Altering Metabolic Profiles of Drugs by Precision Deuteration 2: Discovery of a Deuterated Analog of Ivacaftor with Differentiated Pharmacokinetics for Clinical Development

Authors

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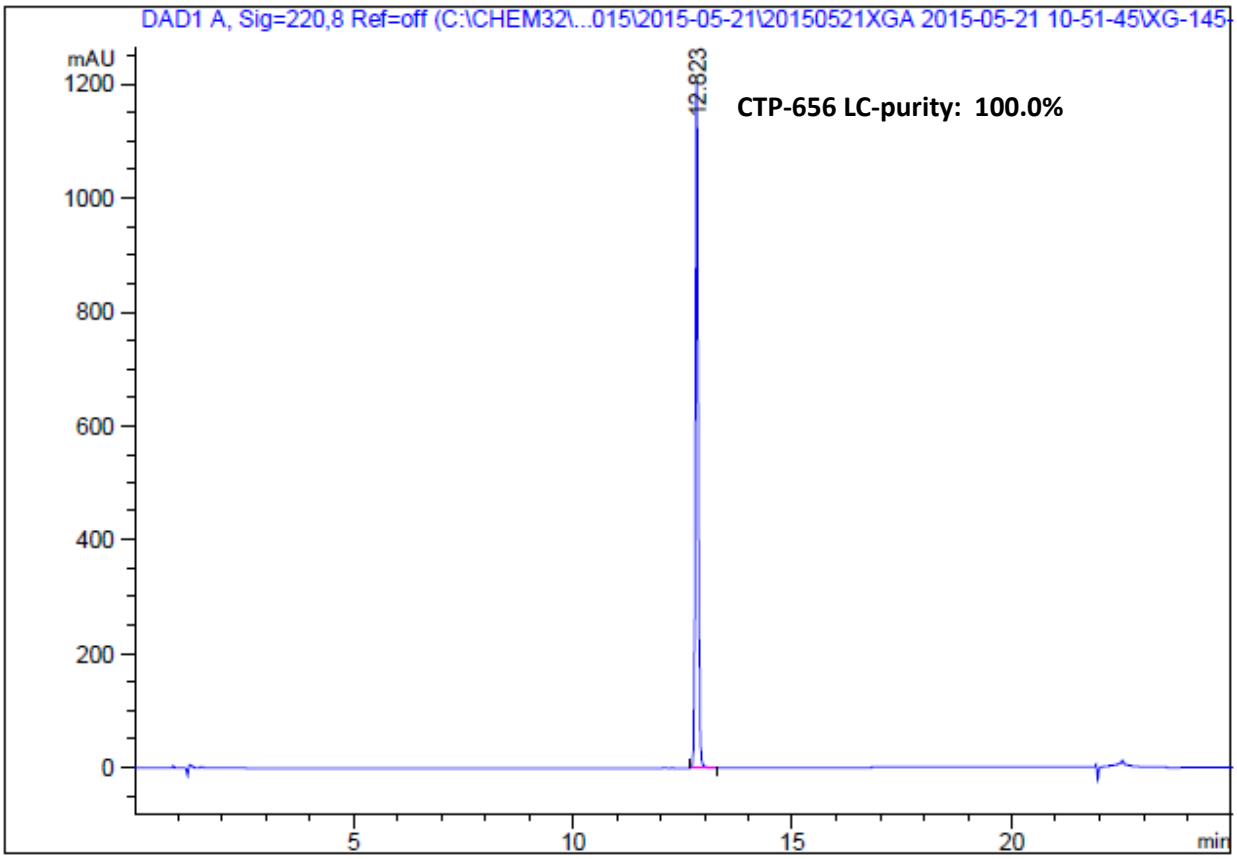
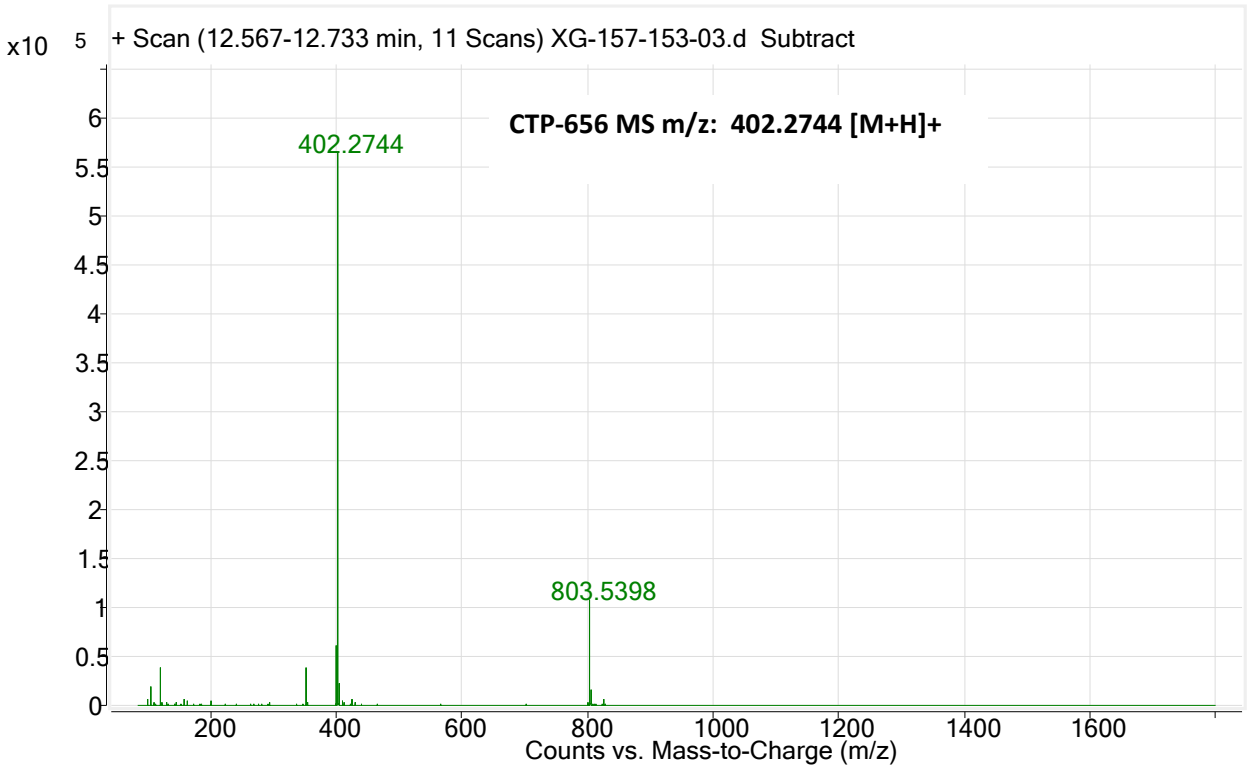
SUPPLEMENTAL DATA SECTION

