METHODS OF TREATMENT FOR CYSTIC FIBROSIS

[0001] This application claims the benefit of priority to U.S. Provisional Application No. 63/123,928, filed December 10, 2020; U.S. Provisional Application No. 63/124,575, filed December 11, 2020; and U.S. Provisional Application No. 63/150,434, filed February 17, 2021, the contents of each of which are incorporated by reference in their respective entireties.

[0002] The invention provides pharmaceutical compositions for and methods of treating cystic fibrosis.

[0003] Cystic fibrosis (CF) is a recessive genetic disease that affects approximately 83,000 children and adults worldwide. Despite progress in the treatment of CF, there is no cure.

[0004] In patients with CF, mutations in CFTR endogenously expressed in respiratory epithelia lead to reduced apical anion secretion causing an imbalance in ion and fluid transport. The resulting decrease in anion transport contributes to enhanced mucus accumulation in the lung and accompanying microbial infections that ultimately cause death in CF patients. In addition to respiratory disease, CF patients typically suffer from gastrointestinal problems and pancreatic insufficiency that, if left untreated, result in death. In addition, the majority of males with cystic fibrosis are infertile, and fertility is reduced among females with cystic fibrosis.

[0005] Sequence analysis of the CFTR gene has revealed a variety of disease-causing mutations (Cutting, G. R. et al. (1990) Nature 346:366-369; Dean, M. et al. (1990) Cell 61:863:870; and Kerem, B-S. et al. (1989) Science 245:1073-1080; Kerem, B-S et al. (1990) Proc. Natl. Acad. Sci. USA 87:8447-8451). To date, greater than 2000 mutations in the CF gene have been identified. CF mutations are listed in the "Cystic Fibrosis Mutation Database," located at http://www.genet.sickkids.on.ca/app, which is incorporated herein by reference in its entirety. The most prevalent disease-causing mutation is a deletion of phenylalanine at position 508 of the CFTR amino acid sequence and is commonly referred to as the *F508del* mutation. This mutation occurs in approximately 90% of the cases of cystic fibrosis and is associated with severe disease.

[0006] The deletion of residue 508 in CFTR prevents the nascent protein from folding correctly. This results in the inability of the mutant protein to exit the endoplasmic

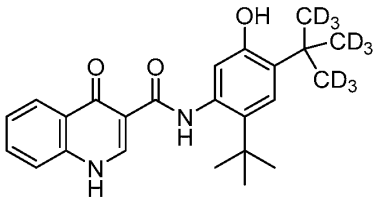
reticulum (ER) and traffic to the plasma membrane. As a result, the number of CFTR channels for anion transport present in the membrane is far less than observed in cells expressing wild-type CFTR, i.e., CFTR having no mutations. In addition to impaired trafficking, the mutation results in defective channel gating. Together, the reduced number of channels in the membrane and the defective gating lead to reduced anion and fluid transport across epithelia. (Quinton, P. M. (1990), FASEB J. 4: 2709-2727). The channels that are defective because of the F508del mutation are still functional, albeit less functional than wild-type CFTR channels. (Dalemans et al. (1991), Nature Lond. 354: 526-528; Pasyk and Foskett (1995), J. Cell. Biochem. 270: 12347-50). In addition to F508del, other disease-causing mutations in CFTR that result in defective trafficking, synthesis, and/or channel gating could be up- or down-regulated to alter anion secretion and modify disease progression and/or severity.

[0007] CFTR is a cAMP/ATP-mediated anion channel that is expressed in a variety of cell types, including absorptive and secretory epithelia cells, where it regulates anion flux across the membrane, as well as the activity of other ion channels and proteins. In epithelial cells, normal functioning of CFTR is critical for the maintenance of electrolyte transport throughout the body, including respiratory and digestive tissue. CFTR is composed of 1480 amino acids that encode a protein which is made up of a tandem repeat of transmembrane domains, each containing six transmembrane helices and a nucleotide binding domain. The two transmembrane domains are linked by a large, polar, regulatory (R)-domain with multiple phosphorylation sites that regulate channel activity and cellular trafficking.

[0008] Chloride transport takes place by the coordinated activity of ENaC (epithelial sodium channel) and CFTR present on the apical membrane and the Na^+-K^+-ATPase pump and Cl^- channels expressed on the basolateral surface of the cell. Secondary active transport of chloride from the luminal side leads to the accumulation of intracellular chloride, which can then passively leave the cell via Cl^- channels, resulting in a vectorial transport. Arrangement of $Na^+/2Cl^-/K^+$ co-transporter, Na^+-K^+-ATPase pump and the basolateral membrane K^+ channels on the basolateral surface and CFTR on the luminal side coordinate the secretion of chloride. Because water is probably never actively transported itself, its flow across epithelia depends on tiny transepithelial osmotic gradients generated by the bulk flow of sodium and chloride.

[0009] A number of CFTR modulating compounds have recently been identified. However, compounds that can treat or reduce the severity of the cystic fibrosis and other CFTR mediated diseases, and particularly the more severe forms of these diseases, are still needed.

[0010] Thus, one aspect of the disclosure provides pharmaceutical composition comprising 250 mg of CFTR potentiator compound, *N*-(2-(*tert*-butyl)-5-hydroxy-4-(2-(methyl-d3)propan-2-yl-1,1,1,3,3,3-d6)phenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (Compound **I**) or an equivalent amount of a pharmaceutically acceptable salt thereof. Compound **I** can be depicted as having the following structure:



(Compound **I**)

[0011] It has been discovered that 250 mg of Compound I administered once daily may improve the therapeutic profile as measured by sweat chloride (SwCl), compared to 150 mg q12h of ivacaftor (i.e., twice daily at 12 hour intervals) and 150 mg qd of Compound I (i.e., once daily).

[0012] Other aspects of the disclosure provide pharmaceutical compositions comprising 250 mg of Compound **I** or an equivalent amount of a pharmaceutically acceptable salt thereof, that further include at least one additional active pharmaceutical ingredient and/or at least one carrier. Yet other aspects of the disclosure are methods of treating the CFTR-mediated disease cystic fibrosis comprising administering Compound **I** or an equivalent amount of a pharmaceutically acceptable salt thereof, optionally as part of a pharmaceutical composition comprising at least one additional component, to a subject in need thereof. In some embodiments, the pharmaceutical compositions of the invention comprise 250 mg of Compound **I** (or an equivalent amount of a pharmaceutically acceptable salt thereof), 21.24 mg of Compound **II**, calcium salt hydrate Form D, and 100 mg of Compound **III** (or an equivalent amount of a pharmaceutically acceptable salt thereof). In some embodiments, the pharmaceutical compositions of the invention comprise 125 mg of Compound **I** (or an equivalent amount of a pharmaceutically acceptable salt thereof), 10.62 mg of Compound **II**, calcium salt hydrate Form D, and 50

mg of Compound **III** (or an equivalent amount of a pharmaceutically acceptable salt thereof).

[0013] One embodiment provides a method of treating the CFTR-mediated disease cystic fibrosis comprising administering 250 mg of *N*-(2-(*tert*-butyl)-5-hydroxy-4-(2-(methyl-d3)propan-2-yl-1,1,1,3,3,3-d6)phenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (Compound **I**), alone or in combination with 21.24 mg of (14S)-8-[3-(2-{Dispiro[2.0.2.1]heptan-7-yl}ethoxy)-1H-pyrazol-1-yl]-12,12-dimethyl-2λ6-thia-3,9,11,18,23-pentaazatetracyclo [17.3.1.111,14.05,10]tetracosa-1(22),5,7,9,19(23),20-hexaene-2,2,4-trione (Compound **II**) calcium salt hydrate Form D, and/or 50-100 mg of (*R*)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-N-(1-(2,3-dihydroxypropyl)-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide (Compound **III**).

[0014] In some embodiments, 250 mg of Compound **I** (or an equivalent amount of a pharmaceutically acceptable salt thereof) is administered in the same composition with 20 mg of Compound **II** calcium salt hydrate Form D. In some embodiments, 250 mg of Compound **I** is administered in the same composition with 21.24 mg of Compound **II** calcium salt hydrate Form D and 100 mg of Compound **III** (or an equivalent amount of a pharmaceutically acceptable salt thereof). In some embodiments, a composition comprising 250 mg of Compound **I** (or an equivalent amount of a pharmaceutically acceptable salt thereof) is co-administered with a separate composition comprising 21.24 mg of Compound **II** calcium salt hydrate Form D and/or 100 mg of Compound **III** (or an equivalent amount of a pharmaceutically acceptable salt thereof). In some embodiments 250 mg of Compound **I** is administered in the same composition with 21.24 mg of Compound **II** calcium salt hydrate Form D and 100 mg of Compound **III** (or an equivalent amount of a pharmaceutically acceptable salt thereof). In some embodiments, the 250 mg of Compound **I**, 21.24 mg of Compound **II** calcium salt hydrate Form D. and 100 mg of Compound **III** (or an equivalent amount of a pharmaceutically acceptable salt thereof) are administered once daily in two equivalent dose compositions.

Brief Description of the Figures

[0015] **Fig. 1** provides an XRPD pattern of crystalline Compound **I** (free form) Form A.

[0016] **Fig. 2** shows a ^{13}C solid state NMR spectrum of crystalline Compound **I** (free form) Form A.

[0017] **Fig. 3** provides an XRPD pattern of crystalline Compound **I** calcium salt hydrate Form A.

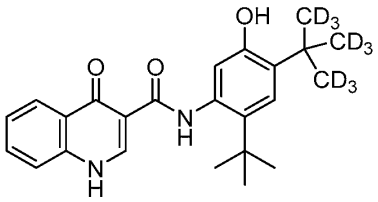
[0018] **Fig. 4** shows a ^{13}C solid state NMR spectrum of Compound **I** calcium salt hydrate Form A.

[0019] **Fig. 5** provides an XRPD pattern of crystalline Compound **I** calcium salt hydrate Form D.

[0020] **Fig. 6** shows a ^{13}C solid state NMR spectrum of Compound **I** calcium salt hydrate Form D.

Definitions

[0021] "Compound **I**" as used throughout this disclosure refers to *N*-(2-(*tert*-butyl)-5-hydroxy-4-(2-(methyl-d3)propan-2-yl-1,1,1,3,3,3-d6)phenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide, which can be depicted as having the following structure:



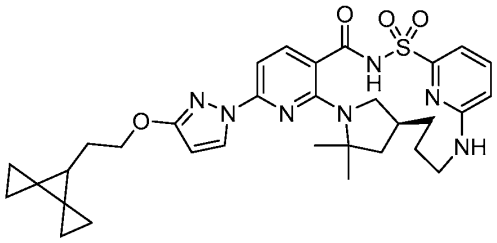
(Compound **I**).

Compound **I** may be in the form of a pharmaceutically acceptable salt. Compound **I** and its pharmaceutically acceptable salts have been previously described in United States Patent Nos. 8,865,902, 9,181,192, and 9,512,079, and International Patent Publication Nos. WO 2012/158885, WO 2014/078842, WO 2017/053455, and WO 2018/080591, each of which are incorporated herein by reference.

[0022] In some embodiments, the isotopic enrichment factor of each deuterium in Compound I may vary. The term "isotopic enrichment factor" refers to the ratio between the isotopic abundance and the natural abundance of a specified isotope. In some embodiments, the isotopic enrichment factor for each designated deuterium atom in Compound I is at least 3500 (52.5% deuterium incorporation at each designated deuterium atom), at least 4000 (60% deuterium incorporation), at least 4500 (67.5% deuterium incorporation), at least 5000 (75% deuterium incorporation), at least 5500 (82.5%

deuterium incorporation), at least 6000 (90% deuterium incorporation), at least 6333.3 (95% deuterium incorporation), at least 6466.7 (97% deuterium incorporation), at least 6600 (99% deuterium incorporation), or at least 6633.3 (99.5% deuterium incorporation).

[0023] "Compound **II**" as used herein, refers to (14*S*)-8-[3-(2-{Dispiro[2.0.2.1]heptan-7-yl}ethoxy)-1H-pyrazol-1-yl]-12,12-dimethyl-2λ6-thia-3,9,11,18,23-pentaazatetracyclo[17.3.1.111,14.05,10]tetracosa-1(22),5,7,9,19(23),20-hexaene-2,2,4-trione, which can be depicted with the following structure:



(Compound **II**).

Compound **II** and deuterated derivatives and pharmaceutically acceptable salts thereof were first described in International Patent Publication WO 2019/161078 (incorporated herein by reference).

[0024] In some embodiments, Compound **II** is in the form of calcium salt hydrate Form D. 20 mg of Compound **II** is equivalent to 21.24 mg of Compound **II** calcium salt hydrate From D. In some embodiments, Compound **II** calcium salt hydrate Form D is characterized by an X-ray powder diffractogram having signals at 6.1 ± 0.2 degrees two-theta, 16.2 ± 0.2 degrees two-theta, and 22.8 ± 0.2 degrees two-theta. In some embodiments, Compound **II** calcium salt hydrate Form D is characterized by an X-ray powder diffractogram having (a) signals at 6.1 ± 0.2 degrees two-theta, 16.2 ± 0.2 degrees two-theta, and 22.8 ± 0.2 degrees two-theta; and (b) one or more signals selected from 5.5 ± 0.2 degrees two-theta, 15.5 ± 0.2 degrees two-theta, 19.7 ± 0.2 degrees two-theta, 21.5± 0.2 degrees two-theta, 22.1 ± 0.2 degrees two-theta, 23.0 ± 0.2 degrees two-theta, and 27.6 ± 0.2 degrees two-theta.

[0025] In some embodiments, Compound **II** calcium salt hydrate Form D is characterized by an X-ray powder diffractogram having (a) signals at 6.1 ± 0.2 degrees two-theta, 16.2 ± 0.2 degrees two-theta, and 22.8 ± 0.2 degrees two-theta; and (b) two or more signals selected from 5.5 ± 0.2 degrees two-theta, 15.5 ± 0.2 degrees two-theta, 19.7 ± 0.2 degrees two-theta, 21.5± 0.2 degrees two-theta, 22.1 ± 0.2 degrees two-theta, 23.0 ±

0.2 degrees two-theta, and 27.6 ± 0.2 degrees two-theta. In some embodiments, Compound **II** calcium salt hydrate Form D is characterized by an X-ray powder diffractogram having (a) signals at 6.1 ± 0.2 degrees two-theta, 16.2 ± 0.2 degrees two-theta, and 22.8 ± 0.2 degrees two-theta; and (b) three or more signals selected from 5.5 ± 0.2 degrees two-theta, 15.5 ± 0.2 degrees two-theta, 19.7 ± 0.2 degrees two-theta, 21.5± 0.2 degrees two-theta, 22.1 ± 0.2 degrees two-theta, 23.0 ± 0.2 degrees two-theta, and 27.6 ± 0.2 degrees two-theta. In some embodiments, Compound **II** calcium salt hydrate Form D is characterized by an X-ray powder diffractogram having (a) signals at 6.1 ± 0.2 degrees two-theta, 16.2 ± 0.2 degrees two-theta, and 22.8 ± 0.2 degrees two-theta; and (b) four or more signals selected from 5.5 ± 0.2 degrees two-theta, 15.5 ± 0.2 degrees two-theta, 19.7 ± 0.2 degrees two-theta, 21.5± 0.2 degrees two-theta, 22.1 ± 0.2 degrees two-theta, 23.0 ± 0.2 degrees two-theta, and 27.6 ± 0.2 degrees two-theta.

[0026] In some embodiments, Compound **II** calcium salt hydrate Form D is characterized by an X-ray powder diffractogram having signals at 6.1 ± 0.2 degrees two-theta, 16.2 ± 0.2 degrees two-theta, and 22.8 ± 0.2 degrees two-theta, and 27.6 ± 0.2 degrees two-theta. In some embodiments, Compound **II** calcium salt hydrate Form D is characterized by an X-ray powder diffractogram having signals at 6.1 ± 0.2 degrees two-theta, 15.5 ± 0.2 degrees two-theta, 16.2 ± 0.2 degrees two-theta, 19.7 ± 0.2 degrees two-theta, 22.8 ± 0.2 degrees two-theta, and 27.6 ± 0.2 degrees two-theta. In some embodiments Compound **II** calcium salt hydrate Form D is characterized by an X-ray powder diffractogram substantially similar to **Fig. 5**.

[0027] In some embodiments, Compound **II** calcium salt hydrate Form D is characterized as having a ^{13}C solid state nuclear magnetic resonance (^{13}C ssNMR) spectrum with one or more peaks selected from: 179.8 ± 0.2 ppm, 130.2 ± 0.2 ppm, 125.6 ± 0.2 ppm, 120.9 ± 0.2 ppm, 55.2 ± 0.2 ppm, 44.3 ± 0.2 ppm, 35.0 ± 0.2 ppm, and 1.6 ± 0.2 ppm. In some embodiments, Compound **II** calcium salt hydrate Form D is characterized as having a ^{13}C ssNMR spectrum with two or more peaks selected from 179.8 ± 0.2 ppm, 130.2 ± 0.2 ppm, 125.6 ± 0.2 ppm, 120.9 ± 0.2 ppm, 55.2 ± 0.2 ppm, 44.3 ± 0.2 ppm, 35.0 ± 0.2 ppm, and 1.6 ± 0.2 ppm. In some embodiments, Compound **II** calcium salt hydrate Form D is characterized as having a ^{13}C ssNMR spectrum with three or more peaks selected from 179.8 ± 0.2 ppm, 130.2 ± 0.2 ppm, 125.6 ± 0.2 ppm, 120.9 ± 0.2 ppm, 55.2 ± 0.2 ppm, 44.3 ± 0.2 ppm, 35.0 ± 0.2 ppm, and 1.6 ± 0.2 ppm. In some

embodiments, Compound **II** calcium salt hydrate Form D is characterized as having a ^{13}C ssNMR spectrum with four or more peaks selected from: 179.8 ± 0.2 ppm, 130.2 ± 0.2 ppm, 125.6 ± 0.2 ppm, 120.9 ± 0.2 ppm, 55.2 ± 0.2 ppm, 44.3 ± 0.2 ppm, 35.0 ± 0.2 ppm, and 1.6 ± 0.2 ppm. In some embodiments, Compound **II** calcium salt hydrate Form D is characterized as having a ^{13}C ssNMR spectrum with five or more peaks selected from 179.8 ± 0.2 ppm, 130.2 ± 0.2 ppm, 125.6 ± 0.2 ppm, 120.9 ± 0.2 ppm, 55.2 ± 0.2 ppm, 44.3 ± 0.2 ppm, 35.0 ± 0.2 ppm, and 1.6 ± 0.2 ppm. In some embodiments, Compound **II** calcium salt hydrate Form D is characterized as having a ^{13}C ssNMR spectrum with six or more peaks selected from 179.8 ± 0.2 ppm, 130.2 ± 0.2 ppm, 125.6 ± 0.2 ppm, 120.9 ± 0.2 ppm, 55.2 ± 0.2 ppm, 44.3 ± 0.2 ppm, 35.0 ± 0.2 ppm, and 1.6 ± 0.2 ppm.

[0028] In some embodiments, Compound **II** calcium salt hydrate Form D is characterized as having a ^{13}C ssNMR spectrum with one or more peaks selected from 130.2 ± 0.2 ppm, 125.6 ± 0.2 ppm, and 35.0 ± 0.2 ppm. In some embodiments, Compound **II** calcium salt hydrate Form D is characterized as having a ^{13}C ssNMR spectrum with two or more peaks selected from 130.2 ± 0.2 ppm, 125.6 ± 0.2 ppm, and 35.0 ± 0.2 ppm. In some embodiments, Compound **I** calcium salt hydrate Form D is characterized as having a ^{13}C ssNMR spectrum with peaks at 130.2 ± 0.2 ppm, 125.6 ± 0.2 ppm, and 35.0 ± 0.2 ppm.

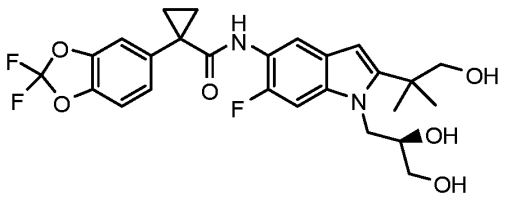
[0029] In some embodiments, Compound **II** calcium salt hydrate Form D is characterized as having a ^{13}C ssNMR spectrum with (a) a peak at 130.2 ± 0.2 ppm, 125.6 ± 0.2 ppm, and/or 35.0 ± 0.2 ppm; and (b) a peak at 176.9 ± 0.2 ppm, 160.9 ± 0.2 ppm, 142.0 ± 0.2 ppm, and/or 98.6 ± 0.2 ppm. In some embodiments, Compound **II** calcium salt hydrate Form D is characterized as having a ^{13}C ssNMR spectrum with (a) a peak at 130.2 ± 0.2 ppm, 125.6 ± 0.2 ppm, and/or 35.0 ± 0.2 ppm; and (b) peaks at 176.9 ± 0.2 ppm, 160.9 ± 0.2 ppm, 142.0 ± 0.2 ppm, and 98.6 ± 0.2 ppm. In some embodiments, Compound **II** calcium salt hydrate Form D is characterized as having a ^{13}C ssNMR spectrum with (a) peaks at 130.2 ± 0.2 ppm, 125.6 ± 0.2 ppm, and 35.0 ± 0.2 ppm; and (b) a peak at 176.9 ± 0.2 ppm, 160.9 ± 0.2 ppm, 142.0 ± 0.2 ppm, and/or 98.6 ± 0.2 ppm. In some embodiments, Compound **II** calcium salt hydrate Form D is characterized as having a ^{13}C ssNMR spectrum with peaks at 130.2 ± 0.2 ppm, 125.6 ± 0.2 ppm, 35.0 ± 0.2 ppm, 176.9 ± 0.2 ppm, 160.9 ± 0.2 ppm, 142.0 ± 0.2 ppm, and 98.6 ± 0.2 ppm.

[0030] In some embodiments, Compound **II** calcium salt hydrate Form D is characterized by a ^{13}C ssNMR spectrum substantially similar to **Fig. 6**.

[0031] In some embodiments, Compound **II** calcium salt hydrate Form D is characterized by a triclinic crystal system, a P1 space group, and the following unit cell dimensions measured at by 100 K on a Bruker diffractometer equipped with Cu K_α radiation (λ=1.5478 Å) and a Complementary metal-oxide-semiconductor (CMOS) detector:

a	12.78 ± .01 Å	α	64.93 ± .02 °
b	16.64 ± .01 Å	β	75.10 ± .02 °
c	18.19 ± .01 Å	γ	68.22 ± .02 °.

[0032] "Compound **III**" as used throughout this disclosure refers (*R*)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)-*N*-(1-(2,3-dihydroxypropyl)-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl)cyclopropanecarboxamide:



(Compound **III**).

In some embodiments, Compound **III** is in the form of a pharmaceutically acceptable salt. Compound **III** and its pharmaceutically acceptable salts were previously disclosed in International Patent Publication WO 2010/053471 (incorporated herein by reference).

[0033] As used herein, "CFTR" means cystic fibrosis transmembrane conductance regulator.

[0034] As used herein, "mutations" can refer to mutations in the *CFTR* gene or the CFTR protein. A "*CFTR* gene mutation" refers to a mutation in the *CFTR* gene, and a "CFTR protein mutation" refers to a mutation in the CFTR protein. A genetic defect or mutation, or a change in the nucleotides in a gene in general results in a mutation in the CFTR protein translated from that gene, or a frame shift(s).

[0035] The term "F508del" refers to a mutant CFTR protein which is lacking the amino acid phenylalanine at position 508.

[0036] As used herein, a patient who is "homozygous" for a particular gene mutation has the same mutation on each allele.

[0037] As used herein, a patient who is "heterozygous" for a particular gene mutation has the particular mutation on one allele, and a different mutation on the other allele.

[0038] As used herein, the term "modulator" refers to a compound that increases the activity of a biological compound such as a protein. For example, a CFTR modulator is a compound that increases the activity of CFTR. The increase in activity resulting from a CFTR modulator includes but is not limited to compounds that correct, potentiate, stabilize and/or amplify CFTR.

[0039] As used herein, the term "CFTR corrector" refers to a compound that facilitates the processing and trafficking of CFTR to increase the amount of CFTR at the cell surface. Compounds **II** and **III** disclosed herein are CFTR correctors.

[0040] As used herein, the term "CFTR potentiator" refers to a compound that increases the channel activity of CFTR protein located at the cell surface, resulting in enhanced ion transport. Compound **I** disclosed herein is a CFTR potentiator.

[0041] As used herein, the term "active pharmaceutical ingredient" or "therapeutic agent" ("API") refers to a biologically active compound.

[0042] As used herein, the term "pharmaceutically acceptable salt" refers to a salt form of a compound of this disclosure wherein the salt is nontoxic. Pharmaceutically acceptable salts of the compounds of this disclosure include those derived from suitable inorganic and organic acids and bases. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, *et al.* describes pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 1977, *66*, 1-19.

[0043] As used herein, the term "amorphous" refers to a solid material having no long-range order in the position of its molecules. Amorphous solids are generally supercooled liquids in which the molecules are arranged in a random manner so that there is no well-defined arrangement, e.g., molecular packing, and no long-range order. Amorphous solids are generally isotropic, i.e. exhibit similar properties in all directions and do not have definite melting points. For example, an amorphous material is a solid material having no sharp characteristic crystalline peak(s) in its X-ray power diffraction (XRPD) pattern (i.e., is not crystalline as determined by XRPD). Instead, one or several broad peaks (e.g., halos) appear in its XRPD pattern. Broad peaks are characteristic of an amorphous solid. See, US 2004/0006237 for a comparison of XRPDs of an amorphous material and

crystalline material. In some embodiments, a solid material may comprise an amorphous compound, and the material may, for example, be characterized by a lack of sharp characteristic crystalline peak(s) in its XRPD spectrum (i.e. the material is not crystalline, but is amorphous, as determined by XRPD). Instead, one or several broad peaks (e.g. halos) may appear in the XRPD pattern of the material. See US 2004/0006237 for a comparison of XRPDs of an amorphous material and crystalline material. A solid material, comprising an amorphous compound, may be characterized by, for example, a wider temperature range for the melting of the solid material, as compared to the range for the melting of a pure crystalline solid. Other techniques, such as, for example, Raman spectroscopy, infrared spectroscopy, and solid-state NMR may be used to characterize crystalline or amorphous forms.

[0044] In some embodiments, a solid material may comprise a mixture of crystalline solids and amorphous solids. A solid material prepared to comprise an amorphous compound may also, for example, contain up to 30% of a crystalline solid. In some embodiments, a solid material prepared to comprise an amorphous compound may also, for example, contain up to 25%, 20%, 15%, 10%, 5%, or 2% of a crystalline solid. In embodiments wherein the solid material contains a mixture of crystalline solids and amorphous solids, the characterizing data, such as XRPD, may contain indicators of both crystalline and amorphous solids.

[0045] As used herein, the term "substantially amorphous" refers to a solid material having little or no long-range order in the position of its molecules. For example, substantially amorphous materials have less than 15% crystallinity (e.g., less than 10% crystallinity, less than 5% crystallinity, or less than 2% crystallinity). It is also noted that the term 'substantially amorphous' includes the descriptor, 'amorphous', which refers to materials having no (0%) crystallinity.

