration (4.508g, 63%), the structure of which was verified by ^{1}H NMR. TLC (R_f = 0.35 (EtOAc:MeOH:TEA; 80:15:5)); Melting point (mp): 58 – 60 ˚C. ^{1}H NMR (500 MHz, CD_3OD): δ 1.35 – 1.47 (12H, m, CH_2), 1.69 – 1.73 (4H, m, CH_2), 2.17 (3H, s, CH_3), 2.29 – 2.31 (4H, m, CH_2), 3.30 (4H, s(br), CH_2), 6.45 (2H, s(br), ArH), 7.34 – 7.37 (2H, m, ArH), 7.75 (2H, s, ArH), 8.06 – 8.08 (2H, m, ArH), 8.32 (2H, s(br), ArH). ^{13}C NMR (125 MHz, CD_3OD): δ 26.2, 26.6, 27.0, 27.9, 41.0, 42.5, 57.1, 98.2, 117.4, 122.9, 124.5, 126.2, 134.8, 148.3, 151.0, 151.3. FTIR (thin film) λ (cm^{-1}): 3683, 3455, 3019, 2936, 2859, 1582, 1215, 852. HRMS (ESI) $C_{31}H_{39}Cl_2N_5$: Calculated for [M+H] $C_{31}H_{40}N_5Cl_2$, 552.2655; found: 552.2654.



4

Lys 05 (Water Soluble Salt)

Synthesis of Lys 05 the water-soluble salt of DC221, was performed according to literature precedent. NMR of the water-soluble salt was found to match the reported literature precedent.[2] ^{1}H NMR (500 MHz, D_2O): δ 3.23 (s, 3H), 3.76 (s, 4H), 4.01 (s, 4H), 6.75 (d, *J* = 7.0 Hz, 2H), 7.36 – 7.42 (m, 2H), 7.71 (d, *J* = 2.2 Hz, 2H), 7.86 (d, *J* = 9.0 Hz, 2H), 8.26 (d, *J* = 6.9 Hz, 2H). ^{13}C NMR (126 MHz, D_2O): δ 155.43, 142.80, 140.01, 136.94, 127.86, 123.86, 118.78, 114.49, 98.68, 52.91, 42.65, 38.15. Purity of the final compound was determined by UPLC-MS analysis. Lys05 was found to be a single peak, with a mass corresponding to the reported literature precedent, [M+2H-3HCl]/2 = 220.8. (Page 34)

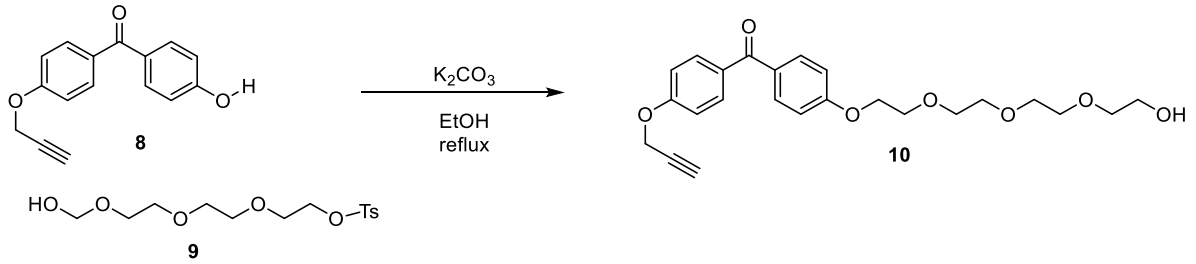


7

DC661 (Water Soluble Salt)

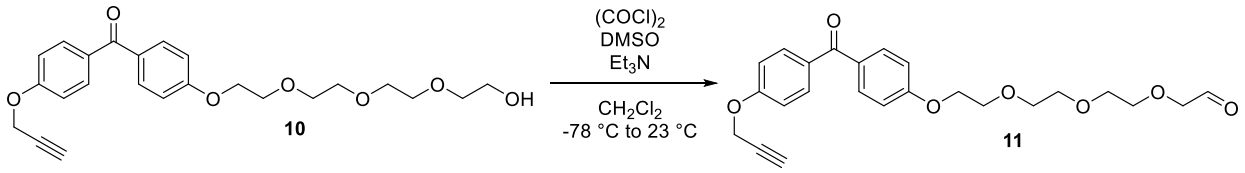
DC661 (126 mg, 0.23 mmol, 1.0 eq) was added to a round bottom flask. MeOH (3 mL) was added to dissolve DC661, and the solution was stirred at 23 °C. A solution of HCl in methanol (3 mL, 0.25 M) was added to the reaction vessel, and the resulting solution was stirred for 1 hour at 23 °C. The solvent and excess HCl was removed by concentration under reduced pressure to afford a yellow foam (150 mg, 99%). The foam was found to be a hygroscopic solid, which must be stored under a dry inert atmosphere. Attempts to determine a melting point result in phase transition of the foam to a paste. ^{1}H NMR (500 MHz, CD_3OD) δ 8.45 (d, *J* = 9.1 Hz, 2H), 8.35 (d, *J* = 7.1 Hz, 2H), 7.83 (d, *J* = 2.1 Hz, 2H), 7.60 (dd, *J* = 9.1, 2.1 Hz, 2H), 6.86 (d, *J* = 7.2 Hz, 2H), 3.58 (t, *J* = 7.3 Hz, 4H), 3.25 – 3.02 (m, 4H), 2.83 (s, 3H), 1.87 – 1.70 (m, 8H), 1.57 – 1.40 (m, 8H). ^{13}C NMR (126 MHz, CD_3OD) δ 157.47, 143.64, 140.84, 139.91, 128.53, 126.26, 120.18, 116.83, 99.81, 57.23, 44.69, 40.44, 28.80, 27.35, 27.16, 25.08. FTIR (thin film) λ (cm^{-1}): 3220, 3020, 2938, 2729, 1612, 1591, 1453, 1212. HRMS (ESI) $C_{31}H_{42}Cl_5N_5$: Calculated for [M+H-3HCl] $C_{31}H_{40}N_5Cl_2$, 552.2661; found: 552.2656. Purity of the final compound was determined by UPLC-MS analysis. DC661-3HCl was found to be a single peak, with a mass corresponding to the reported literature precedent, [M+2H-3HCl]/2 = 277.8. (Page 35)

Synthesis of photoaffinity analogs



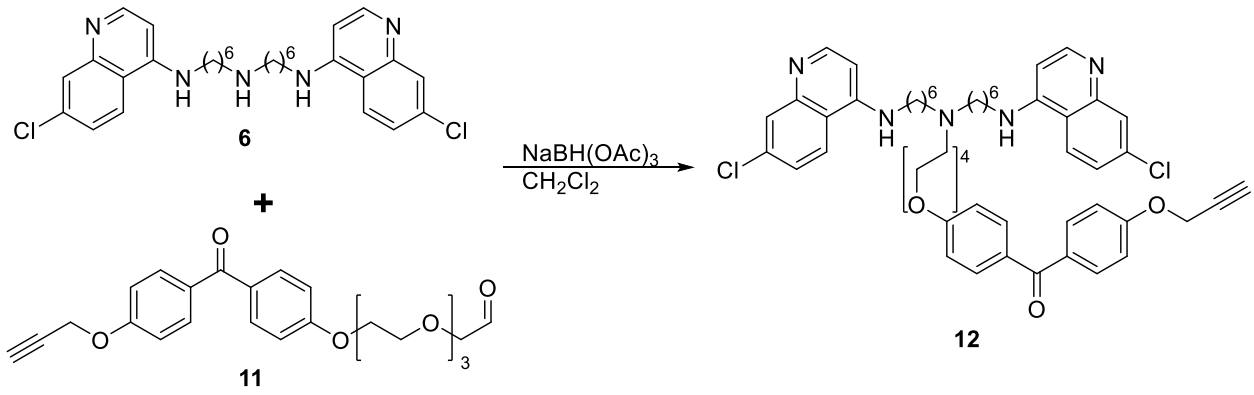
Synthesis of Compound **10** [3]

(4-hydroxyphenyl)(4-(prop-2-yn-1-yloxy)phenyl)methanone[4] (compound **8**) (1.02g, 4.05 mmol) was added to a round bottom flask, under an argon atmosphere, followed by K_2CO_3 (Fisher Scientific) (1.10g, 8.1 mmol, 2.0 eq.). 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate[5] (compound **9**) (1.55g, 4.46 mmol, 1.1 eq.) was dissolved in absolute ethanol (8 mL) and added to the reaction vessel. The reaction was heated to reflux under a condenser for 16 hours. The reaction was halted upon consumption of the tosylate starting material as determined by TLC (R_f = 0.2, EtOAc). The reaction was concentrated under reduced pressure to remove ethanol. The crude paste was dissolved in 30 mL of a 1:1 mixture of water and EtOAc. The layers were separated, and the aqueous layer was washed with EtOAc (2 x 25 mL). The combined organic layers were washed once with brine and dried over anhydrous Na_2SO_4. Evaporation under reduced pressure yielded a yellow clear paste (2.06g). The crude material was purified by column chromatography (45mm x 150mm, SiO_2, EtOAc) which yielded a translucent paste (1.314g, 76%), the structure of which was verified by ^{1}H NMR to match the reported literature.[3] TLC (R_f = 0.2, EtOAc) ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.56 (1H, s, CH), 3.76-3.61 (m, 12H, CH_2), 3.90 (2H, t, *J*= 4.84, CH_2), 4.22 (2H, t, *J*=4.81, CH_2), 4.78 (2H, d, *J*=2.4 Hz, CH_2), 6.98 (2H, d, *J*= 8.7 Hz, ArH), 7.04 (2H, d, *J*= 8.7 Hz, ArH), 7.80-7.77 (4H, m, ArH). ^{13}C NMR (126 MHz, $CDCl_3$): δ 194.47, 162.27, 160.77, 132.35, 132.22, 131.68, 130.85, 114.49, 114.24, 78.05, 76.27, 71.00, 70.81, 70.73, 70.48, 69.70, 67.75, 61.87, 56.00. FTIR λ (neat/cm^{-1}): 2873, 1644, 1600, 1507. HRMS (ESI) Calculated for [M+H] $C_{24}H_{29}O_7$,429.1913; found: 429.1933.



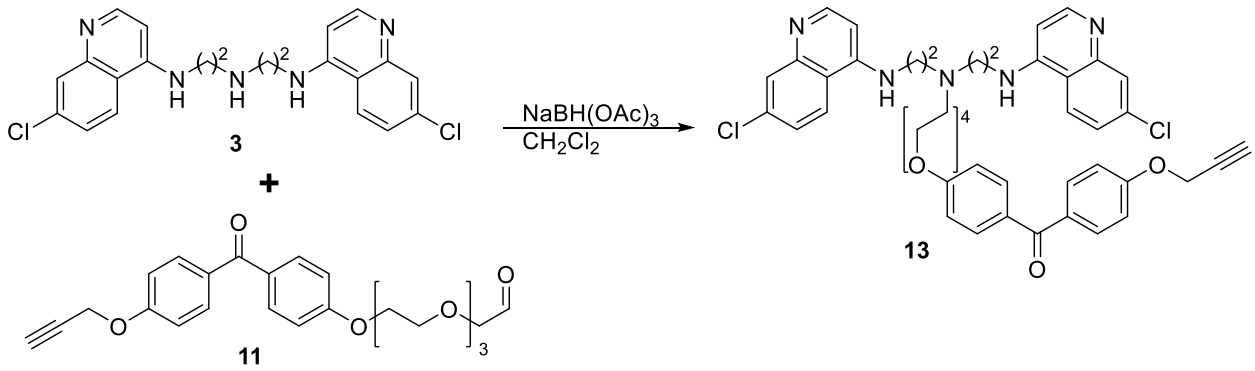
Synthesis of Compound **11**[3]

In a flame-dried round bottom flask under an argon atmosphere, a solution of freshly distilled oxalyl chloride (0.31 mL, 3.6 mmol, 1.2 eq.) in dichloromethane (20 mL), was cooled to -78°C. Anhydrous DMSO (Acros Organics) (0.51 mL, 7.2 mmol, 2.4 eq.) was added to the flask, causing the evolution of gas. The reaction mixture was stirred for 30 min at -78°C, then a solution of compound **10** (1.28g, 3.0 mmol, 1.0 eq.) in CH_2Cl_2 was added slowly. Prior to addition, the alcohol was dried azeotropically with benzene and then placed under high vacuum for 16 hours to remove water. The resulting solution was stirred at -78°C for 1 hour, at which time freshly distilled triethylamine (Acros Organics) (2.0 mL, 15.0 mmol, 5.0 eq.) was added to the reaction. The resulting mixture was slowly warmed to 23 °C and stirred for 16 hours. The progress of the reaction was determined via TLC by consumption of the starting alcohol (TLC R_f = 0.18, EtOAc) and the appearance of a new TLC spot for the aldehyde product (R_f = 0.6, EtOAc). Upon completion, the reaction mixture was then washed with saturated aqueous NH_4Cl (3 x 20 mL), brine (1 x 20 mL), and the resulting organic solution dried over anhydrous Na_2SO_4. The filtrate was concentrated under reduced pressure yielding a crude paste, the presences of the product was verified by ^{1}H NMR to match the literature precedent.[3] Due to stability concerns, the crude aldehyde was used without further purification in the next reaction. TLC (R_f = 0.6, EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.56(1H, s, CH), 3.76-3.61, (10H, m, CH_2), 3.87 (2H, t, J= 3.86, CH_2), 4.13 (2H, s, CH_2), 4.18 (2H, t, J= 4.35, CH_2), 4.75 (2H, d, J= 2.0 Hz, CH_2), 6.98 (2H, d, J=8.0 Hz, ArH), 7.04 (2H, d, J= 9.0 Hz, ArH), 7.8-7.5 (4H, m, ArH), 9.69 (1H, s, CHO).



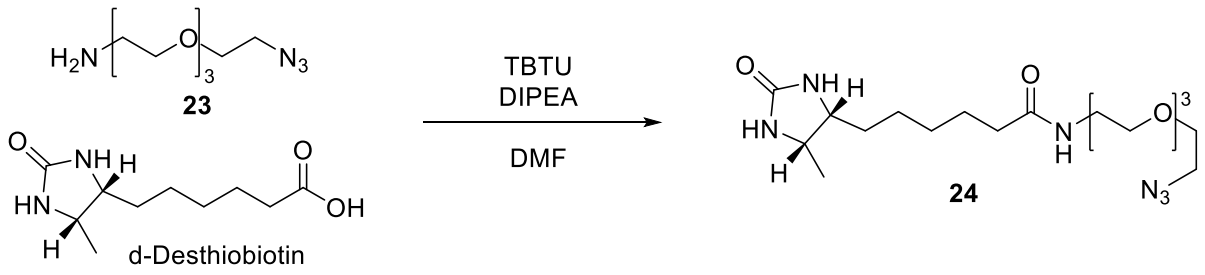
DC661-Alkyne (12)

To a round bottom flask, solid DC660 inhibitor (compound **6**) was added (307 mg, 0.57 mmol, 1.0 eq.). The solid was then placed under an atmosphere of argon. The aldehyde (505mg, 1.2 mmol, 2.1 eq.) was then dissolved in CH_2Cl_2 (7mL). The aldehyde solution was then added to the reaction vessel via a syringe to give a reaction concentration of 0.1 M. The reaction was stirred at 23 °C for 10 minutes. Next, sodium triacetoxyborohydride was added to the reaction as a solid (483mg, 2.4 mmol, 4.0 eq.). The reaction was stirred for 22 hours at 23 °C, when the starting DC660 was consumed as observed via TLC (R_f = 0.10 (EtOAc:MeOH:TEA; 80:15:5). Upon completion of the reaction, CH_2Cl_2 was added (10 mL) to the reaction, and an equal volume of 2N sodium hydroxide was added (10 mL). The biphasic solution was stirred for 60 min to quench the remaining sodium triacetoxyborohydride. After stirring, the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL), and the resulting organic layers were combined. The combined organic layers were washed once with an equal volume of brine (50 mL) and dried over anhydrous Na_2SO_4. The organic phase was concentrated under reduced pressure to afford a crude paste (580 mg), which was purified by flash chromatography (SiO_2, gradient starting 3:1:96 MeOH:triethylamine:EtOAc, moving to 3:1:50:44 MeOH:triethylamine:EtOAc:$CHCl_3$) to afford the pure product as a yellow paste (200mg, 51%), the structure of which was verified by ^{1}H NMR. TLC (R_f = 0.25, 5:1:50:44, MeOH:triethylamine:EtOAc:$CHCl_3$,UV 254 nm) ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.27 – 1.50 (m, 12H), 1.72 (p, *J* = 7.2 Hz, 4H), 2.44 (t, *J* = 7.4 Hz, 4H), 2.56 (t, *J* = 2.3 Hz, 1H), 2.65 (t, *J* = 6.4 Hz, 2H), 3.28 (td, *J* = 7.1, 5.3 Hz, 4H), 3.49 – 3.73 (m, 10H), 3.83 – 3.89 (m, 2H), 4.17 (t, *J* = 4.8 Hz, 2H), 4.76 (d, *J* = 2.3 Hz, 2H), 5.15 (s, 2H), 6.39 (d, *J* = 5.4 Hz, 2H), 6.91 – 6.98 (m, 2H), 7.03 (d, *J* = 9.0 Hz, 2H), 7.34 (dd, *J* = 8.9, 2.2 Hz, 2H), 7.69 – 7.80 (m, 7H), 7.95 (d, *J* = 2.1 Hz, 2H), 8.52 (d, *J* = 5.4 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 194.50, 162.27, 160.82, 151.90, 150.10, 149.08, 135.00, 132.34, 132.22, 131.59, 130.83, 128.62, 125.31, 121.46, 117.31, 114.51, 114.21, 99.08, 78.01, 76.29, 71.02, 70.82, 70.75, 70.54, 69.68, 67.76, 56.02, 54.70, 53.43, 43.29, 28.81, 27.21, 27.10, 26.98. FTIR (thin film) λ (cm^{-1}): 2921.63, 2848.35, 1756.83, 1719.23, 1627.63, 1594.84, 1558.2. HRMS (ESI) $C_{54}H_{63}Cl_2N_5O_6$: Calculated for [M+H] $C_{54}H_{64}Cl_2N_5O_6$, 948.4234; found: 948.4229.



