METHODS OF TREATING CFTR-MEDIATED DISEASES OR DISORDERS

Background

[1] Cystic fibrosis (CF) results from CF transmembrane conductance regulator (CFTR) mutations, the most prevalent being ΔF508-CFTR. Approved CFTR modulators increase its function, providing eligible patients with clinical benefits. Despite advances, current modulators do not provide most people with CF with normal levels of CFTR function, indicated by the fact that, in most eligible patient groups, mean sweat chloride levels do not reach the normal range. ΔF508-CFTR results in loss of phenylalanine F508 within CFTR's first nucleotide binding domain (NBD1). ΔF508 causes NBD1 destabilization: a key driver of the impaired folding, trafficking, half-life, and function of ΔF508-CFTR. F508 also participates in the interface of NBD1 and the fourth intracellular loop (ICL4) of CFTR's second transmembrane domain (TMD). ΔF508 weakens this interface, adding to ΔF508-CFTR's molecular pathology. There remains a need to find methods of treating CFTR-mediated diseases or disorders.

Summary

[2] The present disclosure includes methods of treating CFTR-mediated diseases or disorders comprising administering an NBD1 stabilizer in combination with one or more additional therapeutic agents, including, but not limited to, a TMD1 corrector and an ICL4 corrector. As shown herein, and without being bound by any particular theory, full ΔF508-CFTR correction may require NBD1 stabilization, and without NBD1 stabilization, correction is significantly less. Compounds and methods described herein stabilize NBD1 and thus may improve patient health.

Brief Description of the Drawings

[3] FIGs. 1, 2, 3, 4, 5 and 6 depict western blot diagrams showing impact of Compounds 1–7 on ΔF508-CFTR maturation. Western blot of CFTR expressing CFSMEo- cells. Biological replicates comparing both ΔF508-CFTR and WT-CFTR expressing cells, treated as indicated, are shown. The combination of Compounds 1, 4, 5, 6 or 7 with Compound 2 and/or Compound 3 improves ΔF508-CFTR maturation to levels exceeding WT, further demonstrating the synergy between NBD1 stabilizers and modulators that address ΔF508-CFTR domain-domain assembly defects.

[4] FIGs. 7, 8, 9, 10 and 11 depict bar graphs illustrating the impact of Compounds 1–7 on ΔF508-CFTR maturation. CFTR activity of ΔF508-CFTR homozygous CFHBEs treated

for 48 hours with Compounds 1, 4, 5, 6, or 7 alone and/or in combination with Compound 2 and/or Compound 3, compared with that of TEZ/IVA/ELX at its E_{max}. CFTR-dependent chloride transport (vehicle-subtracted FSK peak) is expressed as a relative percentage of the average forskolin-stimulated CFTR current (FSK response) across a panel of 8 non-CF HBE donors (green horizontal bar). Each vertical bar represents the mean +/- standard error of 9 CFHBE donors with 6-8 replicates per donor. CFTR activity achieved the levels of non-CF HBE cells when Compounds 1, 4, 5, 6 or 7 are combined with ICL4- and/or TMD1-directed correctors, or the combination thereof.

Detailed Description

Definitions

[5] As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge et al., describe pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 1977, 66, 1-19, incorporated herein by reference. Pharmaceutically acceptable salts of the compounds of this disclosure include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other 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, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like.

[6] Salts derived from appropriate bases include alkali metal, alkaline earth metal,

ammonium and $N(C_{1-4}alkyl)_4$ salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate and aryl sulfonate.

[7] As used herein, a "therapeutically effective amount" means an amount of a substance (e.g., a therapeutic agent, composition, and/or formulation) that elicits a desired biological response. In some embodiments, a therapeutically effective amount of a substance is an amount that is sufficient, when administered as part of a dosing regimen to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, diagnose, prevent, and/or delay the onset of the disease, disorder, and/or condition. As will be appreciated by those of ordinary skill in this art, the effective amount of a substance may vary depending on such factors as the desired biological endpoint, the substance to be delivered, the target cell or tissue, etc. For example, the effective amount of a provided compound in a formulation to treat a disease, disorder, and/or condition is the amount that alleviates, ameliorates, relieves, inhibits, prevents, delays onset of, reduces severity of and/or reduces incidence of one or more symptoms or features of the disease, disorder, and/or condition. I

[8] As used herein, the terms "treatment," "treat," and "treating" refer to partially or completely alleviating, inhibiting, delaying onset of, preventing, ameliorating and/or relieving a disorder or condition, or one or more symptoms of the disorder or condition, as described herein. In some embodiments, treatment may be administered after one or more symptoms have developed. In some embodiments, the term "treating" includes preventing or halting the progression of a disease or disorder. In other embodiments, treatment may be administered in the absence of symptoms. For example, treatment may be administered to a susceptible individual prior to the onset of symptoms (e.g., in light of a history of symptoms and/or in light of genetic or other susceptibility factors). Treatment may also be continued after symptoms have resolved, for example to prevent or delay their recurrence. Thus, in some embodiments, the term "treating" includes preventing relapse or recurrence of a disease or disorder.

[9] The term “subject”, as used herein, means an animal, preferably a mammal, and most preferably a human.

[10] The term “pharmaceutically acceptable excipient” refers to a non-toxic carrier, adjuvant, or vehicle that does not destroy the pharmacological activity of the compound(s) with which it is formulated. Pharmaceutically acceptable carriers, adjuvants or vehicles that

may be used in the compositions of the compounds disclosed herein 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, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

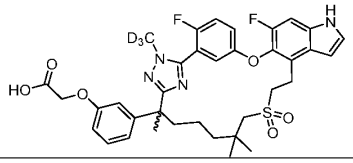
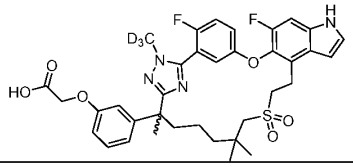
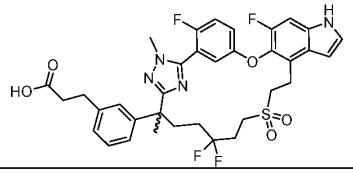
[11] A "pharmaceutically acceptable derivative" means any non-toxic salt, ester, salt of an ester or other derivative of a compound of this disclosure that, upon administration to a recipient, is capable of providing, either directly or indirectly, a compound of this disclosure or an inhibitorily active metabolite or residue thereof.

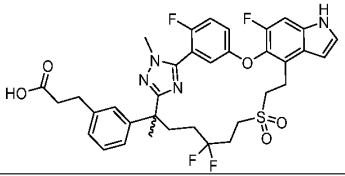
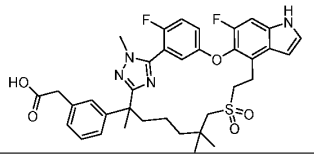
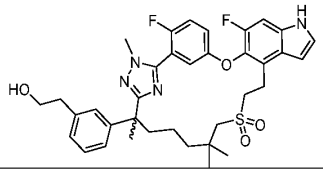
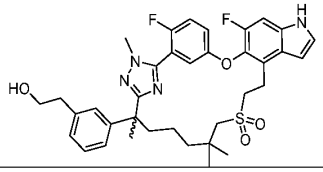
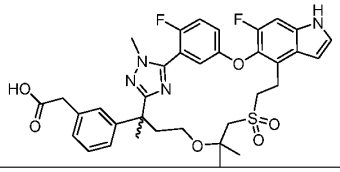
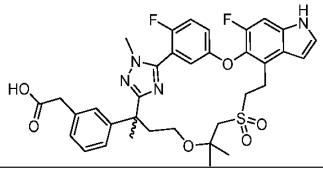
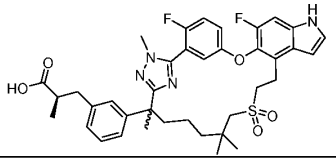
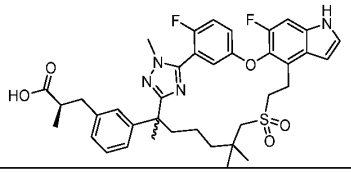
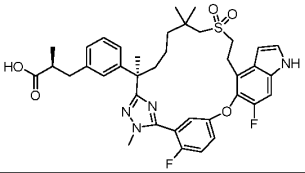
Compounds of the Present Disclosure

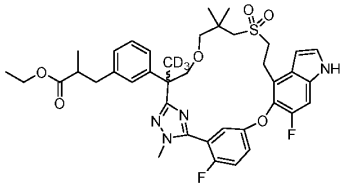
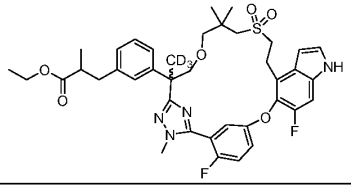
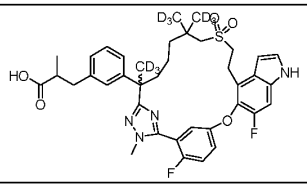
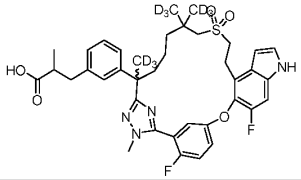
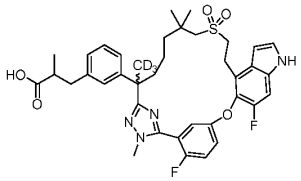
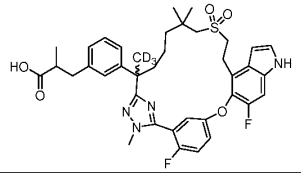
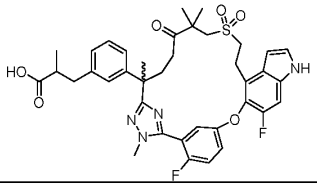
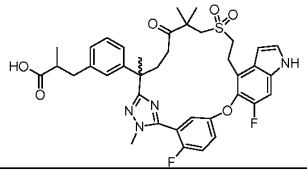
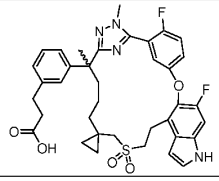
[12] In some embodiments, a compound of the present disclosure is an NBD1 stabilizer.

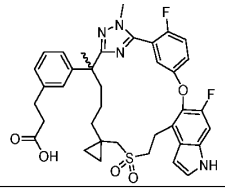
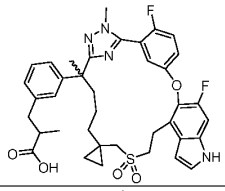
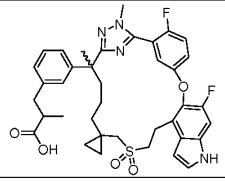
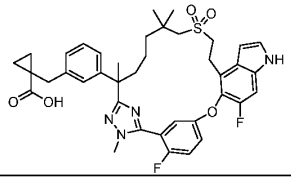
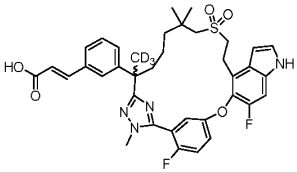
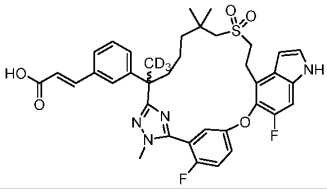
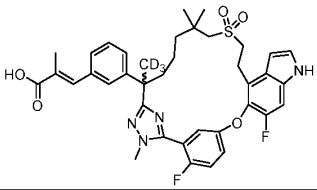
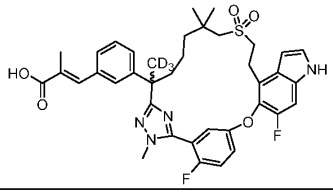
[13] In some embodiments, an NBD1 stabilizer is a compound selected from those in table 1 or a pharmaceutically acceptable salt thereof.

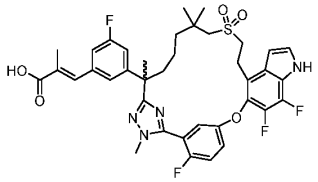
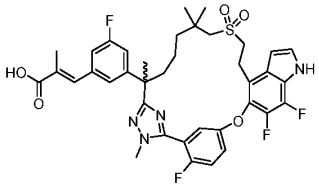
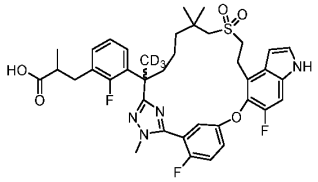
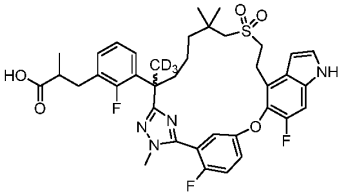
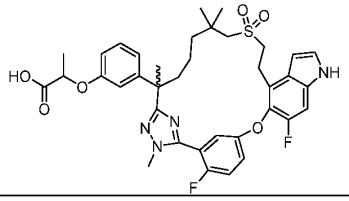
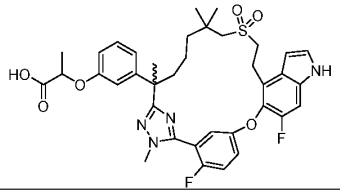
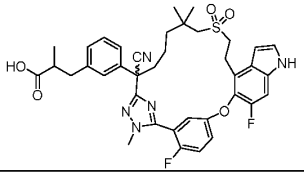
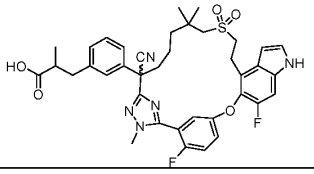
Table 1

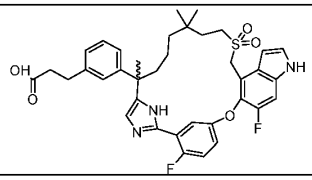
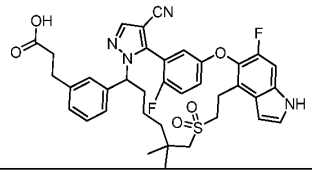
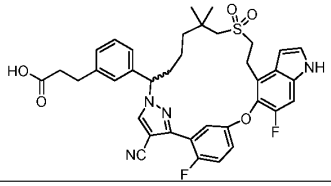
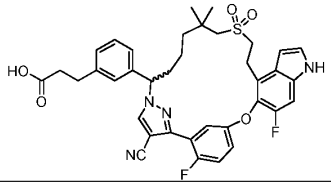
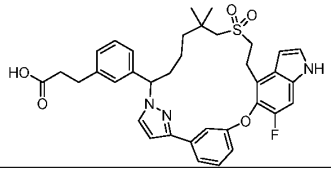
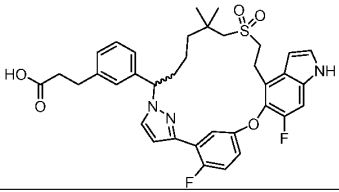
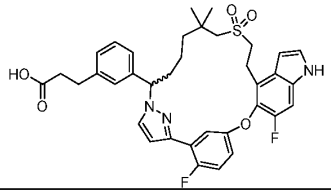
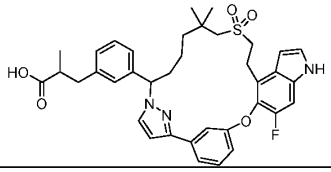
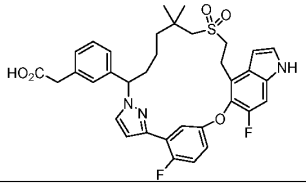
Compound No.	Structures
1A	
1B	
2A	

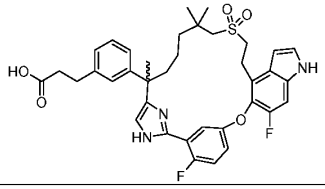
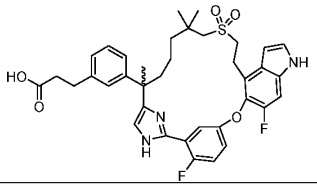
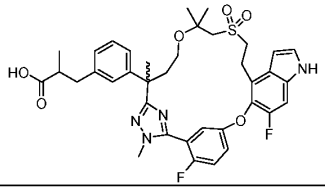
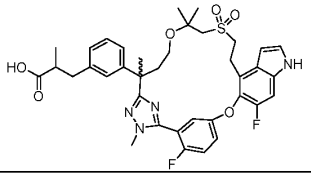
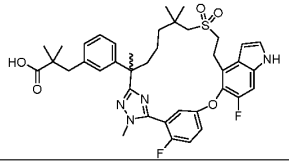
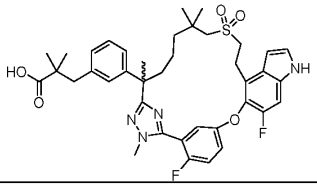
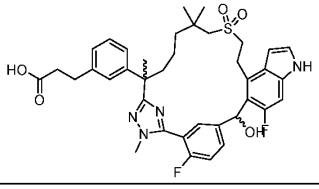
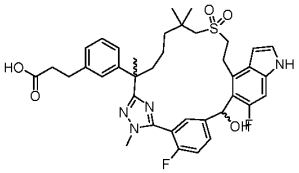
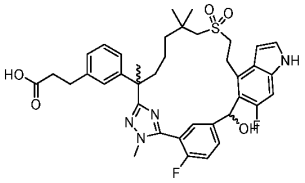
2B	
3A	
4A	
4B	
5A	
5B	
6A	
6B	
7	

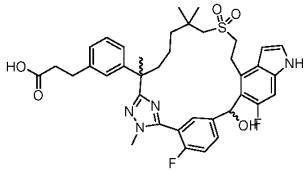
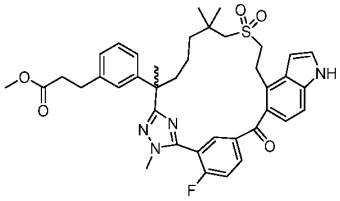
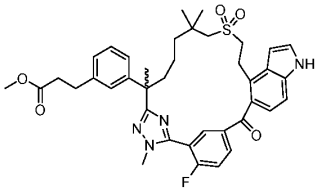
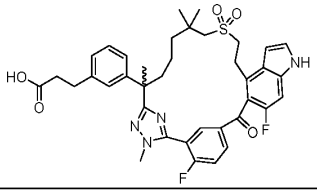
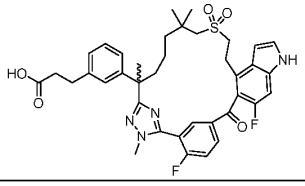
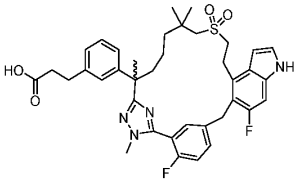
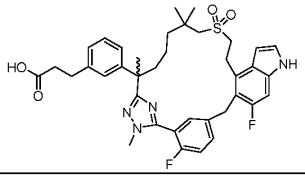
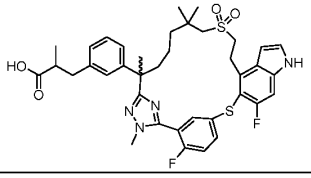
8A	
8B	
9A	
9B	
10A	
10B	
11A	
11B	
12A	

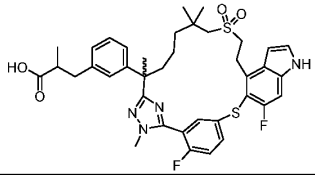
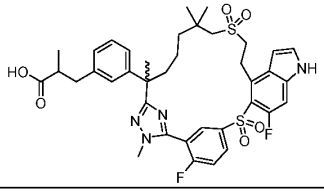
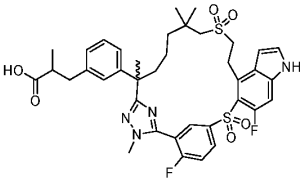
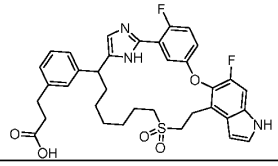
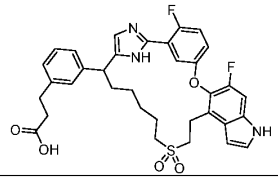
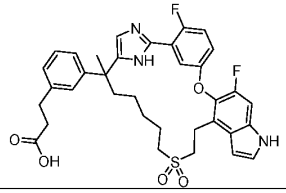
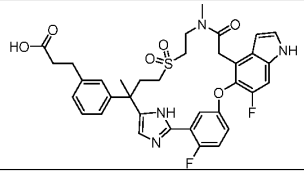
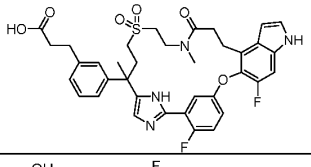
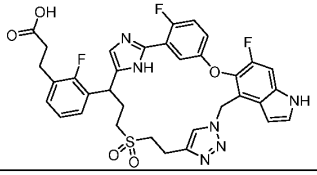
12B	
13A	
13B	
14	
15A	
15B	
16A	
16B	

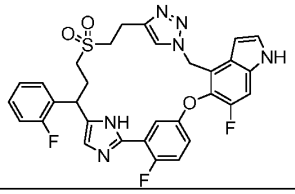
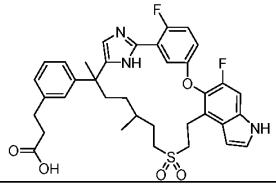
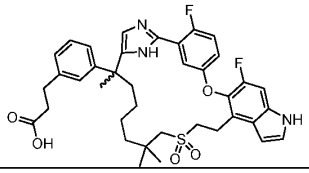
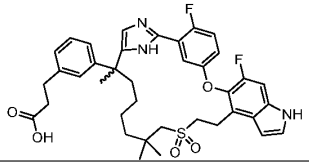
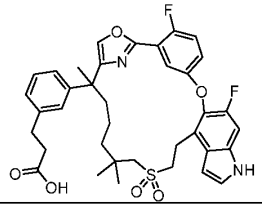
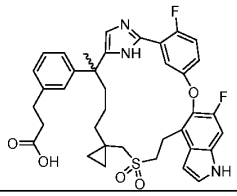
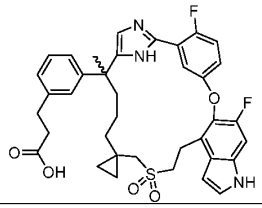
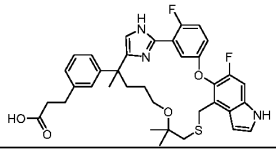
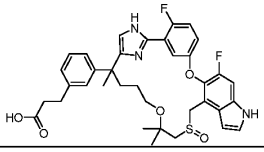
17A	
17B	
18A	
18B	
19A	
19B	
20A	
20B	

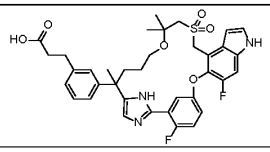
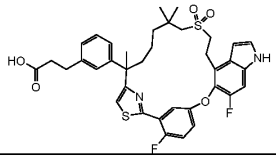
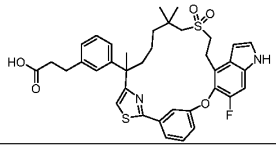
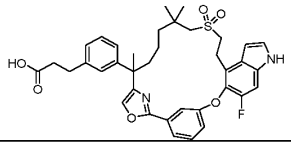
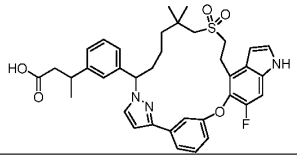
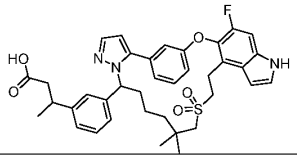
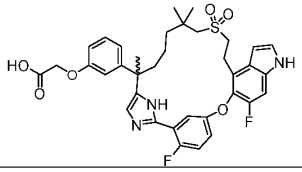
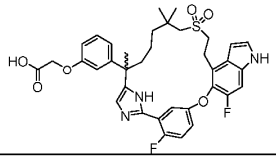
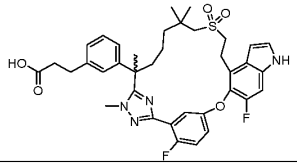
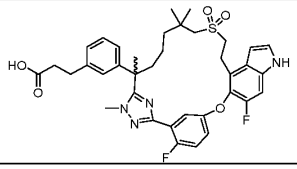
21	
22A	
22B	
22C	
23	
24A	
24B	
25	
26	

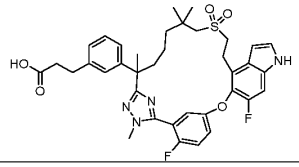
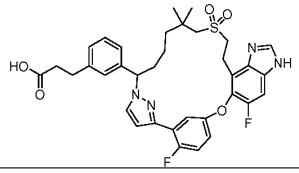
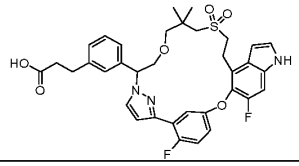
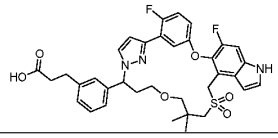
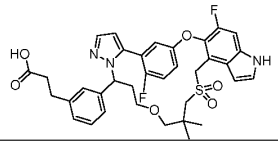
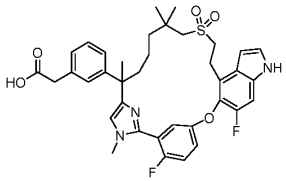
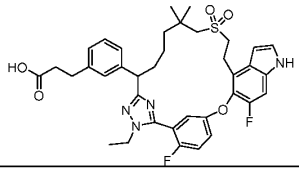
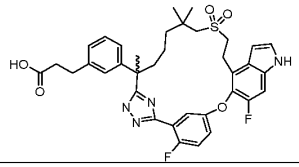
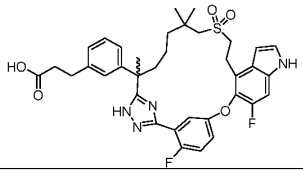
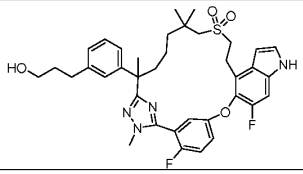
27A	
27B	
28A	
28B	
29A	
29B	
30A	
30B	
30C	

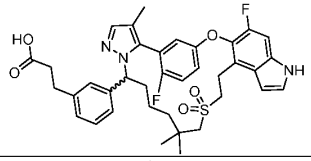
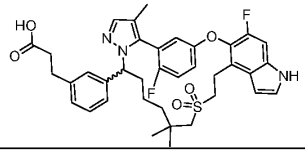
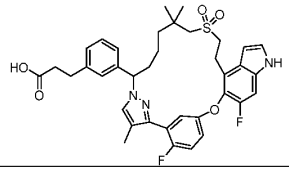
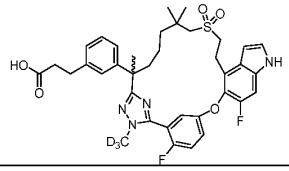
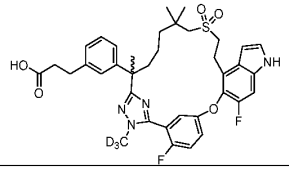
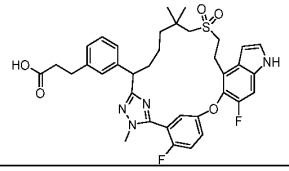
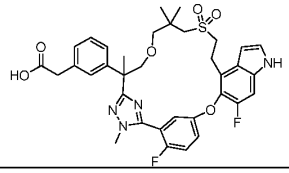
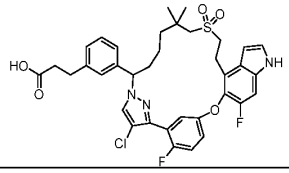
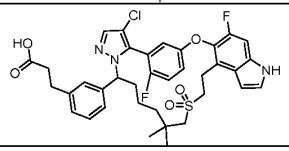
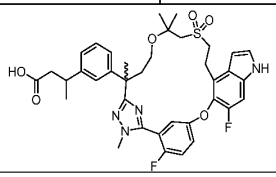
30D	
31A	
31B	
31C	
31D	
32A	
32B	
33A	

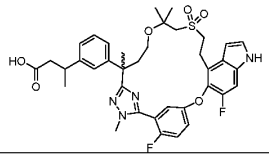
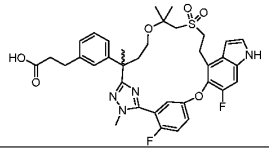
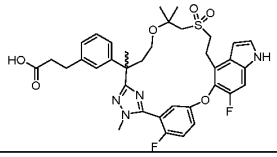
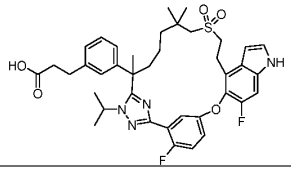
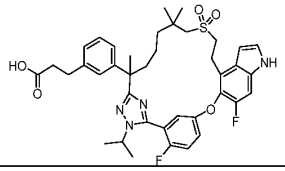
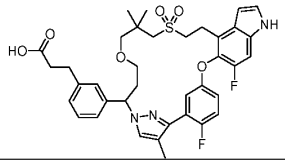
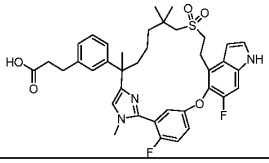
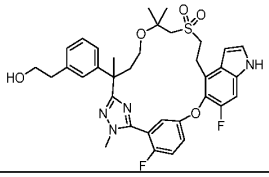
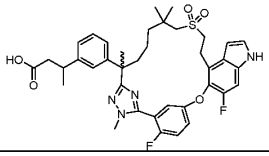
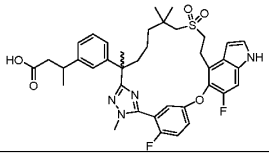
33B	
34A	
34B	
35	
36	
37	
38	
39	
40	

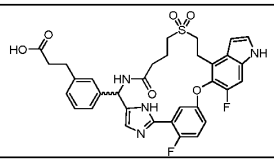
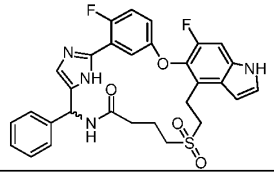
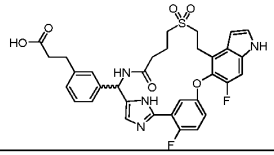
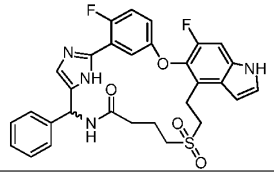
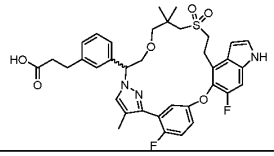
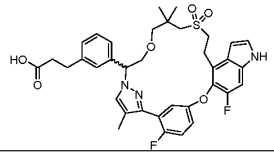
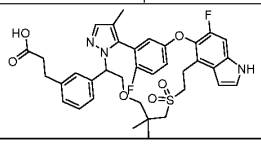
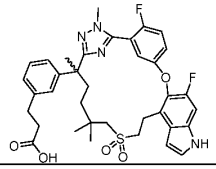
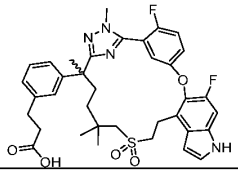
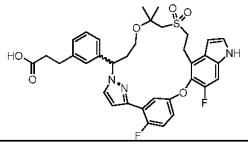
41	
42	
43A	
43B	
44	
45A	
45B	
46	
47	

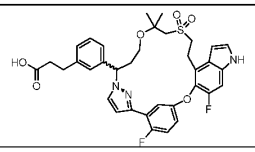
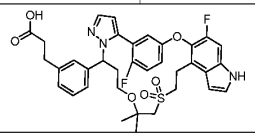
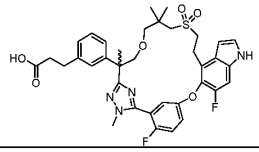
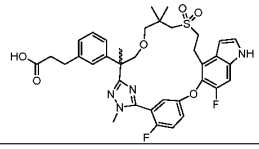
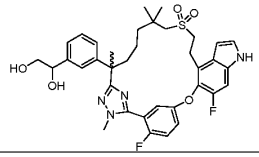
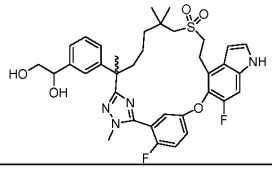
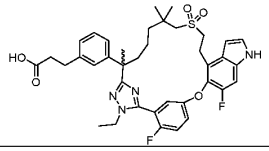
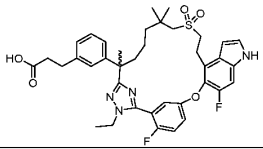
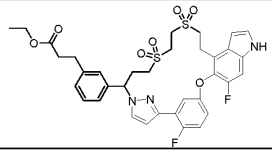
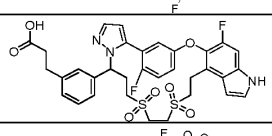
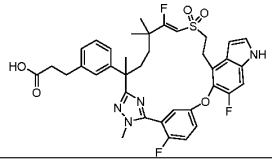
48	
49	
50	
51	
52	
53	
54A	
54B	
55A	
55B	

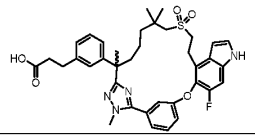
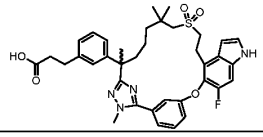
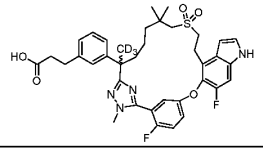
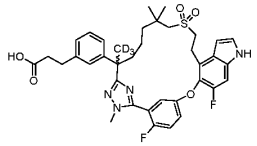
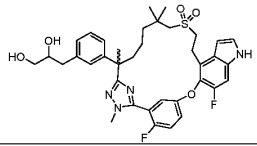
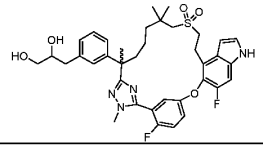
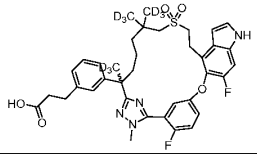
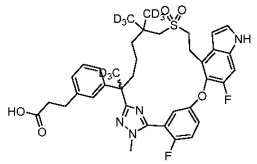
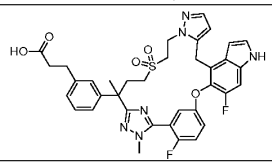
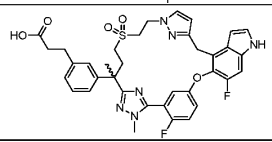
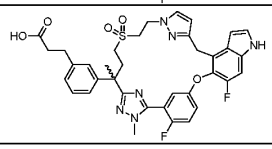
56	
57	
58	
59	
60	
61	
62	
63A	
63B	
64	

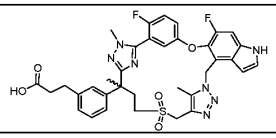
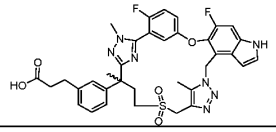
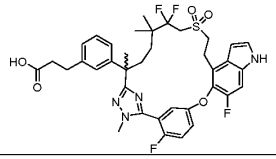
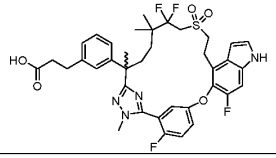
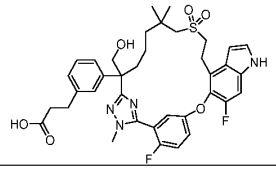
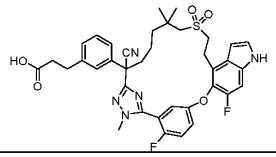
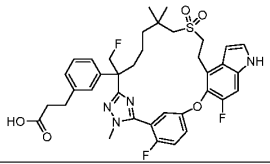
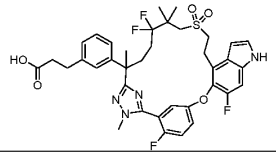
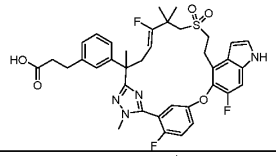
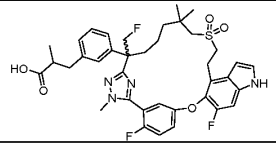
65A	
65B	
66	
67A	
67B	
68	
69	
70	
71	
72A	

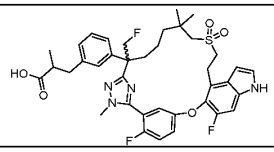
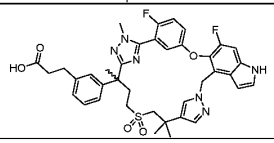
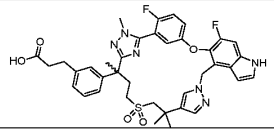
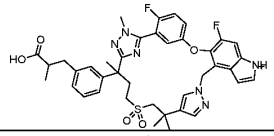
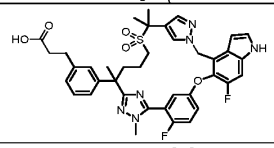
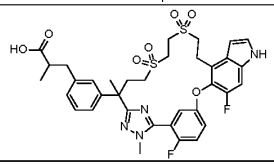
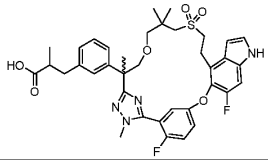
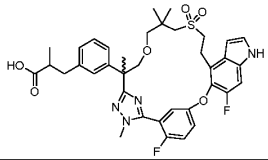
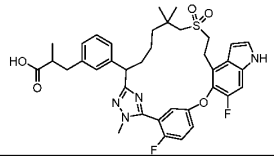
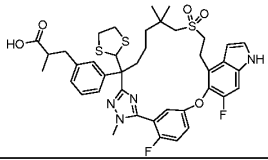
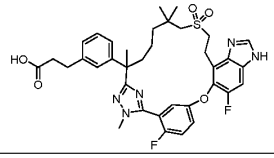
72B	
73A	
73B	
74	
75	
76	
77	
78	
79A	
79B	

