CHEMISTRY
A European Journal

Supporting Information

Design, Synthesis, and Characterization of Brequinar Conjugates as Probes to Study DHODH Inhibition**

Joseph T. Madak,[a] Christine R. Cuthbertson,[a] Wenmin Chen,[a] Hollis D. Showalter,*[b] and Nouri Neamati*[a]

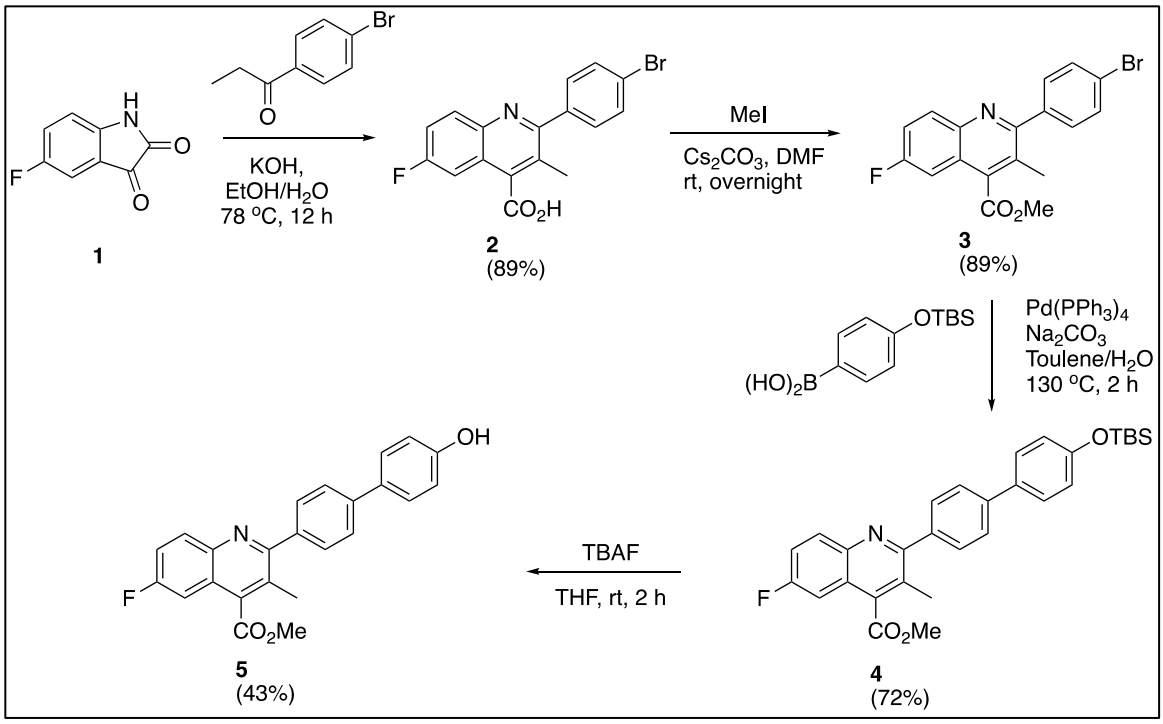
chem_201702999_sm_miscellaneous_information.pdf

Contents:

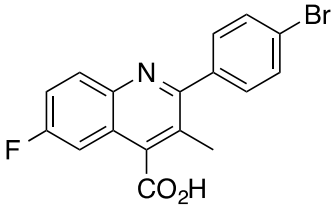
General method:

All reactions were performed under a nitrogen or argon atmosphere using standard inert gas techniques. Glassware for reactions were oven/flame dried in preparation for usage. All reagents and anhydrous solvents were purchased from commercial sources and used without further purification. Microwave catalyzed reactions were performed on a Biotage Initiator+ and sealed vials were purged with argon gas. The progresses of reactions were monitored using analytical thin-layer chromatography (TLC) on aluminum-backed pre-coated silica plates (Silicycle, SiliaPlate 200 μM thickness, F_{254}) and visualized by UV absorbance. Flash chromatography purifications were performed using a Biotage® Isolera Chromatography system with 10g and 25g Ultra-SNAP Cartridge columns (25 μm spherical silica). ^{1}H NMR spectra were obtained using a Bruker (300 or 400 MHz) or

a Varian (400 or 500 MHz). Chemicals shifts are reported in ppm and calibrated based on known solvent peaks (^{1}H using $CDCl_3$ = 7.26 ppm, MeOD = 3.31 ppm, DMSO, 2.50 ppm; ^{13}C using $CDCl_3$ = 77.16 ppm, MeOD = 49.00 ppm, DMSO, 39.52 ppm).[1] Spectral data was reported using the following abbreviations: (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets), coupling constants are reported in Hz, followed by integration. ^{13}C NMR spectra were obtained at 126 MHz on a Varian 500 MHz instrument with a proton decoupled probe. ^{13}C spectra are reported with observed carbon-fluorine splitting and couplings constants are reported with relation to CF bond (J_{CF1} = CF bond, J_{CF2} = *ortho* to CF bond, J_{CF3} = *meta* to CF bond). Data from MS spectrometry and HPLC traces were obtained using a Shimadzu LCMS 20-20 system, which was equipped with photo diode UV detector and a Kinetex® 2.6 μm, XB-C18 100 Å, 75 x 4.6 mm column. HPLC traces were obtained at room temperature using a gradient method from 1% to 90% MeCN in H_2O with 0.01% formic acid over 20 minutes. The flow rate was 0.50 mL/min. Semi-preparative purifications were performed at room temperature on a Shimadzu LC-20 modular HPLC system, which was equipped with a photo diode UV detector and a Kinetex® 5 μm XB-C18 100 Å, 150 x 21.2 mm column. Semi-preparative purification was performed using a gradient method from 10% to 90% MeCN in H_2O with 0.01% trifluoroacetic acid over 30 minutes.

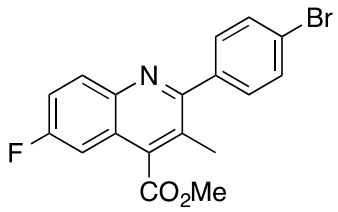


Supplemental scheme 1: Synthesis of intermediate **5**



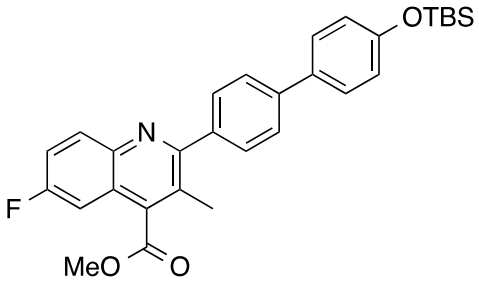
2-(4-Bromophenyl)-6-fluoro-3-methylquinoline-4-carboxylic acid (**2**): 5-Fluoroindoline-2,3-dione (1.90 g, 11.5 mmol) and KOH (3.89 g, 69.6 mmol) were dissolved in 19 mL EtOH and the solution was stirred at room temperature. After 15 min, 1-(4-bromophenyl)propan-1-one (2.45 g, 11.5 mmol) was added and the mixture was heated at reflux for 18 h. Upon completion, the mixture was cooled to room temperature and EtOH was removed *in vacuo.* The solution was washed with ethyl acetate (3x) and then acidified with aqueous HCl until pH 2-3 was reached. Product precipitation was observed and the solid was collected over a frit. 2-(4-Bromophenyl)-6-fluoro-3-methylquinoline-4-carboxylic acid was isolated as a tan solid and used without further

purification (3.70 g, 10.3 mmol, 89%). **^{1}H NMR** (500 MHz, DMSO-d_6) δ = 8.14 – 8.10 (m, 1H), 7.73 – 7.67 (m, 3H), 7.57 (d, J = 8.1 Hz, 2H), 7.51 – 7.47 (m, 1H), 2.39 (s, 3H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ = 168.70, 160.82 (d, J_{CF1}= 247.0 Hz)., 159.03, 143.31, 140.91, 139.38, 132.75 (d, J_{CF3}= 9.6 Hz), 131.67 (2C), 131.57 (2C), 125.76, 123.70 (d, J_{CF3} = 10.2 Hz), 122.53, 120.15 (d, J_{CF2} = 25.7 Hz), 108.15 (d, J_{CF2} = 23.1 Hz), 18.11. **MS** (ESI+) 360.00, 361.95 [M+H] 358.10, 360.15 [M-H].



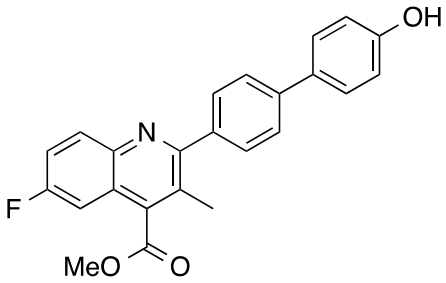
Methyl 2-(4-bromophenyl)-6-fluoro-3-methylquinoline-4-carboxylate (**3**): 2-(4-Bromophenyl)-6-fluoro-3-methylquinoline-4-carboxylic acid (6.50 g, 18.1 mmol) and Cs_2CO_3 (7.06 g, 21.7 mmol) were dissolved in 90 mL anhydrous DMF and the solution was stirred for 15 min at room temperature under an argon atmosphere. Methyl iodide (2.27 mL, 36.5 mmol) was added drop-wise to the solution and the reaction was stirred at room temperature for an additional 12 hours. The mixture was diluted with brine and product was extracted with EtOAc (3x). The combined EtOAc was washed with brine (6x), dried with magnesium sulfate, and concentrated *in vacuo*. Purification via flash chromatography (2:1 hexane/EtOAc) yielded methyl 2-(4-bromophenyl)-6-fluoro-3-methylquinoline-4-carboxylate as white powder (6.02 g, 16.1 mmol, 89%). **^{1}H NMR** (500 MHz, Chloroform-*d*) δ = 8.11 (dd, J = 9.2, 5.4 Hz, 1H), 7.64 (d, J = 8.3 Hz, 2H), 7.50 – 7.41 (m, 3H), 7.37 (d, J = 9.5, 2.7 Hz, 1H), 4.09 (s, 3H), 2.40 (s, 3H). **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 168.01, 161.27 (d, J_{CF1}= 249.5 Hz), 158.94, 143.53, 139.07, 139.04, 132.44 (d, J_{CF3}= 9.4 Hz), 131.76 (2C), 130.73 (2C), 126.64, 124.31 (d, J_{CF3}= 10.3

Hz), 123.13, 119.91 (d, J_{CF2}= 25.8 Hz), 108.00 (d, J_{CF2}= 23.4 Hz), 52.95, 18.18. **MS** (ESI+) 373.95, 375.95 [M+H]

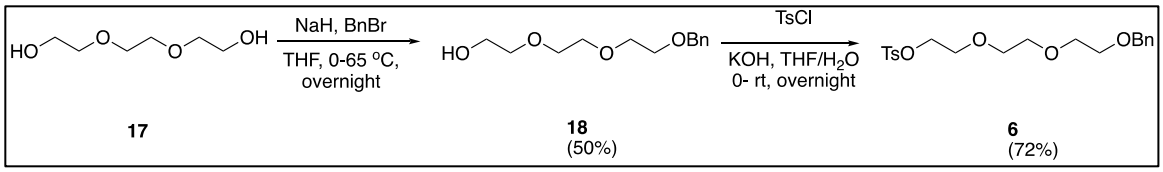


Methyl 2-(4'-((*tert*-butyldimethylsilyl)oxy)-[1,1'-biphenyl]-4-yl)-6-fluoro-3-methylquinoline-4-carboxylate (**4**)**:** Methyl 2-(4-bromophenyl)-6-fluoro-3-methylquinoline-4-carboxylate **(**652 mg, 1.75 mmol) and (4-((*tert*-butyldimethylsilyl)oxy)phenyl)boronic acid (661 mg, 2.62 mmol) were dissolved in a degassed mixture of 10 mL toluene/5 mL H_2O in a microwave vial. $Pd(PPh_3)_4$ (202 mg, 0.17 mmol) and Na_2CO_3 (1.06 g, 10.0 mmol) were added to the mixture, the vial was sealed, and then heated at 130 °C for 2 h. Toluene was removed under reduced pressure and the mixture was extracted with EtOAc (3x), dried with magnesium sulfate, filtered, and concentrated. The residue was loaded onto a silica column and eluted in a gradient of 9:1 hexane/EtOAc to yield methyl 2-(4'-((*tert*-butyldimethylsilyl)oxy)-[1,1'-biphenyl]-4-yl)-6-fluoro-3-methylquinoline-4-carboxylate as a yellow oil (627 mg, 1.25 mmol, 72%). **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 8.16 (dd, J = 3.8, 1.1 Hz, 1H), 7.72 – 7.67 (m, 2H), 7.64 – 7.60 (m, 2H), 7.57 – 7.52 (m, 2H), 7.50 – 7.45 (m, 1H), 7.42 – 7.37 (m, 1H), 6.95 (d, J = 7.4 Hz, 2H), 4.11 (s, 3H), 2.48 (s, 3H), 1.03 (s, 9H), 0.26 (s, 6H). **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 168.20, 161.15 (d, J_{CF1}= 248.9 Hz)., 159.94, 155.70, 143.59, 141.26, 138.87, 138.82, 138.44, 133.76, 132.46 (d, J_{CF3} = 9.2 Hz), 129.44 (2C), 128.25 (2C), 126.95, 126.85 (2C), 124.18 (d, J_{CF3} = 10.2 Hz), 120.55 (2C), 119.65 (d, J_{CF2} = 25.7

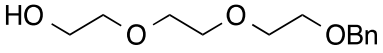
Hz), 107.93 (d, J_{CF2} = 23.4 Hz), 52.87, 25.82 (3C), 18.36, -4.24 (2C). **MS** (ESI) 502.25 [M+H]



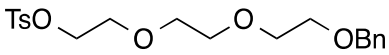
Methyl 6-fluoro-2-(4'-hydroxy-[1,1'-biphenyl]-4-yl)-3-methylquinoline-4-carboxylate (**5**): Methyl 2-(4'-((*tert*-butyldimethylsilyl)oxy)-[1,1'-biphenyl]-4-yl)-6-fluoro-3-methylquinoline-4-carboxylate (627 mg, 1.25 mmol) was dissolved in 12 mL THF. TBAF (392 mg, 1.50 mmol) was slowly added to the solution and the mixture was stirred at room temperature for 2 hour. Upon completion, the reaction mixture was concentrated, the residue was washed with a saturated solution of aqueous NH_4Cl, and the product was extracted with EtOAc. The organic layer was dried with sodium sulfate, filtered, concentrated, and purified via flash chromatography using 1:1 EtOAc/Hexane to yield methyl 6-fluoro-2-(4'-hydroxy-[1,1'-biphenyl]-4-yl)-3-methylquinoline-4-carboxylate (209 mg, 0.54 mmol, 43%). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 9.63 (s, 1H), 8.16 – 8.12 (m, 1H), 7.75 – 7.68 (m, 2H), 7.68 – 7.64 (m, 2H), 7.61 – 7.53 (m, 3H), 6.89 (d, J = 8.5, 1.5 Hz, 2H), 4.07 (s, 3H), 2.41 (s, 3H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 167.25, 160.44 (d, J_{CF1} = 246.5 Hz), 159.39, 157.43, 142.92, 140.30, 138.64, 138.60, 137.52, 132.27 (d, J_{CF3} = 9.5 Hz), 129.64 (2C), 127.85 (2C), 126.45, 125.58 (2C), 123.27 (d, J_{CF3} = 11.0 Hz), 119.77 (d, J_{CF2} = 25.9 Hz), 115.82 (2C), 107.82 (d, J_{CF2} = 23.2 Hz), 53.14, 17.84. **MS** (ESI) 388.10 [M+H]



Supplemental scheme 2: Synthesis of intermediate **6.**

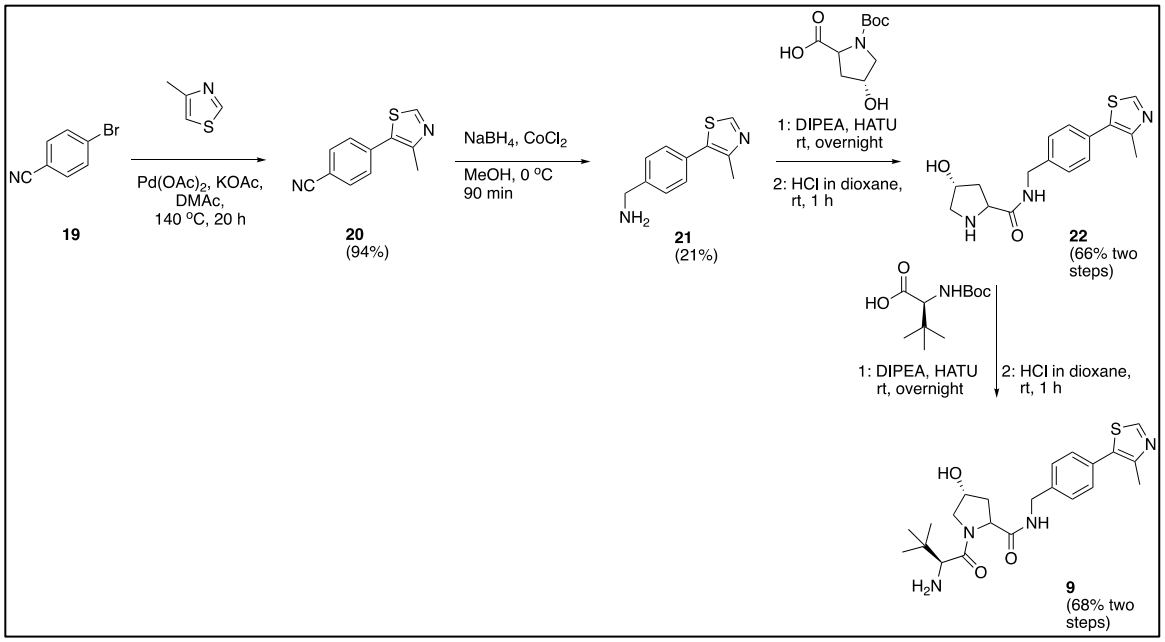


2-(2-(2-(Benzyloxy)ethoxy)ethoxy)ethan-1-ol (**18**): Compound **18** was prepared following a modified protocol described by Jiang & Yu.[2] Triethylene glycol (**17**) (3.97 mL, 29.8 mmol) was dissolved in THF (46 mL) and the solution was chilled to 0 °C. After 15 min, NaH (1.20 g, 30.1 mmol) was added and the mixture was stirred for an additional 15 min before adding benzyl bromide (2.10 mL, 17.5 mmol). The reaction mixture was warmed to room temperature overnight. Upon quenching with water, the mixture was concentrated and then extracted with EtOAc. The dried extracts were concentrated to a residue that was purified via flash chromatography eluting with 1:1 hexane/EtOAc to yield 2-(2-(2-(benzyloxy)ethoxy)ethoxy)ethan-1-ol as a clear oil (2.09 g, 8.74 mmol, 50%). **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 7.37 – 7.31 (m, 4H), 7.28 – 7.20 (m, 1H), 4.54 (s, 2H), 3.76 – 3.51 (m, 12H). **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 138.06, 128.41 (2C), 127.83 (2C), 127.70, 73.28, 72.60, 70.63, 70.57, 70.32, 69.36, 61.67. **MS** (ESI) 241.70 [M+H]

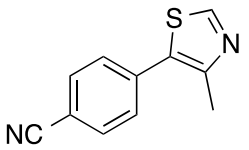


2-(2-(2-(Benzyloxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**6**): 2-(2-(2-(Benzyloxy)ethoxy)ethoxy)ethan-1-ol (**18**) (2.09 g, 8.74 mmol)) was dissolved in 60 mL of THF and 20 mL of H_2O. KOH (1.71 g, 30.5 mmol) was added to the mixture, which was stirred at room temperature for 15 min before TsCl (2.00 g, 10.5 mmol) was added

and the mixture for stirred overnight. Upon completion, the mixture was poured into a saturated solution of ammonium hydroxide and extracted with dichloromethane (3x). The extract was pooled, dried with magnesium sulfate, filtered, and concentrated to yield 2-(2-(2-(benzyloxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate as a orange oil **(**2.50 g, 6.33 mmol, 72%), which was used without further purification. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 7.79 (d, J = 8.3 Hz, 2H), 7.38 – 7.21 (m, 7H), 4.55 (s, 2H), 3.71 – 3.57 (m, 12H), 2.43 (s, 3H). **^{13}C NMR** (126 MHz, Chloroform-*d*)) δ 144.91, 138.36, 129.94 (2C), 128.51 (2C), 128.13 (2C), 127.89 (2C), 127.76 (2C), 73.39, 70.93, 70.83, 70.73, 69.55, 69.38, 68.85, 21.78. **MS** (ESI) 395.15 [M+H].