Supplemental Figure 1. CTP-656 LC purity assessment

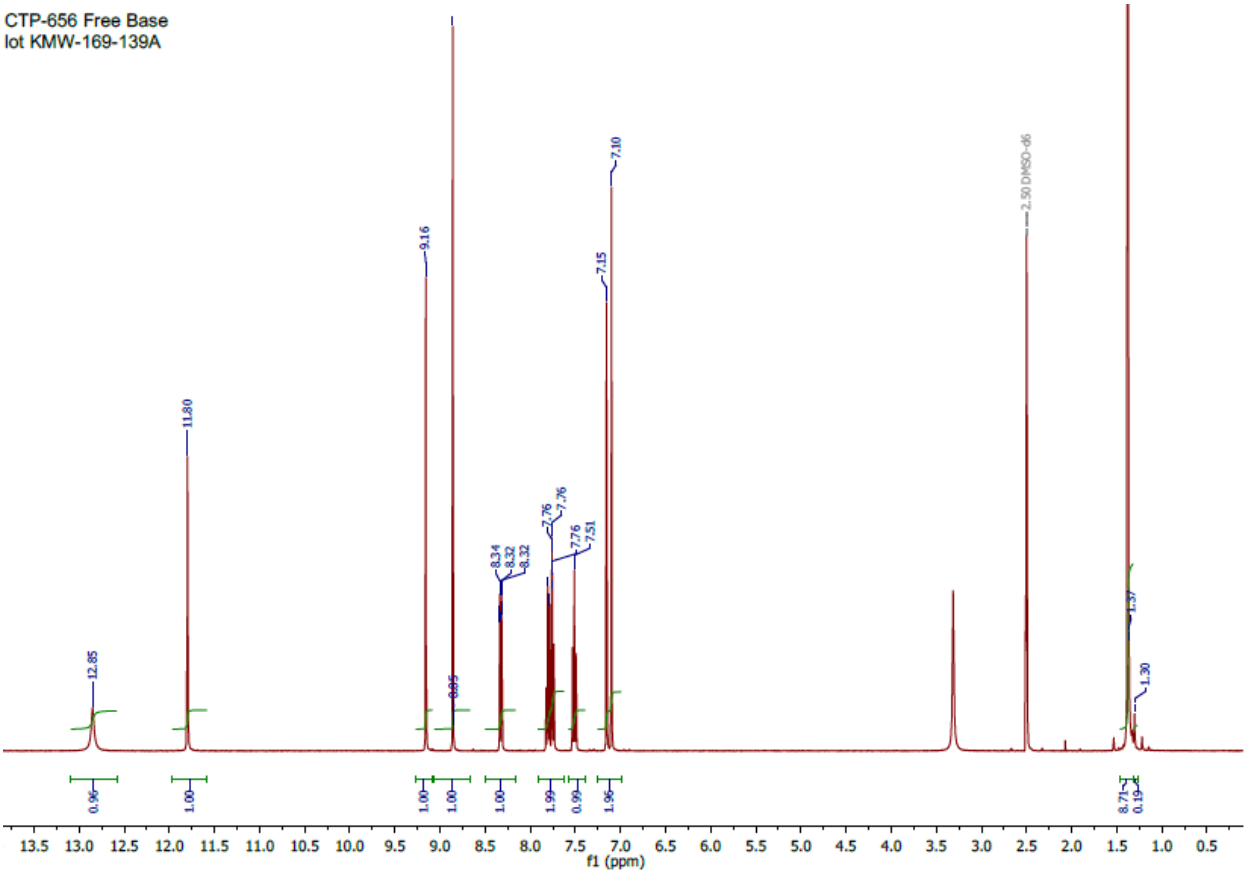


Elution Time (min)	Peak Area	Percentage of Total
12.823	5490.28857	100%

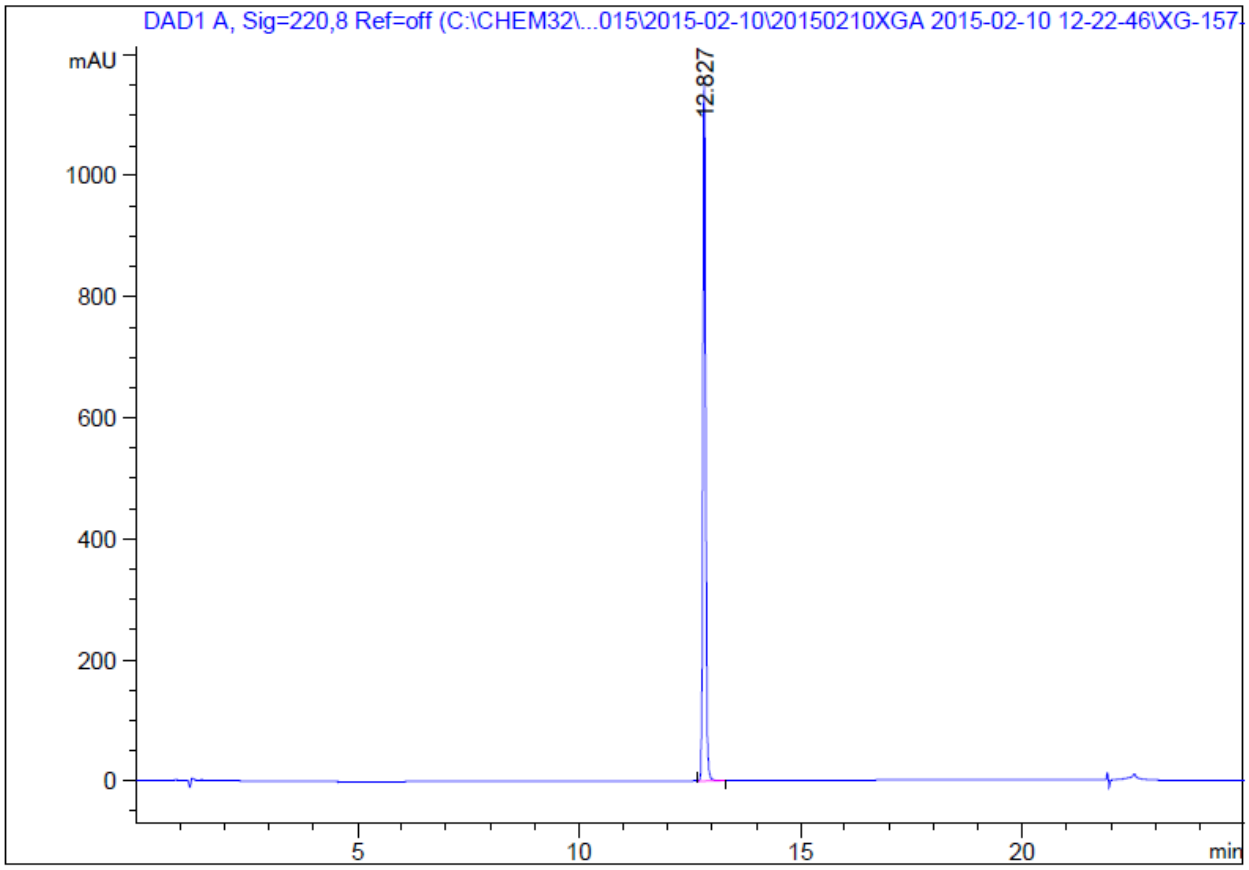
Supplemental Figure 2. CTP-656 MS identity assessment



Supplemental Figure 3. CTP-656 ^{1}H-NMR spectrum



Supplemental Figure 4. d_{18}-Ivacaftor LC purity assessment



Elution Time (min)	Peak Area	Percentage of Total
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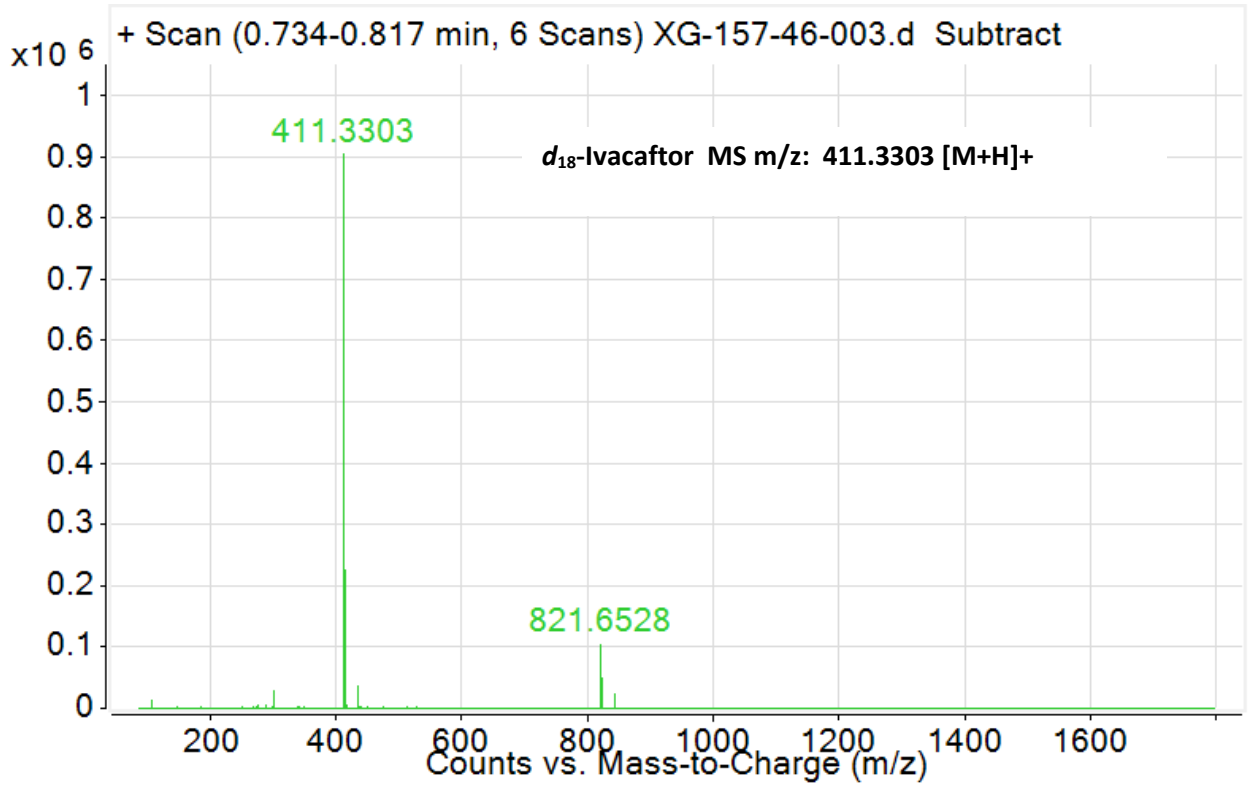
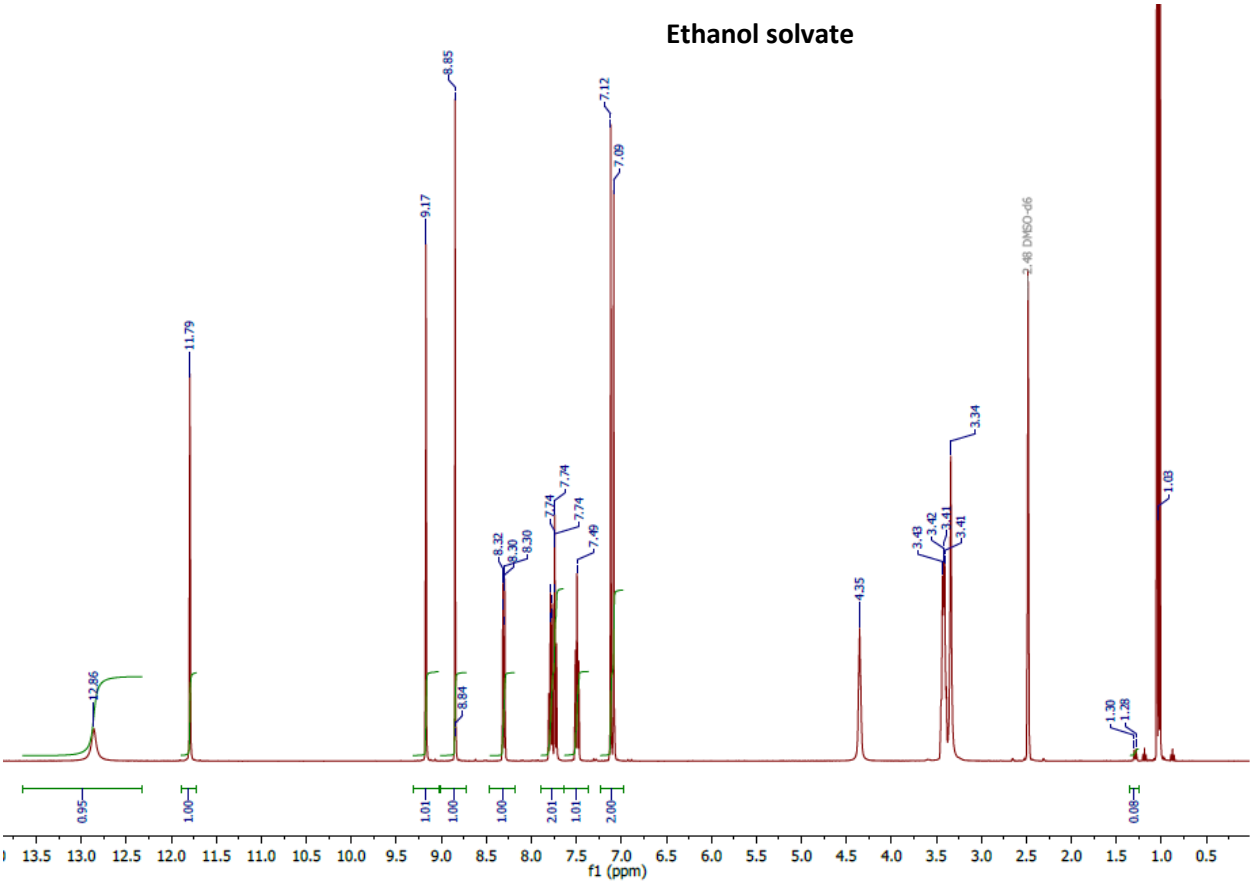
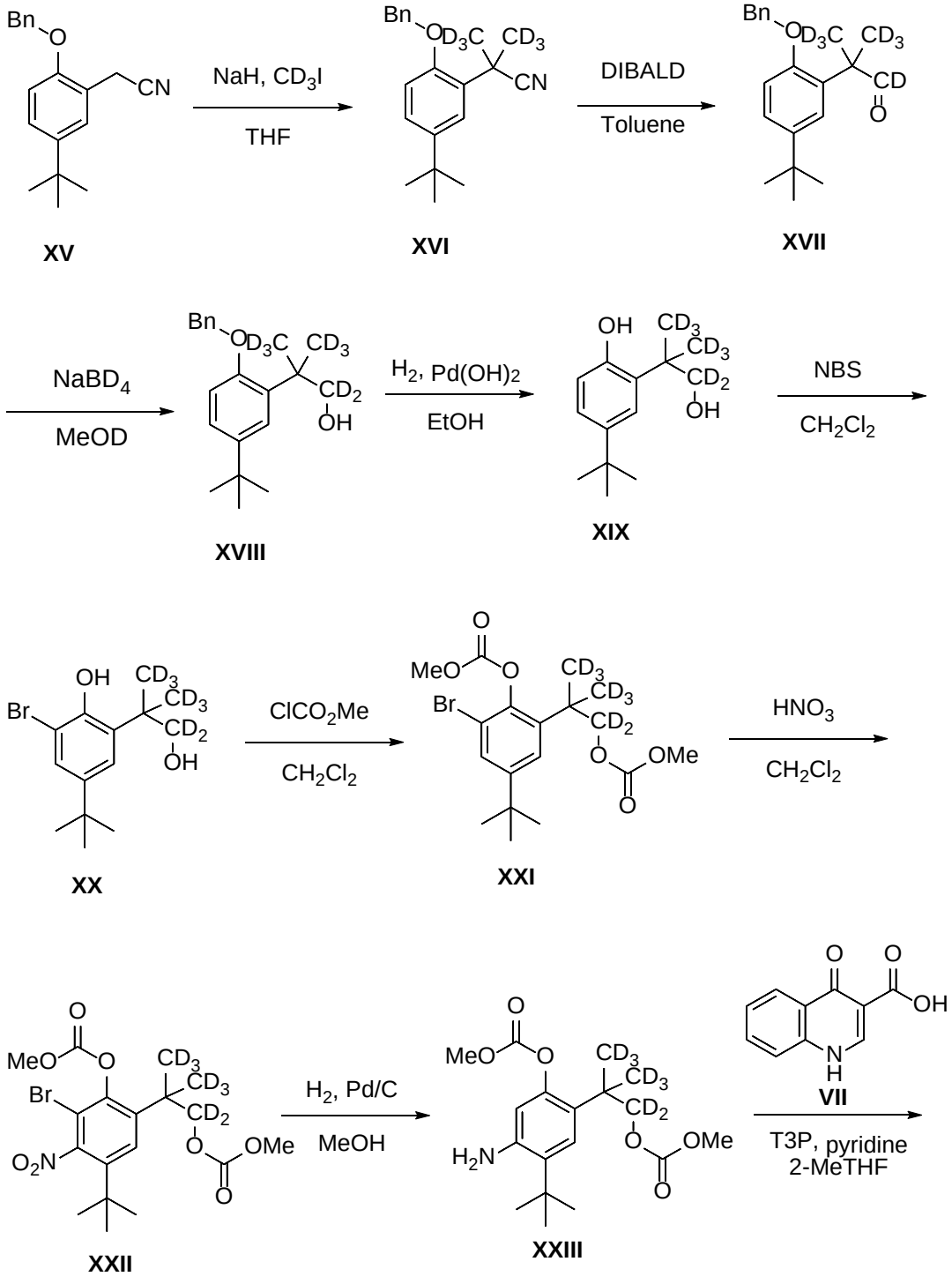
Supplemental Figure 5. d_{18}-Ivacaftor MS identity assessment