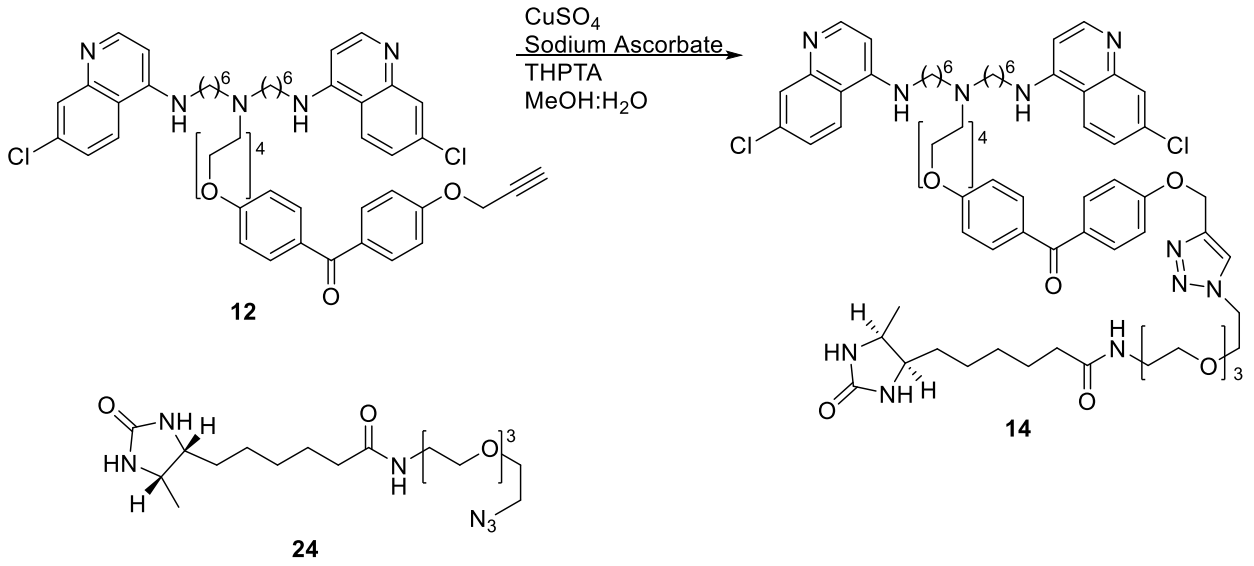
DC221-Alkyne (13)

To a round bottom flask, solid DC220 was added (95 mg, 0.22 mmol, 1.0 eq.). The solid was then placed under an atmosphere of argon. The aldehyde (189mg, 0.44 mmol, 2.0 eq.) was then dissolved in CH_2Cl_2 (2.2 mL). The aldehyde solution was then added to the reaction vessel via a syringe, to give a total reaction concentration of 0.1 M. The reaction was stirred at 23 °C for 10 minutes. Next, sodium triacetoxyborohydride was added to the reaction as a solid (190mg, 0.89 mmol, 4.0 eq.). The reaction was stirred for 22 hours at 23 °C, when the starting DC inhibitor was consumed as observed via TLC (R_f = 0.2, 10:89:1, MeOH:CH_2Cl_2;NH_4OH, UV 254 nm). Upon completion of the reaction, CH_2Cl_2 was added (10 mL) to the solution, and an equal volume of 2N sodium hydroxide was added (10 mL). The biphasic solution was stirred for 60 min to quench the remaining sodium triacetoxyborohydride. After stirring, the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 x 15 mL), and the resulting organic layers were combined. The combined organic layers were washed once with an equal volume of brine (40 mL) and dried over anhydrous Na_2SO_4. The organic phase was concentrated under reduced pressure to afford a crude paste (185 mg), which was purified by flash chromatography (SiO_2, 25 mm x 150 mm, 7.5:1:92, MeOH:NH_4OH:CH_2Cl_2) to yield the product as a yellow paste (55 mg, 38%), the structure of which was verified by ^{1}H NMR. TLC (R_f = 0.5, 7.5:1:92, MeOH:NH_4OH:CH_2Cl_2, UV 254 nm). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.57 (d, *J* = 2.4 Hz, 1H), 2.84 (t, 2H), 2.97 (dd, *J* = 6.6, 4.4 Hz, 4H), 3.23 – 3.28 (m, 4H), 3.44 – 3.53 (m, 4H), 3.55 – 3.59 (m, 2H), 3.64 – 3.73 (m, 6H), 4.04 (t, 2H), 4.77 (d, *J* = 2.4 Hz, 2H), 6.19 (s, 2H), 6.24 (d, *J* = 5.4 Hz, 2H), 6.75 (dd, *J* = 8.9, 2.2 Hz, 2H), 6.87 (d, *J* = 8.8 Hz, 2H), 7.03 (d, *J* = 8.9 Hz, 2H), 7.55 (d, *J* = 8.9 Hz, 2H), 7.74 (dd, *J* = 17.5, 8.8 Hz, 3H), 7.87 (d, *J* = 2.2 Hz, 2H), 8.41 (d, *J* = 5.3 Hz, 2H).^{13}C NMR ($CDCl_3$): δ 41.0, 53.0, 53.3, 56.0, 67.6, 69.6, 70.1, 70.2, 70.6 (C2), 70.8, 76.3, 78.0, 99.1, 114.1, 114.5, 117.3, 121.7, 125.3, 128.3, 130.9, 131.6, 132.3 (2C), 135.2, 148.7, 150.0, 151.6, 160.8, 162.2, 194.5. FTIR (thin film) λ (cm^{-1}): 3435.56, 2871.49, 1644.98, 1600.63, 1581.34. HRMS (ESI) $C_{46}H_{47}Cl_2N_5O_6$: Calculated for [M+H] $C_{46}H_{48}Cl_2N_5O_6$, 836.2982; found: 836.2982.



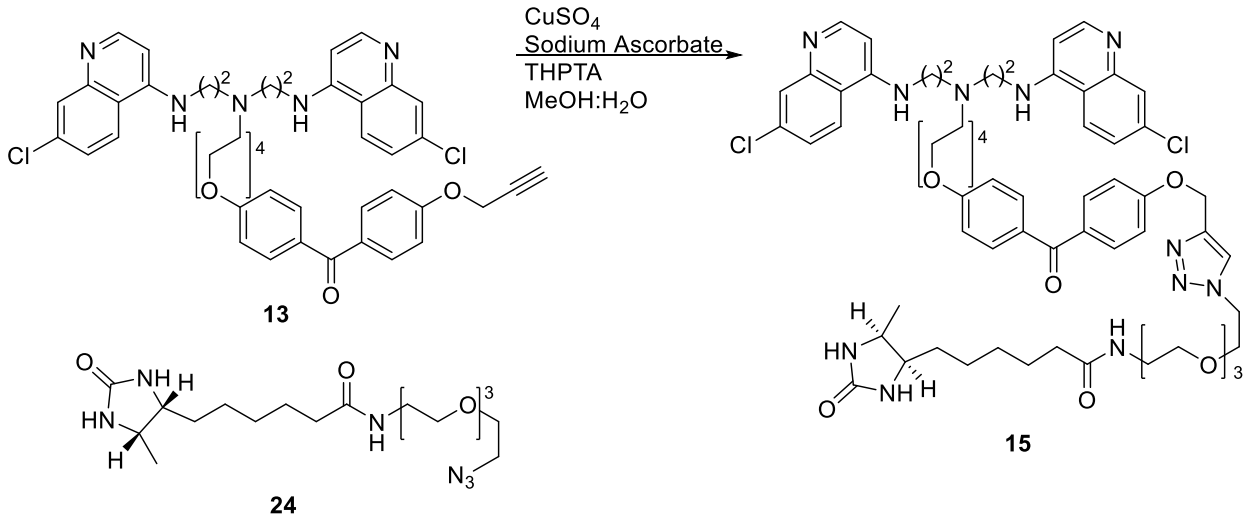
Desthiobiotin Azide (compound **24**)

To a flame-dried round bottom flask, d-desthiobiotin (Sigma Aldrich) (200 mg, 0.94 mmol, 1.0 eq.) was added, followed by TBTU (449mg, 1.4 mmol, 1.5 eq.). The reactants were placed under an atmosphere of argon and dissolved in dimethylformamide (DMF) (2 mL). The solution was stirred and (2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-amine)[4] (305mg, 1.4 mmol,1.5 eq.) was added as a solution in DMF (1mL). Then after stirring for 5 min, DIPEA (490 uL, 3.7 mmol, 4.0 eq.) was added to the reaction via micro-syringe. The reaction was stirred for 24 hours until the starting desthiobiotin was consumed, as observed by TLC (R_f = 0.1, 10:1 $CHCl_3$:MeOH, $KMnO_4$ stain). The reaction was quenched by pouring onto 30 mL of brine. The opaque solution was extracted with EtOAc (3 x 30mL). The combined organic extracts were then washed with water (5 x 10mL), resulting in a second aqueous fraction. TLC analysis (R_f = 0.35, 1:10, MeOH:$CHCl_3$, $KMnO_4$ stain) revealed the product was found exclusively in the second aqueous fraction. The second aqueous fraction was then extracted with EtOAc (5 x 50mL). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated under reduced pressure to yield a crude brown oil. The crude product was then purified by column chromatography [SiO_2, 20 mm x 180 mm, gradient elution of increasing MeOH in $CHCl_3$ (MeOH: 2% for 100 mL, 4% for 100 mL, and 6% for 200 mL)]. The product was isolated as a purple-translucent oil (163mg, 42%). TLC (R_f = 0.35, 1:10, MeOH:$CHCl_3$, $KMnO_4$ stain). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.14 (d, *J* = 6.5 Hz, 3H), 1.56 – 1.23 (m, 7H), 1.66 (t, *J* = 7.3 Hz, 2H), 2.19 (t, *J* = 7.6 Hz, 2H), 3.50 – 3.36 (m, 4H), 3.74 – 3.54 (m, 13H), 3.91 – 3.82 (m, 1H), 6.16 (s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 15.7, 25.2, 25.8, 28.6, 29.5, 35.84, 39.1, 50.7, 51.4, 56.1, 70.0(2C), 70.1, 70.5, 70.7, 77.4, 164.2, 173.2. FTIR λ (neat/cm^{-1}): 3287.07, 2 2933.2, 2106.85, 1699.94, 1545.67. HRMS (ESI) $C_{18}H_{35}N_6O_5$: Calculated for [M+H], $C_{18}H_{36}N_6O_5$, 415.2669; found: 415.2666.



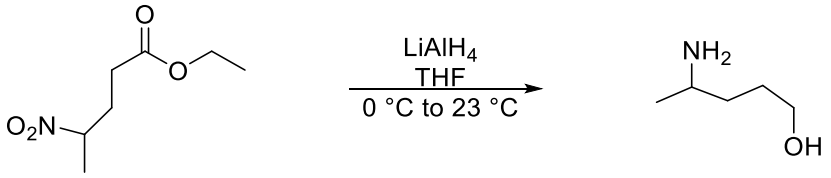
DC661 Pulldown (14)

Alkyne **12** (50mg, 0.053 mmol, 1.0 eq.) and desthiobiotin azide **24** (23mg, 0.080 mmol, 1.5 eq.), were added to a screwcap vial. The vial contents were dissolved in 500 uL of a 1:2:1 mixture of acetone:water:DMSO and placed under an argon atmosphere. $CuSO_4$ was added from a 1M aqueous solution (38uL, 0.05 mmol, 1.0 eq.), followed by the addition of solid sodium ascorbate (37mg, 0.2 mmol, 5.0 eq.). The reaction was complete after 30 min when the starting alkyne was consumed, as observed by TLC (R_f = 0.25, 5:1:50:44, MeOH:triethylamine:EtOAc:$CHCl_3$, UV 254 nm). The reaction was concentrated to a paste and partitioned between water (1mL) and $CHCl_3$ (1.5 mL), which caused a film to deposit on the wall of the vial. The aqueous layer was removed, and methanol was added to the organic layer to solubilize the film. The aqueous layer was extracted with a 1:3 mixture of 2-propanol:$CHCl_3$ (2 x 2.5 mL). The combined organic layers were then dry loaded onto silica for flash chromatography. Crude material was chromatographed (SiO_2, 15 mm x 150 mm, 1:10 MeOH:$CHCl_3$ gradient to 10:1:100 MeOH:NH_4OH:$CHCl_3$) to afford the product as a yellow translucent paste (35mg, 51%), the structure of which was verified by ^{1}H NMR. TLC (R_f= 0.3, 10:1:100 MeOH:NH_4OH:$CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.09 (d, J = 6.5 Hz, 3H), 1.15 – 1.51 (m, 19H), 1.61 (p, J = 7.2 Hz, 2H), 1.71 (p, J = 7.3 Hz, 4H), 2.14 (t, J = 7.5 Hz, 2H), 2.42 (t, J = 7.4 Hz, 4H), 2.63 (t, J = 6.3 Hz, 2H), 3.27 (td, J = 7.2, 5.3 Hz, 4H), 3.37 – 3.74 (m, 28H), 3.75 – 3.82 (m, 1H), 3.87 (dt, J = 15.5, 4.9 Hz, 4H), 4.17 (dd, J = 5.6, 4.0 Hz, 2H), 4.51 (s, 1H), 4.55 (t, J = 5.0 Hz, 2H), 5.04 (s, 1H), 5.27 (s, 4H), 6.38 (d, J = 5.4 Hz, 2H), 6.90 – 6.97 (m, 2H), 7.04 (d, J = 8.8 Hz, 1H), 7.32 (dd, J = 8.9, 2.2 Hz, 2H), 7.69 – 7.78 (m, 6H), 7.85 (s, 1H), 7.93 (d, J = 2.2 Hz, 2H), 8.50 (d, J = 5.4 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 194.60, 173.37, 163.53, 162.28, 161.62, 151.94, 150.18, 149.08, 143.41, 135.02, 132.37, 131.29, 130.80, 128.58, 125.32, 124.46, 121.57, 117.35, 114.48, 114.26, 99.07, 70.97, 70.82, 70.72, 70.66, 70.59, 70.49, 70.47, 70.23, 70.15, 69.67, 69.55, 69.52, 67.74, 62.19, 56.13, 54.66, 53.57, 51.53, 50.53, 43.27, 39.29, 36.21, 29.59, 28.96, 28.76, 27.22, 27.07, 26.77, 26.10, 25.44, 15.91. FTIR (thin film) λ (cm^{-1}): 3352.64, 2929.34, 2854.13, 1696.09, 1645.95, 1596.77, 1578.45, 1539.88. HRMS (ESI) $C_{67}H_{87}Cl_2N_{11}O_{11}$: Calculated for [M+2H]/2 $C_{67}H_{89}Cl_2N_{11}O_{11}$, 646.8060; found: 646.8046.



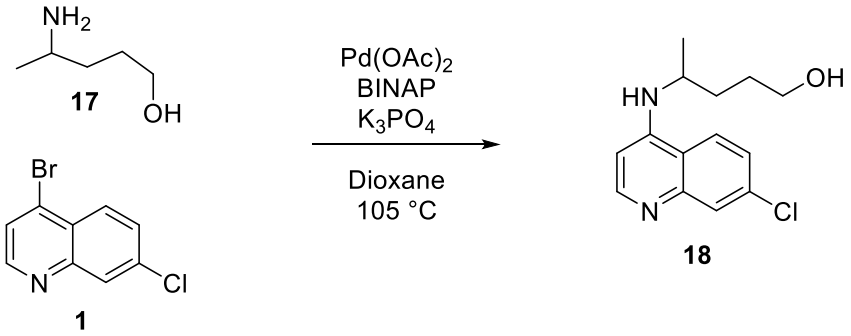
DC221 Pulldown (15)

Alkyne **13** (16mg, 0.019 mmol, 1.0 eq.) was added to a screwcap vial, followed by desthiobiotin azide **24** (12mg, 0.029 mmol, 1.5 eq.). The contents of the vial were dissolved in MeOH (200 uL) to give a 0.1 M reaction concentration. In a separate vial, THPTA and $CuSO_4$ dissolved in water to form a dark blue solution (100 mM $CuSO_4$ and 200 mM THPTA). This aqueous solution was used to deliver THPTA and $CuSO_4$ (0.0036 mmol and 0.0018 mmol respectively) to the screwcap vial. While stirring, sodium ascorbate was added to the vial (20 mg, 0.10 mmol, 5.3 eq.). The reaction was monitored by RPHPLC (C_{18}, 4.6 mm x 250 mm, gradient elution: 10% acetonitrile in water (0.1% TFA) to 60% acetonitrile in water (0.1% TFA) over 40 min). The reaction was complete when no starting alkyne remains. The reaction was concentrated to remove the methanol and dissolved in $CHCl_3$ (5 mL). The $CHCl_3$ washed with saturated sodium bicarbonate (3 x 5 mL). The organic layer was separated and dried over anhydrous Na_2SO_4. The organic layer was then concentrated under reduced pressure to afford a film. The film was then solubilized in MeOH and DMSO (300 uL and 60 uL respectively) and purified by RPHPLC (C_{18}, 19 mm x 250 mm, gradient elution: 10% acetonitrile in water (0.1% TFA) to 60% acetonitrile in water (0.1% TFA) over 40 min). The pH of the fractions was brought to 12 by addition of NH_4OH (1 mL), and then saturated with NaCl. The aqueous layer was then extracted with $CHCl_3$ (3 x 5 mL). The $CHCl_3$ was dried over sodium sulfate and concentrated under reduced pressure to afford a film on a vial (8mg, 34%), the structure of which was verified by ^{1}H NMR. TLC (R_f= 0.2, 10:1:100 MeOH:NH_4OH:$CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.44 (d, *J* = 5.3 Hz, 2H), 7.89 – 7.85 (m, 3H), 7.73 (dd, *J* = 14.1, 8.9 Hz, 4H), 7.53 (d, *J* = 8.8 Hz, 2H), 7.05 (d, *J* = 8.9 Hz, 2H), 6.88 (d, *J* = 8.8 Hz, 2H), 6.76 (dd, *J* = 8.9, 2.2 Hz, 2H), 6.31 (s, 1H), 6.26 (d, *J* = 5.6 Hz, 2H), 6.06 (s, 2H), 4.95 (s, 1H), 4.57 (t, *J* = 5.1 Hz, 2H), 4.45 (s, 1H), 4.05 (t, *J* = 4.6 Hz, 2H), 3.90 (t, *J* = 5.1 Hz, 2H), 3.81 (p, *J* = 6.4 Hz, 1H), 3.73 – 3.39 (m, 27H), 3.27 (q, *J* = 5.5 Hz, 4H), 2.98 (t, *J* = 5.3 Hz, 4H), 2.84 (t, *J* = 4.7 Hz, 2H), 2.16 – 2.13 (m, 2H), 1.61 (q, *J* = 7.1 Hz, 2H), 1.50 – 1.28 (m, 8H), 1.10 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 16.0, 25.5, 26.3, 29.1, 29.7, 36.4, 39.3, 41.1, 50.6, 51.5, 53.2, 53.6, 56.2, 62.3, 67.7, 69.6 (2C), 70.1, 70.2, 70.3, 70.6 70.7 (3C), 70.9, 99.2, 114.2, 114.5, 117.4, 121.5, 124.4, 125.3, 128.7, 130.9, 131.4, 132.4, 135.1, 143.5, 149.0, 149.9, 152.0, 161.6, 162.2, 163.3, 173.1, 194.5. FTIR (thin film) λ (cm^{-1}): 3350.71, 2933.2, 2865.7, 1697.05, 1684.52, 1650.77, 1599.66, 1578.45. HRMS (ESI) $C_{64}H_{81}Cl_2N_{11}O_{11}$: Calculated for [M+2H]/2 $C_{64}H_{83}Cl_2N_{11}O_{11}$, 625.7825; found: 625.7823.