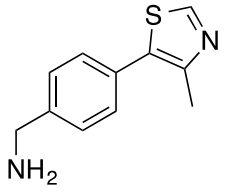


Supplemental scheme 3: Synthesis of intermediate **9.**



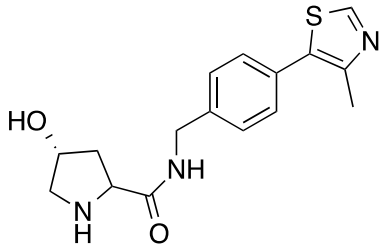
4-(4-Methylthiazol-5-yl)benzonitrile (**20**): Compound **20** was prepared following a protocol that was reported by Buckley[3] and Galdeno[4]. A mixture containing 4-

bromobenzonitrile (5.00 g, 27.5 mmol), 4-methylthiazole (4.98 mL, 54.7 mmol), KOAc (5.40 g, 55.0 mmol), and palladium acetate (62.0 mg, 0.27 mmol) were dissolved in 16 mL DMAc. The mixture was heated to 140 °C overnight, cooled, and then poured into EtOAc. The solution was washed with H_2O (6x), dried with magnesium sulfate, filtered, and concentrated. The resulting oil was crystallized by the addition of hexanes and used without further purification. 4-(4-Methylthiazol-5-yl)benzonitrile was isolated as a yellow solid (5.15 g, 25.8 mmol, 94%) and matched the reported spectral data.[4] **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 8.76 (s, 1H), 7.75 – 7.71 (m, 2H), 7.60 – 7.55 (m, 2H), 2.59 – 2.56 (m, 3H). **MS** (ESI) 200.90 [M+H].

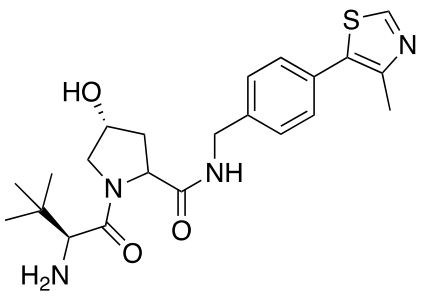


(4-(4-Methylthiazol-5-yl)phenyl)methanamine (**21**): Compound **21** was prepared following a protocol reported by Buckley[3] and Galdeno.[4] Compound **20** (5.15 g, 25.8 mmol) was dissolved in 280 mL anhydrous MeOH. Cobalt chloride hexahydrate (9.90 g, 41.7 mmol) was added and the solution was placed on an ice bath for 30 min. $NaBH_4$ (5.22 g, 138 mmol) was slowly added over 20 min, bubbling was observed, and the solution turned black. The mixture was stirred for 90 min and quenched with cold H_2O. The quenched solution was poured over a frit to remove insoluble by-products and the filtrate was washed with H_2O then extracted with EtOAc. The organic layer was dried with sodium sulfate, filtered, concentrated, and purified via flash chromatography using a gradient of 1-10% 0.5 M methanolic ammonia in DCM. Compound **21** was isolated (1.12 g, 5.49 mmol, 21%) and matched the reported spectral data.[3] **^{1}H NMR** (400 MHz,

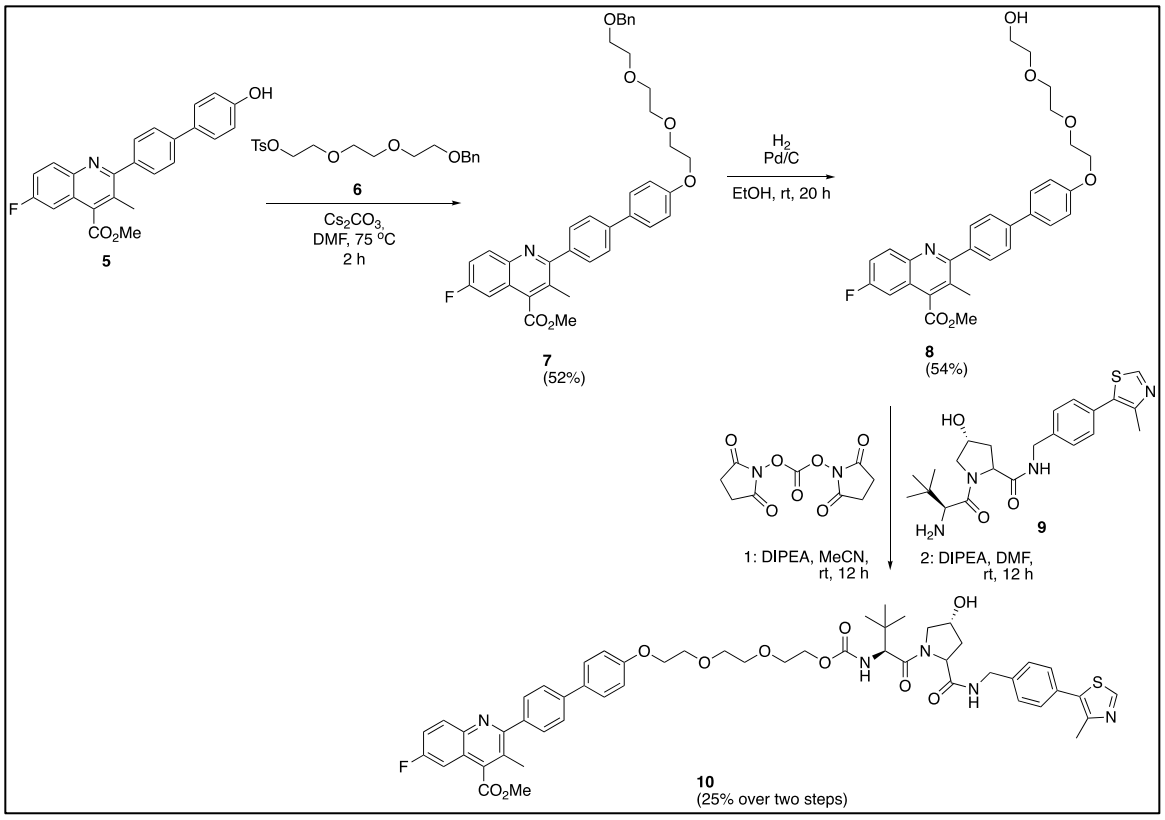
Chloroform-*d*) δ 8.65 (s, 1H), 7.41 – 7.35 (m, 4H), 3.90 (s, 2H), 2.50 (s, 3H). **MS** (ESI) 204.90, 187.80 (fragmentation product reported by Galdeno also observed)[3-4] [M+H].



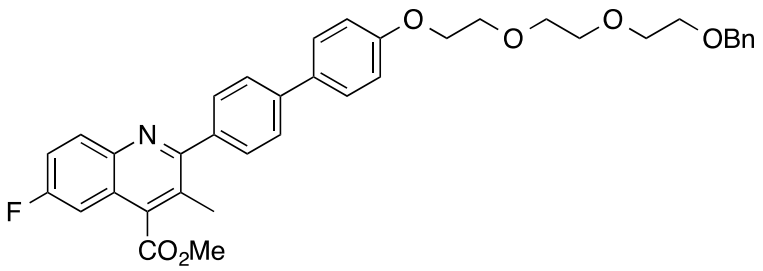
(4*R*)-4-Hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**22**): Compound **22** was prepared following a protocol reported by Galdeno[4]. Anhydrous DMF (55 ml) was added to a round-bottom flask containing compound **21 (**1.12 g, 5.49 mmol)**,** Boc-Hyp-OH (1.27 g, 5.49 mmol), DIPEA (3.85 mL, 22.0 mmol), and HATU (2.30 g, 6.05 mmol). The mixture was stirred at room temperature overnight. The solution was diluted with H_2O and extracted with EtOAc. The EtOAc layer was washed with brine (6x), dried with magnesium sulfate, and concentrated. The residue was purified via flash chromatography using a gradient from 1-10% 0.5 M methanolic ammonia in DCM. (**MS** (ESI) 418.15 [M+H]). The isolated oil was re-dissolved in 10 mL of 4.0 N HCl in dioxane and stirred at room temperature for 1 h. The solvent was concentrated and used without further purification. Compound **22** was isolated as an oil (1.16 g, 3.66 mmol, 66% two steps) and matched the reported spectral data.[3] **^{1}H NMR** (300 MHz, Chloroform-*d*) δ 8.69 (s, 1H), 8.28 – 8.04 (m, 1H), 7.45 – 7.32 (m, 4H), 4.52 – 4.44 (m, 3H), 4.10 (t, J = 8.4 Hz, 1H), 3.10 – 3.00 (m, 1H), 2.88 – 2.77 (m, 1H), 2.54 (s, 3H), 2.41 – 2.32 (m, 1H), 2.07 – 1.93 (m, 1H). **MS** (ESI) 317.90 [M+H]



(4*R*)-1-((*S*)-2-Amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**9**): A mixture containing compound **22** (1.16 g, 3.29 mmol), Boc-L-*tert*-leucine (847 mg, 3.67 mmol), HATU (1.67 g, 4.40 mmol), and DIPEA (2.56 mL, 14.7 mmol) was dissolved in 55 mL DMF. The mixture was stirred at room temperature overnight, diluted with H_2O, and extracted with EtOAc.. The EtOAc layer was washed with a saturated $NaHCO_3$ solution (2x), brine (6x), dried with magnesium sulfate, filtered, and concentrated. (**MS (**ESI**)** 531.10 [M+H]) Residue containing the intermediate was dissolved in 10 mL of 4.0 N HCl in dioxane, stirred at room temperature for 1 hour, and followed the workup described by Buckner.[3] The solution was concentrated to an orange oil and matched the reported spectra of (4*R*)-1-((*S*)-2-Amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (970 mg, 2.25 mmol, 68% yield two steps) and used without further purification. **^{1}H NMR** (300 MHz, Methanol-d_4) δ 8.90 (s, 1H), 7.50 – 7.41 (m, 4H), 4.66 – 4.34 (m, 4H), 3.82 – 3.65 (m, 1H), 3.49 (s, 1H), 2.50 (s, 3H), 2.30 – 2.17 (m, 1H), 2.16 – 2.07 (m, 1H), 1.04 (s, 9H). **MS** (ESI) 431.15 [M+H].

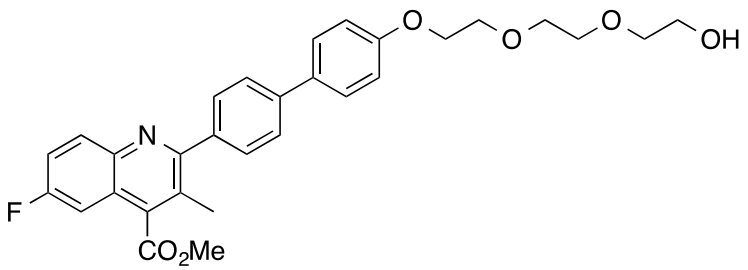


Supplemental scheme 4: Synthesis of probe **10.**



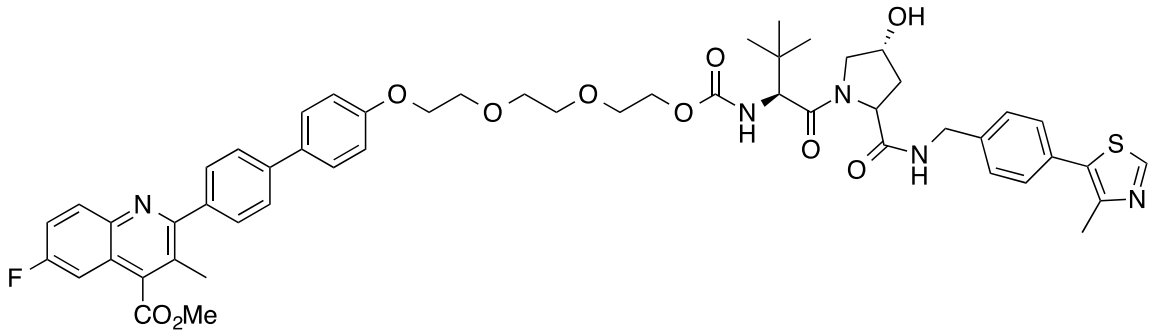
Methyl 2-(4'-(2-(2-(2-(benzyloxy)ethoxy)ethoxy)ethoxy)-[1,1'-biphenyl]-4-yl)-6-fluoro-3-methylquinoline-4-carboxylate (**7**): Compound **5** (209 mg, 0.54 mmol) was dissolved in 11 mL DMF. Cs_2CO_3 (790 mg, 2.42 mmol) was added to the solution and the mixture was stirred at room temperature for 5 min. 2-(2-(2-(Benzyloxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**6**) **(**255 mg, 0.65 mmol**),** was slowly added and the mixture was heated at 75 °C for 2 h. The solution was concentrated to an oil, re-dissolved in DCM, washed with brine (6x), dried with

magnesium sulfate, and re-concentrated to an oil. The crude was purified by silica chromatography eluting with a gradient from 10% to 40% EtOAc in hexane. Methyl 2-(4'-(2-(2-(2-(benzyloxy)ethoxy)ethoxy)ethoxy)-[1,1'-biphenyl]-4-yl)-6-fluoro-3-methylquinoline-4-carboxylate was isolated (173 mg**,** 0.28 mmol, 52%) and used without further purification. **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 8.20 – 8.15 (m, 1H), 7.71 – 7.66 (m, 2H), 7.61 (d, J = 8.3 Hz, 2H), 7.60 – 7.56 (m, 2H), 7.51 – 7.46 (m, 1H), 7.41 – 7.25 (m, 6H), 7.04 – 7.00 (m, 2H), 4.58 (s, 2H), 4.22 – 4.17 (m, 2H), 4.11 (s, 3H), 3.93 – 3.88 (m, 2H), 3.80 – 3.63 (m, 8H), 2.48 (s, 3H). **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 168.23, 162.20, 161.21 (d, J_{CF1} = 249.1 Hz). 159.93, 158.79, 143.53, 141.23, 138.42, 133.39, 132.43 (d, J_{CF3} = 9.7 Hz), 129.49 (2C), 128.50 (2C), 128.30 (2C), 127.87 (2C), 127.72, 127.00, 126.88 (2C), 124.23 (d, J_{CF3} = 10.1 Hz), 119.76 (d, J_{CF2} = 25.7 Hz), 115.17 (2C), 110.14, 107.97 (d, J_{CF2} = 23.4 Hz)., 73.39, 71.04, 70.87 (2C), 69.91, 69.60, 67.70, 52.94, 18.34. **MS** (ESI) 610.45 [M+H].



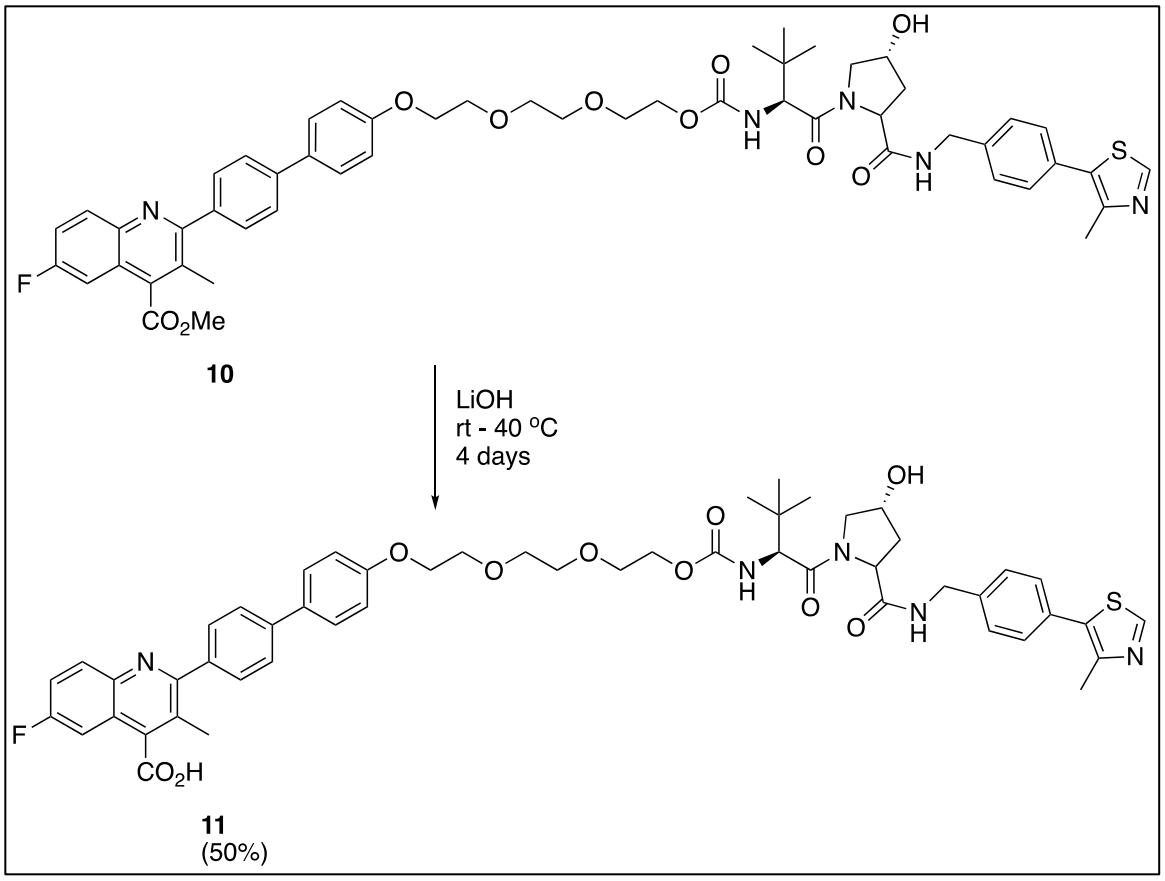
Methyl 6-fluoro-2-(4'-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)-[1,1'-biphenyl]-4-yl)-3-methylquinoline-4-carboxylate (**8**): Compound **7** (173 mg, 0.28 mmol) was dissolved in 30 mL anhydrous EtOH and the solution was purged with argon for 30 min. Activated Pd on charcoal (10%) (60 mg) was added to the solution, (excess EtOH was used to submerge all Pd/C) and the mixture was stirred under an H_2 environment overnight. The mixture was filtered over EtOAc washed celite and eluted with EtOAc. Filtrate was

concentrated and loaded onto silica column eluting with a gradient of 20 – 66% EtOAc in hexane. Compound **8** was isolated (79 mg, 0.15 mmol, 54%). **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 8.21 (s, 1H), 7.69 (d, J = 8.3 Hz, 2H), 7.64 – 7.56 (m, 4H), 7.52 – 7.45 (m, 1H), 7.38 (dd, J = 9.5, 2.7 Hz, 1H), 7.07 – 6.99 (m, 2H), 4.24 – 4.18 (m, 2H), 4.11 (s, 3H), 3.94 – 3.88 (m, 2H), 3.79 – 3.69 (m, 6H), 3.69 – 3.61 (m, 2H), 2.48 (s, 3H). **^{13}C NMR** (126 MHz, Chloroform-*d*) δ 168.16, 161.25 (d, J_{CF1} = 249.2 Hz), 159.85, 158.71, 143.29, 141.31, 138.16, 133.47 (2C, overlap), 132.26 (d, J_{CF3} = 10.1 Hz), 129.54 (2C), 128.35 (2C), 127.08, 126.90 (2C), 124.28 (d, J_{CF3} = 9.9 Hz), 119.90 (d, J_{CF2} = 25.5 Hz), 115.17 (2C), 108.00 (d, J_{CF2} = 23.7 Hz), 72.64, 71.02, 70.55, 69.91, 67.64, 61.94, 52.98, 18.33. **MS** (ESI) 520.30 [M+H].