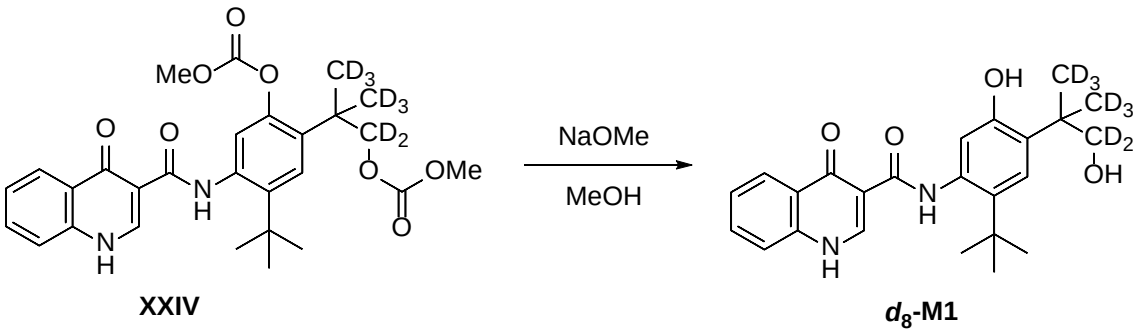


Supplemental Figure 6. d_{18}-Ivacaftor ^{1}H-NMR spectrum



Supplemental Figure 7. Synthesis of CTP-656 Metabolite d_8-M1

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CD3
CD3
CD2
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OMe
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NaOMe
MeOH
OH
CD3
CD3
CD2
OH
O
O
N
H
N
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XXIV **d_8-M1**

Supplemental Materials: Synthesis of CTP-656 d_8-M1 Metabolite

2-(2-(Benzyloxy)-5-(tert-butyl)phenyl)-2-(methyl-d_3)propanenitrile-3,3,3-d_3 (XVI) – 60% dispersion of sodium hydride in mineral oil (4.3 g, 107.5 mmol, 6 equiv) was added to THF (75 mL, anhydrous) and stirred for 15 minutes. **XV** (5.0 g, 17.9 mmol, 1 equiv) was added and the mixture was stirred at room temperature for 1 hour. Iodomethane-d3 (4.6 mL, 71.7 mmol, 4 equiv, Cambridge Isotopes) was added and the mixture was stirred at room temperature overnight. TLC analysis (30% ethyl acetate in heptanes, SM R_f = 0.6, product R_f = 0.65, stained with $KMnO_4$) indicated the reaction was complete. The mixture was cooled to 0 °C and quenched by the slow addition of 0.5 N HCl (200 mL). The biphasic mixture was transferred to a separatory funnel and extracted with ethyl acetate (2 x 100 mL). The combined organic layers were washed with brine (1 x 100 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified on an Analogix automated chromatography system, eluting with a gradient of 0 to 25% ethyl acetate in heptanes, over 45 minutes to give **XVI** (6.4 g, >100% yield) as a yellow oil.

2-(2-(Benzyloxy)-5-(tert-butyl)phenyl)-2-(methyl-d_3)propanal-1,3,3,3-d_4 (XVII) – A solution of **XVI** (6.4 g, 17.9 mmol, assumed 100 % yield in previous step, 1 equiv) in toluene (60 mL, anhydrous) was cooled to -78 °C. A 0.7 M solution of diisobutylaluminum deuteride (28.1 mL, 19.7 mmol, 1.1 equiv, Aldrich, 98 atom% D) was added drop-wise over 15 minutes. The mixture was stirred at -78 °C for 3 hours, then at room temperature overnight, at which time GC/MS analysis indicated ~40% conversion. The reaction was cooled to -78 °C and additional 0.7 M solution of diisobutylaluminum deuteride (28.1 mL, 19.7 mmol, 1.1 equiv) was added drop-wise. The mixture was warmed to room temperature and stirred for 6 hours at which time GC/MS analysis indicated the reaction was complete. The mixture was left stirring overnight then cooled to 0 °C and quenched by the addition of 1 N DCl in D_2O (100 mL). The mixture was

stirred for 30 minutes at room temperature, transferred to a separatory funnel and extracted with heptanes (100 mL). The layers were separated and the organic layer was washed with 1 N HCl (1 x 100 mL), water (1 x 100 mL), saturated aqueous sodium bicarbonate (1 x 100 mL) and brine (1 x 100 mL). The organic layer was dried over sodium sulfate and Celite (10 g), filtered, and concentrated under reduced pressure to give crude **XVII** (8.1 g) as a yellow oil which was used without purification.

2-(2-(Benzyloxy)-5-(*tert*-butyl)phenyl)-2-(methyl-d_3)propan-1,1,3,3,3-d_5-1-ol (XVIII) – A solution of **XVII** (8.1 g, 17.9 mmol, assumed 100% yield in previous step, 1 equiv) in methanol-OD (80 mL, CDN, 99.6 atom% D) was cooled to 0 °C and sodium borodeuteride (1.50 g, 35.8 mmol, 2 equiv, Cambridge Isotopes, 99 atom% D) was added in portions over 5 minutes. The mixture was stirred at room temperature for 90 minutes at which time TLC analysis (30% ethyl acetate in heptanes, SM R_f = 0.65, product R_f = 0.3) indicated the reaction was complete. The mixture was quenched by adding 1 N DCl in D_2O (100 mL, Cambridge Isotopes, 99 atom% D). The mixture was partially concentrated under reduced pressure to remove methanol. The remaining aqueous mixture was extracted with ethyl acetate (1 x 150 mL). The organic layer was washed with brine (1 x 100 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude material was purified on an Analogix automated chromatography system, eluting with 0 to 50 % ethyl acetate in heptanes, over 40 minutes to give **XVIII** (3.3 g, 58% yield over 2 steps) as a colorless oil which solidified upon standing.

4-(*tert*-Butyl)-2-(1-hydroxy-2-(methyl-d_3)propan-2-yl-1,1,3,3,3-d_5)phenol (XIX). A 500-mL Parr shaker bottle was charged with 20% $Pd(OH)_2$ on carbon (325 mg), **XVIII** (3.25 g, 10.2 mmol, 1 equiv), and methanol (50 mL). The mixture was shaken at 20 psi H_2 for 7 hours at which time GC/MS analysis indicated the reaction was complete. The mixture was filtered

through a pad of Celite (washing with methanol) and the filtrate was concentrated under reduced pressure to give **XIX** (1.77 g, 75% yield) as an off-white solid.

2-Bromo-4-(*tert*-butyl)-6-(1-hydroxy-2-(methyl-d_3)propan-2-yl-1,1,3,3,3-d_5)phenol (XX) – *N*-bromosuccinimide (1.37 g, 7.70 mmol, 1 equiv) was added to a solution of **XIX** (1.77 g, 7.70 mmol, 1 equiv) in dichloromethane (30 mL) at 0 °C. The mixture was stirred for 2 hours while warming to room temperature, at which time GC/MS analysis indicated the reaction was complete. Additional dichloromethane (100 mL) was added and the mixture was washed with saturated aqueous sodium bicarbonate solution (1 x 100 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to give **XX** (2.7 g, >100% yield) as a light yellow oil which solidified on standing.