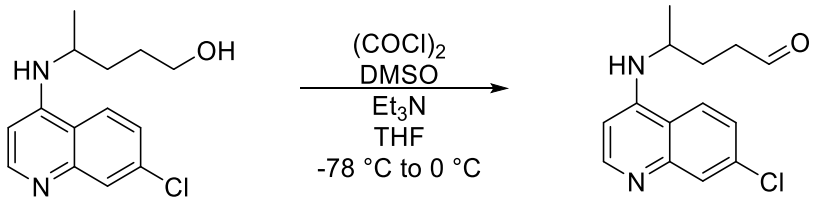


16 **17**

Tetrahydrofuran (THF) (70 mL) was added to a flame-dried round bottom flask, placed under an atmosphere of argon, and cooled to 0°C. Lithium aluminum hydride was added (7.09g, 186 mmol, 3.5 eq.) to the reaction vessel, causing the evolution of gas. Ethyl 4-nitropentanoate[6] **16** (9.334g, 53.3 mmol, 1.0 eq.) was dissolved in THF (70 mL) and added to the reaction mixture using a syringe pump at a rate of 1mL/min. The reaction was stirred for 30 min at 0 °C then allowed to warm to 23 °C. The reaction was then stirred at 23°C for 14 hours. The reaction was cooled to 0 °C and quenched by the addition of three solutions: First, 8 mL of water were added via a syringe pump at a rate of 0.5 mL/min. Second, 8 mL of 15% (w/v) NaOH (aq) were added at rate of 0.5 mL/min. Third, 24 mL of water were added to the reaction at a rate of 1 mL/min. The reaction was then warmed to room temperature and filtered through a pad of Celite™ using 100 mL of EtOAc. The combined organic layer was then dried over anhydrous sodium sulfate and concentrated to a translucent oil (4.77 g, 87%), the structure of which was verified by ^{1}H NMR. TLC (R_f = 0.0, regardless of solvent choice, $KMnO_4$ stain). ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.66 – 3.56 (m, 2H), 2.98 – 2.90 (m, 1H), 1.77 – 1.68 (m, 1H), 1.66 – 1.56 (m, 2H), 1.41 – 1.32 (m, 1H), 1.13 (d, *J* = 6.5 Hz, 3H). ^{NO} N-H showed up. ^{13}C NMR (126 MHz, $CDCl_3$): δ 25.32, 30.45, 37.77, 47.17, 63.04. FTIR (thin film) λ (cm^{-1}): 3345.89, 2938.98, 2360.44, 1567.84. HRMS (ESI) $C_5H_{13}NO$: Calculated for [M+H], $C_5H_{14}NO$, 104.1075; found: 104.1050

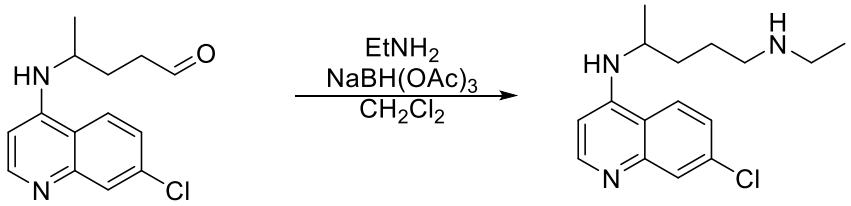


4-aminopentan-1-ol, **17**, (1.00 g, 9.71 mmol, 1.0 eq.), 4-bromo-7-chloroquinoline[1] (1.80 g, 7.28 mmol, 0.75 eq.), $Pd(OAc)_2$ (109 mg, 0.485 mmol, 0.05 eq.), BINAP (605 mg, 0.971 mmol, 0.1 eq.), and K_3PO_4 (3.10 g, 14.6 mmol, 1.5 eq.) were added to a flame-dried round bottom flask. The flask contents under a reflux condenser were then placed under an argon atmosphere. 1,4-dioxane, which was degassed, was then added to the round bottom flask. The reaction was heated to reflux in a bath preset to 105 °C for 2 hours. After the first 20 minutes, the reaction takes on a deep red color. The reaction was complete when the 4-bromo-7-chloroquinoline was consumed as observed by TLC (R_f = 0.65, 4:3:1 Hex:CH_2Cl_2:EtOAc, UV 254 nm). The reaction was then filtered through a pad of Celite™ using a 100 mL of a 1:10 mixture, MeOH:$CHCl_3$. Removal of solvent under reduced pressure affords a red paste which was then dry loaded on silica gel and purified by flash chromatography (SiO_2, 5:94:1 MeOH:CH_2Cl_2:NH_4OH (aq)) to afford a brown white solid. This solid required triturated with refluxing CH_2Cl_2 to yield a white solid which still contains an inseparable impurity (~10% by ^{1}H NMR) (695 mg, 36%). TLC (R_f = 0.25, 5:94:1 MeOH:CH_2Cl_2:NH_4OH). ^{1}H NMR (500 MHz, CD_3OD): δ 8.33 (d, *J* = 5.6 Hz, 1H), 8.17 (dd, *J* = 9.0, 0.5 Hz, 1H), 7.76 (dd, *J* = 2.2, 0.5 Hz, 1H), 7.38 (dd, *J* = 9.0, 2.2 Hz, 1H), 6.54 (dd, *J* = 5.7, 0.7 Hz, 1H), 3.81 (h, *J* = 6.4 Hz, 1H), 3.59 (t, *J* = 6.4 Hz, 2H), 1.86 – 1.60 (m, 3H), 1.32 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD): δ 150.81, 150.66, 148.26, 134.80, 125.97, 124.31, 122.93, 117.29, 98.34, 61.23, 47.98, 32.12, 28.76, 18.78. FTIR (thin film) λ (cm^{-1}): 3319, 2937, 1578. HRMS (ESI) $C_{14}H_{17}Cl_1N_2O$: Calculated for [M+H] $C_{14}H_{18}Cl_1N_2O$, 265.1108; found: 265.1101.



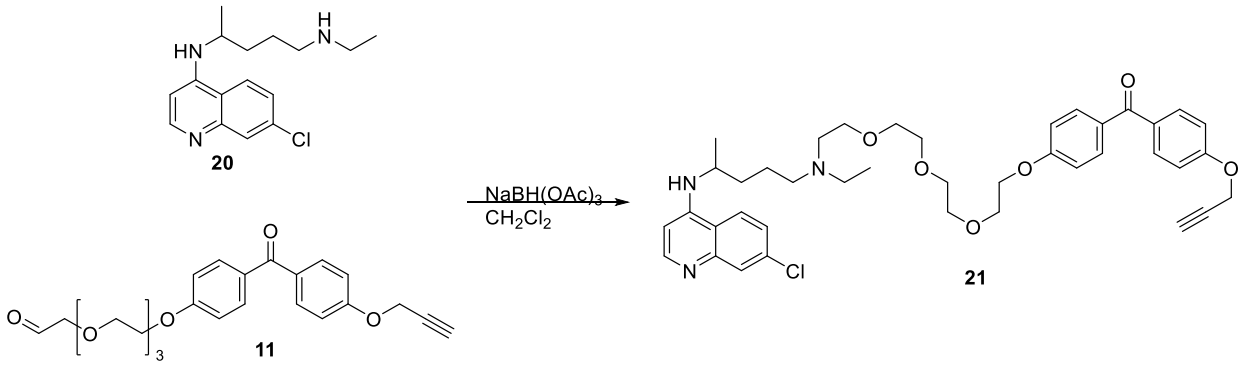
18 **19**

THF (4.7 mL) was added to a flame-dried round bottom flask under an atmosphere of argon. Freshly distilled oxalyl chloride (190 uL, 2.3 mmol 1.2 eq.) was added to the round bottom flask, and the solution was cooled to -78°C. While stirring at -78°C, DMSO (320 uL, 4.5 mmol, 2.4 eq.) was added via a syringe, causing the evolution of gas. In a separate flame-dried round bottom flask, 4-((7-chloroquinolin-4-yl)amino)pentan-1-ol (compound **18**) under an argon atmosphere, was dissolved in tetrahydrofuran(20 mL) by heating to reflux and cooling to 25° C. After 30 minutes, the 4-((7-chloroquinolin-4-yl)amino)pentan-1-ol (compound **18**) solution was added dropwise via a syringe to the oxalyl chloride/DMSO solution. The combined reaction was then stirred for 30 minutes at -78 °C. At this time, triethylamine (1.3 mL, 9.45 mmol, 5.0 eq.) was added to the reaction via a syringe. The reaction was stirred at -78°C for 60 min before being moved to an ice bath at 0°C. The reaction was stirred at 0°C for 4 hours, until the consumption of the starting alcohol was observed by TLC (R_f= 0.10, 2:1:1 Hex:THF:CH_2Cl_2 with 3 drops of triethylamine). The reaction was then concentrated under reduced pressure to afford a crude paste. The structure of the aldehyde was confirmed by ^{1}H NMR of the crude reaction mixture, with ~10% of starting alcohol remaining. Previous experiments demonstrated purification the aldehyde caused decomposition. The crude mixture was carried on to the next step without further purification. TLC (R_f= 0.15. 2:1:1 Hex:THF:CH_2Cl_2 with 1% v/v triethylamine). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.74 (s, 1H), 8.37 (d, *J* = 5.3 Hz, 1H), 7.82 (d, *J* = 2.3 Hz, 1H), 7.73 (d, *J* = 9.0 Hz, 1H), 7.28 – 7.21 (m, 1H), 6.32 (d, *J* = 5.5 Hz, 1H), 5.48 (d, *J* = 7.2 Hz, 1H), 3.70 – 3.64 (m, 3H), 1.78 (t, *J* = 3.6 Hz, 2H), 1.26 (d, *J* = 6.4 Hz, 3H).



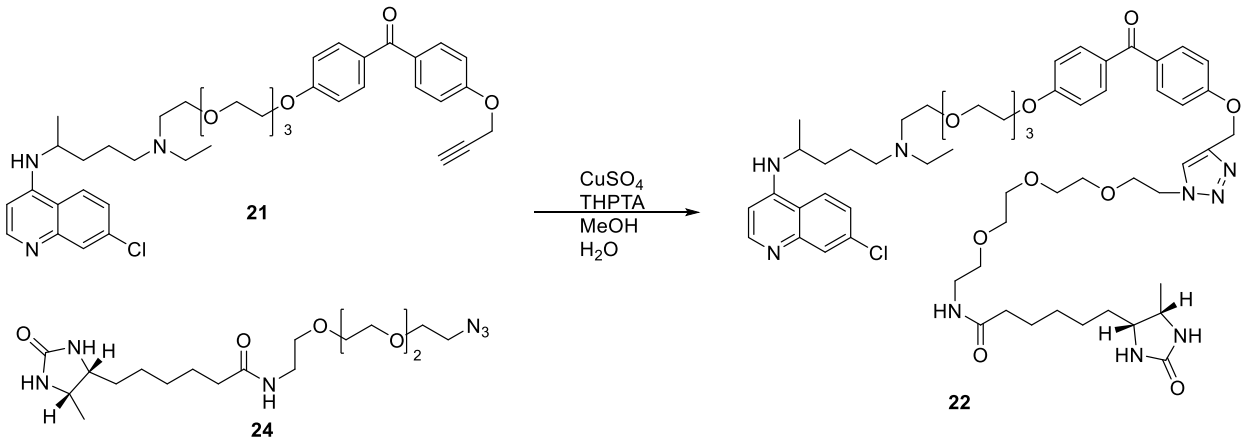
19 **20**

Crude 4-((7-chloroquinolin-4-yl)amino)pentanal, compound **19**, (450mg, 1.88mmol, 1.0 eq.) was added to a round bottom flask with a stir bar and placed under an atmosphere of argon. The flask was then charged with CH_2Cl_2 (7mL) and allowed to stir at 23°C until the solution was homogenous. A solution of ethylamine (70% w/v) (230 uL, 2.8 mmol, 1.5 eq.) was prepared in CH_2Cl_2 (2 mL) and added via a syringe to the reaction vessel. The reaction was stirred at 23°C for 5 minutes, and sodium triacetoxyborohydride (1.20 g, 5.63 mmol, 3.0 eq.) was added in one portion. The reaction was stirred at 23 °C for two hours until the aldehyde starting material was consumed as observed by TLC (R_f = 0.25, 2:1:1 Hex: CH_2Cl_2:THF with 2% triethylamine (v/v)). The reaction was then diluted with CH_2Cl_2 (5mL) and an equal volume of 2N NaOH (5mL). The reaction was stirred for 60 minutes at 23°C to quench any remaining sodium triacetoxyborohydride. The layers were then separated in a separatory funnel, and the aqueous layer was extracted with CH_2Cl_2 (2 x 15 mL). The combined organic layers were dried over anhydrous sodium sulfate. The organic layers were then concentrated under reduced pressure to afford a translucent green paste (400 mg). The green paste was purified by flash chromatography (SiO_2, 25mm x 100 mm, 10:90:1 MeOH:CH_2Cl_2:NH_4OH) to afford (155 mg, 0.53 mmol, 28% over 2 steps) of a green paste. TLC (R_f = 0.3, 10:90:1 MeOH:CH_2Cl_2:NH_4OH). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.48 (dd, *J* = 5.4, 0.8 Hz, 1H), 7.94 – 7.91 (m, 1H), 7.70 (d, *J* = 8.9 Hz, 1H), 7.31 (ddd, *J* = 8.9, 2.2, 0.9 Hz, 1H), 6.38 (dd, *J* = 5.5, 0.7 Hz, 1H), 5.54 (d, *J* = 7.0 Hz, 1H), 3.73 – 3.64 (m, *J* = 5.9 Hz, 1H), 2.69 – 2.60 (m, 4H), 1.83 – 1.58 (m, 3H), 1.30 (dd, *J* = 6.4, 0.9 Hz, 3H), 1.12 (td, *J* = 7.2, 0.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 152.13, 149.52, 149.29, 134.88, 128.88, 125.07, 121.46, 117.54, 99.30, 49.45, 48.50, 44.36, 34.27, 26.64, 20.29, 15.37. FTIR (thin film) λ (cm^{-1}): 2966, 2927, 1577. HRMS (ESI) $C_{16}H_{22}ClN_3$: Calculated for [M+H] $C_{16}H_{22}ClN_3$, 292.1581; found: 292.1575.



CQ-Alkyne (21)

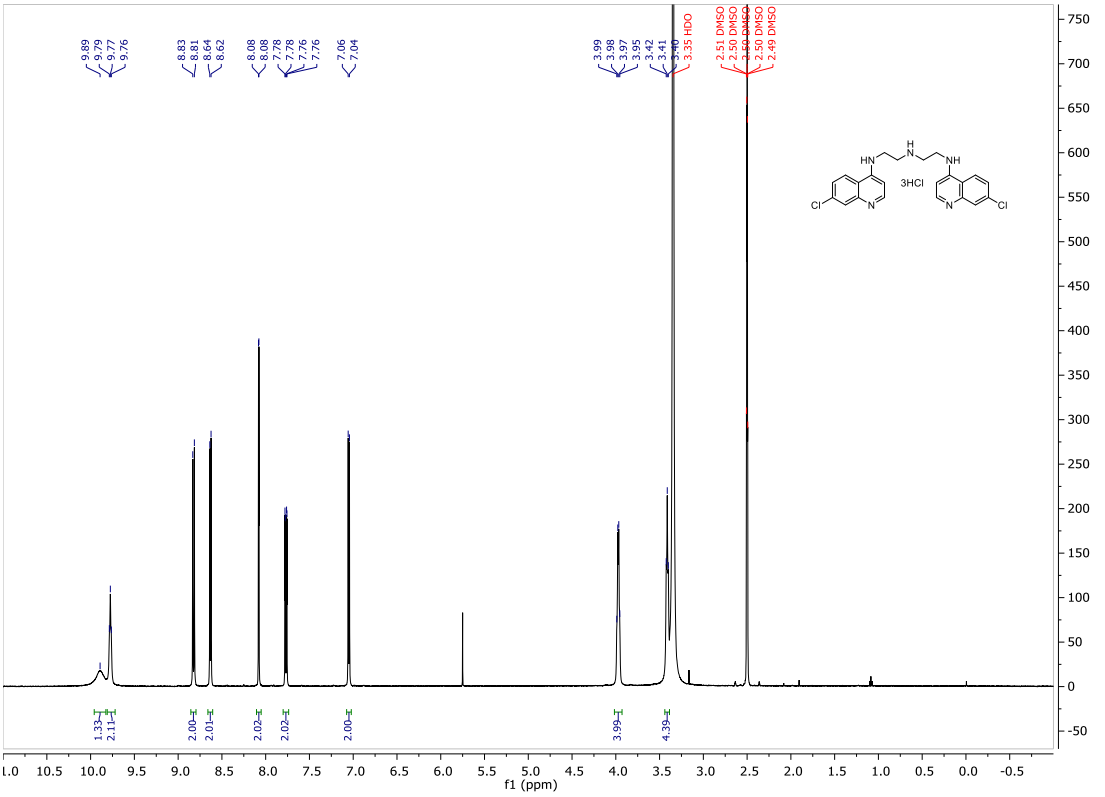
Compound **11** (57 mg, 0.13 mmol, 1.0 eq.) was added to a flame-dried screwcap vial, followed by compound **20** (33mg, 0.1 mmol, 0.8 eq.) in a CH_2Cl_2 solution (500 uL). The reaction was stirred at 23°C for 5 minutes before sodium triacetoxyborohydride was added in one portion (85 mg, 0.40 mmol, 3.0 eq.). The reaction was complete in 30 minutes when the aldehyde was consumed as observed by TLC (R_f = 0.5, EtOAc, UV 254 nm). The reaction was diluted with 1 mL of CH_2Cl_2, and 1 mL 2N NaOH. The reaction was vortexed for 1 minute, and the resulting emulsion was placed into a freezer set to -20 °C, where after two hours the emulsion had separated. The layers were then separated, and the aqueous layer was extracted with CH_2Cl_2 (2 x 1mL). The combined CH_2Cl_2 layers were washed with 3 mL of brine. The resulting organic layer was then dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford a white paste (89 mg). The white paste was dissolved in a solution of 10:20:70 DMSO:CH_2Cl_2:MeOH and purified by preparative HPLC (C_{18}, 19 mm x 250 mm, gradient elution: 10% acetonitrile in water (0.1% TFA) to 60% acetonitrile in water (0.1% TFA) over 40 min). Lyophilization of the collected fractions yields a white film on a vial (21mg). The vials contents were then dissolved in a immiscible mixture of $CHCl_3$:NH_4OH (aq), vortexed for 1 min, the layers were separated, and the resulting organic layer was dried over anhydrous sodium sulfate. The dried organic solution was concentrated under reduced pressure to yield a film on a vial (19 mg, 24%), the structure of which was verified by ^{1}H NMR. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.50 (d, *J* = 5.4 Hz, 1H), 7.93 (d, *J* = 2.1 Hz, 1H), 7.81 – 7.71 (m, 5H), 7.33 (dd, *J* = 8.9, 2.2 Hz, 1H), 7.04 (d, *J* = 8.9 Hz, 1H), 6.94 (d, *J* = 8.9 Hz, 1H), 6.40 (d, *J* = 5.4 Hz, 1H), 5.29 (d, *J* = 7.3 Hz, 1H), 4.77 (d, *J* = 2.4 Hz, 2H), 4.16 (t, *J* = 4.8 Hz, 2H), 3.83 (t, 2H), 3.72 – 3.51 (m, 11H), 2.64 (hept, *J* = 6.6, 6.1 Hz, 2H), 2.58 – 2.51 (m, 3H), 2.48 (t, *J* = 6.8 Hz, 2H), 1.78 – 1.53 (m, 4H), 1.29 (d, *J* = 6.4 Hz, 3H), 0.98 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 11.5, 20.5, 24.1, 34.5, 48.4, 48.5, 52.9, 53.7, 56.0, 67.8, 69.7, 69.9, 70.6, 70.8, 70.9, 71.0, 76.3, 78.1, 99.4, 114.3, 114.5, 117.5, 121.6, 125.1, 129.0, 130.9, 131.7, 132.3, 132.4, 134.9, 149.3, 149.6, 152.2, 160.8, 162.3, 194.5. FTIR (thin film) λ (cm^{-1}): 2922.59, 2869.56, 1646.91, 1599.66, 1576.52, 1538.92 HRMS (ESI) $C_{40}H_{48}ClN_3O_6$: Calculated for [M+H] $C_{40}H_{49}ClN_3O_6$, 702.3310; found: 702.3304.