[0046] As used herein, the term "substantially crystalline" refers to a solid material having little or no amorphous molecules. For example, substantially crystalline materials have less than 15% amorphous molecules (e.g., less than 10% amorphous molecules, less than 5% amorphous molecules, or less than 2% amorphous molecules). It is also noted that the term “substantially crystalline” includes the descriptor “crystalline,” which refers to materials that are 100% crystalline form.

[0047] As used herein, the term "XRPD" refers to the analytical characterization method of X-ray powder diffraction. XRPD patterns disclosed herein were recorded at ambient conditions in transmission or reflection geometry using a diffractometer.

[0048] As used herein, the term "ambient conditions" means room temperature, open air condition and uncontrolled humidity condition. The terms "room temperature" and "ambient temperature" mean 15 ºC to 30 ºC.

[0049] As used herein, the terms "X-ray powder diffractogram," "X-ray powder diffraction pattern," "XRPD pattern," "XRPD spectrum" interchangeably refer to an experimentally obtained pattern plotting signal positions (on the abscissa) versus signal intensities (on the ordinate). For an amorphous material, an X-ray powder diffractogram may include one or more broad signals; and for a crystalline material, an X-ray powder diffractogram may include one or more signals, each identified by its angular value as measured in degrees 2θ (° 2θ), depicted on the abscissa of an X-ray powder diffractogram, which may be expressed as "a signal at … degrees two-theta," "a signal at [a] two-theta value(s)of …" and/or "a signal at at least … two-theta value(s) selected from …."

[0050] A "signal" or "peak" as used herein refers to a point in the XRPD pattern where the intensity as measured in counts is at a local maximum. One of ordinary skill in the art would recognize that one or more signals (or peaks) in an XRPD pattern may overlap and may, for example, not be apparent to the naked eye. Indeed, one of ordinary skill in the art would recognize that some art-recognized methods are capable of and suitable for determining whether a signal exists in a pattern, such as Rietveld refinement.

[0051] As used herein, "a signal at … degrees two-theta" refer to X-ray reflection positions as measured and observed in X-ray powder diffraction experiments (° 2θ).

[0052] The repeatability of the measured angular values is in the range of ±0.2° 2θ, i.e., the angular value can be at the recited angular value + 0.2 degrees two-theta, the angular value - 0.2 degrees two-theta, or any value between those two end points (angular value +0.2 degrees two-theta and angular value -0.2 degrees two-theta).

[0053] The terms "signal intensities" and "peak intensities" interchangeably refer to relative signal intensities within a given X-ray powder diffractogram. Factors that can affect the relative signal or peak intensities include sample thickness and preferred orientation (e.g., the crystalline particles are not distributed randomly).

[0054] As used herein, an X-ray powder diffractogram is "substantially similar to that in [a particular] Figure" when at least 90%, such as at least 95%, at least 98%, or at least 99%, of the signals in the two diffractograms overlap. In determining "substantial similarity," one of ordinary skill in the art will understand that there may be variation in the intensities and/or signal positions in XRPD diffractograms even for the same crystalline form. Thus, those of ordinary skill in the art will understand that the signal maximum values in XRPD diffractograms (in degrees two-theta) generally mean that value is identified as ±0.2 degrees two-theta of the reported value, an art-recognized variance.

[0055] As used herein, a ^{13}C ssNMR spectrum is "substantially similar to that in [a particular] Figure" when at least 90%, such as at least 95%, at least 98%, or at least 99%, of the signals in the two spectra overlap. In determining "substantial similarity," one of ordinary skill in the art will understand that there may be variation in the intensities and/or signal positions in ssNMR spectra even for the same crystalline form. Thus, those of ordinary skill in the art will understand that the chemical shifts in ssNMR spectra (in parts per million (ppm) referred to herein) generally mean that value is identified as ± 0.2 ppm of the reported value, an art-recognized variance.

[0056] The term "X-ray powder diffractogram having a signal at … two-theta values" as used herein refers to an XRPD pattern that contains X-ray reflection positions as measured and observed in X-ray powder diffraction experiments (° two-theta).

[0057] As used herein, the term "DSC" refers to the analytical method of Differential Scanning Calorimetry.

[0058] As used herein, the term "solvent" refers to any liquid in which the product is at least partially soluble (solubility of product >1 g/l).

[0059] As used herein, the term "dispersion" refers to a disperse system in which one substance, the dispersed phase, is distributed, in discrete units, throughout a second substance (the continuous phase or vehicle). The size of the dispersed phase can vary considerably (e.g. colloidal particles of nanometer dimension, to multiple microns in size). In general, the dispersed phases can be solids, liquids, or gases. In the case of a solid dispersion, the dispersed and continuous phases are both solids. In pharmaceutical applications, a solid dispersion can include a crystalline drug (dispersed phase) in an

amorphous polymer (continuous phase); or alternatively, an amorphous drug (dispersed phase) in an amorphous polymer (continuous phase). In some embodiments, a solid dispersion includes the polymer constituting the dispersed phase, and the drug constitute the continuous phase. Or, a solid dispersion includes the drug constituting the dispersed phase, and the polymer constituting the continuous phase.

[0060] The terms “patient” and “subject” are used interchangeably and refer to an animal including humans.

[0061] As used herein, the terms "treatment," "treating," and the like generally mean the improvement of CF or its symptoms or lessening the severity of CF or one or more of the symptoms of CF in a subject. “Treatment,” as used herein, includes, but is not limited to, the following: increased growth of the subject, increased weight gain, reduction of mucus in the lungs, improved pancreatic and/or liver function, reduction of chest infections, and/or reductions in coughing or shortness of breath. Improvements in or lessening the severity of any of these symptoms can be readily assessed according to standard methods and techniques known in the art.

[0062] As used herein, the term “in combination with,” when referring to two or more compounds, agents, or additional active pharmaceutical ingredients, means the administration of two or more compounds, agents, or active pharmaceutical ingredients to the patient prior to, concurrently with, or subsequent to each other.

[0063] The terms “about” and “approximately”, when used in connection with doses, amounts, or weight percent of ingredients of a composition or a dosage form, include the value of a specified dose, amount, or weight percent or a range of the dose, amount, or weight percent that is recognized by one of ordinary skill in the art to provide a pharmacological effect equivalent to that obtained from the specified dose, amount, or weight percent. The terms “about” and “approximately” may refer to an acceptable error for a particular value as determined by one of skill in the art, which depends in part on how the values is measured or determined. In some embodiments, the terms “about” and “approximately” mean within 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1%, or 0.5% of a given value or range.

[0064] One of ordinary skill in the art would recognize that, when an amount of “a compound or a pharmaceutically acceptable salt thereof” is disclosed, the amount of the

pharmaceutically acceptable salt form of the compound is the amount equivalent to the concentration of the free base of the compound. It is noted that the disclosed amounts of the compounds or their pharmaceutically acceptable salts thereof herein are based upon their free base form. For example, "100 mg of at least one compound chosen from Compound **I** and pharmaceutically acceptable salts thereof" includes 100 mg of Compound **I** and a concentration of a pharmaceutically acceptable salt of Compound **I** equivalent to 100 mg of Compound **I**.

[0065] Suitable pharmaceutically acceptable salts are, for example, those disclosed in S. M. Berge, *et al. J. Pharmaceutical Sciences*, 1977, *66*, 1-19. For example, Table 1 of that article provides the following pharmaceutically acceptable salts:

Table 1:

Acetate	Iodide	Benzathine
Benzenesulfonate	Isethionate	Chloroprocaine
Benzoate	Lactate	Choline
Bicarbonate	Lactobionate	Diethanolamine
Bitartrate	Malate	Ethylenediamine
Bromide	Maleate	Meglumine
Calcium edetate	Mandelate	Procaine
Camsylate	Mesylate	Aluminum
Carbonate	Methylbromide	Calcium
Chloride	Methylnitrate	Lithium
Citrate	Methylsulfate	Magnesium
Dihydrochloride	Mucate	Potassium
Edetate	Napsylate	Sodium
Edisylate	Nitrate	Zinc
Estolate	Pamoate (Embonate)	
Esylate	Pantothenate	

Fumarate	Phosphate/diphosphate	
Gluceptate	Polygalacturonate	
Gluconate	Salicylate	
Glutamate	Stearate	
Glycollylarsanilate	Subacetate	
Hexylresorcinate	Succinate	
Hydrabamine	Sulfate	
Hydrobromide	Tannate	
Hydrochloride	Tartrate	
Hydroxynaphthoate	Teociate	
	Triethiodide	

[0066] Non-limiting examples of pharmaceutically acceptable acid addition salts include: salts formed with inorganic acids, such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, or perchloric acid; salts formed with organic acids, such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid; and salts formed by using other methods used in the art, such as ion exchange. Non-limiting examples of pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, and valerate salts. Pharmaceutically acceptable salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium, and $N^+(C_{1-4}alkyl)_4$ salts. This disclosure also envisions the quaternization of any basic nitrogen-containing groups of the compounds disclosed

herein. Suitable non-limiting examples of alkali and alkaline earth metal salts include sodium, lithium, potassium, calcium, and magnesium. Further non-limiting examples of pharmaceutically acceptable salts include ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate and aryl sulfonate. Other suitable, non-limiting examples of pharmaceutically acceptable salts include besylate and glucosamine salts.

Methods of Treatment

[0067] A CFTR mutation may affect the CFTR quantity, i.e., the number of CFTR channels at the cell surface, or it may impact CFTR function, i.e., the functional ability of each channel to open and transport ions. Mutations affecting CFTR quantity include mutations that cause defective synthesis (Class I defect), mutations that cause defective processing and trafficking (Class II defect), mutations that cause reduced synthesis of CFTR (Class V defect), and mutations that reduce the surface stability of CFTR (Class VI defect). Mutations that affect CFTR function include mutations that cause defective gating (Class III defect) and mutations that cause defective conductance (Class IV defect). Some CFTR mutations exhibit characteristics of multiple classes.

[0068] In some embodiments, disclosed herein methods of treating cystic fibrosis in a patient comprising administering an effective amount of a compound, pharmaceutically acceptable salt thereof, or a deuterated analog of any of the foregoing; or a pharmaceutical composition, of this disclosure to a patient, such as a human, wherein said patient has cystic fibrosis. In some embodiments, the patient has an F508del/minimal function (MF) genotype, F508del/F508del genotype (homozygous for the F508del mutation), F508del/gating genotype, or F508del/residual function (RF) genotype. In some embodiments the patient is heterozygous and has one F508del mutation.

[0069] As used herein, "minimal function (MF) mutations" refer to CFTR gene mutations associated with minimal CFTR function (little-to-no functioning CFTR protein) and include, for example, mutations associated with severe defects in ability of the CFTR channel to open and close, known as defective channel gating or "gating mutations"; mutations associated with severe defects in the cellular processing of CFTR and its delivery to the cell surface; mutations associated with no (or minimal) CFTR synthesis; and mutations associated with severe defects in channel conductance.

[0070] In some embodiments, the patient is heterozygous and has an F508del mutation on one allele and a mutation on the other allele selected from Table 2:

Table 2: CFTR Mutations

Mutation				
Q2X	L218X	Q525X	R792X	E1104X
S4X	Q220X	G542X	E822X	W1145X
W19X	Y275X	G550X	W882X	R1158X
G27X	C276X	Q552X	W846X	R1162X
Q39X	Q290X	R553X	Y849X	S1196X
W57X	G330X	E585X	R851X	W1204X
E60X	W401X	G673X	Q890X	L1254X
R75X	Q414X	Q685X	S912X	S1255X
L88X	S434X	R709X	Y913X	W1282X
E92X	S466X	K710X	Q1042X	Q1313X
Q98X	S489X	Q715X	W1089X	Q1330X
Y122X	Q493X	L732X	Y1092X	E1371X
E193X	W496X	R764X	W1098X	Q1382X
W216X	C524X	R785X	R1102X	Q1411X
185+1G→T	711+5G→A	1717-8G→A	2622+1G→A	3121-1G→A
296+1G→A	712-1G→T	1717-1G→A	2790-1G→C	3500-2A→G
296+1G→T	1248+1G→A	1811+1G→C	3040G→C	3600+2insT
405+1G→A	1249-1G→A	1811+1.6kbA→G	(G970R)	3850-1G→A
405+3A→C	1341+1G→A	1811+1643G→T	3120G→A	4005+1G→A
406-1G→A	1525-2A→G	1812-1G→A	3120+1G→A	4374+1G→T
621+1G→T	1525-1G→A	1898+1G→A	3121-2A→G	
711+1G→T		1898+1G→C		
182delT	1078delT	1677delTA	2711delT	3737delA
306insA	1119delA	1782delA	2732insA	3791delC
306delTAGA	1138insG	1824delA	2869insG	3821delT
365-366insT	1154insTC	1833delT	2896insAG	3876delA
394delTT	1161delC	2043delG	2942insT	3878delG
442delA	1213delT	2143delT	2957delT	3905insT
444delA	1259insA	2183AA→G	3007delG	4016insT
457TAT→G	1288insTA	2184delA	3028delA	4021dupT
541delC	1343delG	2184insA	3171delC	4022insT
574delA	1471delA	2307insA	3171insC	4040delA
663delT	1497delGG	2347delG	3271delGG	4279insA
849delG	1548delG	2585delT	3349insT	4326delTC
935delA	1609del CA	2594delGT	3659delC	
CFTRdele1		CFTRdele16-17b	1461ins4	
CFTRdele2		CFTRdele17a,17b	1924del7	

Mutation			
CFTRdele2,3		CFTRdele17a-18	2055del9→A
CFTRdele2-4		CFTRdele19	2105-2117del13insAGAAA
CFTRdele3-10,14b-16		CFTRdele19-21	2372del8
CFTRdele4-7		CFTRdele21	2721del11
CFTRdele4-11		CFTRdele22-24	2991del32
CFTR50kbdel		CFTRdele22,23	3667ins4
CFTRdup6b-10		124del23bp	4010del4
CFTRdele11		602del14	4209TGTT→AA
CFTRdele13,14a		852del22	
CFTRdele14b-17b		991del5	
A46D	V520F	Y569D	N1303K
G85E	A559T	L1065P	
R347P	R560T	R1066C	
L467P	R560S	L1077P	
I507del	A561E	M1101K	

[0071] In some embodiments, the patient is heterozygous and has an F508del mutation on one allele and a mutation on the other allele selected from Table 3.

Table 3

711+3A>G	L206W	K1060T
2789+5G>A	R347H	A1067T
3272-26A>G	R352Q	G1069R
3849+10kbC>T	A455E	R1070Q
E56K	S549N	R1070W
P67L	S549R	F1074L
R74W	G551D	D1152H
D110E	G551S	G1244E
D110H	D579G	S1251N
R117C	E831X	S1255P
R117H	S945L	D1270N
G178R	S977F	G1349D
E193K	F1052V	

[0072] In some embodiments, the patient has at least one mutation selected from Table 4. In some embodiments, the patient has no F508del mutation and at least one mutation selected from Table 4.

Table 4

3141del9	E822K	G1244E
546insCTA	F191V	G1249R
A46D	F311del	G1349D
A120T	F311L	H139R
A234D	F508C	H199Y
A349V	F508C;S1251N †	H939R
A455E	F575Y	H1054D
A554E	F1016S	H1085P
A1006E	F1052V	H1085R
A1067T	F1074L	H1375P
D110E	F1099L	I148T
D110H	G27R	I175V
D192G	G85E	I336K
D443Y	G126D	I502T
D443Y;G576A;R668C †	G178E	I601F
D579G	G178R	I618T
D614G	G194R	I807M
D836Y	G194V	I980K
D924N	G314E	I1027T
D979V	G463V	I1139V
D1152H	G480C	I1269N
D1270N	G551D	I1366N
E56K	G551S	K1060T
E60K	G576A	L15P
E92K	G576A;R668C †	L165S
E116K	G622D	L206W
E193K	G628R	L320V
E403D	G970D	L346P
E474K	G1061R	L453S
E588V	G1069R	L967S
L997F	R117P	S945L
L1077P	R170H	S977F
L1324P	R258G	S1159F
L1335P	R334L	S1159P
L1480P	R334Q	S1251N
M152V	R347H	S1255P
M265R	R347L	T338I
M952I	R347P	T1036N
M952T	R352Q	T1053I
M1101K	R352W	V201M
P5L	R553Q	V232D
P67L	R668C	V456A
P205S	R751L	V456F
P574H	R792G	V562I
Q98R	R933G	V754M
Q237E	R1066H	V1153E
Q237H	R1070Q	V1240G

Q359R	R1070W	V1293G
Q1291R	R1162L	W361R
R31L	R1283M	W1098C
R74Q	R1283S	W1282R
R74W	S13F	Y109N
R74W;D1270N †	S341P	Y161D
R74W;V201M †	S364P	Y161S
R74W;V201M;D1270N †	S492F	Y563N
R75Q	S549N	Y1014C
R117C	S549R	Y1032C
R117G	S589N	
R117H	S737F	
R117L	S912L	

† Complex/compound mutations where a single allele of the CFTR gene has multiple mutations; these exist independent of the presence of mutations on the other allele.

[0073] In some embodiments, the disclosure also is directed to methods of treatment using isotope-labelled derivatives of the afore-mentioned compounds. In some embodiments, isotope-labelled derivatives of the afore-mentioned compounds or pharmaceutically acceptable salts thereof, wherein one or more atoms therein have been replaced by an atom or atoms having an atomic mass or mass number which differs from the atomic mass or mass number of the atom which usually occurs naturally (isotope labelled). Examples of isotopes which are commercially available and suitable for the disclosure include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, fluorine and chlorine, for example ^{2}H, ^{3}H, ^{13}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F and ^{36}Cl, respectively.

[0074] The isotope-labelled compounds and salts can be used in a number of beneficial ways. They can be suitable for medicaments and/or various types of assays, such as substrate tissue distribution assays. For example, tritium (^{3}H)- and/or carbon-14 (^{14}C)-labelled compounds are particularly useful for various types of assays, such as substrate tissue distribution assays, due to relatively simple preparation and excellent detectability. For example, deuterium (^{2}H)-labelled ones are therapeutically useful with potential therapeutic advantages over the non-^{2}H-labelled compounds. In general, deuterium (^{2}H)-labelled compounds and salts can have higher metabolic stability as compared to those that are not isotope-labelled owing to the kinetic isotope effect described below. Higher metabolic stability translates directly into an increased in vivo half-life or lower dosages,

which could be desired. The isotope-labelled compounds and salts can usually be prepared by carrying out the procedures disclosed in the synthesis schemes and the related description, in the example part and in the preparation part in the present text, replacing a non-isotope-labelled reactant by a readily available isotope-labelled reactant.

[0075] In some embodiments, the isotope-labelled compounds and salts are deuterium (^{2}H)-labelled ones. In some specific embodiments, the isotope-labelled compounds and salts are deuterium (^{2}H)-labelled, wherein one or more hydrogen atoms therein have been replaced by deuterium. In chemical structures, deuterium is represented as "D."

[0076] The deuterium (^{2}H)-labelled compounds and salts can manipulate the oxidative metabolism of the compound by way of the primary kinetic isotope effect. The primary kinetic isotope effect is a change of the rate for a chemical reaction that results from exchange of isotopic nuclei, which in turn is caused by the change in ground state energies necessary for covalent bond formation after this isotopic exchange. Exchange of a heavier isotope usually results in a lowering of the ground state energy for a chemical bond and thus causes a reduction in the rate-limiting bond breakage. If the bond breakage occurs in or in the vicinity of a saddle-point region along the coordinate of a multi-product reaction, the product distribution ratios can be altered substantially. For explanation: if deuterium is bonded to a carbon atom at a non-exchangeable position, rate differences of k_M/k_D = 2-7 are typical. For a further discussion, see S. L. Harbeson and R. D. Tung, *Deuterium In Drug Discovery and Development*, Ann. Rep. Med. Chem. 2011, 46, 403-417, incorporated in its entirety herein by reference.

[0077] The concentration of the isotope(s) (e.g., deuterium) incorporated into the isotope-labelled compounds and salt of the disclosure may be defined by the isotopic enrichment factor. The term "isotopic enrichment factor" as used herein means the ratio between the isotopic abundance and the natural abundance of a specified isotope. In some embodiments, if a substituent in a compound of the disclosure is denoted deuterium, such compound has an isotopic enrichment factor for each designated deuterium atom of at least 3500 (52.5% deuterium incorporation at each designated deuterium atom), at least 4000 (60% deuterium incorporation), at least 4500 (67.5% deuterium incorporation), at least 5000 (75% deuterium incorporation), at least 5500 (82.5% deuterium incorporation), at least 6000 (90% deuterium incorporation), at least 6333.3 (95% deuterium

incorporation), at least 6466.7 (97% deuterium incorporation), at least 6600 (99% deuterium incorporation), or at least 6633.3 (99.5% deuterium incorporation).

[0078] When discovering and developing therapeutic agents, the person skilled in the art attempts to optimize pharmacokinetic parameters while retaining desirable in vitro properties. It may be reasonable to assume that many compounds with poor pharmacokinetic profiles are susceptible to oxidative metabolism.

[0079] One aspect disclosed herein provides methods of treating cystic fibrosis and other CFTR mediated diseases comprising administering 250 mg of Compound **I** (or an equivalent amount of a pharmaceutically acceptable salt). In some embodiments, the 250 mg of Compound **I** (or an equivalent amount of a pharmaceutically acceptable salt) is administered in a single dose daily alone or in combination with another CFTR modulator. In some embodiments, the 250 mg of Compound **I** (or an equivalent amount of a pharmaceutically acceptable salt) is administered as two 125 mg doses once daily alone or in combination with another CFTR modulator.

[0080] One aspect disclosed herein provides methods of treating cystic fibrosis and other CFTR mediated diseases with daily administration of 250 mg of Compound **I** (or an equivalent amount of a pharmaceutically acceptable salt thereof) in combination with 21.24 mg of Compound **II** calcium salt hydrate Form D and 100 mg of Compound **III** (or an equivalent amount of a pharmaceutically acceptable salt thereof). In some embodiments, the 250 mg of Compound **I** (or an equivalent amount of a pharmaceutically acceptable salt thereof), 21.24 mg of Compound **II** calcium salt hydrate Form D, and 100 mg of Compound **III** (or an equivalent amount of a pharmaceutically acceptable salt thereof) are administered daily in separate pharmaceutical compositions. In some embodiments, the 250 mg of Compound **I** (or an equivalent amount of a pharmaceutically acceptable salt thereof), 21.24 mg of Compound **II** calcium salt hydrate Form D, and 100 mg of Compound **III** (or an equivalent amount of a pharmaceutically acceptable salt thereof), are administered in a single pharmaceutical composition daily. In some embodiments the 250 mg of Compound **I** (or an equivalent amount of a pharmaceutically acceptable salt thereof), 21.24 mg of Compound **II** calcium salt hydrate Form D, and 100 mg of Compound **III** (or an equivalent amount of a pharmaceutically acceptable salt thereof) are administered together in two equivalent pharmaceutical compositions once daily.

Pharmaceutical Compositions

[0081] Another aspect of the invention provides pharmaceutical compositions for use in treating cystic fibrosis. In some embodiments, the pharmaceutical composition of the invention comprises 250 mg of Compound **I** (or an equivalent amount of a pharmaceutically acceptable salt thereof). In some embodiments, the pharmaceutical composition of the invention comprises 125 mg of Compound **I** (or an equivalent amount of a pharmaceutically acceptable salt thereof).

[0082] In some embodiments, the pharmaceutical composition of the invention comprises 250 mg of Compound **I**, 21.24 mg of Compound **II** calcium salt hydrate Form D, and 100 mg of Compound **III**. In some embodiments, the pharmaceutical composition of the invention comprises 125 mg of Compound, 10.62 mg of Compound **II** calcium salt hydrate Form D, and 50 mg of Compound **III**.

[0083] In some embodiments, the pharmaceutical compositions (e.g., tablets) disclosed herein comprise a first solid dispersion (e.g., a spray dried dispersion) comprising Compound **I** and a second solid dispersion (e.g., a spray dried dispersion) comprising Compound **III**. Solid dispersions of a non-deuterated analog of Compound **I** and methods of preparing such dispersions are disclosed in PCT Publication No. WO 2007/079139, incorporated herein by reference. These same solid dispersions are suitable for use with Compound **I**. Solid dispersions of Compound **III** and methods of preparing thereof are disclosed in PCT Publication Nos. WO 2011/119984 and WO 2015/160787, incorporated herein by reference.

[0084] In some embodiments, the pharmaceutical composition of the invention comprises about 39.9 wt% by weight of the composition of a solid dispersion comprising Compound **I** (wherein the solid dispersion comprises 80 wt% Compound **I**, 19.5 wt% hypromellose acetate succinate, and 0.5 wt% sodium lauryl sulfate, by weight of the solid dispersion), about 2.7 wt% by weight of the composition of Compound **II** calcium salt hydrate Form D, and about 16.0 wt% by weight of the composition of a solid dispersion comprising Compound **III** (wherein the solid dispersion comprises 80 wt% Compound **I** and 20 wt% hypromellose, by weight of the solid dispersion).

[0085] Any suitable pharmaceutical compositions known in the art can be used for Compound **I**, Compound **II** calcium salt hydrate Form D, and Compound **III**. Some

exemplary pharmaceutical compositions for Compound **I** and its pharmaceutically acceptable salts can be found in US 8,865,902, US 9,181,192, US 9,512,079, WO 2017/053455, and WO 2018/080591, all of which are incorporated herein by reference. Exemplary pharmaceutical compositions comprising Compound **II** and its pharmaceutically acceptable salts are disclosed in WO 2019/161078 and WO 2020/102346. Exemplary pharmaceutical compositions for Compound **III** and its pharmaceutically acceptable salts are disclosed in WO 2011/119984 and WO 2014/014841, incorporated herein by reference.

[0086] Pharmaceutical compositions disclosed herein may optionally further comprise at least one pharmaceutically acceptable carrier. The at least one pharmaceutically acceptable carrier may be chosen from adjuvants and vehicles. The at least one pharmaceutically acceptable carrier, as used herein, includes any and all solvents, diluents, other liquid vehicles, dispersion aids, suspension aids, surface active agents, isotonic agents, thickening agents, emulsifying agents, preservatives, solid binders, and lubricants, as suited to the particular dosage form desired. Remington: *The Science and Practice of Pharmacy*, 21st edition, 2005, ed. D.B. Troy, Lippincott Williams & Wilkins, Philadelphia, and *Encyclopedia of Pharmaceutical Technology*, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York discloses various carriers used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional carrier is incompatible with the compounds of this disclosure, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this disclosure. Non-limiting examples of suitable pharmaceutically acceptable carriers include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins (such as human serum albumin), buffer substances (such as phosphates, glycine, sorbic acid, and potassium sorbate), partial glyceride mixtures of saturated vegetable fatty acids, water, salts, and electrolytes (such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, and zinc salts), colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, wool fat, sugars (such as lactose, glucose and sucrose), starches (such as corn starch and potato starch), cellulose and its derivatives (such as

sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate), powdered tragacanth, malt, gelatin, talc, excipients (such as cocoa butter and suppository waxes), oils (such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil), glycols (such as propylene glycol and polyethylene glycol), esters (such as ethyl oleate and ethyl laurate), agar, buffering agents (such as magnesium hydroxide and aluminum hydroxide), alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, phosphate buffer solutions, non-toxic compatible lubricants (such as sodium lauryl sulfate and magnesium stearate), coloring agents, releasing agents, coating agents, sweetening agents, flavoring agents, perfuming agents, preservatives, and antioxidants.

