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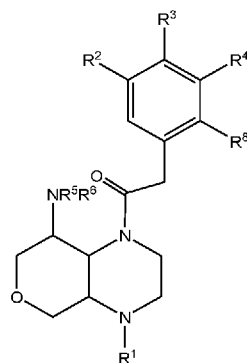
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(54) Title: PYRANO[3,4-B]PYRAZINE KAPPA OPIOID RECEPTOR LIGANDS FOR TREATING ADDICTION, PRURITUS, PAIN, AND INFLAMMATION



(I)

(57) Abstract: 1-Phenylacetyl-8-aminohexahydro-2H-pyrano[3,4-b]pyrazines of formula Formula (I) are disclosed. The compounds are kappa ligands and are useful to treat drug dependency, pruritus, pain, and inflammation.



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PYRANO[3,4-b]PYRAZINE KAPPA OPIOID RECEPTOR LIGANDS FOR TREATING ADDICTION, PRURITUS, PAIN, AND INFLAMMATION

Cross-reference to Related Applications

[001] This application claims priority from US provisional application 62/596,357, filed December 8, 2017, which is incorporated herein by reference in its entirety.

Field of the Invention

[002] The invention relates to kappa opioid receptor ligands of the 1-phenylacetyl-8-aminohexahydro-2H-pyrano[3,4-b]pyrazine family that are useful to treat disorders such as drug dependency, pruritus, pain, inflammation, and mood disorders.

Background of the Invention

[003] One-and-a-half million current (past-month) cocaine users (12 or older) (approximately 0.6% of the U.S. population) were reported in 2014. The 2011 Drug Abuse Warning Network (DAWN) report showed that, of the nearly 1.3 million visits to emergency departments for illicit drug misuse or abuse, cocaine was involved in over 500,000 of these emergency department visits. No medication has been shown to be effective in humans for treating cocaine dependence.

[004] Exposure to cocaine, which inhibits the biogenic amine neurotransmitter transporters, acutely causes increased extracellular dopamine, serotonin, and norepinephrine, and also results in changes in components of the endogenous opioid system. Acutely, cocaine results in increased gene expression of dynorphin in the dorsal and ventral striatum, in animal models. Chronic cocaine exposure also results in changes in mu and kappa opioid receptor binding. Similar alterations have been detected in human postmortem brain following cocaine abuse or dependence. Kappa opioid receptor/dynorphin dysfunction has been observed following experimental stress in animals, with accompanying depressant-like behavioral effects. Additionally, PET imaging has shown brain KOP-r populations to be altered in people exhibiting symptoms of trauma, including anhedonia or dysphoria and anxiety.

[005] Full kappa agonists have the ability to block the rewarding effects of cocaine, but by themselves they have been shown to be aversive [see Zhang et al. *Psychopharmacology* 179(3):

551-558 (2005)]. Similarly, dysphoria and psychotomimetic/hallucinogenic effects result from KOP-r agonist administration in humans [see Pfeiffer et al. *Science* 233(4765): 774-776 (1986)]. Kappa antagonists have been shown to block stress induced reinstatement to cocaine seeking in animal self-administration models, but with no effect on drug-induced reinstatement.

[006] Although multiple selective KOP-r antagonists (with no agonistic efficacy, and full blockade) have been identified to date, no selective partial or differentially efficacious G-protein/beta-arrestin signaling biased KOP-r agonists have been previously reported to be tested in animal models of drug addiction.

[007] Chronic pruritus, which is defined as itch persisting for more than 6 weeks, has a number of causes and is associated with a markedly reduced quality of life. Chronic pruritus is characteristic of several dermatologic diseases (e.g., atopic eczema, psoriasis, lichen planus, and scabies) but also occurs in a variety of noncutaneous disorders. The causes of chronic pruritus can be broadly categorized into four major groups: dermatologic causes, systemic causes (e.g., cholestasis, chronic kidney disease, myeloproliferative disorders, and hyperthyroidism), neuropathic causes (e.g., notalgia paresthetica [a distinctive itch of the upper back] and brachioradial pruritus [a characteristic itch of the arms, probably caused by spinal-nerve impingement]), and psychogenic causes. Kappa agonists such as nalfurafine (TRK-820) have been shown to abolish the chloroquine-induced scratching in murine models [see Inan and Cowan *Eur J Pharmacol.*, 502, 233-237 (2004)]. Thus, KOP-r agonists are expected to be useful for the treatment of chronic pruritus.