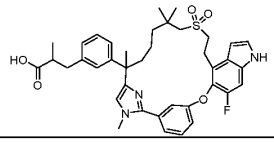
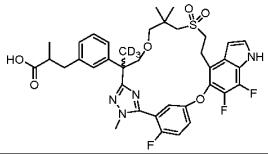
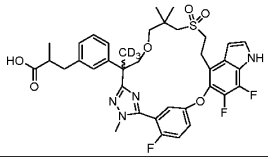
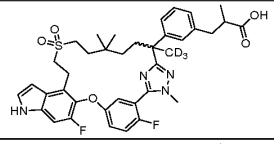
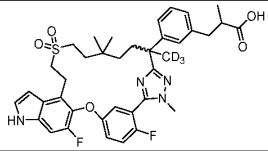
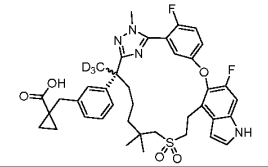
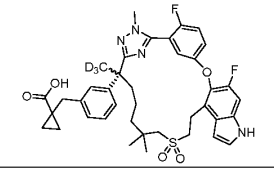
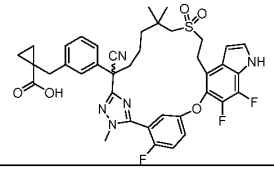
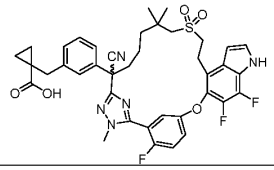
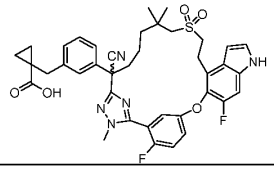
80	
81	
82	
83	
84A	
84B	
85	
86A	
86B	
87A	

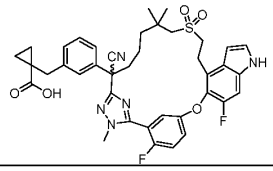
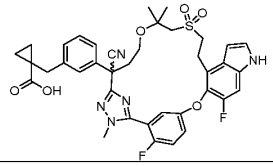
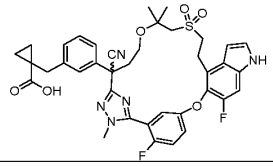
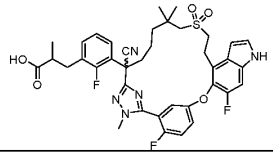
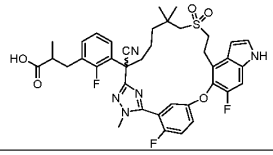
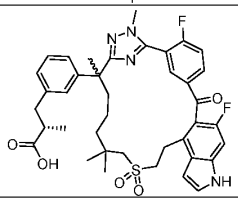
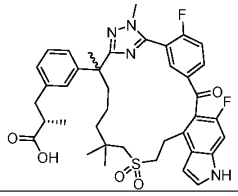
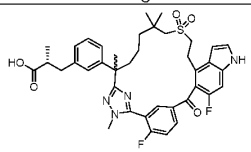
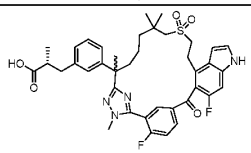
87B	
88	
89A	
89B	
90A	
90B	
91A	
91B	
92	
93	
94	

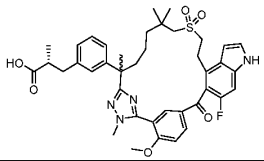
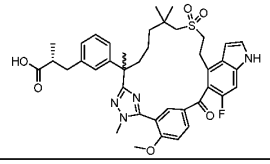
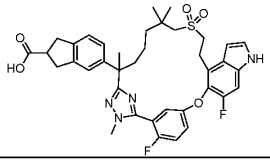
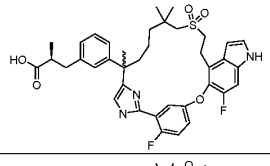
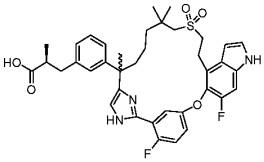
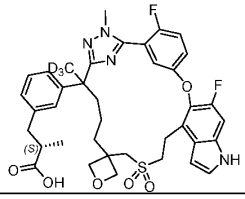
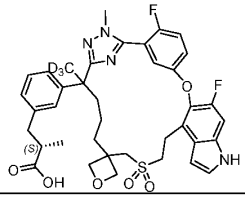
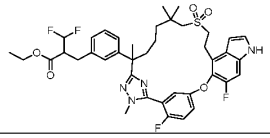
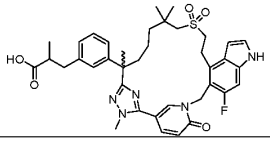
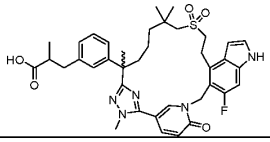
95A	
95B	
96A	
96B	
97A	
97B	
98A	
98B	
99	
100A	
100B	

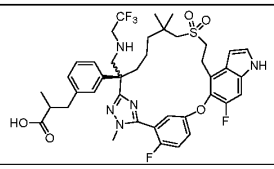
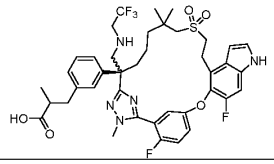
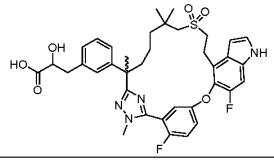
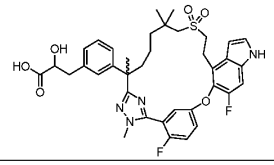
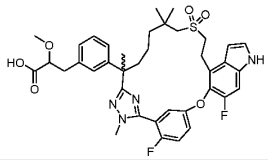
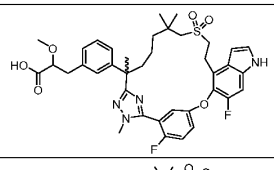
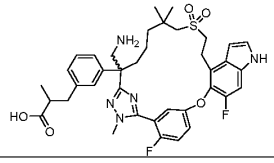
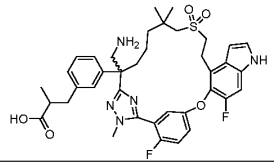
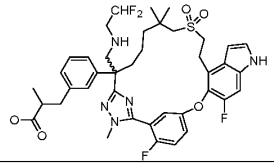
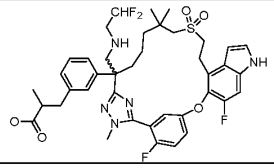
101A	
101B	
102A	
102B	
103	
104	
105	
106	
107	
108A	

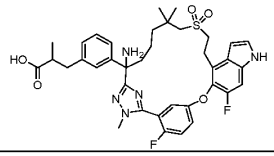
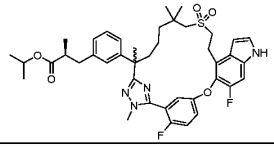
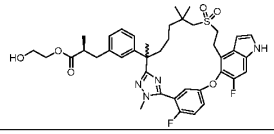
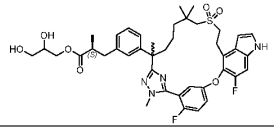
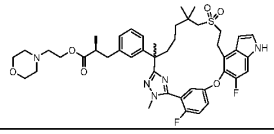
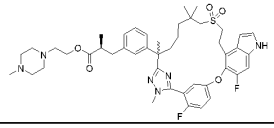
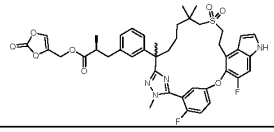
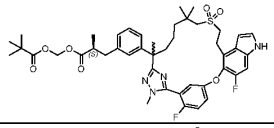
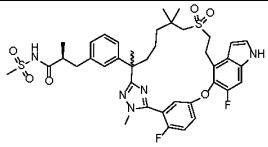
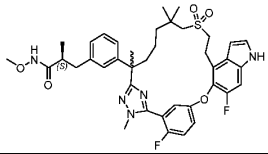
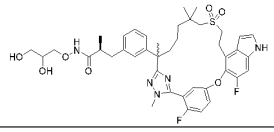
108B	
109A	
109B	
110	
111	
112	
113A	
113B	
114	
115	
116	

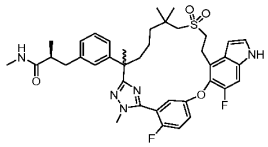
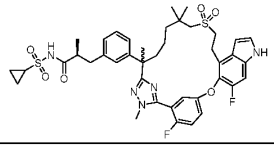
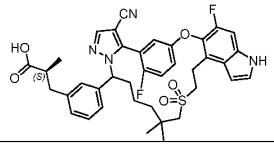
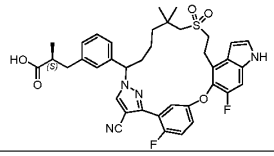
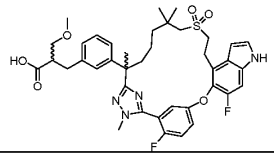
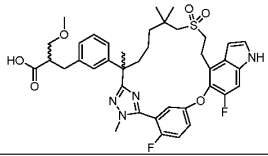
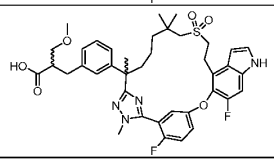
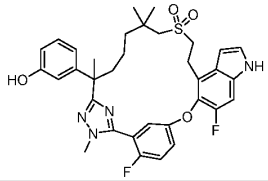
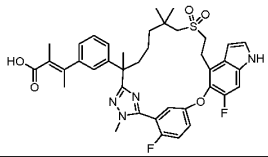
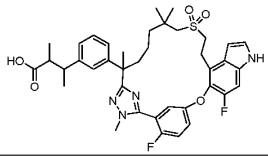
117	
118A	
118B	
119A	
119B	
120A	
120B	
121A	
121B	
122A	

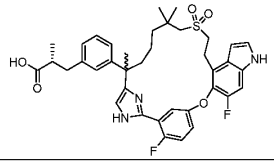
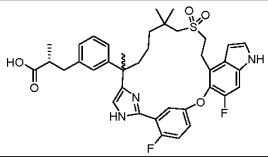
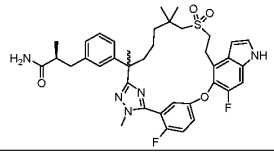
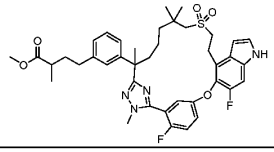
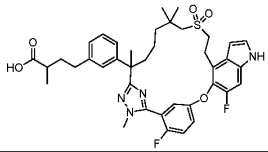
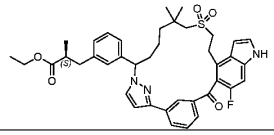
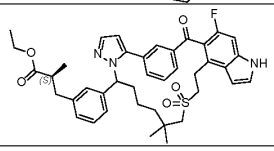
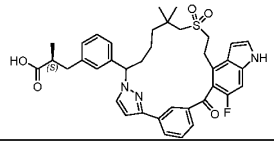
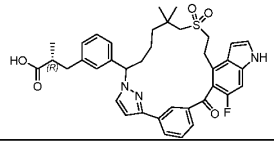
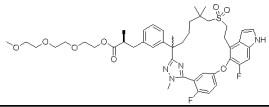
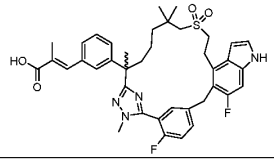
122B	
123A	
123B	
124A	
124B	
125A	
125B	
126A	
126B	

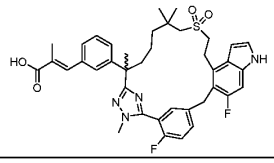
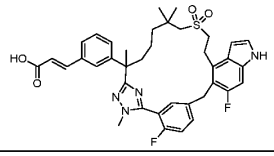
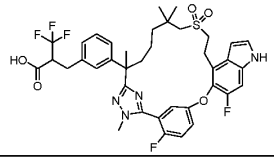
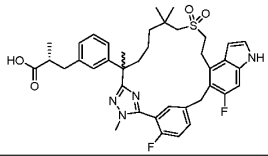
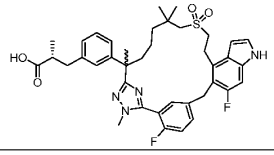
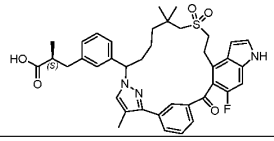
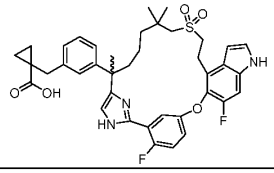
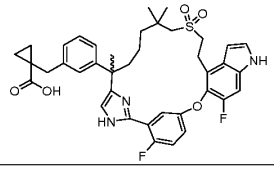
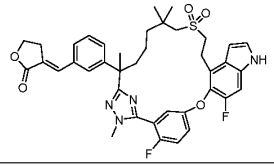
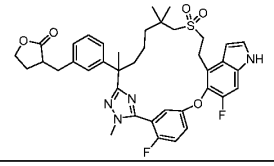
127A	
127B	
128	
129A	
129B	
130A	
130B	
131	
132A	
132B	

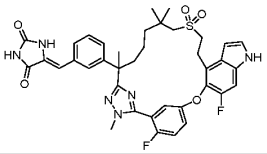
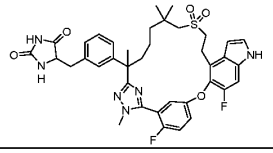
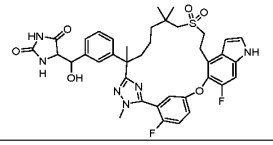
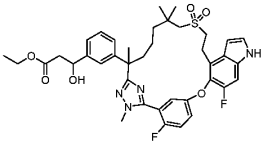
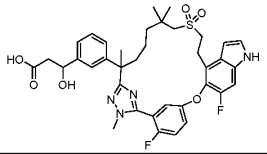
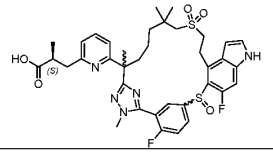
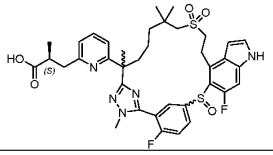
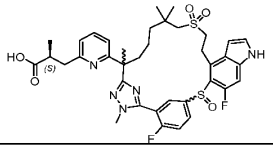
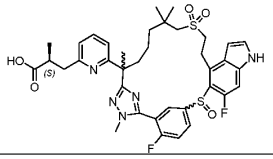
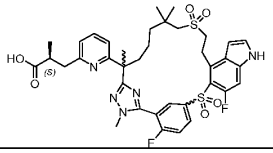
133A	
133B	
134A	
134B	
135A	
135B	
136A	
136B	
137A	
137B	

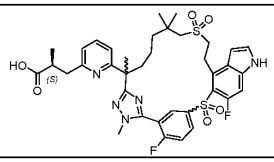
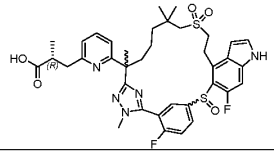
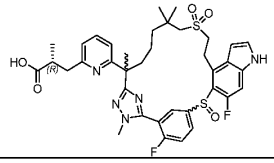
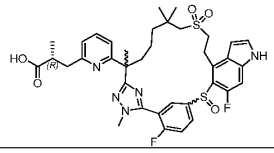
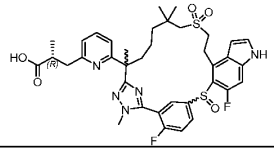
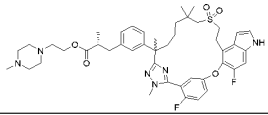
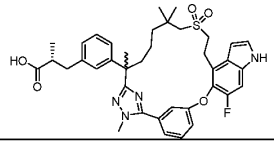
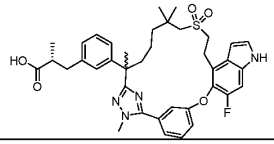
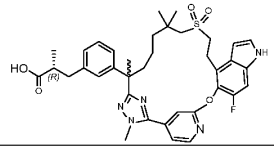
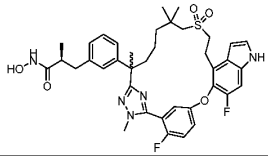
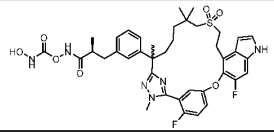
138	
139	
140	
141	
142	
143	
144	
145	
146	
147	
148	

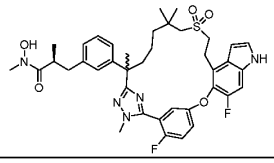
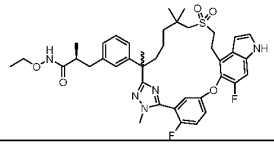
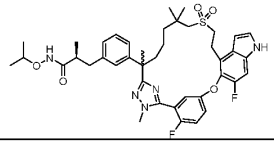
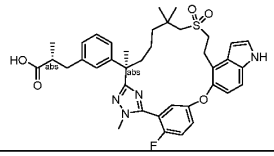
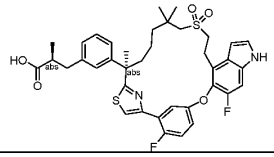
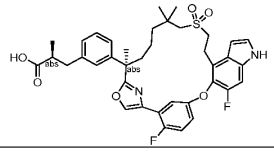
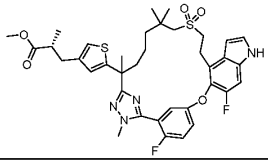
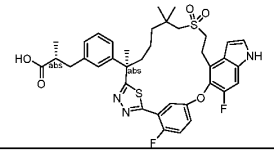
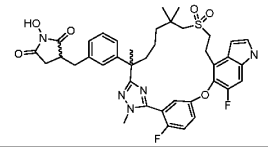
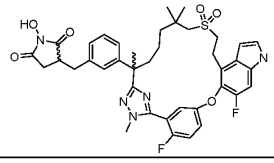
149	
150	
151	
152	
153A	
153B	
153C	
154	
155	
156	

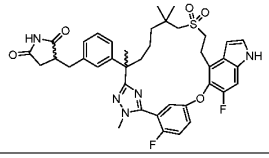
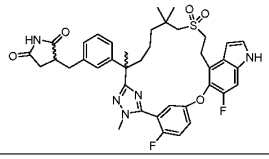
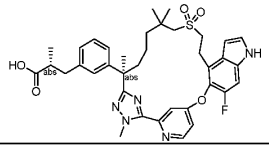
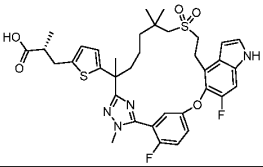
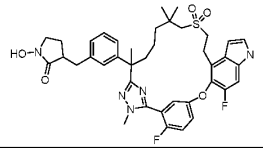
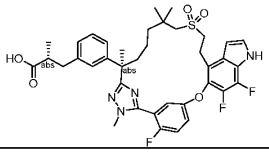
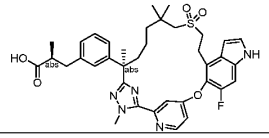
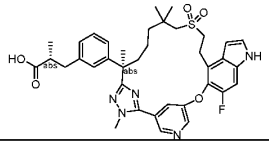
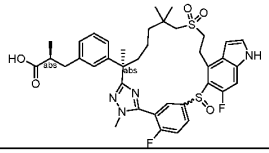
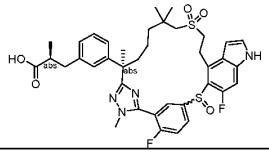
157A	
157B	
158	
159	
160	
161	
162	
163	
164	
165	
166A	

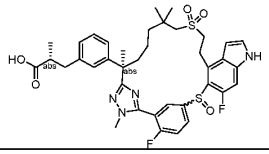
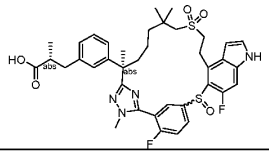
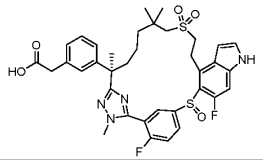
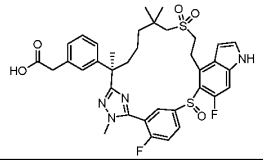
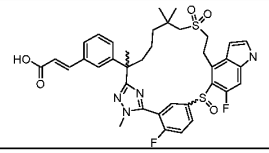
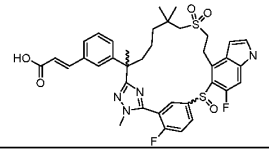
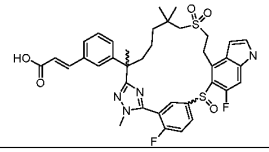
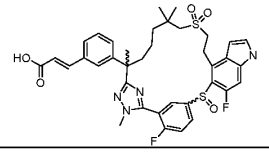
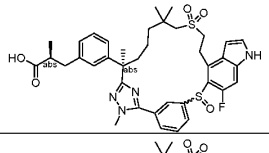
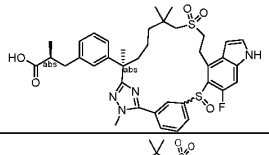
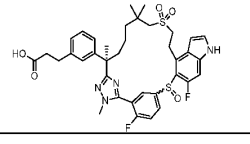
166B	
167	
168	
169A	
169B	
170	
171A	
171B	
172	
173	

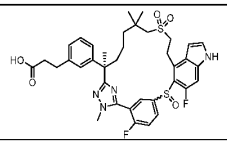
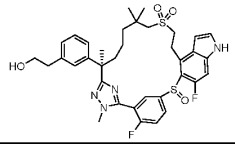
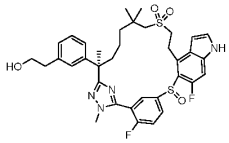
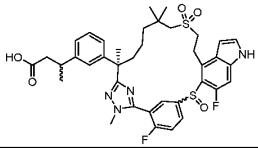
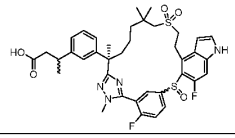
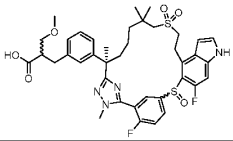
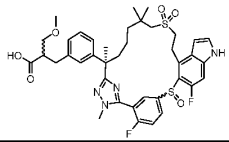
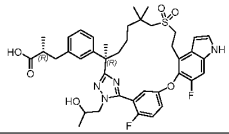
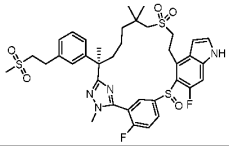
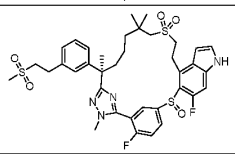
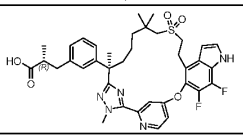
174	
175	
176	
177	
178	
179A	
179B	
179C	
179D	
180A	

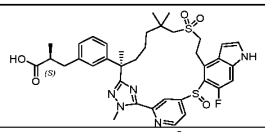
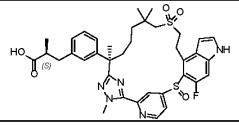
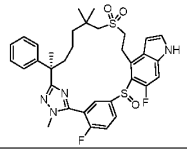
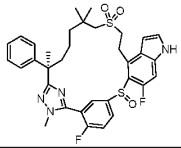
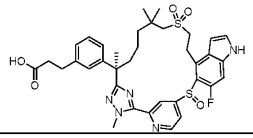
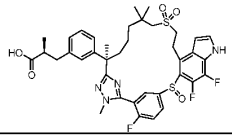
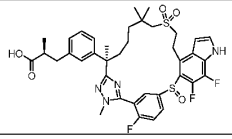
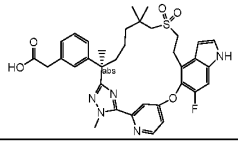
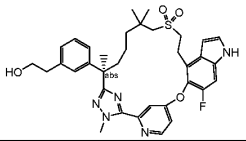
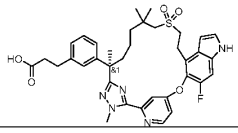
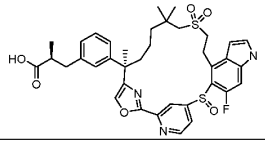
180B	
181A	
181B	
181C	
181D	
182	
183A	
183B	
184	
185	
186	

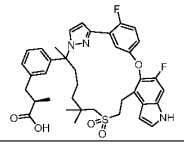
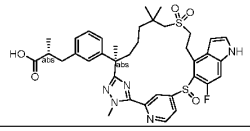
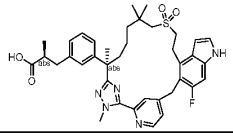
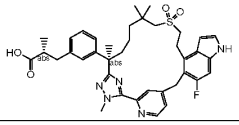
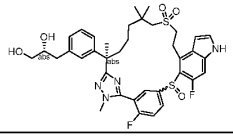
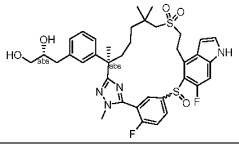
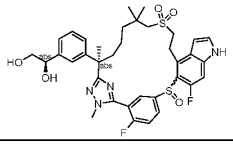
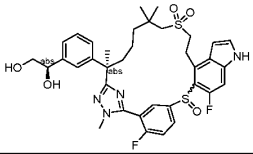
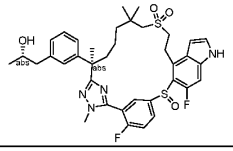
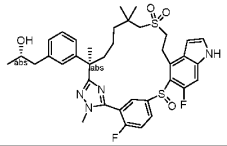
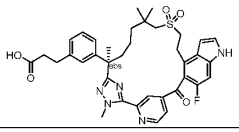
187	
188	
189	
190	
191	
192	
193	
194	
195A	
195B	

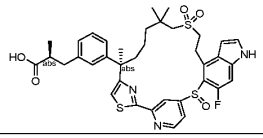
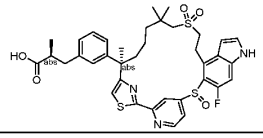
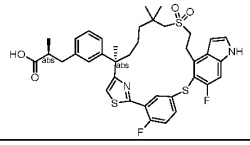
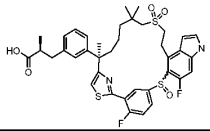
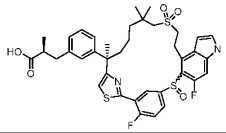
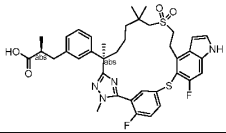
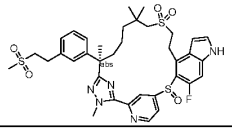
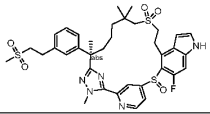
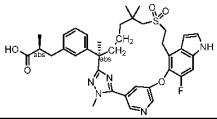
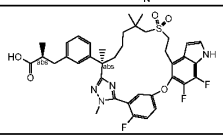
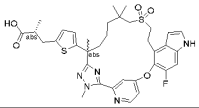
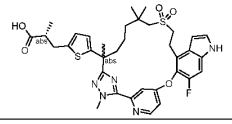
196A	
196B	
197	
198	
199	
200	
201	
202	
204	
205	

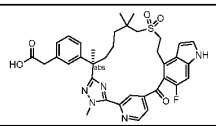
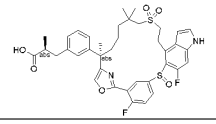
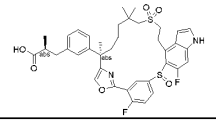
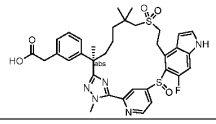
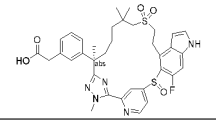
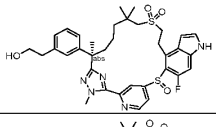
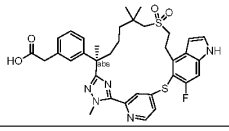
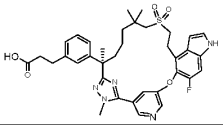
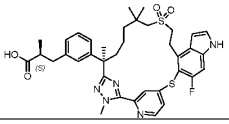
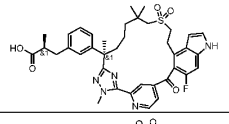
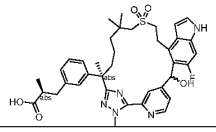
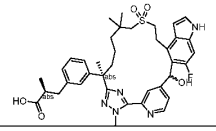
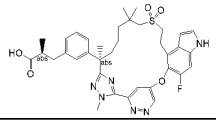
206A	
206B	
209A	
209B	
210A	
210B	
210C	
210D	
211A	
211B	
215A	

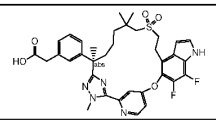
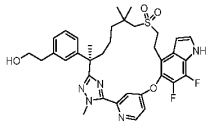
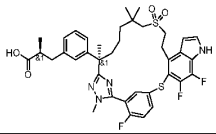
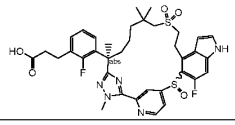
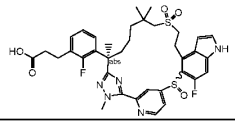
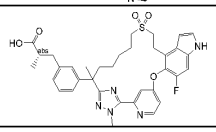
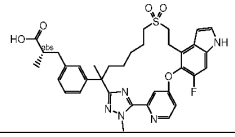
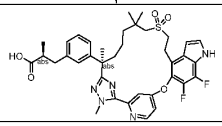
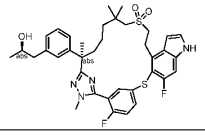
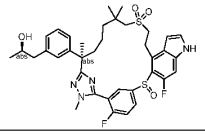
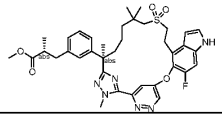
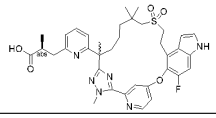
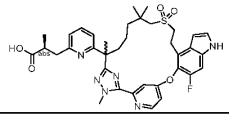
215B	
216A	
216B	
217A	
217B	
218A	
218B	
219	
220A	
220B	
221	

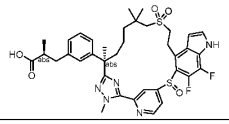
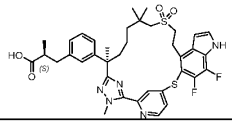
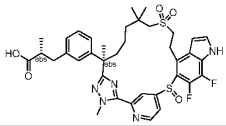
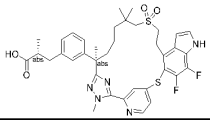
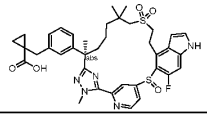
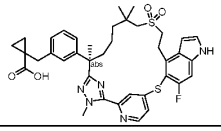
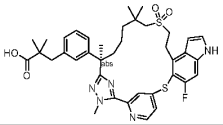
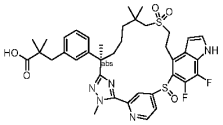
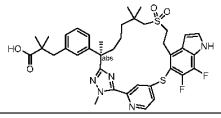
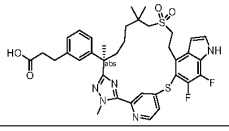
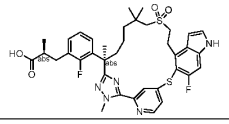
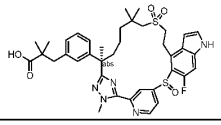
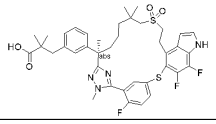
222A	
222B	
223A	
223B	
224	
225A	
225B	
226	
227	
228	
229	

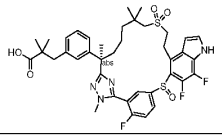
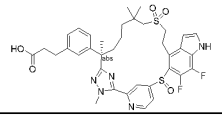
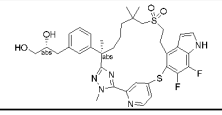
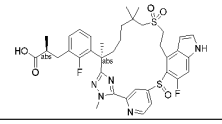
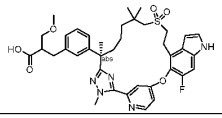
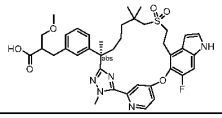
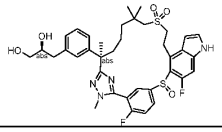
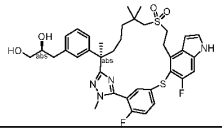
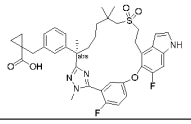
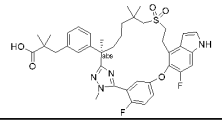
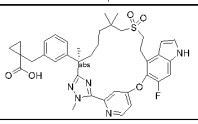
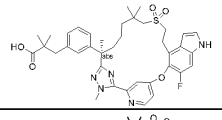
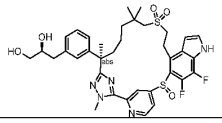
230	
231	
233	
234	
235A	
235B	
236A	
236B	
237A	
237B	
238	

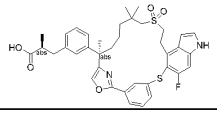
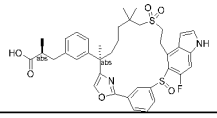
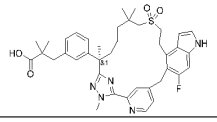
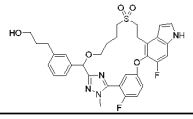
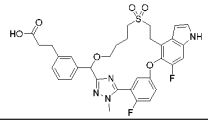
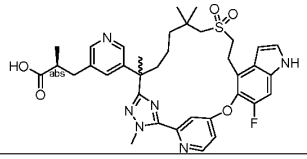
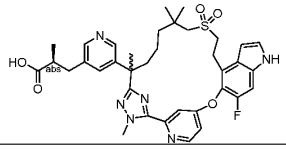
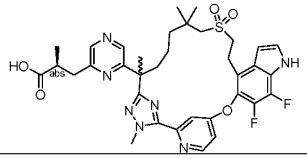
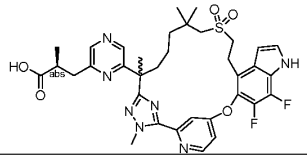
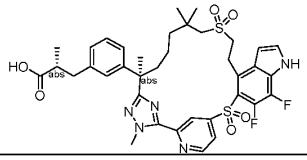
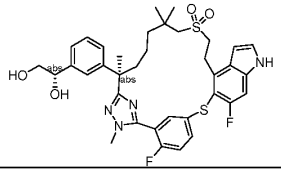
239A	
239B	
240	
241A	
241B	
242	
243A	
243B	
244	
245	
246A	
246B	

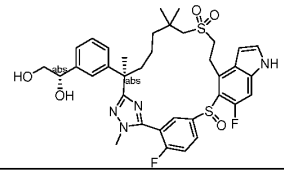
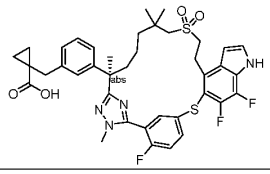
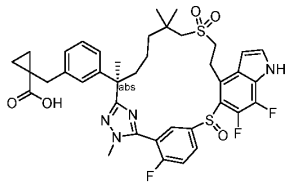
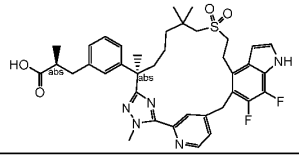
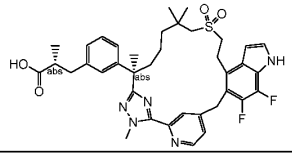
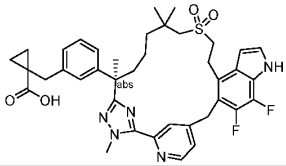
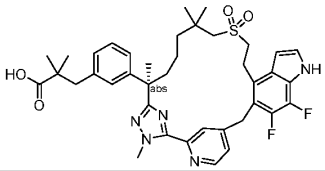
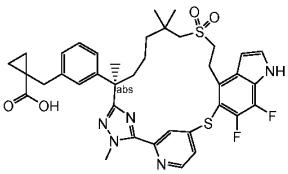
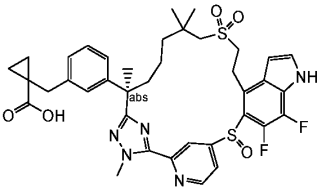
247	
248A	
248B	
249A	
249B	
250	
251	
252	
253	
254	
255A	
255B	
256	

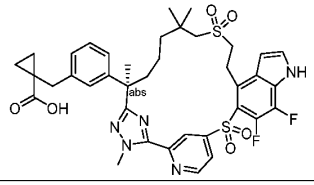
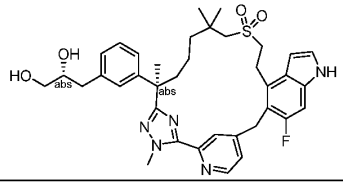
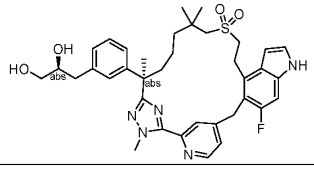
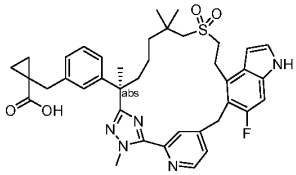
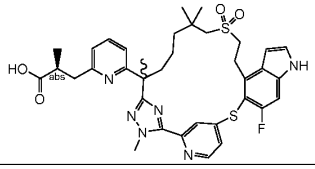
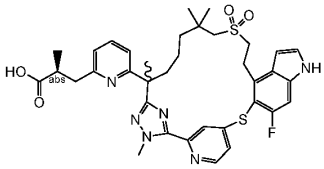
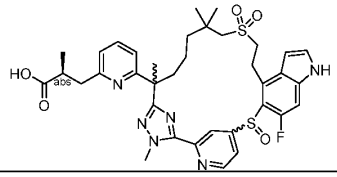
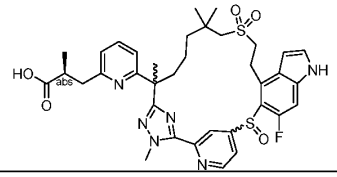
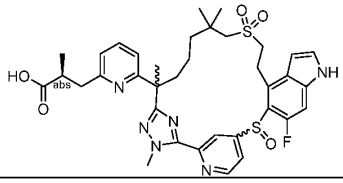
257	
258	
259	
260A	
260B	
261A	
261B	
262	
263	
264	
265	
266A	
266B	