2-Bromo-4-(*tert*-butyl)-6-(1-((methoxycarbonyl)oxy)-2-(methyl-d_3)propan-2-yl-1,1,3,3,3-d_5)phenyl methyl carbonate (XXI) – A solution of **XX** (2.7 g, 7.7 mmol, 1 equiv, assumed 100% yield in previous step), triethylamine (3.2 mL, 23.1 mmol, 3 equiv) and DMAP (100 mg, 0.11 mmol, 0.1 equiv) in dichloromethane (75 mL) was cooled to 0 °C and methyl chloroformate (1.30 mL, 16.9 mmol, 2.2 equiv) was added drop-wise over 5 minutes. The mixture was stirred at 0 °C for 30 minutes, then at room temperature for 2 hours, at which time TLC analysis (20% ethyl acetate in heptanes, SM R_f = 0.3, product R_f = 0.35, stained with $KMnO_4$) indicated the reaction was complete. The reaction mixture was diluted with dichloromethane (100 mL), water (100 mL) and 1 N HCl (100 mL). The layers were separated and the aqueous layer was washed with dichloromethane (1 x 100 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude material was purified on an Analogix automated chromatography system, eluting with 0 to 40 % ethyl acetate in heptanes, over 45 minutes to give **XXI** (2.9 g, 89% yield over 2 steps) as a colorless oil.

2-Bromo-4-(*tert*-butyl)-6-(1-((methoxycarbonyl)oxy)-2-(methyl-d_3)propan-2-yl-1,1,3,3,3-d_5)-3-nitrophenyl methyl carbonate (XXII) – A solution of **XXI** (2.8 g, 6.6 mmol, 1 equiv) in dichloromethane (50 mL) was cooled to 0 °C and sulfuric acid (2.97 g, 30.4 mmol, 4.6 equiv) added drop-wise over 1 minute. The mixture was stirred for 15 minutes at 0 °C before the drop-wise addition of nitric acid (1.19 g, 13.2 mmol, 2 equiv) over 5 minutes. The mixture was stirred at 0 °C for 3 hours, during which time a yellow/brown color developed. Proton NMR analysis of an aliquot indicated the reaction was incomplete; therefore, the mixture was stirred an additional 2 hours at 0 °C. The mixture was quenched by the addition of ice water (100 mL). Dichloromethane (100 mL) was added and the layers were separated. The organic layer was washed with saturated sodium bicarbonate (50 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure to give **XXII** (2.89 g, 93 % yield) as a yellow oil.

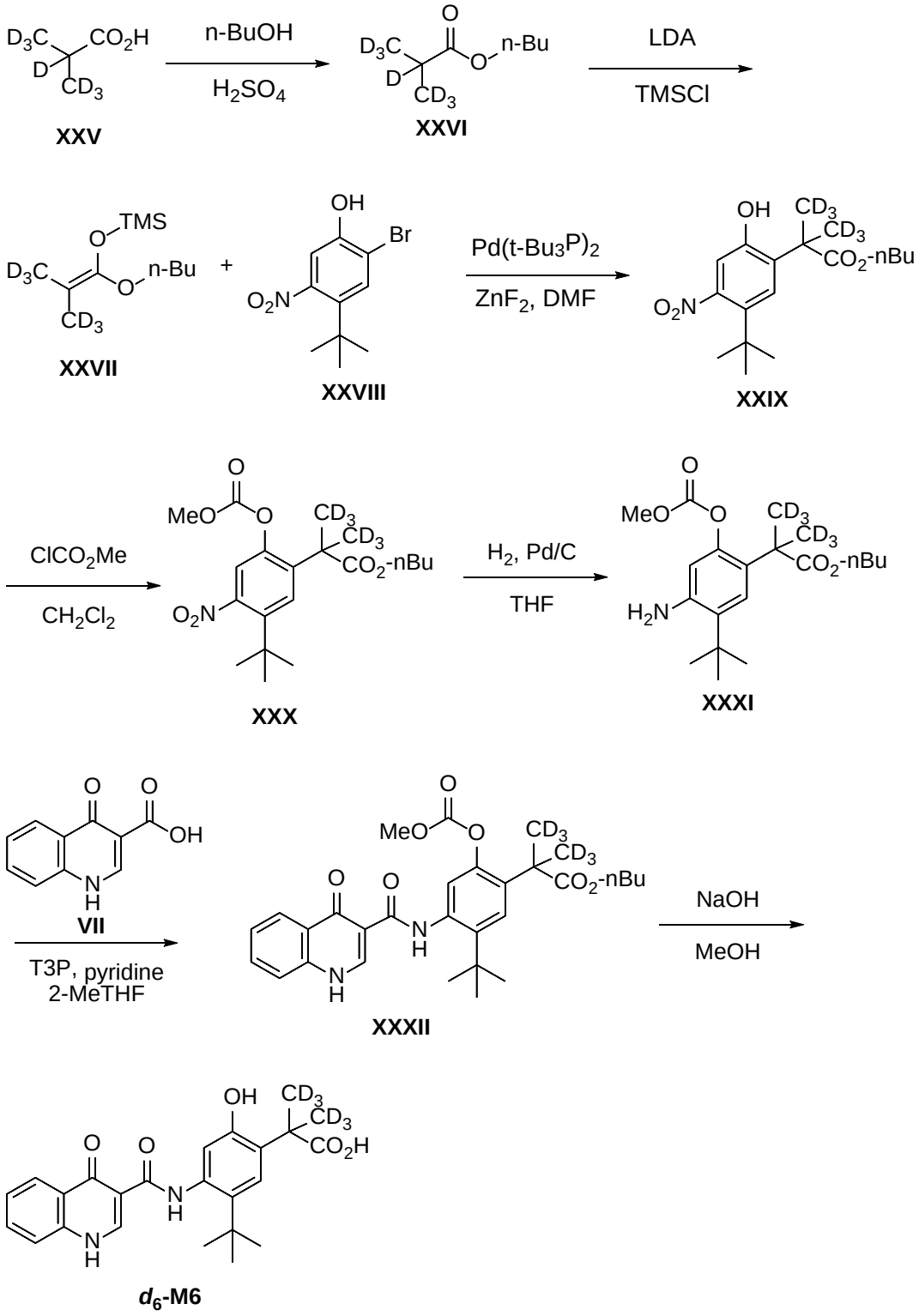
5-Amino-4-(*tert*-butyl)-2-(1-((methoxycarbonyl)oxy)-2-(methyl-d_3)propan-2-yl-1,1,3,3,3-d_5)phenyl methyl carbonate (XXIII) – A 500-mL Parr shaker bottle was charged with 10% Pd/C (720 mg, dry), ethanol (85 mL), **XXII** (2.89 g, 6.15 mmol, 1 equiv), and N,N-diisopropylethyl amine (1.2 mL, 6.76 mmol, 1.1 equiv). The mixture was shaken at 50 psi H_2 overnight at which time TLC and LC/MS analysis confirmed reduction was complete. The mixture was filtered through a pad of Celite (washing with ethanol). The filtrate was partially concentrated under reduced pressure to a volume of approximately 15 mL. The remaining solution was cooled to 0 °C and water (50 mL) was added drop-wise. The resulting solid was filtered, washed with water and dried on the filter to give **XXIII** (1.60 g, 72% yield) as an off-white solid.

4-(*tert*-Butyl)-2-(1-((methoxycarbonyl)oxy)-2-(methyl-d_3)propan-2-yl-1,1,3,3,3-d_5)-5-(4-oxo-1,4-dihydroquinoline-3-carboxamido)phenyl methyl carbonate (XXIV) – A 50% solution of propylphosphonic anhydride in ethyl acetate (5.73 g, 9.0 mmol, 2.5 equiv) and pyridine (570

mg, 7.2 mmol, 2 equiv) were added to a suspension of **VII** (680 mg, 3.6 mmol, 1 equiv) and **XXIII** (1.3 g, 3.6 mmol, 1 equiv) in 2-methyltetrahydrofuran (25 mL). The mixture was heated at 55 °C for 16 hours; after 2 hours most of the solids had dissolved. The mixture was cooled to room temperature and ethyl acetate (50 mL) was added. The mixture was washed with 10% aqueous sodium carbonate solution (2 x 50 mL) and brine (1 x 50 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to give **XXIV** (1.9 g, 99% yield) as a tan foam which was used without purification.

***N*-(2-(*tert*-butyl)-5-hydroxy-4-(1-hydroxy-2-(methyl-d_3)propan-2-yl-1,1,3,3,3-d_5)phenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (*d*$_8$-M1) –** A 25% solution of sodium methoxide in methanol (1.54 g, 7.2 mmol, 2 equiv) was added to a solution of **XXIV** (1.9 g, 3.6 mmol, 1 equiv) in 2-methyltetrahydrofuran (50 mL). The mixture was stirred at room temperature for 4 hours, during which time a dark brown color developed. LC/MS analysis indicated the reaction was complete. The mixture was cooled to 0 °C, 1 N HCl (50 mL) was added and the mixture was extracted with ethyl acetate (100 mL). The organic layer was washed with brine, dried over sodium sulfate, filtered and concentrated under reduced pressure to afford a tan foam. The foam was dissolved in ethyl acetate (50 mL) and stirred, resulting in the formation of white crystals. Heptanes (50 mL) were added, and the solids were filtered and dried in a vacuum oven at room temperature for 2 hours to give ***d*$_8$-M1** (1.25 g, 83% yield) as white solid.

Supplemental Figure 8. Synthesis of CTP-656 Metabolite d_6-M6



Supplemental Materials: Synthesis of CTP-656 d_6-M6 Metabolite

Butyl 2-(methyl-d3)propanoate-2,3,3,3-d4 (XXVI) – Methylpropionic-*d*7 acid (**XXV**, 5 g, 52.6 mmol, 1.0 equiv, CDN, 98 atom% D) was dissolved in n-butanol (30 mL) and sulfuric acid (1 mL, 18.8 mmol, 0.35 equiv) was added. The mixture was stirred at 80 °C overnight then poured onto ice (300 g). The mixture was extracted with MTBE (3 x 50 mL). The combined organic layers were washed with saturated sodium bicarbonate solution (50 mL), water (50 mL), brine (50 mL), dried over sodium sulfate, filtered and concentrated to give crude **XXVI** (5.2 g, 65% yield) as a colorless liquid.