[008] Kappa agonists exert potent analgesic activity in a wide variety of visceral pain models. These effects are mediated at peripherally located κ -receptors and possibly through additional nonopioid action at sodium channels located on peripheral nerve endings of primary sensory afferents. The analgesic potency of κ -agonists in visceral pain is enhanced in the presence of inflammation. Thus, KOP-r agonists are expected to be useful for the treatment of pain and inflammation.

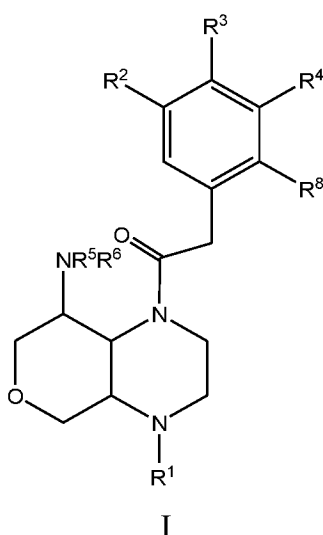
[009] Recent research suggests that kappa full agonists are potentially useful for mania, and kappa partial agonists may find use for mood stabilization. However, currently available agents have some unfavorable properties. The development of kappa-selective agonists with improved

drug-like characteristics may provide mood-modulating medications.

Summary of the Invention

[010] It has now been found that 1-phenylacetyl-8-aminohexahydro-2H-pyrano[3,4-b]pyrazine derivatives are KOP-r ligands with differential agonistic activity, making them useful to treat dependence on cocaine as well as other psychostimulants and alcohol. They are also useful to treat pruritus, pain, inflammation, and mood disorders.

[011] In one aspect, the invention relates to compounds of Formula I



wherein

R^1 is chosen from hydrogen, (C_1-C_{10}) hydrocarbyl, $-C(=O)(C_1-C_{10})$ hydrocarbyl, $-C(=O)O(C_1-C_{10})$ hydrocarbyl and $-SO_2(C_1-C_{10})$ hydrocarbyl;

R^2 , R^3 , R^4 , and R^8 are chosen independently from hydrogen, halogen, (C_1-C_4) alkyl, fluoro (C_1-C_4) alkyl, nitro, $-SO_3H$ and $-N^+HR^5R^6$; with the provisos that (1) at least one of R^2 , R^3 , R^4 , and R^8 must be other than hydrogen; and (2) when R^2 or R^4 is fluorine, at least one of the remaining substituents on phenyl must be other than hydrogen; and

R^5 and R^6 are chosen from (C_1-C_{10}) hydrocarbyl, optionally substituted with fluoro; or, taken together with the nitrogen to which they are attached, R^5 and R^6 form a five-, six- or seven-membered non-aromatic heterocycle, which may be optionally substituted with fluoro or (C_1-C_4) alkyl.

[012] In another aspect, the invention relates to a method for activating a kappa opioid receptor. The method comprises contacting a kappa opioid receptor with a compound as described herein.

[013] In another aspect, the invention relates to a method for treating addiction, particularly cocaine addiction. The method comprises administering to a patient a compound as described herein.

[014] In another aspect, the invention relates to a method for treating mood disorders. The method comprises administering to a patient a compound as described herein.

[015] In another aspect, the invention relates to a method for treating pruritus. The method comprises administering to a patient a compound as described herein.

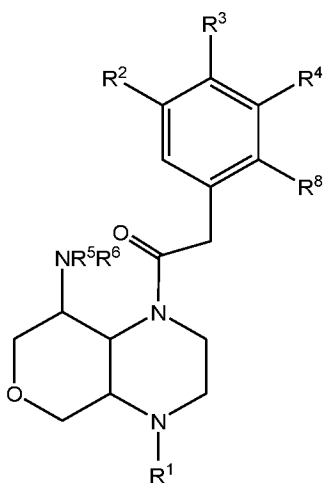
[016] In another aspect, the invention relates to a method for treating pain. The method comprises administering to a patient a compound as described herein.

[017] In another aspect, the invention relates to a method for treating inflammation. The method comprises administering to a patient a compound as described herein.

[018] In another aspect, the invention relates to pharmaceutical compositions comprising a pharmaceutically acceptable carrier and a compound as described herein.

Detailed Description of the Invention

[019] In one aspect, the invention relates to compounds of formula I:



I

[020] In some embodiments, R^1 is hydrogen. In some embodiments, R^1 is (C₁-C₁₀)hydrocarbyl. In some embodiments, R^1 is (C₁-C₇)hydrocarbyl. In some embodiments, R^1 is (C₅-C₇)hydrocarbyl. In some embodiments, R^1 is (C₁-C₇)alkyl. In some embodiments, R^1 is benzyl. In some embodiments, R^1 is (C₃-C₄)alkyl. In some embodiments, R^1 is methyl. In some embodiments, R^1 is -CH₂R¹⁰, wherein R¹⁰ is (C₄-C₆)carbocycle.