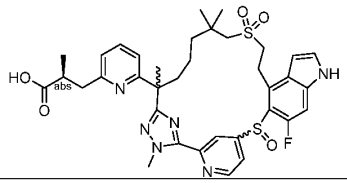
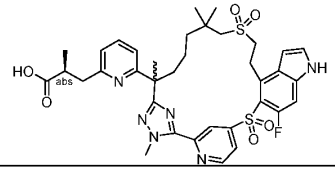
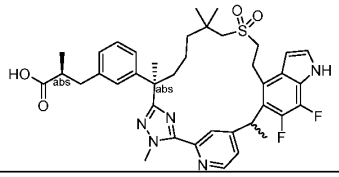
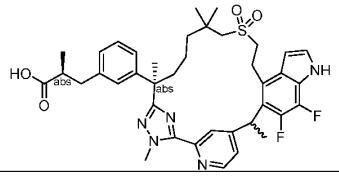
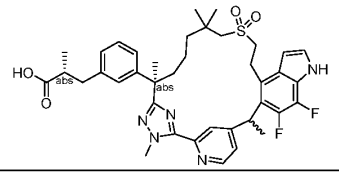
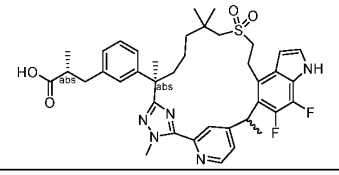
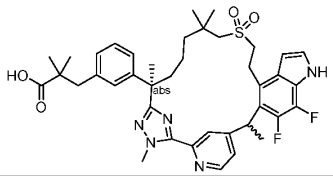
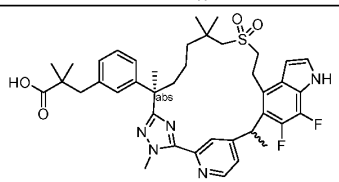
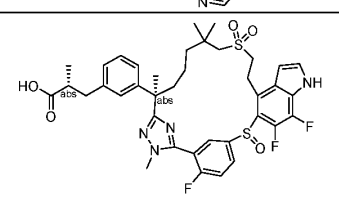
267	
268	
269	
270	
271	
272	
273	
274	
275	
276	
277	
278	
279	

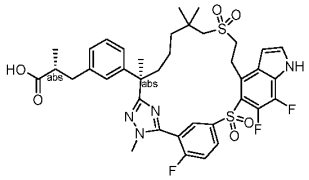
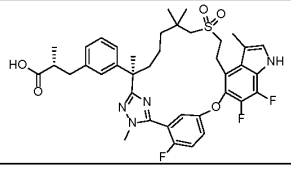
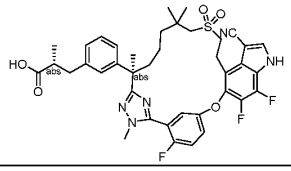
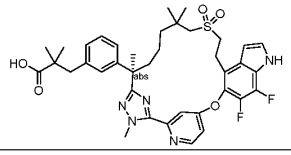
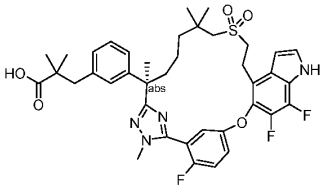
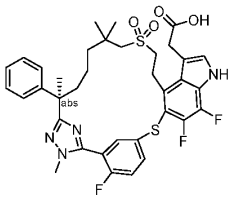
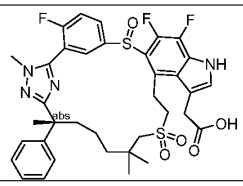
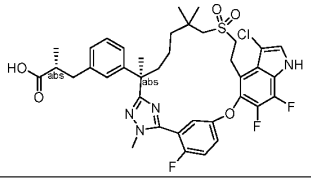
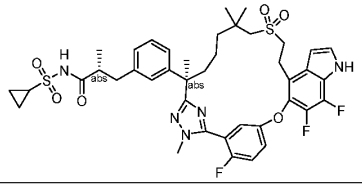
280	
281	
282	
283	
284	
285	
286	
287	
288	
289	
290	
291	
292	

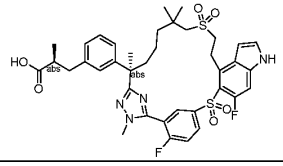
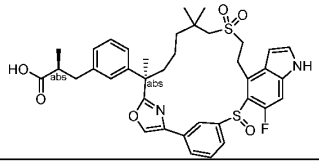
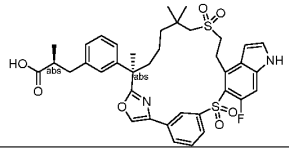
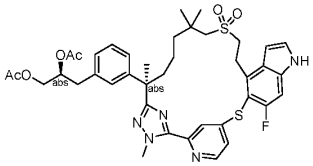
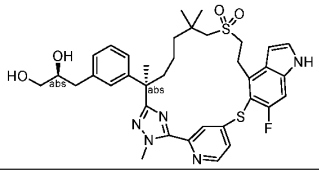
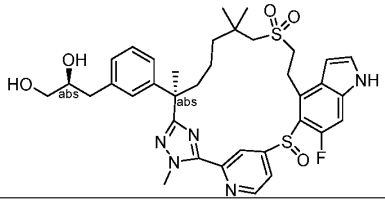
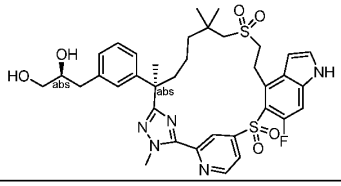
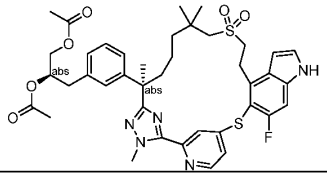
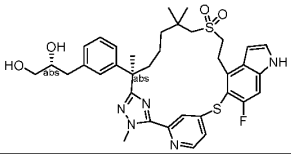
293	
294	
295	
296	
297	
299A	
299B	
300A	
300B	
301	
302A	

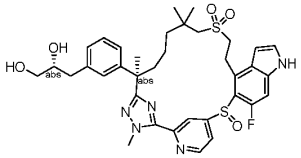
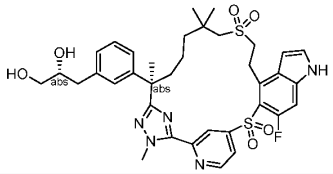
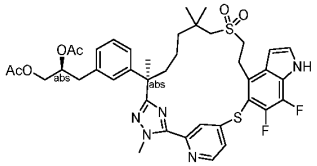
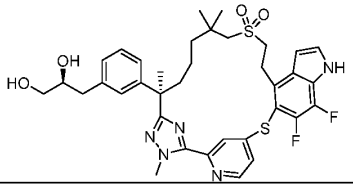
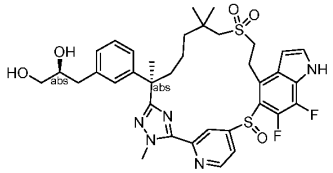
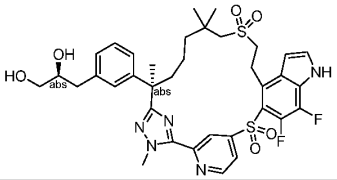
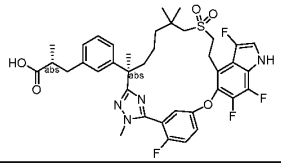
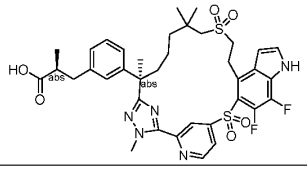
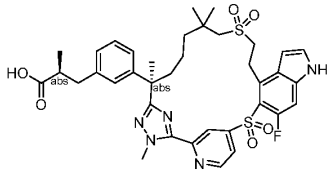
302B	
303A	
303B	
304	
305	
306	
307	
308	
309	

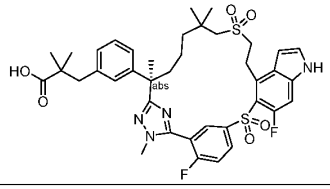
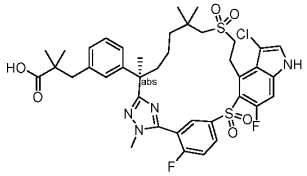
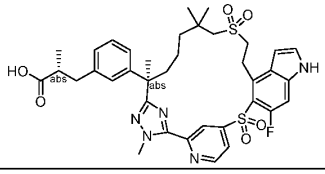
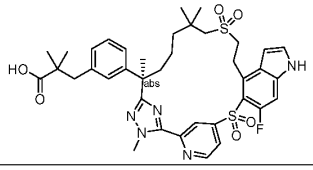
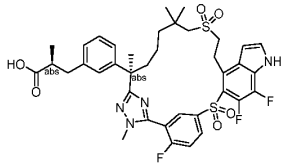
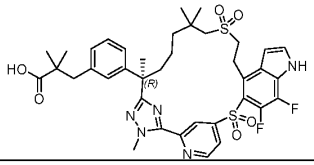
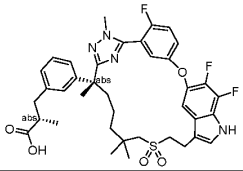
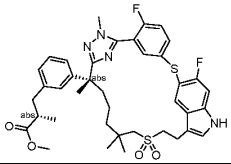
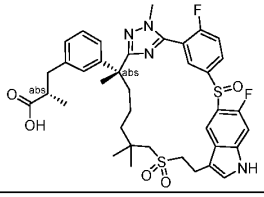
310	
311	
312	
313	
314A	
314B	
315A	
315B	
315C	

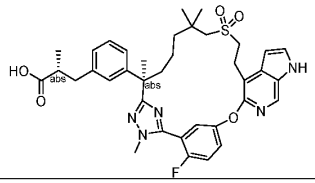
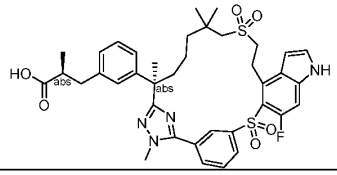
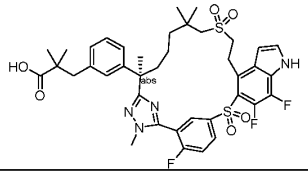
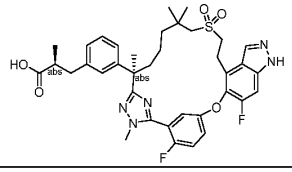
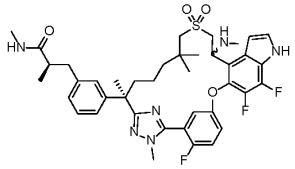
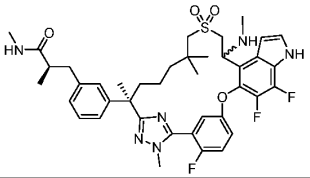
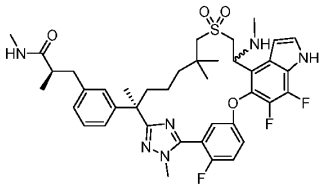
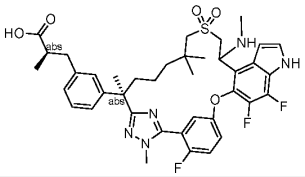
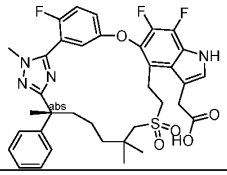
315D	
315E	
316A	
316B	
317A	
317B	
318A	
318B	
319	

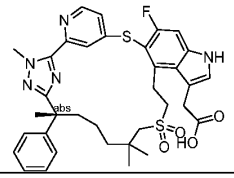
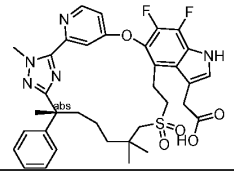
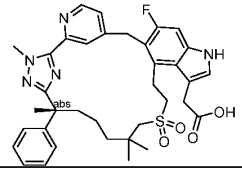
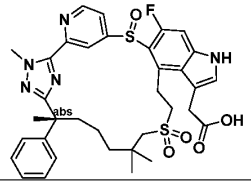
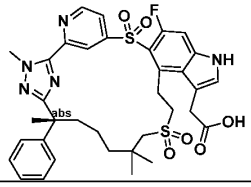
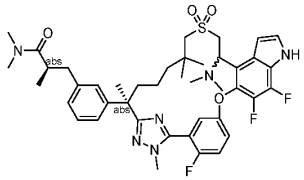
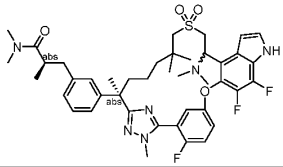
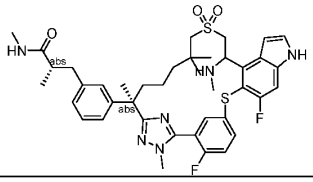
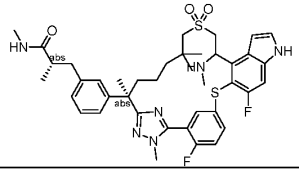
320	
321	
322	
323	
324	
325	
326	
327	
328	

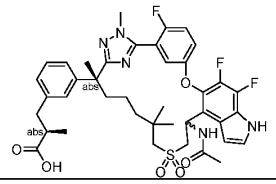
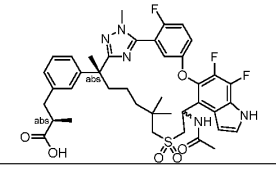
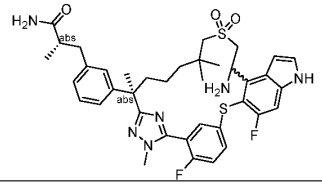
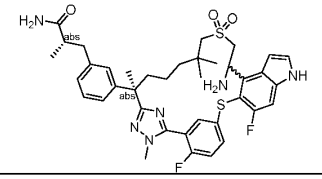
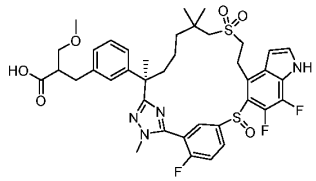
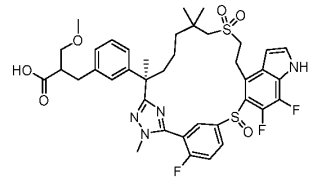
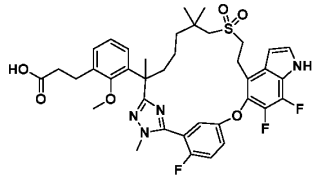
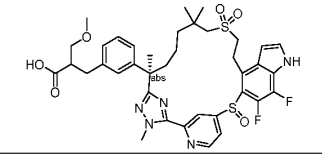
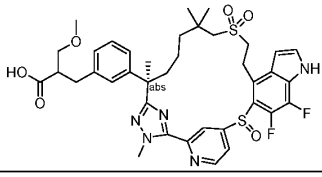
329	
330	
331	
332	
333	
334	
335	
336A	
336B	

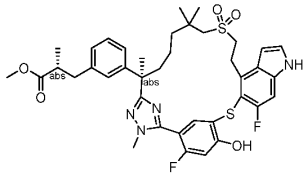
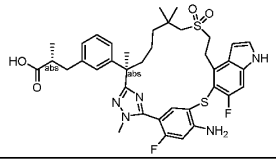
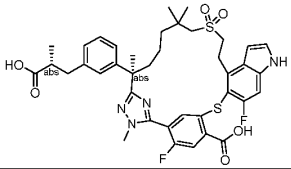
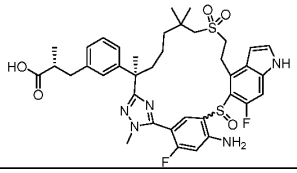
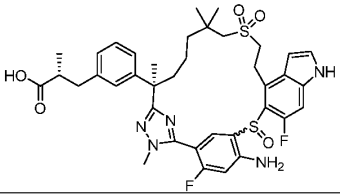
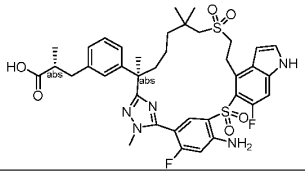
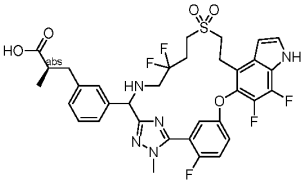
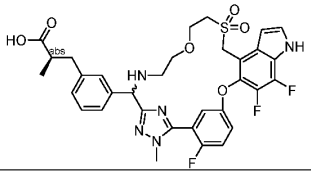
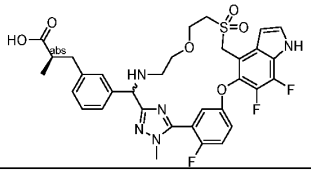
337	
338	
339	
340	
341	
342	
345	
346	
347	

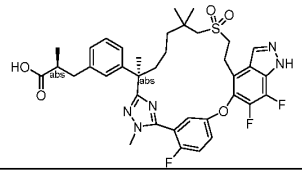
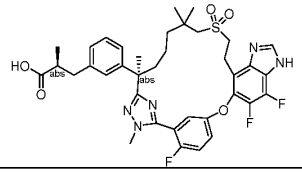
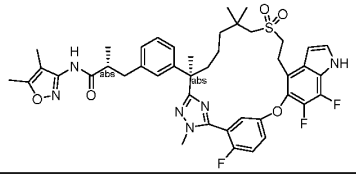
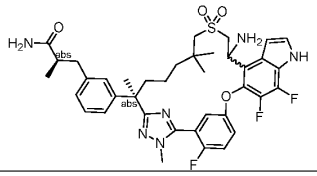
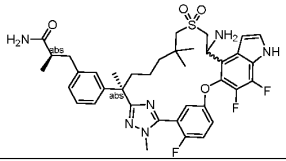
348A	
348B	
349	
350	
351	
352	
353	
354	
355	

356	
357	
358	
359	
360A	
360B	
360C	
360D	
361	

362	
363	
364	
365	
366	
367A	
367B	
368A	
368B	

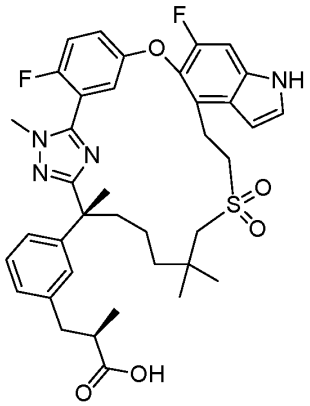
369A	
369B	
370A	
370B	
371	
372	
373	
374	
375	

376	
377	
378	
379A	
379B	
379C	
380	
381A	
381B	

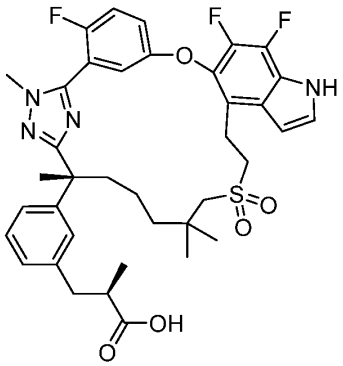
382	
383	
384	
385A	
385B	

[14] In some embodiments, an NBD1 stabilizer is a compound of a formula described in International Patent Application No. PCT/US2023/073543, which is incorporated herein in its entirety.

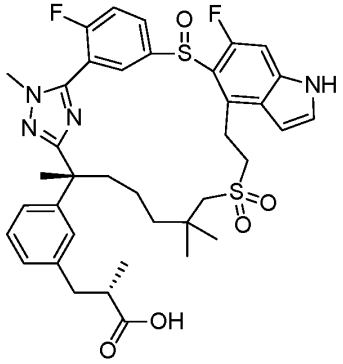
[15] In some embodiments, an NBD1 stabilizer is Compound 1, Compound 4, Compound 5, Compound 6, or Compound 7:



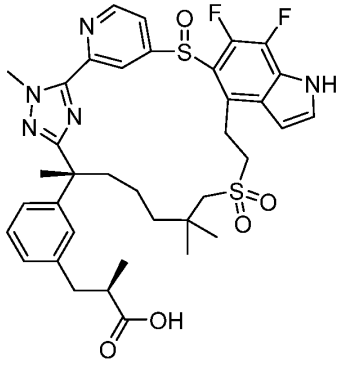
1



4



5



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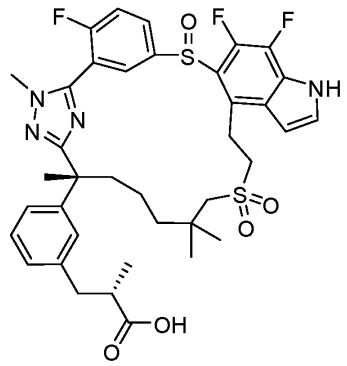
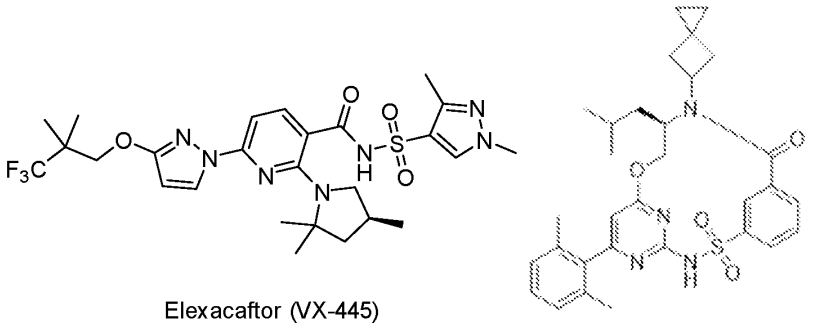


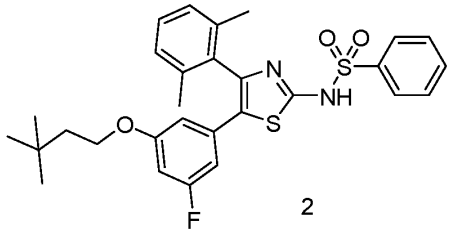
7

or a pharmaceutically acceptable salt thereof.

[16] In some embodiments, a compound of the present disclosure is an ICL4 corrector. In some embodiments, an ICL4 corrector is elexacaftor (VX-445), vanzacaftor (VX-121) or Compound 2:

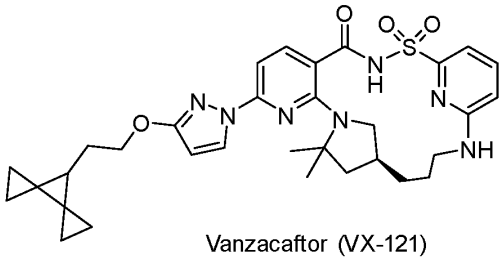


Elexacaftor (VX-445)



2

or

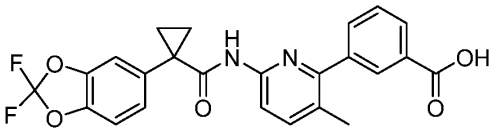


Vanzacaftor (VX-121)

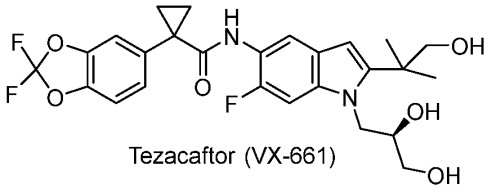
or a pharmaceutically acceptable salt thereof.

[17] In some embodiments, a compound of the present disclosure is a TMD1 corrector. In

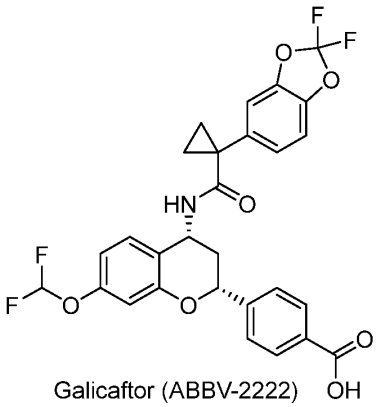
some embodiments, a TMD1 corrector is a compound selected from the group consisting of lumacaftor (VX-809), Tezacaftor (VX-661), Galicaftor (ABBV-2222), Compound A and Compound 3:



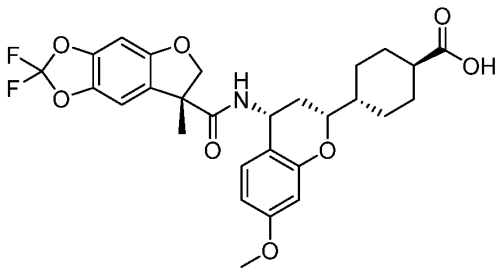
Lumacaftor (VX-809)



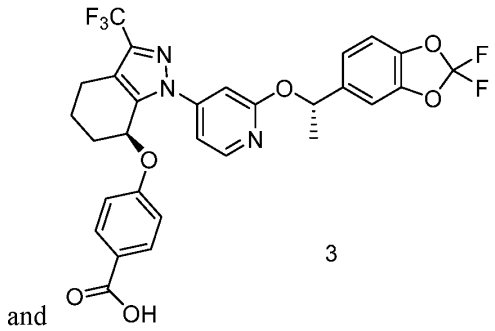
Tezacaftor (VX-661)



Galicaftor (ABBV-2222)



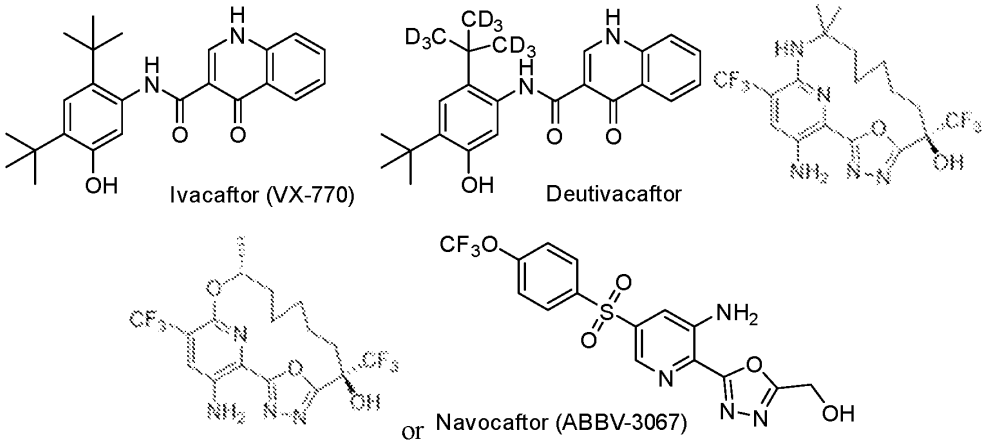
A



and 3

or a pharmaceutically acceptable salt thereof.

[18] In some embodiments, a compound of the present disclosure is a CFTR potentiator. In some embodiments, a CFTR potentiator is



or a pharmaceutically acceptable salt thereof.

[19] In other embodiments, a compound of the present disclosure is a CFTR potentiator. In some embodiments, the CFTR potentiator is selected group consisting of ivacaftor (VX-770), deutivacaftor (VS-5612), GLPG1837 (AbbVie/Galapagos), GLPG2451 (AbbVie/Galapagos), navocaftor (ABBV-3067), icenticaftor (QBW251/6), and dirocaftor (PTI-808).

[20] In other embodiments, a compound of the present disclosure is a CFTR corrector. In some embodiments, the CFTR corrector is selected from the group consisting of lumcaftor (VS-809), tezacaftor (VX-661), galicaftor (ABV-2222), and FDL169 (Flatley Discovery). In other embodiments, the CFTR corrector is selected from the group consisting of olacaftor (VX-440), bamocaftor (VX-659), elexacaftor (VS-445), vanzacaftor (VX-121), ABBV-3221 (AbbVie/Galapagos), GLPG/ABBV-2727 (Galapagos/AbbVie), ABBV-3748 (AbbVie), and posenacaftor.

[21] In other embodiments, a compound of the present disclosure is a CFTR amplifier. In some embodiments, the CFTR amplifier is nesolicaftor.

[22] In some embodiments, a compound of the present disclosure is a compound of a formula described in international patent application publications WO2021/097057, WO2023/034992, WO2024/054840, WO2016/069757, WO2017/009804, and WO2017/208115, whose contents are incorporated herein by reference in their entirety.

Alternative Embodiments

[23] In an alternative embodiment, compounds described herein may also comprise one or more isotopic substitutions. For example, hydrogen may be ^{2}H (D or deuterium) or ^{3}H (T or tritium); carbon may be, for example, ^{13}C or ^{14}C; oxygen may be, for example, ^{18}O; nitrogen

may be, for example, ^{15}N, and the like. In other embodiments, a particular isotope (*e.g.*, ^{3}H, ^{13}C, ^{14}C, ^{18}O, or ^{15}N) can represent at least 1%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or at least 99.9% of the total isotopic abundance of an element that occupies a specific site of the compound.

Pharmaceutical Compositions

[24] In some embodiments, the present disclosure provides a composition comprising a compound disclosed herein and a pharmaceutically acceptable carrier, adjuvant, or vehicle. In some embodiments, the amount of compound in compositions contemplated herein is such that is effective to measurably treat a disease or disorder in a biological sample or in a subject. In certain embodiments, the amount of compound in compositions of this disclosure is such that is effective to measurably treat a disease or disorder in a biological sample or in a subject. In certain embodiments, a composition contemplated by this disclosure is formulated for administration to a subject in need of such composition. In some embodiments, a composition contemplated by this disclosure is formulated for oral administration to a patient.

[25] In some embodiments, compositions of the present disclosure may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. In some preferred embodiments, compositions are administered orally, intraperitoneally or intravenously. In some embodiments, sterile injectable forms of the compositions comprising one or more compounds disclosed herein may be aqueous or oleaginous suspension. In some embodiments, suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. In some embodiments, sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. In some embodiments, among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In some embodiments, additional examples include, but are not limited to, sterile, fixed oils are conventionally employed as a solvent or suspending medium.

[26] The term “parenteral” as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques.

[27] Pharmaceutically acceptable compositions comprising one or more compounds

disclosed herein may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In some embodiments, carriers used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. In some embodiments, useful diluents include lactose and dried cornstarch. In some embodiments, when aqueous suspensions are required for oral use, an active ingredient is combined with emulsifying and suspending agents. In some embodiments, certain sweetening, flavoring or coloring agents may also be added.

[28] Pharmaceutically acceptable compositions comprising a compound disclosed herein may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs. In some embodiments, pharmaceutically acceptable compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of compounds of this disclosure include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, provided pharmaceutically acceptable compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

[29] Pharmaceutically acceptable compositions comprising a compound of disclosed herein may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

[30] In some embodiments, an amount of a compound of the present disclosure that may be combined with the carrier materials to produce a composition in a single dosage form will vary depending upon the host treated, the particular mode of administration. Preferably, provided compositions should be formulated so that a dosage of between 0.01-100 mg/kg body weight/day of the inhibitor can be administered to a patient receiving these compositions.

[31] The presently disclosed compounds can be formulated into pharmaceutical

compositions along with a pharmaceutically acceptable carrier or excipient. According to this aspect, there is provided a pharmaceutical composition comprising a compound disclosed herein in association with a pharmaceutically acceptable excipient, diluent or carrier.

[32] The formulations of Compounds of disclosed herein include those suitable for the administration routes detailed herein. They may conveniently be presented in unit dosage form and can be formulated in accordance with standard pharmaceutical practice as a pharmaceutical composition. Techniques and formulations generally and suitable for use herein are found in Remington's Pharmaceutical Sciences (16th edition, Osol, A. Ed. (1980); Mack Publishing Co., Easton, PA). Such methods include the step of bringing into association the active ingredient with the excipient or carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid excipients or carriers or finely divided solid excipients or carriers or both, and then, if necessary, shaping the product.

[33] Acceptable diluents, carriers, excipients and stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3- pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). The active pharmaceutical ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxy methylcellulose or gelatin-microcapsules and poly-(methyl methacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[34] In particular embodiments the pharmaceutical composition comprising the presently

disclosed compounds further comprise a chemotherapeutic agent. In some of these embodiments, the chemotherapeutic agent is an immunotherapeutic agent.

Methods of Using Compounds of the Present Disclosure

[35] The present disclosure includes methods of treating CFTR-mediated diseases or disorders comprising administering a NBD1 stabilizer in combination with one or more additional therapeutic agents, including, but not limited to, a TMD1 corrector, a CFTR potentiator and an ICL4 corrector.

[36] In some embodiments, the present disclosure includes method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an NBD1 stabilizer in combination with an ICL4 corrector. In some embodiments, an NBD1 stabilizer and an ICL4 corrector are administered simultaneously. In some embodiments, an NBD1 stabilizer is administered prior to an ICL4 corrector. In some embodiments an NBD1 stabilizer is administered after a ICL4 corrector.

[37] In some embodiments, the present disclosure includes method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an NBD1 stabilizer in combination with a ICL4 corrector and a CFTR potentiator. In some embodiments, an NBD1 stabilizer, a TMD1 corrector, and a CFTR potentiator are administered simultaneously. In some embodiments, an NBD1 stabilizer is administered prior to a ICL4 corrector and/or a CFTR potentiator. In some embodiments an NBD1 stabilizer is administered after a ICL4 corrector and/or a CFTR potentiator.

[38] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administered to the subject a therapeutically effective amount of an NBD1 stabilizer, wherein the subject has previously received treatment with an ICL4 corrector. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administered to the subject a therapeutically effective amount of an ICL4 corrector, wherein the subject has previously received treatment with an NBD1 stabilizer.

[39] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1, Compound 4, Compound 5,

Compound 6, or Compound 7 in combination with compound 2. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1, Compound 4, Compound 5, Compound 6, or Compound 7 in combination with compound 3. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1, Compound 4, Compound 5, Compound 6, or Compound 7 in combination with elexacaftor (ELX), or vanzacaftor (VX-121).

[40] In some embodiments, the present disclosure includes method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an NBD1 stabilizer in combination with a TMD1 corrector. In some embodiments, an NBD1 stabilizer and a TMD1 corrector are administered simultaneously. In some embodiments, an NBD1 stabilizer is administered prior to a TMD1 corrector. In some embodiments an NBD1 stabilizer is administered after a TMD1 corrector.

[41] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administered to the subject a therapeutically effective amount of an NBD1 stabilizer, wherein the subject has previously received treatment with a TMD1 corrector. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administered to the subject a therapeutically effective amount of TMD1 corrector, wherein the subject has previously received treatment with an NBD1 stabilizer.

[42] In some embodiments, the present disclosure includes method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an NBD1 stabilizer in combination with a TMD1 corrector and a CFTR potentiator. In some embodiments, an NBD1 stabilizer, a TMD1 corrector, and a CFTR potentiator are administered simultaneously. In some embodiments, an NBD1 stabilizer is administered prior to a TMD1 corrector and/or a CFTR potentiator. In some embodiments an NBD1 stabilizer is administered after a TMD1 corrector and/or a CFTR potentiator.

[43]

[44] In some embodiments, the present disclosure includes a method of treating CFTR-

mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1, Compound 4, Compound 5, Compound 6, or Compound 7 in combination with Compound A. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1, Compound 4, Compound 5, Compound 6, or Compound 7 in combination with lumacaftor (LUM), tezacaftor (TEZ), or galicaftor (ABV-2222).

[45] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an NBD1 stabilizer in combination with an ICL4 corrector and a TMD1 corrector. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1, Compound 4, Compound 5, Compound 6, or Compound 7 in combination with compound 2 and compound 3. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1, Compound 4, Compound 5, Compound 6, or Compound 7 in combination with elexacaftor or vanzacaftor and compound 3. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1, Compound 4, Compound 5, Compound 6, or Compound 7 in combination with compound 2 and Compound A or tezacaftor (TEZ) or lumacaftor (LUM) or Galicaftor (ABBV-2222). In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1, Compound 4, Compound 5, Compound 6, or Compound 7 in combination with elexacaftor (ELX) or vanzacaftor (VX-121) and Compound A or tezacaftor (TEZ) or lumacaftor (VX-809) or galicaftor (ABBV-2222).

[46] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an NBD1 stabilizer in combination with an ICL4 corrector, and a CFTR potentiator. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1,

Compound 4, Compound 5, Compound 6, or Compound 7 in combination with elexacaftor (ELX), vanzacaftor (VX-121), Compound 2, or Compound 3, and a therapeutically effective amount of ivacaftor, deutivacaftor, navocaftor, or icenticaftor.

[47] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an NBD1 stabilizer in combination with an TMD1 corrector, and a CFTR potentiator. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1, Compound 4, Compound 5, Compound 6, or Compound 7 in combination with Compound A, lumacaftor (LUM), tezacaftor (TEZ), or Galicaftor, and a therapeutically effective amount of ivacaftor, deutivacaftor, navocaftor, or icenticaftor.In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an NBD1 stabilizer in combination with an ICL4 corrector, a TMD1 corrector, and a CFTR potentiator. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1, Compound 4, Compound 5, Compound 6, or Compound 7 in combination with elexacaftor (ELX), tezacaftor (TEZ) or Compound A, and ivacaftor (IVA), or vanzacaftor (VX-121), tezacaftor (TEZ) and deutivacaftor. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1, Compound 4, Compound 5, Compound 6, or Compound 7 in combination with a compound 2, tezacaftor (TEZ), and ivacaftor (IVA).

[48] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an NBD1 stabilizer in combination with Trikafta. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1, Compound 4, Compound 5, Compound 6, or Compound 7 in combination with Trikafta.

[49] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the

subject a therapeutically effective amount of an NBD1 stabilizer in combination with a TMD1 corrector. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an NBD1 stabilizer in combination with Compound 3.

[50] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 5 in combination with an ICL4 corrector. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 5 in combination with Compound 2.

[51] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 5 in combination with a TMD1 corrector. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 5 in combination with an a ABBV-2222. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 5 in combination with an a Compound 3. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 5 in combination with an a Compound A.

[52] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 5 in combination with an ICL4 corrector and a TMD1 corrector. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 5 in combination with Compound 2 and Galicaftor. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 5 in combination with Compound 2 and ABBV-2222. In some embodiments, the present

disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 5 in combination with Compound 2 and compound 3.

[53] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 4 in combination with an ICL4 corrector. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 4 in combination with Compound 2.

[54] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 4 in combination with a TMD1 corrector. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 4 in combination with an a ABBV-2222. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 4 in combination with an a Compound 3. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 4 in combination with an a Compound A.

[55] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 4 in combination with an ICL4 corrector and a TMD1 corrector. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 5 in combination with Compound 4 and Galicaftor. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 4 in combination with Compound 2 and ABBV-2222. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of

Compound 4 in combination with Compound 2 and compound 3.