[0087] In one embodiment, the pharmaceutical compositions of the disclosure comprise one or more fillers, a disintegrant, and a lubricant.

[0088] Fillers suitable for the pharmaceutical compositions disclosed herein are compatible with the other ingredients of the pharmaceutical compositions, i.e., they do not substantially reduce the solubility, the hardness, the chemical stability, the physical stability, or the biological activity of the pharmaceutical compositions. Exemplary fillers include: celluloses, modified celluloses, (e.g. sodium carboxymethyl cellulose, ethyl cellulose hydroxymethyl cellulose, hydroxypropylcellulose), cellulose acetate, microcrystalline cellulose, calcium phosphates, dibasic calcium phosphate, starches (e.g. corn starch, potato starch), sugars (e.g., mannitol, lactose, sucrose, or the like), or any combination thereof. In some embodiments, the filler is microcrystalline cellulose.

[0089] In some embodiments, the pharmaceutical compositions comprise one or more fillers in an amount of at least 25 wt% (e.g., at least 27 wt% or at least 30 wt%) by weight of the pharmaceutical composition. For example, the pharmaceutical compositions comprise from 25 wt% to 40 wt% (e.g., from 25 wt% to 35 wt% or from 30 wt% to 33 wt%) of filler, by weight of the pharmaceutical composition. In another example, the pharmaceutical compositions comprise at least 25 wt% (e.g., at least 27 wt% or at least 30 wt%) of microcrystalline cellulose, for example Avicel PH102 or Avicel PH101, by weight of the pharmaceutical composition. In another example, the pharmaceutical compositions comprise from 25 wt% to 40 wt% (e.g., from 25 wt% to 35 wt% or from 30 wt% to 33 wt%) of microcrystalline cellulose, by weight of the pharmaceutical composition. In another example, the pharmaceutical compositions comprise about 31.7 wt% of microcrystalline cellulose, by weight of the pharmaceutical composition. In

another example, the pharmaceutical compositions comprise about 32.6 wt% of microcrystalline cellulose, by weight of the pharmaceutical composition.

[0090] Disintegrants suitable for the pharmaceutical compositions disclosed herein can enhance the dispersal of the pharmaceutical compositions and are compatible with the other ingredients of the pharmaceutical compositions, i.e., they do not substantially reduce the chemical stability, the physical stability, the hardness, or the biological activity of the pharmaceutical compositions. Exemplary disintegrants include croscarmellose sodium, sodium starch glycolate, crospovidone or a combination thereof. In some embodiments, the disintegrant is croscarmellose sodium.

[0091] In some embodiments, the pharmaceutical compositions discosed herein comprise disintegrant in an amount of 10 wt% or less (e.g., 8 wt% or less or 7 wt% or less) by weight of the pharmaceutical composition. For example, the pharmaceutical compositions comprise from 1 wt% to 10 wt% (e.g., from 2 wt% to 8 wt% or from 3 wt% to 7 wt%) of disintegrant, by weight of the pharmaceutical composition. In another example, the pharmaceutical compositions comprise 10 wt% or less (e.g., 8 wt% or less or 7 wt% or less) of croscarmellose sodium, by weight of the pharmaceutical composition. In another example, the pharmaceutical compositions comprise from 1 wt% to 10 wt% (e.g., from 2 wt% to 8 wt% or from 3 wt% to 7 wt%) of croscarmellose sodium, by weight of the pharmaceutical composition. In another example, the pharmaceutical compositions comprise about 5.8 wt% of croscarmellose sodium, by weight of the pharmaceutical composition. In another example, the pharmaceutical compositions comprise about 6.0 wt% of croscarmellose sodium, by weight of the pharmaceutical composition.

[0092] In some embodiments, the pharmaceutical compositions disclosed herein comprise a lubricant. A lubricant can prevent adhesion of a mixture compoent to a surface (e.g., a surface of a mixing bowl, a granulation roll, a compression die and/or punch). A lubricant can also reduce interparticle friction within the granulate and improve the compression and ejection of compressed pharmaceutical compositions from a granulator and/or die press. A suitable lubricant for the pharmaceutical compositions disclosed herein is compatible with the other ingredients of the pharmaceutical compositions, i.e., they do not substantially reduce the solubility, the hardness, or the biological activity of the pharmaceutical compositions. Exemplary lubricants include magnesium stearate, sodium stearyl fumarate, calcium stearate, zinc stearate, sodium stearate, stearic acid,

aluminum stearate, leucine, glyceryl behenate, hydrogenated vegetable oil or any combination thereof. In some embodiments, the lubricant is magnesium stearate.

[0093] In one embodiment, the pharmaceutical compositions comprise a lubricant in an amount of 5 wt% or less (e.g., 4 wt% or less, 3 wt% or less, or 2 wt% or less) by weight of the pharmaceutical composition. For example, the pharmaceutical compositions comprise from 0.10 wt% to 5 wt% (e.g., from 0.5 wt% to 3 wt% or from 0.75 wt% to 2 wt%) of lubricant, by weight of the pharmaceutical composition. In another example, the pharmaceutical compositions comprise 5 wt% or less (e.g., 4 wt% or less, 3 wt% or less, or 2 wt% or less) of magnesium stearate, by weight of the pharmaceutical composition. In another example, the pharmaceutical compositions comprise from 0.10 wt% to 5 wt% (e.g., from 0.5 wt% to 3 wt% or from 0.75 wt% to 2 wt%) of magnesium stearate, by weight of the pharmaceutical composition. In another example, the pharmaceutical compositions comprise about 1.0 wt% of magnesium stearate, by weight of the pharmaceutical composition.

[0094] In some embodiments, the pharmaceutical compositions disclosed herein are tablets. In some embodiments, the tablets comprise a film coat. In some embodiments, the film coat is Opadry 20A100021.

[0095] In some embodiments, the tablets disclosed herein comprise:

Component	**mg per tablet**
intragranular:	
solid dispersion containing 80 wt% Compound **I**, 19.5 wt% hypromellose acetate succinate, and 0.5 wt% sodium lauryl sulfate	156.3
Compound **II**, calcium salt hydrate Form D	10.6
solid dispersion containing 80 wt% Compound **III**, 20 wt% hypromellose	62.5

[0096] In some embodiments, the tablets disclosed herein comprise:

Component	**mg per tablet**
intragranular:	
solid dispersion containing 80 wt% Compound **I**, 19.5 wt% hypromellose acetate succinate, and 0.5 wt% sodium lauryl sulfate	156.3
Compound **II**, calcium salt hydrate Form D	10.6
solid dispersion containing 80 wt% Compound **III**, 20 wt% hypromellose	62.5

Component	mg per tablet
Microcrystalline cellulose	70-170 mg

[0097] In some embodiments, the tablets disclosed herein comprise:

Component	mg per tablet
intragranular:	
solid dispersion containing 80 wt% Compound **I**, 19.5 wt% hypromellose acetate succinate, and 0.5 wt% sodium lauryl sulfate	156.3
Compound **II**, calcium salt hydrate Form D	10.6
solid dispersion containing 80 wt% Compound **III**, 20 wt% hypromellose	62.5
Croscarmellose sodium	10-40 mg

[0098] In some embodiments, the tablets disclosed herein comprise:

Component	mg per tablet
intragranular:	
solid dispersion containing 80 wt% Compound **I**, 19.5 wt% hypromellose acetate succinate, and 0.5 wt% sodium lauryl sulfate	156.3
Compound **II**, calcium salt hydrate Form D	10.6
solid dispersion containing 80 wt% Compound **III**, 20 wt% hypromellose	62.5
Croscarmellose sodium	10-40 mg
Microcrystalline cellulose	70-170 mg

[0099] In some embodiments, the tablets disclosed herein comprise:

Component	mg per tablet
intragranular:	
solid dispersion containing 80 wt% Compound **I**, 19.5 wt% hypromellose acetate succinate, and 0.5 wt% sodium lauryl sulfate	156.3
Compound **II**, calcium salt hydrate Form D	10.6
solid dispersion containing 80 wt% Compound **III**, 20 wt% hypromellose	62.5
microcrystalline cellulose	55.1
croscarmellose sodium	22.8
extragranular:	
microcrystalline cellulose	68.9
magnesium stearate	3.8

[00100] In some embodiments, the tablets disclosed herein comprise:

Component	mg per tablet
intragranular:	
solid dispersion containing 80 wt% Compound **I**, 19.5 wt% hypromellose acetate succinate, and 0.5 wt% sodium lauryl sulfate	156.3
Compound **II**, calcium salt hydrate Form D	10.6
solid dispersion containing 80 wt% Compound **III**, 20 wt% hypromellose	62.5
microcrystalline cellulose	55.1
croscarmellose sodium	22.8
extragranular:	
microcrystalline cellulose	68.9
magnesium stearate	3.8
coating:	
film coat	11.4

[00101] In some embodiments, the tablets disclosed herein comprise:

Component	**Composition (% w/w)** (based on the total weight of the tablet)
intragranular:	
solid dispersion containing 80 wt% Compound **I**, 19.5 wt% hypromellose acetate succinate, and 0.5 wt% sodium lauryl sulfate	41.1
Compound **II**, calcium salt hydrate Form D	2.8
solid dispersion containing 80 wt% Compound **III**, 20 wt% hypromellose	16.4
microcrystalline cellulose	14.5
croscarmellose sodium	6.0
extragranular:	
microcrystalline cellulose	18.1
magnesium stearate	1.0

[00102] In some embodiments, the tablets disclosed herein comprise:

Component	**Composition (% w/w)** (based on the total weight of the tablet)
intragranular:	
solid dispersion containing 80 wt% Compound **I**, 19.5 wt% hypromellose acetate succinate, and 0.5 wt% sodium lauryl sulfate	39.9
Compound **II**, calcium salt hydrate Form D	2.7
solid dispersion containing 80 wt% Compound **III**, 20 wt% hypromellose	16.0

Component	Composition (% w/w) (based on the total weight of the tablet)
microcrystalline cellulose	14.1
croscarmellose sodium	5.8
extragranular:	
microcrystalline cellulose	17.6
magnesium stearate	1.0
coating:	
film coat	2.9

[00103] In some embodiments, the tablets disclosed herein comprise:

Component	mg per tablet
intragranular:	
solid dispersion containing 80 wt% Compound **I**, 19.5 wt% hypromellose acetate succinate, and 0.5 wt% sodium lauryl sulfate	156.3
Compound **II**, calcium salt hydrate Form D	10.6
solid dispersion containing 80 wt% Compound **III**, 20 wt% hypromellose	62.5
microcrystalline cellulose	55.1
croscarmellose sodium	22.8
extragranular:	
microcrystalline cellulose	69.9
magnesium stearate	2.9

[00104] In some embodiments, the tablets disclosed herein comprise:

Component	Composition (% w/w) (based on the total weight of the tablet)
intragranular:	
solid dispersion containing 80 wt% Compound **I**, 19.5 wt% hypromellose acetate succinate, and 0.5 wt% sodium lauryl sulfate	41.1
Compound **II**, calcium salt hydrate Form D	2.8
solid dispersion containing 80 wt% Compound **III**, 20 wt% hypromellose	16.5
microcrystalline cellulose	14.5
croscarmellose sodium	6.0
extragranular:	
microcrystalline cellulose	18.4
magnesium stearate	0.75

[00105] In some embodiments, the tablets disclosed herein comprise:

Component	mg per tablet
core tablet (combined intragranular and extragranular):	
solid dispersion containing 80 wt% Compound **I**, 19.5 wt% hypromellose acetate succinate, and 0.5 wt% sodium lauryl sulfate	156.3
Compound **II**, calcium salt hydrate Form D	10.6
solid dispersion containing 80 wt% Compound **III**, 20 wt% hypromellose	62.5
microcrystalline cellulose	124.5
croscarmellose sodium	22.8
magnesium stearate	3.8
coating:	
film coat	15.9

[00106] In some embodiments, the tablets disclosed herein comprise:

Component	**Composition (% w/w)** (based on the total weight of the tablet)
core tablet (combined intragranular and extragranular):	
solid dispersion containing 80 wt% Compound **I**, 19.5 wt% hypromellose acetate succinate, and 0.5 wt% sodium lauryl sulfate	39.4
Compound **II**, calcium salt hydrate Form D	2.7
solid dispersion containing 80 wt% Compound **III**, 20 wt% hypromellose	15.8
microcrystalline cellulose	31.4
croscarmellose sodium	5.8
magnesium stearate	1.0
coating:	
film coat	4.0

General Experimental Procedures

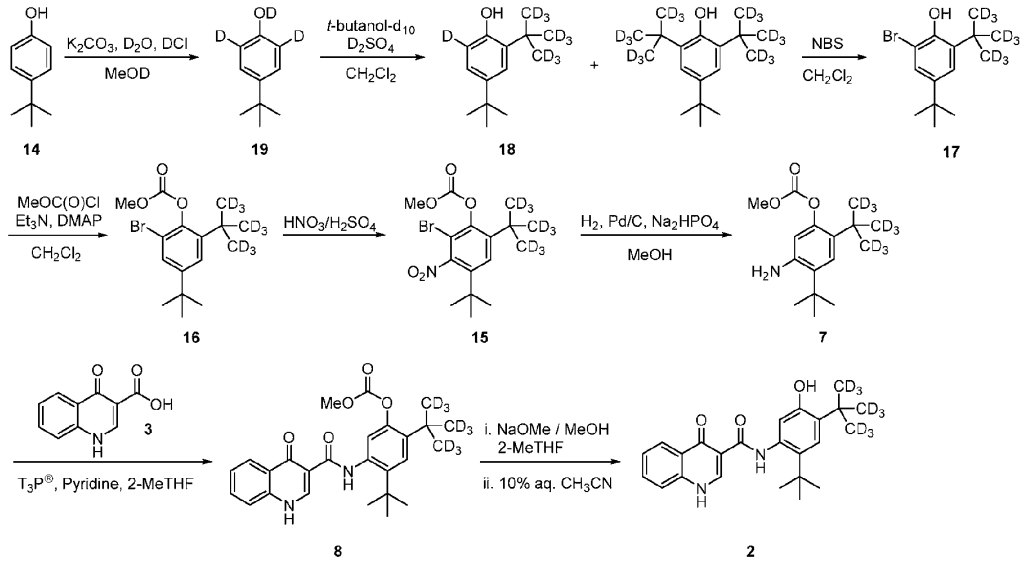
[00107] Reagents and starting materials were obtained by commercial sources unless otherwise stated and were used without purification. Proton and carbon NMR spectra were acquired on either of a Bruker Biospin DRX 400 MHz FTNMR spectrometer operating at a ^{1}H and ^{13}C resonant frequency of 400 and 100 MHz respectively, or on a 300 MHz NMR spectrometer. One dimensional proton and carbon spectra were acquired using a broadband observe (BBFO) probe with 20 Hz sample rotation at 0.1834 and 0.9083 Hz/Pt digital resolution, respectively. All proton and carbon spectra were acquired with temperature control at 30 °C using standard, previously published pulse sequences

and routine processing parameters. Final purity of compounds was determined by reversed phase UPLC using an Acquity UPLC BEH C_{18} column (50 × 2.1 mm, 1.7 μm particle) made by Waters (pn: 186002350), and a dual gradient run from 1-99% mobile phase B over 3.0 minutes. Mobile phase A = H_2O (0.05 % CF_3CO_2H). Mobile phase B = CH_3CN (0.035 % CF_3CO_2H). Flow rate = 1.2 mL/min, injection volume = 1.5 μL, and column temperature = 60 °C. Final purity was calculated by averaging the area under the curve (AUC) of two UV traces (220 nm, 254 nm). Low-resolution mass spectra were obtained using a single quadrupole mass spectrometer with a mass accuracy of 0.1 Da and a minimum resolution of 1000 amu across the detection range using electrospray ionization (ESI) using the hydrogen ion (H^+). Optical purity of methyl (2S)-2,4-dimethyl-4-nitro-pentanoate was determined using chiral gas chromatography (GC) analysis on an Agilent 7890A/MSD 5975C instrument, using a Restek Rt-βDEXcst (30m x 0.25mm x 0.25um_df) column, with a 2.0 mL/min flow rate (H_2 carrier gas), at an injection temperature of 220°C and an oven temperature of 120°C, 15 minutes. Purity of Compound **I** was determined by reverse phase HPLC using an Poroshell 120 EC-C8 column (4.6 × 150 mm, 2.7 μm particle, and a dual gradient run from 30-95% mobile phase B over 40 minutes. Mobile phase A = 5 mM Ammonium Acetate pH 4.50 and Mobile phase B = Acetonitrile. Flow rate = 1.0 mL/min, injection volume = 5 μL, 254 nm, and column temperature = 30 °C.

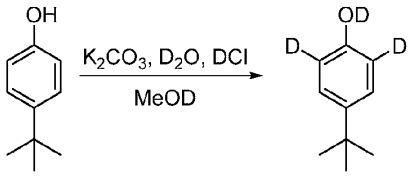
[00108] Compounds **I**, **II**, and **III** can be prepared by any suitable method in the art. Methods of making Compound **I** can be found in WO 2019/109021 and U.S. Patent 9,512,079; methods of making Compound **II** and its pharmaceutically acceptable salts thereof are disclosed in WO 2019/161078 and PCT/US2020/046116; methods of making Compound **III** and pharmaceutically acceptable salts thereof are disclosed in WO 2011/119984 and WO 2011/133751, all of which are incorporated herein by reference.

Example 1: Synthesis of *N*-(2-(*tert*-butyl)-5-hydroxy-4-(2-(methyl-d3)propan-2-yl-1, 1, 1, 3, 3, 3- d6)phenyl)-4-oxo-1, 4-dihydroquinoline-3-carboxamide (Compound I)

[00109] The overall scheme of the synthesis of (2) (Compound I) is shown below, followed by the procedure for the synthesis of each intermediate.

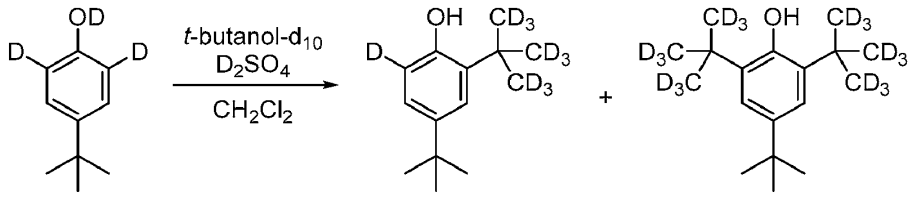


Procedure for the synthesis of 4-(tert-butyl)phen-2,6-d2-ol-d (19)



[00110] To a 5 L round bottom flask equipped with overhead stirrer was charged 4-/er/-butylphenol (14, 503.2 g), K_2CO_3 (46.3 g), D_2O (1949 g, 1761 mL, 3.5 vol), and MeOD (409 g, 503 mL, 1.0 vol). The mixture was heated to reflux under a nitrogen atmosphere. The reaction mixture was aged at reflux for 16 hours. The reaction mixture was cooled to room temperature and sampled for conversion (%D incorporation). The reaction was cooled to 5 °C and 35% DCl solution (90 g, 71.2 mL) was added. The reaction mixture was aged at 5 °C for 2 hours. The resultant slurry was filtered and the cake washed with D_2O (836 g, 755 mL, 1.5 vol). This process was repeated until the target %D incorporation is met (normally two exchanges required). The wet cake is dried in a vacuum oven with a nitrogen bleed at 40 °C until a constant weight is obtained. Yield of 4-(tert-butyl)phen-2,6-d^2-ol-d (19) is 506 g of a white solid (98%) with a purity of 99.6% and %D incorporation of 99.3%.

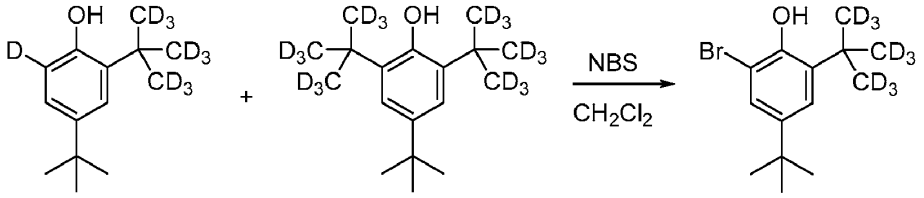
Procedure for the synthesis of 4-(tert-butyl)-2-(2-(methyl-d3)propan-2-yl-1,1,1,3,3,3- d6)phen-6-d-ol-d and 4-(tert-butyl)-2, 6-bis(2-(methyl-d3)propan-2-yl-1, 1,1, 3,3,3- d6)phenol-d (18)



19 18

[00111] 4-(tert-butyl)phen-2,6-d^2-ol-d (19) (101.8 g, 0.66 mol, 1.0 equiv.) was dissolved in CH_2Cl_2 (400 mL) in a 2 L reactor. /er/-Butanol-£¾o (43.0 g, 0.51 mol, 0.77 equiv.) was dissolved in CH_2Cl_2 (100 mL) in a 250 mL flask. The solution of tert- butanol-r / was charged to the 2 L reactor at room temperature. The reaction mixture was cooled to -5 °C. D_2SO_4 (51.1 g, 0.51 mol, 0.77 equiv.) was charged dropwise via an addition funnel while maintaining a temperature range of -4 to -2 °C. The reaction mixture was stirred at -2 °C for 3-4 hours. Upon complete conversion the reaction was quenched by adding water (28 mL) and warmed to 18-20 °C. The bottom aqueous layer was drained and discarded. The CH_2Cl_2 layer was treated with sat. aq. NaHCCh solution (approximately 200 mL), adjusting the pH to 6-8. NaCl (sat.) solution (400 mL) was charged to the mixture. The resulting solution was stirred for 5 min, and settled for 5 min. The lower CH_2Cl_2 layer was drained into a 1 L flask. The aqueous layer was discarded. The CH_2Cl_2 solution was concentrated to minimal volume and n-heptane (200 mL) was charged. The solution was concentrated to minimal volume and nheptane charged to a final volume of 800 mL. 0.5 N NaOH solution 600 mL (6 vol) was charged to the reactor and the resulting mixture was stirred for 5 min, and settled for at least 5 min. The aqueous layer was drained and discarded. 1.0 N NaOH solution 300 mL (3 vol) was charged to the reactor and the resulting mixture was stirred for 5 min, and settled for at least 5 min. The aqueous layer was drained and discarded. 1.0 N NaOH solution 300 mL (3 vol) was charged to the reactor and the resulting mixture was stirred for 5 min, and settled for at least 5 min. The aqueous layer was drained and discarded. The remaining n-heptane solution was concentrated to dryness to afford the desired product, 4-(tert-butyl)-2-(2-(methyl-d^2)propan-2-yl-1,1,1,3,3,3-d^6)phen-6-d-ol-d (18) as a clear oil, 104.5 g, which was carried forward into the next step without further purification.

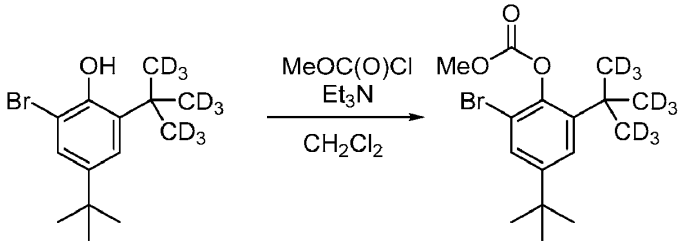
Procedure for the synthesis of 2-bromo-4-(tert-butyl)-6-(2-(methyl-d3)propan-2-yl- 1,1,1,3,3,3-d6)phenol (17)



18 17

[00112] 4-(tert-butyl)-2-(2-(methyl-d^3)propan-2-yl-1,1,1,3,3,3-d^6)phen-6-d-ol-d (18) (100 g, 0.462 mol, 1.0 equiv.) was dissolved in CH2CI2 (800 mL, 7 vol) in a 2 L reactor and the solution was stirred. The batch was cooled down to 0 ± 3 °C. To the batch was charged portion-wise /V-bromosuccinimide (84.4 g, 0.462 mol, 1.0 equiv) over 30 min. The batch was stirred at 0 ± 2 °C for at least 30 minutes. The batch was then heated to 20 ± 2 °C over a period of 2 hours and stirred at 20 ± 2 °C for at least 12 hours. Upon complete conversion, sat. aq. NaHCCh solution (500 mL, 5 vol) was charged and the batch stirred for at least 10 minutes. The agitation was stopped to allow the phases to separate for at least 5 minutes and the CH2CI2 layer was drained, followed by removal of the aqueous layer. The CTLChlayer was charged back to the vessel. To the batch was charged sat. aq. NaHCCh bicarbonate solution (500 mL, 5 vol), and the batch was stirred for at least 10 minutes. The agitation was stopped to allow the phases to separate for at least 5 minutes and the CH_2CI_2 layer was drained, followed by removal of the aqueous layer. The CH_2CI_2 layer was charged back to the vessel and diluted with an additional CH2CI2 (300 mL, 3 vol). The batch was distilled (removal of 300 mL) and checked by KF to achieve dryness. The resulting clear yellow solution of 17 was carried forward into the next step without further purification.

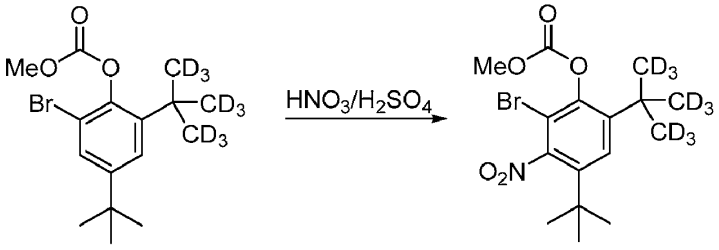
Procedure for the synthesis of 2-bromo-4-(tert-butyl)-6-(2-(methyl-d3)propan-2-yl- 1,1,1,3,3,3-d6)phenyl methyl carbonate (16)



17 16

[00113] To a clean reactor was charged the CH_2Cl_2 solution of 4-(tert-butyl)-2- (2-(methyl-d3)propan-2-yl-1,1,1,3,3,3-d6)phen-6-d-ol-d (17) (136 g, 0.462 mol, 1.0 equiv.) followed by additional CH_2Cl_2 (130 mL, 1 vol), and this solution was stirred. To the batch was charged 4-(dimethylamino)pyridine (2.8 g, 0.023 mol, 0.05 equiv) and triethylamine (70.1 g, 0.693 mol, 1.5 equiv). The batch was cooled to 0 ± 3 °C. To the batch was charged drop- wise methyl chloroformate (48.0 g, 0.508 mol, 1.1 equiv) over 40 minutes while maintaining a batch temperature < 5 °C. The batch was stirred at 3 ± 2 °C for at least 30 minutes, and then warmed to 20 ± 2 °C over a period of 1 hour. Upon complete conversion, 1 N HCl (400 mL, 3 vol) was charged. The batch was stirred for at least 10 minutes, and then the layers were allowed to separate for at least 5 minutes. The lower organic layer was drained followed by the aqueous layer (I^{st} aqueous layer). The organic layer was charged back to the reactor, along with 1 N HCl solution (400 mL, 3 vol). The batch was stirred for at least 10 minutes, and then the layers were allowed to separate for at least 5 minutes. The lower organic layer was drained. The first aqueous layer was charged to the reactor, along with CH_2Cl_2 (300 mL, 2.2 vol). The batch was stirred for at least 10 minutes, and then the layers were allowed to separate for at least 5 minutes. The lower organic layer was drained and combined with the I^{st} organic layer, followed by removal of the aqueous layer. Charge the vessel with the contents of both organic layers. The reactor was charged with water (500 mL, 3.7 vol). The batch was stirred for at least 10 minutes, and then the layers were allowed to separate for at least 5 minutes. The lower organic layer was drained, followed by the aqueous layer. The organic layer was charged back to the reactor, along CH_2Cl_2 (400 mL, 3 vol). The batch was distilled to remove 800 mL and checked by KF to ensure dryness. The resulting clear yellow solution of 16 was telescoped into the next step without further purification.