((1-Butoxy-2-(methyl-d3)prop-1-en-1-yl-3,3,3-d3)oxy)trimethylsilane (XXVII) – Diisopropylamine (7.3 mL, 53 mmol, 1.2 equiv) was added to anhydrous THF (200 mL) and the solution was cooled to -40 °C. 2.5 M *n*-butyllithium in hexane (17.6 mL, 44 mmol, 1.1 equiv) was added over 15 minutes. The mixture was stirred at -30 to -20 °C for 30 minutes then cooled to -78 °C. A solution of **XXVI** (5.2 g, 40 mmol, 1.0 equiv) in anhydrous THF (10 mL) was added over 30 minutes. The mixture was stirred for 30 minutes at -78 °C then chlorotrimethylsilane (10 mL, 80 mmol, 2.0 equiv) was added over 15 minutes. The mixture was stirred at -78 °C for 2 hours, then at room temperature for 2 hours. The mixture was poured into a mixture of saturated sodium bicarbonate solution (300 mL) and ice (100 g) and extracted with hexanes (200 mL). The organic phase was washed with water (3 x 100 mL), dried over sodium sulfate, filtered and concentrated. The residue was distilled (60-70 °C at 15 mmHg) to give **XXVII** (6.2 g, 70% yield) as a colorless liquid.

Butyl 2-(5-(tert-butyl)-2-hydroxy-4-nitrophenyl)-2-(methyl-d3)propanoate-3,3,3-d3 (XXIX) – To a solution of **XXVIII** (1.7 g, 6.2 mmol, 1.0 equiv) in anhydrous DMF (20 mL) was added zinc fluoride (640 mg, 6.2 equiv, 1.0 equiv). The mixture was sparged with nitrogen for 15

minutes, then fresh bis(tri-tert-butylphosphine)palladium(0) (160 mg, 0.31 mmol, 0.05 equiv) and **XXVII** (2.7 g, 12,4 mmol, 2.0 equiv) were added. The mixture was heated to 80 °C and stirred for 16 hours. The mixture was cooled to room temperature and MTBE (50 mL) and water (100 mL) were added. The mixture was stirred for 1 hour and extracted with MTBE (100 mL). The organic layer was washed with 1 N HCl (50 mL), saturated sodium bicarbonate solution (50 mL), and brine (50 mL), dried over sodium sulfate, filtered and concentrated. The residue was purified on an Analogix automated chromatography system, eluting with a gradient of 0 to 35% ethyl acetate in heptanes, to give **XXIX** (900 mg, 42% yield) as a white solid.

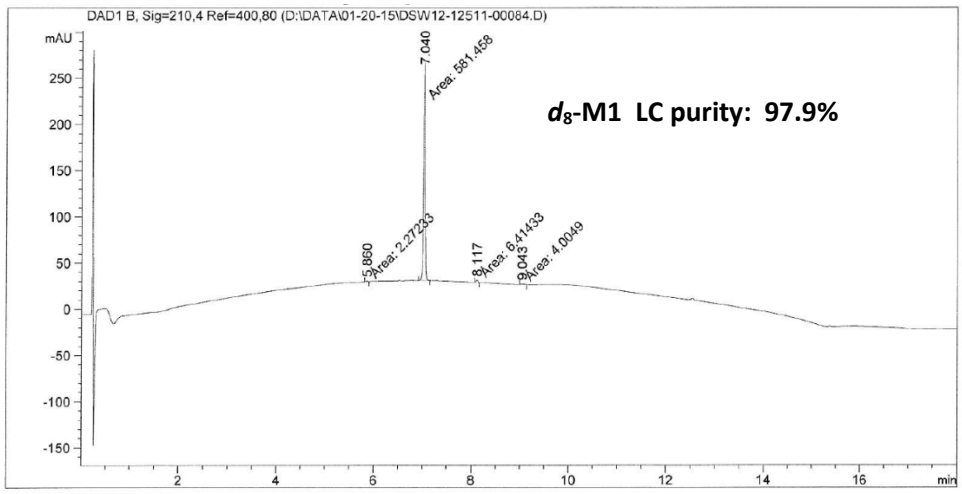
Butyl 2-(5-(tert-butyl)-2-((methoxycarbonyl)oxy)-4-nitrophenyl)-2-(methyl-d3)propanoate-3,3,3-d3 (XXX) – A solution of **XXIX** (900 mg, 2.6 mmol, 1.0 equiv) and triethylamine (0.76 mL, 5.4 mmol, 2.0 equiv) in dichloromethane (30 mL) was cooled to 0 °C and methyl chloroformate (0.51 mL, 6.5 mmol, 1.2 equiv) was added. The mixture was stirred at room temperature for 3 hours, quenched with 1 N HCl (50 mL) and stirred for 15 minutes. The organic layer was washed with 1 N HCl (50 mL), saturated sodium bicarbonate solution (100 mL), water (100 mL), and brine (100 mL), dried over sodium sulfate, filtered and concentrated. The residue was purified on an Analogix automated chromatography system, eluting with a gradient of 0 to 10% ethyl acetate in heptanes, to give **XXX** (950 mg, 90% yield) as a yellow solid.

Butyl 2-(4-amino-5-(tert-butyl)-2-((methoxycarbonyl)oxy)phenyl)-2-(methyl-d3)propanoate-3,3,3-d3 (XXXI) – A mixture of **XXX** (950 mg, 2.37 mmol, 1.0 equiv) and 10% palladium on carbon, 50 wt% wet (100 mg) in methanol (50 mL) was hydrogenated at 30 psi H_2 overnight. TLC analysis showed reduction was complete. The mixture was filtered through a pad of Celite and the filtrate was concentrated to give **XXXI** (810 mg, 91% yield) as a yellow oil.

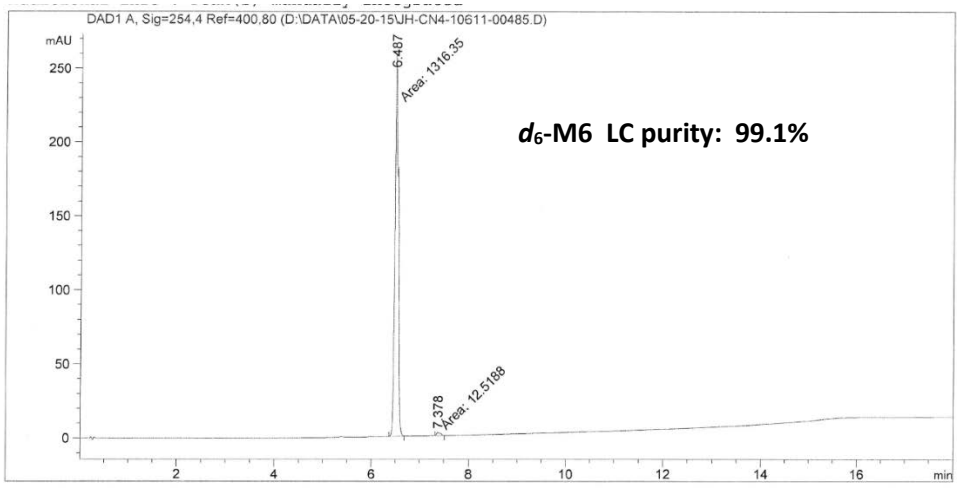
Butyl 2-(5-(tert-butyl)-2-((methoxycarbonyl)oxy)-4-(4-oxo-1,4-dihydroquinoline-3-carboxamido)phenyl)-2-(methyl-d3)propanoate-3,3,3-d3 (XXXII) – To a solution of **XXXI** (810 mg, 2 mmol, 1.0 equiv) in anhydrous 2-methyltetrahydrofuran (10 mL) was added **VII** (455 mg, 2.4 mmol, 1.2 equiv), a 50% solution of propylphosphonic anhydride in DMF (1.9 g, 3 mmol, 1.5 equiv) and pyridine (0.35 mL, 4 mmol, 2.0 equiv). The mixture was stirred at 50 °C for 48 hours then cooled to room temperature. Water (40 mL) and ethyl acetate (40 mL) were added and the layers were separated. The organic layer was washed with saturated sodium bicarbonate solution (2 x 50 mL), water (50 mL), and brine (50 mL), dried over sodium sulfate, filtered and concentrated. The residue was purified on an Analogix automated chromatography system, eluting with a gradient of 0 to 35% ethyl acetate in dichloromethane, to give **XXXII** (800 mg, 74% yield) as a white solid.

2-(5-(*tert*-Butyl)-2-hydroxy-4-(4-oxo-1,4-dihydroquinoline-3-carboxamido)-phenyl)-2-(methyl-d3)propanoic-3,3,3-d3 acid (*d*$_6$-M1) – 1 N sodium hydroxide solution (5.9 mL, 5.9 mmol, 4 equiv) was added to a solution of **XXXII** (800 mg, 1.47 mmol, 1.0 equiv) in methanol (30 mL) and the mixture was stirred for 6 hours at room temperature. The mixture was concentrated, diluted with water (30 mL), and extracted with dichloromethane (20 mL). The aqueous solution was acidified with 6 N HCl to pH 2 at 0 °C, and stirred for 30 minutes at 0 °C. The solid was filtered, washed with water (10 mL) and dried in vacuum oven at 60 °C to give ***d*$_6$-M1**(520 mg, 81% yield) as a white solid.

Supplemental Figure 9. d_8-M1 and d_6-M6 LC purity assessments

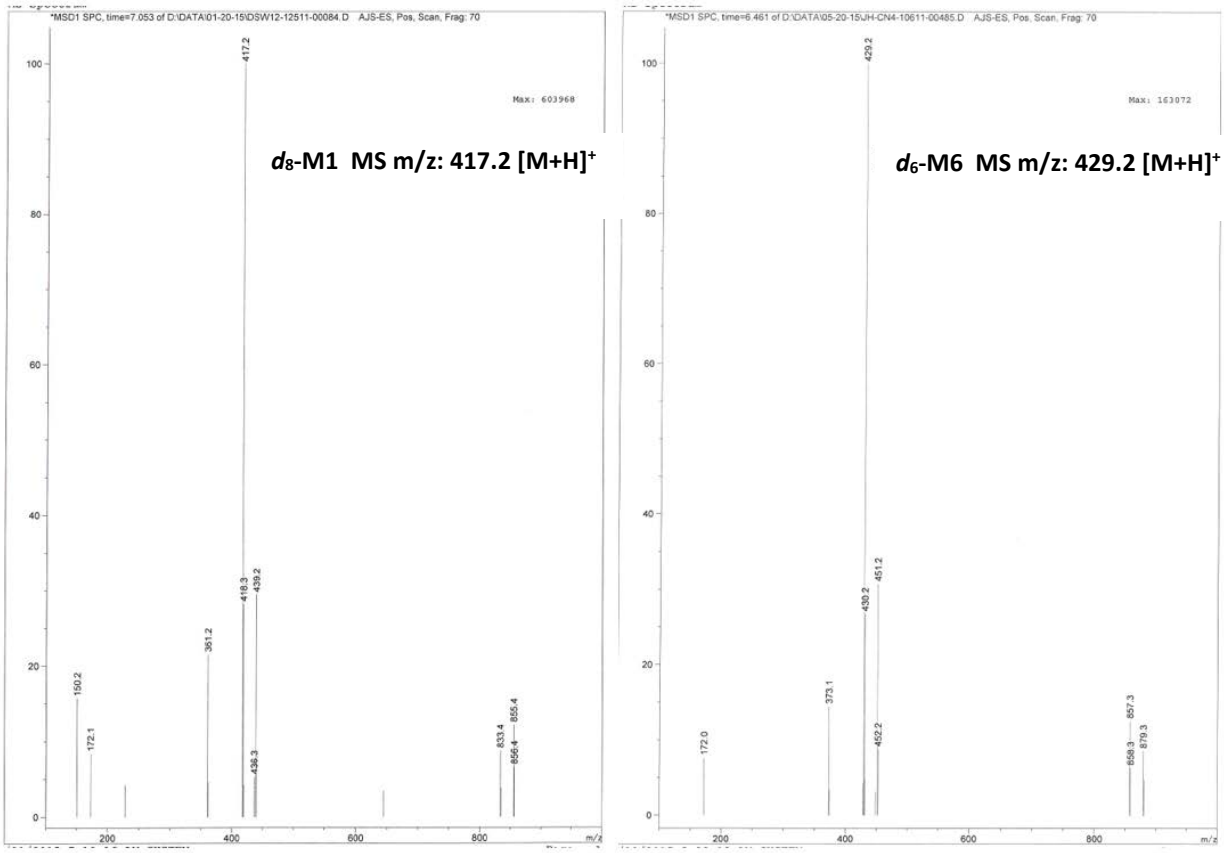


Elution Time (min)	Peak Area	Percentage of Total
5.860	2.27233	0.3825%
7.040	581.45795	97.8639% (d_8-M1)
8.117	6.41433	1.0796%
9.043	4.00490	0.6741%