[56] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 6 in combination with an ICL4 corrector. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 6 in combination with Compound 2.

[57] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 6 in combination with a TMD1 corrector. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 6 in combination with an a ABBV-2222. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 6 in combination with an a Compound 3. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 6 in combination with an a Compound A.

[58] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 6 in combination with an ICL4 corrector and a TMD1 corrector. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 6 in combination with Compound 2 and Galicaftor. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 6 in combination with Compound 2 and ABBV-2222. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 6 in combination with Compound 2 and compound 3.

[59] In some embodiments, the present disclosure includes a method of treating CFTR-

mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 7 in combination with an ICL4 corrector. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 7 in combination with Compound 2.

[60] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 7 in combination with a TMD1 corrector. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 7 in combination with an a ABBV-2222. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 7 in combination with an a Compound 3. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 7 in combination with an a Compound A.

[61] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 7 in combination with an ICL4 corrector and a TMD1 corrector. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 7 in combination with Compound 2 and Galicaftor. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 7 in combination with Compound 2 and ABBV-2222. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 7 in combination with Compound 2 and compound 3.

[62] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 5 in combination with an ICL4

corrector. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 5 in combination with Compound 2.

[63] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1 in combination with a TMD1 corrector. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1 in combination with an a ABBV-2222. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1 in combination with an a Compound 3. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1 in combination with an a Compound A.

[64] In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1 in combination with an ICL4 corrector and a TMD1 corrector. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1 in combination with Compound 2 and Galicaftor. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1 in combination with Compound 2 and ABBV-2222. In some embodiments, the present disclosure includes a method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of Compound 1 in combination with Compound 2 and compound 3.

Exemplification

ANALYTICAL PROCEDURES

[65] High Pressure Liquid Chromatography-Mass Spectrometry (LC-MS) to determine compound retention times (RT) and associated mass ions were performed using one of the following methods.

[66] LC-MS **Method 1**: Mobile Phase: A: water (0.01 % TFA). B: ACN (0.01 % TFA). Gradient: 5% - 95% B in 1.5 min. Flow Rate: 2.0 mL/min. Column: Sunfire C18, 4.6×50 mm, 3.5 µm. Oven Temperature: 50 °C. Mass Range: 110-1000. UV (214 nm, 254 nm).

[67] LC-MS **Method 2**: Column: Xbridge C18(2) (4.6 × 50 mm, 3.5µm). Mobile phase: H_2O (10 mmol NH_4HCO_3) (A) / ACN (B). Elution program: Gradient from 10 to 95% of B in 1.5 min at 1.8 mL/min. Temperature: 50 °C. Detection: UV (214 nm, 254 nm) and MS (ESI, Positive mode, 103 to 800 amu).

[68] LC-MS **Method 3**: Mobile Phase: A: water (0.01% trifluoroacetic acid), B: acetonitrile (0.01% trifluoroacetic acid); Gradient: 5% B increased to 95% B within 1.3 min, 95% B for 1.7 min, back to 5% B within 0.01 min; Flow Rate: 2 ml/min; Column: Sunfire, 50 x 4.6 mm, 3.5 um; Column Temperature: 50 °C; Detection: UV (214.4 nm) and MS (ESI, Pos mode, 110 to 1000 amu).LC-MS Method 4: Mobile Phase: A: water (0.01% trifluoroacetic acid) B: acetonitrile (0.01% trifluoroacetic acid); Gradient: 5% B increase to 95% B within 1.5 minutes, 95% B for 1.5 minutes, back to 5% B within 0.01minutes. Flow Rate: 1.8 mL/minute; Column : Sunfire C18, 4.6*50mm, 3.5 µm; Oven Temperature : 50 °C.

[69] LC-MS Method 5: Mobile phase: water (10 mM ammonium bicarbonate) (A)/acetonitrile (B); Gradient: B = 5% B increase to 95% B within 1.4 minutes, 95% B for 1.6 minutes, back to 5% B within 0.01 minute; Flow rate: 1.8 mL/minute; Column: Xbridge-C18, 50 x 4.6 mm, 3.5 µm. Column Temperature: 50 °C.

[70] LC-MS Method 6: Mobile Phase: A: water (0.1% formic acid) B: acetonitrile (0.1% formic acid), Gradient: 10% B for 0. 2min, increase to 90% B within 1.3 min, 90% B for 1.5 min; Flow Rate: 2 mL/min; Column: Sunfire C18, 4.6 x 50 mm, 3.5 um; Oven Temperature: 50 °C; Detection: UV (214.4 nm) and MS (ESI, Pos mode,110 to 1000 amu).

LC-MS Method 7: Column: SUNFIRE C18 (4.6 x 50 mm, 3.5 µm); Mobile phase: A: water (0.01% trifluoroacetic acid) (A) / acetonitrile (0.01% trifluoroacetic acid)(B); Elution program: Gradient from 5 to 95% of B in 3 minutes, 95% 2 minutes at 2.0 ml/minute. Oven Temperature: 50 °C; Detection: UV (214.4 nm) and MS (ESI, Pos mode,110 to 1000 amu)

[71] LC-MS Method 8: Column: SunFire C18 (4.6x 50 mm,3.5 µm); Mobile phase: A: water (0.01% trifluoroacetic acid) B: Acetonitrile (0.01% trifluoroacetic acid); Gradient: 10 to 95% of B in 1.4 minutes, 95% of B for 1.6 minutes; Flow rate: 2 mL/minute; Temperature: 50 °C; Detection: UV (214 , 4 nm) and MS (ESI, Positive mode ,110 to 1000 amu).

[72] LC-MS Method 9: Mobile Phase : A: water (0.01% trifluoroacetic acid) B: Acetonitrile (0.01% trifluoroacetic acid) Gradient:5% B increase to 95% B within 1.3 minutes, 95% B for 1.78 min Flow Rate :1.6 mL/minute; Column: Agilent Poroshell, 30*3.0

mm, 2.7 µm; Column Temperature: 50 °C; Detection: UV (214, 4 nm) and MS (ESI, Pos mode, 110 to 1000 amu).

[73] LC-MS Method 10: Mobile Phase: A: water (0.01% trifluoroacetic acid) B: Acetonitrile (0.01% trifluoroacetic acid); Gradient: 5% increase to 95% B within 1.5 minutes, 95% B for 1.7 minutes; Flow Rate: 2.0 mL/minute; Column: Sunfire C18, 4.6 * 50 mm, 3.5 µm; Column Temperature: 50 °C; Detection: UV (214, 4 nm) and MS (ESI, Pos mode, 110 to 1000 amu).

[74] LC-MS Method 11: Mobile Phase: A: water (0.01% trifluoroacetic acid) B: Acetonitrile (0.01% trifluoroacetic acid); Gradient: 5% increase to 95% B within 1.05 minutes, 95% B to 2.90 minutes, back to 5% B within 0.05 minutes; Flow Rate: 1.6 mL/minute; Column: Proshell 120 EC-C18, 30*3 mm, 2.7 µm; Oven Temperature: 50 °C; Detection: UV214, MASS 103-1000 amu.

[75] LC-MS Method 12: Mobile Phase : A: water (0.01% trifluoroacetic acid) B: Acetonitrile (0.01% trifluoroacetic acid); Gradient: 5% B increase to 95% B within 9 minutes, 95% B for 6 minutes; Flow Rate :1 ml/minute; Column: Sunfire C18 150 * 4.6 mm, 3.5 µm; Column Temperature:45 °C; Detection: UV (214 nm, 4 nm) and MS (ESI, POS Mode, 110-1300 amu).

[76] The ^{1}H NMR spectra were collected at 400 MHz on a Gemini 400 or Varian Mercury 400 spectrometer (unless noted otherwise) with an ASW 5 mm probe, and usually recorded at ambient temperature in a deuterated solvent, such as D_2O, DMSO-D_6, CH_3OH-d_4 or $CDCl_3$ unless otherwise noted. Chemical shift values (δ) are indicated in parts per million (ppm) with reference to tetramethylsilane (TMS) as the internal standard.

Abbreviations:

ACN: acetonitrile

Boc: tert-butyloxycarbonyl

DEA: diethyl amine

DBU: 1,8-Diazabicyclo[5.4.0]undec-7-ene

DCE: 1,2-dichloroethane

DCM: dichloromethane

DIAD: diisopropyl azodicarboxylate

DMAP: 4-dimethylaminopyridine

DMSO: dimethyl sulfoxide

dppf: 1,1'-Bis(diphenylphosphino)ferrocene

EA: ethyl acetate

ee: enantiomeric excess

ESI: electron spray ionization

HPLC: high performance liquid chromatography

LC-MS: liquid chromatography-mass spectrometry

MsCl: methanesulfonyl chloride

Pd/C: Palladium on carbon

rt: room temperature

PE: petroleum ether

SFC: supercritical fluid chromatography

TBS: *tert*-butyldimethylsilyl

TIPS: triisopropylsilyl

THF: tetrahydrofuran

THP: tetrahydropyran

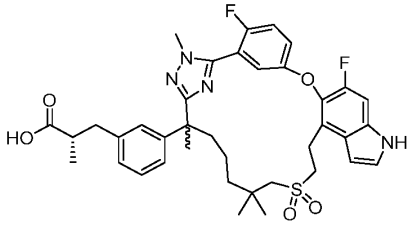
Ts: tosyl

GENERAL DISCLAIMER ABOUT STEREOCHEMISTRY

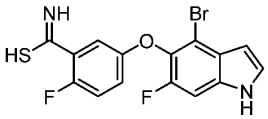
[77] It is understood that absolute stereochemistries for all intermediates and examples described herein have not been determined. The assignments of the chiral center(s) to R or S are completely *arbitrary* and are solely for *the purpose of differentiating* the different fractions (**P1** and **P2**) eluted out from either flash column chromatography or prep-HPLC, or chiral HPLC, or SFC. There is *no association* of **P1** or **P2** with the S or R designations.

[78] Both ChemDraw and Mol2Nam from OpenEye Scientific software have been used to generate compounds' names. If not consistent, the structure should govern.

Example 1. Diastereomers of 2-[3-(22,28-Difluoro-3,6,10,10-tetramethyl-12,12-dioxo-9,24-dioxa-12λ6-thia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl)phenyl]acetic acid (Compound 1)

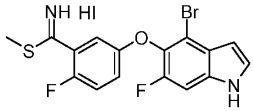


2-fluoro-5-((6-fluoro-4-bromo-1H-indol-5-yl)oxy)benzothioamide



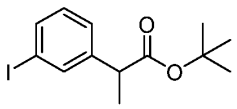
[79] To a stirred solution of 2-fluoro-5-((6-fluoro-4-bromo-1H-indol-5-yl)oxy)benzonitrile (30 mmol) and sodium hydrosulfide (10.1 g, 180 mmol) in *N,N*-dimethylformamide (100 mL) was added magnesium chloride (8.58 g, 90.1 mmol) in water (20 mL). The mixture was stirred at room temperature for 1 hour, then quenched with water (300 mL). The mixture was extracted with ethyl acetate (2 x 200 mL). The combined organic phases were washed with brine, dried over sodium sulfate, filtered, and concentrated to give the title compound.

methyl 5-((4-bromo-6-fluoro-1H-indol-5-yl)oxy)-2-fluorobenzimidothioate hydroiodide



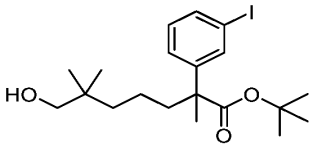
[80] To a stirred solution of 2-fluoro-5-((6-fluoro-4-bromo-1H-indol-5-yl)oxy)benzothioamide (10.6 mmol) in acetone (40 mL) was added iodomethane (3.30 mL, 7.52 g, 53.0 mmol). The mixture was heated to 40 °C and maintained at this temperature overnight. The reaction was then cooled to room temperature and concentrated. The crude title compound was obtained, which was used without purification

tert-Butyl 2-(3-iodophenyl)propanoate



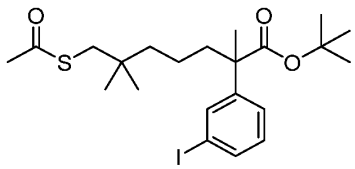
[81] To a stirred and cooled (-78 °C) solution of tert-butyl 2-(3-iodophenyl)acetate (115 g, 361 mmol) in tetrahydrofuran (800 mL) was added, dropwise over 30 minutes, a 2.0 M solution of lithium diisopropylamide in tetrahydrofuran (217 mL, 434 mmol). The mixture was maintained at -78 °C for two hours before adding, dropwise over 10 minutes, iodomethane (23.6 mL, 53.8 g, 379 mmol). The reaction mixture was warmed to room temperature and stirred for an additional two hours. After this time, the reaction was quenched with the slow addition of water (800 mL) and then concentrated to remove the organic solvent. The remaining water/oil mixture was extracted with ethyl acetate (1 x 500 mL) and the organic layer was washed with brine (2 x 500 mL), dried over anhydrous sodium sulfate and concentrated. The residue was purified by automated flash chromatography (330 g silica gel column, 0-5% ethyl acetate in petroleum ether) to afford the title compound as a colorless oil (97.9 g, 82%). MS (ESI): 355 m/z $[M+Na]^+$.

tert-Butyl 7-hydroxy-2-(3-iodophenyl)-2,6,6-trimethylheptanoate



[82] To a stirred and cooled (-78 °C) solution of *tert*-butyl 2-(3-iodophenyl)propanoate (97.9 g, 295 mmol) in tetrahydrofuran (800 mL) was added, dropwise over 20 minutes, a 2.0 M solution of lithium diisopropylamide in tetrahydrofuran (177 mL, 354 mmol. The mixture was maintained at -78 °C for two hours before adding, dropwise over 20 minutes, *tert*-butyl((5-iodo-2,2-dimethylpentyl)oxy)dimethylsilane (110 g, 309 mmol). The reaction mixture was allowed to warm to room temperature and stirred overnight. After this time, the reaction was quenched with the slow addition of water (800 mL) and then concentrated to remove the organic solvent. The remaining water/oil mixture was extracted with ethyl acetate (1 x 500 mL) and the organic layer was washed with brine (2 x 500 mL), dried over anhydrous sodium sulfate and concentrated. The residue was purified by automated flash chromatography (330 g silica gel column, 0-5% ethyl acetate in petroleum ether) to afford the title compound as a pale amber oil (160 g, 97%). A portion of this material (100 g, 178 mmol) was taken up in tetrahydrofuran (400 mL), stirred at room temperature and treated with a 1.0 M solution of tetrabutylammonium fluoride in tetrahydrofuran (357 mL, 357 mmol). The mixture was stirred overnight at room temperature and then partitioned between ethyl acetate (500 mL) and water (800 mL). The organic layer was washed with additional portions of water (2 x 800 mL), dried over magnesium sulfate and concentrated. The residue was purified by automated flash chromatography (330 g silica gel column, 0-30% ethyl acetate in petroleum ether) to afford the title compound as a colorless oil (65.1 g, 79% overall, two steps). MS (ESI): 469 m/z $[M+Na]^+$.

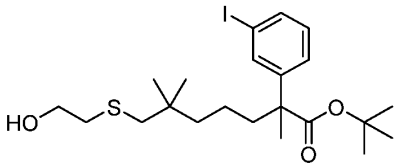
tert-Butyl 7-(acetylthio)-2-(3-iodophenyl)-2,6,6-trimethylheptanoate



[83] To a stirred and cooled (0 °C) solution of triphenylphosphine (115 g, 438 mmol) in tetrahydrofuran (800 mL) was added, dropwise over 10 minutes, diisopropyl azodicarboxylate (86.0 mL, 88.3 g, 437 mmol). The reaction was maintained at 0 °C and monitored for the appearance of a precipitate. Upon the observation of a white solid, a

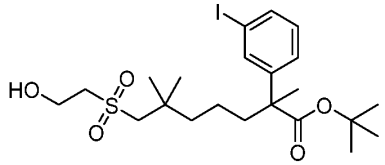
previously prepared solution of *tert*-butyl 7-hydroxy-2-(3-iodophenyl)-2,6,6-trimethylheptanoate (65.0 g, 146 mmol) and thioacetic acid (31.2 mL, 33.2 g, 437 mmol) in tetrahydrofuran (200 mL) was added, dropwise over 30 minutes. Following the addition, the reaction was stirred at 0 °C for one hour, allowed to warm to room temperature and stirred for another one hour. The reaction was then concentrated, and the crude residue was subjected to automated flash chromatography (330 g silica gel column, 0-10% ethyl acetate in petroleum ether). The title compound was obtained as a viscous, pale amber oil (51.0 g, 69%). MS (ESI): 527 *m/z* $[M+Na]^+$.

tert-Butyl 7-((2-hydroxyethyl)thio)-2-(3-iodophenyl)-2,6,6-trimethylheptanoate



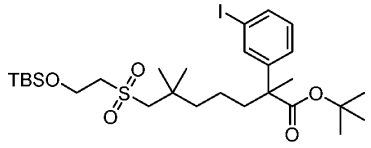
[84] To a stirred solution of *tert*-butyl 7-(acetylthio)-2-(3-iodophenyl)-2,6,6-trimethylheptanoate (51.0 g, 101 mmol) in ethanol (300 mL) was added 2-bromoethanol (9.32 mL, 16.4 g, 131 mmol) followed by sodium ethoxide (10.3 g, 151 mmol). The reaction was stirred for one hour before partitioning between ethyl acetate (500 mL) and water (800 mL). The organic layer was washed with additional portions of water (2 x 800 mL), dried over magnesium sulfate and concentrated. The residue was purified by automated flash chromatography (330 g silica gel column, 0-20% ethyl acetate in petroleum) to afford the title compound as light amber gum (47.2 g, 92%). MS (ESI): 529 *m/z* $[M+Na]^+$.

tert-Butyl 7-((2-hydroxyethyl)sulfonyl)-2-(3-iodophenyl)-2,6,6-trimethylheptanoate



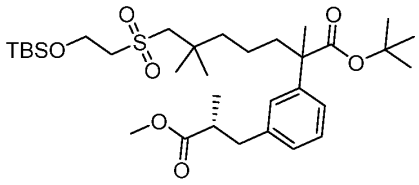
[85] To a stirred solution of *tert*-Butyl 7-((2-hydroxyethyl)thio)-2-(3-iodophenyl)-2,6,6-trimethylheptanoate (34.0 g, 67.1 mmol) in methanol (1.2 L) was added a solution of ammonium molybdate tetrahydrate (34.0 g, 27.5 mmol) dissolved in 30% aqueous hydrogen peroxide solution (150 mL). After two hours at room temperature, the reaction was partitioned between ethyl acetate (1.5 L) and water (2 L). The organic layer was washed with a second portion of water (1 x 2 L), dried over sodium sulfate and concentrated. Crude title compound was afforded as a pale amber gum (32.8 g, 91%). MS (ESI): 561 *m/z* $[M+Na]^+$.

tert-Butyl 7-((2-((tert-butyldimethylsilyl)oxy)ethyl)sulfonyl)-2-(3-iodophenyl)-2,6,6-trimethylheptanoate



[86] To a stirred solution of *tert*-butyl 7-((2-hydroxyethyl)sulfonyl)-2-(3-iodophenyl)-2,6,6-trimethylheptanoate (32.0 g, 59.4 mmol) in dichloromethane (500 mL) was added imidazole (8.09 g, 119 mmol) and *tert*-butyldimethylsilyl chloride (10.7 g, 71.0 mmol). After two hours at room temperature, the reaction mixture was partitioned between dichloromethane (400 mL) and water (500 mL). The organic layer was washed with a second portion of water (1 x 500 mL), dried over magnesium sulfate and concentrated. The residue was purified by automated flash chromatography (330 g silica gel column, 0-20% ethyl acetate in petroleum ether) to afford the title compound as a faint amber gum (34.0 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (t, *J* = 1.9 Hz, 1H), 7.55 (dt, *J* = 7.8, 1.9 Hz, 1H), 7.28-7.26 (m, 1H), 7.04 (t, *J* = 8.0 Hz, 1H), 4.04 (t, *J* = 5.2 Hz, 2H), 3.09-3.05 (m, 4H), 1.99-1.92 (m, 1H), 1.83-1.75 (m, 1H), 1.54-1.50 (m, 2H), 1.45 (s, 3H), 1.39 (s, 9H), 1.28-1.23 (m, 2H), 1.17 (s, 3H), 1.15 (s, 3H), 0.90 (s, 9H), 0.09 (s, 6H) ppm.

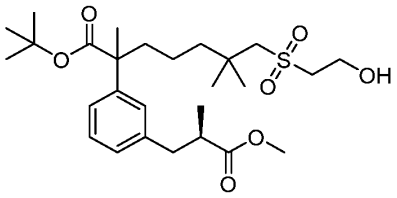
tert-Butyl 7-((2-((tert-butyldimethylsilyl)oxy)ethyl)sulfonyl)-2-(3-((R)-3-methoxy-2-methyl-3-oxopropyl)phenyl)-2,6,6-trimethylheptanoate



[87] The reaction was carried out in a glove box under positive pressure with dry nitrogen. To a stirred suspension of zinc dust (12.0 g, 184 mmol) in *N,N*-dimethylformamide (200 mL) was added iodine (0.778 g, 3.07 mmol). After 40 minutes at room temperature, the mixture was treated with methyl (*S*)-3-iodo-2-methylpropanoate (14.0 g, 61.4 mmol). The organozinc iodide reagent was allowed another 40 minutes to form before adding, in order: SPhos (2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (1.26 g, 3.07 mmol), tris(dibenzylideneacetone)dipalladium(0) (1.40 g, 1.53 mmol) and *tert*-butyl 7-((2-((*tert*-butyldimethylsilyl)oxy)ethyl)sulfonyl)-2-(3-iodophenyl)-2,6,6-trimethylheptanoate (20.0 g, 30.6 mmol). The reaction was left to stir overnight at room temperature. After this time, the mixture was removed from the glovebox and suction filtered through a plug of Celite. The

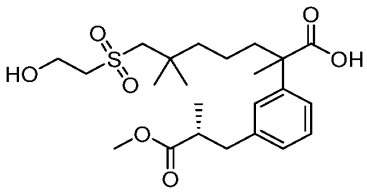
filtering agent was rinsed with ethyl acetate (~200 mL) and the combined filtrate was partitioned between water (600 mL) and ethyl acetate (200 mL). The organic layer was combined with additional extracts (ethyl acetate, 2 x 200 mL), washed with brine (2 x 200 mL) and dried over sodium sulfate. The solution was then concentrated to afford a residue which was subjected to automated flash chromatography (330 g silica gel column, 0-20% ethyl acetate in petroleum ether). The title compound was obtained as a pale amber gum (16.0 g, yield 83%). MS (ESI): 649 *m/z* $[M+Na]^+$.

tert-Butyl 7-((2-hydroxyethyl)sulfonyl)-2-(3-((R)-3-methoxy-2-methyl-3-oxopropyl)phenyl)-2,6,6-trimethylheptanoate



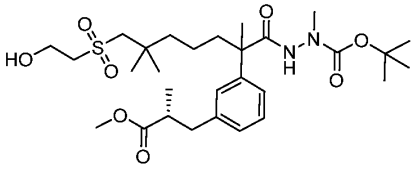
[88] To a stirred solution of *tert*-butyl 7-((2-((*tert*-butyldimethylsilyl)oxy)ethyl)sulfonyl)-2-(3-((*R*)-3-methoxy-2-methyl-3-oxopropyl)phenyl)-2,6,6-trimethylheptanoate (16.0 g, 25.5 mmol) in 1,4-dioxane (100 mL) was added a 4 M solution of hydrogen chloride in 1,4-dioxane (63.8 mL, 255 mmol). After three hours at room temperature, the reaction was concentrated. The residue was purified by automated flash chromatography (120 g silica gel column, 0-40% ethyl acetate in petroleum ether) to afford the title compound as tacky solid (12.0 g, 92%). MS (ESI): 535 *m/z* $[M+Na]^+$.

7-((2-Hydroxyethyl)sulfonyl)-2-(3-((R)-3-methoxy-2-methyl-3-oxopropyl)phenyl)-2,6,6-trimethylheptanoic acid



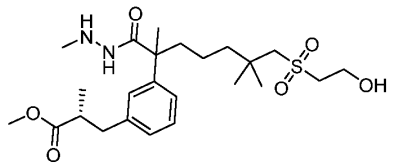
[89] To a stirred solution *tert*-butyl 7-((2-hydroxyethyl)sulfonyl)-2-(3-((*R*)-3-methoxy-2-methyl-3-oxopropyl)phenyl)-2,6,6-trimethylheptanoate (12.0 g, 23.4 mmol) in dichloromethane (100 mL) was added trifluoroacetic acid (17.4 mL, 25.9 g, 227 mmol). After two hours at room temperature, the mixture was concentrated. The residue was subjected to automated flash chromatography (120 g silica gel column, 0-60% ethyl acetate in petroleum for 40 minutes). The title compound was obtained as a pale amber solid (9.11 g, 85%). MS (ESI). 479 *m/z* $[M+Na]^+$.

tert-Butyl 2-(7-((2-hydroxyethyl)sulfonyl)-2-(3-((R)-3-methoxy-2-methyl-3-oxopropyl)phenyl)-2,6,6-trimethylheptanoyl)-1-methylhydrazine-1-carboxylate



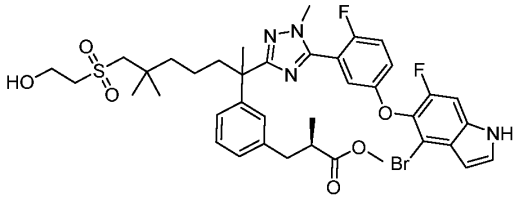
[90] To a stirred solution of 7-((2-hydroxyethyl)sulfonyl)-2-(3-((*R*)-3-methoxy-2-methyl-3-oxopropyl)phenyl)-2,6,6-trimethylheptanoic acid (9.10 g, 19.9 mmol) and *tert*-butyl 1-methylhydrazine-carboxylate (3.25 mL, 3.20 g, 21.9 mmol) in acetonitrile (100 mL) was added 1-methylimidazole (5.73 g, 69.9 mmol) followed by chloro-*N*,*N*,*N*',*N*'-tetramethylformamidinium hexafluorophosphate (5.59 g, 19.9 mmol). After one hour at room temperature, the reaction was partitioned between ethyl acetate (200 mL) and water (300 mL). The organic layer was washed with an additional portion of water (1 x 300 mL), dried over magnesium sulfate and concentrated. The residue was purified by automated flash chromatography (120 g silica gel column, 0-60% ethyl acetate in petroleum ether) to afford the title compound as a light amber gum (10.0 g, yield 86%). MS (ESI): 607 *m/z* $[M+Na]^+$.

Methyl (2R)-3-(3-(7-((2-hydroxyethyl)sulfonyl)-2,6,6-trimethyl-1-(2-methylhydrazineyl)-1-oxoheptan-2-yl)phenyl)-2-methylpropanoate



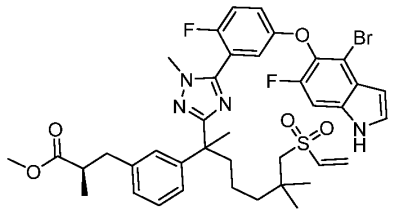
[91] To a stirred solution of *tert*-butyl 2-(7-((2-hydroxyethyl)sulfonyl)-2-(3-((*R*)-3-methoxy-2-methyl-3-oxopropyl)phenyl)-2,6,6-trimethylheptanoyl)-1-methylhydrazine-1-carboxylate (10.0 g, 17.1 mmol) in dichloromethane (100 mL) was added trifluoroacetic acid (12.4 mL, 18.5 mL, 162 mmol). After two hours at room temperature, the reaction mixture was concentrated. The residue was partitioned between ethyl acetate (200 mL) and aqueous sodium bicarbonate solution (200 mL). The organic layer was combined with additional extracts (ethyl acetate, 2 x 100 mL), dried over sodium sulfate and concentrated. The crude material was purified by automated flash chromatography (120 g silica gel column, 0-60% ethyl acetate in petroleum ether) to afford the title compound as a light amber solid (7.83 g, yield 94%). MS (ESI): 485 *m/z* $[M+H]^+$.

Methyl (2R)-3-(3-(2-(5-(5-((4-bromo-6-fluoro-1H-indol-5-yl)oxy)-2-fluorophenyl)-1-methyl-1H-1,2,4-triazol-3-yl)-7-((2-hydroxyethyl)sulfonyl)-6,6-dimethylheptan-2-yl)phenyl)-2-methylpropanoate



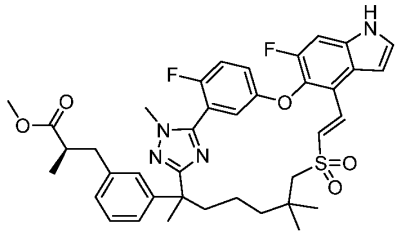
[92] Step A: To a stirred solution of methyl (2*R*)-3-(3-(7-((2-hydroxyethyl)sulfonyl)-2,6,6-trimethyl-1-(2-methylhydrazineyl)-1-oxoheptan-2-yl)phenyl)-2-methylpropanoate (7.81 g, 16.1 mmol) in pyridine (100 mL) was added methyl 5-((4-bromo-6-fluoro-1H-indol-5-yl)oxy)-2-fluorobenzimidothioate hydroiodide (9.30 g, 17.7 mmol). The mixture was heated at 80 °C for four hours and then cooled to room temperature and partitioned between 1.0 M hydrochloric acid (200 mL) and ethyl acetate (300 mL). The organic layer was washed with brine (2 x 300 mL), dried over sodium sulfate, and concentrated to afford a solid which was subjected to automated flash chromatography (80 g silica gel column, 0-60% ethyl acetate in petroleum ether). The title compound was obtained as a tan solid (7.92 g, 60%). MS(ESI): 815, 817 *m/z* $[M+H]^+$.

Methyl (2R)-3-(3-(2-(5-(5-((4-bromo-6-fluoro-1H-indol-5-yl)oxy)-2-fluorophenyl)-1-methyl-1H-1,2,4-triazol-3-yl)-6,6-dimethyl-7-(vinylsulfonyl)heptan-2-yl)phenyl)-2-methylpropanoate



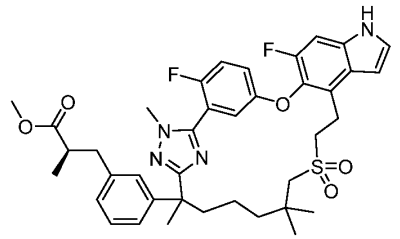
[93] Step B: To a stirred solution of step A product (7.90 g, 9.68 mmol) in dichloromethane (100 mL) was added triethylamine (4.05 mL, 2.94 g, 29.1 mmol) followed by methanesulfonyl chloride (0.825 µL, 1.22 g, 10.7 mmol). After two hours at room temperature, the mixture was partitioned between additional dichloromethane (150 mL) and brine (200 mL). The organic layer was washed with a second portion of brine (1 x 200 mL), dried over sodium sulfate and concentrated. The residue was purified by automated flash chromatography (120 g silica gel column, 0-50% ethyl acetate in petroleum ether) to afford the title compound as tan solid (6.50 g, 84%). MS(ESI): 797, 799 *m/z* $[M+H]^+$.

Methyl (2R)-3-[3-[22,28-difluoro-3,6,10,10-tetramethyl-12,12-dioxo-24-oxa-12λ6-thia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,13,15,17,20,22,25,27-decaen-6-yl]phenyl]-2-methyl-propanoate



[94] Step C: The reaction was carried out in a glove box under a positive pressure of dry nitrogen. To a stirred solution of step B product (6.50 g, 8.15 mmol) in toluene (1 L) was added bis(tri-*tert*-butylphosphine)palladium(0) (0.833 g, 1.63 mmol) and triethylamine (11.4 mL, 8.28 g, 81.8 mmol). The mixture was heated at 100 °C for two hours and then cooled to room temperature and concentrated. The residue was partitioned between ethyl acetate (200 mL) and water (200 mL). The organic layer was washed with a second portion of water (1 x 200 mL), dried over sodium sulfate and concentrated to afford a brown solid. This crude material was subjected to automated flash chromatography (80 g silica gel column, 0-50% ethyl acetate in petroleum ether). The title compound was obtained as a pale amber solid (2.81 g, 48%). MS (ESI): 717 *m/z* $[M+H]^+$.

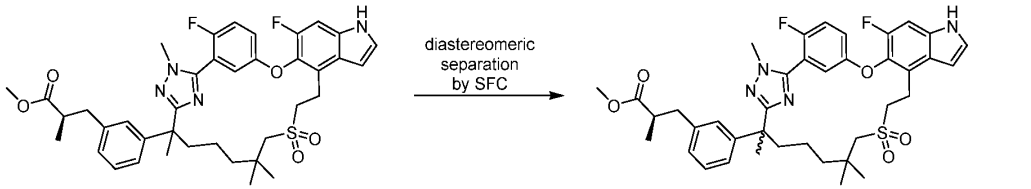
Methyl (2R)-3-[3-(22,28-difluoro-3,6,10,10-tetramethyl-12,12-dioxo-24-oxa-12λ6-thia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl)phenyl]-2-methyl-propanoate



[95] Step D: A stirred suspension of step C product (2.80 g, 3.91 mmol) and 10% palladium on carbon (0.800 g of a 50% aqueous dispersion) in ethanol (50 mL) was cycled between vacuum and a nitrogen atmosphere three times. After a final evacuation, the reaction vessel was backfilled with hydrogen (*via* balloon). The mixture was heated at 50 °C for two hours and then cooled to room temperature and opened to air. The catalyst was removed by suction filtration through a pad of Celite, which was subsequently rinsed with ethanol (2 x 30 mL). The combined filtrate was concentrated to afford the crude title compound, which was

used without purification, as a white solid (2.62 g, 93%). MS (ESI): 719 *m/z* $[M+H]^+$.

Diastereomers 1 and 2 of Methyl (2R)-3-[3-(22,28-difluoro-3,6,10,10-tetramethyl-12,12-dioxo-24-oxa-12λ6-thia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl)phenyl]-2-methyl-propanoate



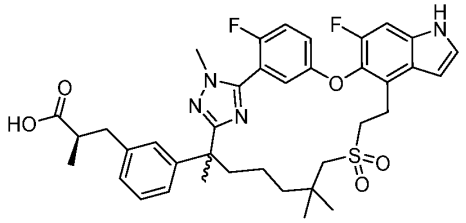
Diastereomer 1 (first eluting isomer)
Diastereomer 2 (second eluting isomer)
(absolute configuration of quaternary chiral center unknown)

[96] Step E: Step D product was separated into its two constituent diastereomers by SFC using a Thar SFC-200 instrument and the following separation conditions. Column: 20 x 250 mm x 10 μm CHIRALPAK AD; sample solution: 2.50 g dissolved in methanol (140 mL); injection volume: 1.5 mL; eluant: 85:15 CO_2/methanol with 0.2% ammonia/methanol additive; flow rate: 130 mL/min; column temperature: 35 °C; back pressure: 100 bar; detection wavelength: 214 nm. The first eluting isomer was designated Diastereomer 1 (1.16 g, 46%) and the second eluting isomer, Diastereomer 2 (1.07 g, 43%). Both were obtained as white solids.

Diastereomer 1: ^{1}H NMR (400 MHz, CD_3OD) δ 7.40-7.30 (m, 3H), 7.26-7.14 (m, 3H), 7.07-7.02 (m, 1H), 7.00-6.93 (m, 2H), 6.63 (dd, *J* = 3.2, 0.7 Hz, 1H), 3.87 (d, *J* = 2.3 Hz, 3H), 3.56 (s, 3H), 3.43-3.35 (m, 2H), 3.31-3.17 (m, 2H), 2.97 (d, *J* = 13.5 Hz, 1H), 2.89-2.78 (m, 2H), 2.71-2.60 (m, 2H), 2.20-2.09 (m, 1H), 1.88-1.79 (m, 1H), 1.69 (s, 3H), 1.67-1.52 (m, 1H), 1.39-1.17 (m, 3H), 1.11-1.01 (m, 9H) ppm.

Diastereomer 2: ^{1}H NMR (400 MHz, CD_3OD) δ 7.40-7.31 (m, 3H), 7.26-7.14 (m, 3H), 7.05-7.00 (m, 1H), 7.00-6.94 (m, 2H), 6.63 (dd, *J* = 3.2, 0.7 Hz, 1H), 3.88 (d, *J* = 2.3 Hz, 3H), 3.55 (s, 3H), 3.43-3.35 (m, 2H), 3.32-3.16 (m, 2H), 2.95 (d, *J* = 13.6 Hz, 1H), 2.91-2.83 (m, 1H), 2.80 (d, *J* = 13.6 Hz, 1H), 2.71-2.60 (m, 2H), 2.21-2.10 (m, 1H), 1.87-1.77 (m, 1H), 1.68 (s, 3H), 1.67-1.55 (m, 1H), 1.39-1.28 (m, 1H), 1.25-1.16 (m, 1H), 1.13-0.98 (m, 10H) ppm.