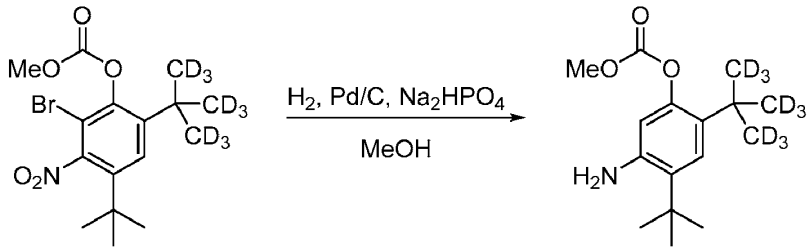
Procedure for the synthesis of 2-bromo-4-(tert-butyl)-6-(2-(methyl-d3)propan-2-yl- 1,1,1,3,3,3-d6)-3-nitrophenyl methyl carbonate (15)



16 15

[00114] To a reactor was charged 2-bromo-4-(tert-butyl)-6-(2-(methyl- d3)propan-2-yl-1,1,1,3,3,3-d6)phenyl methyl carbonate (16) and then the solution was cooled to 0 °C. Sulfuric acid (4.9 equiv) and nitric acid (100%, 2.0 equiv) was charged while maintaining a temperature of not more than 5 °C. The reaction was stirred at 0 °C for 2 hours until complete conversion. The reaction was then quenched with water (8.8 vol) and diluted with CH_2Cl_2 (1.7 vol). The layers were separated and the upper aqueous layer was extracted with CH_2Cl_2 (2.8 vol). After separating the layers, the organic layers were combined, returned to the reactor, and washed with sodium bicarbonate (7.4% w/w, 6.8 vol). After separating the layers, the organic layer was returned to the reactor and washed with sodium chloride (23% w/w, 3.8 vol). After separating the layers, the organic layer was returned to the reactor and concentrated to minimal volume. Methanol (1.2 vol) was charged, followed by concentration to minimal volume. Methanol (1.2 vol) was charged, followed by concentration to minimal volume. Methanol (1.7 vol) was charged, and the slurry was heated to reflux for 30 min and then cooled slowly over 4 hours to 5 °C. The solid product (15) was filtered and the cake washed with cold methanol (1.0 vol). The solid 2-bromo-4-(tert-butyl)-6-(2-(methyl-d3)propan-2-yl-1,1,1,3,3,3-d6)-3- nitrophenyl methyl carbonate (15) was dried under vacuum at 40 - 50 °C to yield an off- white solid, 99.9% purity and 99% D incorporation.

Procedure for the synthesis of 5-amino-4-(tert-butyl)-2-(2-(methyl-d3)propan-2-yl- 1,1,1,3,3,3-d6)phenyl methyl carbonate (7)

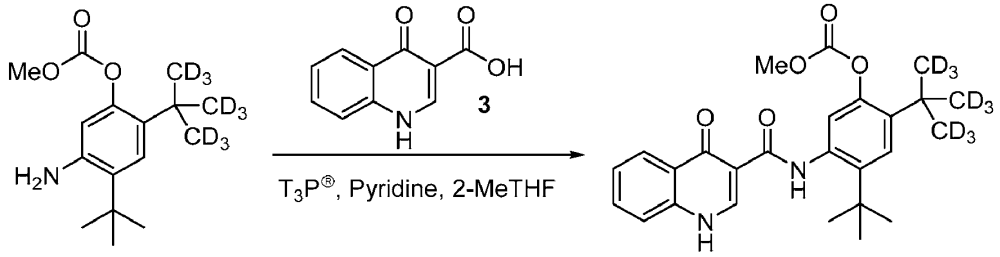


15 7

[00115] Charge 5 wt% (50 - 65 wt% wet, JM Type 37) of 5% Pd/C to a reactor. Charge (4.0 vol) Methanol. Close the system. Purge with N2 (g> at 2.0 Bar. Activate with H2 (g) at 2.0 Bar. Charge the vessel to 2.0 Bar with H2 (g> at25°C +/- 5°C. Stir for not less than 2 hours while maintaining a temperature of 25°C +/- 5°C. Vent and purge with N2 (g) at 2.0 Bar. Charge compound 15 (1.0 eq) to a reactor, together with Na2HP04 (2.3 eq). Charge (11.0 vol) Methanol. Close the system. Purge with N2 (g> at 2.0 Bar. Activate with H2 (g) at 2.0 Bar. Charge the vessel to 2.0 Bar with H2 (g> at25°C +/- 5°C. Stir for about 24 hours while maintaining a reaction temperature of 25°C +/- 5°C. Upon complete conversion, dilute reaction mixture by adding 7.7 vol of MeOH. Heat reaction mixture to 35.0°C +/- 5°C. Filter off catalyst and Na2HP04. Wash the reactor and filter cake with Methanol (4.0 vol), and filter, combining with the initial filtrate. Check Pd content and if needed perform resin treatment (resin treatment is: Charge SPM-32 resin (5 wt%). Stir the resin treated solution for not less than 3 hours at 35.0°C +/- 5°C. Filter off resin.

[00116] Wash the reactor and filter cake with Methanol (2.0 vol), and filter, combining with the initial filtrate). Charge Norit CASP active carbon (3 wt%,). Stir for not less than 3 hours at 35.0°C +/- 5°C. Filter off active carbon. Wash the reactor and filter cake with Methanol (2.0 vol), and filter, combining with the initial filtrate. Distill under vacuum at not more than 50°C to 8.0 vol. Charge water (2.0 vol) while maintaining a temperature of 45°C +/- 5°C. Cool the resultant slurry to 0°C +/- 5°C over 2 hours. Hold and stir the slurry at 0°C +/- 5°C for not less than 1 hour. Filter and wash the cake with 2.0 volumes Methanol / Water (8:2) at 0°C +/- 5°C. Dry 5-amino-4-(tert-butyl)-2-(2-(methyl-d3)propan-2-yI-I, /, 1 ,3,3,3-d6)phenyl methyl carbonate (7) under vacuum at not more than 40°C to give a yield of a white solid, >99.5% purity.

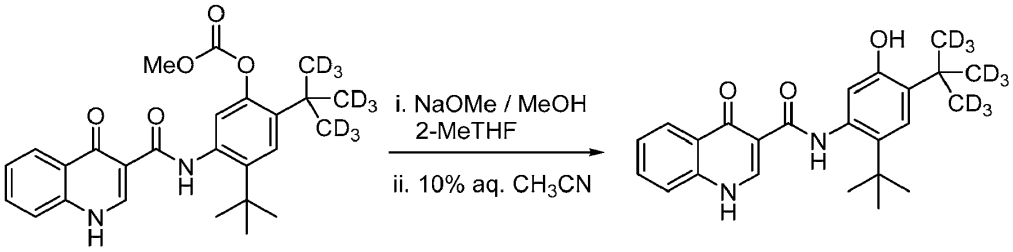
Procedure for the synthesis of 4-(tert-butyl)-2-(2-(methyl-d3)propan-2-yl-1, 1,1, 3,3,3- d6)-5-(4-oxo-1,4-dihydroquinoline-3-carboxamido)phenyl methyl carbonate (8)



7 8

[00117] The procedure for the conversion of compound 7 into compound 8 may be performed according to the analogous procedure for compound 5.

*Procedure for the **synthesis** of N-(2-(tert-butyl)-5-hydroxy-4-(2-(methyl-d3)propan-2- yl-1,1,1,3,3,3-d6)phenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (2) (Compound I)*

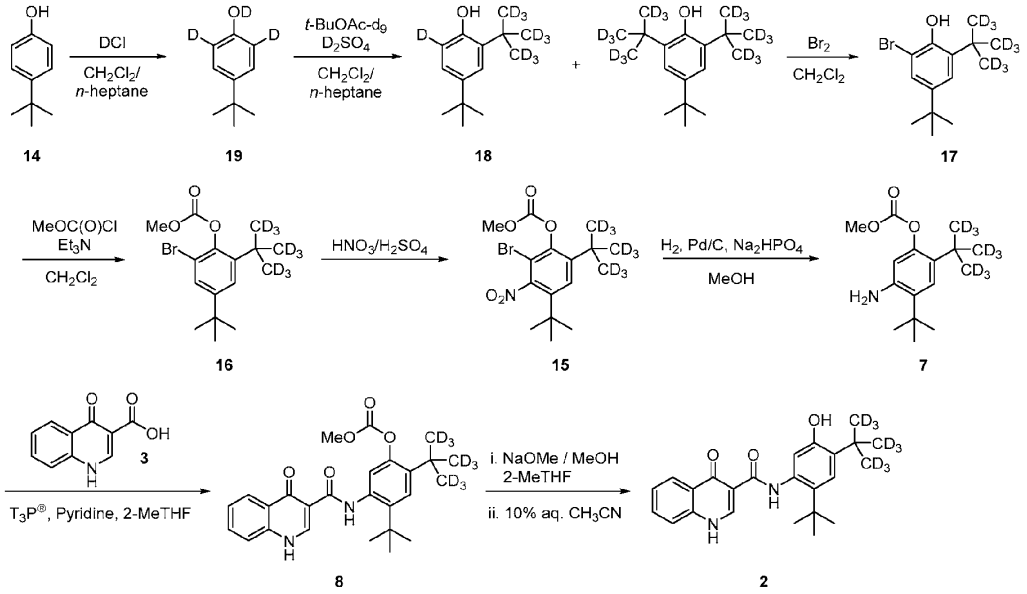


8 2

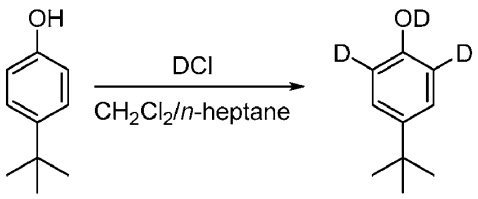
[00118] The procedure for the conversion of compound 8 into compound 2 may be performed according to the analogous procedure for the synthesis of compound 1.

Example 2: Synthesis of 5-amino-4-(tert-buiyl)-2-(2-(methyl-d3)propan-2-yl-1,1,1,3,3,3-d6)phenyl methyl carbonate (7)

[00119] An alternative overall scheme of the synthesis of compound 7 is shown below, followed by the procedure for the synthesis of each synthetic intermediate.



Procedure for the synthesis of 4-(tert-butyl)phen-2,6-d2-ol-d (19)

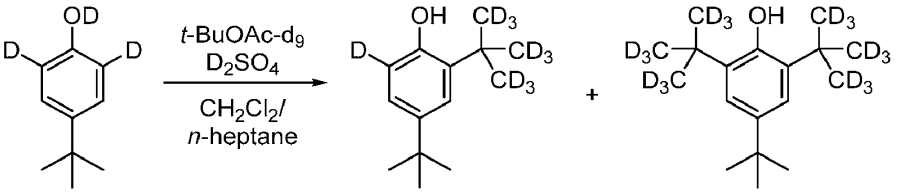


(19)

[00120] To a clean and dry 500-mL reactor was charged 4-/c/7-butyl phenol (14) (24.6 g, 0.162 mmol, 1.00 equiv), CH_2Cl_2 (64 mL, 2.6 vol), and heptane (64 mL, 2.6 vol), and this mixture was warmed to 25 °C and stirred until all solids dissolved. To this solution was charged deuterium chloride (35% w/w in deuterium oxide, 25 mL, 1.0 vol), and this mixture was agitated for at least 3.5 hours. The agitation was stopped and the phases were allowed to separate, and then the aqueous layer (bottom) was drained from the reactor. To the reactor was charged deuterium chloride (35% w/w in deuterium oxide, 25 mL, 1.0 vol), and this mixture was agitated for at least 3.5 hours. The agitation was stopped and the phases were allowed to separate, and then the aqueous layer (bottom) was drained from the reactor. To the reactor was charged deuterium chloride (35% w/w in deuterium oxide,

25 mL, 1.0 vol), and this mixture was agitated for at least 3.5 hours. The agitation was stopped and the phases were allowed to separate, and then the aqueous layer (bottom) was drained from the reactor. The resulting solution was sampled and confirmed to be at least 99% of the desired deuterium incorporation product 4-(tert-butyl)phen-2,6-d2-ol-d (19) relative to starting material 4-/er/-butylphenol. The solution in the reactor was carried on to the next step described below.

Procedure for the synthesis of 4-(tert-butyl)-2-(2-(methyl-d3)propan-2-yl-1,1,1,3,3,3- d6)phen-6-d-ol (18)

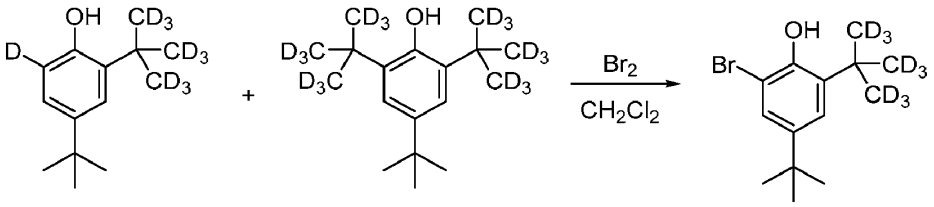


19 **18**

[00121] To the methylene chloride solution containing the reaction mixture of 4- (tert-butyl)phen-2,6-d2-ol-d (19) was charged CH_2Cl_2 (125 mL, 5 vol). Approximately 125 mL of the reaction solution was distilled from the reactor using a distillation head and heating the reactor to 60 °C. To the reactor was charged CH_2Cl_2 (125 mL, 5 vol). Approximately 100 mL of the reaction solution was then distilled from the reactor, and at this time the solution was sampled to confirm water content (KF) was less than 300 ppm and determine the CH_2Cl_2 and heptane content. After measuring the batch volume, CH_2Cl_2 (8 mL, 0.24 vol) was charged to adjust the total CH_2Cl_2 content to 3 vol and heptane (68 mL, 2.8 vol) was charged to adjust the heptane content to 4.5 vol. To the solution was charged /cvv-butyl acetat e-dg (30.2 g, 1.46 equiv), and the resulting solution was cooled to 0 °C. To the solution was charged sulfuric acid -J2 (8.12 g, 0.49 equiv) over at least 15 min, and the solution was agitated for 2 hours while maintaining the temperature at 0-5 °C. After this time, the temperature was set to ramp up to 20 °C over two hours and the solution was agitated for another 14 hours. The solution was sampled to confirm 4-/c77-butyl phenol (14) or 4-(tert-butyl)phen-2,6-d2-ol-d (19) were present at less than 3%. To the reactor was charged CH_2Cl_2 (58 mL, 2.4 vol) and heptane (90 mL, 3.7 vol), and the solution was cooled to 0-5 °C before charging water (125 mL, 5 vol). The mixture was agitated for 15 min before agitation was stopped and the phases were allowed to separate. After the

aqueous phase (bottom) was drained from the reactor, 0.5 N aqueous NaOH (125 mL, 5 vol) was charged and the temperature was adjusted to 20 °C. The mixture was agitated for 20 min before agitation was stopped and the phases were allowed to separate. The organic phase (top) was sampled to confirm 4-tert- butylphenol (14) or 4-(tert-butyl)phen-2,6-d2-ol-d (18) were present at less than 0.5%. The aqueous phase (bottom) was drained from the reactor. The solution in the reactor was carried on to the next step described below.

Procedure for the synthesis of 2-bromo-4-(tert-butyl)-6-(2-(methyl-d3)propan-2-yl- 1,1,1,3,3,3-d6)phenol (17)

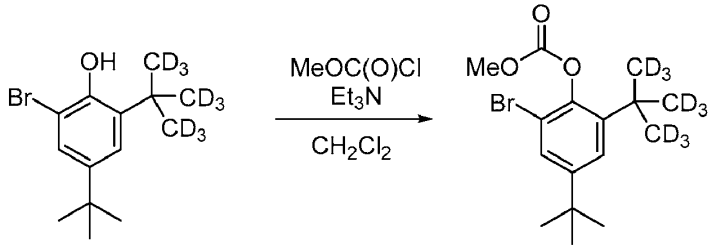


18 **17**

[00122] After the agitated solution of the alkylation reaction to produce 4-(tert- butyl)-2-(2-(methyl-d3)propan-2-yl-1,1,1,3,3,3-d6)phen-6-d-ol-d (18) was brought to 0- 5 °C, bromine (38.4 g, 1.45 equiv) was charged over at least 1 hour, maintaining the temperature below 5 °C. The solution was sampled to confirm 4-(tert-butyl)-2-(2- (methyl-d3)propan-2-yl-1,1,1,3,3,3-d6)phen-6-d-ol was present at less than 1%. To the solution was charged sodium metabisulfite (20% w/w aqueous solution, 147 g, 0.95 equiv) over at least 1 hour, maintaining the temperature below 10 °C. After adjusting the temperature to 20 °C, the mixture was agitated for another 1 hour. Agitation was stopped and the phases were allowed to separate. The aqueous phase (bottom) was drained from the reactor, and water (125 mL, 5 vol) was charged to the reactor. The mixture was agitated for 15 min before stopping agitation and allowing the phases to separate. The aqueous phase (bottom) was drained from the reactor. The solution of 17 in the reactor was carried on to the next step described below.

[00123] Surprisingly, this bromination reaction significantly improved the selectivity of the nitration reaction. Another unexpected advantage to this process was that bromination converted the mixture of compound 18 and 4-(tert-butyl)-2,6-bis(2- (methyl-d3)propan-2-yl-1,1,1,3,3,3-d6)phenol to the same desired product (17). This significantly improved the overall yield.

Procedure for the synthesis of 2-bromo-4-(tert-butyl)-6-(2-(methyl-d3)propan-2-yl- 1,1,1,3,3,3-d6)phenyl methyl carbonate (16)

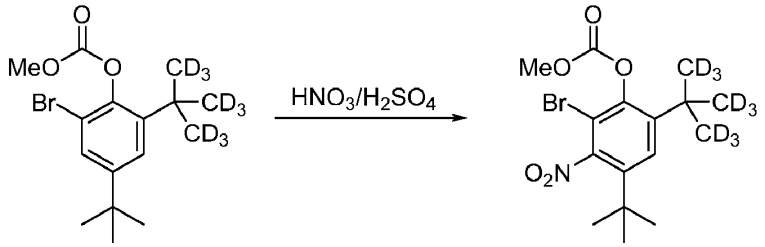


17 16

[00124] To the solution of the bromination reaction to produce 2-bromo-4-(tert- butyl)-6-(2-(methyl-d3)propan-2-yl-1,1,1,3,3,3-d6)phenol (17) was charged CH_2Cl_2 (125 mL, 5 vol). Approximately 125 mL of the reaction solution was distilled from the reactor using a distillation head and heating the reactor to 60 °C. To the reactor was charge CH_2Cl_2 (125 mL, 5 vol). Approximately 125 mL of the reaction solution was distilled from the reactor. To the reactor was charged CH_2Cl_2 (125 mL, 5 vol). Approximately 125 mL of the reaction solution was then distilled from the reactor, and at this time the solution was sampled to confirm water content (KF) was less than 300 ppm and determine the CH2Cl2 and heptane content. After measuring the batch volume, CFLChwas charged to adjust the total CH_2Cl_2 content to 5.3 vol and heptane was charged to adjust the heptane content to 8 vol. To the solution was charged triethylamine (31.7 g, 1.91 equiv), and the solution was cooled to 0-5 °C. To the solution was charged methyl chloroformate (24.1 g, 1.56 equiv) over at least 1 hour, maintaining the temperature below 10 °C. The solution was agitated for 1 hour, and a sample of the solution was taken to confirm 2-bromo-4-(tert-butyl)-6-(2-(methyl-d3)propan-2-yl- 1,1,1,3,3,3-d6)phenol (17) was present at less than 1%. To the solution was charged 1 N aqueous hydrochloric acid (125 mL, 0.76 equiv) over at least 30 min, maintaining the temperature below 10 °C. The temperature was then adjusted to 20 °C, and agitation was stopped and the phases were allowed to separate. After the aqueous phase (bottom) was drained from the reactor, water (125 mL, 5 vol) was charged to the reactor. The mixture was agitated for 15 min before agitation was stopped and the phases were allowed to separate. After the aqueous phase (bottom) was drained from the reactor, water (125 mL, 5 vol) was charged to the reactor. The mixture was agitated for 15 min before agitation was stopped and the phases were allowed to separate. The aqueous phase

(bottom) was drained from the reactor. The solution of (16) in the reactor was carried on to the next step described below.

Procedure for the synthesis of 2-bromo-4-(tert-butyl)-6-(2-(methyl-d3)propan-2-yl- l,l,l,3,3,3-d6)-3-nitrophenyl methyl carbonate (15)

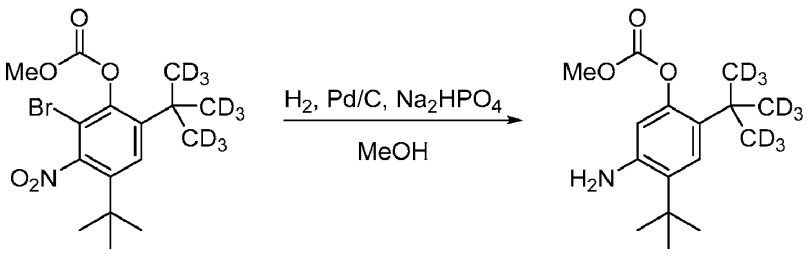


16 **15**

[00125] To the solution of the protection reaction to produce 2-bromo-4-(tert- butyl)-6-(2-(methyl-d3)propan-2-yl-l,l,l,3,3,3-d6)phenyl methyl carbonate (16) was charged CH_2Cl_2 (125 mL, 5 vol). Approximately 125 mL of the reaction solution was distilled from the reactor using a distillation head and heating the reactor to 60 °C. To the reactor was charged CH_2Cl_2 chloride (125 mL, 5 vol). Approximately 125 mL of the reaction solution was distilled from the reactor. To the reactor was charged CH_2Cl_2 (125 mL, 5 vol). To the reactor was charged CH_2Cl_2 (125 mL, 5 vol). Approximately 125 mL of the reaction solution was distilled from the reactor. Approximately 125 mL of the reaction solution was then distilled from the reactor, and at this time the solution was sampled to confirm water content (KF) was less than 300 ppm and determine the CH_2Cl_2 and heptane content. After measuring the batch volume, CH_2Cl_2 was charged to adjust the total CH_2Cl_2 content to 6 vol and heptane was charged to adjust the heptane content to 9 vol. After cooling the solution to 0-5 °C, sulfuric acid (172 g, 10.3 equiv) was charged over at least 30 min, maintaining the temperature below 5 °C. To the mixture was charged nitric acid (70% w/w, 19.1 g, 1.31 equiv) over at least 30 min, maintaining the temperature below 10 °C. After agitating the mixture for 1 hour, a sample was taken and analyzed to confirm 2-bromo-4-(tert-butyl)-6-(2-(methyl-d3)propan-2-yl-l, 1,1, 3,3,3- d6)phenyl methyl carbonate (16) was present at less than 1%. To the mixture was charged water (100 mL, 4 vol) over at least 1 hour, maintaining the temperature below 10 °C. Agitation was stopped and the phases were allowed to separate, and the aqueous phase (bottom) was drained from the reactor. After resuming agitation, sodium bicarbonate (8% w/w aqueous solution, 100 mL, 4 vol, 0.62 equiv) was charged over at least 10 min, maintaining the temperature

below 10 °C. The temperature was adjusted to 20 °C, agitation was stopped, and the phases were allowed to separate. After draining the aqueous phase (bottom) from the reactor, water (100 mL, 4 vol) was charged to the reactor and the mixture was agitated for 15 min. Agitation was stopped, the phases were allowed to separate, and the aqueous phase (bottom) was drained from the reactor. To the mixture was charged water (100 mL, 4 vol), and this mixture was agitated for 15 min. Agitation was stopped, the phases were allowed to separate, and the aqueous phase (bottom) was drained from the reactor. After marking the solvent level on the reactor, a distillation head was attached and the temperature was set to 80 °C. To the solution was charged methanol (570 mL, 23 vol) while distilling at the same time, matching the addition rate to the distillation rate by keeping the solvent level at the mark. Distillation was continued until the batch volume was approximately 264 mL (11 vol) and approximately 1.10 kg of distillate had been removed. The mixture was sampled and analyzed to confirm heptane was present at less than 1% v/v. The temperature was adjusted to 0 °C over 4 hours. The mother liquor was sampled and analyzed to determine the concentration of 2-bromo-4-(tert-butyl)-6-(2-(methyl-d3)propan-2-yl-1,1,1,3,3,3-d6)- 3-nitrophenyl methyl carbonate (15), and the mixture was filtered. To the reactor was charged methanol (51.1 mL, 2 vol), and this was agitated until the temperature reached 0-5 °C. This solution was used to wash the filter cake, and the filter cake was then dried by suction for at least 1 hour. The solid was then submitted to vacuum drying to produce 2-bromo-4-(tert-butyl)-6-(2-(methyl-d3)propan-2-yl- 1 , 1 , 1 , 3 ,3 , 3 -d6)-3 -nitrophenyl methyl carbonate (15) as 41.5 g of an off-white solid (98.4% pure w/w, 63% yield after purity correction).

Procedure for the synthesis of 5-amino-4-(tert-butyl)-2-(2-(methyl-d3)propan-2-yl- 1,1,1,3,3,3-d6)phenyl methyl carbonate (7)



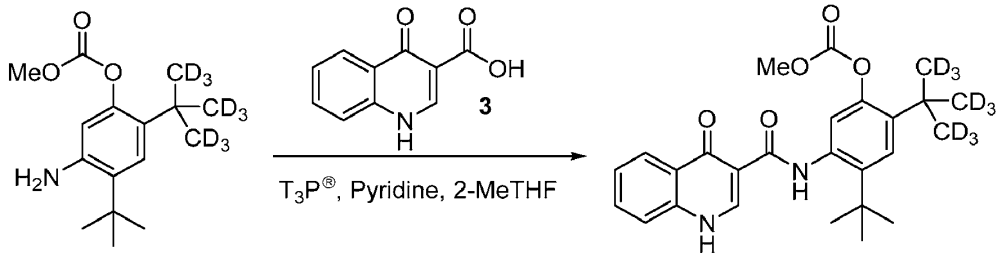
15 **7**

[00126] Charge 5 wt% (50 - 65 wt% wet, JM Type 37) of 5% Pd/C to a reactor. Charge (4.0 vol) Methanol. Close the system. Purge with $N2_{(g)}$ at 2.0 Bar. Activate with $H2_{(g)}$ at

2.0 Bar. Charge the vessel to 2.0 Bar with $H2_{(g)}$ at25°C +/- 5°C. Stir for not less than 2 hours while maintaining a temperature of 25°C +/- 5°C. Vent and purge with $N2_{(g)}$ at 2.0 Bar. Charge compound 15 (1.0 eq) to a reactor, together with Na_2HP0_4 (2.3 eq). Charge (11.0 vol) Methanol. Close the system. Purge with $N2_{(g)}$ at 2.0 Bar. Activate with $H2_{(g)}$ at 2.0 Bar. Charge the vessel to 2.0 Bar with $H2_{(g)}$ at25°C +/- 5°C. Stir for about 24 hours while maintaining a reaction temperature of 25°C +/- 5°C. Upon complete conversion, dilute reaction mixture by adding 7.7 vol of MeOH. Heat reaction mixture to 35.0°C +/- 5°C. Filter off catalyst and Na_2HP0_4. Wash the reactor and filter cake with Methanol (4.0 vol), and filter, combining with the initial filtrate. Check Pd content and if needed perform resin treatment (resin treatment is: Charge SPM-32 resin (5 wt%). Stir the resin treated solution for not less than 3 hours at 35.0°C +/- 5°C. Filter off resin.