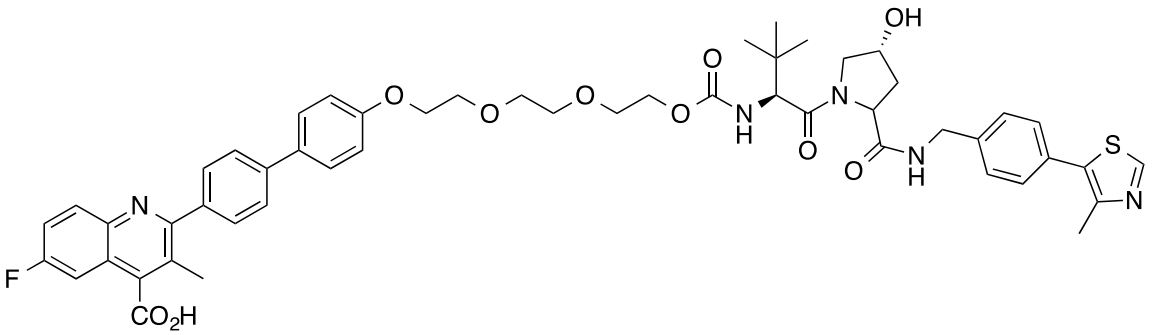


Methyl 6-fluoro-2-(4'-(((12*S*)-12-((4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-13,13-dimethyl-10-oxo-3,6,9-trioxa-11-azatetradecyl)oxy)-[1,1'-biphenyl]-4-yl)-3-methylquinoline-4-carboxylate (**10**): Compound **8** (40 mg, 0.08 mmol) was dissolved in 3 mL anhydrous MeCN. DIPEA (0.07 mL, 0.39 mmol) was added and the mixture was stirred at room temperature for 15 min. N,N'-disuccinimidyl carbonate (102 mg, 0.40 mmol) was added and the mixture was stirred at room temperature for 12 hours before being concentrated. The residue was dissolved in DCM and washed with H_2O (3x). The organic layer was dried with

magnesium sulfate, filtered, concentrated, and used without further purification. In a separate round-bottom flask, compound **9** (34 mg, 0.08 mmol) was dissolved in DMF (2.0 mL) and DIPEA (0.07 mL, 0.39 mmol). The organic residue containing the succimidyl intermediate was dissolved in 1.5 mL DMF and the mixture was slowly added to the basic solution containing **9.** The combined solution was stirred at room temperature for 12 hours. Upon completion, the mixture was concentrated to an oil, re-dissolved in EtOAc, and washed with brine (6x). The organic layer was dried with magnesium sulfate, filtered, concentrated, and loaded onto silica column, eluting in a slow gradient towards 90/10 DCM/MeOH with 0.5 M NH_4. Compound **10** was isolated as a clear oil (16 mg, 0.02 mmol, 25% yield, two-steps). **^{1}H NMR** (500 MHz, Methanol-*d*4) δ 8.83 (s, 1H), 8.10 (dd, *J* = 9.3, 5.4 Hz, 1H), 7.72 (d, *J* = 8.3 Hz, 2H), 7.64 – 7.57 (m, 5H), 7.47 – 7.36 (m, 5H), 7.04 (d, *J* = 8.7 Hz, 2H), 4.58 (t, *J* = 8.3 Hz, 1H), 4.54 – 4.47 (m, 2H), 4.36 – 4.31 (m, 2H), 4.23 – 4.13 (m, 4H), 4.10 (s, 3H), 3.91 – 3.83 (m, 3H), 3.78 (dd, *J* = 10.9, 3.9 Hz, 1H), 3.74 – 3.65 (m, 6H), 2.44 (s, 3H), 2.41 (s, 3H), 2.25 – 2.17 (m, 1H), 2.13 – 2.04 (m, 1H), 1.01 (s, 9H). **^{13}C NMR** (126 MHz, Methanol-d_4) δ 174.40, 172.65, 169.00, 162.55 (d J_{CF1} = 244.4 Hz), 161.55, 160.25, 158.57, 152.77, 149.00, 144.32, 142.56, 140.91, 140.21, 139.20, 134.16, 133.38, 132.58 (d, J_{CF3} = 9.6 Hz), 131.48, 130.61 (2C), 130.35 (2C), 129.15 (2C), 128.93, 128.46 (2C), 127.47 (2C), 125.51 (d, J_{CF3} = 10.1 Hz), 120.94 (d, J_{CF2} = 26.2 Hz), 116.16 (2C), 108.98 (d, J_{CF2} = 23.9 Hz), 71.78, 71.59, 71.09, 70.90, 70.51, 68.72, 65.44, 60.95, 60.81, 57.99, 53.43, 43.70, 38.90, 36.68, 26.95 (3C), 18.32, 15.83. **LCMS** (ESI) 976.45 [M+H], 95% purity based on HPLC chromatograph at 254 nm.

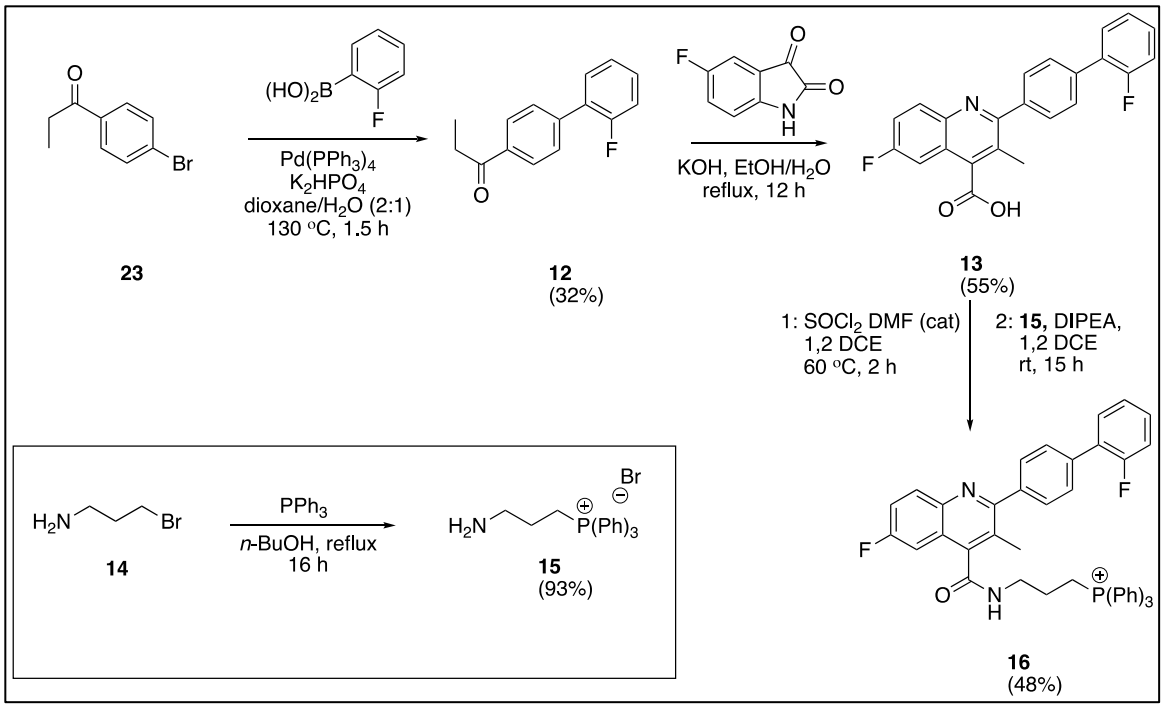


Supplemental scheme 5: Synthesis of probe **11.**

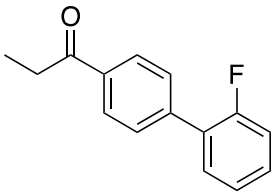


6-Fluoro-2-(4'-(((12*S*)-12-((4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-13,13-dimethyl-10-oxo-3,6,9-trioxa-11-azatetradecyl)oxy)-[1,1'-biphenyl]-4-yl)-3-methylquinoline-4-carboxylic acid (**11**): Compound **10** (10 mg, 0.01 mmol) was dissolved in a 1 mL solution of 1:1 THF/H_2O. LiOH (5 mg, 0.21 mmol) was added to the solution and the mixture was stirred at room temperature overnight. LCMS indicated pre-dominantly starting material remained.

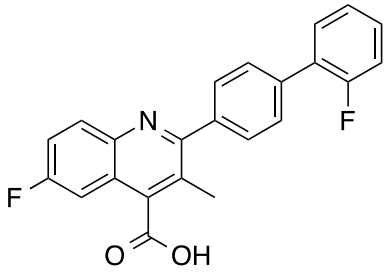
Additional LiOH (20 mg, 0.83 mmol) was added and the solution was heated to 40 °C for three additional days (reaction progress monitored by LCMS). The mixture was concentrated and purified via reverse phase chromatography eluting with a gradient from 0 to 100 MeCN in H_2O. Compound **11** was isolated as a clear oil (5 mg, 0.01 mmol, 50%). **^{1}H NMR** (300 MHz, Methanol-d_4) δ 8.87 (s, 1H), 8.08 – 8.00 (m, 1H), 7.76 (d, J = 7.9 Hz, 2H), 7.70 – 7.56 (m, 5H), 7.57 – 7.39 (m, 5H), 7.09 (d, J = 8.3 Hz, 2H), 4.65 – 4.49 (m, 3H), 4.41 – 4.33 (m, 2H), 4.26 – 4.13 (m, 4H), 3.96 – 3.84 (m, 3H), 3.84 – 3.62 (m, 7H), 2.52 – 2.38 (m, 6H), 2.30 – 2.06 (m, 1H), 2.06 – 2.02 (m, 1H), 1.04 (s, 9H). **^{13}C NMR** (126 MHz, Methanol-d_4) δ 174.43, 172.66, 161.92 (d, J_{CF1} = 247.0 Hz), 161.76, 160.94, 160.16, 158.58, 152.82, 149.02, 144.26, 142.25, 140.23, 140.05, 135.47 (d, J_{CF2} = 12.6 Hz), 134.39, 133.41, 131.57, 131.49, 130.50 (2C), 130.37 (2C), 129.15 (2C), 128.94 (2C), 127.39 (2C), 125.78 (d, J_{CF3} = 10.0 Hz), 124.87, 120.16 (d, J_{CF2} = 26.2 Hz), 116.13 (2C), 110.22 (d, J_{CF2} = 22.8 Hz)., 71.78, 71.59, 71.10, 70.91, 70.53, 68.71, 65.44, 60.97, 60.82, 57.98, 43.70, 38.92, 36.68, 26.94 (3C), 18.14, 15.81. **LCMS** (ESI) 962.45. [M+H], 960.35 [M-H], 96% purity based on HPLC chromatograph at 254 nm.



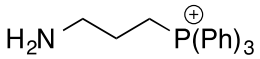
Supplemental scheme 6: Synthesis of intermediate **15** and probe **16.**



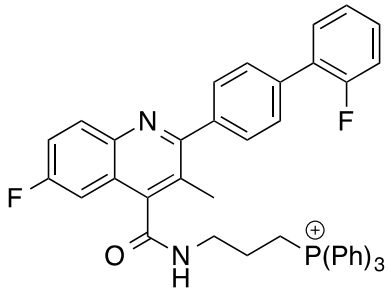
1-(2'-Fluoro-[1,1'-biphenyl]-4-yl)propan-1-one: (12) 1-(4-Bromophenyl)propan-1-one **(**2.00 g, 9.39 mmol), (2-fluorophenyl)boronic acid (1.95 g, 14.1 mmol), K_2HPO_4 (3.89 g, 28.2 mmol), and $Pd(PPh_3)_4$ (0.54 g, 0.47 mmol) were added to a microwave vial. 12 mL of dioxane and 2 mL of H_2O were added and the vial was sealed. The reaction was stirred at 130 °C for 1.5 hour and the mixture was concentrated upon completion. The product was purified from the residue via flash chromatography with a gradient of 1% to 60% EtOAc in hexane to yield compound **12** (679 mg, 2.98 mmol, 32% yield). **^{1}H NMR** (500 MHz, Chloroform-*d*) δ 8.02 (d, *J* = 8.4 Hz, 2H), 7.62 (dd, *J* = 8.4, 1.7 Hz, 2H), 7.43 (td, *J* = 7.8, 1.8 Hz, 1H), 7.36 – 7.30 (m, 1H), 7.21 (td, *J* = 7.6, 1.2 Hz, 1H), 7.18 – 7.12 (m, 1H), 3.00 (q, *J* = 7.2 Hz, 2H), 1.24 (t, *J* = 7.2 Hz, 3H). **MS** (ESI) 229.05 [M+H].



6-Fluoro-2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-3-methylquinoline-4-carboxylic acid (Brequinar) (**13**): 5-fluoroisatin (2.17 g, 13.2 mmol) was dissolved in a 35 mL solution of EtOH/H_2O (2:1 ratio). KOH (2.95 g, 52.6 mmol) were added and the solution was stirred at room temperature for 15 min (solution turns dark when KOH is added). Compound **12** (2.0 g, 8.77 mmol) was added and the mixture was heated at reflux overnight. Upon completion, the mixture was cooled to room temperature, dioxane/EtOH were concentrated *in vacuo*, and the solution was diluted with 1 M KOH. The basic solution was washed with EtOAc (3x) and then acidified to pH 2-3 with HCl (precipitant formation observed). Precipitant was poured over a fritted funnel then triturated with EtOAc and Et_2O, before collection. Compound **13** isolated as a tan solid **(**1.81 g, 4.82 mmol**,** 55%**). ^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.15 (dd, J = 9.2, 5.6 Hz, 1H), 7.79 – 7.69 (m, 5H), 7.65 (t, J = 7.8, 1.7 Hz, 1H), 7.54 – 7.45 (m, 2H), 7.41 – 7.32 (m, 2H), 2.47 (s, 3H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 168.38, 160.34 (d, J_{CF1} = 245.7 Hz), 160.16, 159.35, 159.28, 158.20, 142.94, 139.26, 135.17, 132.32 (d, J_{CF3} = 10.0 Hz), 130.82 (d, J_{CF4} = 3.3 Hz), 129.87 (d, J_{CF3} = 10.1 Hz), 129.38 (2C), 128.59 (d, J_{CF4} = 2.9 Hz), 127.73 (d, J_{CF3} = 13.9 Hz), 125.34, 125.07 (d, J_{CF4} = 3.6 Hz), 119.67 (d, J_{CF2} = 25.7 Hz), 116.21 (d, J_{CF2} = 22.5 Hz), 107.72 (d, J_{CF2} = 23.4 Hz), 17.77. **MS** (ESI) 375.70 [M+H], 373.65 [M-H].



(**3-Aminopropyl)triphenylphosphonium (15);** Compound **15** was prepared following a modified protocol from Zeng et al.[5] 3-bromopropan-1-amine (**14**) (1.0 g 4.56 mmol) was dissolved in 20 mL of *n*-BuOH. Triphenylphosphine (1.19 g, 4.56 mmol) was added and the mixture was heated at reflux overnight. The reaction mixture was cooled to RT for 15 min before the addition of 12 mL of hexanes. The mixture was then stirred for 30 min and filtered over a frit. The white solid was washed with another 12 mL hexane (2x) before triturating with a 50/50 solution of t-butyl methyl ether and hexane (3x). (3-Aminopropyl)triphenylphosphonium was isolated **(**1.69 g, 4.24 mmol, 93%) and used without further purification. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 7.94 – 7.73 (m, 15H), 3.98 – 3.86 (m, 2H), 3.10 – 3.02 (m, 2H), 1.97 – 1.82 (m, 2H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 135.56 (d, J_{CP4} = 3.1 Hz) (3C), 134.06 (d, J_{CP3} = 10.2 Hz) (6C), 130.83 (d, J_{CP2} = 12.5 Hz) (6C), 118.47 (d, J_{CP1} = 86.0 Hz) (3C), 39.24, 20.53, 18.88 (d, J_{CP1} = 52.8 Hz). **MS** (ESI) 320.15 [M+H].



(**3-(6-Fluoro-2-(2'-fluoro-[1,1'-biphenyl]-4-yl)-3-methylquinoline-4-carboxamido)propyl)triphenylphosphonium** (**16**): Compound **13 (**102 mg, 0.27 mmol) was dissolved in 3 mL of anhydrous 1,2 DCE. $SOCl_2$ (0.11 mL, 1.51 mmol) and 1 drop of DMF were added slowly then the mixture was heated at 60 °C for 2 h. (Acid chloride

formation was monitored via TLC; 1 drop of reaction mixture was added to a vial containing TEA and MeOH, then the mixture was checked for the presence of methyl ester). The reaction mixture was cooled to room temperature, concentrated, re-dissolved in 1,2 DCE, and re-concentrated. The acid chloride was re-dissolved in 3 mL 1,2 DCE and slowly added to a separate round bottom flask, which contained a stirring solution of compound **15 (**130 mg, 0.33 mmol), DIPEA (0.30 mL, 1.72 mmol), and 2.00 mL of anhydrous 1,2 DCE chilled over an ice bath. (Gas release was observed when the acid chloride was added to the solution). The mixture was stirred overnight. Upon completion, the mixture was concentrated, loaded onto silica column and eluted in a 1-30 % MeOH gradient in DCM with 0.5 M NH_3. Fractions containing desired compound was concentrated, re-dissolved in MeCN and crystallized by the addition of Et_2O/Hexane. The precipitant was poured over a frit and triturated with cold EtOAc, Et_2O, t-Butyl methyl ether, and hexane. Compound **16** was isolated as a tan solid with a red tint (89 mg, 0.13 mmol, 48%). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.88 (t, J = 5.6 Hz, 1H), 8.12 (dd, J = 9.2, 5.5 Hz, 1H), 7.95 – 7.74 (m, 15H), 7.74 – 7.66 (m, 4H), 7.63 (t, J = 8.0 Hz, 1H), 7.50 – 7.42 (m, 1H), 7.40 – 7.33 (m, 3H), 3.72 – 3.54 (m, 4H), 2.32 (s, 3H), 1.94 – 1.81 (m, 2H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 166.72, 160.67 (d, J_{CF1} = 248.2 Hz), 159.62 (d, J_{CF1} = 247.0 Hz), 159.57, 143.45, 143.41, 139.79, 135.63, 135.51 (d, J_{CP4} = 3.1 Hz) (3C), 134.03 (d, J_{CP3} = 10.3 Hz) (6C), 132.62 (d, J_{CF3} = 10.0 Hz), 131.25 (d, J_{CF4} = 3.3 Hz), 130.81, 130.76 (d, J_{CP2} = 12.4 Hz) (6C), 130.35 (d, J = 8.1 Hz), 129.74, 129.09 (d, J = 3.0 Hz), 128.14 (d, J_{CF3} = 13.0 Hz), 125.91, 125.54 (d, J_{CF4} = 3.4 Hz), 124.54 (d, J_{CF3} = 10.1 Hz), 119.99 (d, J_{CF2} = 25.5 Hz), 118.70 (d, J_{CP1} = 86.0 Hz) (3C), 116.68 (d, J_{CF2} =

22.5 Hz), 108.43, 108.25, 25.94, 22.56, 19.00 (d, J_{CP1} = 51.6 Hz), 17.86. **LCMS** (ESI) 678.15 [M+H], 98% purity based on HPLC chromatograph at 254 nm

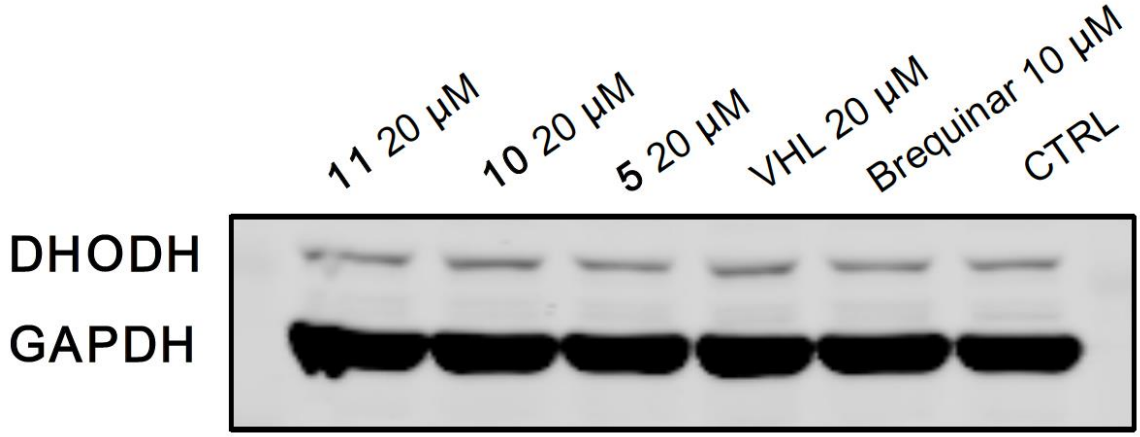
hDHODH expression and purification

The *h*DHODH construct was provided by the De Brabander lab at UT Southwestern.[6] *h*DHODH was expressed in *E. coli* Rosetta 2 (DE3) in LB medium with ampicillin (100 µg/mL) and 0.1 mM FMN. Cells were grown at 37 °C to OD_{600} = 0.6, then induced with 1 mM IPTG for 3 hours. Cells were harvested by centrifugation at 4,000 x g for 15 min at 4 °C. The pellet was re-suspended in lysis buffer (50 mM Tris-HCl, pH 8.5, 300 mM NaCl, 10% glycerol, 5 mM β-mercaptoethanol, 10 mM imidazole, 2% Triton X-100, 0.5 mM FMN, 200 µM PMSF, 1 mg/mL lysozyme). The cell suspension was incubated on ice for 2 hours, followed by sonication. The lysate was clarified by centrifugation at 35,000 x g for 20 min at 4 °C. After the supernatant was incubated with Ni-NTA resin for 1 h at 4 °C, the resin was loaded onto a column. The column was washed with wash buffer (50 mM Tris-HCl, pH 8.5, 300 mM NaCl, 10% glycerol, 5 mM β-mercaptoethanol, 25 mM imidazole, 0.1 mM FMN) and *h*DHODH was eluted with elution buffer (wash buffer containing 300 mM imidazole). Buffer exchange was carried out using an Amicon concentrator into storage buffer (100 mM HEPES, pH 8.0, 150 mM NaCl, 10% glycerol) and *h*DHODH was stored at -80 °C.