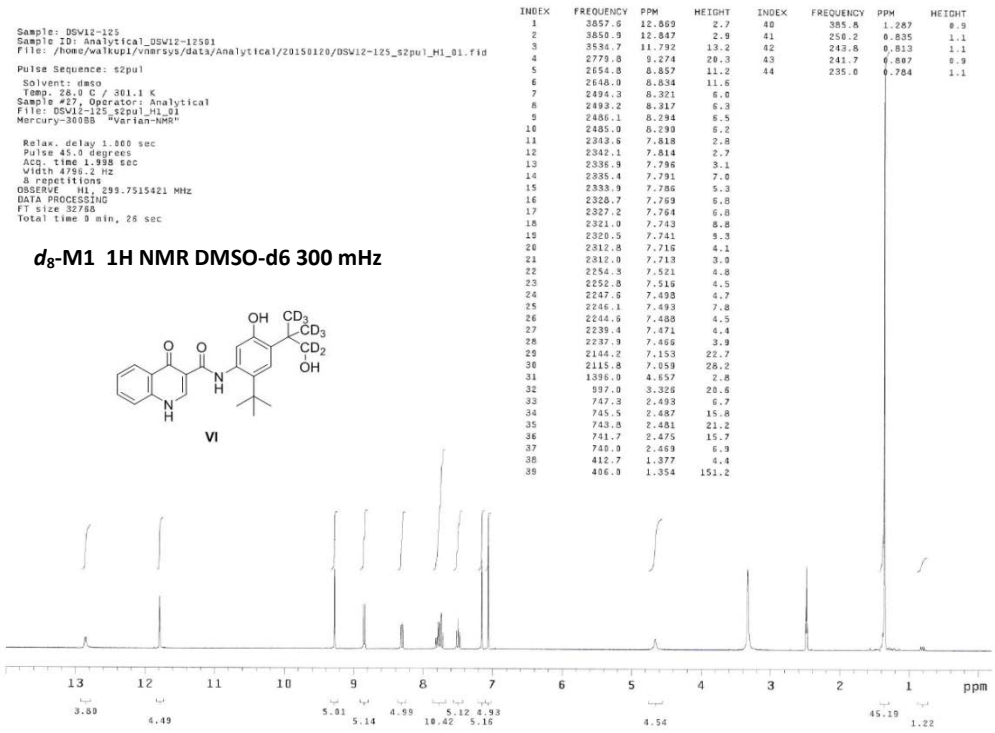


Elution Time (min)	Peak Area	Percentage of Total
6.487	1316.34900	99.0579% (d_8-M6)
7.378	12.51883	0.9421%

Supplemental Figure 10. d_8-M1 and d_6-M6 MS identity assessments

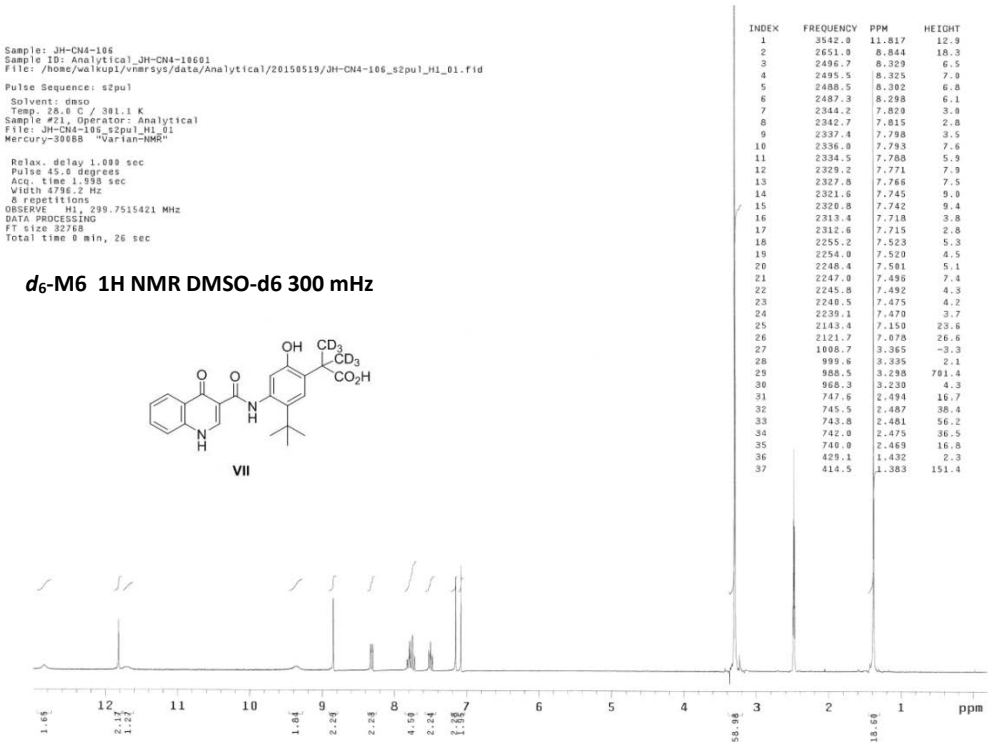


Supplemental Figure 11. d_8-M1 and d_6-M6 ^{1}H-NMR spectra



***d*$_8$-M1 1H NMR DMSO-d6 300 mHz**

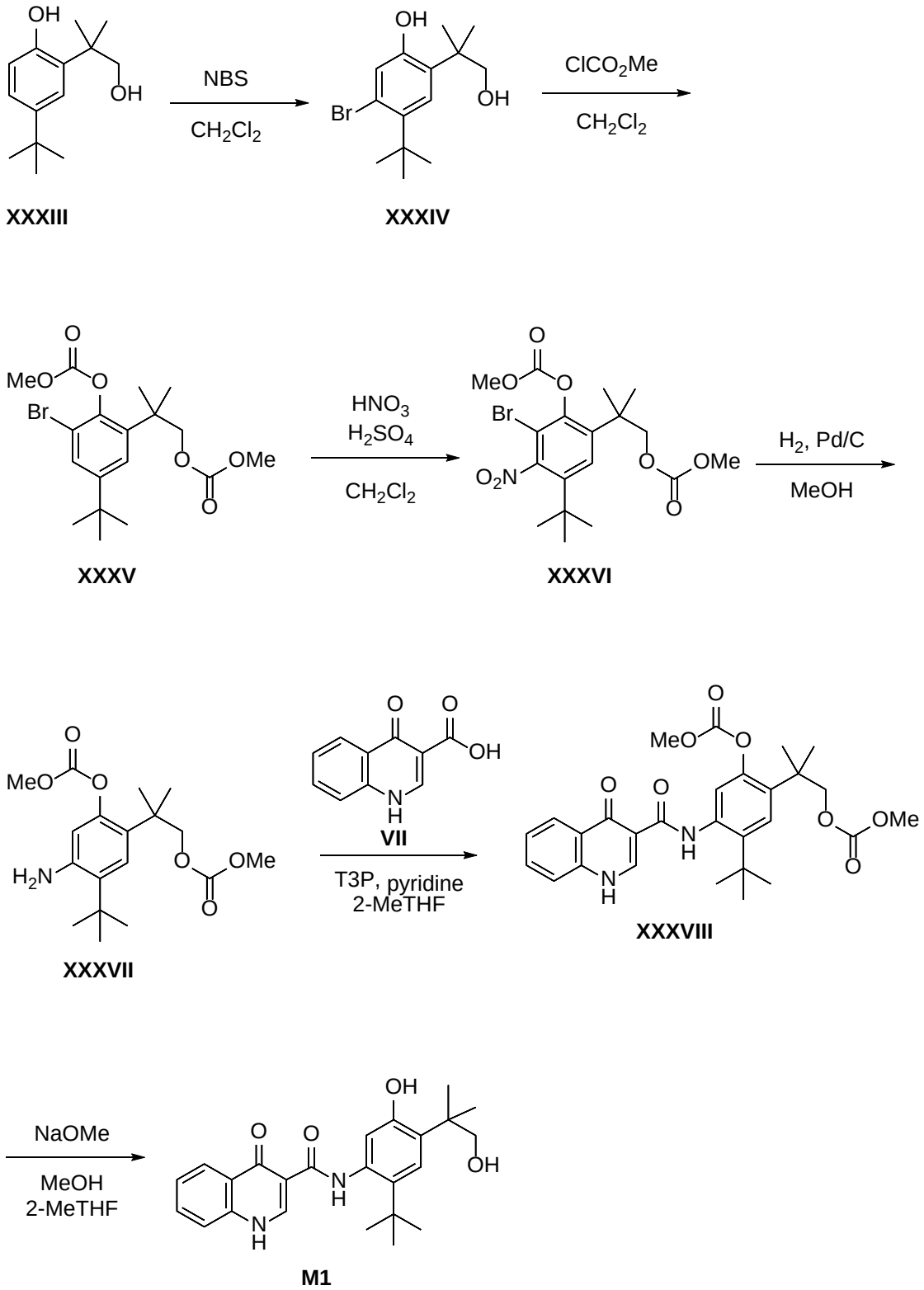
VI



***d*$_6$-M6 1H NMR DMSO-d6 300 mHz**

VII

Supplemental Figure 12. Synthesis of Ivacaftor Metabolite M1.