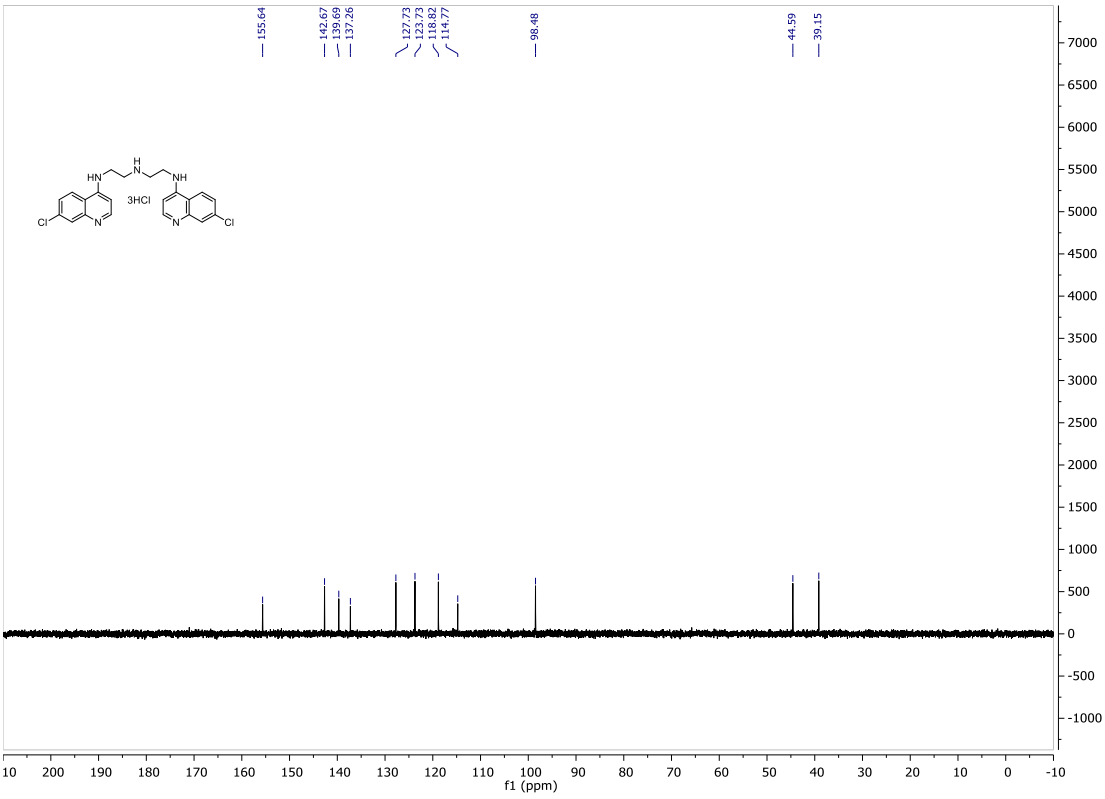


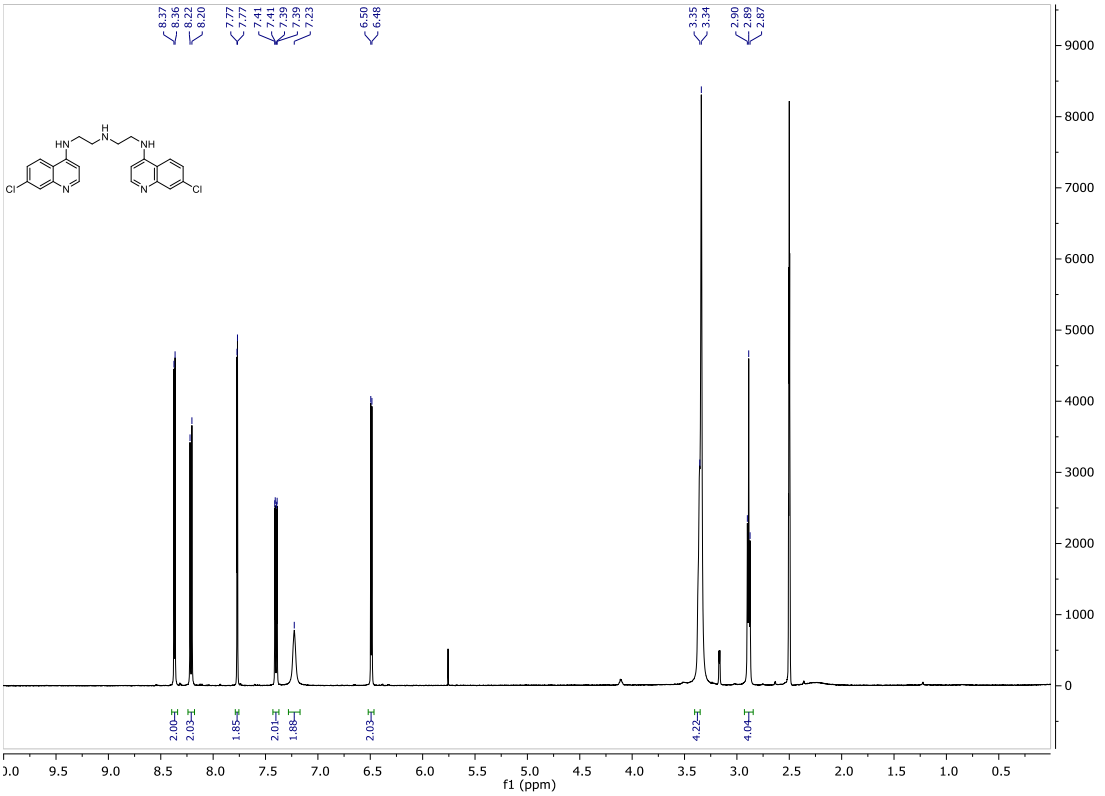
CQ Pulldown (22)

Alkyne **21** (13mg, 0.019 mmol, 1.0 eq.) was added to a screwcap vial and placed under an atmosphere of argon. Desthiobiotin azide **24** was dissolved in MeOH (12 mg/100uL). The whole solution (100 uL) was added to the reaction vessel. A separate aqueous $CuSO_4$ solution was made (100mM) and was used to dissolve the copper (I) ligand THPTA (10mg/100 uL). An aliquot of the $CuSO_4$/THPTA solution (20uL, 0.0018 mmol of Cu and 0.0036 mmol of THPTA) were added to the reaction vessel. Sodium ascorbate (10mg, 0.05 mmol ,2.7 eq.) was then added to the reaction vessel as a solid, and the reaction was stirred for 16 hours at 23 °C. The reaction was monitored by HPLC and was complete when consumption of the alkyne containing starting material was observed (C18, 19 mm x 250 mm, gradient elution: 10% acetonitrile in water (0.1% TFA) to 60% acetonitrile in water (0.1% TFA) over 40 min). The completed reaction was then diluted in 1 mL of methanol and filtered through a 0.45 uM filter. The reaction was then concentrated under reduced pressure to afford 33 mg of a brown film. The film was dissolved in 300 uL of methanol and purified by HPLC (C_{18}, 19 mm x 250 mm, gradient elution: 10% acetonitrile in water (0.1% TFA) to 60% acetonitrile in water (0.1% TFA) over 40 min, retention time = 21.7 min). The product containing fractions were combined and their pH was brought to 12 by the addition of NH_4OH (aq) (1 mL). NaCl was then added to saturate the aqueous solution. The aqueous solution was then extracted with $CHCl_3$ (3 x 5 mL). The $CHCl_3$ extracts were dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford a film on a vial (8mg, 39%), the structure of which was confirmed by ^{1}H NMR. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.98 (t, J = 7.1 Hz, 3H), 1.11 (d, J = 6.5 Hz, 3H), 1.21 – 1.52 (m, 6H), 1.60 (dt, J = 22.0, 7.1 Hz, 4H), 1.76 (d, J = 22.5 Hz, 8H), 2.15 (t, J = 7.5 Hz, 2H), 2.48 (t, J = 6.8 Hz, 2H), 2.55 (q, J = 7.1 Hz, 2H), 2.58 – 2.71 (m, 2H), 3.42 (q, J = 5.3 Hz, 2H), 3.48 – 3.74 (m, 20H), 3.78 – 3.85 (m, 3H), 3.87 – 3.92 (m, 2H), 4.13 – 4.18 (m, 2H), 4.38 (s, 1H), 4.54 – 4.60 (m, 2H), 4.86 (s, 1H), 5.29 (s, 2H), 5.38 (d, J = 7.4 Hz, 1H), 6.26 (s, 1H), 6.40 (d, J = 5.5 Hz, 1H), 6.94 (d, J = 8.8 Hz, 1H), 7.06 (d, J = 9.0 Hz, 1H), 7.33 (dd, J = 9.0, 2.2 Hz, 1H), 7.73 – 7.80 (m, 4H), 7.86 (s, 1H), 7.92 (d, J = 2.2 Hz, 1H), 8.49 (d, J = 5.4 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 194.53, 173.11, 163.24, 162.32, 161.58, 152.16, 149.56, 149.34, 143.50, 134.90, 132.39, 131.41, 130.82, 128.86, 125.10, 124.39, 121.70, 117.54, 114.47, 114.26, 99.35, 71.02, 70.85, 70.77, 70.73, 70.64, 70.61, 70.59, 70.30, 70.11, 69.78, 69.69, 69.57, 67.76, 62.26, 56.14, 53.70, 52.90, 51.54, 50.57, 48.54, 48.43, 39.30, 36.36, 34.52, 29.65, 29.11, 26.26, 25.49, 24.09, 20.46, 15.98, 11.49. FTIR (thin film) λ (cm^{-1}): 3433.64, 1644.98, 1604.48, 1578.45. HRMS (ESI) $C_{58}H_{82}ClN_9O_{11}$: Calculated for [M+2H]/2 $C_{58}H_{84}ClN_9O_{11}$, 558.7977; found: 558.7990.