DHODH activity assay

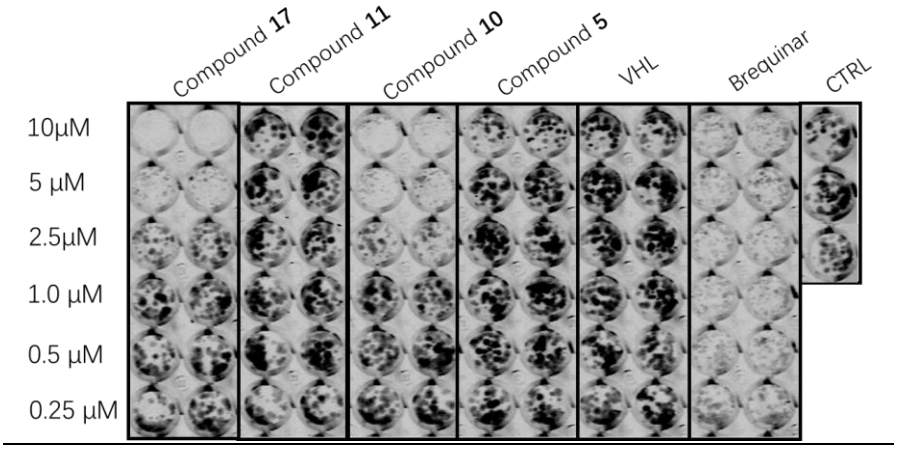
DHODH activity was monitored as previously described with modifications.[7] First, 1 µL of test compound (50x) or DMSO, 60 nM DHODH, 100 µM DCIP, and 20 µM CoQ_{10} (final concentrations for 50 µL) in the assay buffer (100 mM HEPES pH 8.0, 150 mM NaCl, 10% glycerol, 0.1% Triton X-100) in a total of 40 µL were incubated together for

30 min. The assay began with the addition of 10 µL of dihydroorotate to a final concentration of 200 µM. The reduction of DCIP was measured by monitoring the absorbance at 600 nm over 1 hr at room temperature using a microplate reader (BMG Labtech). Data were exported to Microsoft Excel for analysis and IC_{50} values were determined using Prism 6 software.



Supplemental Figure 1: Western blot analysis of HCT116 p53+/+ cells, untreated or treated with compounds.

No change of DHODH protein expression was observed in HCT116 p53+/+ cells after treatment of PROTAC compounds, precursor compound **5**, VHL, or brequinar for 12 h.



Supplemental Figure 2: Clonogenic assay performed on HCT116 p53+/+ cells after continuous treatment with varying doses of **10**, **11**, **5**, VHL, or brequinar for 7d.

Microsomal stability studies to determine $t_{1/2}$

Assays to determine microsomal stability and $t_{1/2}$ were performed by the University of Michigan Pharmacokinetics Core. A microsome solution was created, which contained 10 µL of mouse liver microsome (20 mg/mL) in 330 µL 0.1 M phosphate buffer (3.3 mM $MgCl_2$). Aliquots (40 µL) from stock solutions of test compounds (10 µM) were added and the mixture was incubated at 37 ℃ for 3 min. The enzymatic reactions were initiated by addition of 20 µL NADPH solution (freshly prepared containing 4 mg NADPH in 240 µL of 0.1M phosphate buffer (3.3 mM $MgCl_2$)). Aliquots of 40 µL were removed from reaction solutions and stopped by the addition of chilled acetonitrile, which contained 50 ng/mL of CE302, an internal standard, at the designated time points (0, 5, 10, 15, 30, 45, and 60 min). Verapamil was used as positive control with the same method. The incubated solution was centrifuged at 3500 g for 15 minutes and the supernatant was used for LC/MS/MS analysis. A ratio of the natural log peak area (compound peak area/ internal standard peak area) was plotted against time and used for data extrapolation. Chromatographic conditions utilized a 5 cm x 2.1 mm I.D. 3.5 µm XBridge column from Waters and utilized a gradient method of 5-95% MeCN in H_2O containing 0.1% formic acid.

Cell culture

HCT116 P53 +/+ and MiaPaca-2 cells were maintained in RPMI-1640 (Gibco) supplemented with 10% heat-inactivated FBS (Gibco). Cells were grown at 37 °C in a humidified atmosphere of 5% CO_2. For subculture and counting, cells were washed with DPBS (Gibco) without calcium or magnesium, incubated with 0.25% trypsin-EDTA solution (Gibco) for 5 min, neutralized with full medium, centrifuged, re-suspended with

culture medium and counted by Countess II FL. All experiments were performed using cells in exponential growth. Cells were routinely checked for Mycoplasma contamination.

Western blot

HCT116 p53+/+ cells were seeded into 6-well microtiter plates for 4 x 10^5 cells per well, and allowed to attach overnight before the addition of the dilution of compound (10X). After 12h treatment, the cells are lysated with RIPA buffer with the presence of protease inhibitors and phosphatase inhibitors. The cells are collected and centrifuged. The pellet was discarded. Protein concentration of whole-cell lysate in the supernatant was determined by BCA protein assay kit (Thermo Scientific). Proteins were resolved in 10% SDS/PAGE and electrotransferred to transfer membrane (Immobilon®-FL). After blocking with TBS blocking buffer (Thermo Scientific), membranes were probed with DHODH primary antibody (Santa Cruz Biotechnology; rabbit; 1:1000) and GAPDH primary antibody (Signaling; rabbit; 1:4000) in 5% BSA (EMD Millipore corporation) in TBST (Tris-buffered saline, 0.1% Tween 20) and then washed and incubated with goat anti-rabit IgG (H&L) secondary antibody (Dylight 800 4x PEG conjugated; Thermo Scientific; 1:4000). The membrane was imaged by Odyssey® CLx Imaging System.

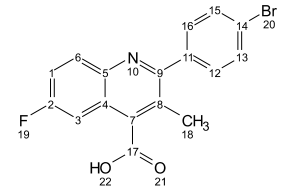
Clonogenic assay

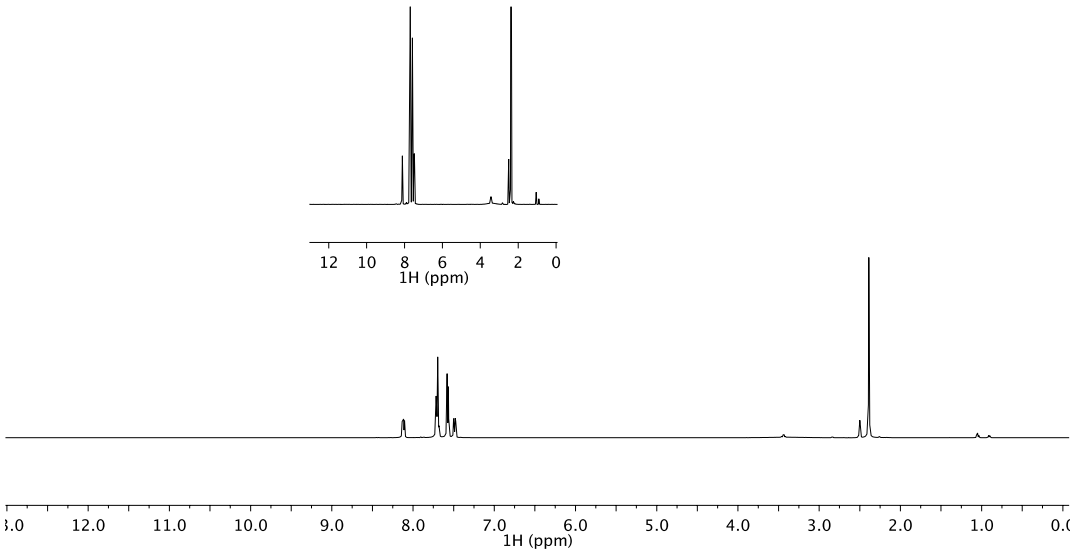
HCT116 p53+/+ cells were seeded 500 cells per well into 24-well plates for or 200 cells per well into 96-well plates, and allowed to attach overnight before the addition of compounds. After 7-day contentious treatment, the medium was removed and crystal violet solution was added to fix and stain the colonies for 20 min. Crystal violet was

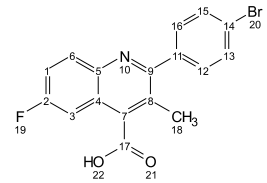
removed and the colonies were washed with ddH2O for three times. The colonies were imaged by Odyssey® CLx Imaging System.

Growth inhibition assay

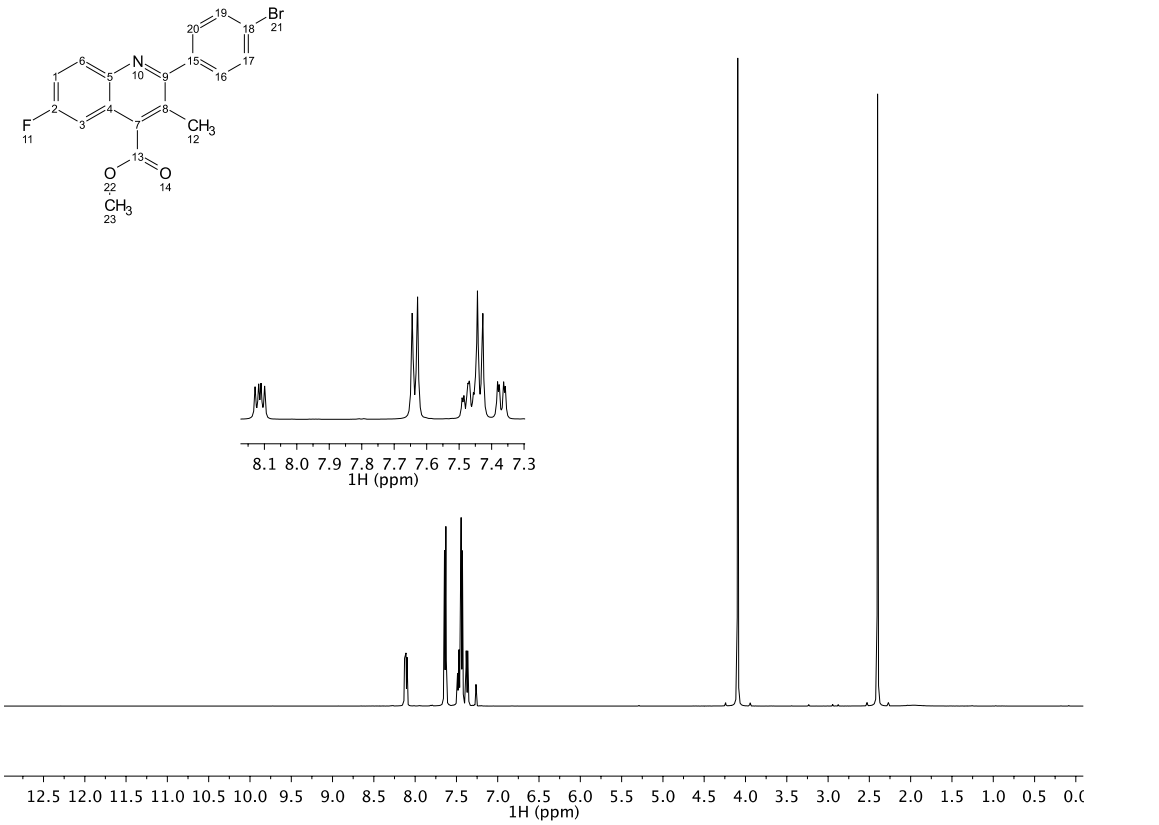
HCT116 p53+/+ cells or MiaPaCa-2 cells were seeded 2500-3000 cells per well in 96-well microtiter plates, and allowed to attach overnight before the addition of the serial dilution of compounds (10X). After 72h, cells were incubated with 0.3 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (VWR) for an additional 3h at 37 °C. After removal of the supernatant, DMSO was added to the wells, and the absorbance was read at 570 nm. All assays were performed in triplicate. Percentage of cell growth inhibition was expressed as $(1 - A/C) \times 100\%$ (A and C were the absorbance values from experimental and control cells, respectively). IC_{50} values were determined for each drug from nonlinear regression analysis of log (drug concentration) vs. percentage of cell growth inhibition using Prism 7.0. SD or SEM was calculated based on the IC_{50} values obtained from at least three independent experiment.

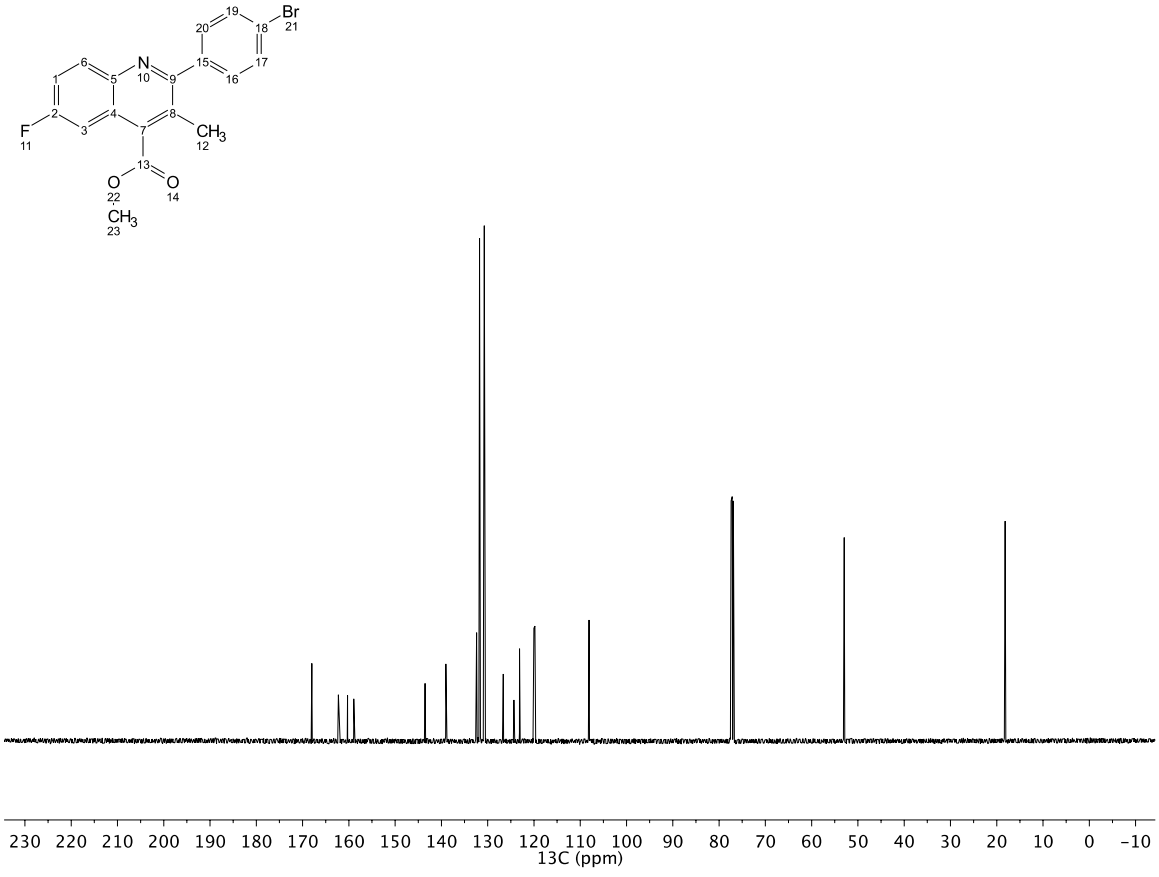

Br
20
15
16
14
N
10
6
5
9
11
13
12
1
2
4
8
F
19
3
7
CH3
18
17
HO
22
O
21

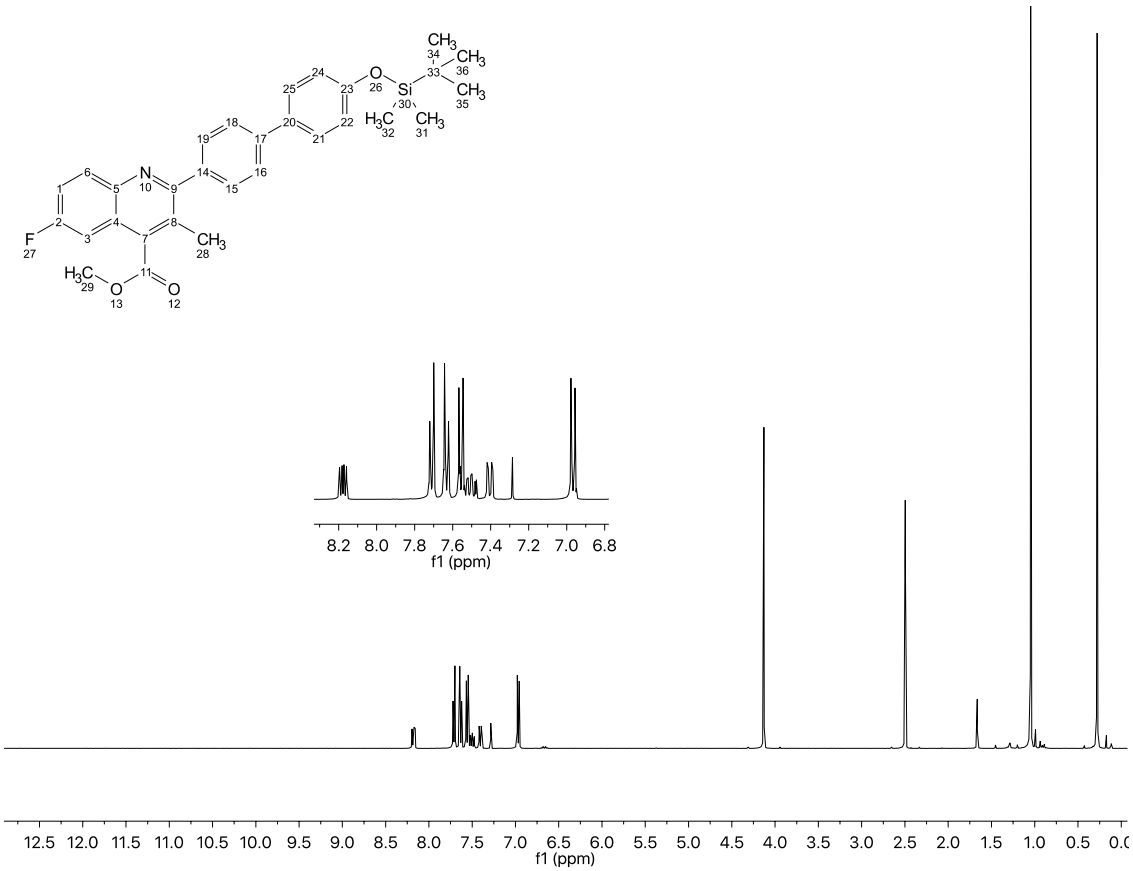

12 10 8 6 4 2 0
1H (ppm)
12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0
1H (ppm)