[021] In some embodiments, R^1 is -C(=O)(C₁-C₁₀)hydrocarbyl. In some embodiments, R^1 is -C(=O)O(C₁-C₁₀)hydrocarbyl. In some embodiments, R^1 is -C(=O)O(C₁-C₃)hydrocarbyl. In some embodiments, R^1 is -SO₂(C₁-C₁₀)hydrocarbyl. In some embodiments, R^1 is -SO₂(C₁-C₈)hydrocarbyl. In some embodiments, R^1 is -SO₂(C₁-C₃)alkyl.

[022] In some embodiments, R^2 is hydrogen. In some embodiments, R^2 is halogen. In some embodiments, R^2 is (C₁-C₄)alkyl. In some embodiments, R^2 is fluoro(C₁-C₄)alkyl. In some embodiments, R^2 is nitro. In some embodiments, R^2 is -SO₃H. In some embodiments, R^2 is -N⁺HR⁵R⁶. In some embodiments, R^2 is (C₁-C₃)alkyl. In some embodiments, R^2 is chloro. In some embodiments, R^2 is fluoro. In some embodiments, R^2 is trifluoromethyl. In some embodiments, R^2 is methyl. In some embodiments, R^2 is fluoro(C₁-C₃)alkyl.

[023] In some embodiments, R^3 is hydrogen. In some embodiments, R^3 is halogen. In some embodiments, R^3 is (C₁-C₄)alkyl. In some embodiments, R^3 is fluoro(C₁-C₄)alkyl. In some embodiments, R^3 is nitro. In some embodiments, R^3 is -SO₃H. In some embodiments, R^3 is -N⁺HR⁵R⁶. In some embodiments, R^3 is (C₁-C₃)alkyl. In some embodiments, R^3 is chloro. In some embodiments, R^3 is fluoro. In some embodiments, R^3 is trifluoromethyl. In some embodiments, R^3 is methyl. In some embodiments, R^3 is fluoro(C₁-C₃)alkyl.

[024] In some embodiments, R^4 is hydrogen. In some embodiments, R^4 is halogen. In some embodiments, R^4 is (C₁-C₄)alkyl. In some embodiments, R^4 is fluoro(C₁-C₄)alkyl. In some embodiments, R^4 is nitro. In some embodiments, R^4 is -SO₃H. In some embodiments, R^4 is -N⁺HR⁵R⁶. In some embodiments, R^4 is (C₁-C₃)alkyl. In some embodiments, R^4 is chloro. In some embodiments, R^4 is fluoro. In some embodiments, R^4 is trifluoromethyl. In some embodiments, R^4 is methyl. In some embodiments, R^4 is fluoro(C₁-C₃)alkyl.

[025] In some embodiments, R⁵ is (C₁-C₁₀)hydrocarbyl. In some embodiments, R⁵ is fluoro(C₁-C₁₀)hydrocarbyl. In some embodiments, R⁵ is (C₁-C₆)hydrocarbyl. In some embodiments, R⁵ is methyl.

[026] In some embodiments, R⁶ is (C₁-C₁₀)hydrocarbyl. In some embodiments, R⁶ is fluoro(C₁-C₁₀)hydrocarbyl. In some embodiments, R⁶ is (C₁-C₆)hydrocarbyl. In some embodiments, R⁶ is methyl. In some embodiments, R⁶ is cyclopropylmethyl.

[027] In some embodiments, R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a five-membered non-aromatic heterocycle. In some embodiments, R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a six-membered non-aromatic heterocycle. In some embodiments, R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a seven-membered non-aromatic heterocycle.

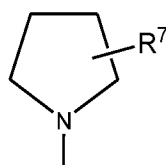
[028] In some embodiments, R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a five-membered non-aromatic heterocycle, which is substituted with fluoro. In some embodiments, R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a six-membered non-aromatic heterocycle, which is substituted with fluoro. In some embodiments, R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a seven-membered non-aromatic heterocycle, which is substituted with fluoro.


[029] In some embodiments, R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a five-membered non-aromatic heterocycle, which is substituted with (C₁-C₄)alkyl. In some embodiments, R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a six-membered non-aromatic heterocycle, which is substituted with (C₁-C₄)alkyl. In some embodiments, R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a seven-membered non-aromatic heterocycle, which is substituted with (C₁-C₄)alkyl.