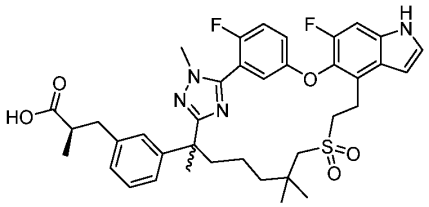
Compound 1 *(Diastereomer 1 of (2R)-3-[3-(22,28-Difluoro-3,6,10,10-tetramethyl-12,12-dioxo-24-oxa-12λ6-thia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl)phenyl]-2-methyl-propanoic acid*



[97] Step F: To a stirred solution of the Diastereomer 1 product of step E (1.10 g, 1.53 mmol) in 3:1 tetrahydrofuran/methanol (20 mL) was added a 1.0 M aqueous lithium hydroxide solution (4.6 mL, 4.6 mmol). After four hours at room temperature, the reaction was acidified with the addition of 1.0 M hydrochloric acid (10 mL). The resulting suspension was extracted with ethyl acetate (1 x 50 mL) and the organic layer was washed with brine (2 x 20 mL), dried over sodium sulfate and concentrated. The crude product was purified by automated flash chromatography (12 g silica gel column, 0-70% ethyl acetate in petroleum ether) to afford **1** as a white solid (0.902 g, 84%). To confirm that the propanoic acid chiral center of the molecule had not epimerized following its incorporation into the molecule (Negishi coupling reaction with (*S*)-3-methoxy-2-methyl-3-oxopropylzinc iodide), the compound was examined by chiral HPLC using a method capable of analytically resolving **1** from its enantiomer and its propanoic chiral center inverted epimer. Chiral purity was determined to be ≥97.4%, with 1.6% contamination from the α-carbonyl epimerized isomer and 1% or less from the enantiomer. Given the stated purity of the methyl (*R*)-3-hydroxy-2-methylpropionate starting material (99%) used to prepare the zinc iodide reagent (steps A and H), this result indicated minimal erosion of chiral purity induced by subsequent synthetic steps. The chiral analysis was carried out using an Agilent 1200 HPLC system and the following separation conditions. Column: 4.6 x 250 mm x 5 µm Chiralpak AD-H; sample solution: 1.0 mg/mL; injection volume: 10 µL; eluant: 85:15 heptane/isopropanol with 0.1% trifluoroacetic acid; flow rate: 1.0 mL/min; column temperature: 23 °C; detection wavelength: 254 nm. Observed retention times: **1**, 16.75 minutes; epimer, 12.81 minutes; enantiomer, 20.56 minutes.

Compound 1: ^{1}H NMR (400 MHz, CD_3OD) δ 7.39-7.30 (m, 3H), 7.25-7.13 (m, 3H), 7.07-6.97 (m, 3H), 6.63 (d, J = 3.2 Hz, 1H), 3.87 (d, J = 2.1 Hz, 3H), 3.44-3.37 (m, 2H), 3.36-3.27 (m, 1H), 3.26-3.16 (m, 1H), 2.99-2.88 (m, 2H), 2.81 (d, J = 13.6 Hz, 1H), 2.68-2.55 (m, 2H), 2.16 (dt, J = 12.8, 3.6 Hz, 1H), 1.84 (dt, J = 12.8, 4.4 Hz, 1H), 1.75-1.51 (m, 4H), 1.40-1.28 (m, 1H), 1.28-1.14 (m, 1H), 1.14-0.95 (m, 10H) ppm. MS (ESI): 705 *m/z* $[M+H]^+$. LC R_t, purity (LC-MS Method 01): 1.44 minutes, >99.9%/96.8% (210/254 nm).

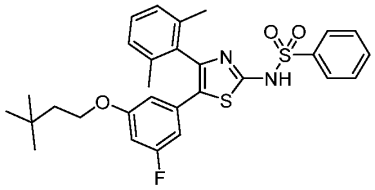
Compound 1A *(Diastereomer 2 of (2R)-3-[3-(22,28-Difluoro-3,6,10,10-tetramethyl-12,12-dioxo-24-oxa-12λ6-thia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl)phenyl]-2-methyl-propanoic acid*



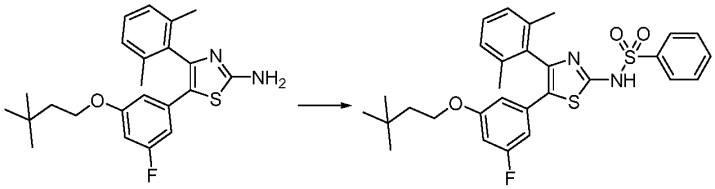
[98] Step G: Using a procedure identical to that described in step F, Diastereomer 2 product of step E (1.00 g, 1.39 mmol) was hydrolyzed to afford the title compound as a white solid (0.846 g, 86%).

Compound 1A: ^{1}H NMR (400 MHz, CD_3OD) δ 7.40-7.29 (m, 3H), 7.27-7.12 (m, 3H), 7.09-6.94 (m, 3H), 6.63 (d, J = 3.1 Hz, 1H), 3.87 (d, J = 2.0 Hz, 3H), 3.47-3.35 (m, 2H), 3.33-3.16 (m, 2H), 3.02-2.88 (m, 2H), 2.82 (d, J = 13.6 Hz, 1H), 2.69-2.53 (m, 2H), 2.16 (dt, J = 12.7, 3.2 Hz, 1H), 1.83 (dt, J = 12.7, 4.2 Hz, 1H), 1.76-1.52 (m, 4H), 1.41-1.27 (m, 1H), 1.27-1.14 (m, 1H), 1.14-0.95 (m, 10H) ppm. MS (ESI): 705 m/z $[M+H]^+$. LC R_t, purity (LC-MS Method 01): 1.44 minutes, >99.9%/>99.9% (210/254 nm).

Example 2. Synthesis of Compound 2: N-(5-(3-(3,3-Dimethylbutoxy)-5-fluorophenyl)-4-(2,6-dimethylphenyl)thiazol-2-yl)benzenesulfonamide (Compound 2)



Step 1.



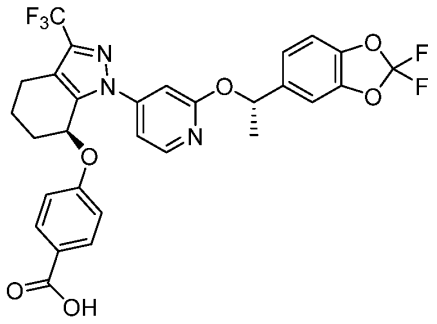
[99] To a mixture of 5-[3-(3,3-dimethylbutoxy)-5-fluoro-phenyl]-4-(2,6-dimethylphenyl)thiazol-2-amine (**Intermediate C-6a as described in WO2021/097057**) (300 mg, 0.75 mmol) and pyridine(3.0 mL) was added benzenesulfonyl chloride (0.192 mL, 1.51 mmol). The reaction was stirred at 130 °C in a microwave oven for 3 h. The reaction was cooled to rt and then diluted with brine (20 mL). The aqueous solution was extracted

with ethyl acetate (40 mL × 2). The combined organics were dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by Prep-HPLC to afford the title compound, N-[5-[3-(3,3-dimethylbutoxy)-5-fluoro-phenyl]-4-(2,6-dimethylphenyl)thiazol-2-yl]benzenesulfonamide (206 mg, 51%) as a white solid.

LCMS: LC retention time 1.76 min. MS (ESI) *m/z* 539 $[M+H]^+$.

^{1}H NMR (400 MHz, chloroform-*d*) δ 9.73 (s, 1H), 7.91 (d, *J* = 7.5 Hz, 2H), 7.52 (dt, *J* = 32.1, 7.3 Hz, 3H), 7.35–7.04 (m, 3H), 6.55–6.21 (m, 3H), 3.66 (t, *J* = 7.2 Hz, 2H), 2.13 (s, 6H), 1.61 (t, *J* = 7.1 Hz, 2H), 0.95 (s, 9H) ppm.

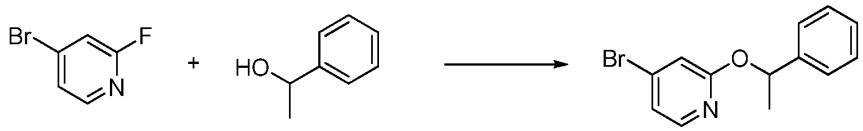
Example 3. Synthesis of 4-[[(7S)-1-[2-[(1S)-1-(2,2-difluoro-1,3-benzodioxol-5-yl)ethoxy]-4-pyridyl]-3-(trifluoromethyl)-4,5,6,7-tetrahydroindazol-7-yl]oxy]benzoic acid (Compound 3)



3

Synthetic method 1 of the synthesis of Example 3, Compound 3.

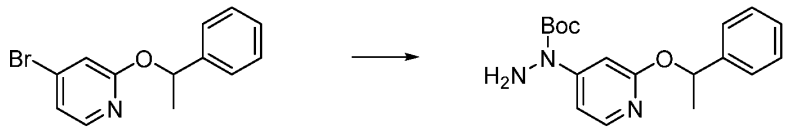
Step 1. Synthesis of 4-bromo-2-(1-phenylethoxy)pyridine



[100] To a solution of 4-bromo-2-fluoro-pyridine (20 gm, 114 mmol) and 1-phenylethanol (13.90 gm, 114 mmol) in DMF (200 mL) was added cesium carbonate (111.08 gm, 341 mmol). Then the mixture was stirred at 100 °C overnight. The crude product was then purified by flash chromatography eluted with 10% EtOAc in isohexane to get the title compound (29.0 gm, 92% yield) as a yellow solid.

LC-MS (Method 1): Retention time = 2.45 min. MS (ESI) m/z 174.1 / 176.1 $(M-PhCHMe+H)^+$.

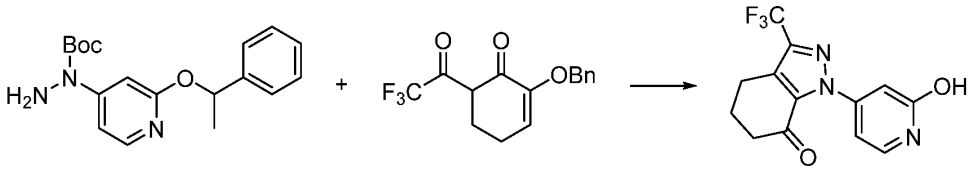
Step 2. Synthesis of tert-butyl 1-(2-(1-phenylethoxy)pyridine-4-yl)hydrazinecarboxylate



[101] To a solution of 4-bromo-2-(1-phenylethoxy)pyridine (15.0 gm, 53.9 mmol) in 1,4-dioxane (150 mL) was added tert-butyl N-aminocarbamate (8.55 gm, 64.7 mmol), (9,9-dimethyl-9H-xanthene-4,5-diyl)bis(diphenylphosphine) (3.12 gm, 5.39 mmol), tris(dibenzylideneacetone) dipalladium (4.94 gm, 5.39 mmol), and cesium carbonate (52.71 gm, 162 mmol). Then the mixture was stirred at 100 °C overnight under argon. The crude product was then purified by flash column chromatography eluted with 10% EtOAc in isohexane to get the title compound (16.0 gm, 90% yield) as a yellow solid.

LC-MS (Method 1): Retention time = 2.11 min. MS (ESI) m/z 330 $(M+H)^+$.

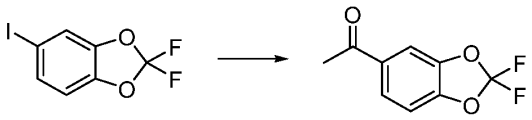
Step 3. Synthesis of 1-(2-hydroxypyridin-4-yl)-3-(trifluoromethyl)-1,4,5,6-tetrahydro-7H-indazol-7-one



[102] To a solution of tert-butyl 1-(2-(1-phenylethoxy)pyridine-4-yl)hydrazinecarboxylate (10 g, 30.4 mmol) and 2-(benzyloxy)-6-(2,2,2-trifluoroacetyl)cyclohex-2-en-1-one in 2,2,2-trifluoroethanol (100 mL) was added sulfuric acid (25 mL). Then the mixture was stirred at 80 °C overnight. The mixture was poured into water (500 mL), the pH was adjusted to 8-9 with $NaHCO_3$, and then extracted with EA (3 x 50 mL). The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude product was then purified by flash column chromatography eluted with 10% EtOAc in isohexane to get the title compound (5 g, 55% yield) as a yellow solid.

LC-MS (Method 1): Retention time = 1.72 min. MS (ESI) m/z 298.3 $(M+H)^+$

Step 4. Synthesis of 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)ethan-1-one



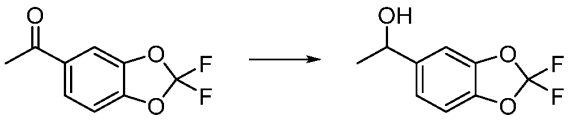
[103] To a solution of 1-ethoxyvinyltri-n-butyltin (1.7 mL, 4.93 mmol) in toluene (20 mL) was added 2,2-difluoro-5-iodo-1,3-benzodioxole (1.40 g, 4.93 mmol) and $Pd(PPh_3)_2Cl_2$ (138 mg, 0.20 mmol). The mixture was stirred at 90 °C for 12 h under N_2, then cooled to rt. HCl (30 mL, 60.0 mmol) was added and stirred for 0.5 hours. The mixture was extracted with EA

(30 mL × 3). The organic layers were combined, washed with brine (30 mL), dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography eluted with 5% EtOAc in isohexane to get the title compound (950 mg, 95% yield) as a colorless oil.

LC-MS (Method 1): Retention time = 2.02 min. MS (ESI) m/z 201.0 $(M+H)^+$.

^1H NMR (500 MHz, $CDCl_3$) δ 7.77 (dd, *J* = 8.3, 1.6 Hz, 1H), 7.69 (d, *J* = 1.6 Hz, 1H), 7.14 (d, *J* = 8.3 Hz, 1H), 2.60 (s, 3H).

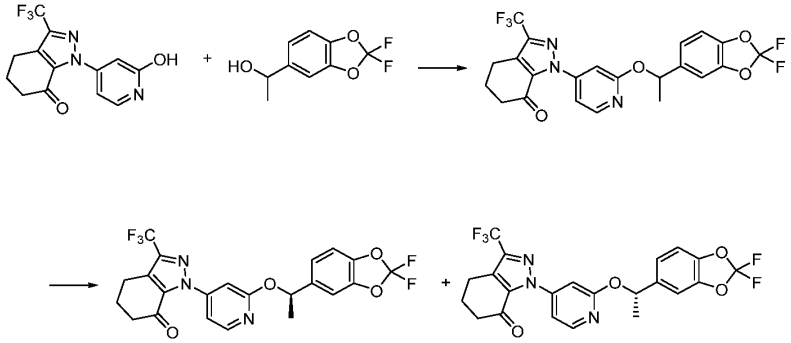
Step 5. Synthesis of 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)ethan-1-ol



[104] To a solution of 1-(2,2-difluoro-1,3-benzodioxol-5-yl)ethanone (900 mg, 4.50 mmol) in methanol (10 mL) was added $NaBH_4$ (340 mg, 8.99 mmol) at 0 °C and stirred for 2 hours. Then the mixture was poured into water (40 mL) and extracted with EA (3 x 30 mL). The organic layers were combined, washed with brine (30 mL), dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash column chromatography eluted with 10% EtOAc in isohexane to get the title compound (860 mg, 94.6% yield) as a colorless oil.

LC-MS (Method 1): Retention time = 1.94 min. MS (ESI) m/z 185.0 $(M-H_2O+H)^+$.

Step 6. Synthesis of 1-(2-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)ethoxy)pyridin-4-yl)-3-(trifluoromethyl)-1,4,5,6-tetrahydro-7H-indazol-7-one

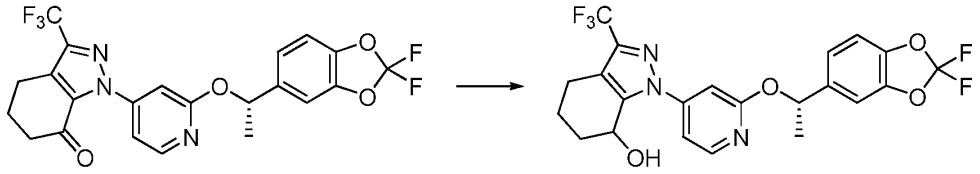


[105] To a solution of 1-(2-hydroxy-4-pyridyl)-3-(trifluoromethyl)-5,6-dihydro-4H-indazol-7-one (4000 mg, 13.5 mmol) in THF (50 mL) were added 1-(2,2-difluoro-1,3-benzodioxol-5-yl)ethanol (3265 mg, 16.1 mmol), and triphenylphosphine (4236 mg, 16.1 mmol). After the solution was cooled to 0 °C, diethyl azodicarboxylate (2.6 mL, 16.1 mmol) was added dropwise at 0 °C and then stirred at 25 °C for 1 hour. The solution was concentrated, and the crude product was purified by flash column chromatography eluted with 10% EtOAc in

isohexane to obtain the racemic product (4500 mg, 69% yield) which was purified by SFC to obtain both enantiomers of the title compound. The enantiomer eluting first (more mobile fractions) was designated as **P1** (2100 mg, 47% yield) and the enantiomer eluting second designated as **P2** (2000 mg, 44% yield).

LC-MS (Method 2): Retention time = 2.36 min. MS (ESI) m/z 482.0 $(M+H)^+$.

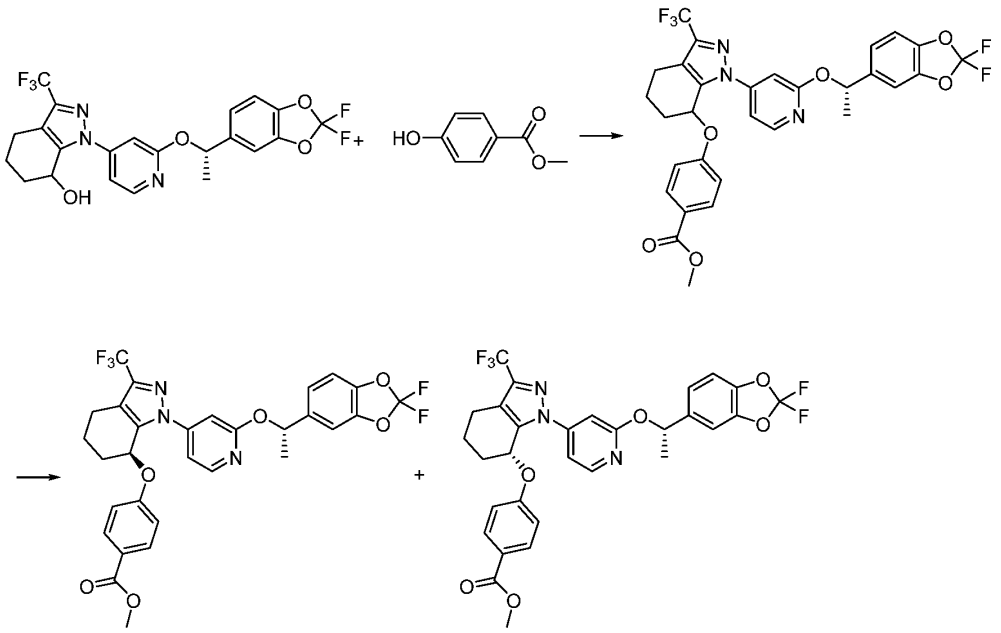
Step 7. Synthesis of 1-(2-((S)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)ethoxy)pyridin-4-yl)-3-(trifluoromethyl)-4,5,6,7-tetrahydro-1H-indazol-7-ol



To a solution of 1-[2-[(1R)-1-(2,2-difluoro-1,3-benzodioxol-5-yl)ethoxy]-4-pyridyl]-3-(trifluoromethyl)-5,6-dihydro-4H-indazol-7-one **(P2** of **Step 6** above**)** (400 mg, 0.831 mmol) in methanol (10 mL) was added sodium borohydride (126 mg, 3.32 mmol). Then the mixture was stirred at rt for 2 h under argon. Then the mixture was quenched with water (50 mL) and extracted with EA (50 mL × 3). The organic layer was washed with brine (20 mL) and dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography (Biotage, 40 g silica gel column @ 60mL/min, eluting with 0-30% ethyl acetate in petroleum for 20 min) to get the title compound (350 mg, 82.8 %) as a light yellow oil.

LCMS (Method 1): Rt 2.38 min. MS (ESI) m/z 300.1 $(M+H)^+$.

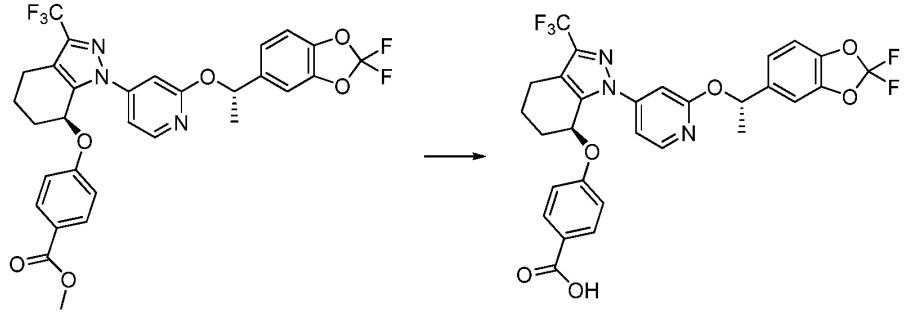
Step 8. Synthesis of methyl 4-((1-(2-((S)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)ethoxy)pyridin-4-yl)-3-(trifluoromethyl)-4,5,6,7-tetrahydro-1H-indazol-7-yl)oxy)benzoate



[106] To the solution of 1-[2-[(1S)-1-(2,2-difluoro-1,3-benzodioxol-5-yl)ethoxy]-4-pyridyl]-3-(trifluoromethyl)-4,5,6,7-tetrahydroindazol-7-ol (product from **Step** 7 above) (800 mg, 1.65 mmol) in THF (10 mL) were added methyl 4-hydroxybenzoate (302 mg, 1.99 mmol) and PPh_3 (521 mg, 1.99 mmol). After the solution was stirred at 0 °C for 5 min, DEAD (0.31 mL, 1.99 mmol) was added dropwise at 0 °C. The solution was then stirred at 25 °C for 1 h. The solution was concentrated, and the crude product was then purified by flash column chromatography eluted with 10% ethyl acetate in isohexane to get the mixture of diastereomers (650 mg, 69% yield) which was further separated by SFC to get the title compounds with the first fraction designated as **P1** (300 mg, 47%; a yellow oil), and the second fractions as **P2** (300 mg, 44%; a yellow oil).

LC-MS (Method 1): Retention time = 2.65 min. MS (ESI) m/z 618.2 $[M+H]^+$.

Step 9. Synthesis of 4-[[(7S)-1-[2-[(1S)-1-(2,2-difluoro-1,3-benzodioxol-5-yl)ethoxy]-4-pyridyl]-3-(trifluoromethyl)-4,5,6,7-tetrahydroindazol-7-yl]oxy]benzoic acid

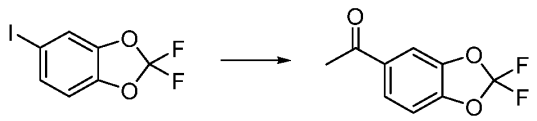


[107] To the solution of methyl 4-(((S)-1-(2-((S)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)ethoxy)pyridine-4-yl)-3-(trifluoromethyl)-4,5,6,7-tetrahydro-1H-indazol-7-yl)oxy)benzoate (product **P1** from **Step 8** above) (300 mg, 0.486 mmol) in methanol (10 mL), THF (10 mL) and water (5 mL) was added lithium hydroxide (47 mg, 1.94 mmol). The solution was then stirred at 25 °C overnight. The solution was concentrated under vacuum and purified by prep-HPLC to get the title compound (185 mg, 48 % yield) as white solid.
LC-MS (Method 1): Retention time = 1.86 min. MS (ESI) m/z 602.0 $[M-H]^+$.
^{1}H NMR (500 MHz, CH_3OH-d_4) δ 8.06 (d, *J* = 7.5 Hz, 1H), 8.03 (d, *J* = 11 Hz, 2H), 7.16 (dd, *J* = 7.0, 2.5 Hz, 1H), 7.12 (dd, *J* = 7.0, 5.0 Hz, 2H), 7.05 (d, *J* = 1.7 Hz, 1H), 7.03 – 6.87 (m, 3H), 6.03 (q, *J* = 8.0 Hz, 1H), 5.74 (t, *J* = 4.5 Hz, 1H), 2.87 (d, *J* = 21 Hz, 1H), 2.63 (ddd, *J* = 16.7, 11.0, 5.7 Hz, 1H), 2.29 (d, *J* = 17 Hz, 1H), 2.00 (dd, *J* = 12.2, 6.9 Hz, 1H), 1.95-1.75 (m, 2H), 1.43 (d, *J* = 8.0 Hz, 3H).

Synthetic method 2 of the synthesis of Example 3, Compound 3.

Synthesis of 4-[[(7S)-1-[2-[(1S)-1-(2,2-difluoro-1,3-benzodioxol-5-yl)ethoxy]-4-pyridyl]-3-(trifluoromethyl)-4,5,6,7-tetrahydroindazol-7-yl]oxy]benzoic acid

Step 1. Synthesis of 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)ethan-1-one



[108] To a solution of 1-ethoxyvinyltri-n-butyltin (1.7 mL, 4.93 mmol) in toluene (20 mL) was added 2,2-difluoro-5-iodo-1,3-benzodioxole (1.40 g, 4.93 mmol) and $Pd(PPh_3)_2Cl_2$ (138 mg, 0.20 mmol). The reaction was stirred at 90 °C for 12 h under N_2, then cooled to rt. HCl (30 mL, 60.0 mmol) was added and stirred for 0.5 h. The mixture was extracted with EA (30 mL × 3). The organic layers were combined, washed with brine (30 mL), dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash column chromatography eluted with 5% ethyl acetate in isohexane to get the title compound (950 mg, 95% yield) as a colorless oil.
LC-MS (Method 1): Retention time = 2.02 min. MS (ESI) m/z 201.0 $(M+H)^+$.
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (dd, *J* = 8.3, 1.6 Hz, 1H), 7.69 (d, *J* = 1.6 Hz, 1H), 7.14 (d, *J* = 8.3 Hz, 1H), 2.60 (s, 3H).

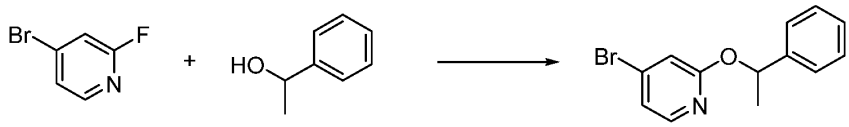
Step 2. Synthesis of (R)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)ethan-1-ol



[109] To a solution of (*S*)-2-methyl-CBSoxazaborolidine (Ref. 1) [(8.25 g, 0.03 mol) in DCM (200 mL) under N_2 was added BH_3·Me_2S (150 mL, 2 M in DCM) at 0°C. The reaction was stirred at room temperature for 0.5 h, and then the solution of 1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)ethan-1-one (60 g, 0.30 mol) in DCM (300 mL) was added dropwise at 0 °C for 1 h. The reaction mixture was stirred at room temperature for 4 h. The reaction was quenched by addition of MeOH at 0 °C, and then concentrated in vacuo to give the residue which was purified by flash silica gel column chromatography (ethyl acetate in petroleum ether from 0% to 10%) to afford the title compound (56 g, 92 % yield) as a light-yellow liquid.

LC-MS: Retention time = 1.75 min. MS (ESI) m/z 185.0 $[M-H_2O+H]^+$.

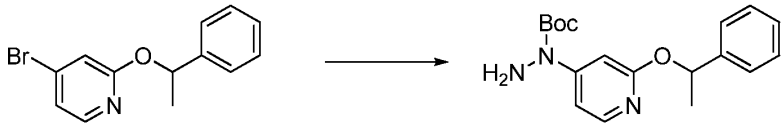
Step 3. Synthesis of 4-bromo-2-(1-phenylethoxy)pyridine



[110] To a solution of 4-bromo-2-fluoro-pyridine (20.0 g, 114 mmol) and 1-phenylethanol (13.9 g, 114 mmol) in DMF (200 mL) was added Cs_2CO_3 (111 g, 342 mmol). The reaction was stirred at 100 °C overnight. The mixture was poured into water (2 L) and extracted with EA (200 mL × 3). The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude was then purified by flash column chromatography eluting with 10% ethyl acetate in PE to get the title compound (29.0 g, 91.8 %) as a yellow solid.

LCMS (Method 1): Retention time = 2.45 min. MS (ESI) m/z 174.1, 176.1 $[M-103]^+$.

Step 4. Synthesis of tert-butyl 1-(2-(1-phenylethoxy)pyridin-4-yl)hydrazinecarboxylate

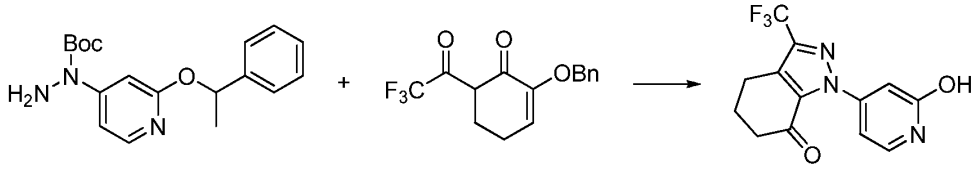


[111] To the solution of 4-bromo-2-(1-phenylethoxy)pyridine (15.0 g, 53.9 mmol) in 1,4-

dioxane (150 mL) were added tert-butyl N-amino carbamate (8.6 g, 64.7 mmol), XantPhos (3.1 g, 5.39 mmol), tris(dibenzylideneacetone) dipalladium (4.9 g, 5.39 mmol), and Cs_2CO_3 (52.8 g, 162 mmol). Then the mixture was stirred at 100 °C overnight under Ar. The mixture was poured into water (2 L) and extracted with EA (200 mL × 3). The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude was then purified by flash column chromatography eluting with 10% ethyl acetate in PE to get the title compound (16.0 mg, 90.1 %) as a yellow solid.

LCMS (Method 1): Retention time = 2.11 min. MS (ESI) m/z 226 $[M-103]^+$.

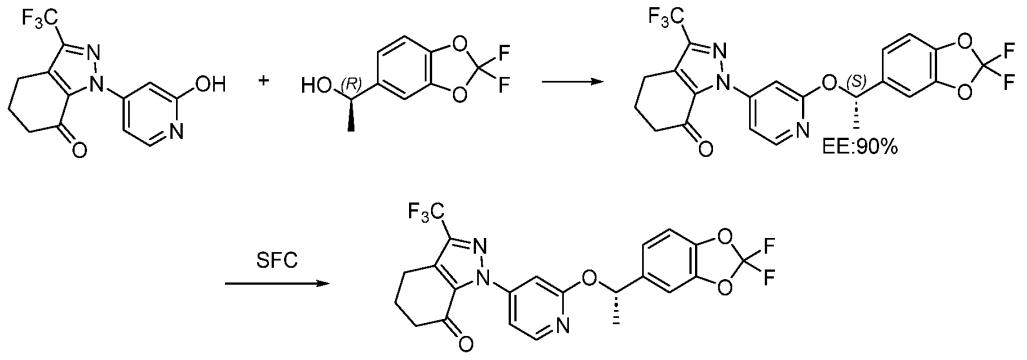
Step 5. Synthesis of 1-(2-hydroxypyridin-4-yl)-3-(trifluoromethyl)-5,6-dihydro-1H-indazol-7(4H)-one



[112] To a solution of tert-butyl N-amino-N-[2-(1-phenylethoxy)-4-pyridyl]carbamate (10.0 g, 30.4 mmol) in 2,2,2-trifluoroethanol (100 mL) was added sulfuric acid (25 mL). Then the mixture was stirred at 80 °C overnight. The mixture was poured into water (500 mL) and adjusted pH to 8-9 with $NaHCO_3$, then extracted with EA (50 mL x 3). The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude was then purified by flash column chromatography eluting with 10% ethyl acetate in PE to get the title compound (5.0 g, 55.4 %) as a yellow solid.

LCMS (Method 1): Retention time = 1.72 min. MS (ESI) m/z 298.3 $[M+H]^+$.

Step 6. Synthesis of (S)-1-(2-(1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)ethoxy)pyridin-4-yl)-3-(trifluoromethyl)-5,6-dihydro-1H-indazol-7(4H)-one

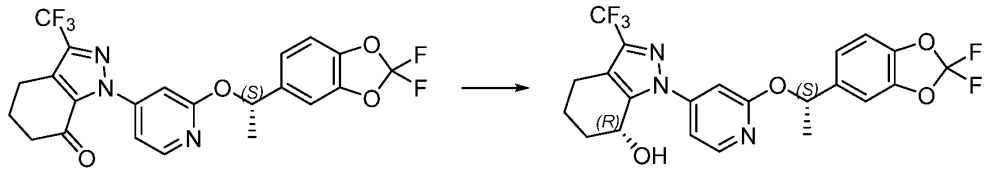


[113] To a solution of 1-(2-hydroxy-4-pyridyl)-3-(trifluoromethyl)-5,6-dihydro-4H-indazol-7-one (4000 mg, 13.5 mmol) in THF (50 mL) were added (R)-1-(2,2-

difluorobenzo[d][1,3]dioxol-5-yl)ethan-1-ol (Product from **Step 2** above) (3265 mg, 16.1 mmol), and PPh_3 (4236 mg, 16.1 mmol). After the mixture was cooled to 0 °C, DEAD (2.6 mL, 16.1 mmol) was added dropwise at 0 °C and then stirred at rt for 1 h under N_2. The mixture was concentrated, and the crude was then purified by flash column chromatography eluting with 10% ethyl acetate in PE to get the crude compound (4.5 g, ee 90%). This crude compound was purified further by SFC to afford the title compound (3.2 g, 49%, ee >99%) as a white solid.

LCMS (Method 2): Retention time = 2.36 min. MS (ESI) m/z 298.0 $(M-183)^+$.

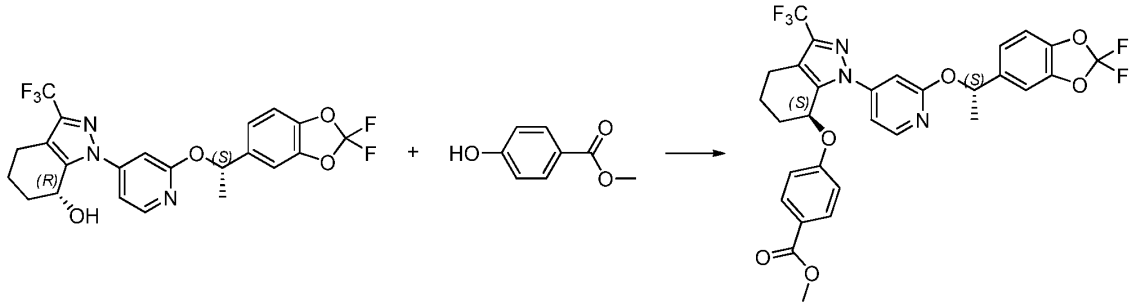
Step 7. Synthesis of (R)-1-(2-((S)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)ethoxy)pyridin-4-yl)-3-(trifluoromethyl)-4,5,6,7-tetrahydro-1H-indazol-7-ol



[114] In a 200 mL reactor, the product from Step 6 (5.0 g, 10.4 mmol) was suspended in IPA (yellow suspension) (10 vol) at room temperature. TEA (0.6 vol) and catalyst (R, R)-TsDPEN-RuCl (p-cymene) (0.006 wt) were added to the mixture, and 3 cycles of vacuum/nitrogen exchange were applied. Formic acid (0.4 vol) was added slowly to the mixture in 10 min. (exothermic reaction from 20 to 25 °C). Temperature was increased to 45 °C and the solution was stirred for 4 h. Reaction mixture was concentrated under vacuum. Water (10 vol) was added and adjusted pH to 7-8 with 5% $NaHCO_3$ aqueous solution, then extracted with EA (2 vol × 3). The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash column chromatography eluting with 20% ethyl acetate in isohexane to get the title compound (3.0 g, 60%) as a yellow solid.

LCMS (Method 1): Retention time = 1.73 min. MS (ESI) m/z 300 $[M-183]^+$.

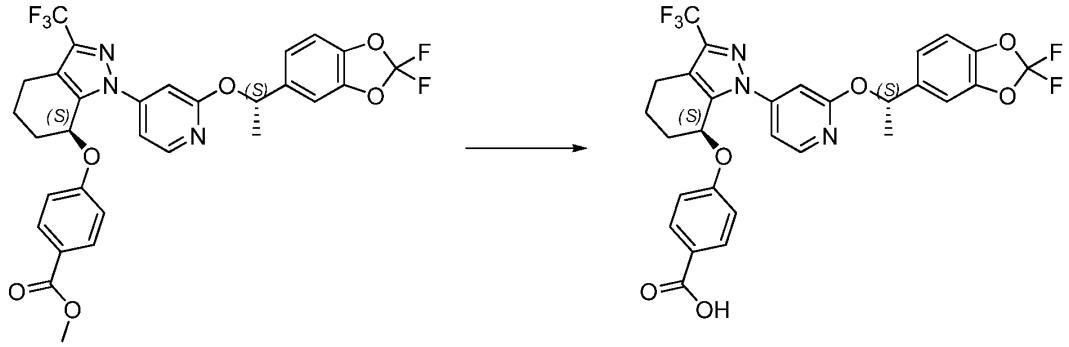
Step 8. Synthesis of methyl 4-(((S)-1-(2-((S)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)ethoxy)pyridin-4-yl)-3-(trifluoromethyl)-4,5,6,7-tetrahydro-1H-indazol-7-yl)oxy)benzoate



[115] To a solution of (R)-1-(2-((S)-1-(2,2-difluorobenzo[d][1,3]dioxol-5-yl)ethoxy)pyridin-4-yl)-3-(trifluoromethyl)-4,5,6,7-tetrahydro-1H-indazol-7-ol (product from **Step 7** above) (3000 mg, 6.2 mmol) in THF (10 mL) were added methyl 4-hydroxybenzoate (1.1 g, 7.4 mmol) and PPh_3 (2.4 g, 9.3 mmol). The mixture was cooled to 0 °C and DEAD (1.6 g, 9.3 mmol) was added dropwise at 0 °C under N_2. Then the mixture was stirred at rt for 1 h. The mixture was concentrated, and the crude was then purified by flash column chromatography eluting with 10% ethyl acetate in PE to get the title compound (3.0 g, 79 %) as a white solid.

LCMS (Method 1): Retention time = 2.65 min. MS (ESI) m/z 434.2 $[M-183]^+$.