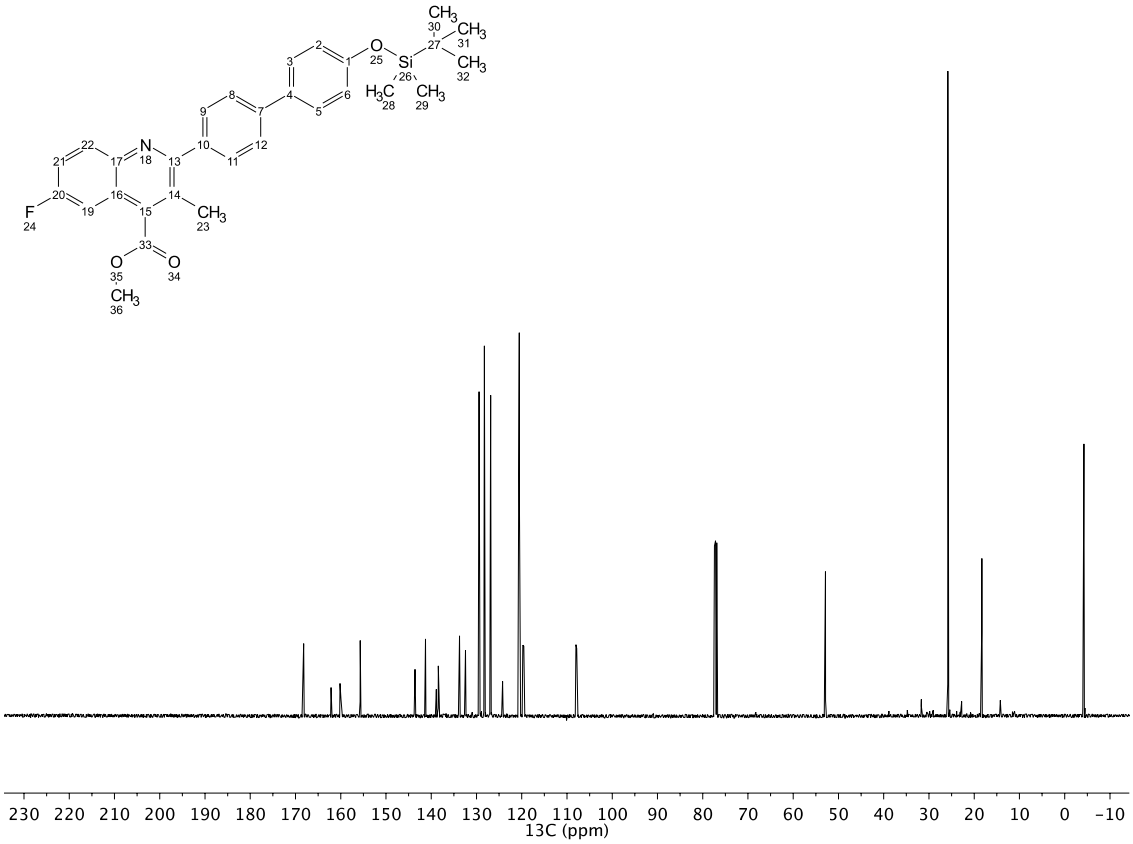

Br
20
15
16
14
N
10
6
5
9
11
13
12
1
2
4
8
F
19
3
7
CH3
18
17
HO
22
O
21

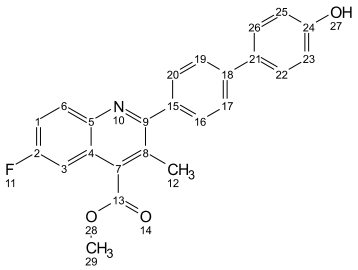
133.0 132.0
13C (ppm)
124 123 122 121 120
13C (ppm)
109 108 107
13C (ppm)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)

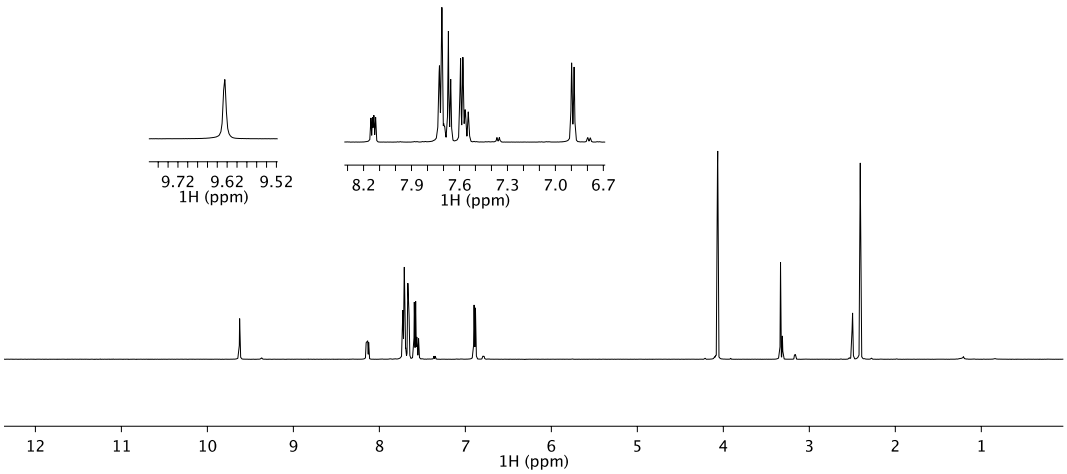

Br
21
19
20
18
N
10
15
16
17
1
6
5
9
2
4
8
F
11
3
7
CH3
12
13
O
22
O
14
CH3
23
8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3
1H (ppm)
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
1H (ppm)

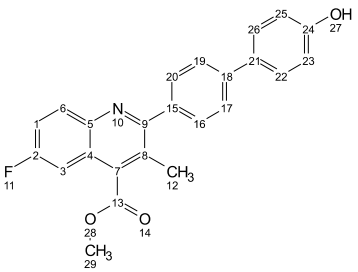

Br
21
19
20
18
N
10
15
16
17
1
6
5
9
2
4
8
F
11
3
7
CH3
12
13
O
22
O
14
CH3
23
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)

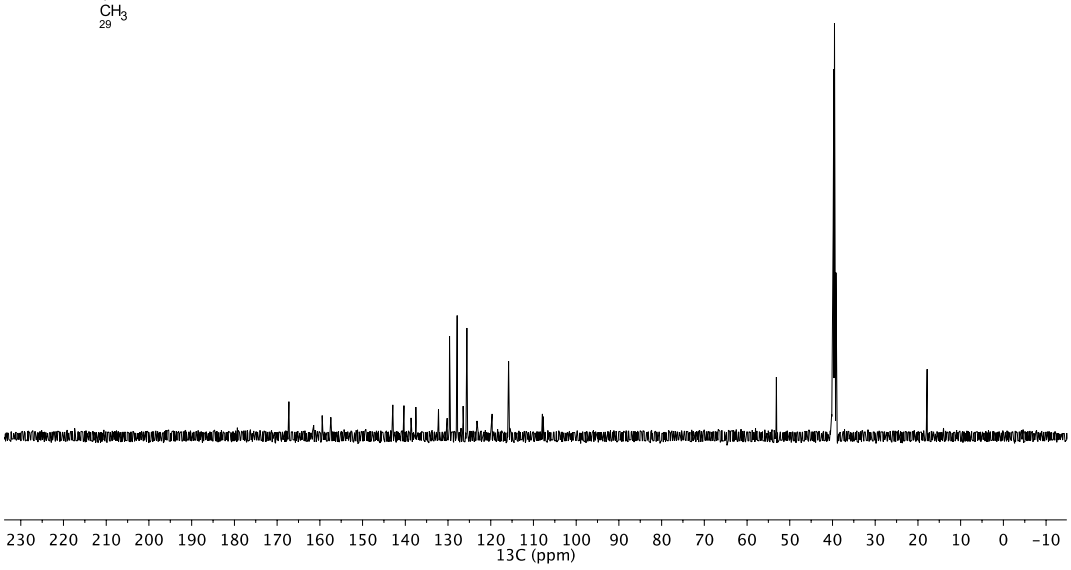

8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8
f1 (ppm)
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

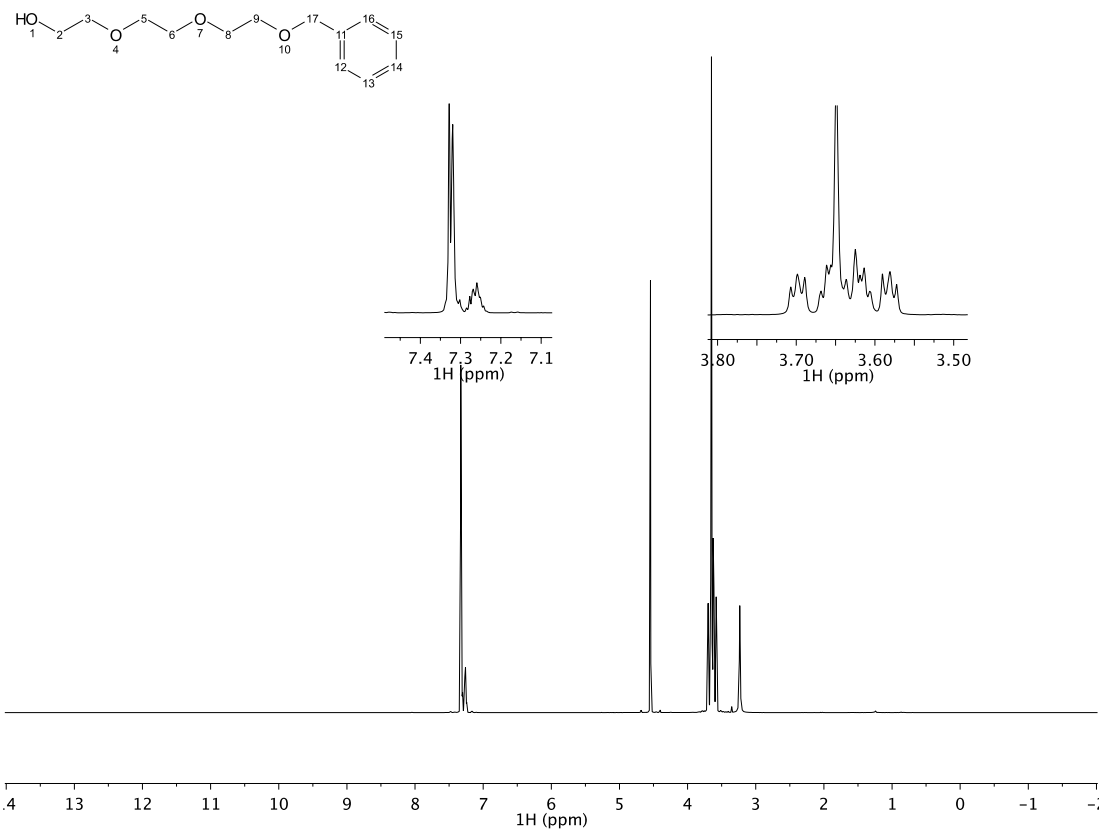

230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)

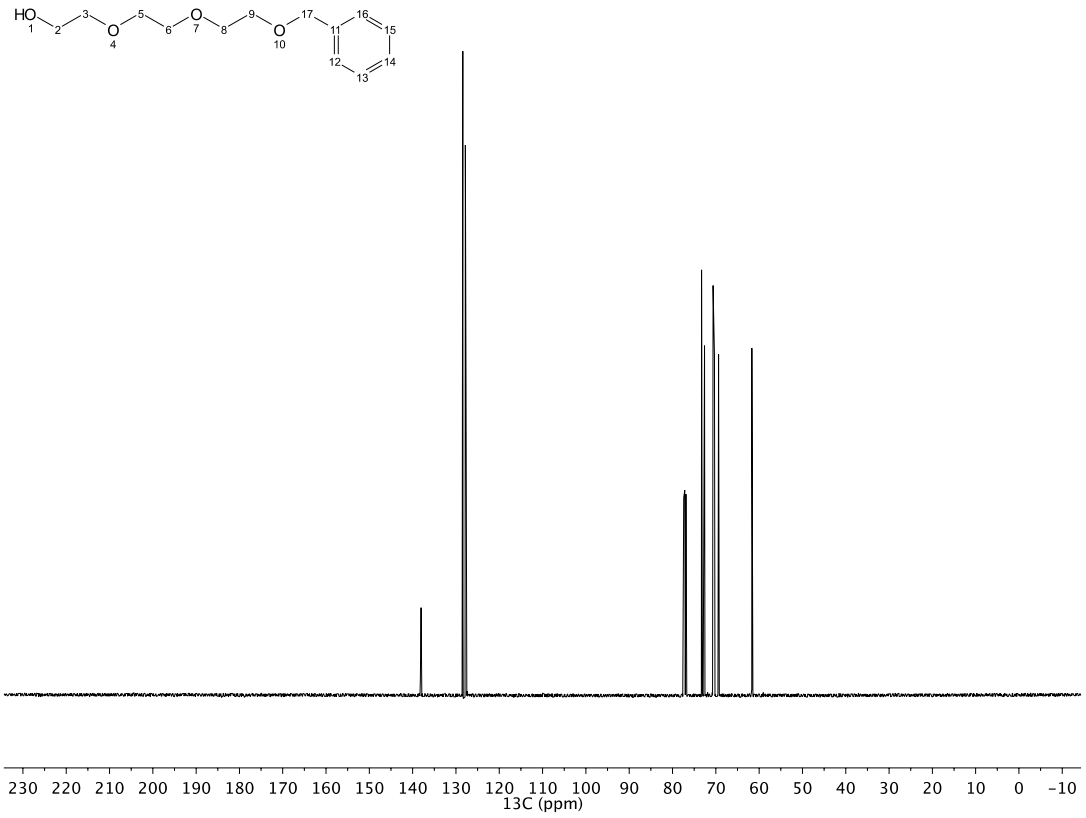

OH
27
25
26
24
23
21
22
19
20
18
15
16
17
N
10
9
6
5
1
2
3
4
7
8
F
11
CH3
12
13
O
28
O
14
CH3
29

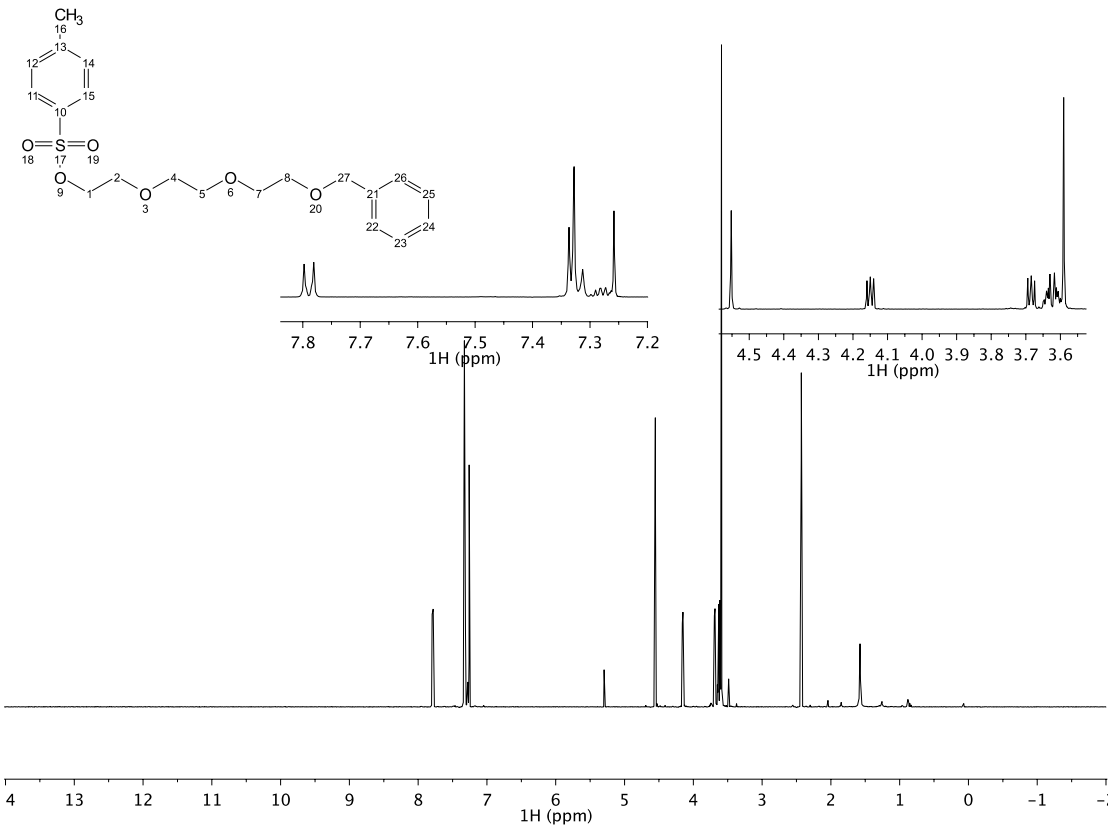

9.72 9.62 9.52
1H (ppm)
8.2 7.9 7.6 7.3 7.0 6.7
1H (ppm)
12 11 10 9 8 7 6 5 4 3 2 1
1H (ppm)