NMR Spectra of compounds



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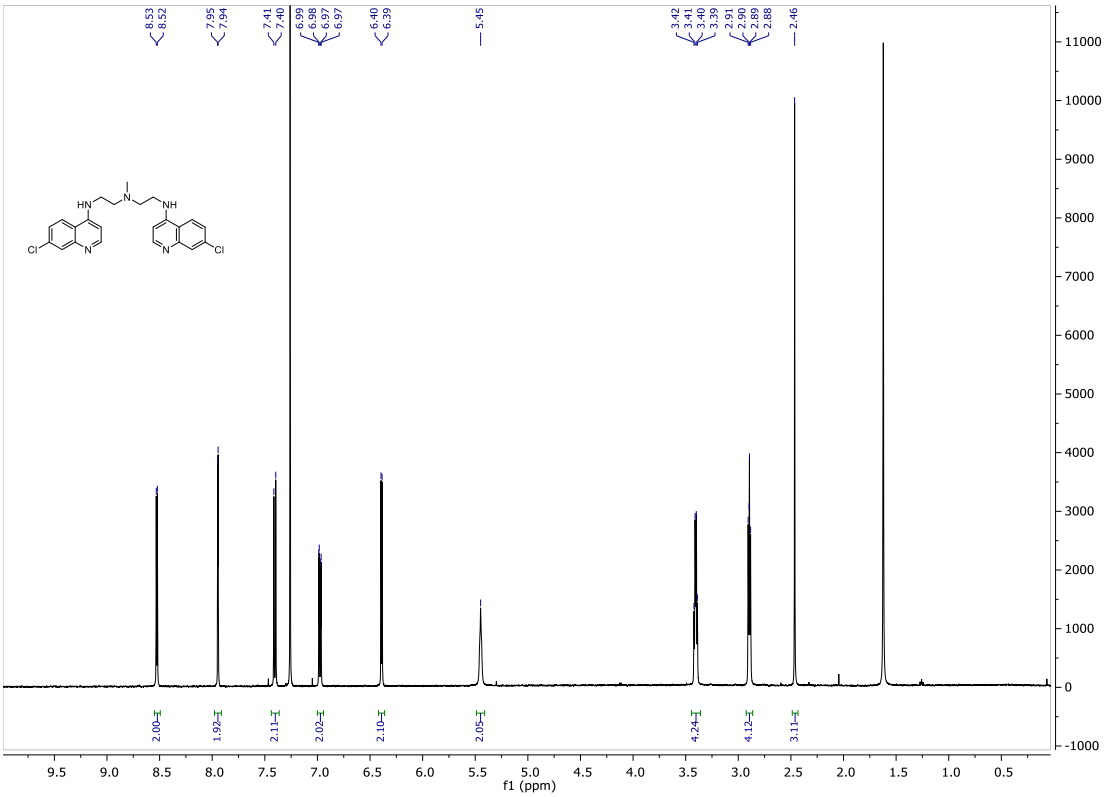
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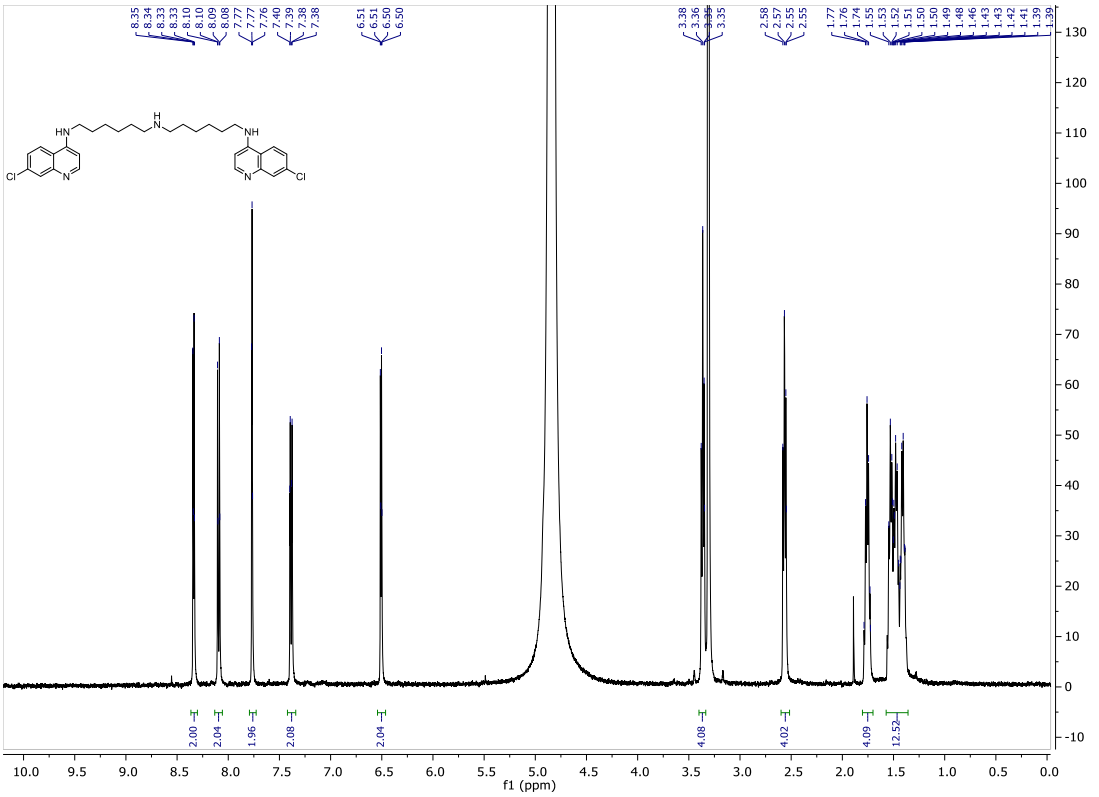
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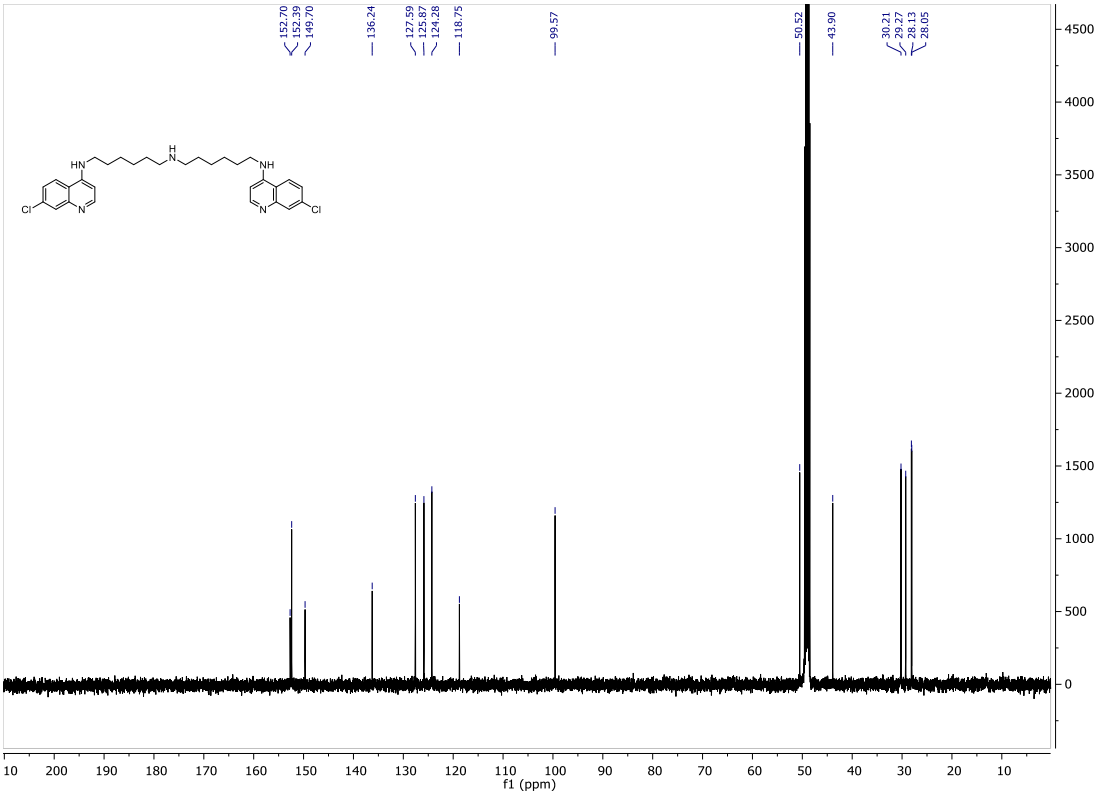
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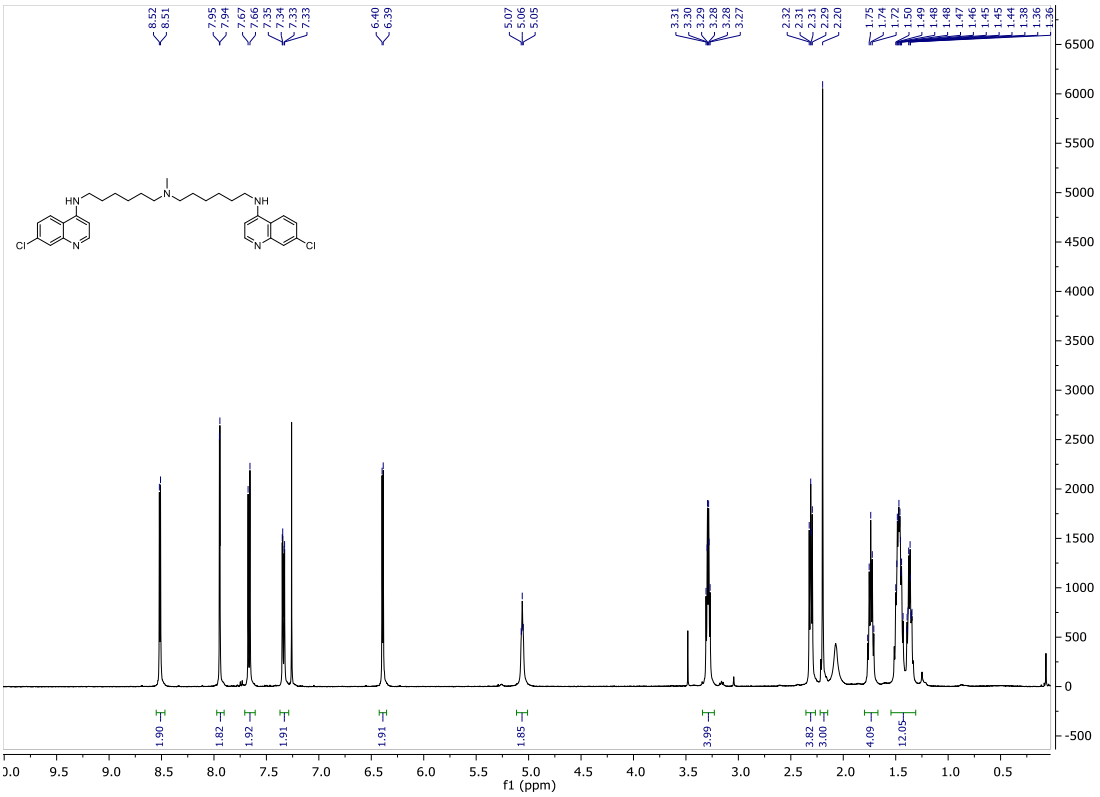
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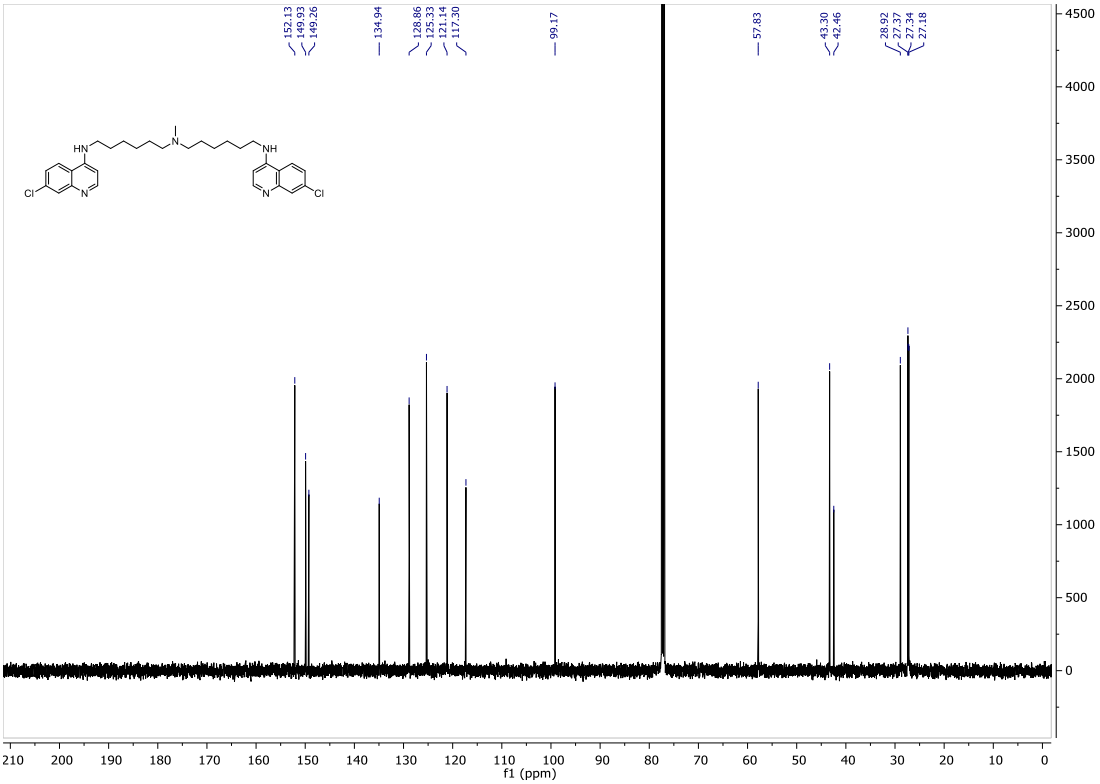
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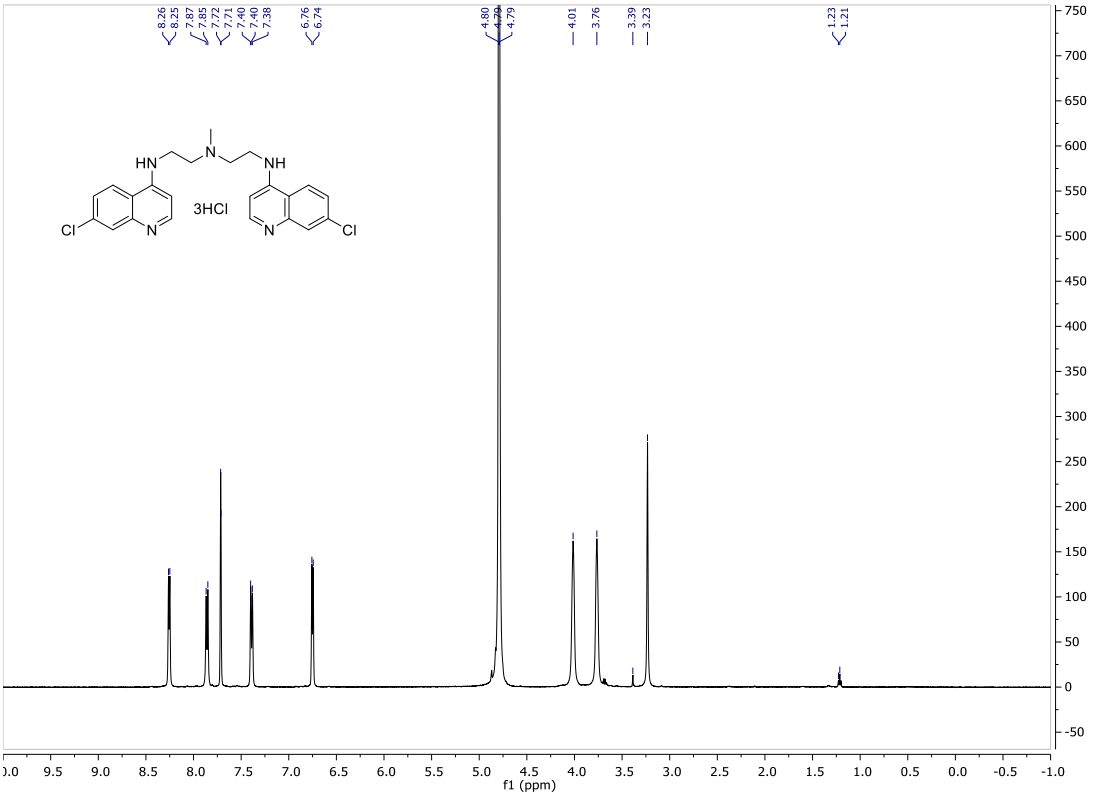
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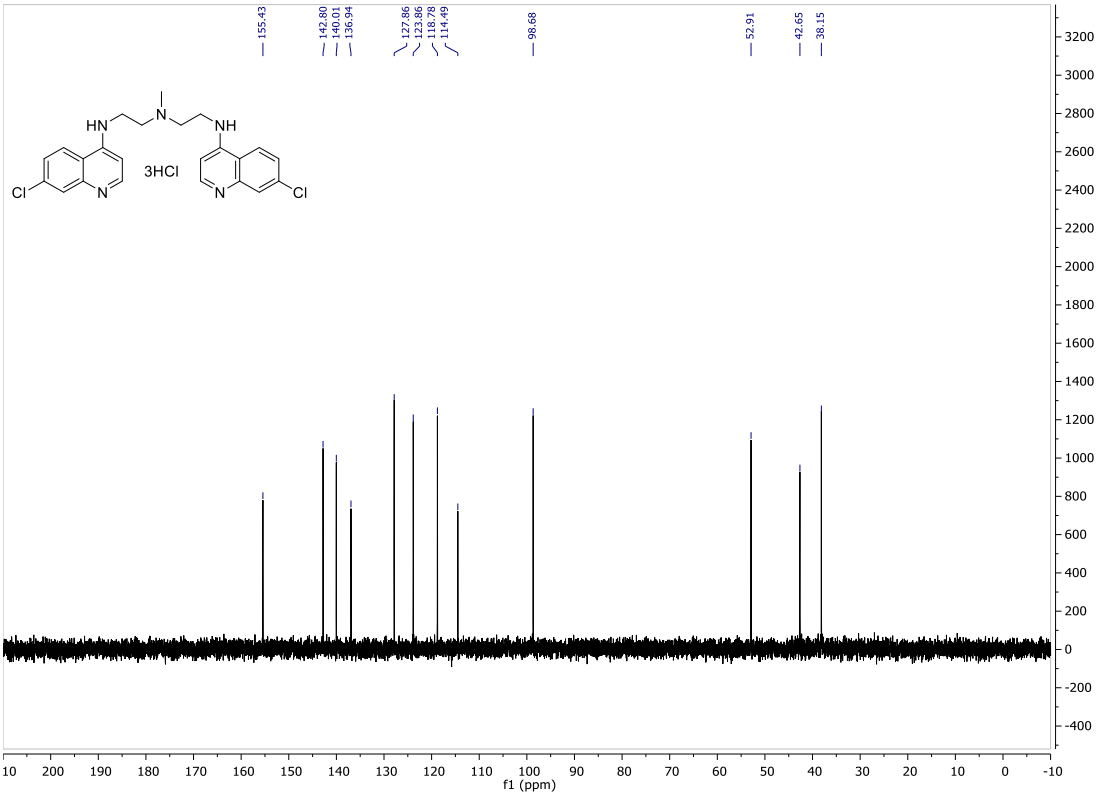
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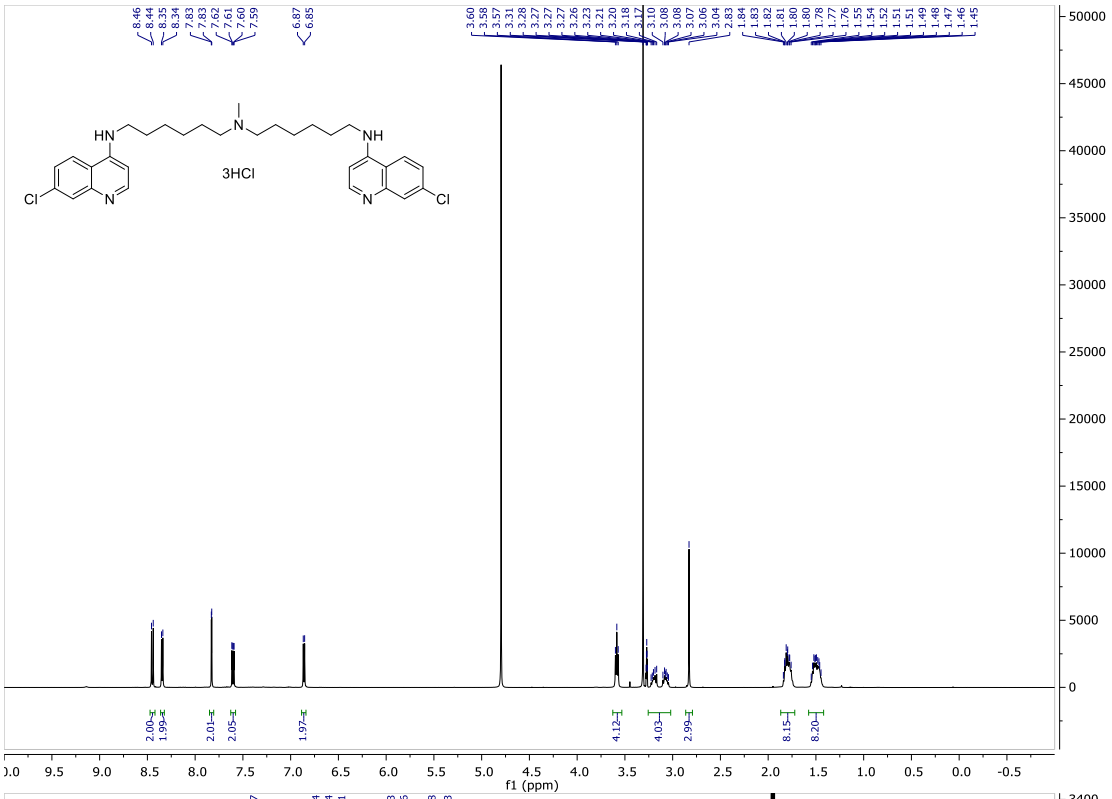
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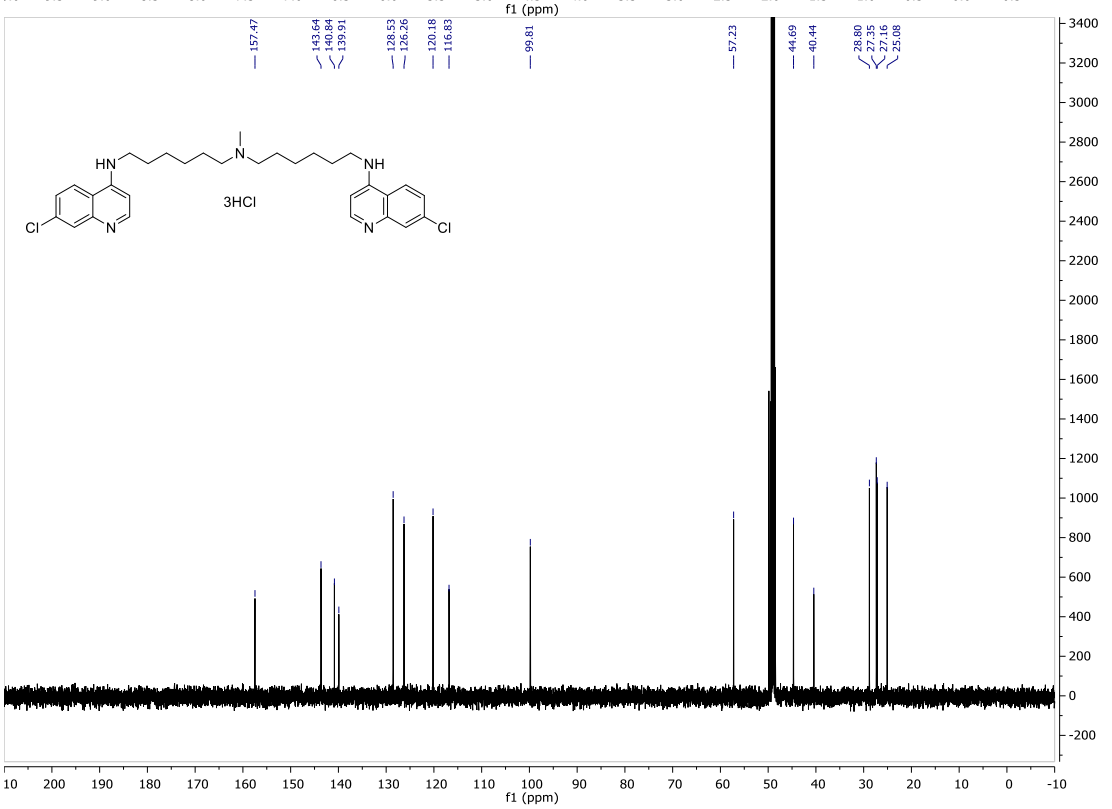
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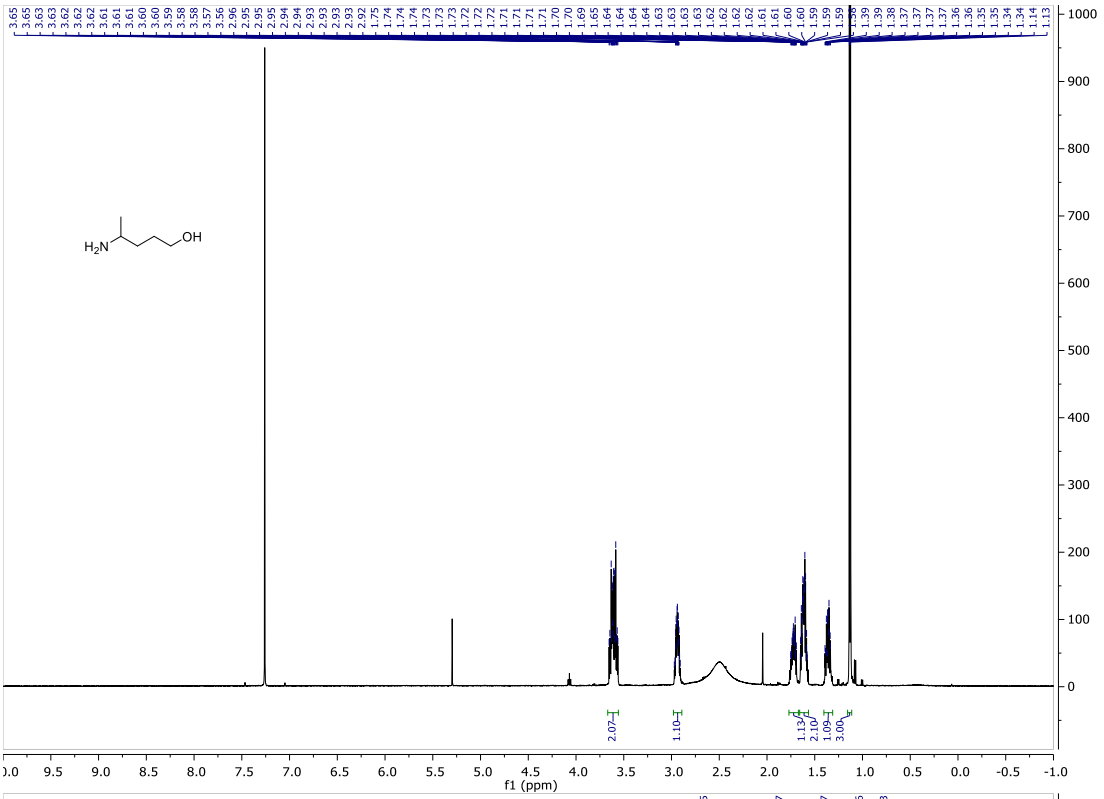
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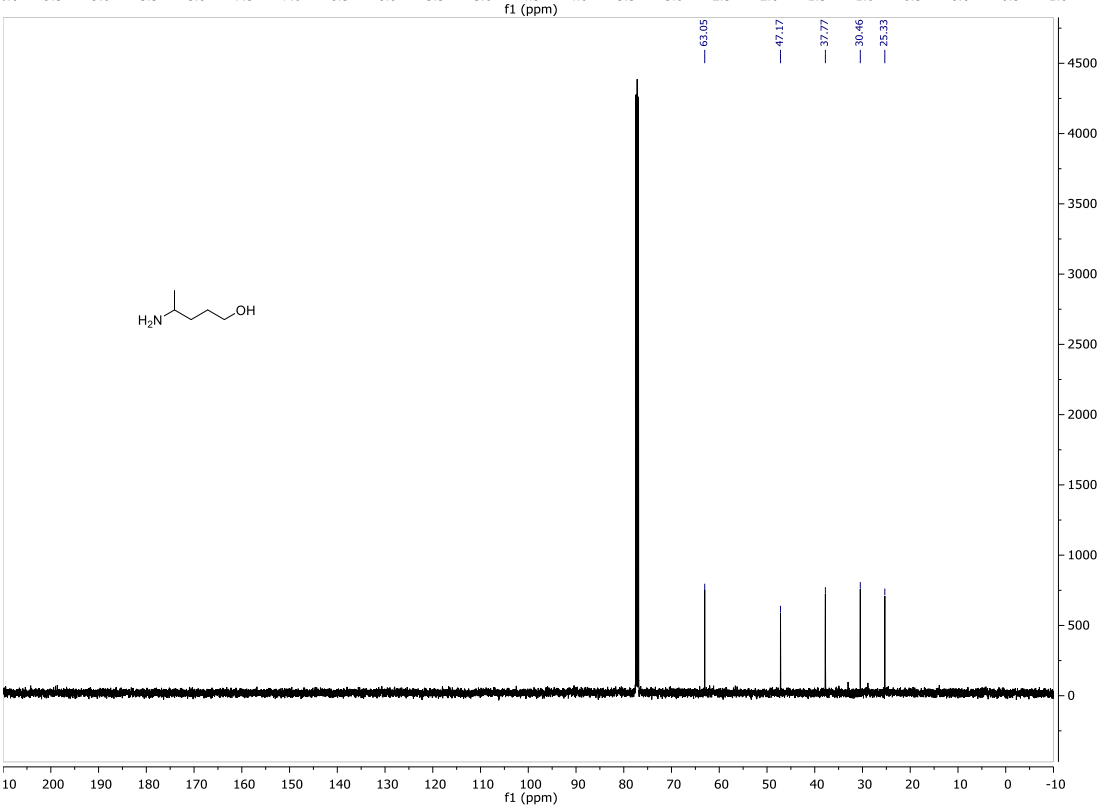
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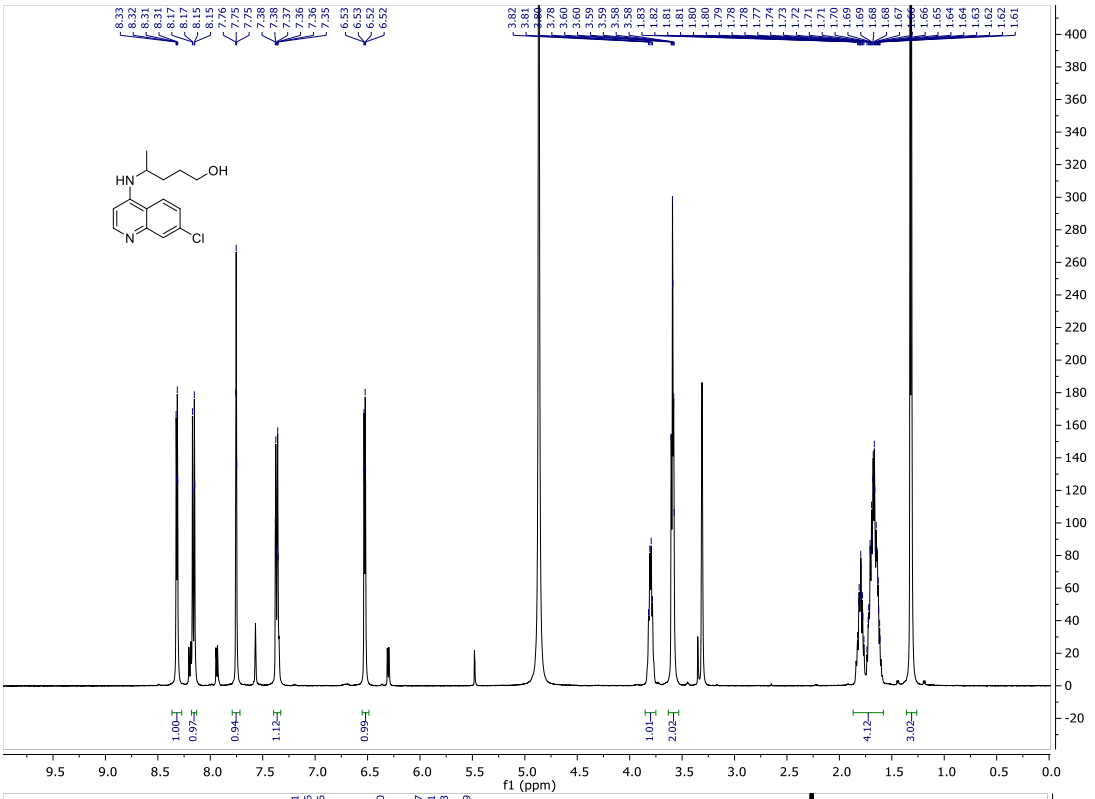
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8.15
7.76
7.75
7.38
7.37
7.36
7.35
6.53
6.52
3.82
3.81
3.78
3.60
3.59
3.58
1.83
1.82
1.81
1.80
1.79
1.78
1.77
1.74
1.73
1.72
1.71
1.70
1.69
1.68
1.67
1.66
1.65
1.64
1.63
1.62
1.61
HN
OH
N
Cl
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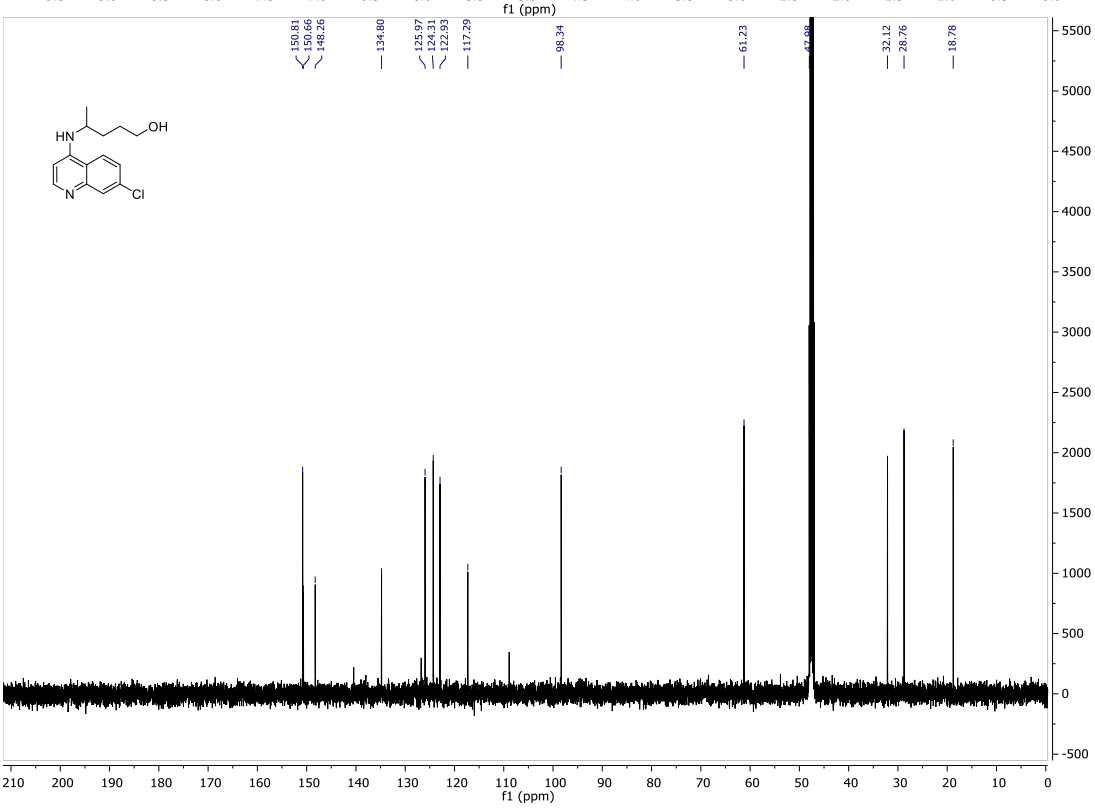
0.97
0.94
1.12
0.99
1.01
2.02
4.12
3.02
f1 (ppm)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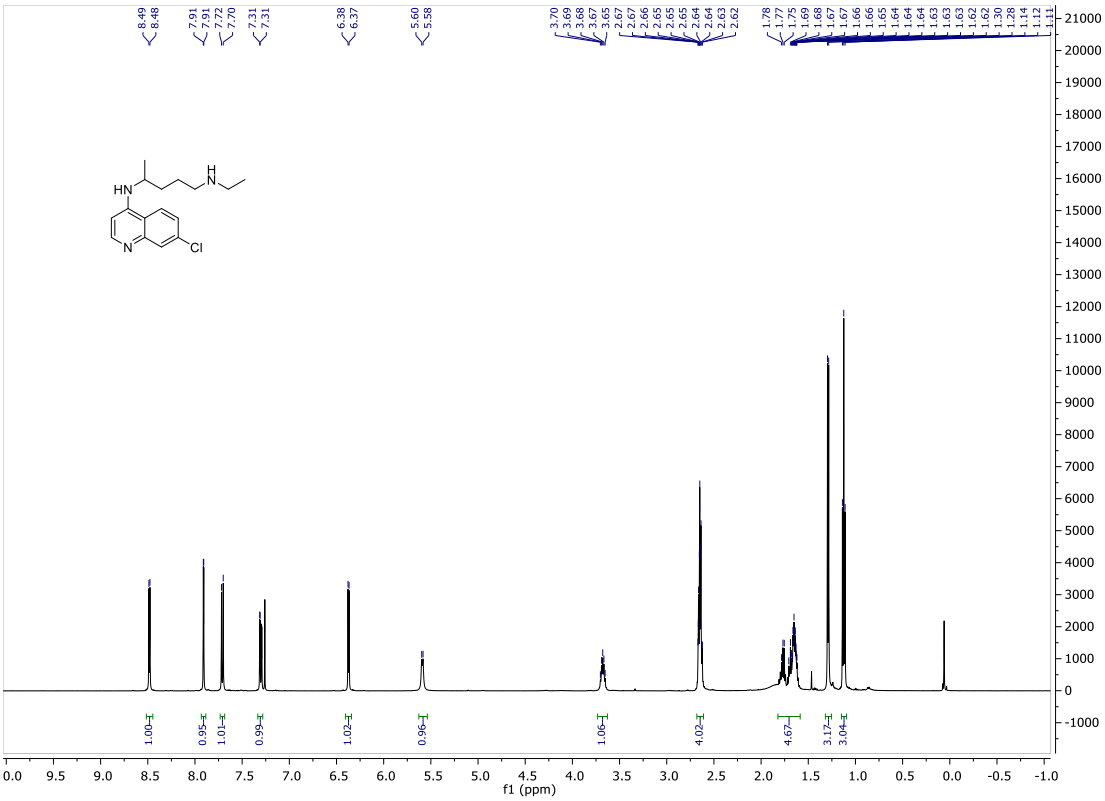
150.66
148.26
134.80
125.97
124.31
122.93
117.29
98.34
61.23
42.08
32.12
28.76
18.78
HN
OH
N
Cl
f1 (ppm)