[00127] Wash the reactor and filter cake with Methanol (2.0 vol), and filter, combining with the initial filtrate). Charge Norit CASP active carbon (3 wt%,). Stir for not less than 3 hours at 35.0°C +/- 5°C. Filter off active carbon. Wash the reactor and filter cake with Methanol (2.0 vol), and filter, combining with the initial filtrate. Distill under vacuum at not more than 50°C to 8.0 vol. Charge water (2.0 vol) while maintaining a temperature of 45°C +/- 5°C. Cool the resultant slurry to 0°C +/- 5°C over 2 hours. Hold and stir the slurry at 0°C +/- 5°C for not less than 1 hour. Filter and wash the cake with 2.0 volumes Methanol / Water (8:2) at 0°C +/- 5°C. Dry 5-amino-4-(tert-butyl)-2-(2-(methyl-d3)propan-2-yl-I, /, 1 ,3,3,3-d6)phenyl methyl carbonate (7) under vacuum at not more than 40 °C to give a yield of a white solid, >99.5% purity.

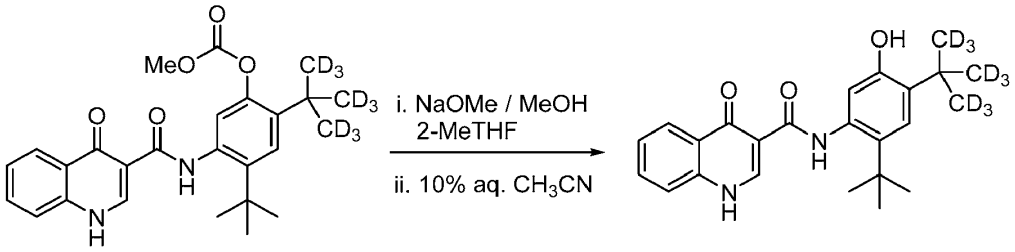
Procedure for the synthesis of 4-(tert-butyl)-2-(2-(methyl-d3)propan-2-yl-1,1,1,3,3,3- d6)-5-(4-oxo-1,4-dihydroquinoline-3-carboxamido)phenyl methyl carbonate (8)



(7) (8)

[00128] The procedure for the conversion of compound 7 into compound 8 may be performed according to the analogous procedure for compound 5.

Procedure for the synthesis of N-(2-(tert-butyl)-5-hydroxy-4-(2-(methyl-d3)propan-2- yl-1,1,1,3,3,3-d6)phenyl)-4-*oxo*-1,4-dihydroquinoline-3-carboxamide (2) (Compound I)

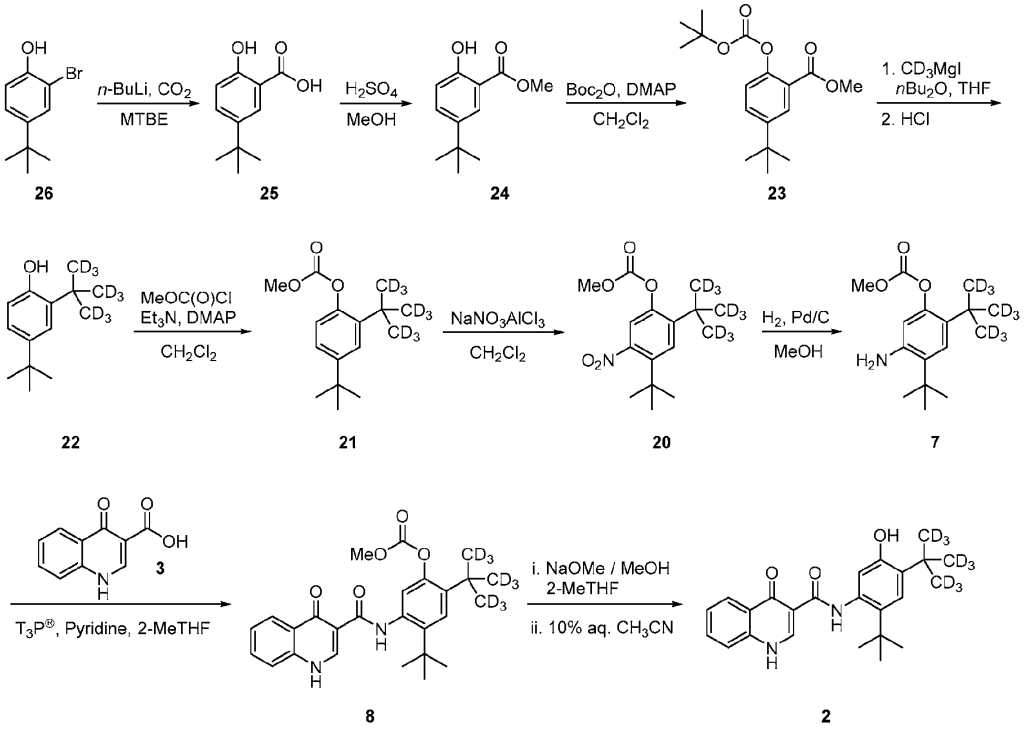


8 2

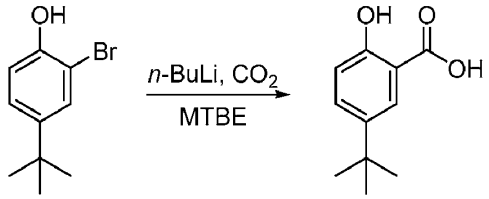
[00129] The procedure for the conversion of compound 8 into compound 2 may be performed according to the analogous procedure for the synthesis of compound 1.

Example 3: Synthesis of 5-amino-4-(tert-buiyl)-2-(2-(methyl-d3)propan-2-yl-1,1,1,3,3,3-d6)phenyl methyl carbonate (7)

[00130] An alternative scheme of the synthesis of compound 7 is shown below, followed by the procedure for the synthesis of each synthetic intermediate.



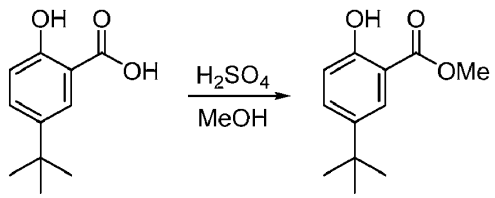
Procedure for the synthesis of 5-(tert-butyl)-2-hydroxybenzoic acid (15)



26 **25**

[00131] nBuLi 1.6 M in hexanes (3.49 g) was added to a round bottom flask equipped with a magnetic stirbar, a thermocouple, and a N2 bubbler. The round bottom flask was cooled down to -20 °C and stirring started. A solution of 2-bromo-4-tert- butylphenol (26) (5.00 g) in MTBE (12.5 mL) was prepared, cooled to - 20 °C, and charged to the round bottom flask drop wise while maintaining the temperature at -20 °C +/- 5 °C. The reaction mixture was stirred at -20 °C +/- 5 °C for 15 min then allowed to warm up to 23 °C. The completeness of the lithiation was measured by ^{1}H NMR (200 pL reaction mixture diluted into 0.75 mL d4-MeOH) after 15 min at room temperature. The reaction was considered complete when less than 1% 2-bromo-4-tert-butylphenol was observed. The reaction mixture was cooled down to 0 °C, dry ice (solid CO_2) was added, and the reaction was stirred at room temperature for 45 min. Water (50.0 mL) was added to quench the reaction. The mixture was transferred into a separatory funnel, the phases were separated, and the organic phase was discarded. The aqueous phase was acidified to pH ~2 with 1 M HC1 (15.0 mL), then extracted with MTBE (25.0 mL) three times. The combined organic extracts were concentrated under reduced pressure to yield 5-(tert- butyl)-2-hydroxybenzoic acid (25) as a yellow solid (2.25 g, 53.15% yield); ¾ NMR (400 MHz, d4-MeOH): 7.86 (1H, d, J = 2.6 Hz), 7.54 (1H, dd, J = 8.7, 2.6 Hz), 6.85 (1H, d, J = 2.7 Hz), 1.30 (9H, s).

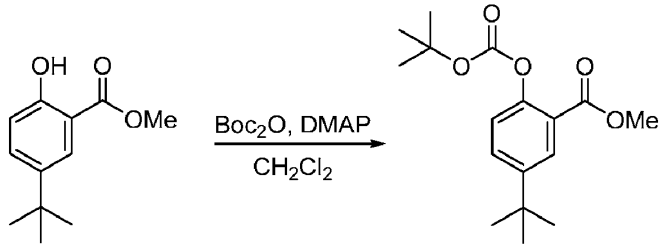
Procedure for the synthesis of methyl 5-(tert-butyl)-2-hydroxybenzoate (24)



25 **24**

[00132] This reaction may be performed according to a procedure disclosed in *Bioorganic and Medicinal Chemistry Letters*, 2005, vol. 15(21), p. 4752 - 4756.

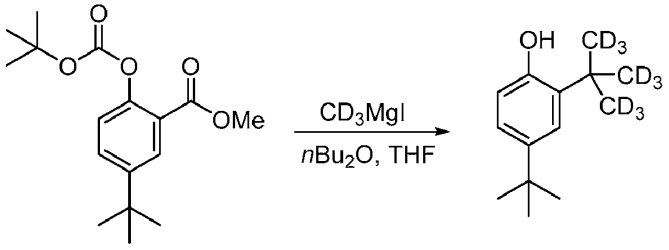
Procedure for the synthesis of methyl 2-((tert-butoxycarbonyl)oxy)-5-(tert-butyl)benzoate (23)



24 **23**

[00133] Di-/e/7-butyl carbonate (230.55 g) and CH_2Cl_2 (400 mL) were charged to a 1 L reactor and the mixture was stirred until the solids dissolved completely. Dimethylamino pyridine (0.587 g) was charged to the stirring solution along with methyl 5-(/er/-butyl)-2-hydroxybenzoate (24) (200 g). The reaction mixture was stirred at 15 - 30 °C and the completeness measured by HPLC (method) with sample aliquots after 60 m. The reaction was considered complete when the peak area of 5-tert-butyl-2- hydroxybenzoate (24) was less than 1%. A half-saturated solution of ammonium chloride was prepared in a separate flask by diluting saturated aqueous ammonium chloride solution (200 mL) with water (200 mL). The reaction mixture was twice washed with half saturated aqueous ammonium chloride solution (200 mL each wash). During each wash, the mixture was stirred for 15 minutes and held for 15 minutes. The organic solution was subsequently washed twice with water (100 mL each wash). During each wash, the mixture was stirred for 15 minutes and held for 15 minutes. The organic solution was transferred to a 1 L round bottom flask and concentrated below 35 °C and under vacuum to yield a white solid (275.51 g and 99.46 %purity as measured by HPLC analysis (method), a 93.0 %yield of methyl 2-((/er/-butoxycarbonyl)oxy)-5-(ter/- butyl)benzoate (23)). ¾ NMR (400 MHz, CDCb): 8.01 (m, 1H), 7.57 (m, 1H); 7.11 (m, 1H); 3.89 (s, 3H); 1.58 (s, 9H); 1.33 (s, 9H).

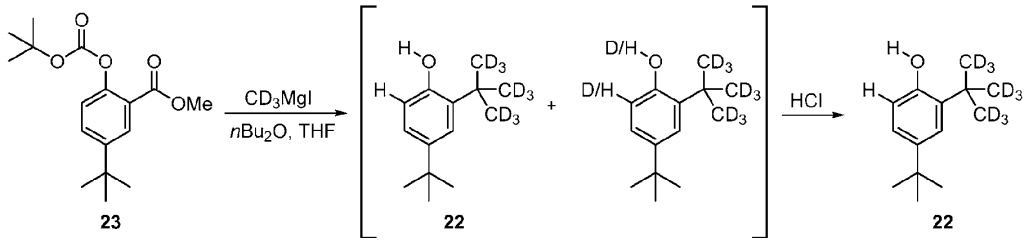
Procedure for the synthesis of 4-(tert-butyl)-2-(2-(methyl-d3)propan-2-yl-1,1,1,3,3,3- d6)phenol (22)



23 22

[00134] THF (176 mL) was charged to a 500 mL jacketed reactor and cooled to 5 °C. To the stirring solvent and at 0 - 35 °C was slowly charged a solution of (methyl-d3)magnesium iodide (60.5 g) in dibutyl ether (145 mL). The resulting slurry was brought to and maintained at 20 - 30 °C while a solution of 2-((tert- butoxycarbonyl)oxy)-5-(/c/7-butyl (benzoate (23) (22 g) in THF (44 mL) was charged over 4 - 6 hours. The reaction mixture was stirred at 20 - 30 °C and the completeness measured by HPLC with sample aliquots after 60 m. The reaction was considered complete when the peak area of 2-((/er/-butoxycarbonyl)oxy)-5-(/er/-butyl)benzoate (23) was less than 1%. A second reactor was charged with 6 N aqueous hydrochloric acid (110 mL) and the stirring solution was cooled to 0 - 10 °C. The reaction slurry was slowly transferred to the acid solution at 0 - 35 °C. The phases were stirred for 15 m and held for 15 m before being separated. The aqueous phase was extracted with dibutyl ether (132 mL). During the extraction the phases were stirred for 15 m and held for 15 m before being separated. The combined organic phases were washed sequentially with water (2 x 77 mL), 5% sodium thiosulfate aqueous solution (77 mL), and water (77 mL). During each wash, the mixture was stirred 15 minutes and held 15 minutes. The organic solution was transferred to a round bottom flask and concentrated below 80 °C and under vacuum to yield 4-(/er/-butyl)-2-(2-(methyl-d3)propan-2-yl-1,1,1,3,3,3-d6)phenol (22) as a crude oil (5.94 g and 83.8 % purity as measured by HPLC analysis with 99.3 %D9 isotopic purity by LC/MS analysis, a 84.9 %yield of methyl 4-(/er/-butyl)-2-(2-(methyl- ¾)propan-2-yl- 1 , 1 , 1 , 3 ,3 , 3 -^phenol (23)). ¾ NMR (400 MHz, CD_3OD): 7.22 (m, 1H); 7.00 (m, 1H), 6.65 (m, 1H); 1.26 (s, 9H).

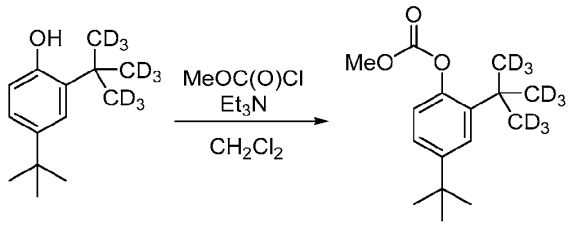
[00135] The Grignard reaction of (23) led to some deuterium incorporation in (22). To effect H/D exchange, the mixture was subjected to a series of HC1 washes:



Procedure for H/D Exchange

[00136] Charge the deuterated analogs of compound 22 (1.00 equiv) to a reactor. Charge DCM (5 vol). Set jacket to 20 °C. Agitate to dissolved solids. Charge 35% hydrochloric acid (5 vol). Agitate to mix the layers for not less than 6 hours. Stop agitation and let the layers settle at least 30 min. Drain the bottom layer (organic) from the reactor. Drain the aqueous layer from the reactor. Charge the organic portion back into the reactor. Repeat HCl wash sequence twice. Charge pre-mixed water (2.5 vol) and sat. aq. NaCl (2.5 vol). Agitate to mix the layers for 30 min. Stop agitation and let the layers settle at least 30 min. Drain the bottom layer (organic) from the reactor. Drain the aqueous from the reactor. Charge the organic portion back into the reactor. Charge water (5 vol). Agitate to mix the layers for 30 min. Stop agitation and let the layers settle at least 30 min. Drain the bottom layer (organic) from the reactor. Drain the aqueous from the reactor. Charge the organic portion back into the reactor. Distill the solvent under reduced pressure to minimal volume (a rotovap with 35 °C bath temperature was used). Charge DCM (5 vol). Distill the solvent under reduced pressure to minimal volume (a rotovap with 35 °C bath temperature was used). Charge DCM (5 vol). Sample the solution and measure water content by KF. Repeat until the water content is less than 300 ppm. Note: This solution was used directly for the next reaction, so the final amount of DCM should be whatever is needed for the alkoxyformylation reaction of compound 22.

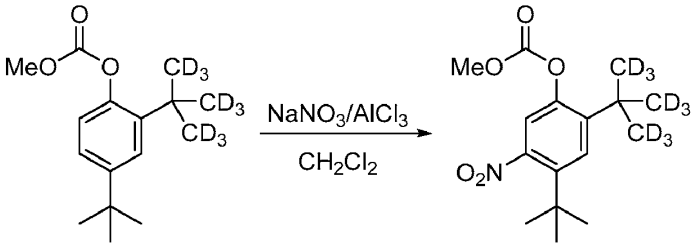
Procedure for the synthesis of 4-(tert-butyl)-2-(2-(methyl-d3)propan-2-yl-1, 1,1, 3,3,3- d6)phenyl methyl carbonate (21)



(21) (22)

[00137] The procedure for the conversion of compound 22 into compound 21 may be performed according to the analogous procedure for compound 12.

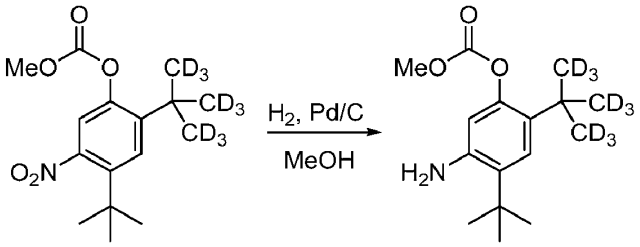
Procedure for the synthesis of 4-(tert-butyl)-2-(2-(methyl-d3)propan-2-yl-1, 1,1, 3,3,3- d6)-5-nitrophenyl methyl carbonate (20)



21 **20**

[00138] The procedure for the conversion of compound 21 into compound 20 may be performed according to the analogous procedure for compound 11 A.

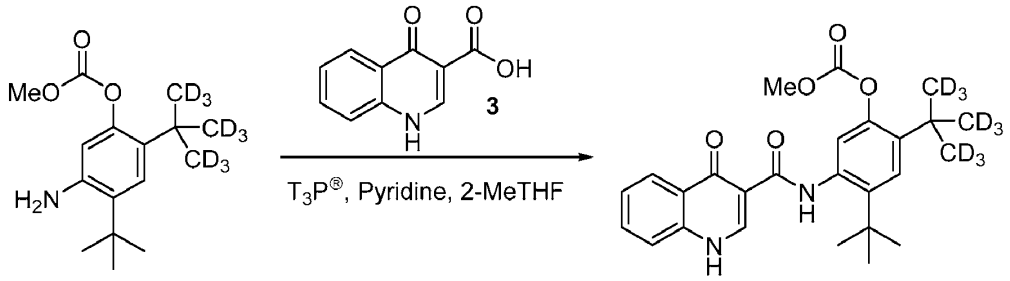
Procedure for the synthesis of 5-amino-4-(tert-butyl)-2-(2-(methyl-d3)propan-2-yl- 1,1,1,3,3,3-d6)phenyl methyl carbonate (7)



20 **7**

[00139] The procedure for the conversion of compound 20 into compound 7 may be performed according to the analogous procedure for compound 4.

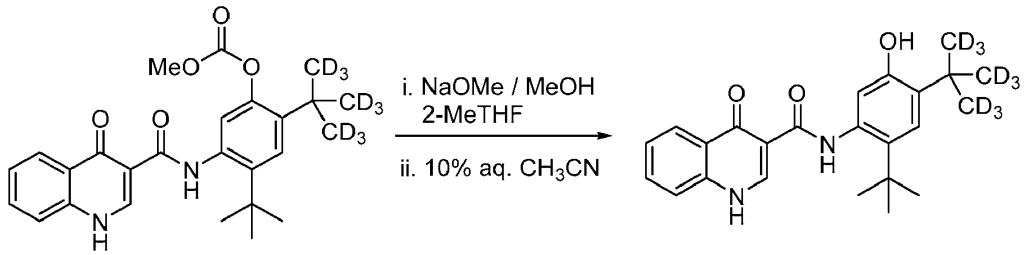
Procedure for the synthesis of 4-(tert-butyl)-2-(2-(methyl-d3)propan-2-yl-1, 1,1, 3,3,3- d6)-5-(4-oxo-1,4-dihydroquinoline-3-carboxamido)phenyl methyl carbonate (8)



7 8

[00140] The procedure for the conversion of compound 7 into compound 8 may be performed according to the analogous procedure for compound 5.

Procedure for the synthesis of N-(2-(tert-butyl)-5-hydroxy-4-(2-(methyl-d3)propan-2- yl-1,1,1,3,3,3-d6)phenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (2) (Compound I)



8 2

[00141] The procedure for the conversion of (8) into (2) may be performed according to the analogous procedure for the synthesis of Compound I.

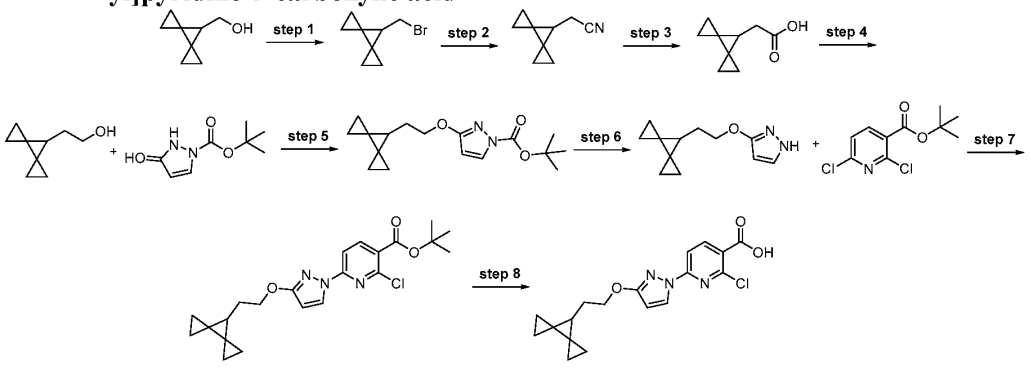
Example 4: Synthesis of (14*S*)-8-[3-(2-{dispiro[2.0.2.1]heptan-7-yl}ethoxy)-1H-pyrazol-1-yl]-12,12-dimethyl-2λ6-thia-3,9,11,18,23-pentaazatetracyclo [17.3.1.111,14.05,10]tetracosa-1(22),5,7,9,19(23),20-hexaene-2,2,4-trione (Compound II)

[00142] Reagents and starting materials were obtained by commercial sources unless otherwise stated and were used without purification.

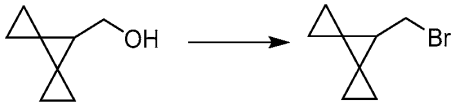
[00143] Proton and carbon NMR spectra were acquired on either of a Bruker Biospin DRX 400 MHz FTNMR spectrometer operating at a ^{1}H and ^{13}C resonant frequency of 400

and 100 MHz respectively, or on a 300 MHz NMR spectrometer. One dimensional proton and carbon spectra were acquired using a broadband observe (BBFO) probe with 20 Hz sample rotation at 0.1834 and 0.9083 Hz/Pt digital resolution respectively. All proton and carbon spectra were acquired with temperature control at 30 °C using standard, previously published pulse sequences and routine processing parameters.

Part A: Synthesis of 2-Chloro-6-[3-(2-dispiro[2.0.2.1]heptan-7-ylethoxy)pyrazol-1-yl]pyridine-3-carboxylic acid



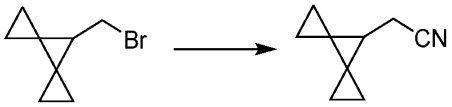
Step 1: 7-(Bromomethyl)dispiro[2.0.2.1]heptane



[00144] A 1000 mL, 3-neck round bottom flask was fitted with a mechanical stirrer, a cooling bath, an addition funnel, a J-Kem temperature probe and a nitrogen inlet/outlet. The vessel was charged under a nitrogen atmosphere with triphenylphosphine (102.7 mL, 443.2 mmol) and dichloromethane (1 L) which provided a clear colorless solution. Stirring was commenced and the cooling bath was charged with acetone. Dry ice was added in portions to the cooling bath until a pot temperature of -15 °C was obtained. The addition funnel was charged with a solution of bromine (22.82 mL, 443.0 mmol) in dichloromethane (220 mL, 10 mL/g) which was subsequently added dropwise over 1 h. Dry ice was added in portions to the cooling bath during the addition to maintain the pot temperature at -15 °C. After the addition of bromine was completed, the pale yellow suspension was continued to stir at -15 °C for 15 min at which point the suspension was cooled to -30 °C. The addition funnel was charged with a solution of dispiro[2.0.2.1]heptan-7-yl methanol (50 g, 402.6 mmol), pyridine (35.82 mL, 442.9 mmol) and dichloromethane (250 mL, 5 mL/g). The clear pale yellow solution was then added dropwise over 1.5 h maintaining the pot temperature at -30 °C. The resulting clear

light yellow reaction mixture was allowed to gradually warm to a pot temperature of -5 °C and then continued to stir at -5 °C for 1 h. The reaction mixture then was poured into hexane (2000 mL) which resulted in the formation of a precipitate. The suspension was stirred at room temperature for 30 min and then filtered through a glass frit Buchner funnel with a 20 mm layer of celite. The clear filtrate was concentrated under reduced pressure (water bath temperature at 20 °C) to provide a yellow oil with some precipitate present. The oil was diluted with some hexane, allowed to stand at room temperature for 15 min and then filtered through a glass frit Buchner funnel with a 20 mm layer of celite. The clear filtrate was concentrated under reduced pressure (water bath temperature at 20 °C) to provide 7-(bromomethyl)dispiro[2.0.2.1]heptane (70 g, 93%) as a clear yellow oil. ^{1}H NMR (400 MHz, Chloroform-d) δ 3.49 (d, J = 7.5 Hz, 2H), 1.90 (t, J = 7.5 Hz, 1H), 1.06 - 0.84 (m, 4H), 0.71 (ddd, J = 9.1, 5.1, 4.0 Hz, 2H), 0.54 (dddd, J = 8.6, 4.8, 3.8, 1.0 Hz, 2H).