Supplemental Materials: Synthesis of Ivacaftor Metabolite **M1**

2-Bromo-4-(tert-butyl)-6-(1-hydroxy-2-methylpropan-2-yl)phenol (XXXIV) – *N*-bromosuccinimide (3.64 g, 20.5 mmol, 1 equiv) was added to a solution of **XXXIII** (4.54 g, 20.5 mmol, 1 equiv) in dichloromethane (190 mL) at 0 °C. The reaction mixture was stirred at 0-10 °C for 2 hours. GC/MS analysis indicated that the reaction was complete after 1 hour. The mixture was diluted with dichloromethane (200 mL) and washed with saturated aqueous sodium bicarbonate solution (150 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to give **XXXIV** (6.20 g, quantitative yield) as a yellow oil that was used without purification.

2-Bromo-4-(tert-butyl)-6-(1-((methoxycarbonyl)oxy)-2-methylpropan-2-yl)phenyl methyl carbonate (XXXV) – A solution of **XXXIV** (6.16 g, 20.5 mmol, 1 equiv), triethylamine (8.00 mL, 61.5 mmol, 3 equiv) and DMAP (250 mg, 2.0 mmol, 0.1 equiv) in dichloromethane (150 mL) was cooled to 0 °C followed by the drop-wise addition of methyl chloroformate (3.48 mL, 45.0 mmol, 2.2 equiv) over 5 minutes. The reaction mixture was stirred at 0-10 °C for 2 hours at which time GC analysis indicated the reaction was complete. The reaction mixture was diluted with dichloromethane (100 mL), water (100 mL) was added and the mixture was stirred for 20 minutes. The layers were separated and the organic layer was washed with 1 N HCl (2 x 100 mL), water (100 mL), and brine (100 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude material was purified on an Analogix automated chromatography system, eluting with 0 to 40% ethyl acetate in heptanes, to give **XXXV** (6.12 g, 72% yield) as yellow oil.

2-Bromo-4-(tert-butyl)-6-(1-((methoxycarbonyl)oxy)-2-methylpropan-2-yl)-3-nitrophenyl methyl carbonate (XXXVI)– A solution of **XXXV** (6.12 g, 14.7 mmol, 1 equiv) in

dichloromethane (120 mL) was cooled to 0 °C and concentrated sulfuric acid (6.61 g, 67.5 mmol, 4.6 equiv) was added over 1 minute. The reaction mixture was stirred for 15 minutes at 0 °C followed by the drop-wise addition of nitric acid (1.85 g, 29.3 mmol, 2 equiv) over 5 minutes. The reaction mixture was stirred at 0 °C for 6 hours. Proton NMR analysis of reaction aliquots indicated that the reaction stopped at 60% conversion after approximately 3 hours. The reaction mixture was quenched by adding ice water (100 mL). Dichloromethane (200 mL) was added and the layers were separated. The organic layer was washed with saturated sodium bicarbonate (150 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure. The material was resubjected to the reaction conditions using the same amounts of acids as above. Proton NMR analysis after 3 hours showed that the reaction was complete. Ice water (100 mL) was added and the reaction was worked up as above to give **XXXVI** (6.17 g, 91% yield) as an orange oil that was used without purification.

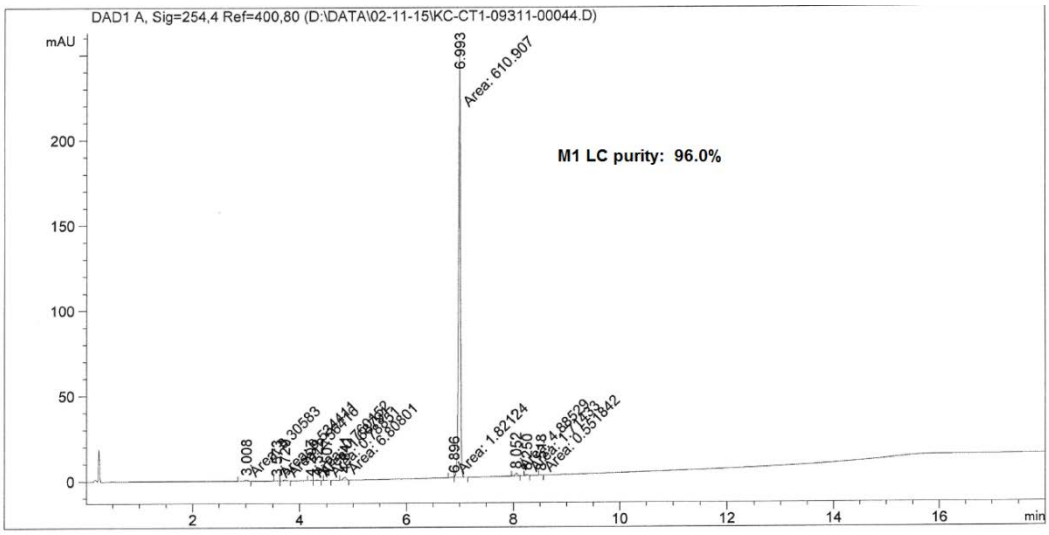
5-Amino-4-(tert-butyl)-2-(1-((methoxycarbonyl)oxy)-2-methylpropan-2-yl)phenyl methyl carbonate (XXXVII) – A 500-mL Parr shaker bottle was charged with 10% Pd/C (1.54 g), ethanol (200 mL), **XXXVI** (6.16 g, 13.3 mmol, 1 equiv), and N,N-diisopropylethylamine (2.55 mL, 14.7 mmol, 1.1 equiv). The reaction was shaken at 50 psi H_2 overnight. The reaction mixture was filtered through a pad of Celite (washing with ethanol). The filtrate was partially concentrated under reduced pressure to a volume of approximately 30 mL. The remaining solution was cooled to 0 °C and water (60 mL) was added drop-wise. The mixture was extracted with dichloromethane and the organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified on an Analogix

automated chromatography system, eluting with a gradient of 0 to 15% ethyl acetate in heptanes, to give **XXXVII** (2.48 g, 53% yield) as an off-white solid.

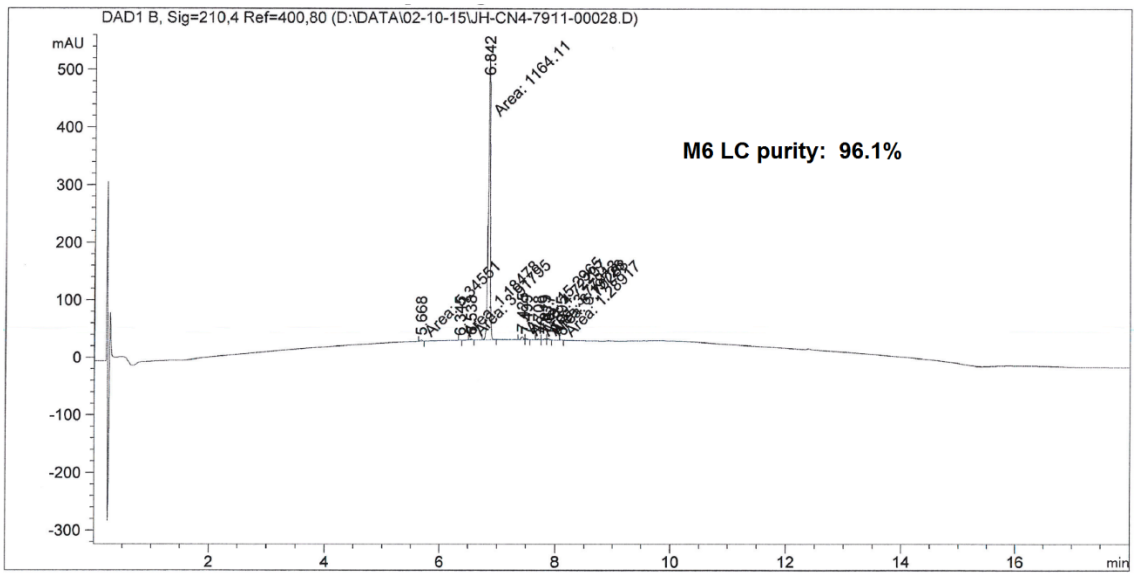
4-(tert-Butyl)-2-(1-((methoxycarbonyl)oxy)-2-methylpropan-2-yl)-5-(4-oxo-1,4-dihydroquinoline-3-carboxamido)phenyl methyl carbonate (XXXVIII) – A 50% solution of propylphosphonic anhydride in ethyl acetate (6.5 mL, 11.0 mmol, 2.5 equiv) and pyridine (0.70 mL, 8.8 mmol, 2 equiv) were added to a suspension of powdered **VII** (0.82 g, 4.4 mmol, 1 equiv) and **XXXVII** (1.55 g, 4.4 mmol, 1 equiv) in 2-methyltetrahydrofuran (35 mL). The reaction mixture was heated at 55 °C for 16 hours. The reaction mixture was cooled to room temperature and ethyl acetate (300 mL) was added. The mixture was washed with 10% sodium carbonate solution (1 x 300 mL, 1 x 200 mL) and brine (200 mL). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to give **XXXVIII** (1.80 g, 78% yield) as a tan foam that was used without purification.

***N*-(2-(tert-Butyl)-5-hydroxy-4-(1-hydroxy-2-methylpropan-2-yl)phenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (M1) –** 25% sodium methoxide in methanol (1.56 mL, 6.82 mmol, 2 equiv) was added to a solution of **XXXVIII** (1.79 g, 3.41 mmol, 1 equiv) in 2-methyltetrahydrofuran (50 mL). The reaction mixture was stirred at room temperature for 2 hours. LC/MS analysis indicated the reaction was complete after 1 hour. The mixture was cooled to 0 °C and 1 N HCl (60 mL) was added. The layers were separated and the aqueous layer was extracted with ethyl acetate (3 x 35 mL). The combined organic layers were washed with brine (30 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure to give a tan foam. The foam was dissolved in ethyl acetate (30 mL), and heptanes (300 mL) was added to afford a solid. The solid was filtered, washed with heptane (15 mL) and dichloromethane (5 mL) and dried in a vacuum oven at room temperature overnight to give **M1** (1.05 g, 76% yield) as a white solid.