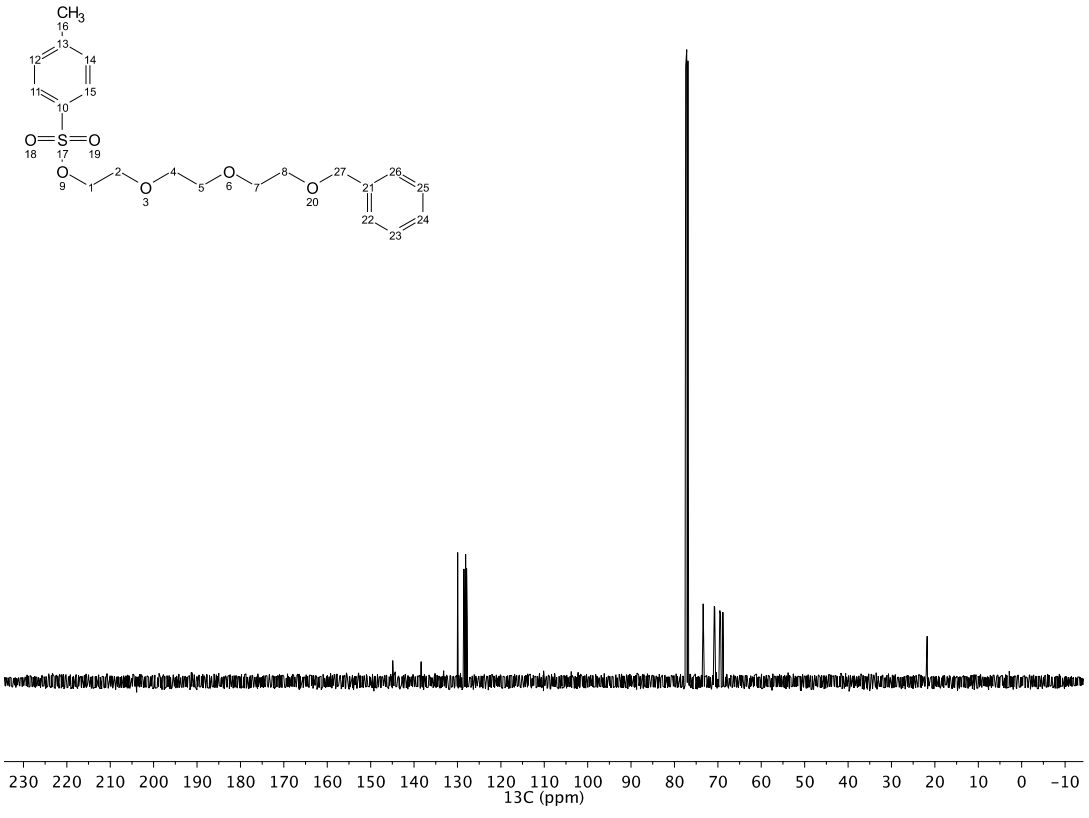

OH
27
25
26
24
23
21
22
19
20
18
15
16
17
N
10
9
6
5
1
2
3
4
7
8
F
11
CH3
12
13
O
28
O
14
CH3
29

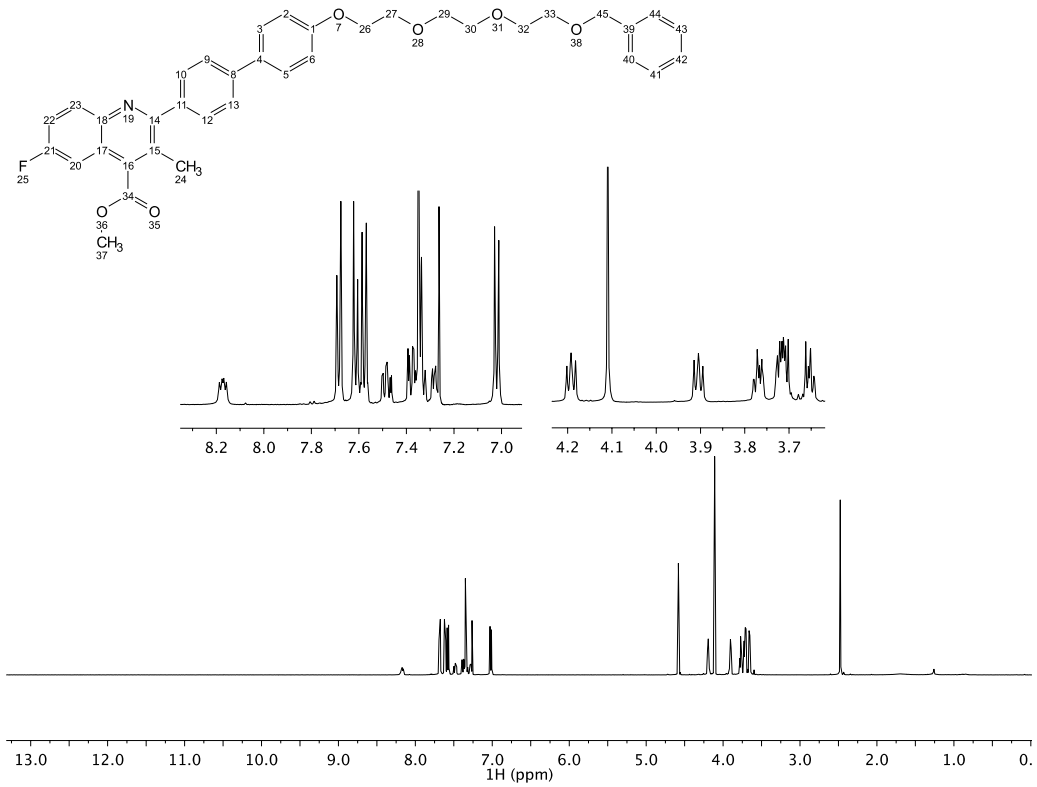

230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)

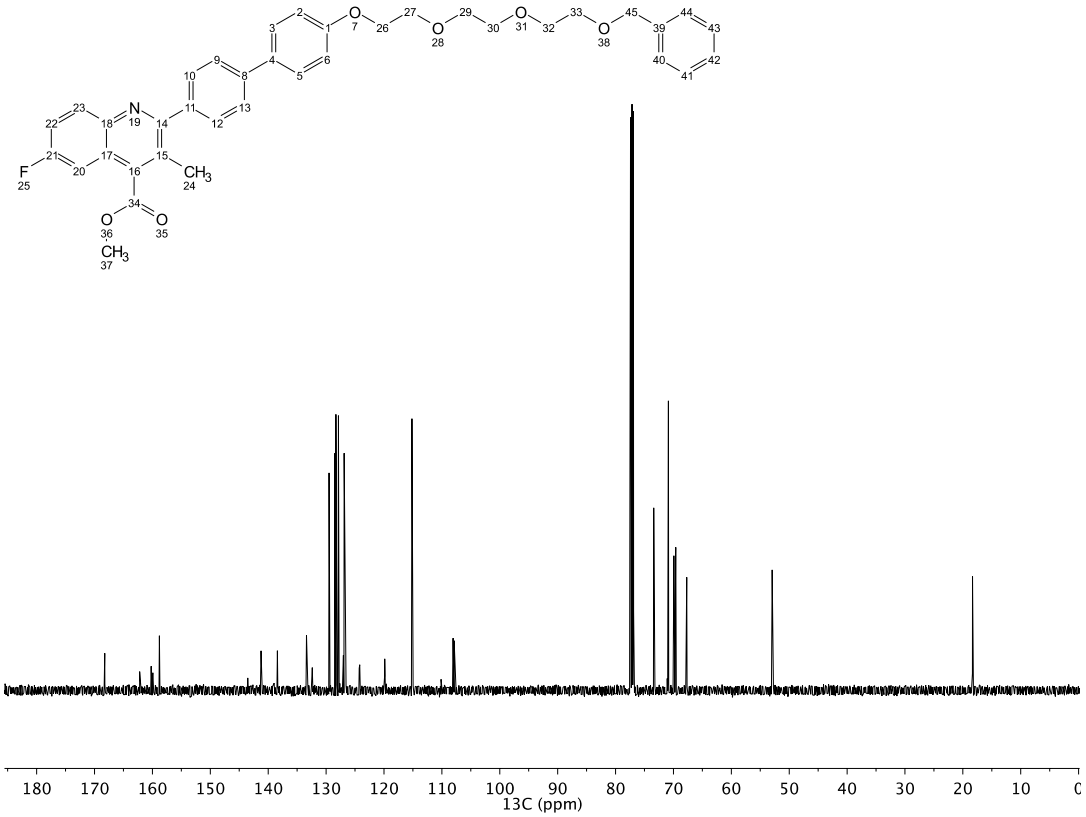

HO
O
O
O
7.4 7.3 7.2 7.1
1H (ppm)
3.80 3.70 3.60 3.50
1H (ppm)
1H (ppm)

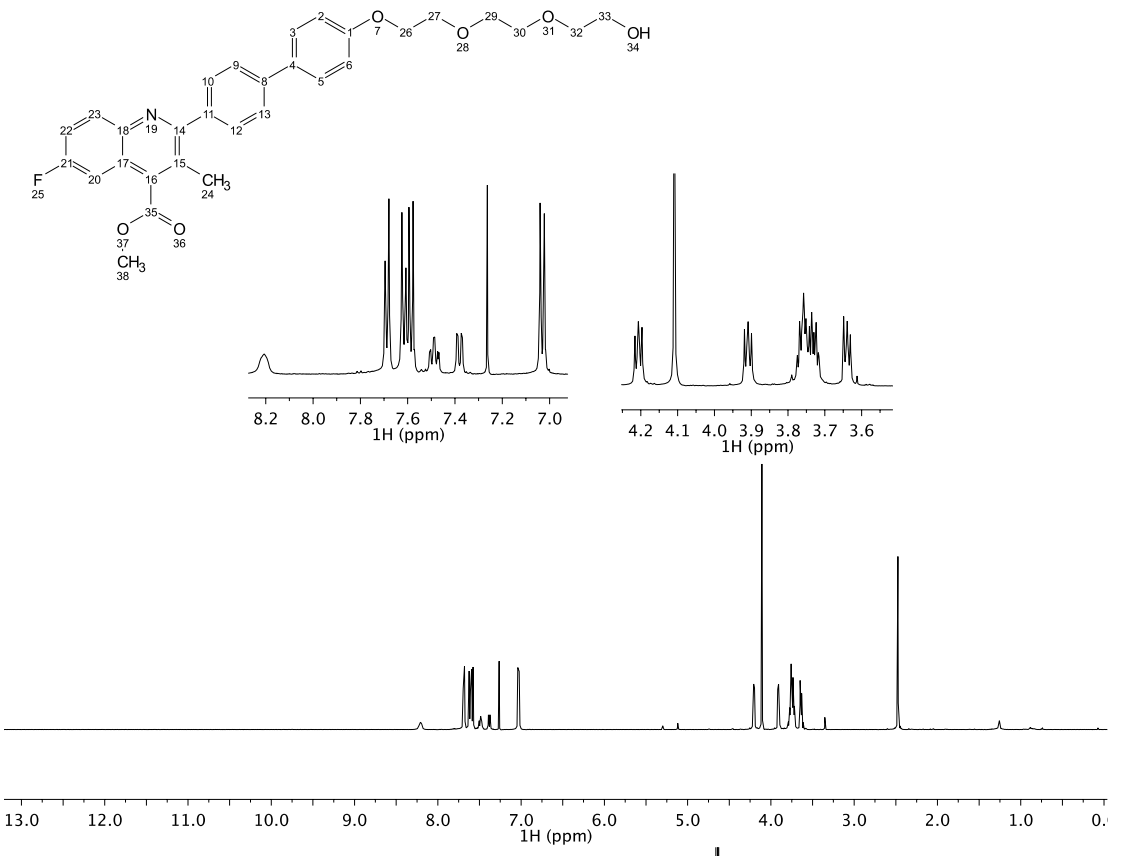

HO
O
O
O
13C (ppm)

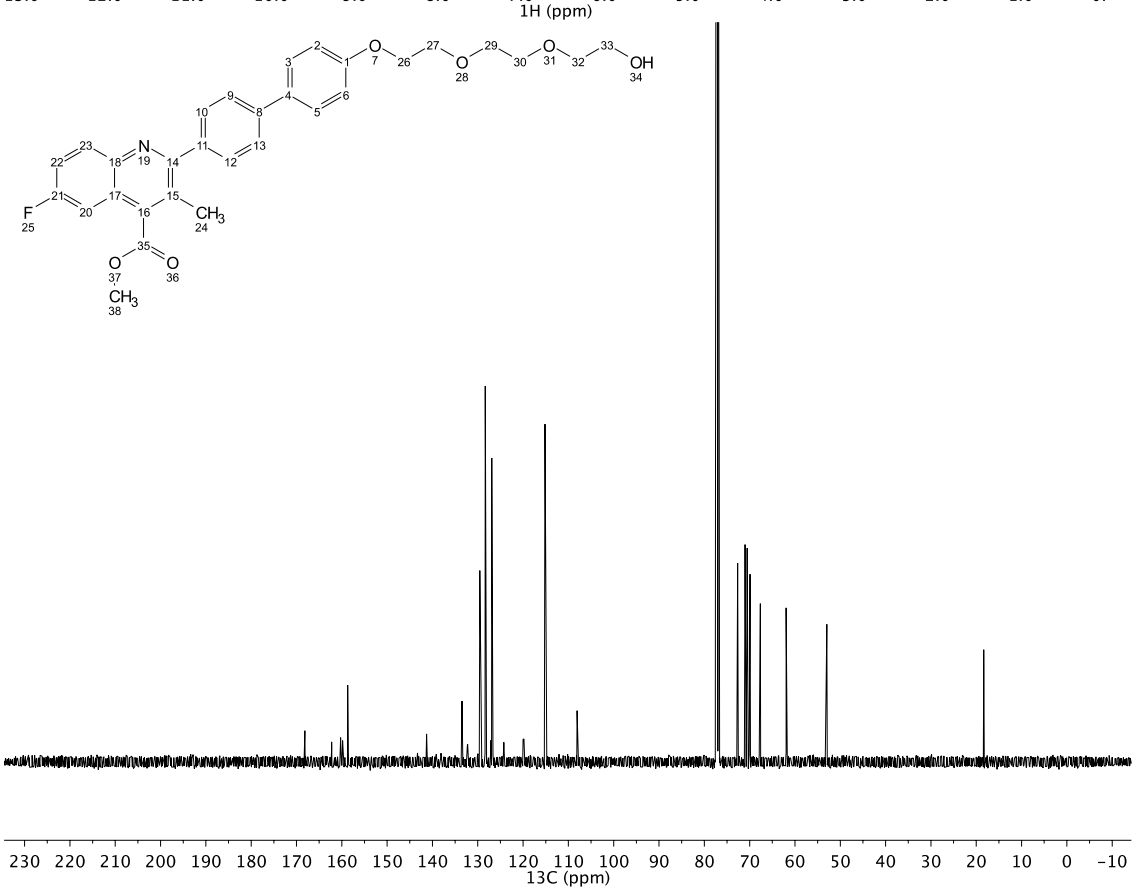

1H (ppm)
1H (ppm)
1H (ppm)

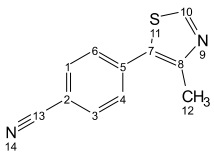

13C (ppm)

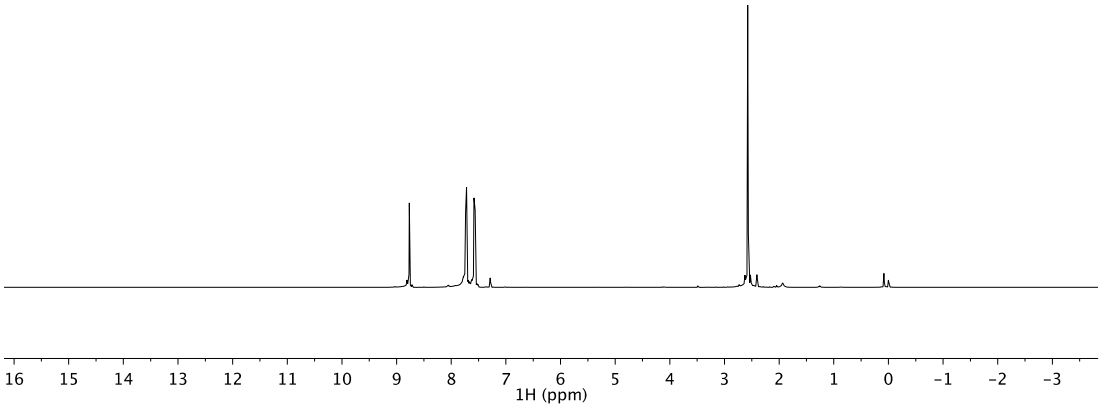

8.2 8.0 7.8 7.6 7.4 7.2 7.0
4.2 4.1 4.0 3.9 3.8 3.7
13.0 12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.
1H (ppm)

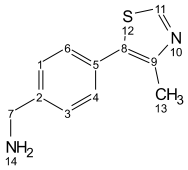

180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
13C (ppm)

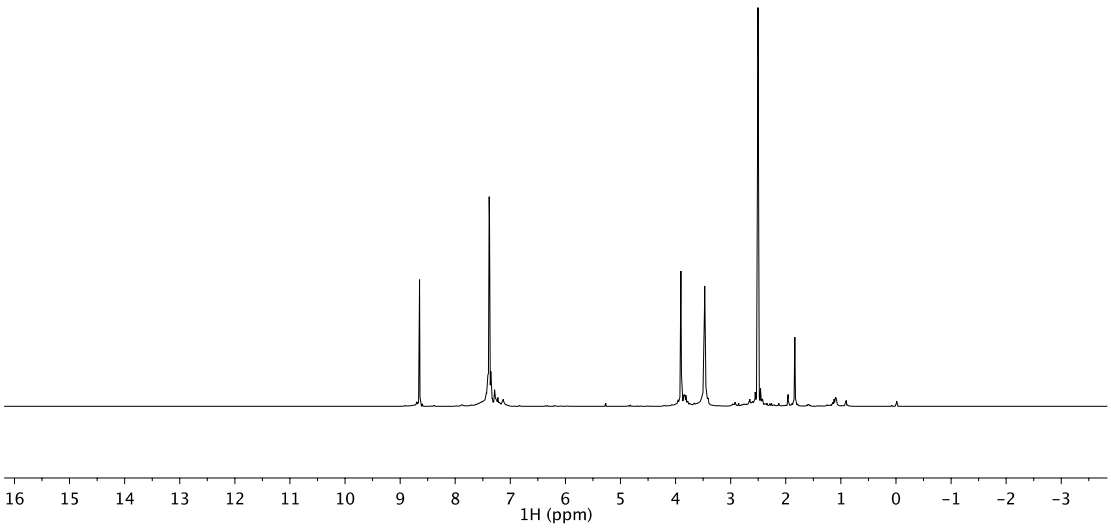

8.2 8.0 7.8 7.6 7.4 7.2 7.0
1H (ppm)
4.2 4.1 4.0 3.9 3.8 3.7 3.6
1H (ppm)
13.0 12.0 11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0
1H (ppm)

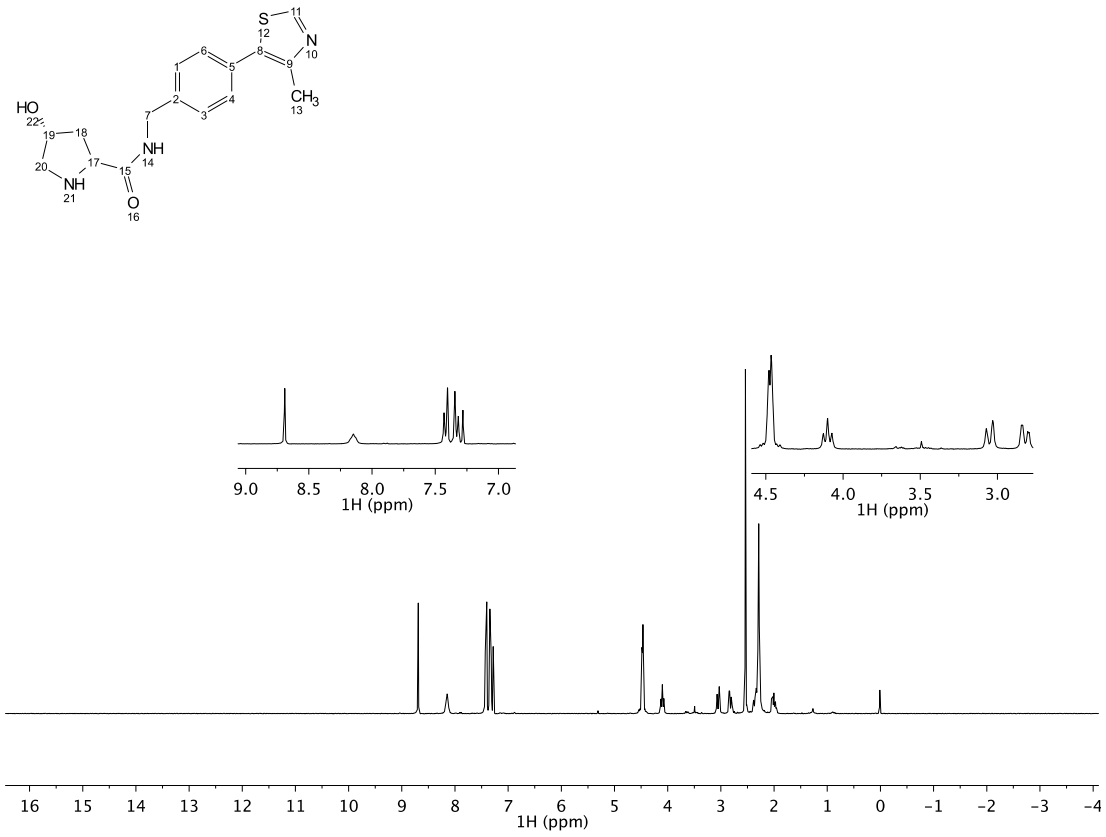

230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)