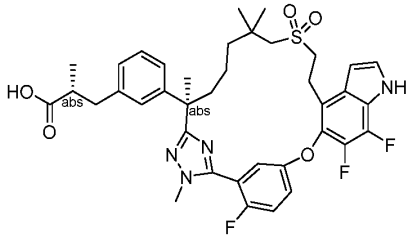
Step 9. Synthesis of 4-[[(7S)-1-[2-[(1S)-1-(2,2-difluoro-1,3-benzodioxol-5-yl)ethoxy]-4-pyridyl]-3-(trifluoromethyl)-4,5,6,7-tetrahydroindazol-7-yl]oxy]benzoic acid



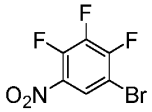
[116] To a solution of methyl 4-[[(7S)-1-[2-[(1S)-1-(2,2-difluoro-1,3-benzodioxol-5-yl)ethoxy]-4-pyridyl]-3-(trifluoromethyl)-4,5,6,7-tetrahydroindazol-7-yl]oxy]benzoate (3.0 g, 4.86 mmol) in methanol (20 mL), THF (20 mL) and water (10 mL) was added lithium hydroxide (465 mg, 19.4 mmol). The reaction was stirred at 25 °C overnight. Then the mixture was concentrated, and the crude was purified by pre-HPLC (NH_4HCO_3) to get the title compound (2204 mg, 75.2 %) as a white solid.

The analytics (LCMS and ^{1}HNMR) are identical as the sample obtained through the synthetic **Method 1**.

Example 4. Synthesis of Compound 4. (2R)-2-methyl-3-[3-[(6R)-21,22,28-trifluoro-3,6,10,10-tetramethyl-12,12-dioxo-24-oxa-12λ6-thia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl]phenyl]propanoic acid

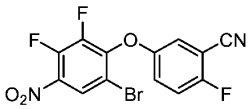


1-Bromo-2,3,4-trifluoro-5-nitrobenzene



[117] Step A: To a stirred solution of 1,2,3-trifluoro-4-nitrobenzene (30 g, 169.5 mmol) in concentrated sulfuric acid (150 mL) was added 1,3-dibromo-5,5-dimethylimidazolidine-2,4-dione (24 g, 84.7 mmol) at 0 °C. The mixture was stirred at room temperature overnight. The mixture was slowly and carefully added to ice water (600 g ice and 100 mL water) to keep the temperature below 30 °C and extracted with heptane (300 mL x 3). The combined organic extracts were washed with water and brine, dried over magnesium sulfate, filtered, and evaporated to dryness. The resulting residue was purified by flash chromatography (silica gel, heptane) to give the title compound (32 g, 75%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (td, J = 7.2, 2.8 Hz, 1H) ppm.

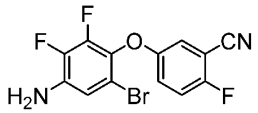
5-(6-Bromo-2,3-difluoro-4-nitrophenoxy)-2-fluorobenzonitrile



[118] **Step B:** To a stirred solution of 1-bromo-2,3,4-trifluoro-5-nitrobenzene (Step A product, 32 g, 125.5 mmol) in N,N-dimethylformamide (250 mL) were added 2-fluoro-5-hydroxybenzonitrile (18.9 g, 138.0 mmol) and potassium carbonate (26 g, 1.5 mmol) at room temperature. The mixture was stirred for one hour, then diluted with water (300 mL) and extracted with ethyl acetate (250 mL x 3). The combined organic extracts were washed with water and brine, dried over magnesium sulfate, filtered, and concentrated. The residue was

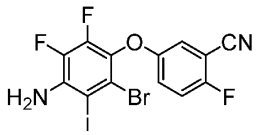
purified by flash chromatography (silica gel) to give the title compound as a yellow solid (15 g, 30%). MS: 373, 375 *m/z* $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ 8.30 (dd, *J* = 7.2, 2.4 Hz, 1H), 7.20-7.25 (m, 2H), 7.15-7.17 (m, 1H) ppm.

5-(4-Amino-6-bromo-2,3-difluorophenoxy)-2-fluorobenzonitrile



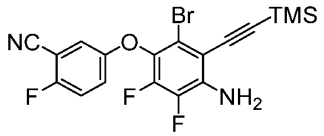
[119] **Step C:** To a stirred solution of 5-(6-bromo-2,3-difluoro-4-nitrophenoxy)-2-fluorobenzonitrile (Step B product, 5 g, 13.4 mmol) in ethanol (100 mL) and water (30 mL) were added iron powder (3 g, 53.6 mmol) and ammonium chloride (5.8 g, 107.5 mmol). The reaction mixture was stirred at 80 °C for four hours, cooled to room temperature, diluted with water (100 mL) and extracted with ethyl acetate (100 mL x 3). The combined organic extracts were washed with water, brine, dried over magnesium sulfate, filtered, and evaporated to dryness. The residue was purified by flash chromatography (silica gel, heptane/ethyl acetate, v/v, 10/1) to give the title compound as a yellow solid (3 g, 65%). MS: 343, 345 *m/z* $[M+H]^+$.

5-(4-Amino-2-bromo-5,6-difluoro-3-iodophenoxy)-2-fluorobenzonitrile



[120] Step D: To a stirred solution of 5-(4-amino-6-bromo-2,3-difluorophenoxy)-2-fluorobenzonitrile (Step C product, 6.7 g, 19.6 mmol) in acetic acid (200 mL) was added NIS (4.4 g, 19.6 mmol). The mixture was stirred at room temperature for three hours, diluted with water (200 mL) and extracted with ethyl acetate (150 mL x 3). The combined organic extracts were washed with water, brine, dried over magnesium sulfate, filtered, and evaporated to dryness. The residue was purified by flash chromatography over silica (petroleum ether/dichloromethane, v/v, 2/1) to give the title compound as a yellow solid (8.2 g, 89%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.16-7.18 (m, 2H), 7.03-7.04 (m, 1H), 4.56 (s, 2H) ppm. MS: 469, 471 *m/z* $[M+H]^+$.

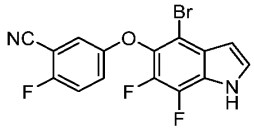
5-(4-Amino-2-bromo-5,6-difluoro-3-((trimethylsilyl)ethynyl)phenoxy)-2-fluorobenzonitrile



[121] **Step E:** To a stirred solution of 5-(4-amino-2-bromo-5,6-difluoro-3-iodophenoxy)-2-

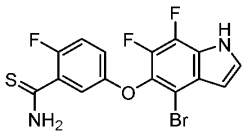
fluorobenzonitrile (Step D product, 8.1 g, 17.3 mmol) in N, N-dimethylformamide (200 mL) were added trimethylsilylacetylene (3.4 g, 34.6 mmol), $Pd(Ph_3P)_2Cl_2$ (1.2 g, 1.7 mmol), CuI (323 mg, 1.7 mmol) and triethylamine (3.5 g, 34.6 mmol). The reaction mixture was stirred at 30 °C under nitrogen for three hours, diluted with water (300 mL) and extracted with ethyl acetate (250 mL x 3). The combined organic extracts were washed with water, brine, dried with sodium sulfate, filtered, and concentrated. The residue was purified by flash chromatography (silica gel, petroleum ether/dichloromethane, v/v, 8/1) to give the title compound as a yellow solid (5 g, 64%). MS: 439, 441 *m/z* $[M+H]^+$.

5-((4-Bromo-6,7-difluoro-1H-indol-5-yl)oxy)-2-fluorobenzonitrile



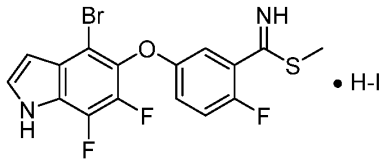
[122] **Step F:** To a stirred solution of 5-(4-amino-2-bromo-5,6-difluoro-3-((trimethylsilyl)ethynyl)phenoxy)-2-fluorobenzonitrile (Step E product, 700 mg, 1.6 mmol) in N,N-dimethylformamide (7 mL) was added CuI (608 mg, 3.2 mmol) and stirred at 100 °C in a glove box for four hours. The reaction mixture was diluted with water and extracted with ethyl acetate (50 mL x 3). The organic phase was washed with water, brine, dried with sodium sulfate, filtered, and concentrated. The residue was purified by flash chromatography over silica to give the title compound as a yellow solid (362 mg, 62%). ^1H NMR (400 MHz, $CDCl_3$) δ 8.63 (s, 1H), 7.37-7.39 (m, 1H), 7.14-7.23 (m, 2H), 7.04-7.06 (m, 1H), 6.67-6.69 (m, 1H) ppm.

5-((4-Bromo-6,7-difluoro-1H-indol-5-yl)oxy)-2-fluorobenzothioamide



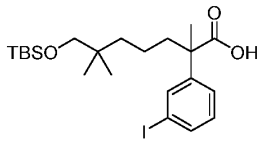
[123] Step G. To a stirred solution of sodium bisulfide (8.73 g, 109 mmol), magnesium chloride hexahydrate (11.08 g, 54.5 mmol) in DMF (260mL) was added 5-((4-Bromo-6,7-difluoro-1H-indol-5-yl)oxy)-2-fluorobenzonitrile (20.00 g, 54.5 mmol). The reaction mixture was stirred at room temperature for 1 hour, then diluted with water (400 mL), and extracted with ethyl acetate (400 mL x 2). The combined organic phases were washed with brine (50 mL), dried over sodium sulfate, filtered, and concentrated to give the title compound (21.00 g, 96 %) as a yellow solid. LC-MS: MS (ESI): 401, 403 *m/z* $[M+H]^+$, retention time: 1.66 minutes; purity: >99% (254 nm) (LC-MS method 2).

Methyl 5-((4-bromo-6,7-difluoro-1H-indol-5-yl)oxy)-2-fluorobenzimidothioate hydroiodide



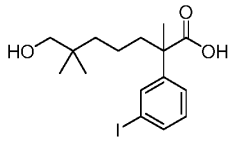
[124] Step H: To a stirred solution of 5-((4-bromo-6,7-difluoro-1H-indol-5-yl)oxy)-2-fluorobenzothioamide (Step G product, 15.50 g, 38.6 mmol) in acetone (120mL) was added methyl iodide (5.3 mL, 84.8 mmol). The reaction mixture was stirring at 50 °C for 3 hours and concentrated to give the title compound (20.00 g, 95 %) as a orange solid. LC-MS: MS (ESI): 415, 417 *m/z* $[M+H]^+$, retention time: 1.77 minutes; purity: 75% (254 nm) (LC-MS method 4).

7-((Tert-butyldimethylsilyl)oxy)-2-(3-iodophenyl)-2,6,6-trimethylheptanoic acid



[125] **Step B:** To a stirred and cooled (-78 °C) solution of diisopropylamide (1.51 g, 15 mmol) in tetrahydrofuran was added n-butyllithium (2.5M in hexanes, 6 mL, 15 mmol). The reaction was stirred for 30 minutes at this temperature, then hexamethylphosphoramide was added (2.68 g, 15 mmol) and stirring continued for another 30 minutes. To this mixture was added 2-(3-iodophenyl)propanoic acid (1.66 g, 6 mmol) in 10 mL of tetrahydrofuran over 5 minutes. The resulting mixture was stirred at -78 °C for 30 minutes. Then tert-butyl((5-iodo-2,2-dimethylpentyl)oxy)dimethylsilane (Intermediate 29C-1, 5.33 g, 15 mmol) was added in one portion. The mixture was allowed to warm to room temperature and stirred for 2 hours. After confirming by LC-MS that the starting material was consumed, the reaction was quenched with a mixture of 1 M hydrochloric acid (20 ml) and saturated ammonium chloride solution (15 mL) and extracted with ethyl acetate (3 x 20 mL). The combined organic extracts were washed with brine (20 mL), dried over sodium sulfate and concentrated to give the crude title product (1.99 g, 65%). MS (ESI): 505 *m/z* $[M+H]^+$.

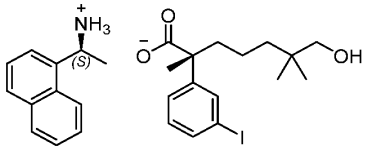
7-Hydroxy-2-(3-iodophenyl)-2,6,6-trimethylheptanoic acid



[126] **Step C:** To a solution of Step B product (4.8 mmol, 2.42 g) in tetrahydrofuran (5 mL) was added 1 M tetrabutylammonium fluoride (9.6 ml, 9.6 mmol). The reaction mixture was

stirred at 60 °C for 16 hours. After confirming by LC-MS that the starting material was consumed, the mixture was acidified with 10 mL 1 M hydrochloric acid and extracted with ethyl acetate (2 x 50 ml). The combined organic phases were washed with water, dried over sodium sulfate, and concentrated. The residue was purified by silica gel column chromatography to afford the title compound (1.03 g, 55%). MS (ESI): 413 *m/z* $[M+Na]^+$.

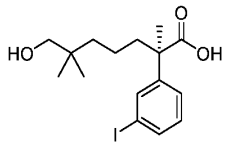
(S)-1-(Naphthalen-1-yl)ethan-1-aminium (R)-7-hydroxy-2-(3-iodophenyl)-2,6,6-trimethylheptanoate



[127] **Step D:** To a stirred solution of (S)-(-)-1-(1-naphthyl)ethylamine (127 mL, 705 mmol) in ethyl acetate (7.5 L) was added 7-hydroxy-2-(3-iodophenyl)-2,6,6-trimethyl-heptanoic acid (Intermediate 17B, 500.00 g, 1281 mmol). The reaction was stirred at room temperature overnight. The solid was collected by filtration and the filter cake was washed with ethyl acetate (800 mL). The wet solid was then suspended into EtOAc (1.7 L) and stirred at room temperature for 20 minutes. The mixture was filtered and washed with ethyl acetate (800 mL). The solid was collected and dried under air to give Enantiomer 1 of (S)-1-(naphthalen-1-yl)ethan-1-aminium 7-hydroxy-2-(3-iodophenyl)-2,6,6-trimethyl-heptanoate (288 g, 40 %). Chiral purity: 95% (90% ee). (Chiral conditions: column: AD-H; Mobile phase: n-hexane (0.1% diethylamine): ethanol (0.1% diethylamine) = 95:5; Column temperature: 40 °C; Flow rate: 1.0 mL/minute; Wavelength: 220 nm; Instrument: Shimadzu; Solid salt were de-salted with 1N hydrochloric acid in acetonitrile before injection).

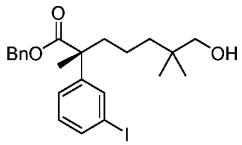
The above ~90% ee of (S)-1-(naphthalen-1-yl)ethan-1-aminium (R)-7-hydroxy-2-(3-iodophenyl)-2,6,6-trimethylheptanoate (186 g, 315 mmol) in acetonitrile (7.65 L) was refluxed for 5 hours and then stirred at room temperature overnight. The mixture was filtered and rinsed with acetonitrile (2 x 450 mL). The solid was collected and dried *in vacuo* to give the title compound (153 g, 86 %). Chiral purity: 99% (98% ee). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (t, *J* = 6.7 Hz, 2H), 7.77 (d, *J* = 8.2 Hz, 1H), 7.67 (t, *J* = 1.6 Hz, 1H), 7.56 (d, *J* = 7.1 Hz, 1H), 7.52-7.37 (m, 4H), 7.19 (d, *J* = 8.5 Hz, 1H), 6.92 (t, *J* = 7.9 Hz, 1H), 4.85 (q, *J* = 6.6 Hz, 1H), 3.19 (dd, *J* = 42.8, 10.4 Hz, 2H), 1.77-1.66 (m, 1H), 1.61-1.57 (m, 1H), 1.54 (d, *J* = 6.7 Hz, 3H), 1.38 (s, 3H), 1.28-0.98 (m, 4H), 0.79 (s, 3H), 0.74 (s, 3H) ppm.

(R)-7-Hydroxy-2-(3-iodophenyl)-2,6,6-trimethylheptanoic acid



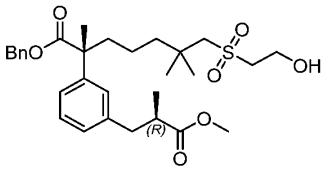
[128] **Step E.** The chiral salt of from Step D (233 g, 415 mmol) was suspended in ethyl acetate (1000 mL). The mixture was washed with 1N HCl (5 x 500 mL), brine, dried over anhydrous sodium sulfate, and concentrated to give the title compound (160.00 g, 99 %) as a light-yellow oil. MS (ESI): 413 *m/z* $[M+Na]^+$, retention time: 1.98 minutes, purity: 89% (214 nm) (LC-MS Method 3).

Benzyl (R)-7-hydroxy-2-(3-iodophenyl)-2,6,6-trimethylheptanoate



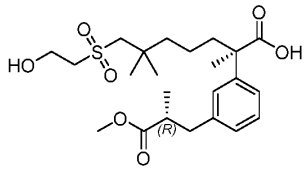
[129] **Step F:** To a stirred solution of Step E product (160.00 g, 410 mmol) in N,N-dimethylformamide (800 mL) was added benzyl bromide (73.6 g, 430 mmol) and potassium carbonate (85.00 g, 615 mmol). The reaction was stirred at room temperature for 16 hours, then diluted with water (1.5 L), and extracted with ethyl acetate (3 x 800 mL). The combined organic phases were washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by automated flash chromatography (330 g silica gel column, eluting with 0-25% ethyl acetate in petroleum) to give the title compound (190.00 g, 96 %) as a light-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (t, *J* = 1.7 Hz, 1H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.36-7.28 (m, 3H), 7.25-7.19 (m, 3H), 7.02 (t, *J* = 7.9 Hz, 1H), 5.20-5.05 (m, 2H), 3.23 (d, *J* = 4.4 Hz, 2H), 2.04-1.97 (m, 1H), 1.86-1.75 (m, 1H), 1.62 (s, 1H), 1.53 (s, 3H), 1.30-1.15 (m, 4H), 0.80-0.77 (m, 6H) ppm. MS (ESI): 503 *m/z* $[M+Na]^+$; Purity: 98% (214 nm); Retention time: 2.14 minutes (LC-MS Method 4). Chiral-HPLC: RT = 1.630 min, 99% (98% ee) (chiral SFC column conditions: Column: OJ-3, 4.6 x 100 mm, 3 µm; mobile phase: carbon dioxide/methanol (0.2% 7M ammonia in methanol) (90:10); injection volume: 3.00 uL; Run time: 6.0 minutes; Flow rate: 3.0 mL/minute; Back-pressure: 2000 psi; Column temperature: 40 °C.)

Benzyl (R)-7-((2-hydroxyethyl)sulfonyl)-2-(3-((R)-3-methoxy-2-methyl-3-oxopropyl)phenyl)-2,6,6-trimethylheptanoate



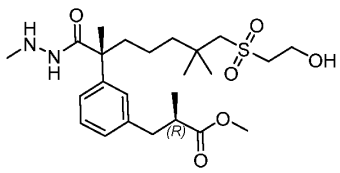
[130] **Step G:** Exchanging tert-Butyl 7-hydroxy-2-(3-iodophenyl)-2,6,6-trimethylheptanoate with the Step F product (190 g, 396 mmol), the reaction procedure sequence used to prepare tert-Butyl 7-((2-hydroxyethyl)sulfonyl)-2-(3-((R)-3-methoxy-2-methyl-3-oxopropyl)phenyl)-2,6,6-trimethylheptanoate in Example 1 (5 steps) was followed to synthesis the title compound (145 g, 265 mmol) as a light yellow oil. MS (ESI): 569 *m/z* $[M+Na]^+$; purity: 97% (214 nm), retention time: 1.92 minutes (LC-MS Method 3). Chiral-HPLC purity: 99.4% (>99% ee, 214 nm), RT = 11.99 minutes (chiral column conditions: Column: OJ-H, 4.6 x 250 mm, 5 µm; mobile phase: Hexanes (0.1% diethylamine): ethanol (0.1% diethylamine) = 80:20); Injection volume: 10.00 uL; Run time: 30.0 minutes; Flow rate: 1.0 mL/minute; Instrument: Shimadzu; Wavelength: 214 nm and 254 nm).

(R)-7-((2-Hydroxyethyl)sulfonyl)-2-(3-((R)-3-methoxy-2-methyl-3-oxopropyl)phenyl)-2,6,6-trimethylheptanoic acid



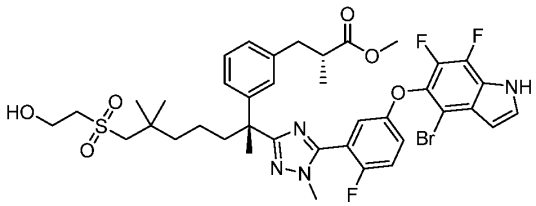
[131] **Step H:** To a stirred solution of benzyl (R)-7-((2-hydroxyethyl)sulfonyl)-2-(3-((R)-3-methoxy-2-methyl-3-oxopropyl)phenyl)-2,6,6-trimethylheptanoate (Step G product, 145 g, 265 mmol) in methanol (1000 mL) was added 10% palladium on carbon (wetted with ca. 55% water, 20 g). The reaction mixture was stirred under hydrogen for 6 hours at 50 °C, cooled to room temperature and filtered through a pad of Celite. The filtrate was concentrated to give the title compound (120 g, 99 %) as colorless oil. MS (ESI): 479 *m/z* $[M+Na]^+$; purity: >99% (214 nm); retention time: 1.66 minutes (LC-MS Method 3). Chiral-HPLC purity: 99.5% (99% ee, 214 nm), RT = 1.61 minutes (chiral SFC column conditions: Column: IG-3, 4.6 x 100 mm, 3 µm; mobile phase: carbon dioxide/methanol (0.2% 7M ammonia in methanol) = 65:35); Injection volume: 5.00 uL; Run time: 6.0 minutes; Flow rate: 3.0 mL/minute; Back pressure: 2000 psi; Wavelength: 214 nm; Column temperature: 40 °C).

Methyl (R)-3-(3-((R)-7-((2-hydroxyethyl)sulfonyl)-2,6,6-trimethyl-1-(2-methylhydrazineyl)-1-oxoheptan-2-yl)phenyl)-2-methylpropanoate



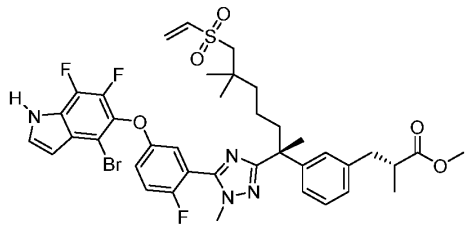
[132] Step I: Exchanging tert-Butyl 7-hydroxy-2-(3-iodophenyl)-2,6,6-trimethylheptanoate with the Step H product (190 g, 396 mmol), the rection procedure sequence used to prepare Methyl (2R)-3-(3-(7-((2-hydroxyethyl)sulfonyl)-2,6,6-trimethyl-1-(2-methylhydrazineyl)-1-oxoheptan-2-yl)phenyl)-2-methylpropanoate in Example 1 (2 steps) was followed to synthesize the title compound (120 g) as a light-yellow oil. MS (ESI): 485 *m/z* $[M+H]^+$; purity: 92% (214 nm); retention time: 1.47 minutes (LC-MS Method 3). Chiral-HPLC purity: 99.4% (>99% ee, 254 nm), RT = 1.75 minutes (chiral SFC column conditions: Column: AD-3, 4.6 x 100 mm, 3 µm; mobile phase: carbon dioxide/methanol (0.2% 7M ammonia in methanol) = 80:20); Injection volume: 5.00 uL; Run time: 6.0 minutes; Flow rate: 3.0 mL/minute; Wavelength: 214 nm, Column temperature: 40 °C).

Methyl (R)-3-(3-((R)-2-(5-(5-((4-bromo-6,7-difluoro-1H-indol-5-yl)oxy)-2-fluorophenyl)-1-methyl-1H-1,2,4-triazol-3-yl)-7-((2-hydroxyethyl)sulfonyl)-6,6-dimethylheptan-2-yl)phenyl)-2-methylpropanoate



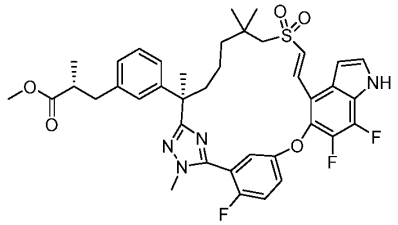
[133] Step J: To a stirred solution of methyl (R)-3-(3-((R)-7-((2-hydroxyethyl)sulfonyl)-2,6,6-trimethyl-1-(2-methylhydrazineyl)-1-oxoheptan-2-yl)phenyl)-2-methylpropanoate (Step I product, 6.60 g, 13.6 mmol) and methyl 5-((4-bromo-6,7-difluoro-1H-indol-5-yl)oxy)-2-fluorobenzimidothioate hydroiodide (Step A product, 7.77 g, 14.3 mmol) in pyridine (80 mL) was added magnesium sulphate (6.0 g). The reaction mixture was stirred at 80 °C overnight. The reaction mixture was quenched with water (400 mL) and extracted with ethyl acetate (3 x 200 mL). The combined organic extracts were washed with brine (400 mL), dried over sodium sulfate, filtered, and concentrated. The residue was purified by automated flash chromatography (120 g silica gel column, eluting with 0-65% ethyl acetate in petroleum ether) to give the title compound (7.00 g, 62 %) as a solid. LC-MS: MS (ESI): 833, 835 *m/z* $[M+H]^+$, retention time: 1.92 minutes; purity: 97% (214 nm) (LC-MS method 4).

Methyl (R)-3-(3-((R)-2-(5-(5-((4-bromo-6,7-difluoro-1H-indol-5-yl)oxy)-2-fluorophenyl)-1-methyl-1H-1,2,4-triazol-3-yl)-6,6-dimethyl-7-(vinylsulfonyl)heptan-2-yl)phenyl)-2-methylpropanoate



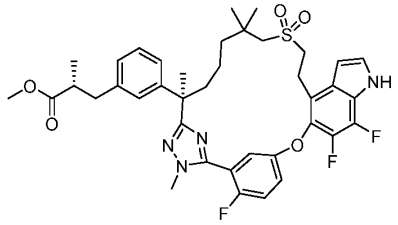
[134] Step K: To a stirred solution of the product from Step J (7.00 g, 8.40 mmol) in dichloromethane (80 mL) was added triethylamine (3.5 mL, 25.2 mmol), followed by methanesulfonyl chloride (0.72 mL, 9.24 mmol). The mixture was stirred at room temperature for 2.0 hours and concentrated. The residue was purified by automated flash chromatography (120 g silica gel column, eluting with 0-65% ethyl acetate in petroleum ether) to afford the title compound (6.20 g, 91 %) as a light-yellow solid. LC-MS: MS (ESI): 815, 817 *m/z* $[M+H]^+$, retention time: 2.01 minutes; purity: 99% (214 nm) (LC-MS method 4)

Methyl (2R)-2-methyl-3-[3-[(6R,13E)-21,22,28-trifluoro-3,6,10,10-tetramethyl-12,12-dioxo-24-oxa-12λ6-thia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,13,15,17,20,22,25,27-decaen-6-yl]phenyl]propanoate



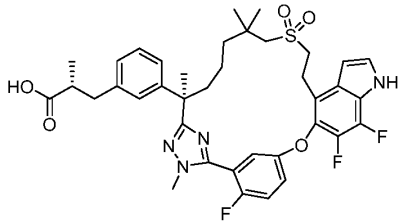
[135] Step L: To a stirred and heated (120 °C) solution of triethylamine (9.7 mL, 69.9 mmol) and bis(tri-t-butylphosphine)palladium(0) (1.07 g, 2.10 mmol) in toluene (600 mL), kept under an argon atmosphere, was added dropwise over 1.5 hours a solution of the product from Step K (5.70 g, 6.99 mmol) in toluene (50 mL) . The mixture was stirred at 120 °C for 2 hours and concentrated. The residue was purified by automated flash chromatography (330 g silica gel column, eluting with 0-50% ethyl acetate in petroleum ether) to give the title compound (3.50 g, 68 %) as a light-yellow solid. LC-MS: MS (ESI): 735 *m/z* $[M+H]^+$, retention time: 2.06 minutes; purity: >99% (214 nm) (LC-MS method 3).

Methyl (2R)-2-methyl-3-[3-[(6R)-21,22,28-trifluoro-3,6,10,10-tetramethyl-12,12-dioxo-24-oxa-12λ6-thia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl]phenyl]propanoate



[136] Step M: To a stirred solution of the product from Step L (3.80 g, 5.17 mmol) in ethanol (100 mL) was added 10% palladium on carbon (wetted with ca. 55% Water, 800 mg). The reaction mixture was stirred under a hydrogen balloon for 4 hours at 50 °C. The catalyst was removed by filtration and the solids washed with ethanol (30 mL x 3). The combined filtrates were concentrated to give the title compound (3.70 g, 97 %) as a white solid. LC-MS: MS (ESI): 737 *m/z* $[M+H]^+$, retention time: 2.04 minutes; purity: 98% (254 nm) (LC-MS method 3).

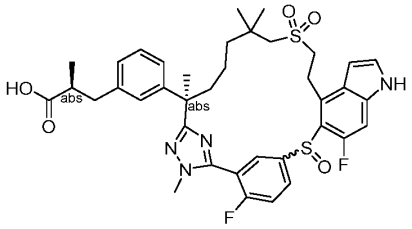
Compound 4: (2R)-2-Methyl-3-[3-[(6R)-21,22,28-trifluoro-3,6,10,10-tetramethyl-12,12-dioxo-24-oxa-12λ6-thia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl]phenyl]propanoic acid



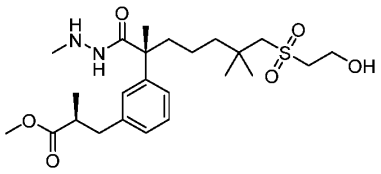
[137] Step N: To a stirred solution of the product from Step M (3.70 g, 5.02 mmol) in tetrahydrofuran (10 mL), methanol (10 mL) and water (10 mL) was added lithium hydroxide monohydrate (421 mg, 10.0 mmol). The reaction was stirred at room temperature for 3 hours, then acidified with 1.0 M hydrochloric acid to pH ~ 6 and extracted with ethyl acetate (3 x 100 mL). The combined organic extracts were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by automated flash chromatography (120 g silica gel column, eluting with 0-40% acetone in petroleum ether) to give the title compound (3.00 g, 83 %) as a white solid. LC-MS: MS (ESI): 723 *m/z* $[M+H]^+$, retention time: 2.01 minutes; purity: 98% (254 nm) (LC-MS method 3). ^{1}H NMR (400 MHz, CD_3OD) δ 7.38-7.34 (m, 3H), 7.24-7.22 (m, 1H), 7.14 (t, *J* = 8.0 Hz, 1H), 7.02-6.96 (m, 3H), 6.67 (t, *J*

= 3.2 Hz, 1H), 3.85 (d, *J* = 2.0 Hz, 3H), 3.35-3.30 (m, 2H), 3.29-3.25 (m, 1H), 3.21-3.15 (m, 1H), 2.99-2.88 (m, 2H), 2.83-2.79 (m, 1H), 2.63-2.54 (m, 2H), 2.20-2.10 (m, 1H), 1.87-1.77 (m, 1H), 1.67 (s, 3H), 1.64-1.55 (m, 1H), 1.39-1.27 (m, 1H), 1.24-1.17 (m, 1H), 1.07-0.98 (m, 10H) ppm

Example 5. Compound 5. Diastereomer 2 of (2S)-3-[3-[(6R)-22,28-difluoro-3,6,10,10-tetramethyl-12,12,24-trioxo-12λ6,24λ4-dithia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl]phenyl]-2-methyl-propanoic acid

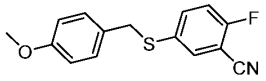


Methyl (S)-3-(3-((R)-7-((2-hydroxyethyl)sulfonyl)-2,6,6-trimethyl-1-(2-methylhydrazineyl)-1-oxoheptan-2-yl)phenyl)-2-methylpropanoate



[138] Step A: Exchanging methyl (S)-3-iodo-2-methylpropanoate with methyl (R)-3-iodo-2-methylpropanoate in the step of preparation of tert-Butyl 7-((2-((tert-butyldimethylsilyl)oxy)ethyl)sulfonyl)-2-(3-((R)-3-methoxy-2-methyl-3-oxopropyl)phenyl)-2,6,6-trimethylheptanoate (Negishi coupling step) in Example 1, the reaction procedure sequence (Step B to Step I) described in Example 4 was followed to prepare the title compound (54 g).

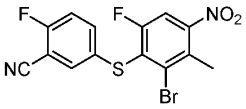
2-Fluoro-5-((4-methoxybenzyl)thio)benzonitrile



[139] Step B: To a stirred solution of 4-bromo-1-fluoro-2-isocyanobenzene (108.00 g, 540 mmol) in 1,4-dioxane (750 mL) was added (4-methoxyphenyl)methanethiol (91 mL, 648 mmol), 4,5-Bis(diphenylphosphino)-9,9-dimethylxanthene (1.87 g, 3.24 mmol), $Pd_2(dba)_3$ (1.48 g, 1.62 mmol), and diisopropylethylamine (139.57 g, 1080 mmol). The reaction was stirred overnight at 100 °C under argon and concentrated. The residue was taken up in

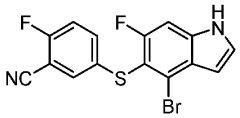
EtOAc (1000 ml), washed with water, brine, dried over magnesium sulfate, filtered, and concentrated. The crude product was triturated with methanol (400 mL) to give the title compound (140.00 g, 512 mmol, 95 %) as a yellow solid. LC-MS: MS (ESI): 296 *m/z* $[M+Na]^+$, retention time: 1.97 minutes; purity: 96% (254 nm) (LC-MS method 4).

5-((2-Bromo-6-fluoro-3-methyl-4-nitrophenyl)thio)-2-fluorobenzonitrile



[140] Step C: To a stirred solution of Step B product (100.22 g, 367 mmol) in trifluoroacetic acid (382 mL, 4956 mmol) was added anisole (382 mL, 3495 mmol). The mixture was stirred at 50 °C for 20 hours, then concentrated in the presence of 2.0 g of triphenylphosphine. The residue was dissolved in DMF (500 mL), treated with 3-bromo-1,2-difluoro-4-methyl-5-nitrobenzene (84.00 g, 333 mmol) and potassium carbonate (92.14 g, 667 mmol). The mixture was stirred at room temperature for 1 hour, quenched with water (1.5 L), and extracted with ethyl acetate (800 mL x 3). The combined organic layers were dried over sodium sulfate, filtered, and concentrated. The residue was purified by flash chromatography (330 g silica gel column x 2, eluting with 0-30% ethyl acetate in petroleum ether) to give the title compound (100.00 g, 260 mmol, 78 %) as a light-yellow solid. LC-MS: MS (ESI): not observed, retention time: 2.07 minutes; purity: 85% (254 nm) (LC-MS method 3). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (d, *J* = 8.4 Hz, 1H), 7.97-7.94 (m, 1H), 7.69-7.65 (m, 1H), 7.52 (t, *J* = 9.2 Hz, 1H), 2.48 (s, 3H) ppm.

5-((4-Bromo-6-fluoro-1H-indol-5-yl)thio)-2-fluorobenzonitrile

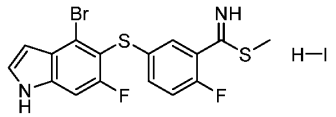


[141] Step D: To a stirred solution of Step C product (108.00 g, 280 mmol) in DMF (20 mL) was added 1,1-ditert-butoxy-N,N-dimethyl-methanamine (171.03 g, 841 mmol). The mixture was stirred at room temperature for 2 days, quenched with water (500 mL), and extracted with ethyl acetate (300 mL x 3). The combined organic extracts were washed with brine (2 x 600 mL), dried over sodium sulfate, filtered, and concentrated to afford (E)-5-((2-bromo-3-(2-(dimethylamino)vinyl)-6-fluoro-4-nitrophenyl)thio)-2-fluorobenzonitrile (110.00 g, 250 mmol, 89 %) as a brown oil. LC-MS: MS (ESI): 440, 442 *m/z* $[M+Na]^+$, retention time: 1.83 minutes; purity: 29% (214 nm) (LC-MS method 3).

[142] To a stirred solution of the above crude (E)-5-((2-bromo-3-(2-(dimethylamino)vinyl)-

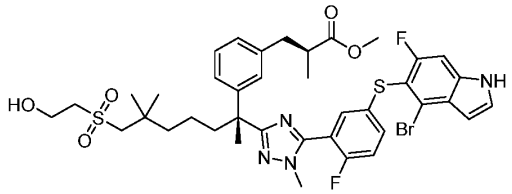
6-fluoro-4-nitrophenyl)thio)-2-fluorobenzonitrile (228.49 g, 519 mmol) in acetic acid (1.1 L) and toluene (2.6 L) was added iron powder (347.83 g, 6228 mmol). The reaction mixture was stirred at 110 °C under argon for 16 hours. The mixture was filtered. The filter cake was washed with toluene. The combined filtrate was concentrated. The residue was purified by flash column chromatography eluting with 50 % dichloromethane in hexanes to afford the title compound (115.00 g, 315 mmol, 61 %) as a pale-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.82 (s, 1H), 7.67 (dd, J = 5.8, 2.5 Hz, 1H), 7.61 – 7.55 (m, 1H), 7.48 (d, J = 9.1 Hz, 1H), 7.44 (t, J = 9.1 Hz, 1H), 7.31 (ddd, J = 8.9, 5.0, 2.5 Hz, 1H), 6.53 – 6.47 (m, 1H).

Methyl 5-((4-bromo-6-fluoro-1H-indol-5-yl)thio)-2-fluorobenzimidothioate hydroiodide



[143] Step E: : Exchanging 2-fluoro-5-((6-fluoro-4-bromo-1H-indol-5-yl)oxy)benzonitrile with 5-((4-bromo-6-fluoro-1H-indol-5-yl)thio)-2-fluorobenzonitrile (Step D product, 35 g, 96 mmol), the reaction procedure sequence described for the synthesis of methyl 5-((4-bromo-6-fluoro-1H-indol-5-yl)oxy)-2-fluorobenzimidothioate hydroiodide (two steps) was followed to prepare the title compound (45 g, 83 mmol) as an orange solid. LC-MS: MS (ESI): 413, 415 m/z $[M+H]^+$, retention time: 1.04 minutes; purity: 97% (254 nm) (LC-MS method 5).