Supplemental Figure 13. M1 and M6 LC purity assessments

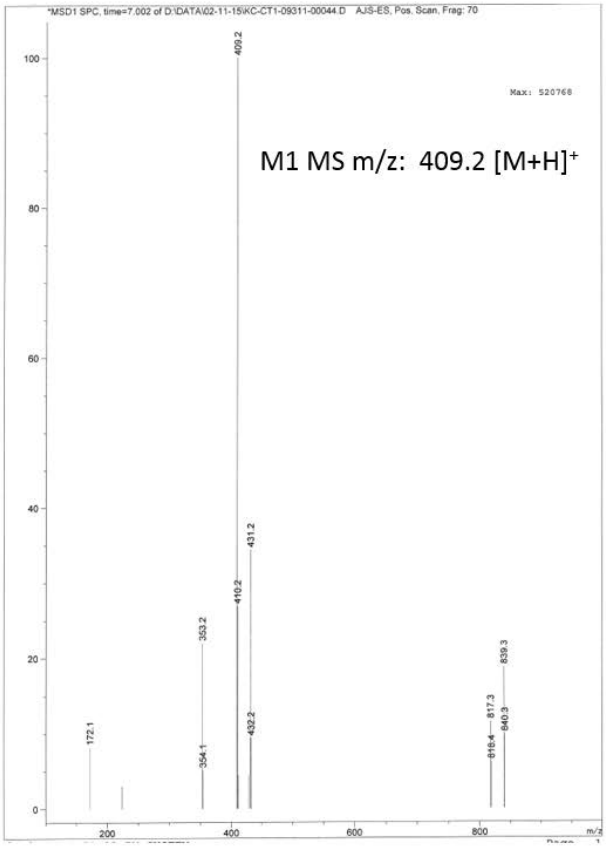


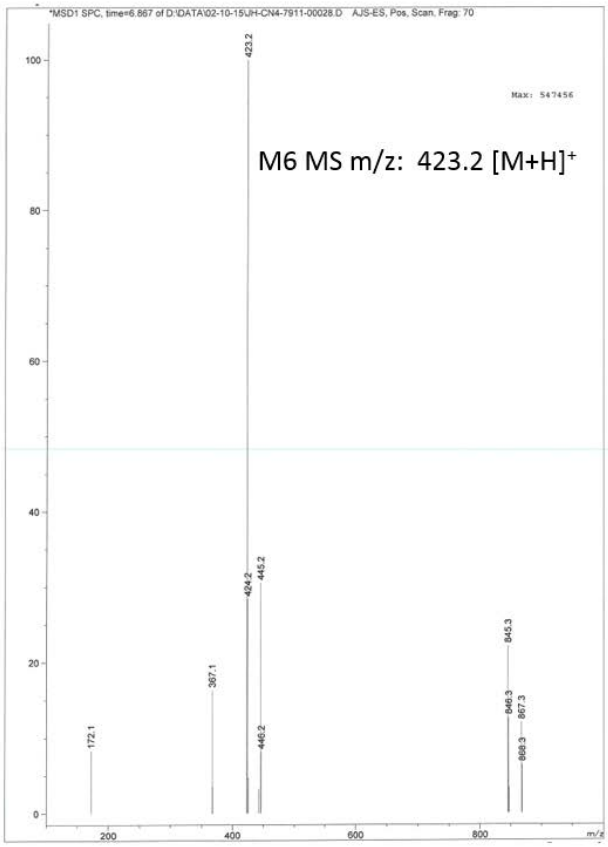
Elution Time (min)	Peak Area	Percentage of Total
3.008	3.30583	0.5195
3.573	5.34411 e-1	0.0840
3.729	2.56416	0.4030
4.207	7.60152 e-1	0.1195
4.322	1.68791	0.2653
4.507	7.88510 e-1	0.1239
4.841	6.80801	1.0699
6.896	1.82124	0.2862
6.993	610.90692	96.0049 (M1)
8.052	4.88529	0.7677
8.250	1.71433	0.2694
8.518	5.51842 e-1	0.0867



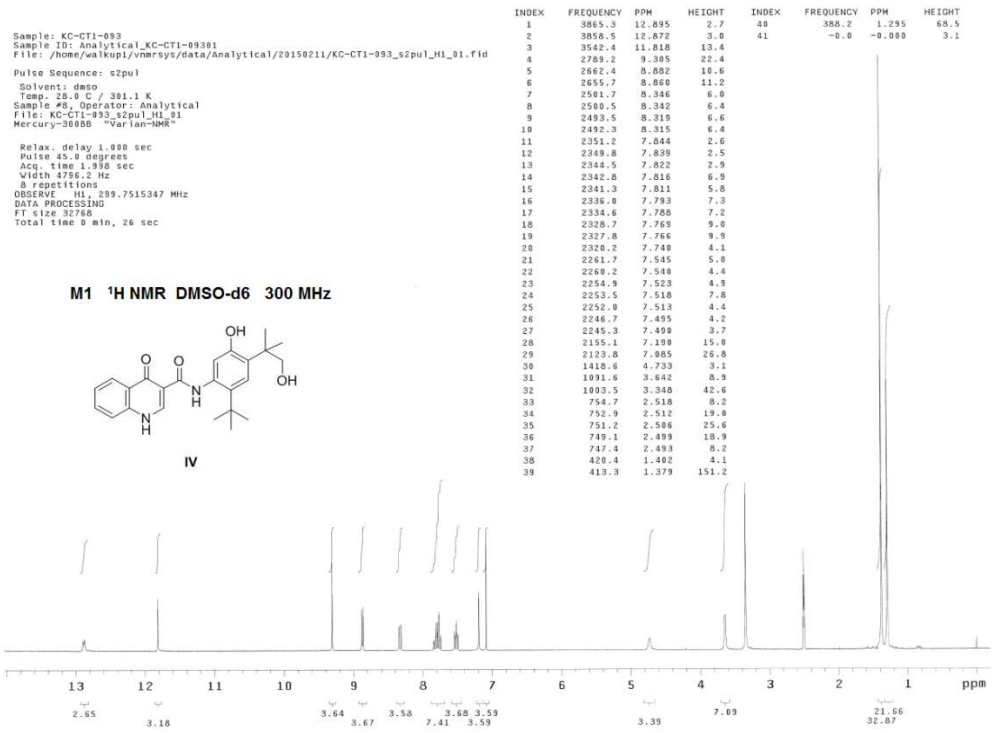
Elution Time (min)	Peak Area	Percentage of Total
5.668	5.34551	0.4412%
6.345	1.18478	0.0978%
6.538	3.91795	0.3234%
6.842	1164.10718	96.0847% (M6)
7.425	15.29650	1.2626%
7.499	4.72207	0.3898%
7.708	3.77913	0.3119%
7.819	6.19726	0.5115%
7.877	5.70288	0.4707%
8.095	1.28917	0.1064%

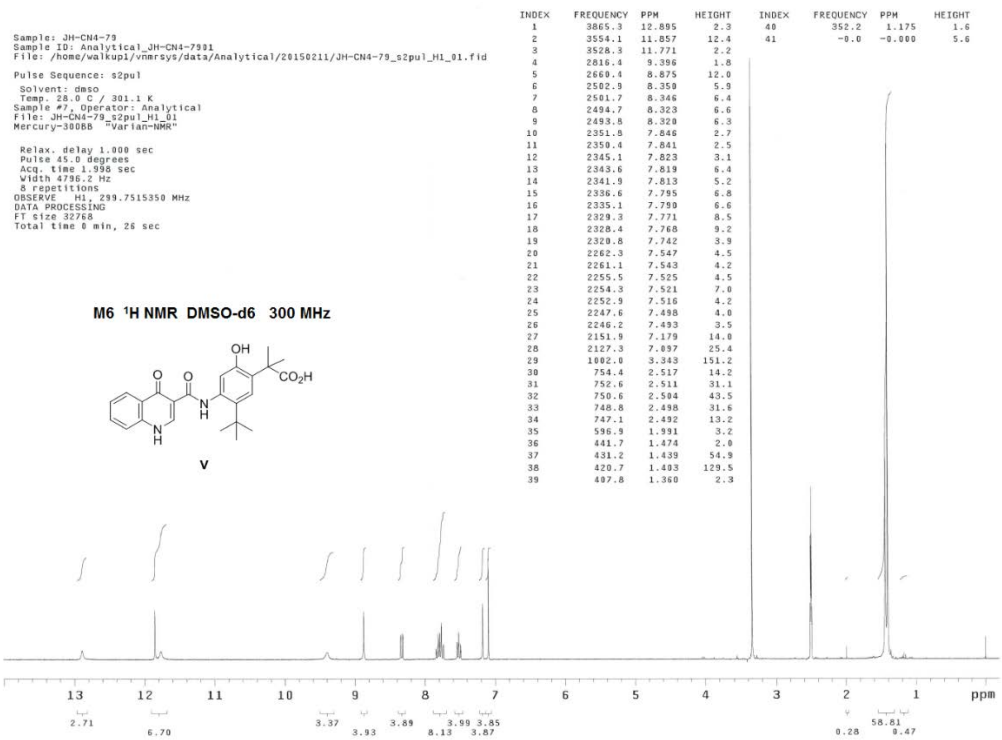
Supplemental Figure 14. M1 and M6 MS identity assessments



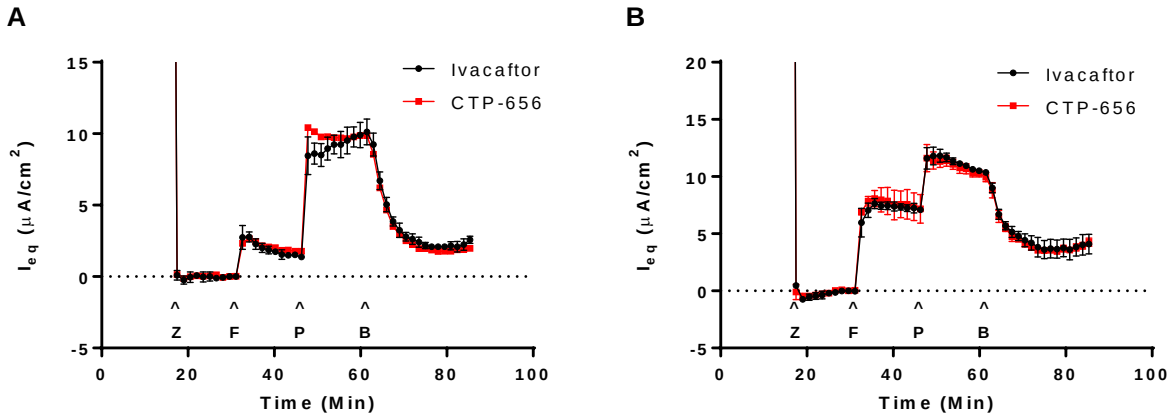


Supplemental Figure 15. M1 and M6 ^{1}H-NMR spectra





Supplemental Figure 16. Current Traces Showing Potentiator Responses of Ivacaftor and CTP-656 in A) G551D/F508del HBE and B) VX-809-corrected F508del/F508del HBE



Mean ± SD, N = 3 replicates. Z = Benzamil (1 μM), F = Forskolin (10 μM); P = Potentiator (300 nM); B = Bumetanide (20 μM). Data for potentiator addition at 300 nM are shown, which is the approximate EC_{50} for VX-770 and CTP-656. Figures are representative of all dose-response comparisons.