[030] In some embodiments, R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a five-membered non-aromatic heterocycle, which may be substituted with fluoro or (C₁-C₄)alkyl. In some embodiments, R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a six-membered non-aromatic heterocycle, which is substituted with fluoro and (C₁-C₄)alkyl. In some embodiments, R⁵ and R⁶, taken together with the nitrogen to which

they are attached, form a seven-membered non-aromatic heterocycle, which is substituted with fluoro and (C₁-C₄)alkyl.

[031] In some embodiments, R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a pyrrolidine ring.



[032] In some embodiments, -NR⁵R⁶ is  and R⁷ is hydrogen; in others R⁷ is fluoro. And in others R⁷ is (C₁-C₃)alkyl.

[033] In some embodiments, R⁸ is hydrogen. In some embodiments, R⁸ is halogen. In some embodiments, R⁸ is (C₁-C₄)alkyl. In some embodiments, R⁸ is fluoro(C₁-C₄)alkyl. In some embodiments, R⁸ is nitro. In some embodiments, R⁸ is -SO₃H. In some embodiments, R⁸ is -N⁺HR⁵R⁶.

[034] In summary, the invention relates to:

- [1]. A compound of formula I.
- [2]. A compound according to [1] above wherein R¹ is hydrogen.
- [3]. A compound according to [1] above wherein R¹ is (C₁-C₁₀)hydrocarbyl.
- [4]. A compound according to [1] above wherein R¹ is (C₁-C₇)hydrocarbyl.
- [5]. A compound according to [1] above wherein R¹ is (C₅-C₇)hydrocarbyl.
- [6]. A compound according to [1] above wherein R¹ is (C₁-C₇)alkyl.
- [7]. A compound according to [1] above wherein R¹ is benzyl.
- [8]. A compound according to [1] above wherein R¹ is (C₃-C₄)alkyl.
- [9]. A compound according to [1] above wherein R¹ is methyl.

- [10]. A compound according to [1] above wherein R^1 is $-CH_2R^{10}$ and R^{10} is (C₄-C₆)carbocycle.
- [11]. A compound according to [1] above wherein R^1 is $-C(=O)(C_1-C_{10})$ hydrocarbyl.
- [12]. A compound according to [1] above wherein R^1 is $-C(=O)O(C_1-C_{10})$ hydrocarbyl.
- [13]. A compound according to [1] above wherein R^1 is $-C(=O)O(C_1-C_3)$ hydrocarbyl.
- [14]. A compound according to [1] above wherein R^1 is $-SO_2(C_1-C_{10})$ hydrocarbyl.
- [15]. A compound according to [1] above wherein R^1 is $-SO_2(C_1-C_8)$ hydrocarbyl.
- [16]. A compound according to [1] above wherein R^1 is $-SO_2(C_1-C_3)$ alkyl.
- [17]. A compound according to any of [1] through [16] above wherein R^2 is hydrogen.
- [18]. A compound according to any of [1] through [16] above wherein R^2 is halogen.
- [19]. A compound according to any of [1] through [16] above wherein R^2 is (C₁-C₄)alkyl.
- [20]. A compound according to any of [1] through [16] above wherein R^2 is fluoro(C₁-C₄)alkyl.
- [21]. A compound according to any of [1] through [16] above wherein R^2 is nitro.
- [22]. A compound according to any of [1] through [16] above wherein R^2 is $-SO_3H$.
- [23]. A compound according to any of [1] through [16] above wherein R^2 is $-N^+HR^5R^6$.
- [24]. A compound according to any of [1] through [16] above wherein R^2 is (C₁-C₃)alkyl.
- [25]. A compound according to any of [1] through [16] above wherein R^2 is chloro.
- [26]. A compound according to any of [1] through [16] above wherein R^2 is fluoro.
- [27]. A compound according to any of [1] through [16] above wherein R^2 is trifluoromethyl.

- [28]. A compound according to any of [1] through [16] above wherein R^2 is methyl.
- [29]. A compound according to any of [1] through [16] above wherein R^2 is fluoro(C_1 - C_3)alkyl.
- [30]. A compound according to any of [1] through [29] above wherein R^3 is hydrogen.
- [31]. A compound according to any of [1] through [29] above wherein R^3 is halogen.
- [32]. A compound according to any of [1] through [29] above wherein R^3 is (C_1 - C_4)alkyl.
- [33]. A compound according to any of [1] through [29] above wherein R^3 is fluoro(C_1 - C_4)alkyl.
- [34]. A compound according to any of [1] through [29] above wherein R^3 is nitro.
- [35]. A compound according to any of [1] through [29] above wherein R^3 is $-SO_3H$.
- [36]. A compound according to any of [1] through [29] above wherein R^3 is $-N^+HR^5R^6$.
- [37]. A compound according to any of [1] through [29] above wherein R^3 is (C_1 - C_3)alkyl.
- [38]. A compound according to any of [1] through [29] above wherein R^3 is chloro.
- [39]. A compound according to any of [1] through [29] above wherein R^3 is fluoro.
- [40]. A compound according to any of [1] through [29] above wherein R^3 is trifluoromethyl.
- [41]. A compound according to any of [1] through [29] above wherein R^3 is methyl.
- [42]. A compound according to any of [1] through [29] above wherein R^3 is fluoro(C_1 - C_3)alkyl.
- [43]. A compound according to any of [1] through [42] above wherein R^4 is hydrogen.
- [44]. A compound according to any of [1] through [42] above wherein R^4 is halogen.