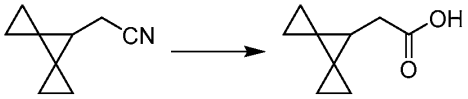
Step 2: 2-Dispiro[2.0.2.1]heptan-7-ylacetonitrile



[00145] A 1000 mL, 3-neck round bottom flask was fitted with a mechanical stirrer, a cooling bath used as secondary containment, a J-Kem temperature probe and a nitrogen inlet/outlet. The vessel was charged under a nitrogen atmosphere with 7-(bromomethyl)dispiro[2.0.2.1]heptane (35 g, 187.1 mmol) and dimethyl sulfoxide (245 mL) which provided a clear amber solution. Stirring was commenced and the pot temperature was recorded at 19 °C. The vessel was then charged with sodium cyanide (11.46 g, 233.8 mmol) added as a solid in one portion which resulted in a dark solution and a gradual exotherm to 49 °C over 15 min. After a few min the pot temperature began to decrease and the mixture was continued to stir at room temperature overnight (about 15 h). The dark reaction mixture was quenched with ice cold saturated sodium carbonate solution (500 mL) and then transferred to a separatory funnel and partitioned with diethyl ether (500 mL). The organic was removed and the residual aqueous was extracted with diethyl ether (2 X 250 mL). The combined organics were washed with water (500 mL), dried over sodium sulfate (200 g) and then filtered through a glass frit Buchner funnel. The clear amber filtrate was concentrated under reduced pressure (water bath temperature 20 °C) to provide 2-dispiro[2.0.2.1]heptan-7-ylacetonitrile (21 g, 84%) as a clear dark

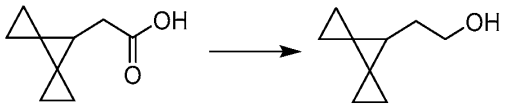
amber oil. ^{1}H NMR (400 MHz, Chloroform-d) δ 2.42 (d, J = 6.6 Hz, 2H), 1.69 (t, J = 6.6 Hz, 1H), 1.02 - 0.88 (m, 4H), 0.79 - 0.70 (m, 2H), 0.66 - 0.55 (m, 2H).

Step 3: 2-Dispiro[2.0.2.1]heptan-7-ylacetic acid



[00146] To a solution of 2-dispiro[2.0.2.1]heptan-7-ylacetonitrile (2.1 g, 14.19 mmol) in EtOH (32 mL) was added sodium hydroxide (5.12 g, 128.0 mmol) followed by water (13 mL) and the resulting solution was stirred and heated to 70 °C overnight. The mixture was then cooled to room temperature, diluted with water and extracted with diethyl ether. The aqueous phase was adjusted to pH = 1 by the addition of 6 N hydrochloric acid (resulting in a cloudy precipitate) and extracted with diethyl ether (3X). The organic phases were dried (magnesium sulfate), filtered and concentrated giving 2-dispiro[2.0.2.1]heptan-7-ylacetic acid (2.19 g, 99% yield, 98% purity) as an orange solid which was used in the next step without further purification. ^{1}H NMR (400 MHz, Chloroform-d) δ 2.44 (d, J = 6.9 Hz, 2H), 1.67 (t, J = 6.9 Hz, 1H), 0.91 (ddd, J = 9.0, 5.2, 3.9 Hz, 2H), 0.81 (dddd, J = 8.9, 5.2, 3.9, 0.5 Hz, 2H), 0.69 (ddd, J = 8.9, 5.2, 3.9 Hz, 2H), 0.56 - 0.44 (m, 2H).

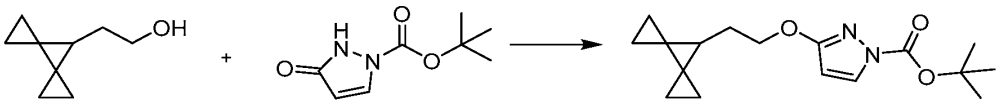
Step 4: 2-Dispiro[2.0.2.1]heptan-7-ylethanol



To lithium aluminum hydride (827.4 mg, 902.3 µL, 21.80 mmol) dissolved in tetrahydrofuran (33.71 mL) cooled in an ice/water bath was added 2-dispiro[2.0.2.1]heptan-7-ylacetic acid (2.552 g, 16.77 mmol) in tetrahydrofuran (7.470 mL) dropwise over 15 min keeping the reaction temperature < 20 °C. The mixture was allowed to stir a total of 18 h, gradually warming to ambient temperature. The mixture was cooled with an ice/water bath and sequentially quenched with slow addition of water (838.4 mg, 838.4 µL, 46.54 mmol), followed by sodium hydroxide (1.006 mL of 5 M, 5.031 mmol), then water (2.493 g, 2.493 mL, 138.4 mmol) affording a white, granular slurry which was filtered over celite. Washed the filtered solid with diethyl ether. The filtrate was concentrated *in vacuo* at ~ 300 mbar and 30 °C water bath. Diluted the residue with diethyl ether, dried (magnesium sulfate), filtered and concentrated *in vacuo* at ~ 300

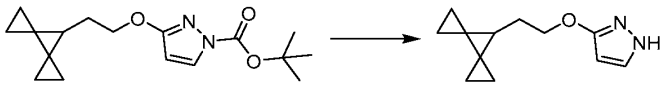
mbar and 30 °C water bath followed by ~ 30 s under vacuum to give 2-dispiro[2.0.2.1]heptan-7-ylethanol (2.318 g, 100%) which was used directly in the ensuing step without further purification. ^{1}H NMR (400 MHz, Chloroform-d) δ 3.64 (s, 2H), 1.68 (d, J = 6.7 Hz, 2H), 1.39 (s, 1H), 1.31 (s, 1H), 0.82 (d, J = 14.0 Hz, 4H), 0.65 (s, 2H), 0.50 (d, J = 3.6 Hz, 2H).

Step 5: *tert*-Butyl 3-(2-dispiro[2.0.2.1]heptan-7-ylethoxy)pyrazole-1-carboxylate



[00147] To a solution of *tert*-butyl 5-oxo-1H-pyrazole-2-carboxylate (2.942 g, 15.97 mmol) and 2-dispiro[2.0.2.1]heptan-7-ylethanol (2.318 g, 16.77 mmol) in tetrahydrofuran (36.78 mL) was added triphenylphosphine (4.399 g, 16.77 mmol). To the mixture was slowly added diisopropyl azodicarboxylate (3.391 g, 3.302 mL, 16.77 mmol) dropwise over 10 min (mild exotherm noted). The reaction mixture was stirred at room temperature for 30 min then at 50 °C for 30 min. The tetrahydrofuran was removed *in vacuo*. To the crude residue was added toluene (23.54 mL) and the mixture was stirred overnight as a precipitate gradually crystallized. Slurried with Celite then the precipitate was filtered off and washed with toluene (8.705 mL) and again with toluene (8.705 mL). The filtrate was concentrated *in vacuo*. The crude product was purified by silica gel chromatography using a shallow gradient from 100% hexanes to 100% ethyl acetate giving *tert*-butyl 3-(2-dispiro[2.0.2.1]heptan-7-ylethoxy)pyrazole-1-carboxylate (3.449 g, 71%). ESI-MS m/z calc. 304.17868, found 305.1 $(M+1)^+$; Retention time: 0.82 min (LC Method A).

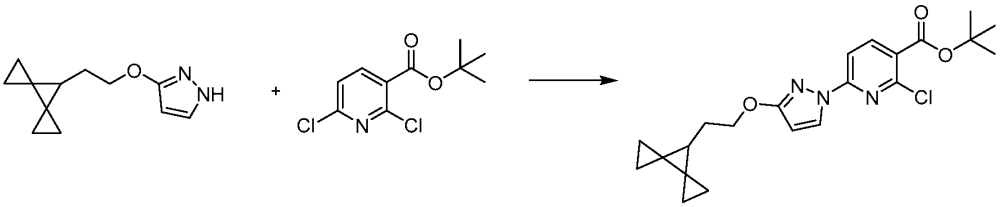
Step 6: 3-(2-Dispiro[2.0.2.1]heptan-7-ylethoxy)-1H-pyrazole



[00148] *tert*-Butyl 3-(2-dispiro[2.0.2.1]heptan-7-ylethoxy)pyrazole-1-carboxylate (5.304 g, 17.43 mmol) was dissolved in dichloromethane (53.04 mL) with trifluoroacetic acid (29.81 g, 20.14 mL, 261.4 mmol) and the reaction was stirred at room temperature for 120 min. The reaction was evaporated and the resulting oil was partitioned between ethyl acetate and a saturated sodium bicarbonate solution and the layers separated. The aqueous portion was extracted two additional times with ethyl acetate, then the organics were

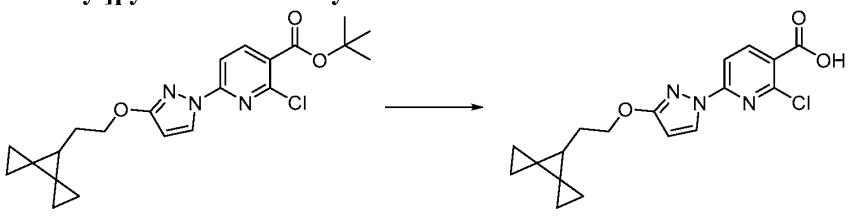
combined, washed with brine, dried over sodium sulfate, filtered and evaporated to give an oil, 3-(2-dispiro[2.0.2.1]heptan-7-ylethoxy)-1H-pyrazole (3.56 g, 100%). ESI-MS m/z calc. 204.12627, found 205.1 $(M+1)^+$; Retention time: 0.59 min (LC Method A).

Step 7: *tert*-Butyl 2-chloro-6-[3-(2-dispiro[2.0.2.1]heptan-7-ylethoxy)pyrazol-1-yl]pyridine-3-carboxylate



[00149] *tert*-Butyl 2,6-dichloropyridine-3-carboxylate (4.322 g, 17.42 mmol), 3-(2-dispiro[2.0.2.1]heptan-7-ylethoxy)-1H-pyrazole (3.559 g, 17.42 mmol) and potassium carbonate (2.891 g, 20.92 mmol) were combined in anhydrous dimethyl sulfoxide (71.18 mL). 1,4-Diazabicyclo[2.2.2]octane (391.1 mg, 3.487 mmol) was added and the mixture was stirred at room temperature under nitrogen for 16 h. The reaction mixture was diluted with water (136.9 mL) and stirred for 15 min. The resulting white solid was filtered and washed with water. The solid was dissolved in dichloromethane and dried over magnesium sulfate. The mixture was filtered and evaporated to give *tert*-butyl 2-chloro-6-[3-(2-dispiro[2.0.2.1]heptan-7-ylethoxy)pyrazol-1-yl]pyridine-3-carboxylate (5.69 g, 79%) as a white solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.35 (d, J = 2.9 Hz, 1H), 8.18 (d, J = 8.4 Hz, 1H), 7.69 (d, J = 8.4 Hz, 1H), 5.94 (d, J = 2.9 Hz, 1H), 4.25 (s, 2H), 1.90 (d, J = 6.8 Hz, 2H), 1.62 (s, 9H), 1.49 (t, J = 6.6 Hz, 1H), 0.85 (d, J = 1.5 Hz, 4H), 0.65 (d, J = 1.5 Hz, 2H), 0.52 (d, J = 1.1 Hz, 2H). ESI-MS m/z calc. 415.16626, found 360.0 (M-tBu)+; Retention time: 2.09 min (LC Method B).

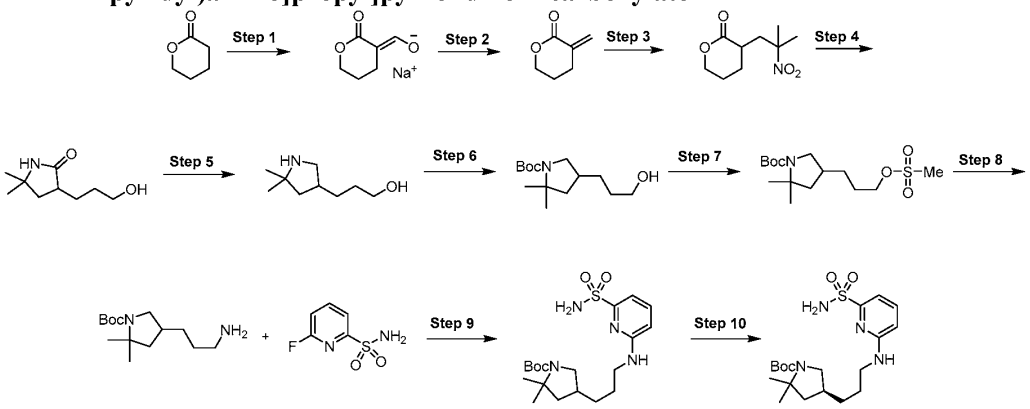
Step 8: 2-Chloro-6-[3-(2-dispiro[2.0.2.1]heptan-7-ylethoxy)pyrazol-1-yl]pyridine-3-carboxylic acid



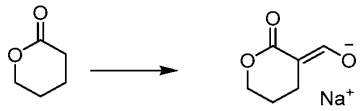
[00150] *tert*-Butyl 2-chloro-6-[3-(2-dispiro[2.0.2.1]heptan-7-ylethoxy)pyrazol-1-yl]pyridine-3-carboxylate (5.85 g, 14.07 mmol) was dissolved in dichloromethane (58.5 mL) with trifluoroacetic acid (16.26 mL, 211.1 mmol) and the reaction was stirred at room

temperature for 16 h. The reaction was evaporated and to the resulting solid was added ether and then removed the ether under reduced pressure. This evaporation from ether was repeated twice more resulting in a white solid, 2-chloro-6-[3-(2-dispiro[2.0.2.1]heptan-7-ylethoxy)pyrazol-1-yl]pyridine-3-carboxylic acid (5.06 g, 100%). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.41 (d, J = 8.5 Hz, 1H), 8.37 (d, J = 2.9 Hz, 1H), 7.75 (d, J = 8.5 Hz, 1H), 5.97 (d, J = 2.9 Hz, 1H), 4.27 (s, 2H), 1.91 (d, J = 6.7 Hz, 2H), 1.50 (s, 1H), 0.85 (d, J = 1.5 Hz, 4H), 0.71 - 0.62 (m, 2H), 0.52 (d, J = 1.1 Hz, 2H). ESI-MS m/z calc. 359.10367, found 360.2 $(M+1)^+$; Retention time: 2.16 min (LC Method B).

Part B: Synthesis of *tert*-Butyl (4*S*)-2,2-dimethyl-4-[3-[(6-sulfamoyl-2-pyridyl)amino]propyl]pyrrolidine-1-carboxylate



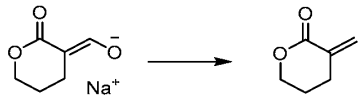
Step 1: (*E*)-(2-Oxotetrahydropyran-3-ylidene)methanolate (sodium salt)



[00151] A 5 L, 3-neck round bottom flask was fitted with a mechanical stirrer, a heating mantle, an addition funnel, a J-Kem temperature probe/controller and a nitrogen inlet/outlet. The vessel was charged under a nitrogen atmosphere with sodium hydride (59.91 g of 60% w/w, 1.498 mol) followed by heptane (1.5 L) which provided a grey suspension. Stirring was commenced and the pot temperature was recorded at 19 °C. The vessel was then charged with ethyl alcohol (3.451 g, 74.91 mmol) added *via* syringe which resulted in gas evolution. The addition funnel was charged with a clear pale yellow solution of tetrahydropyran-2-one (150 g, 1.498 mol) and ethyl formate (111 g, 1.50 mol). The solution was added dropwise over 1 h which resulted in gas evolution and a gradual exotherm to 45 °C. The resulting thick white suspension was then heated to 65 °C for 2 h

and then allowed to cool to room temperature. The mixture was continued to stir at room temperature overnight (about 10 h). The reaction mixture was vacuum filtered through a glass frit Buchner funnel (medium porosity) under a stream of nitrogen. The filter cake was displacement washed with heptane (2 X 250 mL) and pulled for a few min. The slightly heptane wet cake was transferred to a glass tray and dried in a vacuum oven at 45 °C for 15 h to provide a white solid (205 g, 1.36 mol, 91% yield) as the desired product, (*E*)-(2-oxotetrahydropyran-3-ylidene)methanolate (sodium salt).

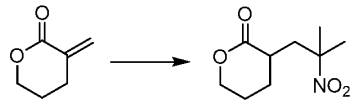
Step 2: 3-Methylenetetrahydropyran-2-one



[00152] A 5 L, 3-neck round bottom flask was fitted with a mechanical stirrer, a heating mantle, an addition funnel, a J-Kem temperature probe/controller and a nitrogen inlet/outlet. The vessel was charged under a nitrogen atmosphere with (*E*)-(2-oxotetrahydropyran-3-ylidene)methanolate (sodium salt) (205 g, 1.366 mol) (205 g, 1.366 mol) and tetrahydrofuran (1640 mL) which provided a white suspension. Stirring was commenced and the pot temperature was recorded at 19 °C. The vessel was then charged with paraformaldehyde (136.6 g, 4.549 mol) added as a solid in one portion. The resulting suspension was heated to 63 °C and the condition was maintained for 15 h. Upon heating the reaction mixture became slightly gelatinous. The white gelatinous mixture was concentrated under reduced pressure to remove most of the tetrahydrofuran. The remaining residue was partitioned with ethyl acetate (1000 mL), saturated sodium chloride (500 mL) and saturated sodium hydrogen carbonate (500 mL) in a separatory funnel. The organic was removed and the residual aqueous was extracted with ethyl acetate (5 X 300 mL). The combined organic was dried over sodium sulfate (500 g) and then vacuum filtered through a glass frit Buchner funnel with a 20 mm layer of celite. The filter cake was displacement washed with ethyl acetate (250 mL). The clear filtrate was concentrated under reduced pressure to provide a clear pale yellow oil (135 g) as the desired crude product. The material was purified by silica gel column flash chromatography (liquid load) eluting with a gradient of 100% hexane to 60% ethyl acetate in hexane over 1 h collecting 450 mL fractions. The product was detected by TLC analysis on silica gel eluting with 3:1 hexanes/ethyl acetate and visualized under UV. The product fractions were combined and

concentrated under reduced pressure to provide a clear, colorless oil (132 g, 1.18 mol, 72% yield containing 16 wt% residual ethyl acetate by NMR) as the desired product, 3-methylenetetrahydropyran-2-one. 1H NMR (400 MHz, dimethyl sulfoxide-d_6) δ 6.18 (q, J = 1.9 Hz, 1H), 5.60 (q, J = 1.9 Hz, 1H), 4.40 - 4.26 (m, 2H), 2.61 (ddt, J = 7.0, 6.3, 2.0 Hz, 2H), 1.90 - 1.75 (m, 2H).

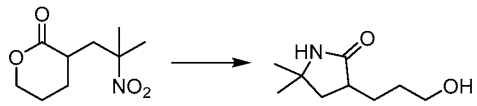
Step 3: 3-(2-Methyl-2-nitro-propyl)tetrahydropyran-2-one



[00153] A 5000 mL, 3-neck round bottom flask was fitted with a mechanical stirrer, a cooling bath used as secondary containment, a J-Kem temperature probe, an addition funnel and a nitrogen inlet/outlet. The vessel was charged under a nitrogen atmosphere with 2-nitropropane (104.9 g, 1.177 mol). Stirring was commenced and the pot temperature was recorded at 19 °C. The vessel was then charged with 1,8-diazabicyclo[5.4.0]undec-7-ene (22.41 g, 147.2 mmol) added neat in one portion which resulted in a clear light yellow solution. No exotherm was observed. The addition funnel was charged with a solution of 3-methylenetetrahydropyran-2-one (110 g, 981.0 mmol) in acetonitrile (1100 mL) which was added dropwise over 1 h which resulted in a clear light yellow solution and a gradual exotherm to 24 °C. The reaction mixture was continued to stir at room temperature for 3.5 h and then concentrated under reduced pressure. The remaining residue was dissolved in dichloromethane (1000 mL) and partitioned with 500 mL of a 3:2 mixture of 1 molar citric acid solution/saturated sodium chloride solution. The resulting organic phase was a clear pale blue solution and the aqueous phase was a slightly cloudy very pale blue solution. The organic was removed and the residual aqueous was extracted with dichloromethane (300 mL). The combined organic was washed with saturated sodium chloride solution (300 mL), dried over sodium sulfate (250 g) and then filtered through a glass frit Buchner funnel. The filtrate was concentrated under reduced pressure to a volume of about 200 mL. The clear pale blue dichloromethane solution was diluted with methyl *tert*-butyl ether (1500 mL) and the cloudy solution was concentrated under reduced pressure to a volume of about 200 mL which provided a suspension. The mixture was again diluted with methyl *tert*-butyl ether (1500 mL) and concentrated under reduced pressure to a volume of about 250 mL. The resulting suspension was allowed to

stand at room temperature overnight (about 12 h). The solid was collected by vacuum filtration in a glass frit Buchner funnel and the filter cake was displacement washed with cold methyl *tert*-butyl ether (2 X 150 mL) and then pulled for 30 min. The material was further dried in a vacuum oven at 45 ℃ for 5 h to provide (160 g, 0.795 mol, 81% yield) of a white solid as the desired product, 3-(2-methyl-2-nitro-propyl)tetrahydropyran-2-one. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ 4.34 (ddd, J = 11.1, 9.3, 4.3 Hz, 1H), 4.20 (dt, J = 11.1, 5.1 Hz, 1H), 2.75 - 2.62 (m, 1H), 2.56 (dd, J = 14.9, 5.2 Hz, 1H), 2.01 - 1.89 (m, 2H), 1.89 - 1.67 (m, 2H), 1.55 (d, J = 6.0 Hz, 6H), 1.44 (dddd, J = 12.8, 11.5, 8.1, 6.6 Hz, 1H).

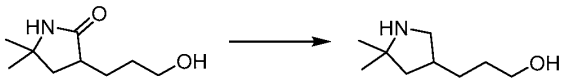
Step 4: 3-(3-Hydroxypropyl)-5,5-dimethyl-pyrrolidin-2-one



[00154] A 1000 mL, 3-neck round bottom flask was fitted with a Teflon stir bar, a heating mantle, a J-Kem temperature probe/controller and rubber septums. The vessel was charged with 3-(2-methyl-2-nitro-propyl)tetrahydropyran-2-one (25 g, 124.2 mmol) and ethyl alcohol (375 mL) which provided a white suspension. Stirring was commenced and the suspension was heated to 40 ℃ for 10 min which provided a clear colorless solution. The vessel was then fitted with a gas dispersion tube and the solution was degased with nitrogen for 15 min. The vessel was then charged with Raney Nickel (8.019 g of 50% w/w, 68.31 mmol) and the vessel was then fitted with the septums. The vessel was evacuated and placed under a hydrogen atmosphere. The process was repeated for three cycles. The vessel was then placed under 1 atmosphere hydrogen and the reaction mixture was gradually heated to 60 ℃. The reaction was continued to stir at 60 ℃ for 24 h. After cooling to room temperature, the vessel was fitted with a gas dispersion tube and the reaction mixture was degased with nitrogen for 15 min. The mixture was vacuum filtered through a glass frit Buchner funnel with a 20 mm layer of celite. The filter cake was displacement washed with ethanol (2 X 100 mL) and pulled until slightly ethyl alcohol wet, then wetted with water and the used Raney nickel catalyst was discarded under water. The clear pale amber filtrate was concentrated under reduced pressure to a clear viscous light amber oil. The oil was diluted with methyl tert-butyl ether (1500 mL) and the cloudy solution was concentrated under reduced pressure to a volume of about 150 mL which

provided a suspension. The mixture was again diluted with methyl tert-butyl ether (1500 mL) and concentrated under reduced pressure to a volume of about 150 mL. The resulting suspension was allowed to stand at room temperature overnight (about 12 h). The solid was collected by vacuum filtration in a glass frit Buchner funnel and the filter cake was displacement washed with cold methyl tert-butyl ether (2 X 50 mL) and then pulled for 30 min. The material was further dried in a vacuum oven at 45 °C for 3 h to provide a white solid (19 g, 0.111 mol, 89% yield) as the product, 3-(3-hydroxypropyl)-5,5-dimethyl-pyrrolidin-2-one. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ 7.63 (s, 1H), 3.38 (t, J = 6.5 Hz, 2H), 2.37 (tdd, J = 9.8, 8.5, 4.4 Hz, 1H), 2.02 (dd, J = 12.3, 8.6 Hz, 1H), 1.72 (tdd, J = 9.6, 7.5, 4.4 Hz, 1H), 1.52 - 1.32 (m, 3H), 1.28 - 1.03 (m, 7H).

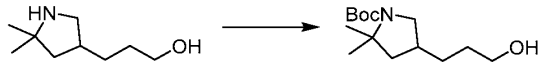
Step 5: 3-(5,5-Dimethylpyrrolidin-3-yl)propan-1-ol



[00155] A 5 L, 3-neck round bottom flask was fitted with a mechanical stirrer, a heating mantle, an addition funnel, a J-Kem temperature probe/controller and a nitrogen inlet/outlet. The vessel was charged under a nitrogen atmosphere with lithium aluminum hydride pellets (19.39 g, 510.9 mmol). The vessel was then charged with tetrahydrofuran (500 mL, 20 mL/g). Stirring was commenced and the pot temperature was recorded at 20 °C. The mixture was allowed to stir at room temperature for 0.5 h to allow the pellets to dissolve. The pot temperature of the resulting grey suspension was recorded at 24 °C. The addition funnel was charged with a solution of 3-(3-hydroxypropyl)-5,5-dimethyl-pyrrolidin-2-one (25 g, 146.0 mmol) in tetrahydrofuran (500 mL) and the clear pale yellow solution was added dropwise over 90 min. Slight heating was required to achieve homogeneity. After the completed addition the pot temperature of the resulting greyish suspension was recorded at 24 °C. The mixture was then heated to a pot temperature of 65 °C and the condition was maintained for 72 h. Analysis of the reaction mixture at this point indicated some residual starting material still remaining and no change in product formation. The reaction was subsequently stopped at this point. The heating mantle was removed and the vessel was fitted with a cooling bath. The suspension was cooled to 0 °C with a crushed ice/water cooling bath and then quenched by the very slow dropwise addition of water (19.93 mL), followed by 15 wt% sodium hydroxide solution (19.93 mL) and then finally with water (59.79 mL). The pot temperature of the resulting white

suspension was recorded at 5 °C. The cooling bath was removed and the vessel was again fitted with a heating mantle. The suspension was warmed to 60 °C and the condition was maintained for 30 min. The warm suspension was vacuum filtered through a glass frit Buchner funnel with a 20 mm layer of celite. The filter cake was then displacement washed with 60 °C tetrahydrofuran (2 X 250 mL) and then pulled for 30 min. The clear filtrate was concentrated under reduced pressure to provide (23.5 g, 0.149 mol, 99% yield) of a clear light yellow viscous oil as the desired product, 3-(5,5-dimethylpyrrolidin-3-yl)propan-1-ol. ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ 3.37 (dt, J = 8.3, 6.4 Hz, 3H), 2.95 (dd, J = 10.6, 7.6 Hz, 1H), 2.40 (dd, J = 10.7, 7.7 Hz, 1H), 2.04 (dt, J = 16.1, 8.1 Hz, 1H), 1.69 (dd, J = 12.2, 8.2 Hz, 1H), 1.50 - 1.24 (m, 5H), 1.11 - 0.94 (m, 7H).