8.49
8.48
7.91
7.91
7.72
7.70
7.31
7.31
6.38
6.37
5.60
5.38
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3.69
3.68
3.67
3.65
2.67
2.67
2.66
2.65
2.65
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1.64
1.64
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1.63
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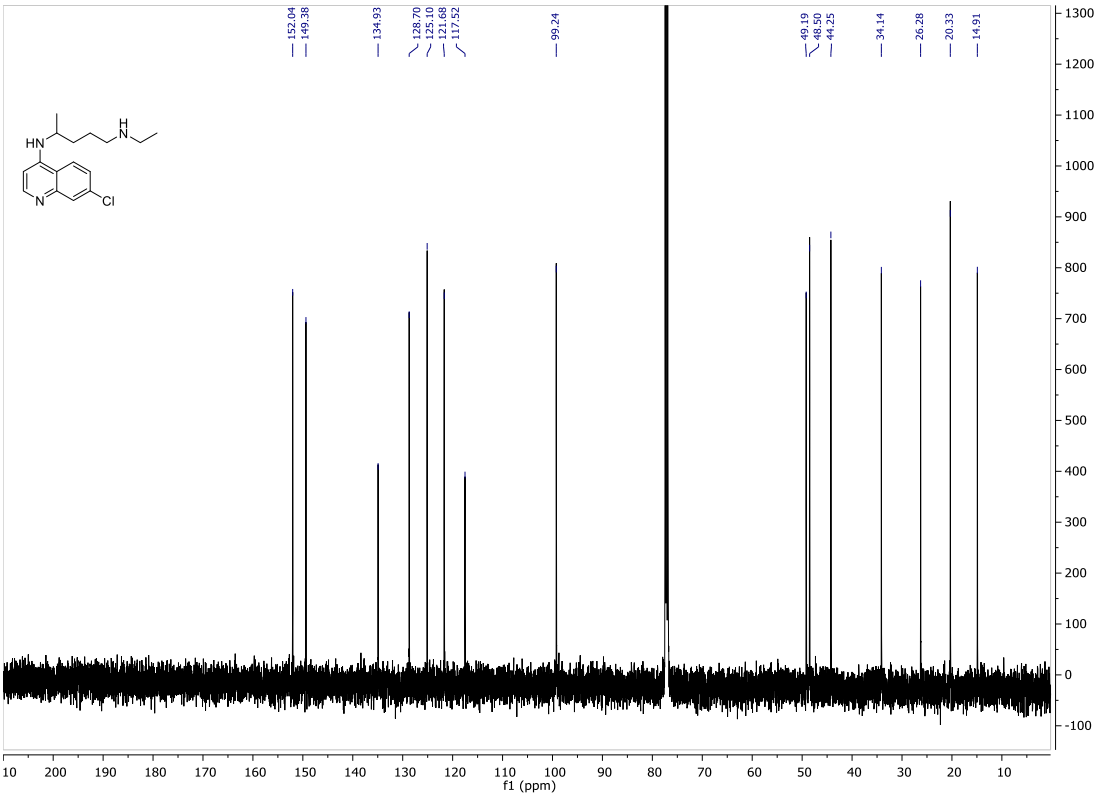
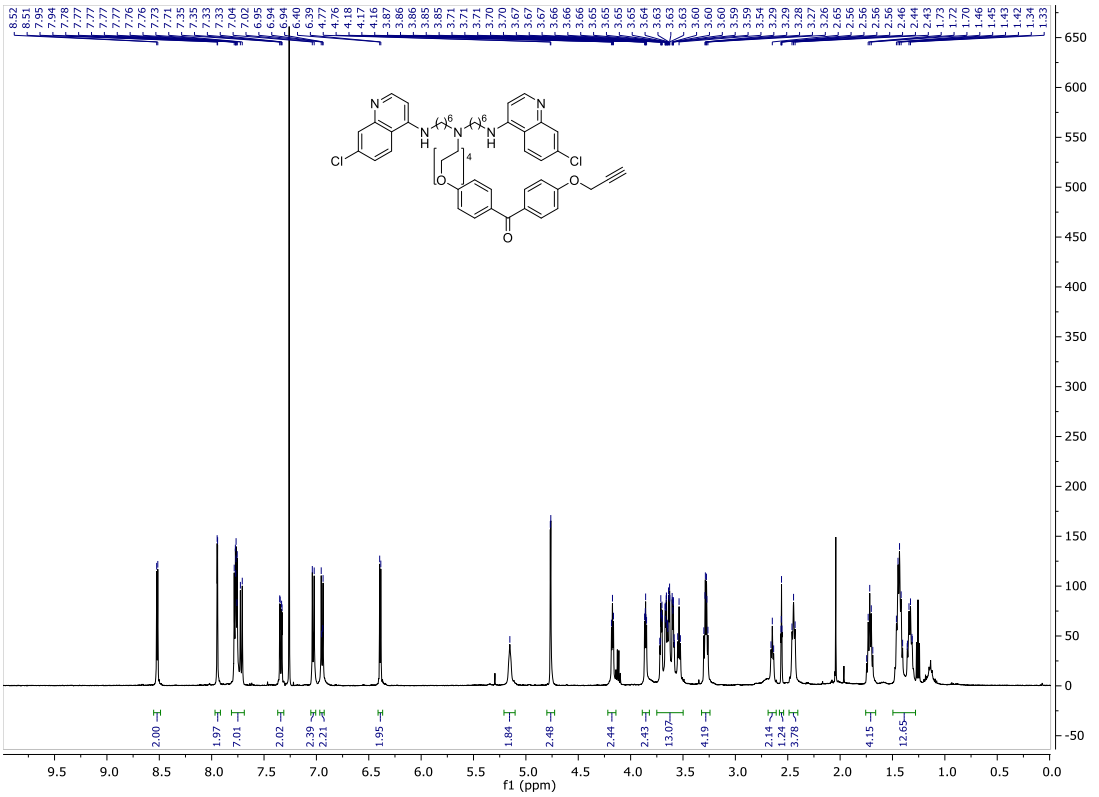
1.63
1.62
1.62
1.30
1.28
1.14
1.12
1.11
HN
H
N
N
Cl
1.00
0.95
1.01
0.99
1.02
0.96
1.06
4.02
4.67
3.17
3.04
f1 (ppm)


152.04
149.38
134.93
128.70
125.10
121.68
117.52
99.24
49.19
48.50
44.25
34.14
26.28
20.33
14.91
HN
H
N
N
Cl
f1 (ppm)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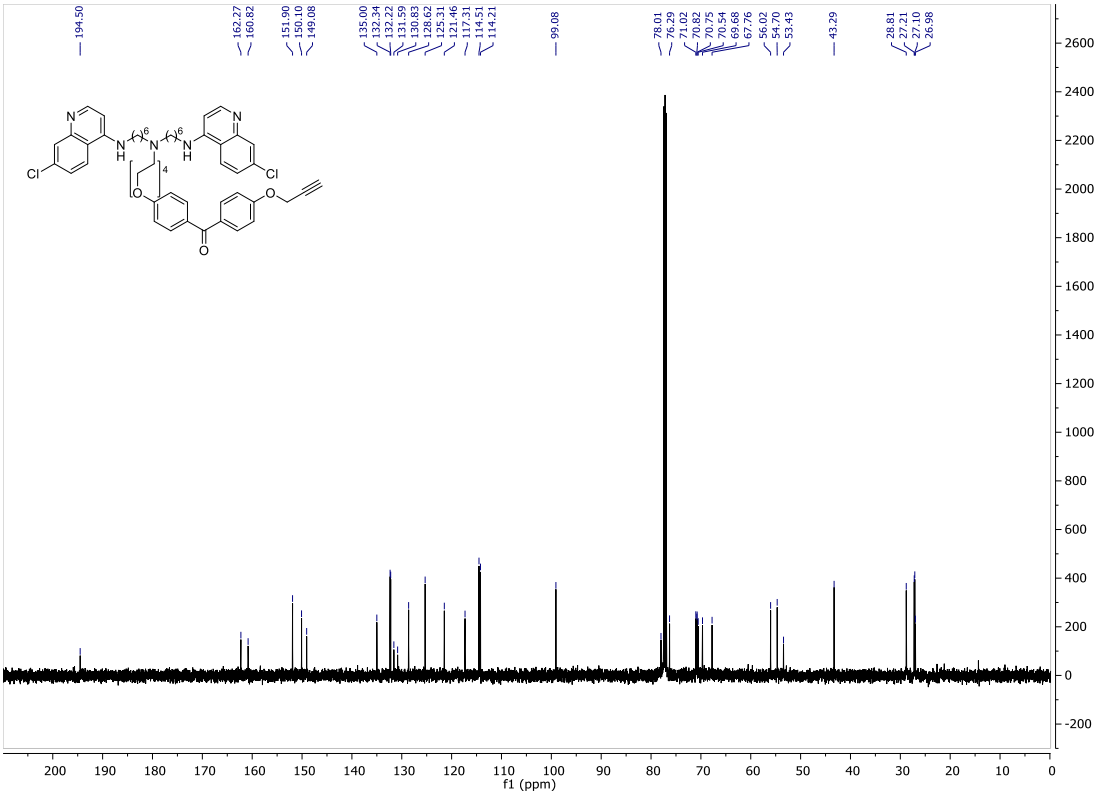
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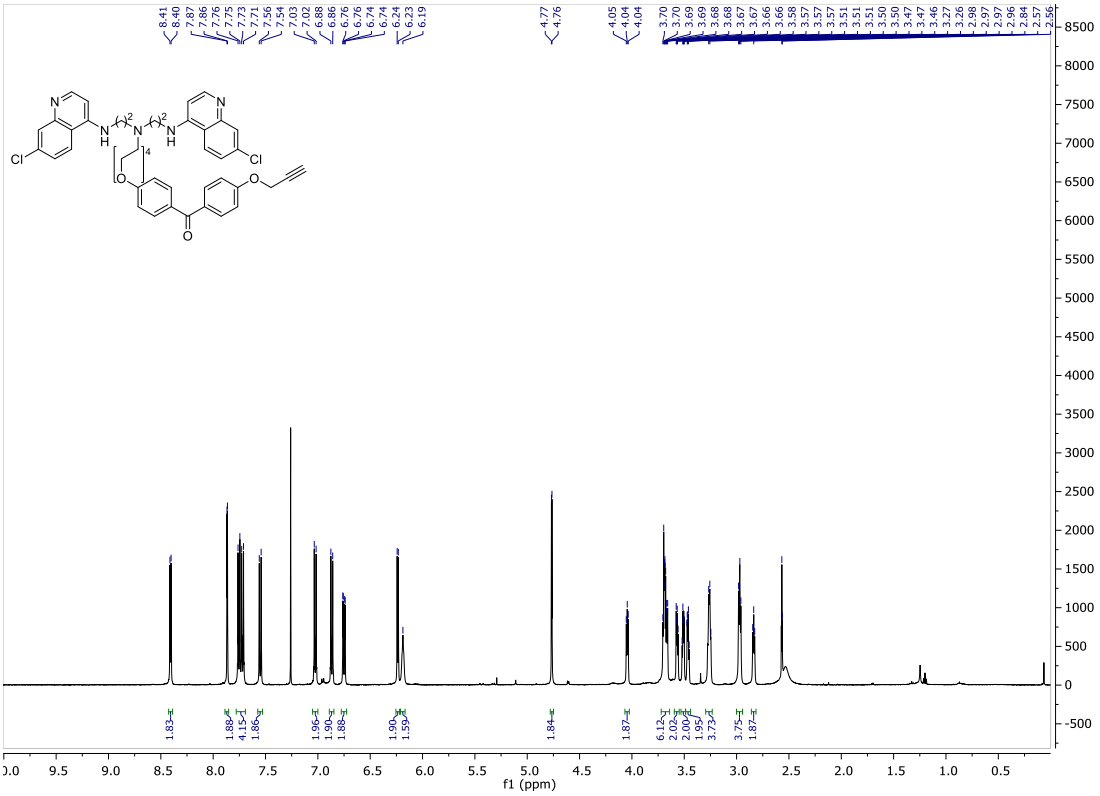
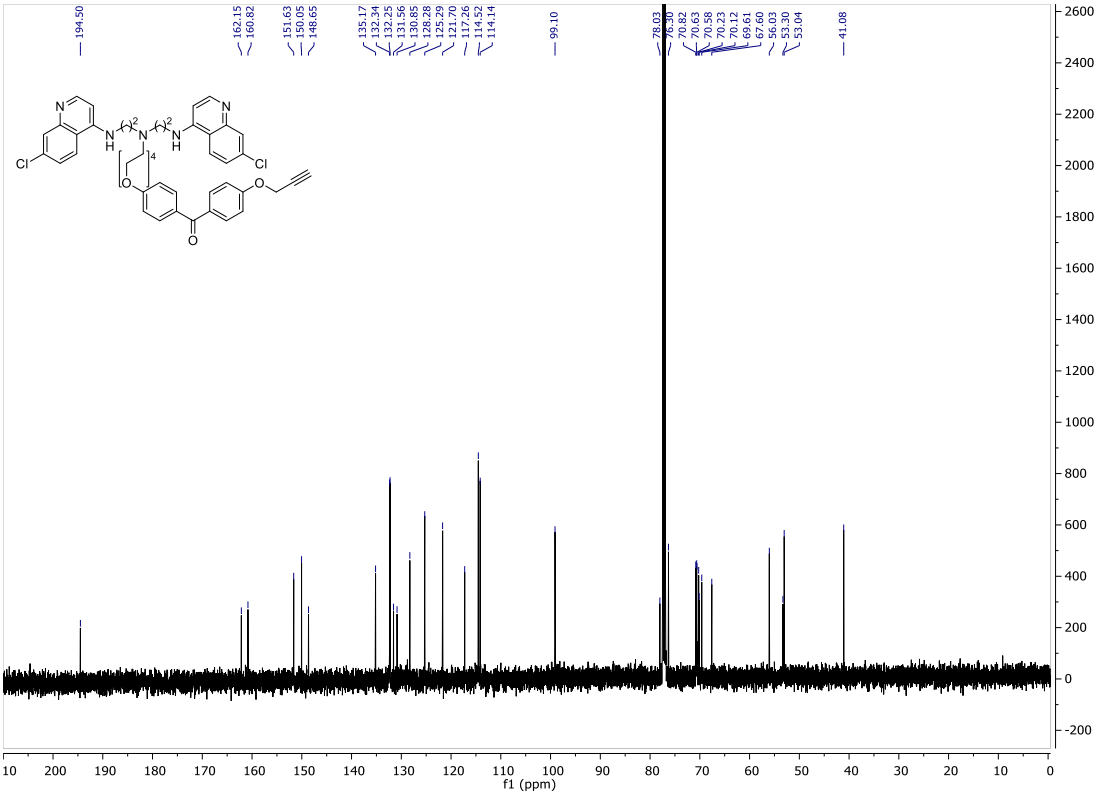