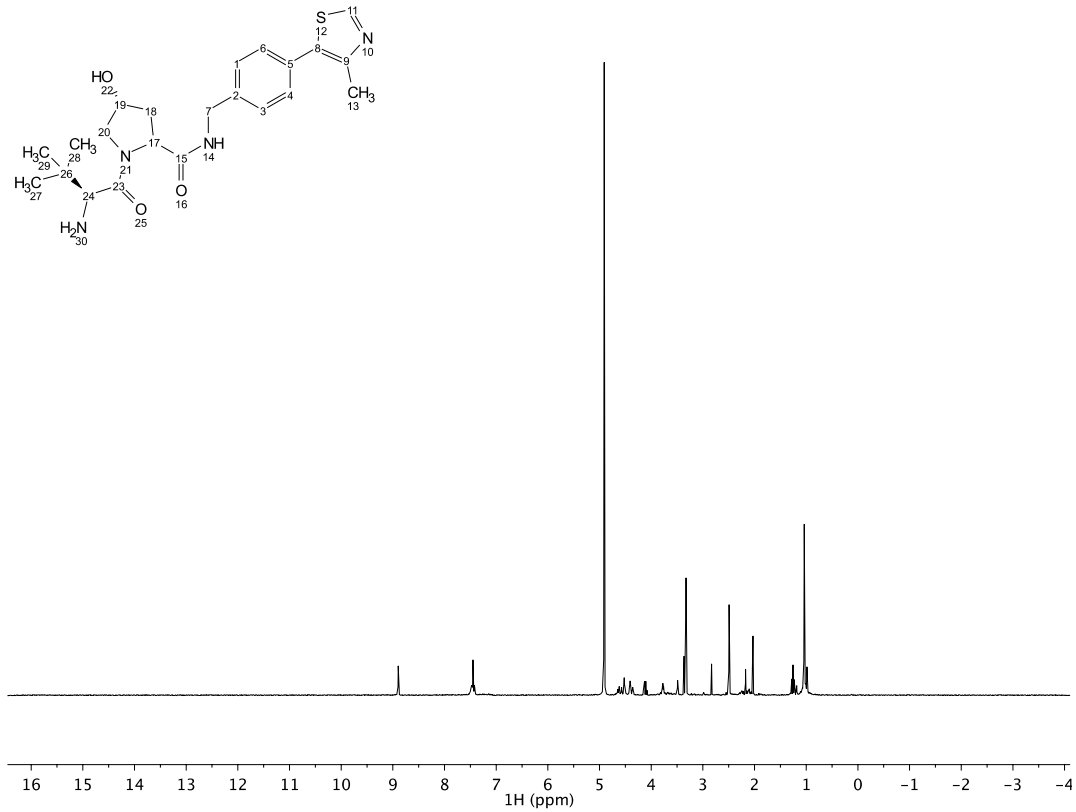

S
10
11
N
9
7
6
5
8
1
2
4
3
CH3
12
13
N
14

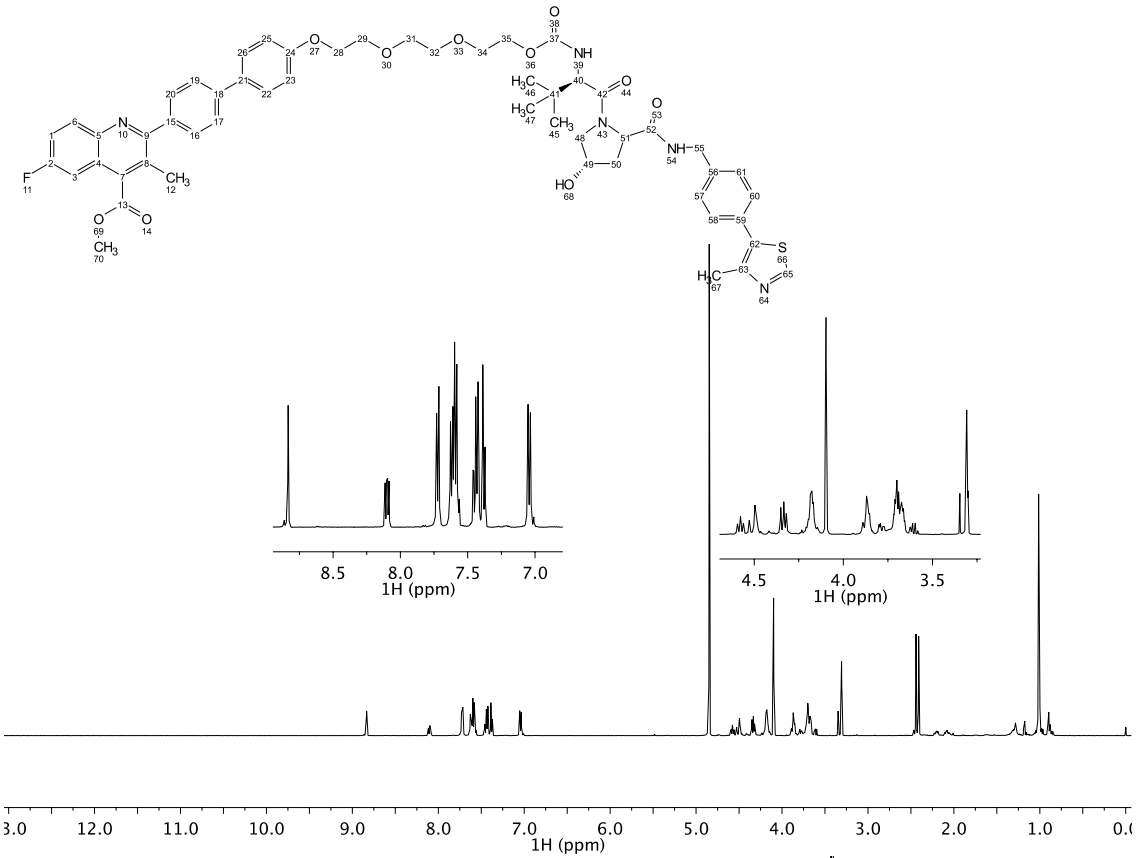

16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2 −3
1H (ppm)

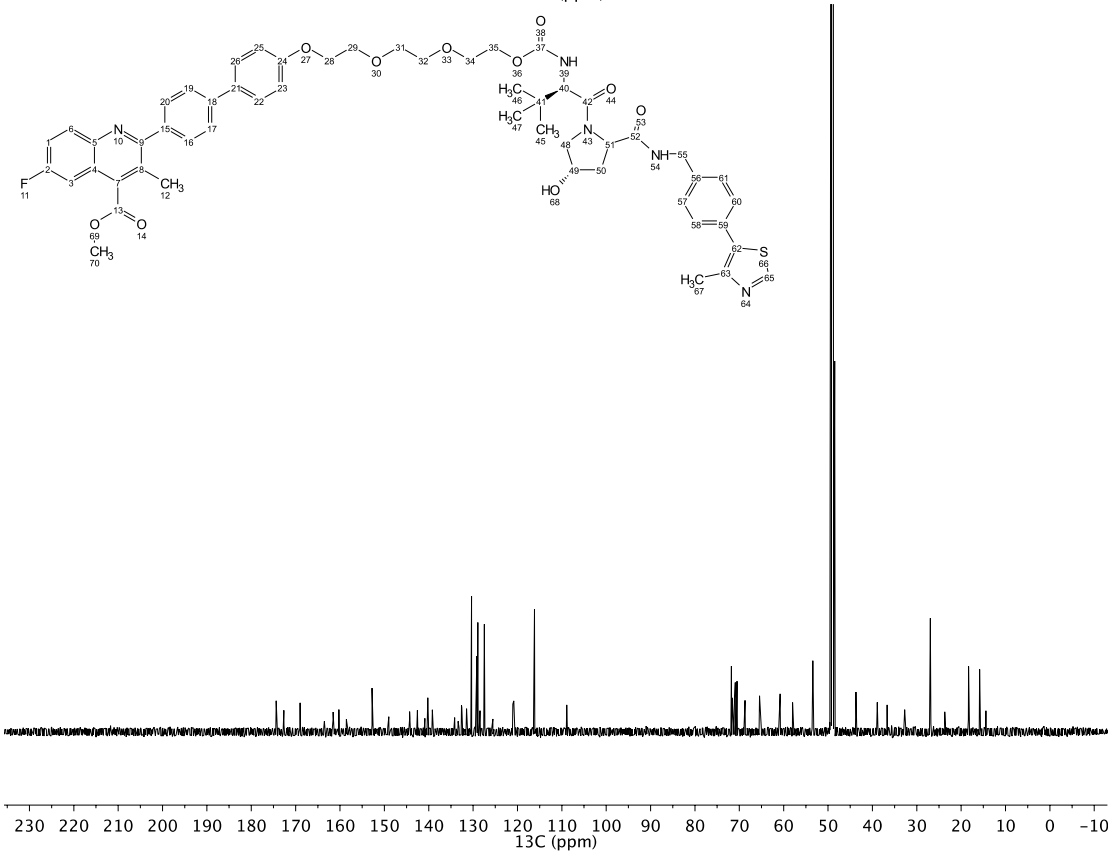

S
11
12
N
10
8
6
5
9
1
2
4
3
CH3
13
7
NH2
14

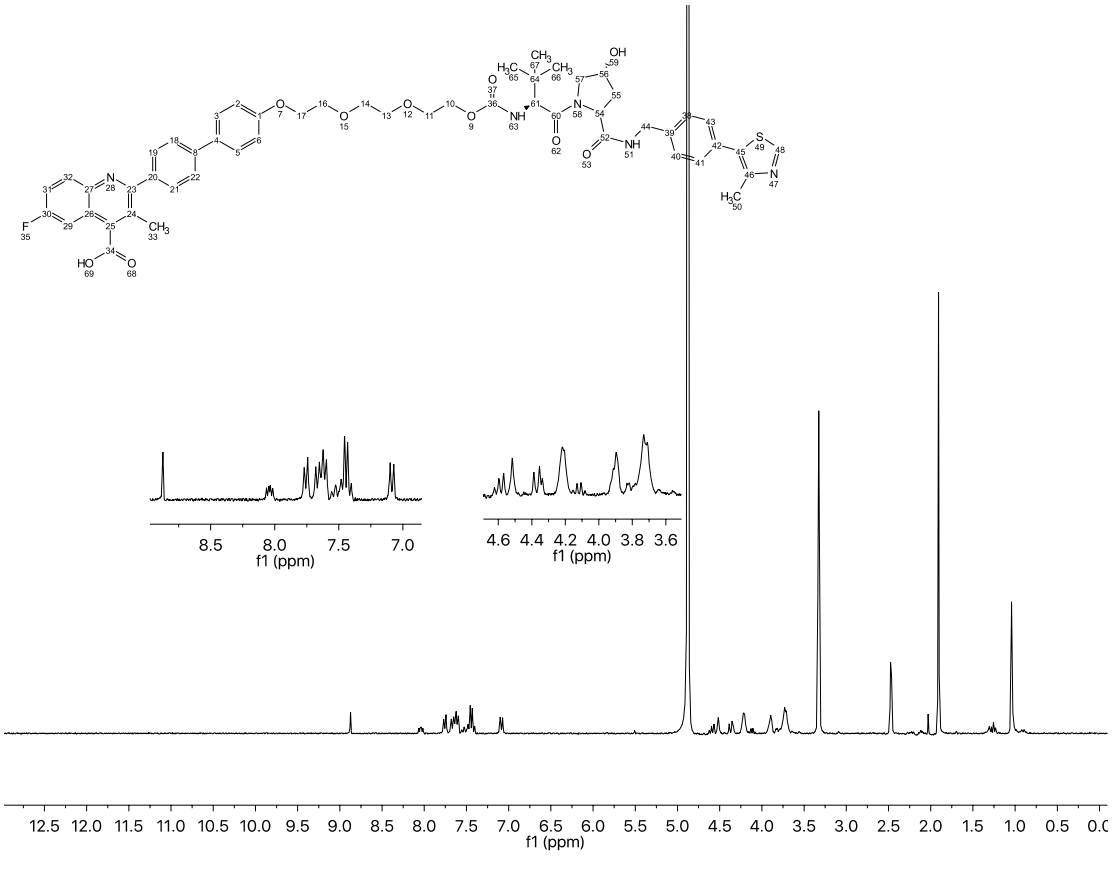

16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2 −3
1H (ppm)

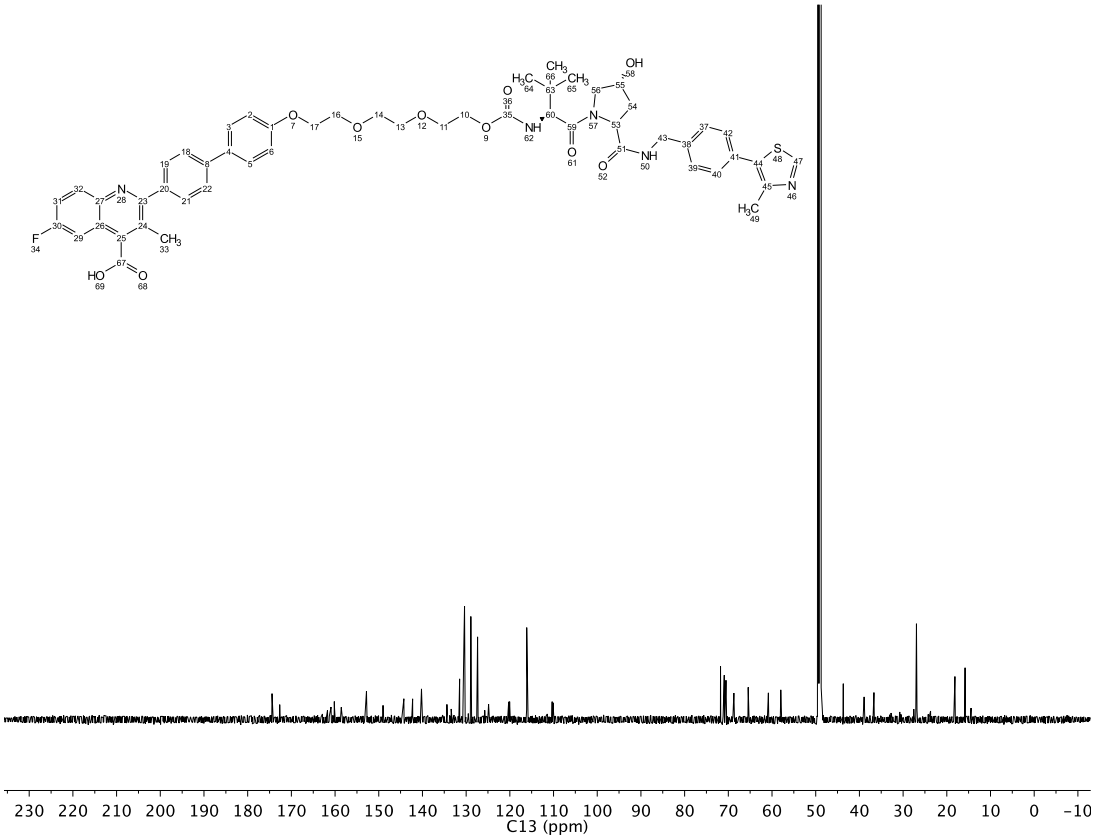

HO
22
19
18
20
17
NH
21
15
14
O
16
7
1
2
3
4
5
6
8
9
S
12
11
N
10
CH3
13
9.0
8.5
8.0
7.5
7.0
1H (ppm)
4.5
4.0
3.5
3.0
1H (ppm)
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
−1
−2
−3
−4
1H (ppm)

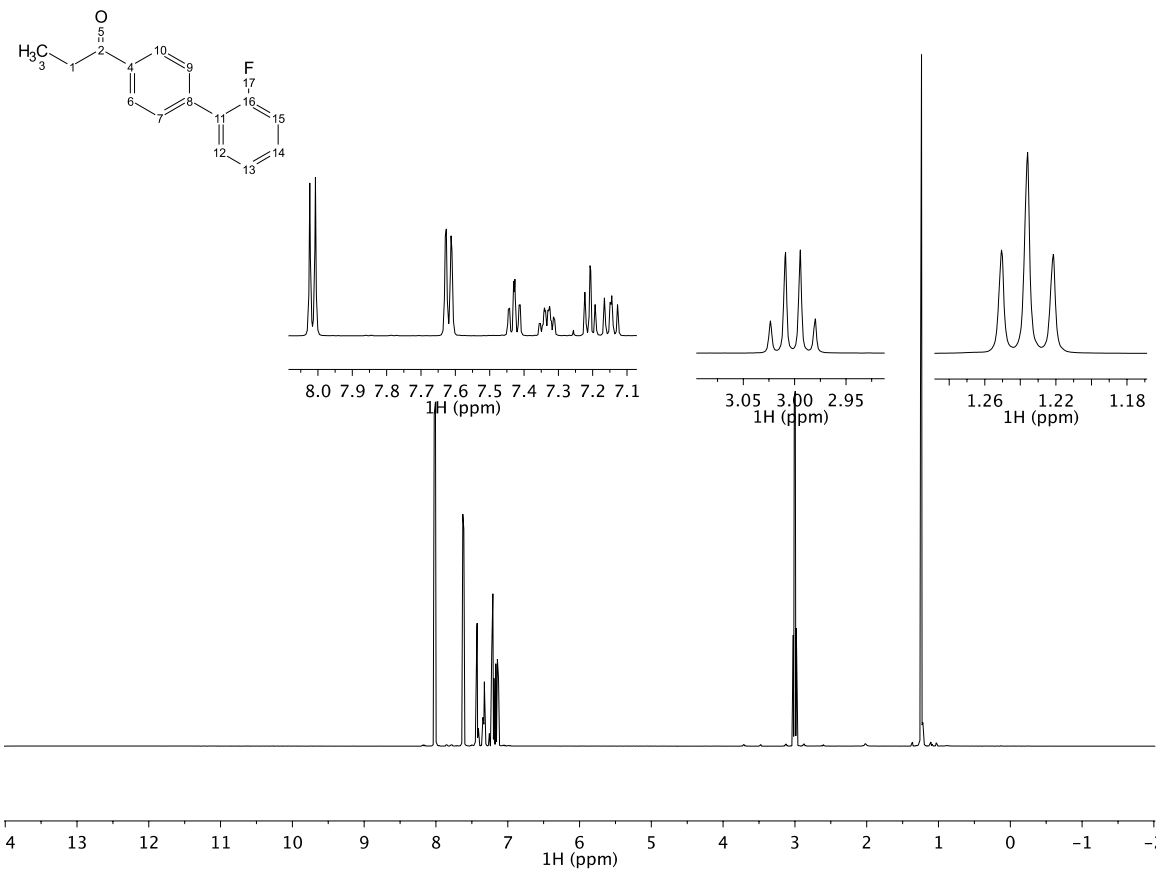

HO
22
19
18
20
17
N
21
CH3
28
H3C
29
H3C
27
26
24
23
O
25
H2N
30
15
NH
14
O
16
7
1
2
3
4
5
6
8
9
S
12
11
N
10
CH3
13
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
−1
−2
−3
−4
1H (ppm)

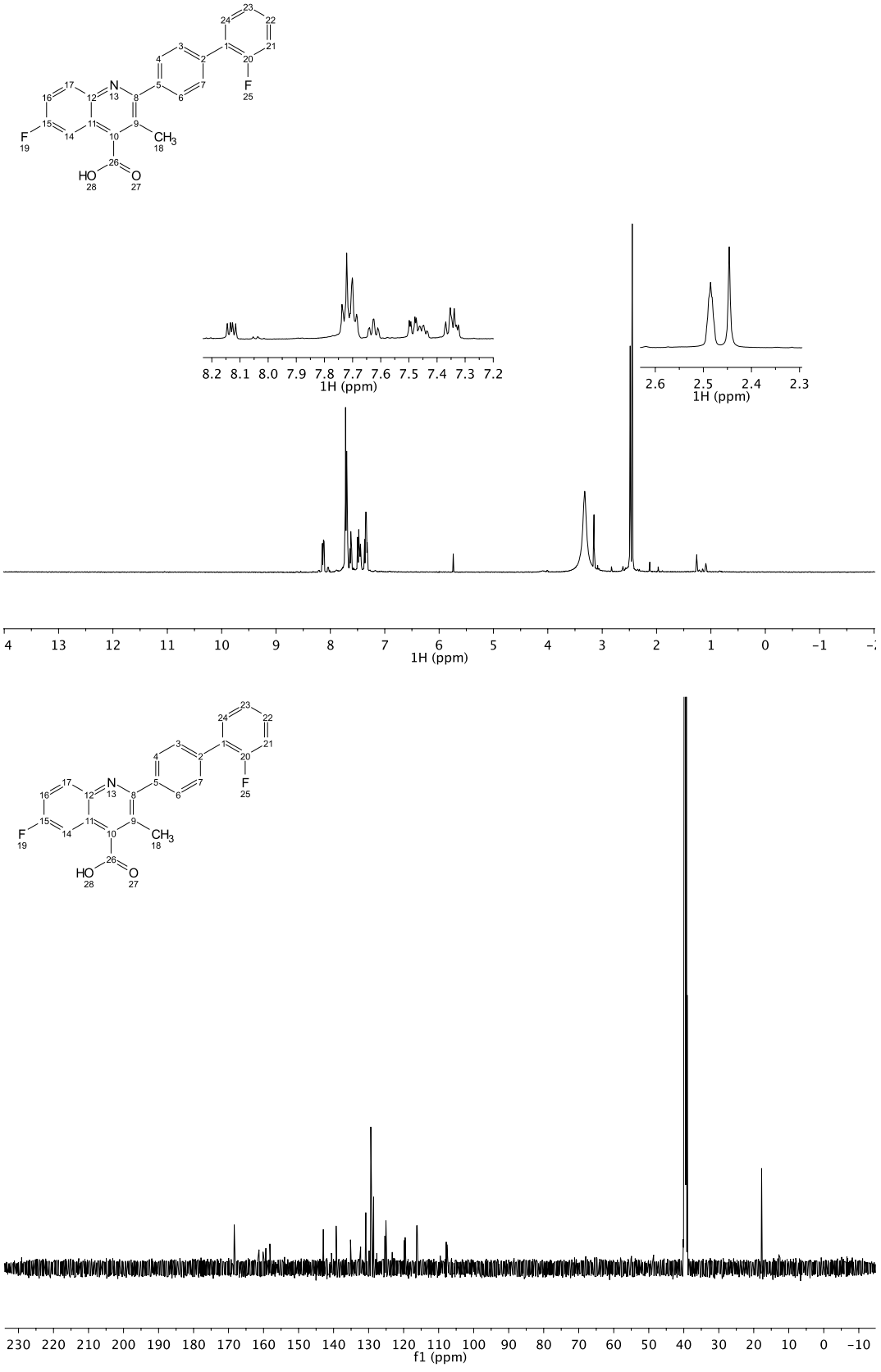

8.5
8.0
7.5
7.0
1H (ppm)
4.5
4.0
3.5
1H (ppm)
3.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
1H (ppm)