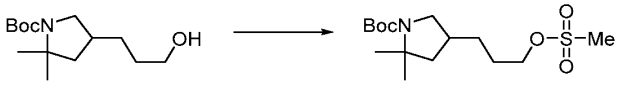
Step 6: *tert*-Butyl 4-(3-hydroxypropyl)-2,2-dimethyl-pyrrolidine-1-carboxylate



[00156] A 1 L, 3-neck round bottom flask was fitted with a mechanical stirrer, a cooling bath, an addition funnel, a J-Kem temperature probe and a nitrogen inlet/outlet. The vessel was charged under a nitrogen atmosphere with 3-(5,5-dimethylpyrrolidin-3-yl)propan-1-ol (15 g, 95.39 mmol) and dichloromethane (225 mL, 15 mL/g) which provided a clear light yellow solution. Stirring was commenced and the pot temperature was recorded at 19 °C. The cooling bath was charged with crushed ice/water and the pot temperature was lowered to 0 °C. The addition funnel was charged with triethylamine (12.55 g, 124.0 mmol) which was subsequently added neat dropwise over 5 min. No exotherm was observed. The addition funnel was then charged with di-*tert*-butyl dicarbonate (22.89 g, 104.9 mmol) dissolved in dichloromethane (225 mL). The clear pale yellow solution was then added dropwise over 30 min which resulted in gentle gas evolution. No exotherm was observed. The cooling bath was removed and the resulting clear light yellow solution was allowed to warm to room temperature and continue to stir at room temperature for 3 h. The reaction mixture was transferred to a separatory funnel and partitioned with water (75 mL). The organic was removed and washed with saturated sodium chloride solution (75 mL), dried over sodium sulfate (150 g) and then filtered through a glass frit Buchner funnel. The filtrate was concentrated under reduced pressure to provide (30 g) of a clear light yellow oil as the desired crude product. The material was purified by silica gel column flash chromatography (liquid load with dichloromethane) eluting with a gradient of 100%

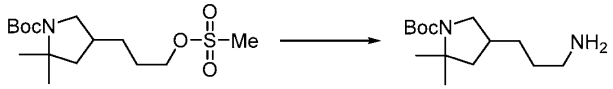
dichloromethane to 10% methyl alcohol in dichloromethane over 60 min collecting 50 mL fractions. The desired product fractions were combined and concentrated under reduced pressure to provide *tert*-butyl 4-(3-hydroxypropyl)-2,2-dimethyl-pyrrolidine-1-carboxylate (22 g, 0.0855 mol, 90% yield) as a clear pale yellow viscous oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.38 (td, J = 5.2, 1.4 Hz, 1H), 3.54 (dt, J = 10.3, 6.7 Hz, 1H), 3.38 (td, J = 6.6, 3.5 Hz, 2H), 2.76 (q, J = 10.3 Hz, 1H), 2.07 (td, J = 11.6, 5.7 Hz, 1H), 1.87 (ddd, J = 16.7, 12.1, 6.0 Hz, 1H), 1.37 (dd, J = 14.2, 10.4 Hz, 17H), 1.24 (s, 3H).

Step 7: *tert*-Butyl 2,2-dimethyl-4-(3-methylsulfonyl oxypropyl)pyrrolidine-1-carboxylate



[00157] *tert*-Butyl 4-(3-hydroxypropyl)-2,2-dimethyl-pyrrolidine-1-carboxylate (50.5 g, 196.22 mmol) and triethylamine (39.711 g, 54.698 mL, 392.44 mmol) were dissolved in dichloromethane (500 mL) and the resulting solution was chilled in an ice water bath for 30 min. Mesyl chloride (24.725 g, 16.706 mL, 215.84 mmol) was added dropwise over a 30 min period, then the ice bath was removed and the mixture stirred at room temperature for one h. The reaction was then quenched with saturated sodium bicarbonate solution (200 mL). The phases were separated and the organic phase was extracted with saturated sodium bicarbonate (200 mL) and water (2 X 100 mL). The aqueous phases were discarded and the organic phase was dried over sodium sulfate, filtered and concentrated *in vacuo* to obtain *tert*-butyl 2,2-dimethyl-4-(3-methylsulfonyl oxypropyl)pyrrolidine-1-carboxylate (64.2 g, 93%) as a pale yellow oil. ESI-MS m/z calc. 335.1766, found 336.4 $(M+1)^+$; Retention time: 5.54 min (LC Method Q).

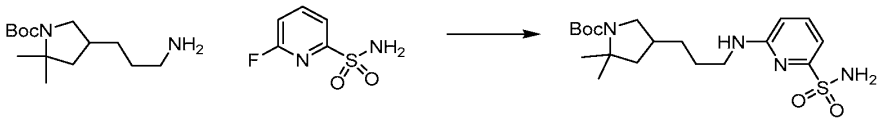
Step 8: *tert*-Butyl 4-(3-aminopropyl)-2,2-dimethyl-pyrrolidine-1-carboxylate



[00158] *tert*-Butyl 2,2-dimethyl-4-(3-methylsulfonyloxypropyl)pyrrolidine-1-carboxylate (64.2 g, 191.38 mmol) was dissolved in dioxane (650 mL) and then ammonium hydroxide (650 mL) was added and the resulting mixture heated to 45 °C for 18 h. After 18 h, the reaction was cooled to room temperature. The solution was diluted with 1M sodium hydroxide (200 mL) and then extracted with diethyl ether (3 X 650 mL). The aqueous phase was discarded and the combined organic phases were extracted with

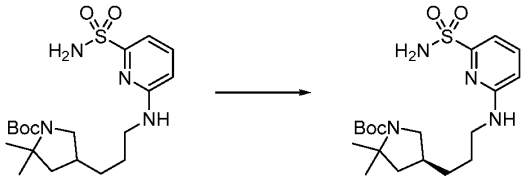
water (2 X 200 mL). The aqueous phases were discarded and the organic phase was dried over sodium sulfate, filtered and concentrated *in vacuo* to afford *tert*-butyl 4-(3-aminopropyl)-2,2-dimethyl-pyrrolidine-1-carboxylate (48.9 g, 95%) as a pale yellow oil. ESI-MS m/z calc. 256.2151, found 257.3 $(M+1)^+$; Retention time: 3.70 min (LC Method Q).

Step 9: *tert*-Butyl 2,2-dimethyl-4-[3-[(6-sulfamoyl-2-pyridyl)amino]propyl]pyrrolidine-1-carboxylate



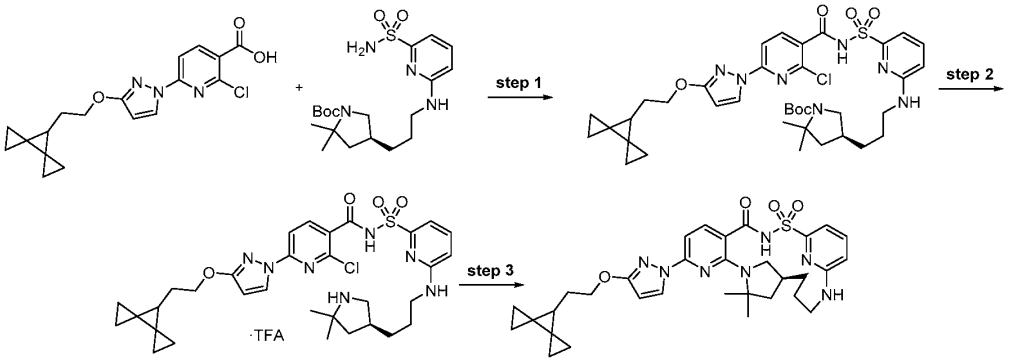
[00159] To *tert*-butyl 4-(3-aminopropyl)-2,2-dimethyl-pyrrolidine-1-carboxylate (8.91 g, 34.8 mmol) and 6-fluoropyridine-2-sulfonamide (6.13 g, 34.8 mmol) in dimethyl sulfoxide (75 mL) was added potassium carbonate (4.91 g, 35.5 mmol) and the mixture stirred at 100 °C for 12 h and then allowed to cool to ambient temperature and stirred for an additional 4 h (16 h total). The reaction mixture was slowly poured into hydrochloric acid (35 mL of 1 M, 35.00 mmol) in water (200 mL) (some foaming) and diluted with ethyl acetate (250 mL). The organic phase was separated and washed with 100 mL of brine. The organic phase was dried over magnesium sulfate, filtered over celite, and concentrated in vacuo to afford a dark yellow oil. The crude product was purified by silica gel chromatography eluting with 0% - 100% ethyl acetate in hexanes. Collected both pure (9.0 g) and impure (3 g) fractions. Purified the impure fractions by silica gel chromatography eluting with 0% - 100% ethyl acetate in hexanes affording, in total, *tert*-butyl 2,2-dimethyl-4-[3-[(6-sulfamoyl-2-pyridyl)amino]propyl]pyrrolidine-1-carboxylate (10.0 g, 69%). ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ 7.52 (dd, J = 8.5, 7.2 Hz, 1H), 7.07 (s, 2H), 6.95 (dd, J = 7.2, 0.7 Hz, 2H), 6.61 (d, J = 8.5 Hz, 1H), 3.55 (q, J = 9.1 Hz, 1H), 3.32 - 3.24 (m, 2H), 2.79 (q, J = 10.0 Hz, 1H), 2.13 (d, J = 16.1 Hz, 1H), 1.96 - 1.82 (m, 1H), 1.51 (dt, J = 18.0, 9.3 Hz, 2H), 1.37 (dd, J = 12.9, 10.6 Hz, 15H), 1.24 (s, 3H). ESI-MS m/z calc. 412.21442, found 413.1 $(M+1)^+$; Retention time: 2.34 min (LC Method D).

Step 10: *tert*-Butyl (4*S*)-2,2-dimethyl-4-[3-[(6-sulfamoyl-2-pyridyl)amino]propyl]pyrrolidine-1-carboxylate

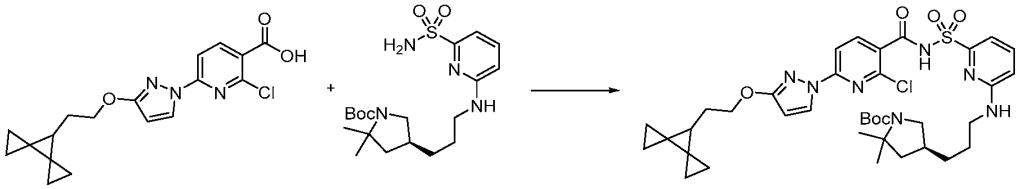


[00160] Subjected racemic *tert*-butyl 2,2-dimethyl-4-[3-[(6-sulfamoyl-2-pyridyl)amino]propyl]pyrrolidine-1-carboxylate (7 g, 16.97 mmol) to chiral separation by SFC chromatography using a ChiralPak IG (250 X 21.2 mm column, 5μm particle size) with 40% methanol/60% carbon dioxide mobile phase at 70 mL/min over 11.0 min (injection volume = 500 μL of 32 mg/mL solution in methanol) giving as the first peak to elute, *tert*-butyl (4*S*)-2,2-dimethyl-4-[3-[(6-sulfamoyl-2-pyridyl)amino]propyl]pyrrolidine-1-carboxylate (3.4481 g, 99%). ESI-MS m/z calc. 412.21442, found 413.2 $(M+1)^+$; Retention time: 0.63 min (LC Method A).

Part C: Synthesis of (14S)-8-[3-(2-{dispiro[2.0.2.1]heptan-7-yl}ethoxy)-1H-pyrazol-1-yl]-12,12-dimethyl-2λ6-thia-3,9,11,18,23-pentaazatetracyclo [17.3.1.111,14.05,10]tetracosa-1(22),5,7,9,19(23),20-hexaene-2,2,4-trione (Compound II)



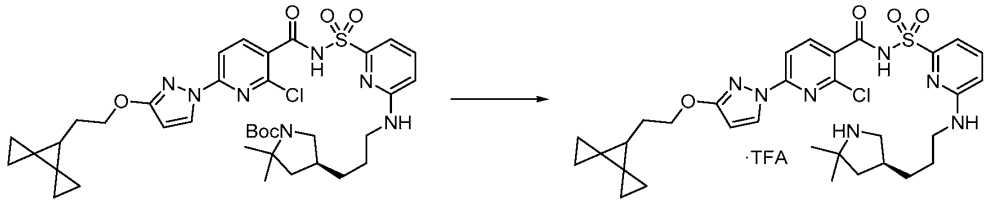
Step 1: *tert*-Butyl (4*S*)-4-[3-[[6-[[2-chloro-6-[3-(2-dispiro[2.0.2.1]heptan-7-ylethoxy)pyrazol-1-yl]pyridine-3-carbonyl]sulfamoyl]-2-pyridyl]amino]propyl]-2,2-dimethyl-pyrrolidine-1-carboxylate



[00161] To a solution of 2-chloro-6-[3-(2-dispiro[2.0.2.1]heptan-7-ylethoxy)pyrazol-1-yl]pyridine-3-carboxylic acid (5.2 g, 14.45 mmol) in tetrahydrofuran (100 mL) was added

carbonyl diimidazole (2.8 g, 16.51 mmol) and the mixture stirred at ambient temperature for 1 h. To this mixture was added *tert*-butyl (4*S*)-2,2-dimethyl-4-[3-[(6-sulfamoyl-2-pyridyl)amino]propyl]pyrrolidine-1-carboxylate (6.0 g, 14.54 mmol) in tetrahydrofuran (15 mL) followed by 1,8-diazabicyclo[5.4.0]undec-7-ene (6.5 mL, 43.47 mmol) and the mixture was stirred at ambient temperature for 16 h. The reaction was diluted with water (150 mL) and the mixture acidified with aqueous hydrochloric acid (15 mL of 6 M, 90.00 mmol). The mixture was extracted with ethyl acetate (300 mL) and the organic phase separated. The organic phase was washed with brine, dried over magnesium sulfate, filtered over Celite and concentrated *in vacuo* affording a white precipitate. The precipitate was slurried with acetonitrile and the solid collected by filtration using a medium glass frit and washed with acetonitrile. The filtrate was concentrated *in vacuo* affording a yellow oil. The crude oil was diluted with acetonitrile and some *N*-methyl-2-pyrrolidone and chromatographed on a 415 g reverse phase C_{18} column eluting with 50% - 100% acetonitrile in water giving *tert*-butyl (4*S*)-4-[3-[[6-[[2-chloro-6-[3-(2-dispiro[2.0.2.1]heptan-7-ylethoxy)pyrazol-1-yl]pyridine-3-carbonyl]sulfamoyl]-2-pyridyl]amino]propyl]-2,2-dimethyl-pyrrolidine-1-carboxylate (4.5 g, 41%). ESI-MS m/z calc. 753.30756, found 754.4 $(M+1)^+$; Retention time: 3.79 min (LC Method D).

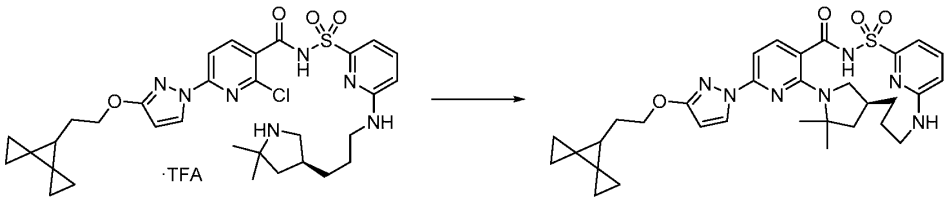
Step 2: 2-Chloro-*N*-[[6-[3-[(3S)-5,5-dimethylpyrrolidin-3-yl]propylamino]-2-pyridyl]sulfonyl]-6-[3-(2-dispiro[2.0.2.1]heptan-7-ylethoxy)pyrazol-1-yl]pyridine-3-carboxamide (trifluoroacetate salt)



[00162] To a solution of *tert*-butyl (4*S*)-4-[3-[[6-[[2-chloro-6-[3-(2-dispiro[2.0.2.1]heptan-7-ylethoxy)pyrazol-1-yl]pyridine-3-carbonyl]sulfamoyl]-2-pyridyl]amino]propyl]-2,2-dimethyl-pyrrolidine-1-carboxylate (5.9 g, 7.821 mmol) in dichloromethane (30 mL) and toluene (15 mL) was added trifluoroacetic acid (6.0 mL, 77.88 mmol) and the mixture stirred at ambient temperature for 18 h. The solvent was removed *in vacuo* with the bath temp set at 45 °C affording a thick, yellow oil. The oil was diluted with toluene (125 mL) and the solvent removed *in vacuo* with the bath temp set at 45 °C. The oil was diluted with toluene and the solvent removed *in vacuo* affording a thick, viscous yellow oil, 2-chloro-*N*-[[6-[3-[(3*S*)-5,5-dimethylpyrrolidin-3-

yl]propylamino]-2-pyridyl]sulfonyl]-6-[3-(2-dispiro[2.0.2.1]heptan-7-ylethoxy)pyrazol-1-yl]pyridine-3-carboxamide (trifluoroacetate salt) (6.0 g, 100%) which was used in the next step without further purification. ESI-MS m/z calc. 653.2551, found 654.3 $(M+1)^+$; Retention time: 2.6 min (LC Method B).

Step 3: (14*S*)-8-[3-(2-{Dispiro[2.0.2.1]heptan-7-yl}ethoxy)-1H-pyrazol-1-yl]-12,12-dimethyl-2λ6-thia-3,9,11,18,23-pentaazatetracyclo [17.3.1.111,14.05,10]tetracosa-1(22),5,7,9,19(23),20-hexaene-2,2,4-trione (Compound II)



[00163] To a solution of 2-chloro-*N*-[[6-[3-[(3*S*)-5,5-dimethylpyrrolidin-3-yl]propylamino]-2-pyridyl]sulfonyl]-6-[3-(2-dispiro[2.0.2.1]heptan-7-ylethoxy)pyrazol-1-yl]pyridine-3-carboxamide (trifluoroacetate salt) (6.0 g, 7.810 mmol) in NMP (140 mL) was added potassium carbonate (5.3 g, 38.35 mmol). The mixture was purged with nitrogen for 5 min. The mixture was then heated at 150 °C for 22 h. The reaction mixture was cooled to room temperature and added to water (300 mL) affording an off-white solid precipitate. The mixture was carefully acidified with aqueous hydrochloric acid (12 mL of 6 M, 72.00 mmol) affording a foamy slurry. The solid was collected by filtration using a medium glass frit. The wet filter cake was dissolved in ethyl acetate (500 mL) and washed with 200 mL of brine. The aqueous phase was slightly cloudy so it was acidified with a small amount of 6N hydrochloric acid and returned to the organic phase. The aqueous phase was separated and the organic phase was dried over magnesium sulfate, filtered and concentrated *in vacuo* affording a light yellow oil. This crude product was diluted with acetonitrile and chromatographed on a 415 g C_{18} reverse phase column eluting with 50% - 100% acetonitrile in water. The product was isolated as a cream colored foam. The foam was dried *in vacuo* at 45 °C for 48 h giving (14*S*)-8-[3-(2-{dispiro[2.0.2.1]heptan-7-yl}ethoxy)-1H-pyrazol-1-yl]-12,12-dimethyl-2λ6-thia-3,9,11,18,23-pentaazatetracyclo[17.3.1.111,14.05,10]tetracosa-1(22),5,7,9,19(23),20-hexaene-2,2,4-trione (Compound **II**) (3.32 g, 68%). ^{1}H NMR (400 MHz, dimethyl sulfoxide-d_6) δ 12.48

(s, 1H), 8.20 (d, J = 2.8 Hz, 1H), 7.81 (d, J = 8.2 Hz, 1H), 7.57 (dd, J = 8.5, 7.2 Hz, 1H), 7.05 (d, J = 7.1 Hz, 1H), 6.97 (d, J = 8.5 Hz, 1H), 6.91 (d, J = 8.2 Hz, 1H), 6.71 (d, J = 8.5 Hz, 1H), 6.08 (d, J = 2.7 Hz, 1H), 4.21 (td, J = 6.7, 1.3 Hz, 2H), 3.92 (d, J = 12.0 Hz, 1H), 3.16 (s, 1H), 2.95 (d, J = 13.3 Hz, 1H), 2.78 - 2.66 (m, 1H), 2.07 (s, 1H), 1.92 - 1.72 (m, 4H), 1.60 (s, 6H), 1.51 (s, 3H), 1.47 (t, J = 6.5 Hz, 1H), 1.31 (q, J = 12.2 Hz, 1H), 0.89 - 0.77 (m, 4H), 0.69 - 0.61 (m, 2H), 0.53 - 0.45 (m, 2H). ESI-MS m/z calc. 617.27844, found 618.4 $(M+1)^+$; Retention time: 10.29 min (LC Method F).

Example 5: Compound II (Free form) Form A

[00164] A reactor was equipped with an overhead stirrer, reflux condenser, N_2 bubble line and outlet, and a temperature probe. A mixture of (14S)-8-bromo-12,12-dimethyl-2λ^6-thia-3,9,11,18,23-pentaazatetracyclo[17.3.1.1^{11,14}.0^{5,10}]tetracosa-1(23),5,7,9,19,21-hexaene-2,2,4-trione (120 g of 86% w/w with IPAc [103.2 g (14S)-8-bromo-12,12-dimethyl-2λ^6-thia-3,9,11,18,23-pentaazatetracyclo[17.3.1.1^{11,14}.0^{5,10}]tetracosa-1(23),5,7,9,19,21-hexaene-2,2,4-trione], 0.21 mol, 1 equiv), 3-(2-(dispiro[2.0.2^4.1^3]heptan-7-yl)ethoxy)-1*H*-pyrazole (42.6 g, 0.21 mol, 1 equiv), 325 mesh K_2CO_3 (63.4 g, 0.46 mol, 2.2 equiv), CuI (3.3 g, 17.2 mmol, 0.083 equiv) and BuOAc (740 mL) were charged into a reactor. The mixture was stirred at ambient temperature. Then DMF (300 mL, 2.9 vol) and *N*,*N*'-dimethylcyclohexane-1,2-diamine (14.6 g or 16.2 ml, 0.1 mol, 0.49 equiv) were charged to the reactor and the mixture was purged with three N_2/vacuum/N_2 cycles. The mixture was then heated to 120 °C for 4 h, then allowed to cool to ambient temperature. 10% aq w/v oxalic acid (860 mL, 0.96 mol, 4.6 equiv) was added dropwise and the mixture stirred for at least 1 h. The mixture was then filtered to remove suspended solids. The removed solids were washed with (2 x 120 mL). The layers from the filtrate were separated. The organic layer was washed with 8% aq. w/v trisodium citrate (600 mL). Brine was added as necessary to aid phase separation. The organic layer was washed with 1:1 v/v water/brine (400 mL). The organic layer was filtered through a pad of Celite. The filter pad was washed with IPAc (150 mL). The filtrate was concentrated, then 800 mL of 1-PrOH (7.8 vol) was added and the mixture concentrated. This step was repeated one more time. Toluene (800 mL) was added and the mixture concentrated. This step was repeated one more time to afford a thick slurry. The crude mixture was concentrated to a volume of 300 mL (2.9 vol) of toluene. After stirring the slurry overnight, the solid was collected by filtration and washing the solid with toluene (2 x 100 mL, 0.97 vol). The solid

was dried under vacuum with a nitrogen bleed at 50 °C until the loss on drying was no more than 1.0% to afford Compound **II** as a white/off-white solid (107.0 g, 83%, 94.5% (AUC) HPLC purity.

[00165] Recrystallization: Compound **II** Form A [22.2 g, 94.6% (AUC) Compound **II** Form A was suspended in toluene (440 mL, 20 vol based on Compound **II** Form A) and the mixture heated to reflux. After holding at reflux for NLT 2 h, the mixture was allowed to cool to ambient over 8 h. After stirring at ambient temperature overnight, the solid was collected by filtration washing the solid with toluene (40 mL, 1.8 vol). The solid was dried under vacuum with a nitrogen bleed at 50 °C until the loss on drying was no more than 1.0% to afford Compound **II** Form A as a white/off-white solid (18.8 g, 84%, 96.8% (AUC) HPLC purity).

[00166] Second Recrystallization: Compound **II** Form A [17.5 g, 97.0% (AUC) Compound **II** Form A] was suspended in toluene (350 mL, 20 vol based on Compound **II** Form A) and the mixture heated to reflux. After holding at reflux for no less than 2 h, the mixture was allowed to cool to ambient temperature over 8 h. After stirring at ambient temperature overnight, the solid was collected by filtration washing the solid with toluene (40 mL, 1.8 vol). The solid was dried under vacuum with a nitrogen bleed at 50 °C until the loss on drying was no more than 1.0% to afford Compound **II** Form A (free form) as a white/off-white solid (15.7 g, 89%, 98.4% (AUC) HPLC purity).

[00167] Compound **II** free Form A is the most stable polymorphic form at water activity ≤0.95 at ambient temperature.

A. X-Ray Powder Diffraction

[00168] The XRPD pattern was acquired at room temperature in reflection mode using a Bruker Advance equipped with Vantec-1 detector. A sample was analyzed on a silicon sample holder from 3-40° 2-theta on continuous mode with step size of 0.0144531° and time per step of 0.25 s. The sample was spinning at 15 rpm. The XRPD diffractogram for Compound **II** (free form) Form A is provided in **Fig. 1** and the XRPD data are summarized below in **Table 5.**

Table 5: XRPD signals for crystalline Form A of Compound II (free form)

XRPD Peaks	Angle (degrees 2-Theta ± 0.2)	Intensity %
1	20.0	100.0
2	23.1	63.9
3	16.6	56.4
4	23.3	52.3
5	9.2	51.2
6	24.3	42.0
7	18.1	41.9
8	16.5	40.7
9	11.2	38.6
10	21.8	37.2
11	11.3	33.3
12	18.0	32.9
13	14.0	31.8
14	24.4	29.5
15	22.9	29.4
16	23.8	29.3
17	5.7	26.2
18	18.7	24.6
19	18.8	24.6
20	18.5	22.4
21	22.5	19.2
22	20.4	17.7
23	15.1	14.9
24	26.5	14.3
25	13.7	13.8
26	22.2	13.8
27	27.3	11.9
28	15.0	11.7
29	14.8	10.4

B. Single Crystal Elucidation

[00169] Single crystals having the Compound **II** (free form) Form A structure were grown from acetone/heptane. X-ray diffraction data were acquired at 298K on a Bruker diffractometer equipped with Mo K_α radiation (λ=0.71073 Å) and a CCD detector. The structure was solved and refined using SHELX programs (Sheldrick, G.M., Acta Cryst., (2008) A64, 112-122) and results are summarized in **Table 6** below.

Table 6: Single crystal elucidation of Compound II (free form) Form A

Crystal System	Monoclinic
Space Group	$P2_1$
a (Å)	15.477(3)
b (Å)	12.741(2)
c (Å)	16.369(3)
α (°)	90
β (°)	99.350(5)
γ (°)	90
V ($Å^3$)	3185.1(9)
Z/Z′	2/2
Temperature	298 K

C. Solid state NMR

1. Solid State NMR experimental (applies to all crystalline forms of Compound II):

[00170] Bruker-Biospin 400 MHz wide-bore spectrometer equipped with Bruker-Biospin 4mm HFX probe was used. Samples were packed into 4mm ZrO_2 rotors and spun under Magic Angle Spinning (MAS) condition with spinning speed typically set to 12.5 kHz. The proton relaxation time was measured using ^1H MAS T_1 saturation recovery relaxation experiment in order to set up proper recycle delay of the ^{13}C cross-polarization (CP) MAS experiment. The CP contact time of carbon CPMAS experiment was set to 2 ms. A CP proton pulse with linear ramp (from 50% to 100%) was employed. The carbon Hartmann-Hahn match was optimized on external reference sample (glycine). Carbon spectra were recorded with proton decoupling using TPPM15 decoupling sequence with the field strength of approximately 100 kHz.