f1 (ppm)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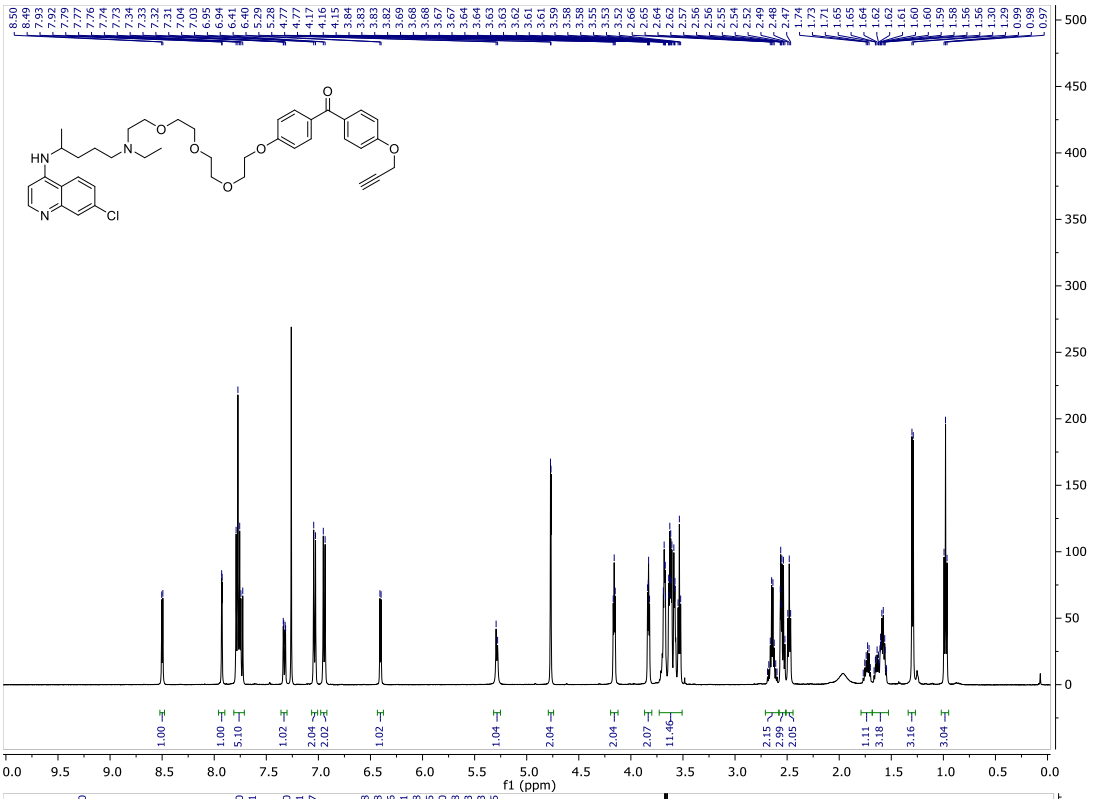
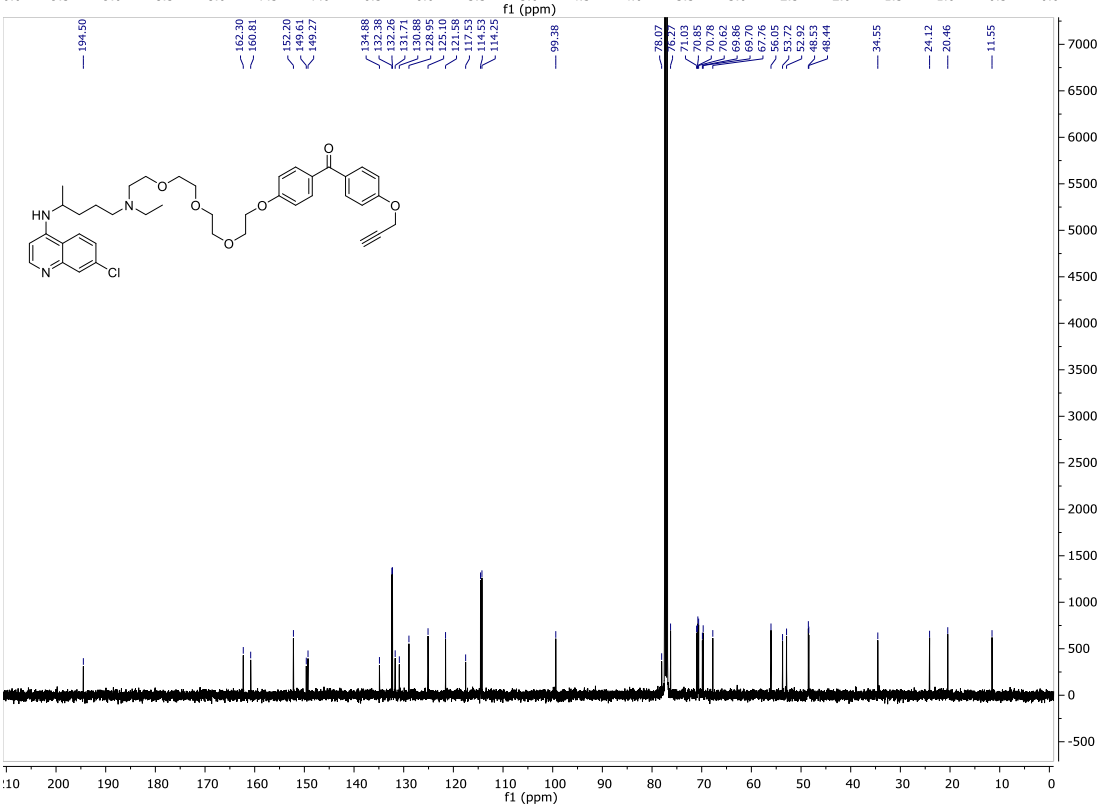
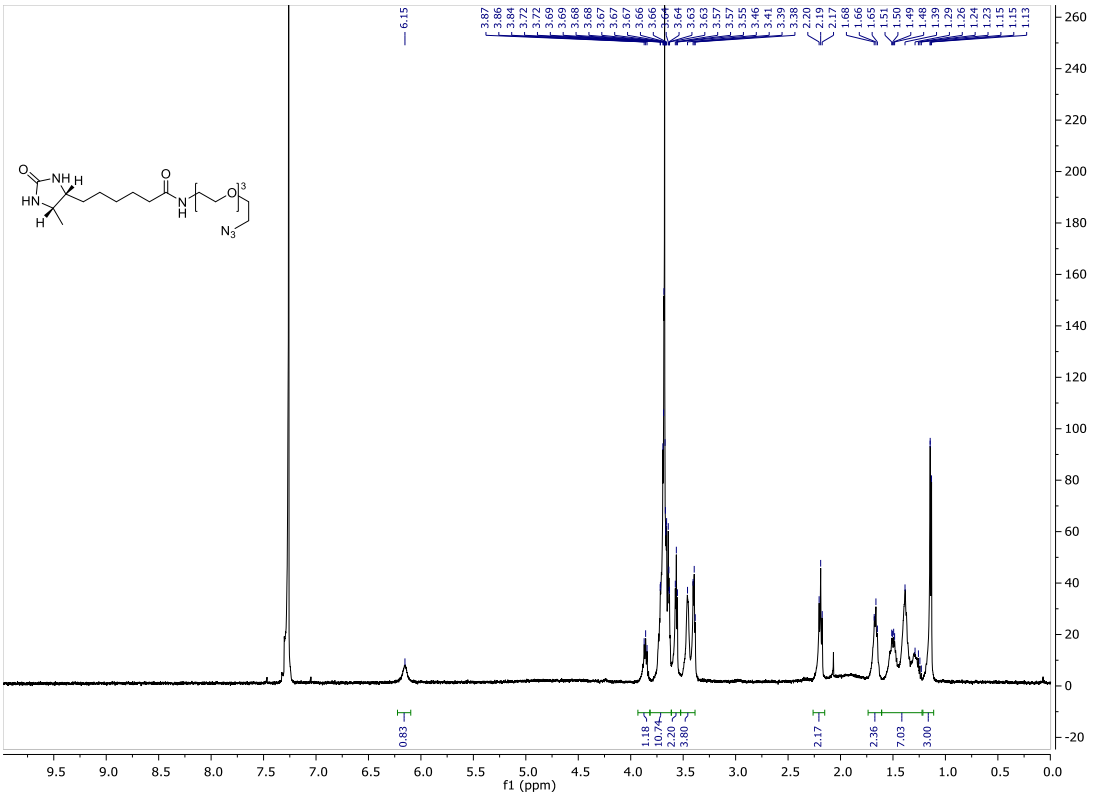
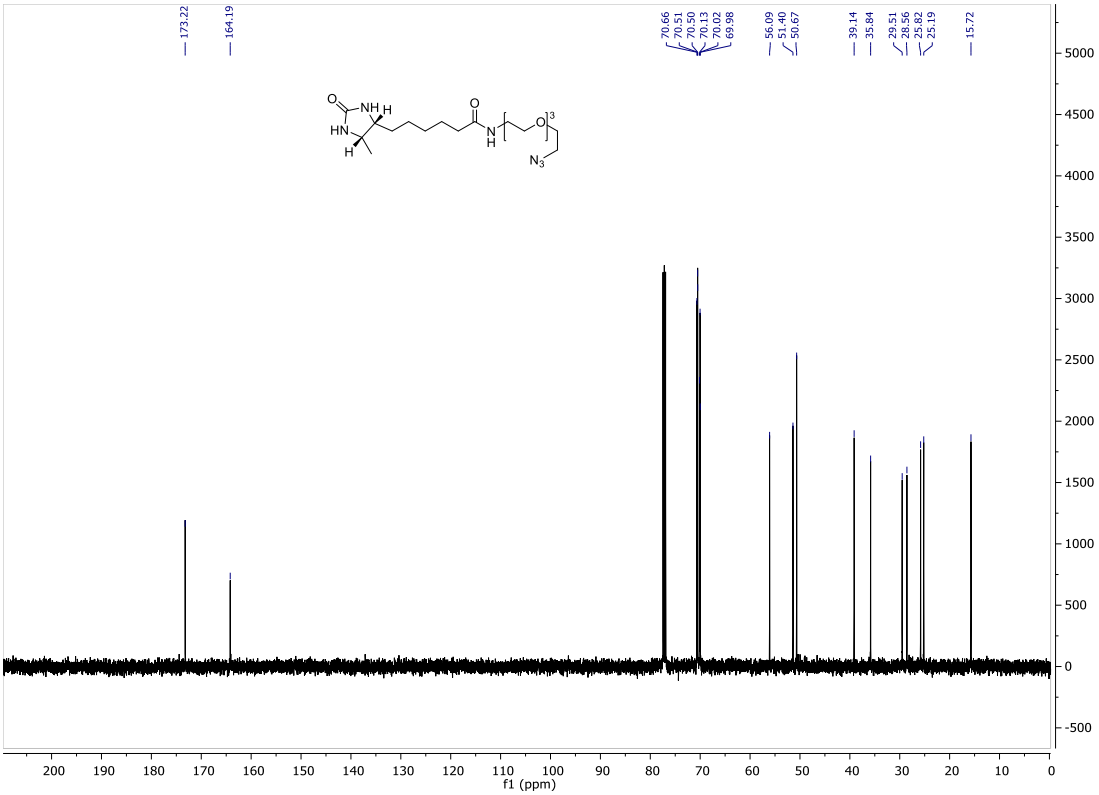
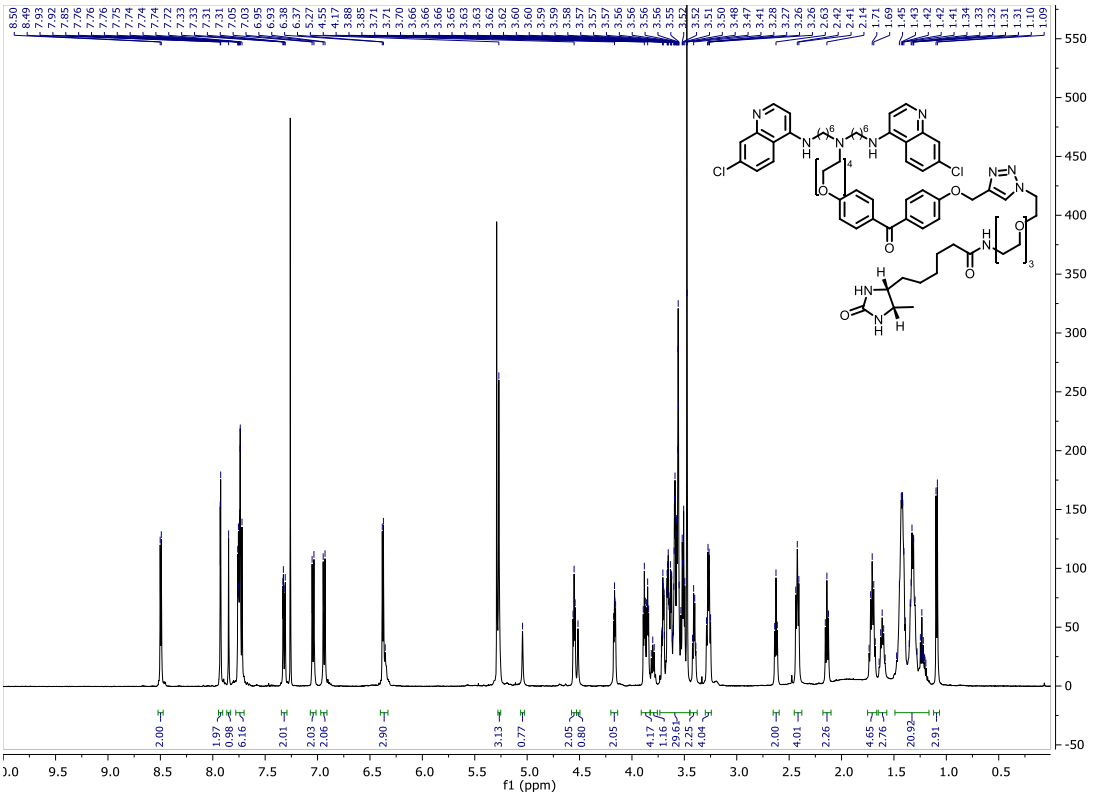
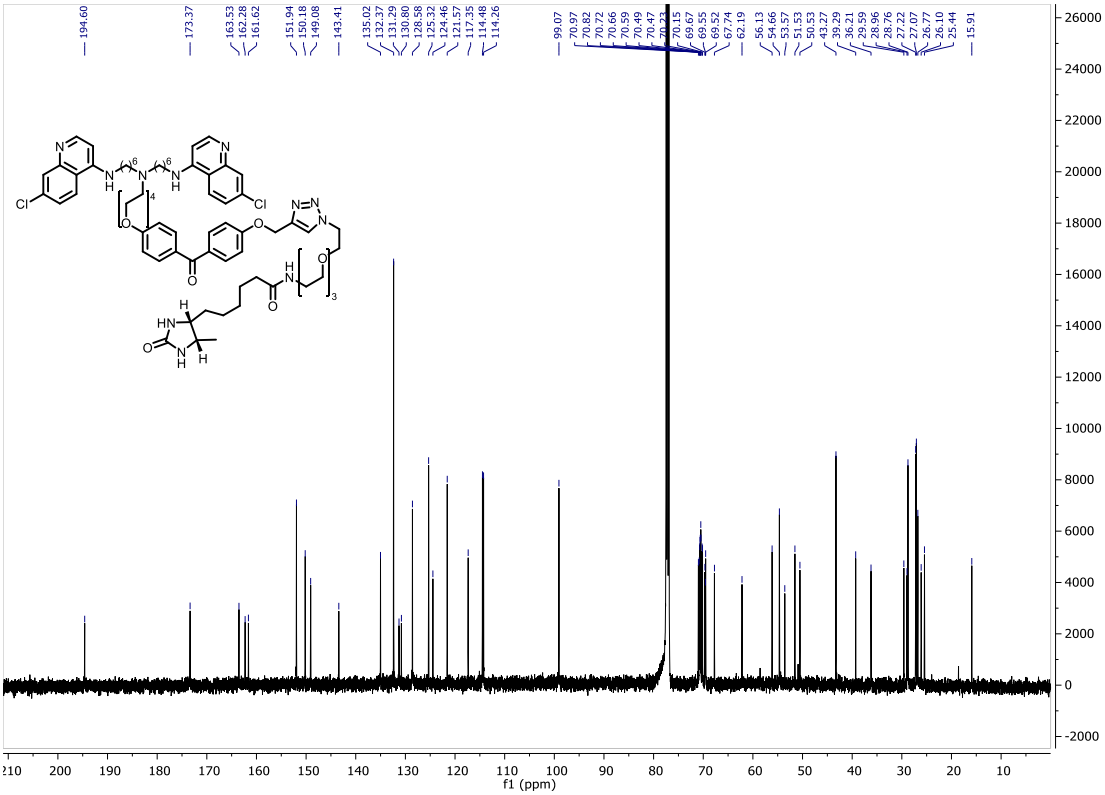

f1 (ppm)

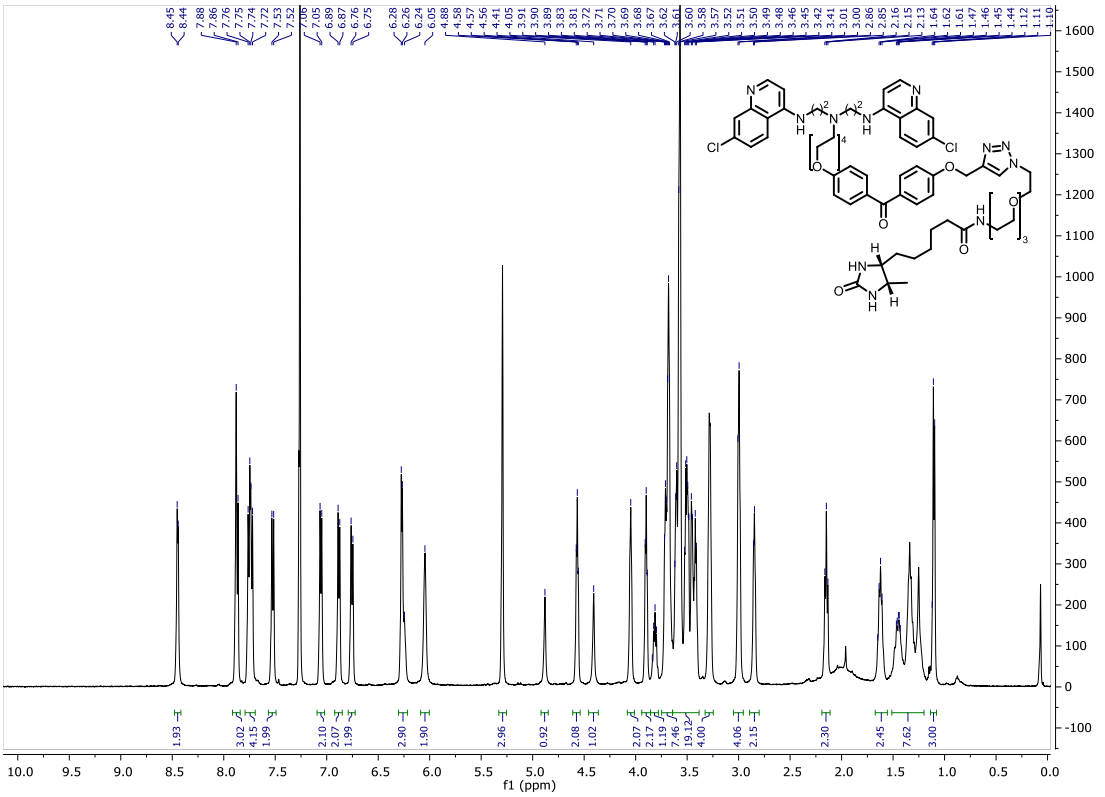

f1 (ppm)