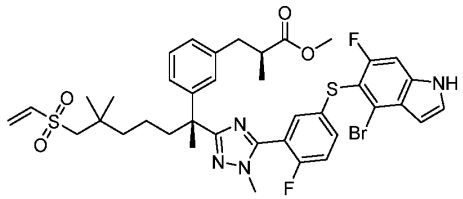
Methyl (2S)-2-methyl-3-[3-[(1R)-1-[5-[5-[(4-bromo-6-fluoro-1H-indol-5-yl)sulfanyl]-2-fluoro-phenyl]-1-methyl-1,2,4-triazol-3-yl]-6-(2-hydroxyethylsulfonyl)-1,5,5-trimethyl-hexyl]phenyl]propanoate



[144] Step F: To a stirred solution of methyl (S)-3-(3-((R)-7-((2-hydroxyethyl)sulfonyl)-2,6,6-trimethyl-1-(2-methylhydrazineyl)-1-oxoheptan-2-yl)phenyl)-2-methylpropanoate (Step A product, 18.00 g, 37.1 mmol) and methyl 5-((4-bromo-6-fluoro-1H-indol-5-yl)thio)-2-fluorobenzimidothioate hydroiodide (Step E product, 20.10 g, 37.1 mmol) in pyridine (150 mL) was added magnesium sulfate (17.88 g, 149 mmol). The mixture was stirred for 16 hours at 80 °C, cooled to room temperature, and poured into 400 mL of water. The solution was extracted with ethyl acetate (2 x 200 mL). The combined organic phases were washed with 1 N hydrochloric acid and brine (300 mL), dried over sodium sulphate, filtered, and

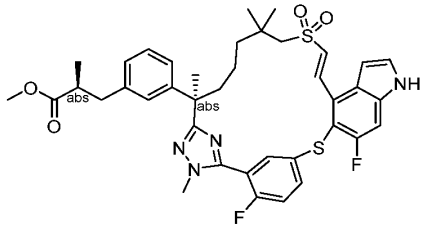
concentrated. The residue was purified by flash chromatography (330 g silica gel column, eluting with 0-70% ethyl acetate in petroleum ether) to give the title compound (19.00 g, 62 %) as a yellow solid. MS (ESI): 831, 833 *m/z* $[M+H]^+$, retention time: 2.02 minutes; purity: 87% (214 nm) (LC-MS method 3).

Methyl (2S)-3-[3-[(1R)-1-[5-[5-[(4-bromo-6-fluoro-1H-indol-5-yl)sulfanyl]-2-fluoro-phenyl]-1-methyl-1,2,4-triazol-3-yl]-1,5,5-trimethyl-6-vinylsulfonyl-hexyl]phenyl]-2-methyl-propanoate



[145] Step G: To a solution of Step F product (18.80 g, 22.6 mmol) in dichloromethane (150 mL) was added triethylamine (9.5 mL, 67.8 mmol) and methane sulfonyl chloride (1.10 eq, 1.9 mL, 24.9 mmol). The mixture was stirred at room temperature for 2.0 hours and concentrated. The residue was purified by automated flash chromatography (330 silica gel column, eluting with 0-50% ethyl acetate in petroleum ether) to afford the title compound (16.00 g, 87 %) as a light-yellow solid. MS (ESI): 813, 815 *m/z* $[M+H]^+$, retention time: 2.13 minutes; purity: 96% (214 nm) (LC-MS method 3).

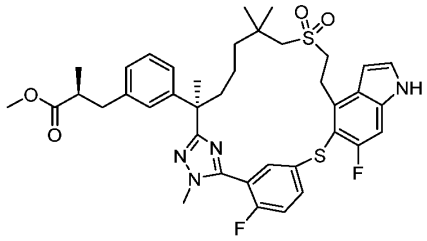
Methyl (2S)-3-[3-[(6R,13E)-22,28-difluoro-3,6,10,10-tetramethyl-12,12-dioxo-12λ6,24-dithia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,13,15,17,20,22,25,27-decaen-6-yl]phenyl]-2-methyl-propanoate



[146] Step H: To a stirred solution of triethylamine (27 mL, 194 mmol) and bis(tri-t-butylphosphine)palladium(0) (1.98 g, 3.88 mmol) in toluene (700 mL) was added dropwise a solution of Step G product (15.80 g, 19.4 mmol) in toluene (50 mL) over 2 hours under argon at 120 °C. The mixture was stirred at 120 °C for another 0.5 hours and concentrated. The residue was purified by automated flash chromatography (330 g silica gel column, eluting with 0-50% ethyl acetate in petroleum ether) to give the title compound (9.80 g, 69 %) as a yellow solid. MS (ESI): 733 *m/z* $[M+H]^+$, retention time: 2.10 minutes; purity: 96% (214 nm)

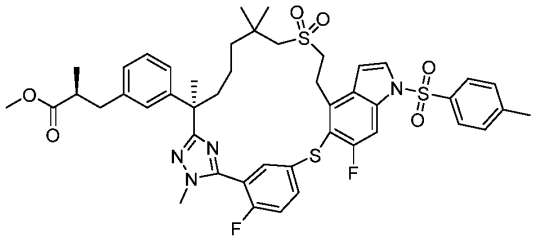
(LC-MS method 3).

Methyl (2S)-3-[3-[(6R)-22,28-difluoro-3,6,10,10-tetramethyl-12,12-dioxo-12λ6,24-dithia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl]phenyl]-2-methyl-propanoate



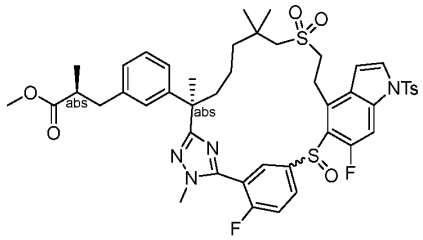
[147] Step I: To a stirred solution of Step H product (9.65 g, 13.2 mmol) in ethanol (150 mL) was added 10% palladium on carbon (wetted with ca. 55% water, 2 g). The reaction mixture was stirred under hydrogen for 4 hours at 50 °C, cooled to room temperature, and filtered through a pad of Celite. The filtrate was concentrated to give the title compound (8.90 g, 92 %) as a solid. MS (ESI): 735 *m/z* $[M+H]^+$, retention time: 2.09 minutes, purity: 84% (214 nm) (LC-MS method 4).

Methyl (2S)-3-[3-[(6R)-22,28-difluoro-3,6,10,10-tetramethyl-12,12-dioxo-19-(p-tolylsulfonyl)-12λ6,24-dithia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl]phenyl]-2-methyl-propanoate



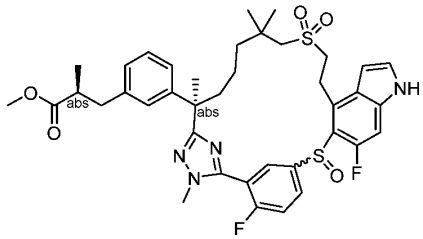
[148] Step J: To a stirred solution of Step I product (8.75 g, 11.9 mmol) in acetonitrile (100 mL) was added 1-(p-tolylsulfonyl)imidazole (5.29 g, 23.8 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (3.6 mL, 23.8 mmol). The mixture was stirred at room temperature overnight, diluted with water (200 mL), and extracted with ethyl acetate (2 x 150 mL). The combined organic phases were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by automated silica gel column chromatography (120 g silica gel column, eluting with 0-50% ethyl acetate in petroleum ether) to give the title compound (8.30 g, 78 %) as a solid. MS (ESI): 889 *m/z* $[M+H]^+$, retention time: 2.27 minutes; purity: 90% (214 nm) (LC-MS method 10).

Diastereomer 2 of Methyl (2S)-3-[3-[(6R)-22,28-difluoro-3,6,10,10-tetramethyl-12,12,24-trioxo-19-(p-tolylsulfonyl)-12λ6,24λ4-dithia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl]phenyl]-2-methyl-propanoate



[149] Step K: To a stirred solution of Step J product (5.00 g, 5.62 mmol) in acetonitrile (100 mL) was added (S,S)-hydrobenzoin (6.02 g, 28.1 mmol), titanium(IV) isopropoxide (4.9 mL, 16.9 mmol) and tert-butyl hydroperoxide (1.52 g, 16.9 mmol). The mixture was stirred at room temperature overnight, poured into 200 mL of water, and extracted with ethyl acetate (2 x 150 mL). The combined organic phases were washed with brine (300 mL), dried over sodium sulphate, filtered, and concentrated. The residue was purified by automated flash chromatography (80 g silica gel column, eluting with 0-60% ethyl acetate in petroleum ether) to give the title compound (4.10 g, 81 %) as a solid. MS (ESI): 905 *m/z* $[M+H]^+$, retention time: 2.15 minutes; purity: 91% (214 nm) (LC-MS method 10).

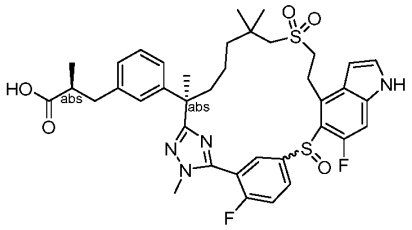
Diastereomer 2 of methyl (2S)-3-[3-[(6R)-22,28-difluoro-3,6,10,10-tetramethyl-12,12,24-trioxo-12λ6,24λ4-dithia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl]phenyl]-2-methyl-propanoate



[150] Step L: To a stirred solution of Step K product (1.50 g, 1.66 mmol) in methanol (20 mL) was added potassium carbonate (0.46 g, 3.31 mmol). The mixture was stirred at room temperature for 3 hours, poured into 80 mL of water, and extracted with ethyl acetate (2 x 50 mL). The combined organic phases were washed with brine, dried over sodium sulphate, filtered, and concentrated. The residue was purified by automated flash chromatography (40 g silica gel column, eluting with 0-80% ethyl acetate in petroleum ether) to give the title compound (1.10 g, 88 %) as a solid. MS (ESI): 751 *m/z* $[M+H]^+$, retention time: 1.99

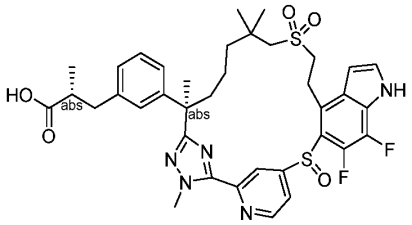
minutes; purity: 97% (214 nm) (LC-MS method 10).

Compound 5: Diastereomer 2 of (2S)-3-[3-[(6R)-22,28-difluoro-3,6,10,10-tetramethyl-12,12,24-trioxo-12λ6,24λ4-dithia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl]phenyl]-2-methyl-propanoic acid

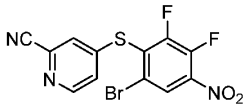


[151] Step M: To a stirred solution of Step L product (1100 mg, 1.46 mmol) in tetrahydrofuran (16 mL) was added lithium hydroxide monohydrate (8.0 mL, 8.00 mmol) (1 M in water). The mixture was stirred at room temperature for 24 hours, then acidified with 1.0 M hydrochloric acid to pH ~ 6 and extracted with ethyl acetate (3 x 40 mL). The combined organic phases were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by automated flash chromatography (40 g silica gel column, eluting with 0-100% ethyl acetate in petroleum ether) to give a white solid. This white solid was triturated with acetonitrile to afford the title compound (700 mg, 65 %) as a white solid. MS (ESI): 737 *m/z* $[M+H]^+$, retention time: 2.37 minutes; purity: 99% (214 nm) (LC-MS method 1). ^{1}H NMR (400 MHz, CD_3OD) δ 8.20-8.10 (m, 1H), 8.00-7.85 (m, 1H), 7.60-7.53 (m, 1H), 7.37 (d, *J* = 3.2 Hz, 1H), 7.19-6.97 (m, 5H), 6.67 (s, 1H), 3.98-3.80 (m, 4H), 3.71-3.64 (m, 1H), 3.56-3.44 (m, 1H), 3.25-3.00 (m, 3H), 2.96-2.88 (m, 1H), 2.64-2.56 (m, 2H), 2.32-2.20 (m, 1H), 2.00-1.90 (m, 1H), 1.71 (s, 3H), 1.50-1.35 (m, 3H), 1.21 (s, 3H), 1.14 (s, 3H), 1.07-1.04 (m, 4H) ppm.

Example 6. Compound 6. Diastereomer 2 of (2R)-3-[3-[(6R)-21,22-difluoro-3,6,10,10-tetramethyl-12,12,24-trioxo-12λ6,24λ4-dithia-3,4,19,28,30-pentazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl]phenyl]-2-methyl-propanoic acid



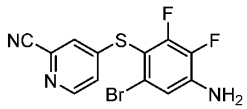
4-((6-Bromo-2,3-difluoro-4-nitrophenyl)thio)picolinonitrile



[152] Step A: To a stirred solution of 4-chloropicolinonitrile (80 g, 577.41 mmol) in ethanol (540 mL) was added thiourea (46.1 g, 606.28 mmol) at room temperature. The reaction mixture was stirring at 100 °C for 1 hour. After cooling, the resultant salt was filtered off and dried to give 2-cyanopyridin-4-yl carbamimidothioate hydrochloride (97 g, 78%) as a solid. LC-MS: MS (ESI): 179 *m/z* $[M+H]^+$, retention time: 0.35 minutes; purity: 95% (254 nm) (LC-MS method 6).

[153] To a stirred solution of the above 2-cyanopyridin-4-yl carbamimidothioate hydrochloride (REQ-00221-1, 97 g, 451.8 mmol) in DMF (1500 mL) was added sodium hydroxide (452 mL, 903.6 mmol) at room temperature. The reaction mixture was stirred at room temperature for 10 minutes, then treated with 1-bromo-2,3,4-trifluoro-5-nitro-benzene (115.6 g, 451.8 mmol). The mixture was stirred at 60 °C for 1 hour, then diluted with ethyl acetate (2 L), washed with water (3 L x 2), dried over magnesium sulfate, filtered, and concentrated. The residue was slurred with petroleum ether (160 mL) and ethyl acetate (400 mL) for 30 minutes and filtered. The solid was dried *in vacuo* to give the title compound (61 g, 36%) as a solid. LC-MS: MS (ESI): 372, 374 *m/z* $[M+H]^+$, retention time: 1.94 minutes; purity: 99% (254 nm) (LC-MS method 6).

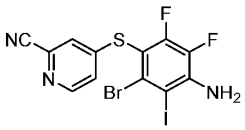
4-((4-Amino-6-bromo-2,3-difluorophenyl)thio)picolinonitrile



[154] Step B: To a stirred solution of 4-(6-bromo-2,3-difluoro-4-nitro-phenyl)sulfanylpyridine-2-carbonitrile (Step A product, 61 g, 163.9 mmol) in acetic acid (500 mL) was added Fe powder (27.5 g, 491.7 mmol) at room temperature. The reaction mixture was stirred at 40 °C for 1 hour and concentrated. The residue was diluted with ethyl acetate (1000 mL) and filtered. The filtrate was washed with water (1000 mL x 2), dried over

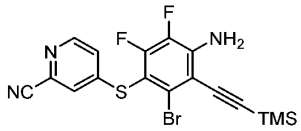
magnesium sulfate, filtered, and concentrated. The crude product was purified by silica gel column chromatography (330 g silica gel column, eluting with 0-30% ethyl acetate in petroleum ether) to give the title compound (48 g, 86%) as a solid. LC-MS: MS (ESI): 342, 344 *m/z* $[M+H]^+$, retention time: 1.92 minutes; purity: 92% (254 nm) (LC-MS method 6).

4-((4-Amino-2-bromo-5,6-difluoro-3-iodophenyl)thio)picolinonitrile



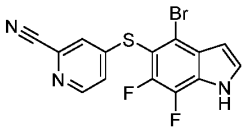
[155] Step C: To a stirred solution of 4-((4-amino-2-bromo-5,6-difluoro-3-iodophenyl)thio)picolinonitrile (Step B product, 48 g, 140.2 mmol) in ethanol (480 mL) was added sulfuric acid (98% in water, 9 mL, 168.3 mmol) and N-Iodosuccinimide (31.6 g, 140.2 mmol) at room temperature. The reaction mixture was stirred at 40 °C for 1 hour. The mixture was diluted with ethyl acetate (2000 mL), washed with water (2000 mL x 2), dried over magnesium sulfate, filtered, and concentrated. The residue was purified by silica gel column chromatography (330 g silica gel column, eluting with 0-50% ethyl acetate in petroleum ether) to give the title compound (51 g, 78%) as a solid. LC-MS: MS (ESI): 468, 470 *m/z* $[M+H]^+$, retention time: 1.24 minutes; purity: 90% (254 nm) (LC-MS method 6).

4-((4-Amino-2-bromo-5,6-difluoro-3-((trimethylsilyl)ethynyl)phenyl)thio)picolinonitrile



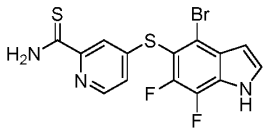
[156] Step D: To a stirred solution of 4-((4-amino-2-bromo-5,6-difluoro-3-iodophenyl)thio)picolinonitrile (Step C product, 51 g, 108.96 mmol) in DMF (500 mL) was added trimethylsilylacetylene (61.1 mL, 435.84 mmol), $(Ph_3P)_2PdCl_2$ (7.64 g, 10.9 mmol), CuI (4.15 g, 21.8 mmol) and triethylamine (45.6 mL, 326.9 mmol) at room temperature. The reaction mixture was stirred at room temperature for 16 hours. The mixture was diluted with ethyl acetate (1000 mL), washed with brine (1000 mL×2), dried over sodium sulfate, filtered, and concentrated. The residue was purified by flash chromatography (330 g silica gel column, eluting with 0-20% ethyl acetate in petroleum ether) to give the title compound (34 g, 71%) as a solid. LC-MS: MS (ESI): 438, 440 *m/z* $[M+H]^+$, retention time: 2.05 minutes; purity: 92% (254 nm) (LC-MS method 6).

4-((4-Bromo-6,7-difluoro-1H-indol-5-yl)thio)picolinonitrile



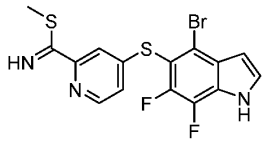
[157] Step E: To a stirred solution of 4-((4-amino-2-bromo-5,6-difluoro-3-((trimethylsilyl)ethynyl)phenyl)thio)picolinonitrile (Step D product, 34 g, 77.5 mmol) in DMF (300 mL) was added CuI (2.95 g, 15.5 mmol) at room temperature. The reaction mixture was stirred at 100 °C for 2 hours, diluted with ethyl acetate (1000 mL), washed with brine (1000 mL×2), dried over sodium sulfate, filtered, and concentrated. The residue was purified by flash chromatography (330 g silica gel column, eluting with 0-50% ethyl acetate in petroleum ether) to give the title compound (17 g, 60%) as a solid. LC-MS: MS (ESI): 366, 368 *m/z* $[M+H]^+$, retention time: 2.02 minutes; purity: 90% (254 nm) (LC-MS method 6).

4-((4-Bromo-6,7-difluoro-1H-indol-5-yl)thio)pyridine-2-carbothioamide



[158] Step F: To a stirred solution of 4-((4-bromo-6,7-difluoro-1H-indol-5-yl)thio)picolinonitrile (Step E product, 17 g, 46.4 mmol) in DMF (170 mL) was added $NaHSxH_2O$ (5.2 g, 92.85 mmol) and $MgCl_2*6H_2O$ (12.9 g, 46.4 mmol) at room temperature. The reaction mixture was stirred at room temperature for 1 hour, then diluted with ethyl acetate (500 mL), washed with water (500 mL x 2), dried over magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography (330 g silica gel column, eluting with 0-60% ethyl acetate in petroleum ether) to give the title compound (17.5 g, 94 %) as a solid. LC-MS: MS (ESI): 400, 402 *m/z* $[M+H]^+$, retention time: 1.57 minutes; purity: >99% (254 nm) (LC-MS method 6).

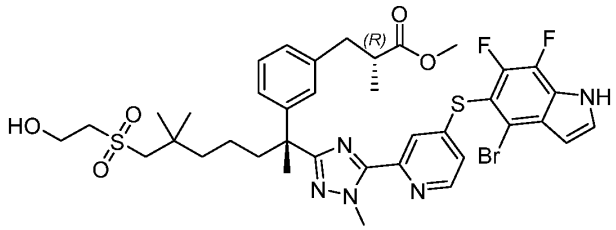
Methyl 4-((4-bromo-6,7-difluoro-1H-indol-5-yl)thio)pyridine-2-carbimidothioate



[159] Step G: To a stirred solution of 4-((4-bromo-6,7-difluoro-1H-indol-5-yl)thio)pyridine-2-carbothioamide (Step F product, 17.5 g, 43.7 mmol) in acetone (200 mL) was added iodomethane (13.6 mL, 218.6 mmol) and sodium bicarbonate (18.4 g, 218.6 mmol) at room temperature. The reaction mixture was stirring at room temperature for 16 hours. The

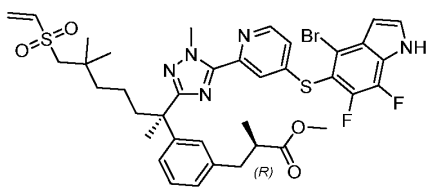
reaction mixture was diluted with dichloromethane (500 mL), washed with water (500 mL x 2), dried over magnesium sulfate, filtered, and concentrated to give the title compound (18 g, 99%) as a solid. LC-MS: MS (ESI): 414, 416 *m/z* $[M+H]^+$, retention time: 1.42 minutes, purity: 86% (254 nm) (LC-MS method 6).

Methyl (R)-3-(3-((R)-2-(5-(4-((4-bromo-6,7-difluoro-1H-indol-5-yl)thio)pyridin-2-yl)-1-methyl-1H-1,2,4-triazol-3-yl)-7-((2-hydroxyethyl)sulfonyl)-6,6-dimethylheptan-2-yl)phenyl)-2-methylpropanoate



[160] Step H: To a stirred solution of methyl (2R)-3-[3-[(1R)-6-(2-hydroxyethylsulfonyl)-1,5,5-trimethyl-1-(methylaminocarbamoyl)hexyl]phenyl]-2-methyl-propanoate (Step I product of Example **4**, 12.28 g, 25.3 mmol) in dichloroethane (100 mL) were added methyl 4-((4-bromo-6,7-difluoro-1H-indol-5-yl)thio)pyridine-2-carbimidothioate (Step G product, 10.50 g, 25.3 mmol), pyridine (10 mL, 127 mmol) and magnesium sulfate (30.51 g, 253 mmol). The reaction was stirred at 80 °C for 5 hours, cooled to room temperature, diluted with 300 mL of water, and extracted with ethyl acetate (300 mL x 3). The combined organic phases were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by automated silica gel column chromatography (300 g silica gel column, eluting with 0~70% of ethyl acetate in petroleum ether) to give the title compound (10.50 g, 50 %) as a yellow solid. LC-MS: MS (ESI): 832, 834 *m/z* $[M+H]^+$, purity: 95% (214 nm), retention time: 1.75 minutes (LC-MS method 10).

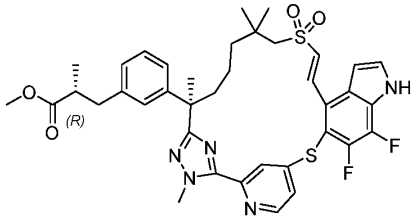
Methyl (R)-3-(3-((R)-2-(5-(4-((4-bromo-6,7-difluoro-1H-indol-5-yl)thio)pyridin-2-yl)-1-methyl-1H-1,2,4-triazol-3-yl)-6,6-dimethyl-7-(vinylsulfonyl)heptan-2-yl)phenyl)-2-methylpropanoate



[161] Step I: To a stirred solution of the product from Step H (10.50 g, 12.6 mmol)

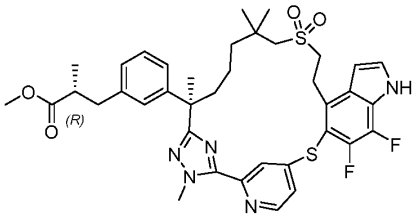
in dichloromethane (100 mL) were added methanesulfonyl chloride (1.1 mL, 13.9 mmol) and triethylamine (5.3 mL, 37.8 mmol). The reaction was stirred at room temperature for 2 hours, diluted with 300 mL of water, and extracted with dichloromethane (3 x 300 mL). The combined organic extracts were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by silica gel column chromatography (eluting with 0~50% of ethyl acetate in petroleum ether) to give the title compound (9.50 g, 93 %) as a yellow solid. LC-MS: MS (ESI): 814, 816 *m/z* $[M+H]^+$, purity: 98% (214 nm), retention time: 1.98 minutes (LC-MS method 11).

Methyl (2R)-3-[3-[(6R,13E)-21,22-difluoro-3,6,10,10-tetramethyl-12,12-dioxo-12λ6,24-dithia-3,4,19,28,30-pentazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,13,15,17,20,22,25,27-decaen-6-yl]phenyl]-2-methyl-propanoate



[162] Step J: To a stirred solution of bis(tri-t-butylphosphine)palladium(0) (1192 mg, 2.33 mmol) and triethylamine (16 mL, 117 mmol) in toluene (500 mL) was added dropwise, at 120 °C under argon, and over 1.5 hours a solution of the product from Step I (9.50 g, 11.7 mmol) in toluene (50 mL). After the addition, the reaction was stirred at 120 °C for 1 hour and concentrated. The residue was purified by automated silica gel column chromatography (120 g silica gel column, eluting with 0~50% of ethyl acetate in petroleum ether) to give the title compound (4.80 g, 56 %) as a light-yellow solid. LC-MS: MS (ESI): 734 *m/z* $[M+H]^+$, purity: 84% (214 nm), retention time: 2,27 minutes (LC-MS method 11).

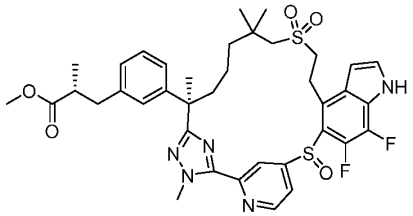
Methyl (2R)-3-[3-[(6R)-21,22-difluoro-3,6,10,10-tetramethyl-12,12-dioxo-12λ6,24-dithia-3,4,19,28,30-pentazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl]phenyl]-2-methyl-propanoate



[163] Step K: To a stirred solution of the product from Step J (4.80 g, 6.54 mmol) in toluene (100 mL) was added p-toluene sulfonyl hydrazide (12.18 g, 65.4 mmol). The reaction was

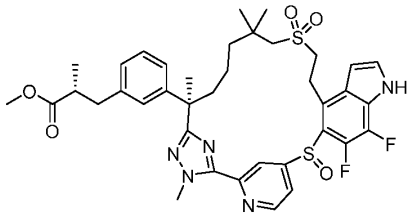
stirred at 110 °C for 2 hours, cooled to room temperature, diluted with 300 mL of water, and extracted with ethyl acetate (3 x 300 mL). The combined organic extracts were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by silica gel column chromatography (eluting with 0~50% of ethyl acetate in petroleum ether) to give the title compound (3.90 g, 81 %) as a white solid. LC-MS: MS (ESI): 736 *m/z* $[M+H]^+$, purity: 96% (214 nm), retention time: 2.10 minutes (LC-MS method 11).

Methyl (2R)-3-[3-[(6R)-21,22-difluoro-3,6,10,10-tetramethyl-12,12,24-trioxo-12λ6,24λ4-dithia-3,4,19,28,30-pentazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl]phenyl]-2-methyl-propanoate



[164] Step L: To a stirred solution of the product from Step K (3.90 g, 5.30 mmol) in acetonitrile (30 mL) were added (1S,2S)-1,2-diphenylethane-1,2-diol (5.68 g, 26.5 mmol), titanium(IV) isopropoxide (4.7 mL, 15.9 mmol) and tert-butyl hydroperoxide (2.2 mL, 15.9 mmol). The reaction was stirred at room temperature for 16 hours, then diluted with 150 mL of water, and extracted with ethyl acetate (3 x 150 mL). The combined organic extracts were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by silica gel column chromatography (eluting with 0~50% of ethyl acetate in petroleum ether) to give the title compound (3.30 g, 83 %) as a yellow solid. LC-MS: MS (ESI): 752 *m/z* $[M+H]^+$, purity: 79% (214 nm), retention time: 2.03 minutes (LC-MS method 11).

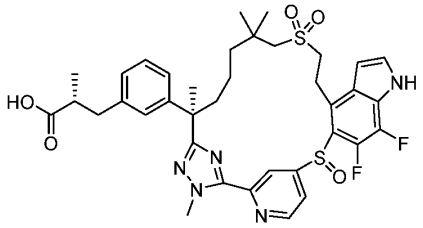
Diastereomer 2 of methyl (2R)-3-[3-[(6R)-21,22-difluoro-3,6,10,10-tetramethyl-12,12,24-trioxo-12λ6,24λ4-dithia-3,4,19,28,30-pentazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl]phenyl]-2-methyl-propanoate



[165] Step M: The diastereomeric mixture of products from Step L (3.30 g, 4.39 mmol) was

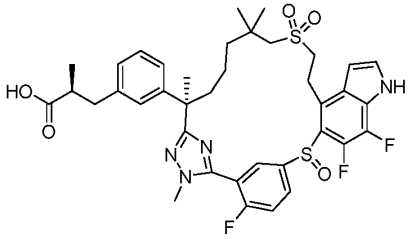
subjected to chiral SFC separation under the following conditions: Instrument: SFC-150 (Waters); Column: IH 20 * 250 mm, 10 μm; Column temperature: 35 °C; Mobile phase: carbon dioxide/methanol[0.2% ammonia (7M in methanol)] = 60/40; Flow rate: 100 g/minute; Back pressure: 100 bar; Detection wavelength: 214 nm; Cycle time: 4.58 minutes; Sample solution: 2700 mg dissolved in 350 ml methanol; Injection volume: 4.5 ml. The major isomer of the title compound (2.10 g, 64 %) was obtained as a white solid. LC-MS: MS (ESI): 752 *m/z* $[M+H]^+$, purity: 99% (214 nm), retention time: 2.14 minutes (LC-MS method 3).

Diastereomer 2 of (2R)-3-[3-[(6R)-21,22-Difluoro-3,6,10,10-tetramethyl-12,12,24-trioxo-12λ6,24λ4-dithia-3,4,19,28,30-pentazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl]phenyl]-2-methyl-propanoic acid

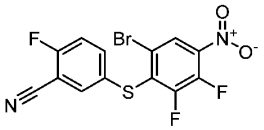


[166] Step N: To a solution of the product from Step M (2.10 g, 2.79 mmol) in tetrahydrofuran (30 mL) and water (15 mL) was added lithium hydroxide monohydrate (586 mg, 14.0 mmol). The reaction was stirred at room temperature for 16 hours, then diluted with 100 mL of water, acidified with hydrochloric acid solution to pH ~ 5, and extracted with ethyl acetate (3 x 150 mL). The combined organic extracts were washed with water, brine, dried over sodium sulfate, filtered, and concentrated. The residue was lyophilized to give (2R)-3-[3-[(6R)-21,22-difluoro-3,6,10,10-tetramethyl-12,12,24-trioxo-12λ6,24λ4-dithia-3,4,19,28,30-pentazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl]phenyl]-2-methyl-propanoic acid (1.52 g, 74 %) as a white solid. LC-MS: MS (ESI): 738 *m/z* $[M+H]^+$, purity: >99% (214 nm), retention time: 1.40 minutes (LC-MS method 5). ^{1}H NMR (400 MHz, CD_3OD) δ 8.93-8.10 (m, 2H), 7.45 (s, 1H), 7.12-6.63 (m, 6H), 4.31 (s, 3H), 3.62-3.48 (m, 2H), 3.09-2.91 (m, 4H), 2.60-2.45 (m, 3H), 1.74 (s, 4H), 1.53-0.89 (m, 14H) ppm.

Example 7. Compounds 7A and 7B. Diastereomers 1 and 2 of (2S)-2-methyl-3-[3-[(6R)-21,22,28-trifluoro-3,6,10,10-tetramethyl-12,12,24-trioxo-12λ6,24λ4-dithia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl]phenyl]propanoic acid

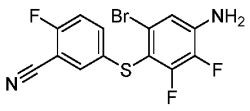


5-((6-Bromo-2,3-difluoro-4-nitrophenyl)thio)-2-fluorobenzonitrile



[167] Step A: To a stirred solution of 2-fluoro-5-((4-methoxybenzyl)thio)benzonitrile (Step B product of Example 5, 250.00 g, 915 mmol) in anisole (10.0 eq, 1000 mL, 9155 mmol) was added trifluoroacetic acid (1000 mL, 12980 mmol) , the reaction mixture was stirred at 50 °C for 72 hours and concentrated. The residue was dissolved in 200 mL of DMF. The solution was added dropwise to a suspension of 1-bromo-2,3,4-trifluoro-5-nitro-benzene (212.00 g, 828 mmol) and sodium bicarbonate (138.98 g, 1654 mmol) in DMF (1000mL) at -60 °C. The reaction mixture was stirred at -60 °C under Argon for 1 hour, quenched with water (1000 mL), and extracted with ethyl acetate (500 mL X 3). The combined organic extracts were washed with brine (500 mL), dried over sodium sulfate, filtered, and concentrated. The residue was purified by flash chromatography (330 g silica gel column, eluting with 0-40% ethyl acetate in petroleum ether) to give 5-(6-bromo-2,3-difluoro-4-nitro-phenyl)sulfanyl-2-fluoro-benzonitrile (241.00 g, 619 mmol, 75 %) which contained 17% of 5-(4-bromo-2,3-difluoro-6-nitro-phenyl)sulfanyl-2-fluoro-benzonitrile as yellow solid. LC-MS: MS (ESI): 389, 391 *m/z* $[M+H]^+$, purity: >99% (214 nm), retention time: 3.06 minutes (LC-MS method 7).

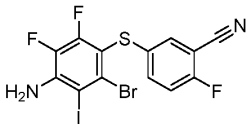
5-((4-Amino-6-bromo-2,3-difluorophenyl)thio)-2-fluorobenzonitrile



[168] Step B: To a stirred solution of 5-(6-bromo-2,3-difluoro-4-nitro-phenyl)sulfanyl-2-fluoro-benzonitrile (144.00 g, 370 mmol) in acetic acid (600 mL) was added iron powder (55.97 g, 1002 mmol), and the reaction mixture was stirred at 40 °C under argon for 2 hours. The mixture was filtered and concentrated to dryness, and the residue was taken up in ethyl

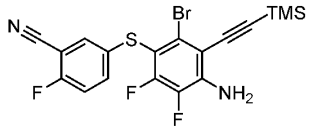
acetate (1000 ml). The solution was washed with water, brine, dried over magnesium sulfate, filtered, and concentrated. The residue was triturated with 10% ethyl acetate in hexanes for a couple of times. The solid was filtered and dried to obtain the title compound (105.00 g, 71%) as a yellow solid. LC-MS: MS (ESI): 359, 361 *m/z* $[M+H]^+$, purity: 90% (214 nm), retention time: 2.76 minutes (LC-MS method 7).

5-((4-Amino-2-bromo-5,6-difluoro-3-iodophenyl)thio)-2-fluorobenzonitrile



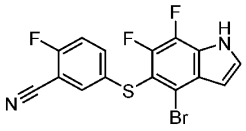
[169] Step C. To a stirred solution of 5-((4-amino-6-bromo-2,3-difluorophenyl)thio)-2-fluorobenzonitrile (Step B product, 60.00 g, 167 mmol) in acetic acid (350 mL) was added N-iodosuccinimide (37.58 g, 167 mmol) at room temperature. The reaction mixture was stirred at room temperature for 3 hours and concentrated. The residue was slurried with ethyl acetate in petroleum ether (10%), then acetonitrile, and dried to afford the title compound (65.00 g, 80%). LC-MS: MS (ESI): 485, 487 *m/z* $[M+H]^+$, purity: 63% (254 nm), retention time: 2.07 minutes (LC-MS method 8).

5-((4-Amino-2-bromo-5,6-difluoro-3-((trimethylsilyl)ethynyl)phenyl)thio)-2-fluorobenzonitrile



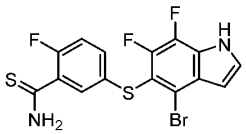
[170] Step D: To a stirred solution of 5-((4-amino-2-bromo-5,6-difluoro-3-iodophenyl)thio)-2-fluorobenzonitrile (Step C product, 50.00 g, 103 mmol) in DMF (500 mL) was added (trimethylsilyl)acetylene (40.50 g, 412 mmol), cuprous iodide (3.93 g, 20.6 mmol), triethylamine (43 mL, 309 mmol), and bis(triphenylphosphine)palladium(II) chloride (7.24 g, 10.3 mmol). The mixture was stirred at 30 °C under argon for 3 hours. The mixture was quenched with water (1000 mL), extracted with ethyl acetate (500 mL × 3).
The combined organic extracts were washed with saturated aqueous LiCl solution, brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography (330 g silica gel column, eluting with 0-20% ethyl acetate in petroleum ether) to give the title compound (39.00 g, 83 %) as a yellow solid. LC-MS: MS (ESI): 455, 457 *m/z* $[M+H]^+$, purity: 84% (254 nm), retention time: 2.40 minutes (LC-MS method 6).

5-((4-Bromo-6,7-difluoro-1H-indol-5-yl)thio)-2-fluorobenzonitrile



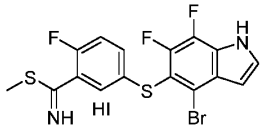
[171] Step E: To a stirred solution of Step D product (33.00 g, 72.5 mmol) in DMF (330 mL) was added cuprous iodide (2.76 g, 14.5 mmol) The reaction mixture was stirred at 100 °C under an argon atmosphere for 3 hours, then quenched with water (1000 mL), and extracted with ethyl acetate (50 mL X 2). The combined organic extracts were washed with brine (50 mL x 3), dried over sodium sulfate, filtered, and concentrated. The residue was purified by flash chromatography (330 g silica gel column, eluting with 0-30% ethyl acetate in petroleum ether) to afford the title compound (22.00 g, 57.4 mmol, 79%) as a yellow solid. LC-MS: MS (ESI): 383, 385 *m/z* $[M+H]^+$, purity: >99% (214 nm), retention time: 2.05 minutes (LC-MS method 8).