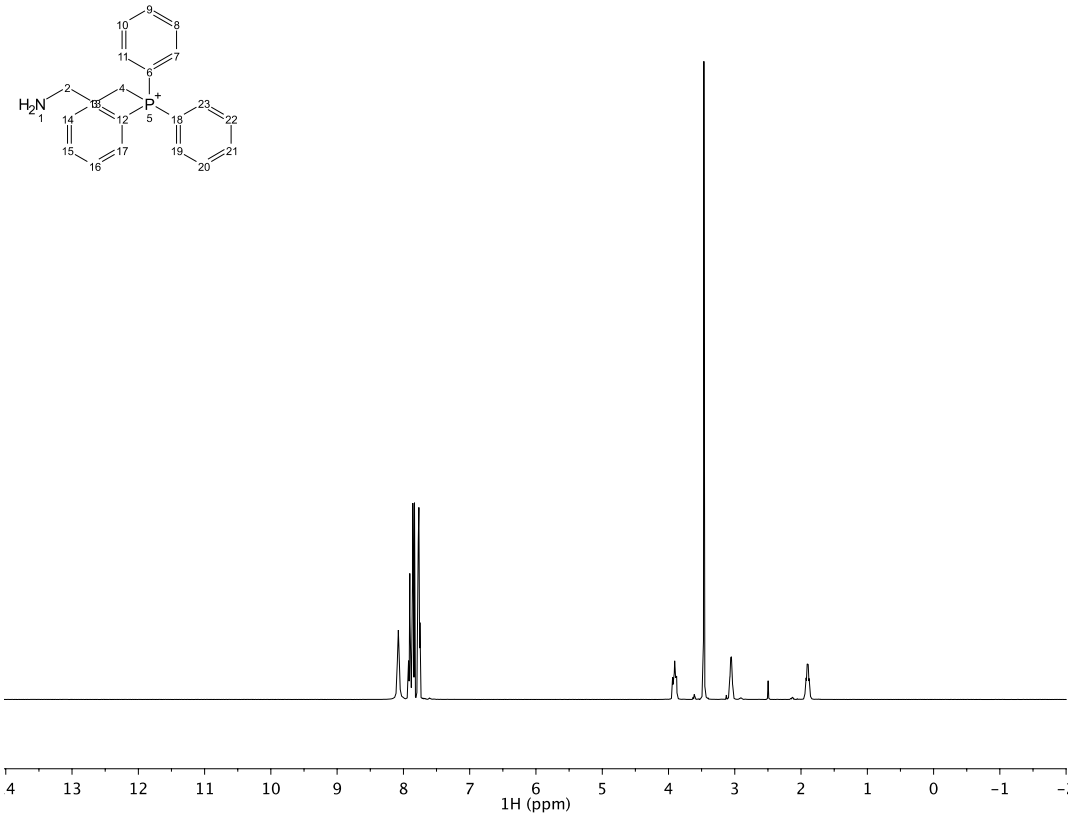

230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
13C (ppm)

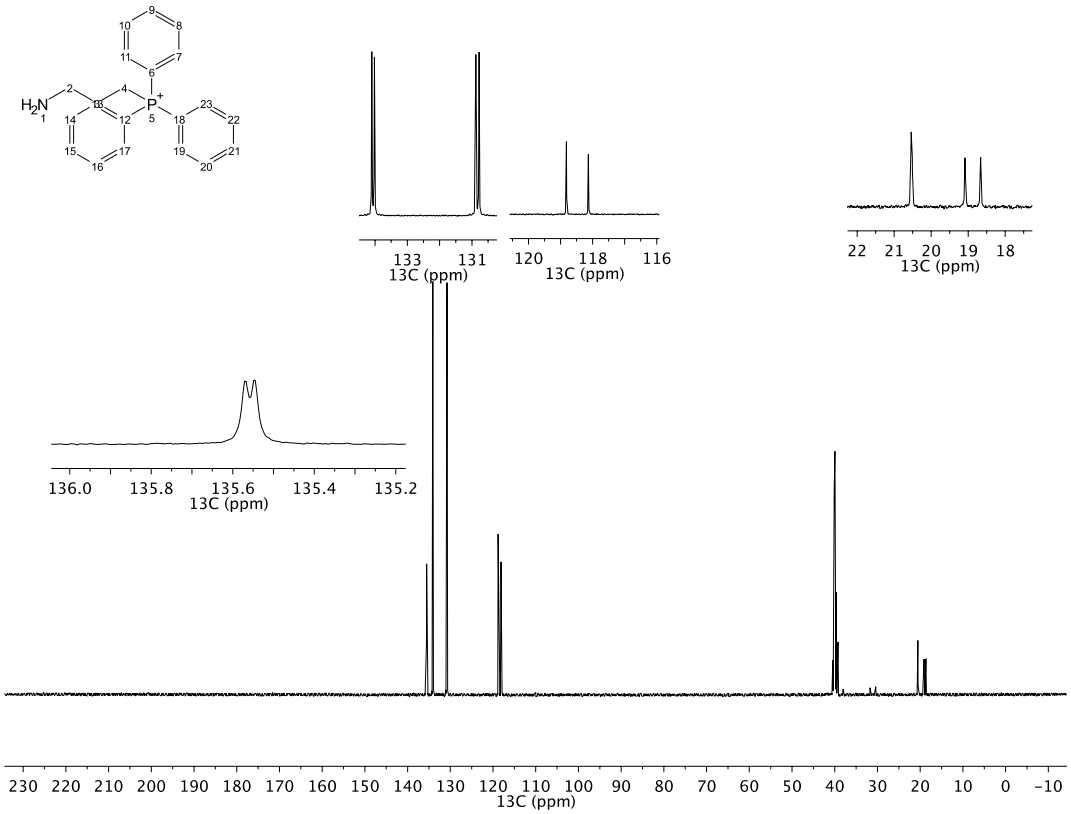

8.5 8.0 7.5 7.0
f1 (ppm)
4.6 4.4 4.2 4.0 3.8 3.6
f1 (ppm)
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

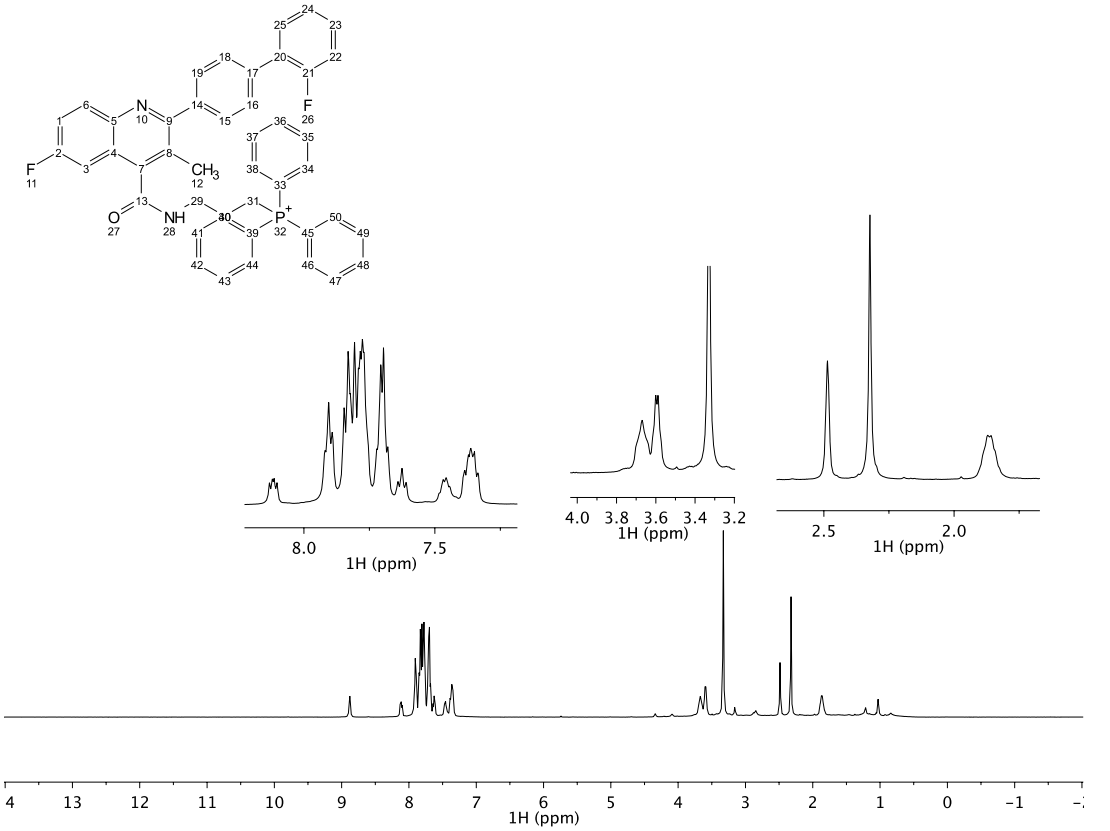

230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
C13 (ppm)

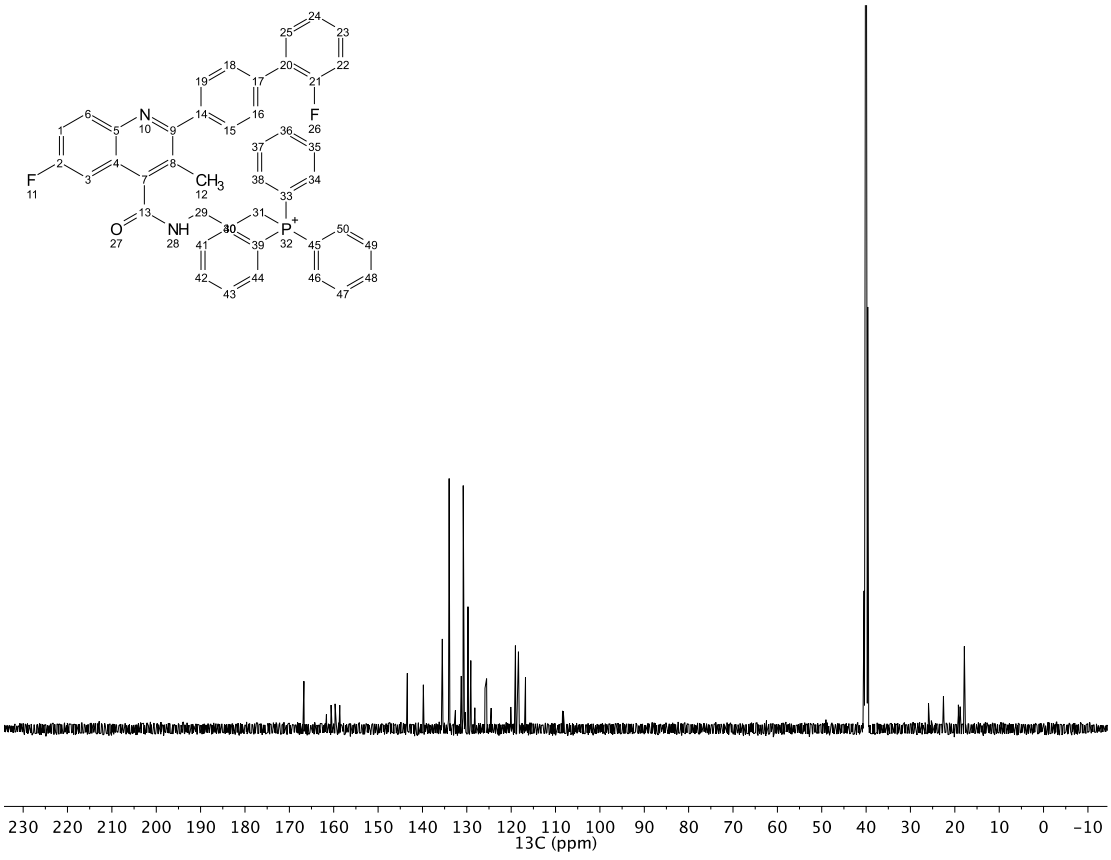

O
5
H3C
3
1
2
4
10
9
F
17
6
7
8
11
16
15
12
14
13
8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1
1H (ppm)
3.05 3.00 2.95
1H (ppm)
1.26 1.22 1.18
1H (ppm)
4 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1
1H (ppm)

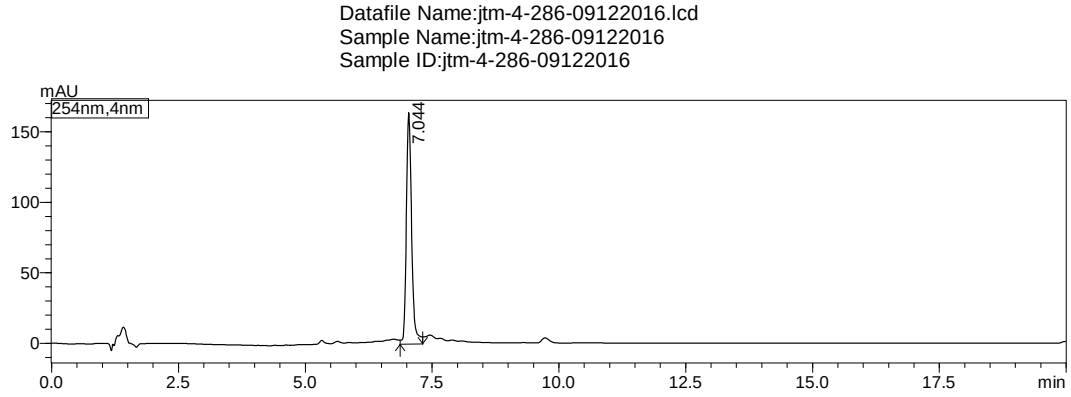

8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2
1H (ppm)
2.6 2.5 2.4 2.3
1H (ppm)
4 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1
1H (ppm)
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)


1H (ppm)

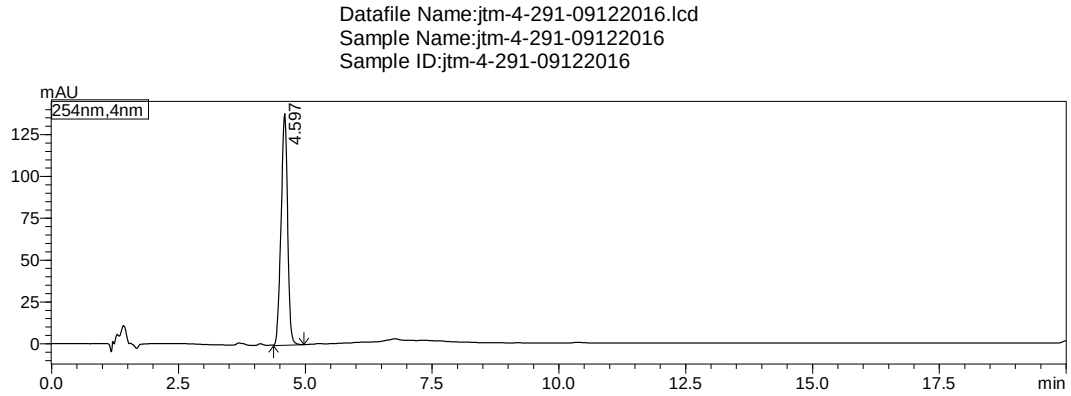

13C (ppm)
13C (ppm)
13C (ppm)
13C (ppm)
13C (ppm)


8.0
7.5
1H (ppm)
4.0
3.8
3.6
3.4
3.2
1H (ppm)
2.5
2.0
1H (ppm)
1H (ppm)

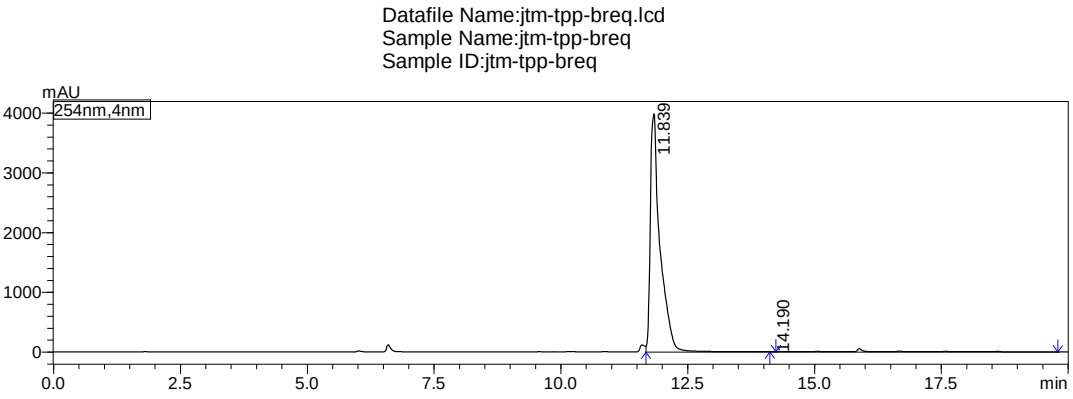

13C (ppm)



Chromatogram at 254 nm for **10**



Chromatogram at 254 nm for **11**



Chromatogram at 254 nm for **16**

References:

[1] H. E. Gottlieb, V. Kotlyar and A. Nudelman, *J Org Chem* **1997**, *62*, 7512-7515.
[2] Z. X. Jiang and Y. B. Yu, *Synthesis (Stuttg)* **2008**, *2008*, 215-220.
[3] D. L. Buckley, J. L. Gustafson, I. Van Molle, A. G. Roth, H. S. Tae, P. C. Gareiss, W. L. Jorgensen, A. Ciulli and C. M. Crews, *Angew Chem Int Ed Engl* **2012**, *51*, 11463-11467.
[4] C. Galdeano, M. S. Gadd, P. Soares, S. Scaffidi, I. Van Molle, I. Birced, S. Hewitt, D. M. Dias and A. Ciulli, *J Med Chem* **2014**, *57*, 8657-8663.
[5] Q. Zeng, Q. Guo, Y. Yuan, Y. Yang, B. Zhang, L. Ren, X. Zhang, Q. Luo, M. Liu, L. S. Bouchard and X. Zhou, *Anal Chem* **2017**, *89*, 2288-2295.
[6] P. Das, X. Deng, L. Zhang, M. G. Roth, B. M. Fontoura, M. A. Phillips and J. K. De Brabander, *ACS Med Chem Lett* **2013**, *4*, 517-521.
[7] J. Baldwin, C. H. Michnoff, N. A. Malmquist, J. White, M. G. Roth, P. K. Rathod and M. A. Phillips, *J Biol Chem* **2005**, *280*, 21847-21853.