2. Solid State NMR for Compound II (free form) Form A

[00171] Solid state ^{13}C NMR data for Compound **II** (free form) Form A is provided in **Fig. 2** and summarized in **Table 7** below.

Table 7: Solid State NMR of Compound II (free form) Form A

Peak #	Chem Shift [ppm] ± 0.2 ppm	Intensity [rel]
1	165.9	42.7
2	164.6	16.9

Peak #	Chem Shift [ppm] ± 0.2 ppm	Intensity [rel]
3	163.2	16.6
4	159.8	29.3
5	158.5	14.9
6	157.6	11.6
7	154.1	17.7
8	153.2	22.5
9	151.3	30.6
10	143.8	51.7
11	136.9	53.8
12	130.2	48.5
13	116.4	52.2
14	115.1	20.7
15	113.7	37.4
16	112.9	26.1
17	104.6	27.2
18	103.9	29.5
19	95.7	49.9
20	69.1	67.4
21	63.6	31.9
22	61.8	47.4
23	58.3	25.8
24	49.7	30.8
25	47.2	26.4
26	43.3	28.8
27	39.6	22.8
28	37.0	32.0
29	33.9	38.8
30	31.9	53.4
31	30.5	94.5
32	29.5	35.6
33	26.9	41.6
34	25.6	100.0
35	19.9	72.7
36	19.0	67.8
37	6.6	82.8
38	3.7	78.1

D. Differential Scanning Calorimetry Analysis

[00172] DSC was performed using TA Discovery differential scanning calorimeter (TA Instruments, New Castle, DE). The instrument was calibrated with indium. Samples of approximately 1-10 mg were weighed into hermetic pans that were crimped using lids with one hole. The DSC samples were scanned from 25 ºC to 300 ºC at a heating rate of

10 °C/min. Data was collected and analyzed by Trios Analysis software (TA Instruments, New Castle, DE). The thermogram showed a single melting endothermic peak at ~227 °C.

Example 6: Compound II Calcium Salt Hydrate Form A

[00173] Compound **II** calcium salt hydrate Form A is the most kinetically favored calcium salt hydrate form, providing higher dissolution, solubility, and exposure than the other calcium salt hydrate forms.

[00174] Compound **II** calcium salt hyrate Form A is prepared by charging 0.2 mmol of Compound **II** (free form) Form A and 0.1 mmol of $Ca(OMe)_2$ dry powder with IPA at ~45 mg/mL and spiked with ~10% of water and heated to 70 °C. Initially, all solids dissolved. After 5 min, white solid precipitated out. The resulting slurry was stirred for 4 d at room temperature. The solid was isolated as Compound **II** calcium salt hydrate Form A by vacuum filtration and dried under vacuum at 40 °C for overnight (~ 78% isolated yield).

[00175] An alternative method of preparing Compound **II** calcium salt hydrate Form A utilized 10 g of Compound **I** (free form Form A) charged with 63 mL IPA and 7 mL water. The slurry was heated to 55-65 °C. The mixture was charged with 1.1 equiv of NaOH. The mixture was stirred until the solution turned homogeneous. The solution was then cooled to 25 °C and seeded with 0.1 g of Compound **II** sodium salt hydrate Form A. The slurry was stirred for 18 h. The solution was then heated to 45 °C. The slurry was seeded with 0.1 g of Compound **II** calcium salt hydrate Form A. A solution of 0.55 equiv $CaCl_2$, 9 mL IPA, and 1 mL water were added over a 5 h period of time. The resulting slurry was stirred for 2 h. The slurry was cooled to 20 °C over a 5 h period of time. The resulting solids were collected by vacuum filtration and the resulting wet cake was washed with 50 mL of water. The washed wet cake was allowed to air-dry for 1 h. The air-dried wet cake was transferred to a vacuum oven at 45 °C with a slight nitrogen bleed for 20 h to yield crystalline Compound **II** calcium salt hydrate Form A (8.5 g, 82% isolated yield).

A. X-Ray Powder Diffraction:

[00176] The XRPD patterns were acquired at room temperature in reflection mode using a Bruker Advance equipped with Vantec-1 detector. A sample was analyzed on a silicon sample holder from 3-40° 2-theta on continuous mode with step size of 0.0144531° and time per step of 0.25 s. The sample was spinning at 15 rpm. The XRPD diffractogram for Compound **II** calcium salt hydrate Form A is shown in **Fig. 3** and summarized in **Table 8**.

Table 8: XRPD signals for crystalline Compound II calcium salt hydrate Form A

XRPD Peaks	Angle (degrees 2-Theta ± 0.2)	Intensity %
1	18.0	100.0
2	4.2	81.7
3	19.7	71.2
4	22.3	59.5
5	17.8	57.1
6	24.4	54.9
7	10.5	52.5
8	10.6	51.9
9	14.2	50.9
10	20.7	48.8
11	25.1	48.1
12	19.6	42.1
13	14.8	36.8
14	17.3	35.8
15	25.3	31.2
16	15.3	29.2
17	21.1	28.9
18	12.2	26.6
19	21.9	26.5
20	22.0	24.5
21	13.6	19.1
22	11.8	16.6
23	28.7	15.9
24	25.8	14.6
25	8.3	10.3

B. Single Crystal Elucidation

[00177] Crystals having the Compound **II** calcium salt hydrate Form A structure were grown by dissolving 1 mg of Compound **II** calcium salt hydrate Form A in 350 μL of a 90/10 mixture of dichloroethane/ethanol and then was vapor diffused with pentane over several days. X-ray diffraction data were acquired at both 100K and 298K on a Bruker diffractometer equipped with Cu K_α radiation (λ=1.5478 Å) and a CCD detector. The structure was solved and refined using SHELX programs (Sheldrick, G.M., Acta Cryst., (2008) A64, 112-122) and results are summarized in **Table 9** below.

Table 9: Single crystal elucidation of Compound II calcium salt hydrate Form A

Crystal System:	Monoclinic	Monoclinic
Space Group:	C2	C2
a (Å)	11.1298(4)	11.1871(10)
b (Å)	13.7688(5)	13.8793(12)
c (Å)	22.2139(8)	22.4114(18)
α (°)	90	90
β (°)	101.9330(10)	101.477(4)
γ (°)	90	90
V ($Å^3$)	3330.6(2)	3410.2(5)
Z/Z'	2/0.5	2/0.5
Temperature	100 K	298 K

C. Solid Sate NMR

[00178] Solid state ^{13}C NMR spectrum for Compound **II** calcium salt hydrate Form A is provided in **Fig. 4** and summarized in **Table 10**.

Table 10: Solid state NMR of Compound II calcium salt hydrate Form A

Peak #	Chem Shift [ppm] ± 0.2	Intensity [rel]
1	178.3	27.0
2	165.2	38.8
3	158.2	27.6
4	155.8	32.7
5	153.1	20.5
6	150.9	29.9
7	143.4	42.1
8	136.8	41.3
9	127.9	31.5
10	116.3	27.4
11	114.6	48.2
12	112.1	47.4
13	98.6	27.4
14	93.6	41.2
15	69.5	33.0
16	68.6	17.0
17	63.8	55.6
18	57.7	43.1
19	51.8	50.2
20	42.9	34.2
21	37.2	49.9

Peak #	Chem Shift [ppm] ± 0.2	Intensity [rel]
22	31.2	16.9
23	29.6	48.1
24	26.4	100.0
25	20.8	77.4
26	17.0	65.8
27	7.8	44.7
28	5.3	48.8
29	2.6	18.7

D. Differential Scanning Calorimetry Analysis:

[00179] A DSC thermogram was obtained using TA Instruments DSC Q2000. Sample was heated at 10 °C/min from 30 °C to 350 °C. The thermogram showed an endothermic peak at ~223 °C.

Example 7: Compound II Calcium Salt Hydrate Form D

[00180] Compound **II** calcium salt hydrate Form D is the most stable form of calcium salt hydrate under certain conditions, such as in mixtures of ethanol and water.

[00181] Approximately 25 mg of Compound **II** calcium salt hydrate Form A was charged with 0.5 mL of EtOH:water 67:33 w/w. The slurry was heated to 65 °C for 8 d. The resulting solid collected by vacuum filtration was Compound **II** calcium salt hydrate Form D.

[00182] Alternatively, Compound **II** calcium salt hydrate Form D was prepared from 89 g of Compound **II** sodium hydrate Form A charged with 1080 mL IPA and 120 mL water. The slurry was heated to 55-65 °C. The slurry was charged with 18 g of Compound **II** calcium salt hydrate Form D seed. The slurry was wet-milled as a solution of 0.55 equiv $CaCl_2$, 81 mL IPA and 9 mL water was added over a 5 h period of time. The wet mill was allowed to run until the X-ray powder diffraction confirmed that the slurry was all Compound **I** calcium salt hydrate Form D. The resulting solids were collected by vacuum filtration and wet cake was washed with 350 mL of water. The washed wet cake was allowed to air-dry for 1 h. The air-dried wet cake was transferred to a vacuum oven at 45 °C with a slight nitrogen bleed for 20 h to yield crystalline Compound **II** calcium salt hydrate Form D (83.15 g, 90.6% isolated yield).

[00183] Compound **II** calcium salt hydrate Form D is the most stable polymorphic form in IPA/water at water activity 0.1-0.95 from ambient temperature to 60 ℃.

A. X-Ray Powder Diffraction:

[00184] X-ray powder diffraction (XRPD) spectra were recorded at room temperature in reflection mode using a PANalytical Empyrean system equipped with a sealed tube source and a PIXcel 1D Medipix-2 detector (Malvern PANalytical Inc, Westborough, Massachusetts). The X-Ray generator operated at a voltage of 45 kV and a current of 40 mA with copper radiation (1.54060 Å). The powder sample was placed in a back filled sample holder and loaded into the instrument. The sample was scanned over the range of about 3° to about 40° 2θ with a step size of 0.0131303° and 49.725 s per step. The XRPD diffractogram for Compound **I** calcium salt hydrate Form D is shown in **Fig. 5** and summarized in **Table 11**.

Table 11: XRPD signals for crystalline Compound II calcium salt hydrate Form D

XRPD Peaks	Angle (degrees 2-Theta ± 0.2)	Intensity %
1	16.2	100.0
2	22.8	79.8
3	6.1	79.3
4	19.7	61.5
5	15.5	53.6
6	15.4	53.0
7	22.1	52.9
8	21.5	49.1
9	5.5	47.0
10	23.0	43.3
11	18.1	41.5
12	18.2	38.9
13	15.8	36.7
14	17.5	34.6
15	25.9	34.1
16	25.4	16
17	12.9	17
18	20.2	18
19	19.4	19
20	23.7	20
21	20.7	21
22	16.4	22

XRPD Peaks	Angle (degrees 2-Theta ± 0.2)	Intensity %
23	20.6	23
24	13.8	30.5
25	7.5	28.4
26	19.03	28.2
27	19.0	27.7
28	29.1	27.5
29	24.6	26.3
30	27.6	25.1
31	29.8	33.6
32	8.8	33.5
33	26.5	33.2
34	14.4	32.4
35	11.3	32.1
36	24.1	31.3
37	28.7	31.2
38	27.3	30.6
39	18.6	17.4
40	23.3	16.3
41	15.0	15.7
42	11.0	15.3
43	9.5	13.4
44	6.5	12.6
45	10.3	12.2

B. Single Crystal Elucidation

[00185] Crystals were selected from Compound **II** calcium salt hydrate Form D seeded process in ethanol/water. X-ray diffraction data were acquired at 100K on a Bruker diffractometer equipped with Cu K_a radiation (l = 1.5478), provided by a Rigaku MM007HF rotating anode, and an CMOS detector. The structure was solved and refined using SHELX program (Sheldrick, G.M., Acta Cryst., (2008) A64, 112-122) and results are summarized in **Table 12**.

Table 12: Single crystal elucidation of Compound II calcium salt hydrate Form D

Crystal System	Triclinic
Space Group	P1
a (Å)	12.783(3)
b (Å)	16.639(3)
c (Å)	18.190(4)

α (°)	64.932(12)
β (°)	75.095(14)
γ (°)	68.220(13)
V (Å^3)	3231.3(13)
Z/Z′	1/1
Temperature	100 K

C. Solid Sate NMR:

[00186] Solid state ^{13}C NMR spectrum for Compound **I** calcium salt hydrate Form C is provided in **Fig. 6** and summarized in **Table 13**.

Table 13: Solid state NMR of Compound II calcium salt hydrate Form D

Peak #	Chem Shift [ppm] ± 0.2	Intensity [rel]
1	179.8	22.0
2	176.9	14.0
3	176.3	13.7
4	165.8	34.1
5	164.4	33.6
6	160.9	33.0
7	159.9	32.8
8	158.5	23.1
9	154.8	22.2
10	154.3	24.4
11	153.3	16.2
12	149.5	33.1
13	147.9	20.4
14	143.8	28.0
15	142.5	27.7
16	142.0	29.6
17	140.4	25.0
18	139.5	19.3
19	137.3	20.4
20	136.7	29.3
21	130.2	29.6
22	127	16.4
23	125.6	28.3
24	120.9	11.5
25	118.5	47.2
26	117.5	27.1
27	115.0	7.6
28	113.8	15.0
29	112.0	10.9
30	110.7	42.5

Peak #	Chem Shift [ppm] ± 0.2	Intensity [rel]
31	108.8	10.6
32	100.1	13.7
33	98.6	36.6
34	95.2	23.9
35	94.7	41.8
36	93.2	26.1
37	92.6	22.0
38	70.1	27.2
39	68.3	42.8
40	63.5	46.0
41	62.3	30.6
42	61.4	24.0
43	58.4	4.2
44	56.7	20.9
45	55.2	28.2
46	52.1	22.1
47	51.8	23.1
48	50.3	16.2
49	49.4	30.2
50	44.3	11.2
51	40.4	9.3
52	39.3	38.6
53	35.0	41.6
54	33.4	35.8
55	32.0	41.7
56	29.8	45.0
57	28.4	45.8
58	26.9	43.7
59	24.7	31.2
60	20.1	100.0
61	18.8	62.3
62	18.5	64.2
63	18.2	58.0
64	6.5	60.3
65	5.1	47.4
66	4.7	47.8
67	3.8	54.4
68	3.3	52.2
69	1.6	22.5

D. Differential Scanning Calorimetry Analysis

[00187] DSC was performed using TA Discovery differential scanning calorimeter (TA Instruments, New Castle, DE). The instrument was calibrated with indium. Samples of approximately 1-10 mg were weighed into hermetic pans that were crimped using lids

with one hole. The DSC samples were scanned from 25 °C to 300 °C at a heating rate of 10 °C/min. Data was collected and analyzed by Trios Analysis software (TA Instruments, New Castle, DE). The thermogram showed multiple endothermic peaks at ~182 °C, and ~208 °C.

Example 8: Efficacy data for 250 mg of Compound I

[00188] In a clinical trial, a comparative study was conducted on absolute changes in SwCl at 12 weeks in subjects with gating mutations where were on stable treatment with ivacaftor. Compound **I** was considered generally safe and well tolerated at 150 mg and 250 mg for 12 weeks. The 250 mg dose demonstrated improvement in SwCl at week 12 compared to the ivacaftor baseline whereas the 150 mg dose of Compound 1 demonstrated decrease in SwCl compared to ivacaftor baseline.

Table 14: Analysis of absolute Change in SwCl

	Ivacaftor 150 mg q12h N = 11	**Compound I 150 mg qd N = 23**	**Compound I 250 mg qd N = 24**
Baseline; Mean (SD)	54.6 (23.1)	52.0 (16.6)	55.0 (26.5)
Absolute change at Week 12:			
LS mean (SE)	**0.9 (5.2)**	**3.3 (3.9)**	**-6.5 (3.8)**
95% CI of LS mean	**(-9.5, 11.3)**	**(-4.6, 11.2)**	**(-14.1, 1.2)**
LS mean difference vs Ivacaftor, 95% CI		2.4 (-10.6, 15.5)	-7.3 (-20.2, 5.6)

Example 9: Preparation of Exemplary Tablet Formulation

[00189] The intragranular components in **Table 15** (Compound **I** spray dried dispersion (SDD), Compound **II** calcium salt hydrate Form D, Compound **III** SDD, microcrystalline cellulose, and croscarmellose sodium) were weighed and sieved through a screen and placed in a bin blender. These components were blended and combined to prepare the intragranular powder blend. The intragranular powder blend was dry granulated using a

roller compactor and then milled into granules. The extragranular microcrystalline cellulose was weighed, passed through a screen, and blended with the milled granules in a bin blender. Magnesium stearate was weighed and sieved, then added to the bin blender and blended. The blended components were compressed using a power-assisted rotary tablet press to prepare a table with the required core weight and hardness. The tablets were then placed in a coater to add a nonfunctional coating.

Table 15. Exemplary Tablet Formulation Comprising 125 mg Compound I, 10.6 mg Compound II calcium salt hydrate Form D, and 50 mg Compound III.

	Ingredient	**Amount per tablet (mg)**
Intragranular	Compound **I** SDD (80 wt% Compound **I**, 19.5 wt% hypromellose acetate succinate, and 0.5 wt% sodium lauryl sulfate)	156.3
	Compound **II** calcium salt hydrate Form D	10.6
	Compound **III** SDD (80 wt% Compound **III**, 20 wt% hypromellose)	62.5
	Microcrystalline cellulose	55.1
	Croscarmellose Sodium	22.8
Extragranular	Microcrystalline cellulose	68.9
	Magnesium Stearate	3.8
Film Coat	Opadry 20A100021	11.4
Total		**391.4**

Other Embodiments

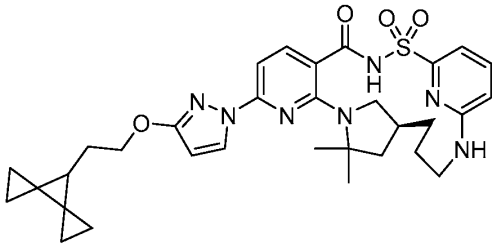
[00190] The foregoing discussion discloses and describes merely exemplary embodiments of this disclosure. One skilled in the art will readily recognize from such discussion and from the accompanying drawings and claims, that various changes, modifications and variations can be made therein without departing from the spirit and scope of this disclosure as defined in the following claims.

CLAIMS

1. A method of treating cystic fibrosis comprising daily administration of:

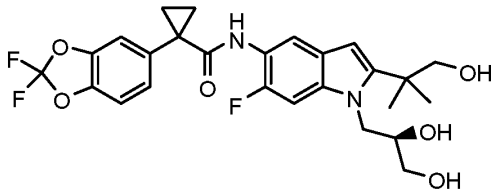
(a) 250 mg of Compound **I** or an equivalent amount of a pharmaceutically acceptable salt therof; and

(b) 21.24 mg Compound **II** calcium salt hydrate Form D



(Compound **II**); and

(c) 100 mg of Compound **III**:



(Compound **III**),

or an equivalent amount of a pharmaceutically acceptable salts thereof.

2. A method of treating cystic fibrosis comprising daily administration of:

(a) 250 mg of Compound **I**.

(b) 21.240 mg of Compound **II** calcium salt hydrate Form D; and

(c) 100 mg of Compound **III**

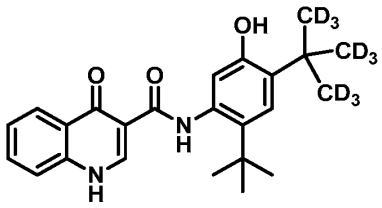
to a patient in need thereof.

3. The method of claim 1 or claim 2, wherein Compounds **I**, **II**, and **III** are administered in separate compositions.

4. The method of claim 1 or claim 2, wherein Compounds **I**, **II**, and **III** are administered in a single composition.

5. The method of claim 1 or claim 2, wherein Compounds **I**, **II**, and **III** are administered as two compositions once daily, each composition comprising 125 mg of Compound **I**, 10.62 mg of Compound **II** calcium salt hydrate Form D, and 50 mg of Compound **III**.

6. A method of treating cystic fibrosis comprising administering 250 mg of Compound **I**:



(Compound **I**),

or an equivalent amount of a pharmaceutically acceptable salt thereof, once per day to a patient in need thereof.

7. The method of any one of claims 1-6, wherein the patient is homozygous for the F508del mutation or has an F508del/minimal function genotype, a F508del/gating genotype, or a F508del/residual function genotype.

8. The method of any one of claims 1-6, wherein the patient has a heterozygous genotype and has one F508del mutation.

9. The method of claim 8, wherein the patient has one mutation selected from Table 3.

10. The method of claim 8, wherein the patient has one mutation selected from Table 4.

11. The method of any one of claims 1-6, wherein the patient has at least one mutation selected from Table 4.

12. A pharmaceutical composition comprising

(a) 250 mg of Compound **I** or an equivalent amount of a pharmaceutically acceptable salt thereof,

(b) 21.24 mg of Compound **II** calcium salt hydrate Form D, and

(c) 100 mg of Compound **III** or an equivalent amount of a pharmaceutically acceptable salt thereof.

13. A pharmaceutical composition comprising

(a) 250 mg of Compound **I**,

(b) 21.24 mg of Compound **II** calcium salt hydrate Form D, and

(c) 100 mg of Compound **III**.

14. A pharmaceutical composition comprising

(a) 125 mg of Compound **I** or an equivalent amount of a pharmaceutically acceptable salt thereof,

(b) 10.62 mg of Compound **II** calcium salt hydrate Form D, and

(c) 50 mg of Compound **III** or an equivalent amount of a pharmaceutically acceptable salt thereof.

15. A pharmaceutical composition comprising

(a) 125 mg of Compound **I**,

(b) 10.62 mg of Compound **II** calcium salt hydrate Form D, and

(c) 50 mg of Compound **III**.

16. A tablet comprising:

(a) 156.3 mg of a solid dispersion comprising Compound **I**, wherein the solid dispersion comprises:

(i) 80 wt% Compound **I**, by weight of the solid dispersion,

(ii) 19.5 wt% hypromellose acetate succinate, by weight of the solid dispersion, and

(iii) 0.5 wt% sodium lauryl sulfate, by weight of the solid dispersion;

(b) 10.6 mg of Compound **II** calcium salt hydrate Form D;

(c) 62.5 mg of a solid dispersion comprising Compound **III**. wherein the solid dispersion comprises:

(i) 80 wt% Compound **III**, by weight of the solid dispersion, and

(ii) 20 wt% hypromellose, by weight of the solid dispersion;

(d) 124.0 mg of microcrystalline cellulose;

(e) 22.8 mg of croscarmellose sodium; and

(f) 3.8 mg of magnesium stearate.

17. A tablet comprising:

(a) 156.3 mg of a solid dispersion comprising Compound **I**, wherein the solid dispersion comprises:

(i) 80 wt% Compound **I**, by weight of the solid dispersion,

(ii) 19.5 wt% hypromellose acetate succinate, by weight of the solid dispersion, and

(iii) 0.5 wt% sodium lauryl sulfate, by weight of the solid dispersion;

(b) 10.6 mg of Compound **II** calcium salt hydrate Form D;

(c) 62.5 mg of a solid dispersion comprising Compound **III**. wherein the solid dispersion comprises:

(i) 80 wt% Compound **III**, by weight of the solid dispersion, and

(ii) 20 wt% hypromellose, by weight of the solid dispersion;

(d) 124.0 mg of microcrystalline cellulose;

(e) 22.8 mg of croscarmellose sodium;

(f) 3.8 mg of magnesium stearate; and

(g) 11.4 mg of a film coat.

18. A tablet comprising:

(a) 156.3 mg of a solid dispersion comprising Compound **I**, wherein the solid dispersion comprises:

(i) 80 wt% Compound **I**, by weight of the solid dispersion,

(ii) 19.5 wt% hypromellose acetate succinate, by weight of the solid dispersion, and

(iii) 0.5 wt% sodium lauryl sulfate, by weight of the solid dispersion;

(b) 10.6 mg of Compound **II** calcium salt hydrate Form D; and

(c) 62.5 mg of a solid dispersion comprising Compound **III**. wherein the solid dispersion comprises:

(i) 80 wt% Compound **III**, by weight of the solid dispersion, and

(ii) 20 wt% hypromellose, by weight of the solid dispersion.

19. The tablet of claim 18, wherein the tablet futher comprises 70-170 mg of microcrystalline cellulose.

20. The tabet of claim 18, wherein the tablet further comprises 10-40 mg of croscarmellose sodium.

21. The tablet of claim 18, wherein the tablet futher comprises 70-170 mg of microcrystalline cellulose and 10-40 mg of croscarmellose sodium.

22. A tablet comprising:

(a) 156.3 mg of a solid dispersion comprising Compound **I**, wherein the solid dispersion comprises:

(i) 80 wt% Compound **I**, by weight of the solid dispersion,

(ii) 19.5 wt% hypromellose acetate succinate, by weight of the solid dispersion, and

(iii) 0.5 wt% sodium lauryl sulfate, by weight of the solid dispersion;

(b) 10.6 mg of Compound **II** calcium salt hydrate Form D;

(c) 62.5 mg of a solid dispersion comprising Compound **III**. wherein the solid dispersion comprises:

(i) 80 wt% Compound **III**, by weight of the solid dispersion, and

(ii) 20 wt% hypromellose, by weight of the solid dispersion;

(d) 125.0 mg of microcrystalline cellulose;

(e) 22.8 mg of croscarmellose sodium; and

(f) 2.9 mg of magnesium stearate.

23. A tablet comprising:

(a) 156.3 mg of a solid dispersion comprising Compound **I**, wherein the solid dispersion comprises:

(i) 80 wt% Compound **I**, by weight of the solid dispersion,

(ii) 19.5 wt% hypromellose acetate succinate, by weight of the solid dispersion, and

(iii) 0.5 wt% sodium lauryl sulfate, by weight of the solid dispersion;

(b) 10.6 mg of Compound **II** calcium salt hydrate Form D;

(c) 62.5 mg of a solid dispersion comprising Compound **III**. wherein the solid dispersion comprises:

(i) 80 wt% Compound **III**, by weight of the solid dispersion, and

(ii) 20 wt% hypromellose, by weight of the solid dispersion;

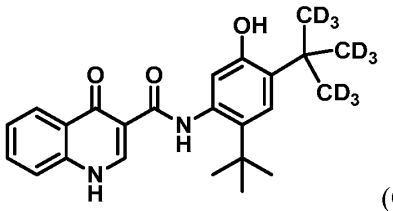
(d) 124.5 mg of microcrystalline cellulose;

(e) 22.8 mg of croscarmellose sodium;

(f) 3.8 mg of magnesium stearate; and optionally

(g) 15.9 mg of a film coat.

24. A pharmaceutical composition comprising 250 mg of Compound **I**:



(Compound **I**),

or an equivalent amount of a pharmaceutically acceptable salt thereof.