5-((4-Bromo-6,7-difluoro-1H-indol-5-yl)thio)-2-fluorobenzothioamide



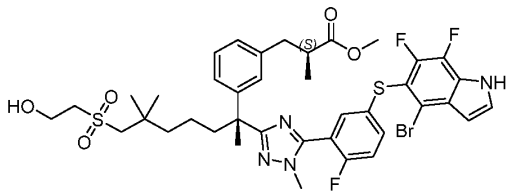
[172] Step F: To a stirred solution of magnesium chloride hexahydrate (21.22 g, 104 mmol) in DMF (400 mL) was added sodium hydrosulfide (11.69 g, 209 mmol) and 5-((4-Bromo-6,7-difluoro-1H-indol-5-yl)thio)-2-fluorobenzonitrile (Step E product, 40.00 g, 104 mmol). The reaction was stirred at room temperature for 2 hours, then quenched with water (400 mL), and extracted with ethyl acetate (400 mL x 2). The combined organic extracts were washed with brine (400 mL x 2), dried over sodium sulfate, filtered, and concentrated to give the title compound (41.00 g, 90 %) as a yellow solid. LC-MS: MS (ESI): 417, 419 *m/z* $[M+H]^+$, purity: 90% (214 nm), retention time: 1.67 minutes (LC-MS method 9).

Methyl 5-((4-bromo-6,7-difluoro-1H-indol-5-yl)thio)-2-fluorobenzimidothioate hydroiodide



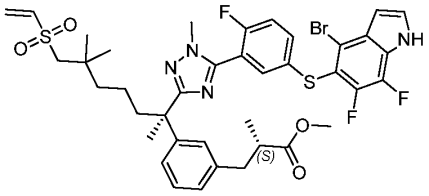
[173] Step G: To a stirred solution of Step F product (41.00 g, 98.3 mmol) in acetone (100 mL) was added methyl iodide (31 mL, 491 mmol) . The reaction was stirred at 50 °C for 2 hours. The mixture was concentrated to give the title compound (56.00 g, 85 %) as a yellow solid. LC-MS: MS (ESI): 431, 433 *m/z* $[M+H]^+$, purity: 83% (214 nm), retention time: 1.60 minutes (LC-MS method 9).

Methyl (S)-3-(3-((R)-2-(5-(5-((4-bromo-6,7-difluoro-1H-indol-5-yl)thio)-2-fluorophenyl)-1-methyl-1H-1,2,4-triazol-3-yl)-7-((2-hydroxyethyl)sulfonyl)-6,6-dimethylheptan-2-yl)phenyl)-2-methylpropanoate



[174] Step H: To a stirred solution of methyl (2S)-3-[3-[(1R)-6-(2-hydroxyethylsulfonyl)-1,5,5-trimethyl-1-(methylaminocarbamoyl)hexyl]phenyl]-2-methyl-propanoate (Step A product of Example 5, 3.20 g, 8.66 mmol) and methyl 5-((4-bromo-6,7-difluoro-1H-indol-5-yl)thio)-2-fluorobenzimidothioate hydroiodide (Step G product, 2.80 g, 5.01 mmol) in pyridine (30 mL) was added magnesium sulphate (5 g). The reaction mixture was stirred at 80 °C overnight, quenched with water (50 mL), and extracted with ethyl acetate (2 x 50 mL). The combined organic extracts were washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by automated flash chromatography (20 g silica gel column, eluting with 0-90% ethyl acetate in petroleum ether) to give the title compound (3.80 g, 68 %) as a solid. LC-MS: MS (ESI): 849, 851 *m/z* $[M+H]^+$, purity: 98% (214 nm), retention time: 2.01 minutes (LC-MS method 4).

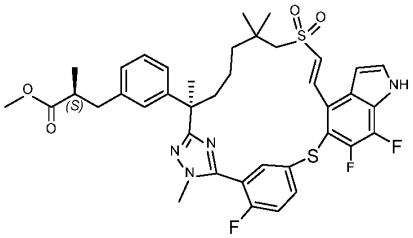
Methyl (S)-3-(3-((R)-2-(5-(5-((4-bromo-6,7-difluoro-1H-indol-5-yl)thio)-2-fluorophenyl)-1-methyl-1H-1,2,4-triazol-3-yl)-6,6-dimethyl-7-(vinylsulfonyl)heptan-2-yl)phenyl)-2-methylpropanoate



[175] Step I: To a stirred solution of the product from Step H (3.80 g, 4.47 mmol) in dichloromethane (40 mL) was added at room temperature triethylamine (1.9 mL, 13.4 mmol) and methanesulfonyl chloride (0.45 mL, 5.81 mmol). The reaction mixture was stirred at room temperature for 2 hours and diluted with dichloromethane (40 mL). The mixture was washed with brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by automated flash chromatography (20 g silica gel column, eluting with 0-80% ethyl acetate in petroleum ether) to give the title compound (3.40 g, 91 %) as a solid. LC-MS: MS (ESI): 831, 833 *m/z* $[M+H]^+$, purity: 81% (214 nm). retention time: 2.11 minutes (LC-MS

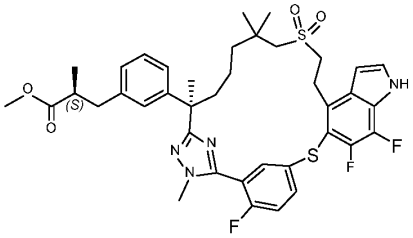
method 4).

Methyl (2S)-2-methyl-3-[3-[(6R,13E)-21,22,28-trifluoro-3,6,10,10-tetramethyl-12,12-dioxo-12λ6,24-dithia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,13,15,17,20,22,25,27-decaen-6-yl]phenyl]propanoate



[176] Step J: A solution of triethylamine (0.96 mL, 6.61 mmol) and bis(tri-tert-butylphosphine)palladium(0) (0.42 g, 0.818 mmol) in toluene (400 mL) was sparged with argon for 15 minutes at room temperature. To this solution was added dropwise the product from Step I (3.40 g, 4.09 mmol) in toluene (20 mL) over 2 hours at 120 °C. After the addition, the mixture was stirred at 100 °C for 2 hours and concentrated. The residue was purified by automated flash chromatography (20 g silica gel column, eluting with 0-65% ethyl acetate in petroleum ether) to give the title compound (2.40 g, 80 %) as solid. LC-MS: MS (ESI): 751 *m/z* $[M+H]^+$, purity: 96% (214 nm). retention time: 2.07 minutes (LC-MS method 4).

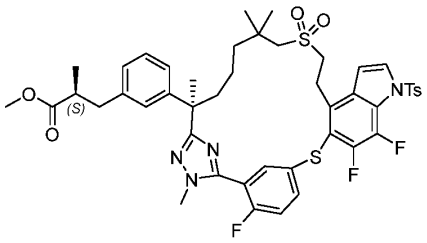
Methyl (2S)-2-methyl-3-[3-[(6R)-21,22,28-trifluoro-3,6,10,10-tetramethyl-12,12-dioxo-12λ6,24-dithia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl]phenyl]propanoate



[177] Step K: To a stirred solution of the product from Step J (2.40 g, 3.60 mmol) in ethanol (40 mL) was added 10% palladium on carbon (wetted with ca. 55% Water, 1 g). The reaction mixture was stirred under hydrogen at 50 °C for 2 hours. The mixture was filtered through a pad of Celite. The filter cake was washed with ethanol (3 x 50 mL). The filtrate was concentrated. The residue was purified by automated flash chromatography (20 g silica gel column, eluting with 0-65% ethyl acetate in petroleum ether) to give the title compound (2.3 g, 85 %) as a solid. LC-MS: MS (ESI): 753 *m/z* $[M+H]^+$, purity: 98% (214 nm). retention

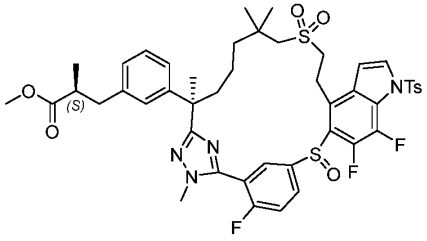
time: 2.10 minutes (LC-MS method 4).

Methyl (2S)-2-methyl-3-[3-[(6R)-21,22,28-trifluoro-3,6,10,10-tetramethyl-12,12-dioxo-19-(p-tolylsulfonyl)-12λ6,24-dithia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl]phenyl]propanoate



[178] Step L: To a stirred solution of the product from Step K (1.50 g, 1.99 mmol) in acetonitrile (20 mL) was added 1-tosyl-1H-imidazole (1.78 g, 7.96 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (1.2 mL, 7.96 mmol). The reaction mixture was stirred at room temperature for 3 days, quenched with water (20 mL), and extracted with ethyl acetate (2 x 50 mL). The combined organic extracts were washed with water, brine, dried over sodium sulfate, filtered, and concentrated. The residue was purified by automated flash chromatography (20 g silica gel column, eluting with 0-80% ethyl acetate in petroleum ether) to give the title compound (900 mg, 49 %) as a solid. LC-MS: MS (ESI): 906 *m/z* $[M+H]^+$, purity: >99% (214 nm). retention time: 2.25 minutes (LC-MS method 4).

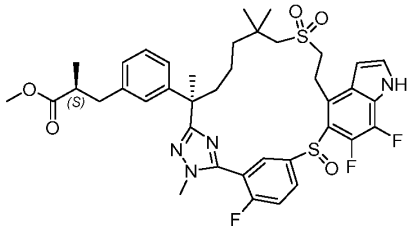
Methyl (2S)-2-methyl-3-[3-[(6R)-21,22,28-trifluoro-3,6,10,10-tetramethyl-12,12,24-trioxo-19-(p-tolylsulfonyl)-12λ6,24λ4-dithia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl]phenyl]propanoate



[179] Step M: To a stirred solution of the product from Step L (900 mg, 0.992 mmol) in acetonitrile (20 mL) was added (S,S)-hydrobenzoin (1.06 g, 4.96 mmol), titanium(IV) isopropoxide (0.87 mL, 2.98 mmol) and tert-butyl hydroperoxide (0.27 g, 2.98 mmol). The mixture was stirred at room temperature for 4 days, poured into 20 mL of water, and extracted with ethyl acetate (2 x 15 mL). The combined organic phases were washed with

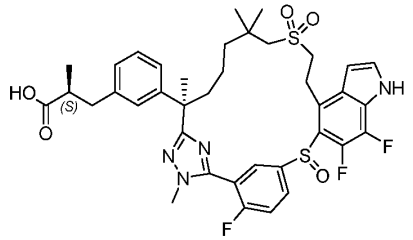
brine, dried over sodium sulphate, filtered, and concentrated. The residue was purified by automated silica gel chromatography (20 g silica gel column, eluting with 0-90% ethyl acetate in petroleum ether) to give the title compound (820 mg, 89 %) as a yellow solid. LC-MS: MS (ESI): 923 *m/z* $[M+H]^+$, purity: 89% (214 nm). retention time: 2.17 minutes (LC-MS method 4).

Methyl (2S)-2-methyl-3-[3-[rac-(6R)-21,22,28-trifluoro-3,6,10,10-tetramethyl-12,12,24-trioxo-12λ6,24λ4-dithia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl]phenyl]propanoate



[180] Step N: To a stirred solution of the product from Step M (820 mg, 0.888 mmol) in tetrahydrofuran (20 mL) was added tetrabutylammonium fluoride (5 ml, 1 M in tetrahydrofuran). The mixture was stirred at room temperature overnight, poured into 20 mL of water, and extracted with ethyl acetate (2 x 15 mL). The combined organic phases were washed brine, dried over sodium sulphate, filtered, and concentrated. The residue was purified by automated silica gel chromatography (20 g silica gel column, eluting with 0-90% ethyl acetate in petroleum ether) to give the title compound (570 mg, 83 %) as a solid. LC-MS: MS (ESI): 769 *m/z* $[M+H]^+$, purity: 92% (214 nm). retention time: 1.98 minutes (LC-MS method 4).

(2S)-2-Methyl-3-[3-[(6R)-21,22,28-trifluoro-3,6,10,10-tetramethyl-12,12,24-trioxo-12λ6,24λ4-dithia-3,4,19,30-tetrazapentacyclo[23.3.1.12,5.015,23.016,20]triaconta-1(29),2(30),4,15,17,20,22,25,27-nonaen-6-yl]phenyl]propanoic acid



[181] Step O: To a stirred solution of Step N product (570 mg, 0.741 mmol) in tetrahydrofuran (2 mL) and water (2 mL) was added lithium hydroxide monohydrate (159 mg, 0.380 mmol). The reaction was stirred at room temperature overnight, acidified with 1N

hydrochloric acid to pH ~ 4, and diluted with ethyl acetate (20 mL), The mixture was washed with brine, dried over sodium sulphate, filtered, and concentrated. The residue was purified by prep-HPLC to give the title compound (335 mg, 60 %, Diastereomer 2) as a solid. LC-MS: MS (ESI): 755 *m/z* $[M+H]^+$, purity: >99% (214 nm). retention time: 8.97 minutes (LC-MS method 12). ^{1}H NMR (500 MHz, CD_3OD) δ 8.24-7.82(m, 2H), 7.61 (d, *J* = 9.0 Hz, 1H), 7.44 (d, *J* = 3.5 Hz, 1H), 7.13 (t, *J* = 7.5Hz, 1H), 7.06(s, 1H), 7.04 - 6.94 (m, 2H), 6.76 (s, 1H), 4.02-3.87 (m, 1H), 3.85 (d, *J* = 2.5 Hz, 3H), 3.72-3.61 (m, 1H), 3.58-3.44 (m, 1H), 3.25-3.21 (m, 1H), 3.12-2.96 (m, 2H), 2.95-2.87 (m, 1H), 2.65-2.53 (m, 2H), 2.31-2.21 (m, 1H), 1.97-1.87 (m, 1H), 1.69 (s, 3H), 1.52-1.34 (m, 3H), 1.19 (s, 3H), 1.11 (s, 3H), 1.06-0.94 (m, 4H) ppm.

Example 8. Analysis of CFTR maturation by western blot

Cystic Fibrosis Submucosal Gland Epithelial Cells

[182] CF submucosal gland epithelial cells (CFSMEo$^-$) were derived from the airways of a CF patient (ΔF508/Q2X) and provided by Dr. Dieter Gruenert, University of California-San Francisco. CFSMEo$^-$ cells were cultured at 37°C with 5% CO_2 in minimum essential medium (MEM) with Earle's salt and nonessential amino acids, supplemented with 10% (volume per volume [v/v]) fetal bovine serum, 2 mM L-glutamine and 1x (v/v) penicillin/streptomycin and grown in tissue culture-treated flasks coated with an extracellular matrix (ECM) cocktail consisting of 10 µg/mL human fibronectin, 30 µg/mL bovine collagen type I, and 100 µg/mL bovine serum albumin in LHC basal medium.

CFTR Mammalian Expression Constructs

[183] Using standard techniques, CFTR and ΔF508-CFTR were each cloned into pcDNA3.0, featuring a cytomegalovirus promoter and SV40 early polyadenylation signal, for expression in mammalian cells (Sambrook et al, 1989).

Nucleofection

[184] CFSMEo$^-$ cells were transiently transfected with CFTR expression constructs using the Lonza 4D-Nucleofector Core unit (Lonza, Catalogue No. AAF-1002B) with the X unit (Lonza, Catalogue No. AAF-1002X) and SF Cell Line 4D-Nucleofector X Kit L (Lonza, Lonza, Catalogue No. V4XC-2024) according to the manufacturer's instructions. In brief, cells were nucleofected with 4-µg plasmid deoxyribonucleic acid (DNA) at a concentration of 5.0×10^7 cells/mL and plated at 2.4×10^5 cells/well onto collagen-coated, 6-well plates (Corning Inc., Corning, NY).

Western Blotting Assay

[185] Transiently transfected CFSMEo$^-$ cells expressing either wild-type CFTR or ΔF508-CFTR were plated at 3.2×10^5 cells/well onto collagen-coated, 6-well plates (Corning Inc.). Twenty-four hours post-transfection, CFTR modulators were applied and allowed to incubate for 48 hours. Cell lysates were then harvested in cold IP lysis buffer (Pierce, Rockford, IL) supplemented with ethylenediaminetetraacetic acid (EDTA)free protease inhibitors (Pierce). Following quantitation with a bicinchoninic acid assay (Pierce), 5μg of each sample were separated on a Novex 4% to 12% Tris Glycine Plus gel (Invitrogen, Waltham, MA) and transferred onto a nitrocellulose membrane using the iBlot 2 Dry Blotting System (Invitrogen). Blots were then incubated with the UNC-596 human CFTR specific monoclonal antibody (lot#s 596TJ100285 and 210628TJ20220317) supplied by the Cystic Fibrosis Foundation, University of North Carolina-Chapel Hill. To control for loading, a second blot was incubated with the murine Na^+/K^+ ATPase monoclonal antibody (Millipore, Catalogue No. 050369). Following normalization of each sample to the Na^+/K^+ ATPase loading control, CFTR maturation levels were quantitated by densitometry using ImageJ software (National Institute of Health, Bethesda, MD).

Example 9. Analysis of CFTR channel function

Study Design

[186] CFhBE cells or CFTR wild-type hBE cells were expanded and seeded onto a semipermeable membrane and cultured for 15 to 20 days at an air-liquid interface to allow for the formation of tight junctions and apically-directed CFTR expression. At this time, CFhBE were treated with the indicated modulators using a Tecan D300 digital dispenser and allowed to incubate for 48 hours at 37°C in a humidified chamber. After 48 hours cells were loaded onto TECC24 platforms (EP Design, Bertem, Belgium) in horizontal orientation and plates were filled with symmetric physiologic saline with glucose ("PS+", in mM: 150 NaCl, 5 KCl, 2 CaCl, 1 MgCl, 10 HEPES, 10 glucose, pH 7.4 with HCl). Transepithelial voltage and resistance were recorded in current clamp mode, and the equivalent current (Ieq) was calculated according to Ohm's Law (V = IR, where V is the voltage across the conductor, I is the current flowing through the conductor, and R is the resistance provided by the conductor to the flow of current). After obtaining a baseline current reading, 10 μM benzamil was added to inhibit the epithelial sodium channel and thus eliminate all non-CFTR conductance. Once a stable baseline had been achieved, forskolin (10 μM) was then added to stimulate CFTR-dependent chloride conductance; finally, 20 μM bumetanide was added to inhibit chloride transport.

Data Analysis

[187] Bar graphs were plotted by subtracting the baseline minimum current after benzamil addition, and the area under the curve (AUC) between forskolin and bumetanide addition was calculated for each well and values are expressed as either fold over tezacaftor/ivacaftor/elexacaftor alone, which was used as the in-plate control, or as a percent of wild type CFTR current. The AUC was used as the measure of CFTR activity and functional restoration of epithelial chloride secretion in response to different therapeutic agents. Bar graphs are displayed as mean ± standard error (SE).

Example 10. Analysis of CFTR maturation by western blot

[188] The effects of a test agent, or of a combination thereof, on CFTR-mediated transepithelial chloride transport was measured using TECC24 recording analysis. Test agents were solubilized in DMSO. Solubilized test agents were mixed with incubation medium containing DMEM/F12, Ultroser G (2%; Crescent Chemical, catalog #67042), Hyclone Fetal Clone II (2%; GE Healthcare, catalog # SH30066.02), bovine brain extract (0.25%; Lonza, catalog #CC-4098), insulin (2.5 µg/mL), IL-13 (10 ng/mL), hydrocortisone (20 nM), transferrin (2.5 µg/mL), triiodothyronine (500 nM), ethanolamine (250 nM), epinephrine (1.5 µM), phosphoethanolamine (250 nM), and retinoic acid (10 nM). Primary human bronchial epithelial cells from a ΔF508 homozygous CF donor (CF-HBE cells; from University of North Carolina Cystic Fibrosis Tissue Procurement Center), grown on Transwell HTS 24-well cell culture inserts (Costar, catalog #3378), were exposed to test agents or controls dissolved in incubation medium. The CF-HBE cells were cultured at 36.5°C for 48 hours before TECC24 recordings were performed in the presence or absence of test agent or agents (used at a final concentration of 1 micromolar or at the concentration indicated), a positive control or vehicle (DMSO).

[189] Following incubation, the transwell cell culture inserts containing the test agent, or combinations thereof, or control-treated CF-HBE cells were loaded onto a TECC24 apparatus (TECC v7 or MTECC v2; EP Design) to record the transepithelial voltage (VT) and resistance (TEER) using 4 AgCl electrodes per well configured in current-clamp mode. The apical and basolateral bath solutions both contained (in mM) 140 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 Hepes, and 10 glucose (adjusted to pH 7.4 with NaOH). To inhibit basal Na+ absorption, the ENaC inhibitor benzamil (10 µM) was added to the bath. Then, the adenylate cyclase activator, forskolin (10 µM), was added to the bath to activate CFTR. The forskolin-stimulated Cl- transport was halted by addition of CFTR inhibitor-172 (20 µM) to the bath at

the end of the experiment to confirm specificity. VT and TEER recordings were digitally acquired at routine intervals using TECC or MTECC software (EP Design). VT and TEER were transformed into equivalent transpeithelial Cl- current (IEQ), and the Area Under the Curve (AUC) of the IEQ timecourse between forskolin and CFTR inhibitor-172 addition is generated using Excel (Microsoft). Efficacy is expressed as the ratio of the test agent AUC divided by vehicle AUC. EC50s based on AUC are generated using the non-linear regression log(agonist) vs. response function in Prism software (Graphpad) with HillSlope fixed = 1.

[190] If a test agent increased the AUC of the forskolin-stimulated IEQ relative to vehicle in CF-HBE cells, and this increase was inhibited by CFTR inhibitor-172, then the test agent was considered a CFTR corrector. The data is shown in Table 2 below.

TABLE 2

NBD1 Stabilizer	Additional Compound(s)	**CFTR-dependent current AUC (relative to vehicle): + = <40; ++ = 40<x<55; +++ = >55**	(+)3uM Navocaftor **CFTR-dependent current AUC (relative to vehicle): + = <40; ++ = 40<x<55; +++ = >55**
Compound 5		+	+
Compound 6		+	+
Compound 7		+	+
Compound 4		+	+
Compound 1		+	+
Compound 5	(+)1uM Compound 2	+	++
Compound 5	(+)1uM Galicaftor	+	++
Compound 5	(+)1uM Compound 3	++	++
Compound 5	(+)1uM Compound A	++	++
Compound 5	(+)1uM Compound 2 (+)1uM Galicaftor	++	++
Compound 5	(+)1uM Compound 2 (+)1uM Compound A	++	+++
Compound 4	(+)1uM Compound 2	++	++
Compound 4	(+)1uM Galicaftor	++	+++
Compound 4	(+)1uM Compound 3	++	+++
Compound 4	(+)1uM Compound A	++	+++
Compound 4	(+)1uM Compound 2 (+)1uM Galicaftor	++	+++
Compound 4	(+)1uM Compound 2 (+)1uM Compound A	++	++
Compound 6	(+)1uM Compound 2	++	++
Compound 6	(+)1uM Galicaftor	++	+++
Compound 6	(+)1uM Compound 3	++	+++
Compound 6	(+)1uM Compound A	++	+++

Compound 6	(+)1uM Compound 2 (+)1uM Galicaftor	++	+++
Compound 6	(+)1uM Compound 2 (+)1uM Compound A	++	++
Compound 7	(+)1uM Compound 2	++	+++
Compound 7	(+)1uM Galicaftor	++	+++
Compound 7	(+)1uM Compound 3	++	+++
Compound 7	(+)1uM Compound A	++	+++
Compound 7	(+)1uM Compound 2 (+)1uM Galicaftor	++	+++
Compound 7	(+)1uM Compound 2 (+)1uM Compound A	+++	+++
Compound 1	(+)1uM Compound 2	+	+++
Compound 1	(+)1uM Galicaftor	++	+++
Compound 1	(+)1uM Compound 3	++	+++
Compound 1	(+)1uM Compound A	++	+++
Compound 1	(+)1uM Compound 2 (+)1uM Galicaftor	++	+++
Compound 1	(+)1uM Compound 2 (+)1uM Compound A	++	+++
Compound 5	(+)1uM Compound 2 (+)1uM Compound 3	++	+++
Compound 6	(+)1uM Compound 2 (+)1uM Compound 3	++	+++
Compound 7	(+)1uM Compound 2 (+)1uM Compound 3	++	+++
Compound 4	(+)1uM Compound 2 (+)1uM Compound 3	++	+++
Compound 1	(+)1uM Compound 2 (+)1uM Compound 3	++	+++

Claims

1. A method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an NBD1 stabilizer in combination with an ICL4 corrector.

2. The method of claim 1, wherein the NBD1 stabilizer and the ICL4 corrector are administered simultaneously.

3. The method of claim 1, wherein the NBD1 stabilizer is administered prior to the ICL4 corrector.

4. The method of claim 1, wherein the NBD1 stabilizer is administered after the ICL4 corrector.

5. A method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administered to the subject a therapeutically effective amount of an NBD1 stabilizer, wherein the subject has previously received treatment with an ICL4 corrector.

6. A method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administered to the subject a therapeutically effective amount of an ICL4 corrector, wherein the subject has previously received treatment with an NBD1 stabilizer.

7. The method of any of claims 1-6, further comprising administering to the subject a TMD1 corrector.

8. A method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an NBD1 stabilizer in combination with an TMD1 corrector.

9. The method of claim 1, wherein the NBD1 stabilizer and the TMD1 corrector are administered simultaneously.

10. The method of claim 1, wherein the NBD1 stabilizer is administered prior to the TMD1 corrector.

11. The method of claim 1, wherein the NBD1 stabilizer is administered after the TMD1 corrector.

12. A method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administered to the subject a therapeutically effective amount of an NBD1 stabilizer, wherein the subject has previously received treatment with an TMD1 corrector.

13. A method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administered to the subject a therapeutically effective amount of an TMD1 corrector, wherein the subject has previously received treatment with an NBD1 stabilizer.

14. The method of any of claims 8-13, further comprising administering to the subject a ICL4 corrector.

15. A method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an NBD1 stabilizer in combination with a CFTR potentiator.

16. The method of claim 1, wherein the NBD1 stabilizer and the CFTR potentiator are administered simultaneously.

17. The method of claim 1, wherein the NBD1 stabilizer is administered prior to the CFTR potentiator.

18. The method of claim 1, wherein the NBD1 stabilizer is administered after the CFTR potentiator.

19. A method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administered to the subject a therapeutically effective amount of an NBD1 stabilizer, wherein the subject has previously received treatment with a CFTR potentiator.

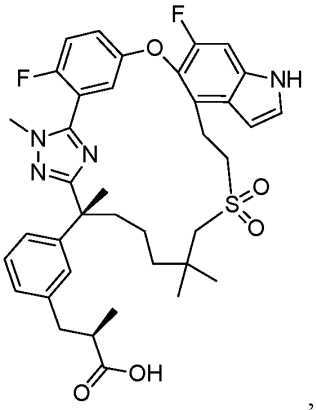
20. A method of treating CFTR-mediated disease or disorder in a subject in need thereof, comprising administered to the subject a therapeutically effective amount of a CFTR

potentiator, wherein the subject has previously received treatment with an NBD1 stabilizer.

21. The method of any of claims 15-20, further comprising administering to the subject a TMD1 corrector.

22. The method of any of claims 15-21, further comprising administering to the subject an ICL4 corrector.

23. The method of any of claims 1-22, wherein the NBD1 stabilizer is a compound selected from the group consisting of



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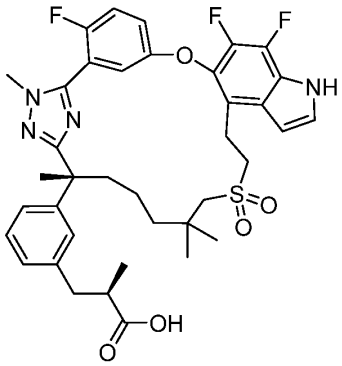

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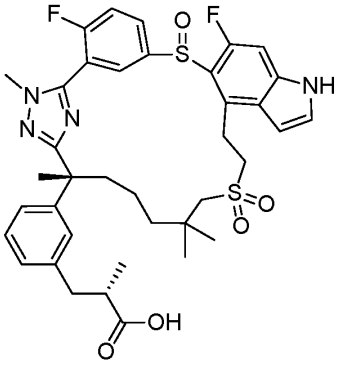

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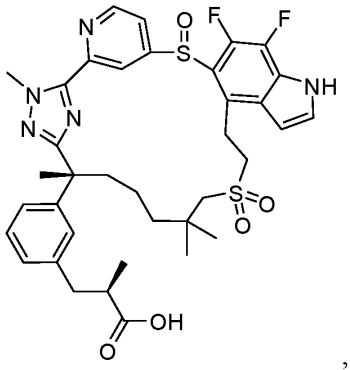

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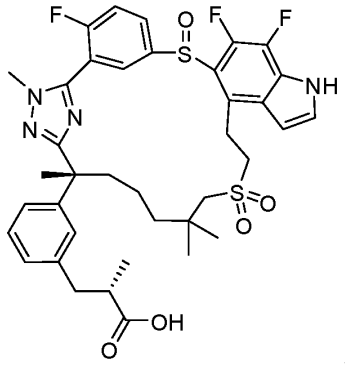

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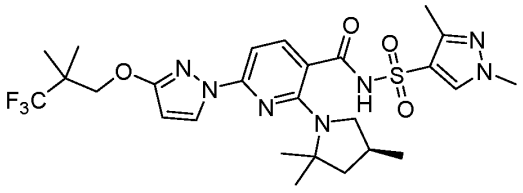
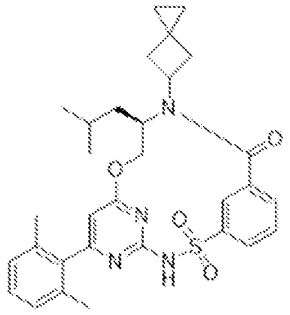
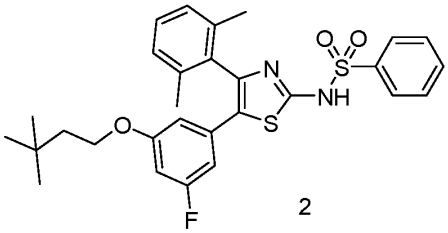
or a pharmaceutically acceptable salt thereof.

24. The method of any of claims 1-23, wherein the ICL4 corrector is a compound selected from the group consisting of





Elexacaftor (VX-445)



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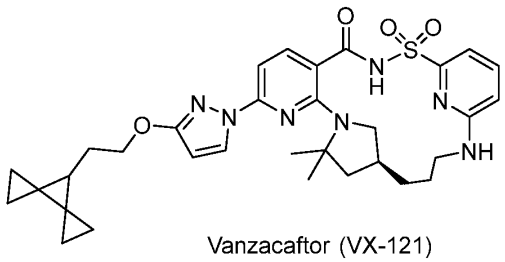
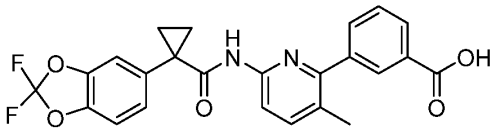


Vanzacaftor (VX-121)

or a pharmaceutically acceptable salt thereof.

25. The method of any of claims 1-23, wherein the TMD1 corrector is a compound selected from the group consisting of



Lumacaftor (VX-809)

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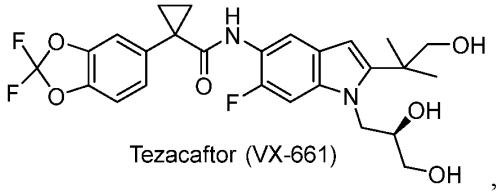

Tezacaftor (VX-661)

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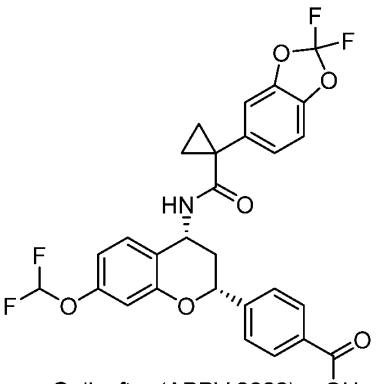

Galicaftor (ABBV-2222)

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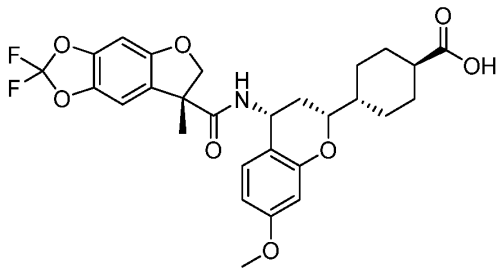

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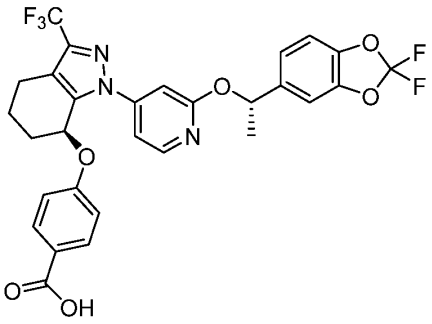
and



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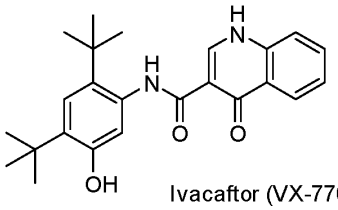
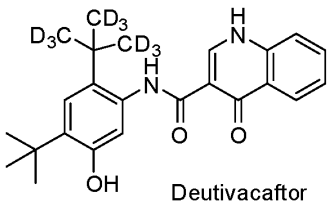
or a pharmaceutically acceptable salt thereof.

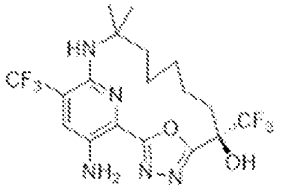
26. The method of any of claims 1-25, wherein the CFTR potentiator is a compound selected from the group consisting of

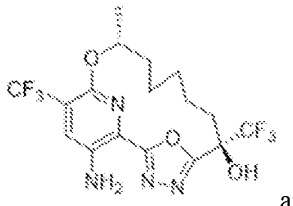


Ivacaftor (VX-770)



Deutivacaftor





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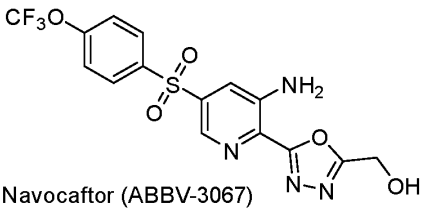

Navocaftor (ABBV-3067)

or a pharmaceutically acceptable salt thereof.

27. The method of any of claims 1-26, wherein the CFTR-mediated disease or disorder is selected from the group consisting of cystic fibrosis, asthma, smoke induced COPD, chronic bronchitis, rhinosinusitis, constipation, pancreatitis, pancreatic insufficiency, male infertility caused by congenital bilateral absence of the vas deferens (CBAVD), mild pulmonary disease, idiopathic pancreatitis, allergic bronchopulmonary aspergillosis (ABPA), liver disease, hereditary emphysema, hereditary hemochromatosis, coagulation-fibrinolysis deficiencies, protein C deficiency, Type 1 hereditary angioedema, lipid processing deficiencies, familial hypercholesterolemia, Type 1 chylomicronemia, abetalipoproteinemia, lysosomal storage diseases, I-cell disease/pseudo-Hurler, mucopolysaccharidoses, Sandhof/Tay-Sachs, Crigler-Najjar type II, polyendocrinopathy/hyperinsulemia, Diabetes mellitus, Laron dwarfism, myeloperoxidase deficiency, primary hypoparathyroidism, melanoma, glycanosis CDG type 1, congenital hyperthyroidism, osteogenesis imperfecta, hereditary hypofibrinogenemia, ACT deficiency, Diabetes insipidus (DI), neurophyseal DI, neprogenic DI, Charcot-Marie Tooth syndrome, Perlizaeus-Merzbacher disease, neurodegenerative diseases, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, progressive supranuclear palsy, Pick's disease, several polyglutamine neurological disorders, Huntington's, spinocerebellar ataxia type I, spinal and bulbar muscular atrophy, dentatorubal pallidoluysian, myotonic dystrophy, spongiform encephalopathies, hereditary Creutzfeldt-Jakob disease, Fabry disease, Straussler-Scheinker syndrome, COPD, dry-eye disease, Sjogren's disease, Osteoporosis,

Osteopenia, bone healing and bone growth, bone repair, bone regeneration, reducing bone resorption, increasing bone deposition, Gorham's Syndrome, chloride channelopathies, myotonia congenita, Bartter's syndrome type III, Dent's disease, hyperekplexia, epilepsy, hyperekplexia, lysosomal storage disease, Angelman syndrome, Primary Ciliary Dyskinesia (PCD), PCD with situs inversus, PCD without situs inversus and ciliary aplasia.

28. The method of claim 27, wherein the CFTR-mediated disease or disorder is selected from the group consisting of disease or condition is selected from cystic fibrosis, congenital bilateral absence of vas deferens (CBAVD), acute, recurrent, or chronic pancreatitis, disseminated bronchiectasis, asthma, allergic pulmonary aspergillosis, chronic obstructive pulmonary disease (COPD), chronic sinusitis, dry eye disease, protein C deficiency, Abetalipoproteinemia, lysosomal storage disease, type 1 chylomicronemia, mild pulmonary disease, lipid processing deficiencies, type 1 hereditary angioedema, coagulation-fibrinolyis, hereditary hemochromatosis, CFTR-related metabolic syndrome, chronic bronchitis, constipation, pancreatic insufficiency, hereditary emphysema, and Sjogren's syndrome.

29. The method of any of claims 1-28, wherein the CFTR-mediated disease or disorder is cystic fibrosis.

30. The method of any of claims 1-29, wherein the subject is human.