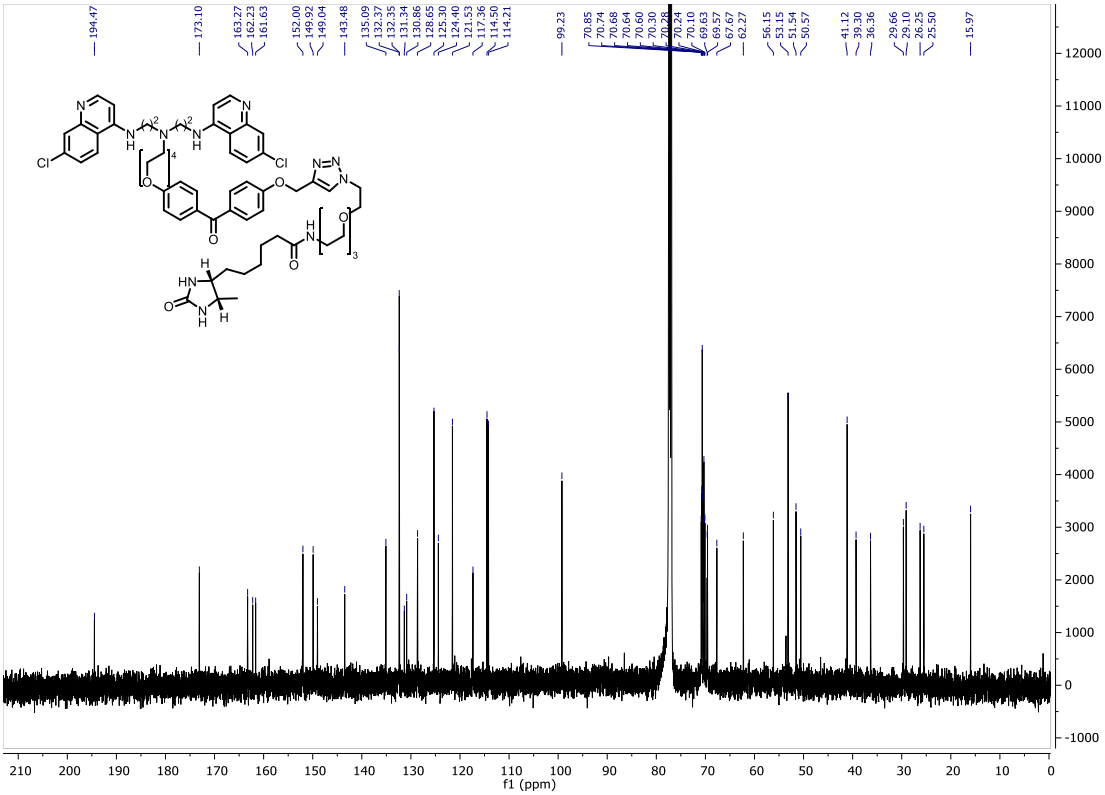

6.15
3.87
3.86
3.84
3.72
3.69
3.68
3.67
3.66
3.64
3.63
3.57
3.55
3.46
3.41
3.39
3.38
2.20
2.19
2.17
1.68
1.66
1.65
1.51
1.50
1.49
1.48
1.39
1.29
1.26
1.24
1.23
1.15
1.13
0.83
1.18
10.74
2.20
3.80
2.17
2.36
7.03
3.00
f1 (ppm)

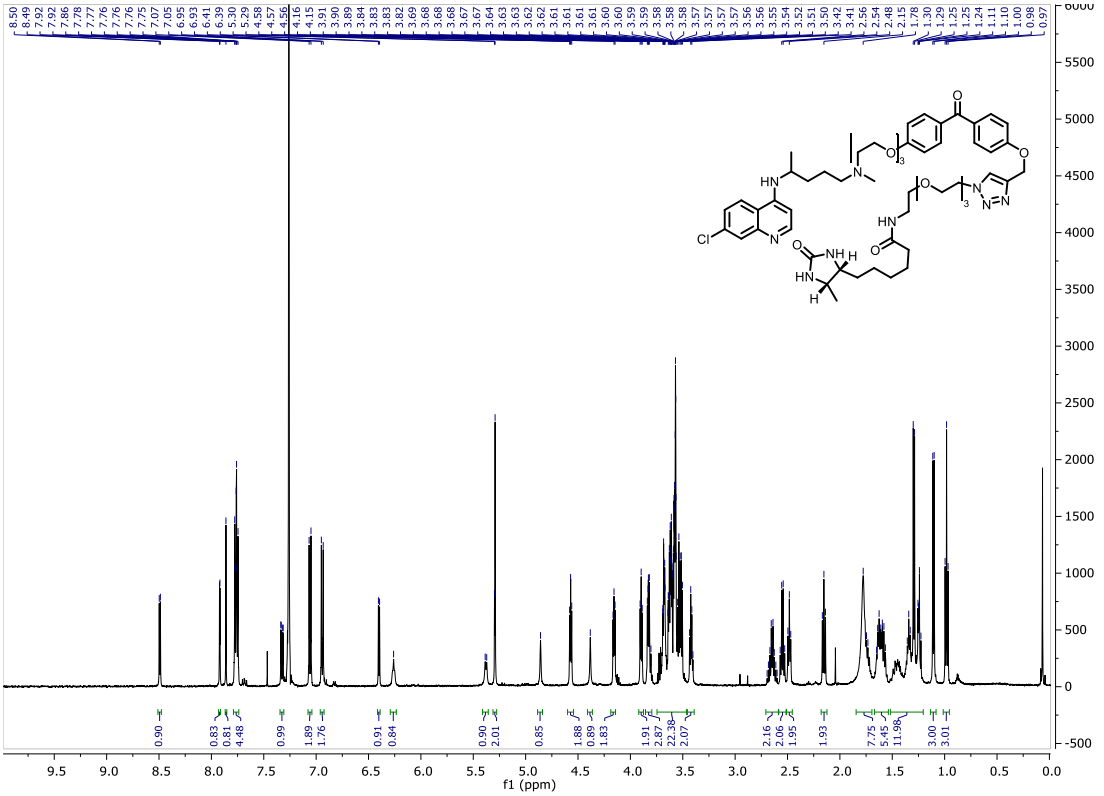

173.22
164.19
70.66
70.51
70.50
70.13
70.02
69.98
56.09
51.40
50.67
39.14
35.84
29.51
28.56
25.82
25.19
15.72
f1 (ppm)

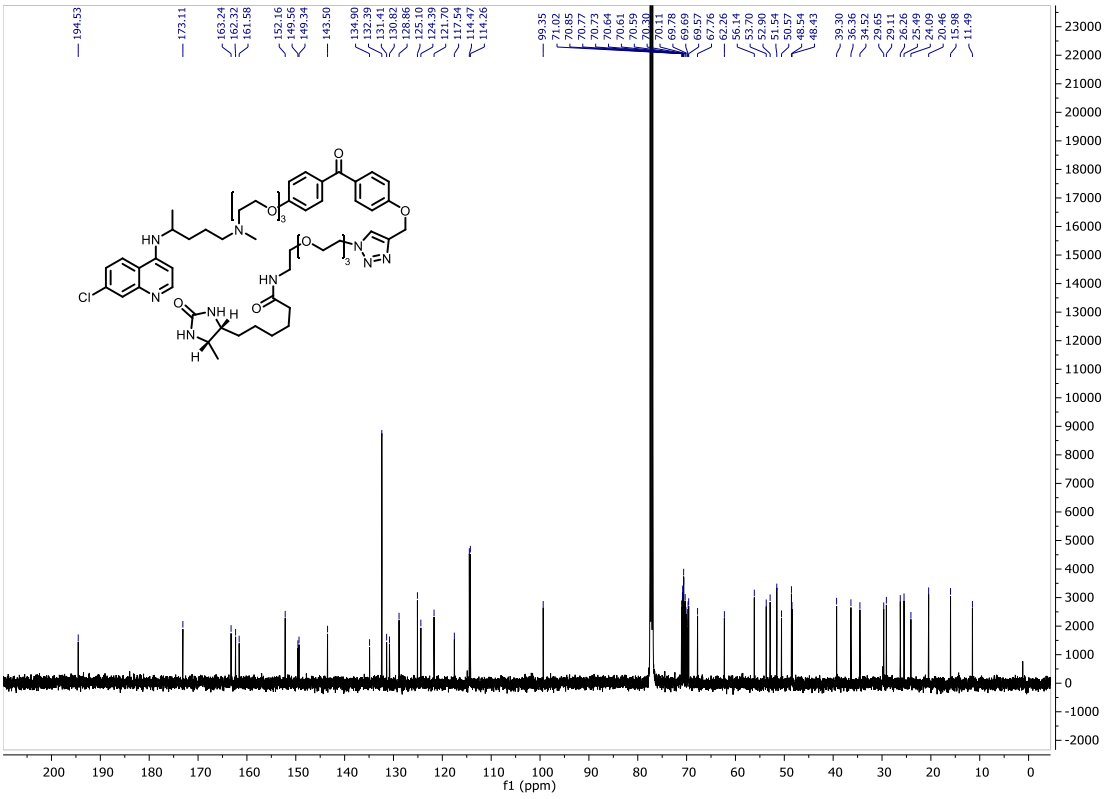

f1 (ppm)


f1 (ppm)


f1 (ppm)


f1 (ppm)

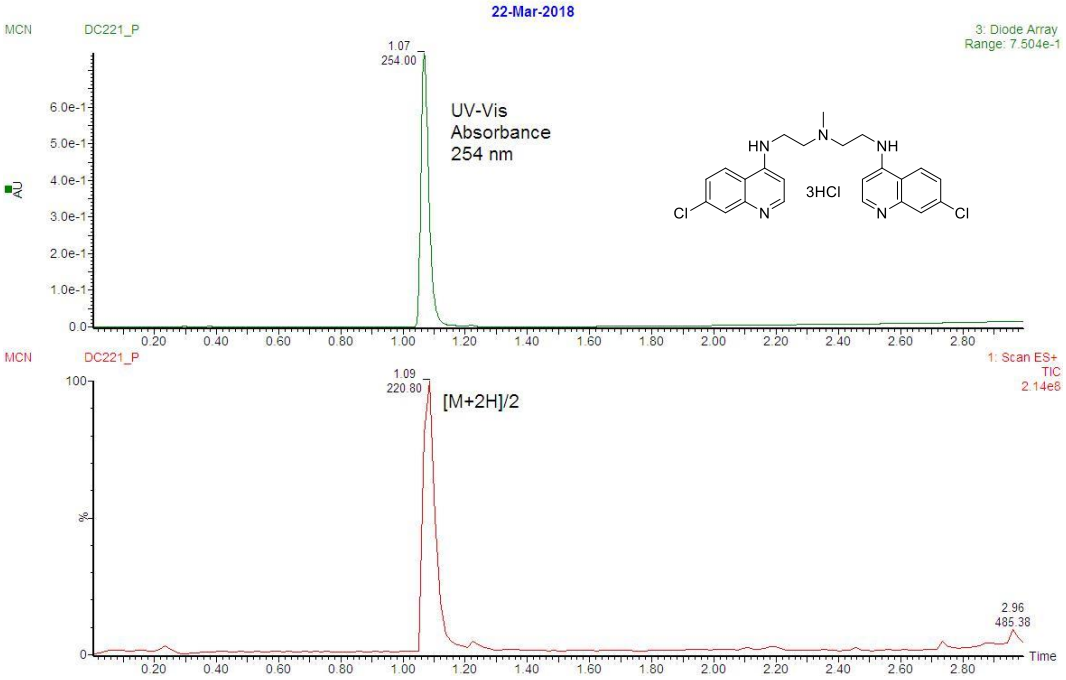


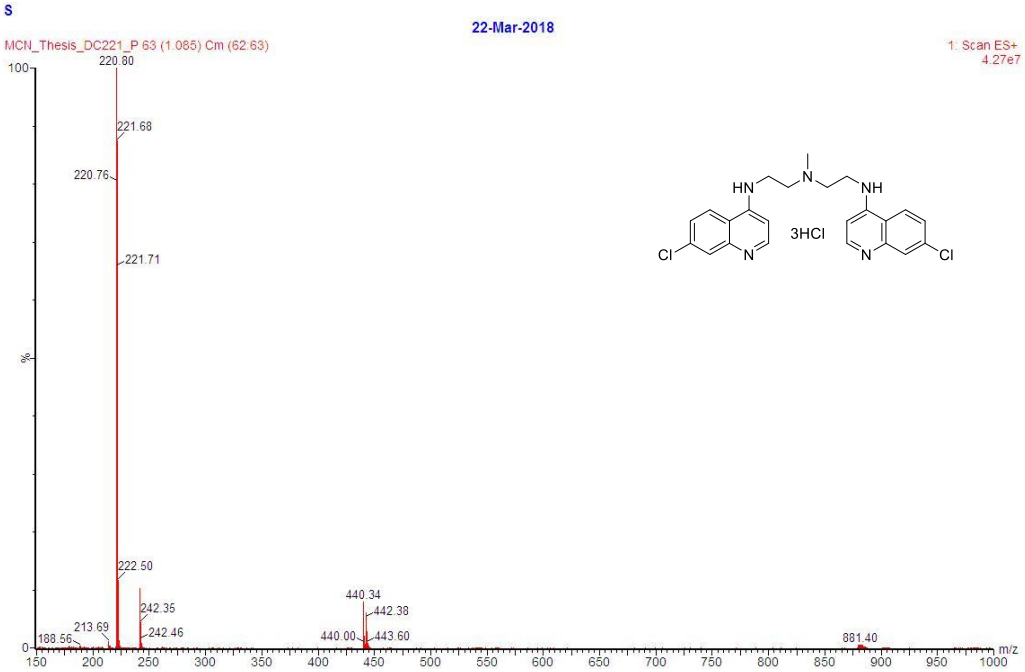


Purity Data for Lysosomal Inhibitors

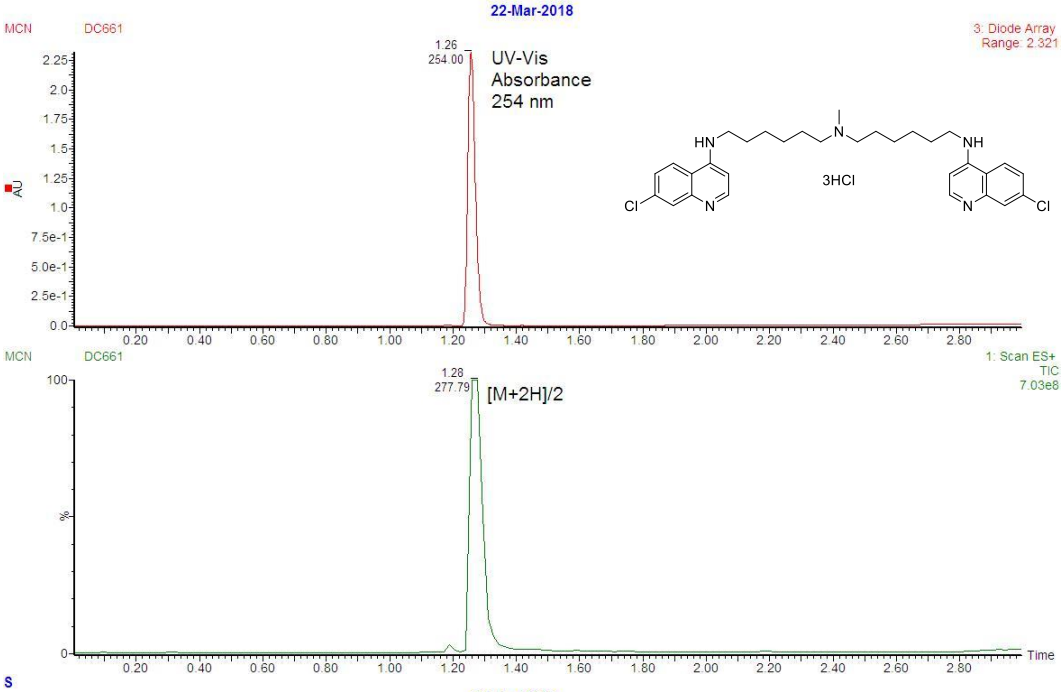
Purity was determined by sample observation by UPLC-MS. Nominal mass accuracy LCMS data were obtained by use of a Waters Acquity UPLC system equipped with a Waters TUV detector (254 nm) and a Waters SQD single quadrupole mass analyzer with electrospray ionization. LC gradient 500 uL/min: 30 second hold 95:5 (water:acetonitrile 0.1% v/v formic acid), 2 minute gradient to 5:95, and 30 second hold. Acquity UPLC BEH C18, 1.7um, 2.1x 50 mm column. Final inhibitors were all observed to be single peaks by absorbance and matched the determined accurate mass measurements.

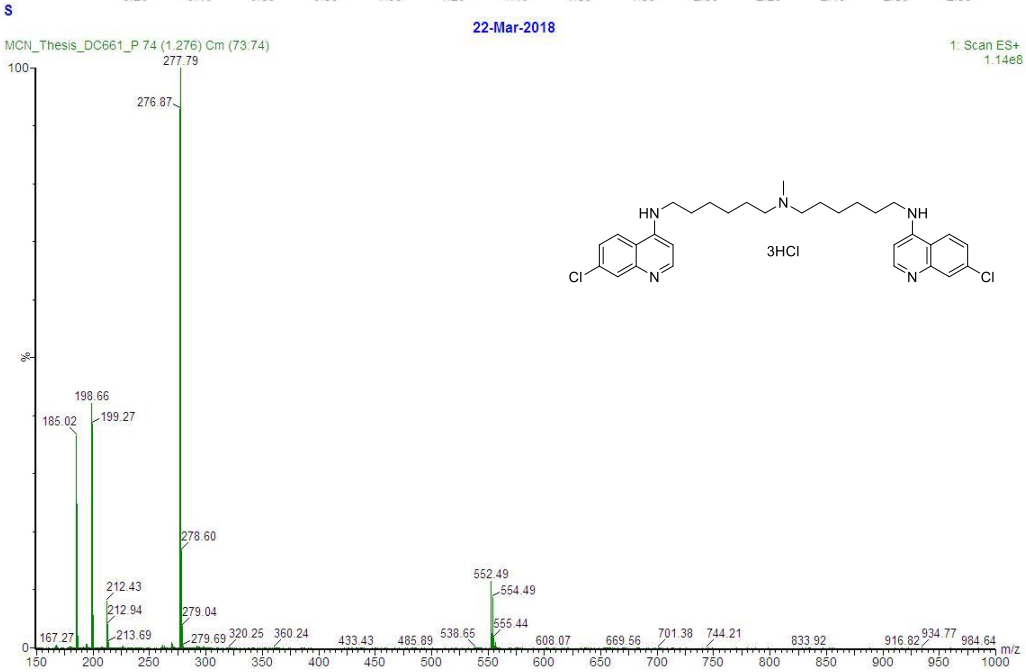
Lys05





DC661


22-Mar-2018
MCN DC661
3: Diode Array
Range: 2.321
1.26
254.00
UV-Vis
Absorbance
254 nm
AU
HN
N
NH
3HCl
Cl
N
N
Cl
MCN DC661
1: Scan ES+
TIC
7.03e8
1.28
277.79
[M+2H]/2
%
Time


S
22-Mar-2018
MCN_Thesis_DC661_P 74 (1.276) Cm (73:74)
1: Scan ES+
1.14e8
277.79
276.87
198.66
185.02
199.27
278.60
212.43
212.94
279.04
213.69
279.69
320.25
360.24
433.43
485.89
538.65
552.49
554.49
555.44
608.07
669.56
701.38
744.21
833.92
916.82
934.77
984.64
167.27
%
m/z
HN
N
NH
3HCl
Cl
N
N
Cl

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