- [45]. A compound according to any of [1] through [42] above wherein R^4 is (C₁-C₄)alkyl.
- [46]. A compound according to any of [1] through [42] above wherein R^4 is fluoro(C₁-C₄)alkyl.
- [47]. A compound according to any of [1] through [42] above wherein R^4 is nitro.
- [48]. A compound according to any of [1] through [42] above wherein R^4 is -SO₃H.
- [49]. A compound according to any of [1] through [42] above wherein R^4 is -N⁺HR⁵R⁶.
- [50]. A compound according to any of [1] through [42] above wherein R^4 is (C₁-C₃)alkyl.
- [51]. A compound according to any of [1] through [42] above wherein R^4 is chloro.
- [52]. A compound according to any of [1] through [42] above wherein R^4 is fluoro.
- [53]. A compound according to any of [1] through [42] above wherein R^4 is trifluoromethyl.
- [54]. A compound according to any of [1] through [42] above wherein R^4 is methyl.
- [55]. A compound according to any of [1] through [42] above wherein R^4 is fluoro(C₁-C₃)alkyl.
- [56]. A compound according to any of [1] through [55] above wherein R^5 is (C₁-C₁₀)hydrocarbyl.
- [57]. A compound according to any of [1] through [55] above wherein R^5 is fluoro(C₁-C₁₀)hydrocarbyl.
- [58]. A compound according to any of [1] through [55] above wherein R^5 is (C₁-C₆)hydrocarbyl.
- [59]. A compound according to any of [1] through [55] above wherein R^5 is methyl.

- [60]. A compound according to any of [1] through [59] above wherein R⁶ is (C₁-C₁₀)hydrocarbyl.
- [61]. A compound according to any of [1] through [59] above wherein R⁵ is fluoro(C₁-C₁₀)hydrocarbyl.
- [62]. A compound according to any of [1] through [59] above wherein R⁵ is (C₁-C₆)hydrocarbyl.
- [63]. A compound according to any of [1] through [59] above wherein R⁵ is methyl.
- [64]. A compound according to any of [1] through [59] above wherein R⁵ is cyclopropylmethyl.
- [65]. A compound according to any of [1] through [55] above wherein R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a five-membered non-aromatic heterocycle.
- [66]. A compound according to any of [1] through [55] above wherein R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a six-membered non-aromatic heterocycle.
- [67]. A compound according to any of [1] through [55] above wherein R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a seven-membered non-aromatic heterocycle.
- [68]. A compound according to any of [1] through [55] above wherein R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a five-membered non-aromatic heterocycle, which is substituted with fluoro.
- [69]. A compound according to any of [1] through [55] above wherein R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a six-membered non-aromatic heterocycle, which is substituted with fluoro.

[70]. A compound according to any of [1] through [55] above wherein R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a seven-membered non-aromatic heterocycle, which is substituted with fluoro.

[71]. A compound according to any of [1] through [55] above wherein R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a five-membered non-aromatic heterocycle, which is substituted with (C₁-C₄)alkyl.

[72]. A compound according to any of [1] through [55] above wherein R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a six-membered non-aromatic heterocycle, which is substituted with (C₁-C₄)alkyl.

[73]. A compound according to any of [1] through [55] above wherein R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a seven-membered non-aromatic heterocycle, which is substituted with (C₁-C₄)alkyl.

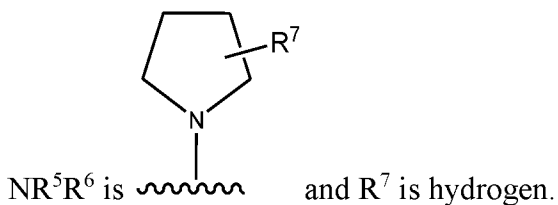
[74]. A compound according to any of [1] through [55] above wherein R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a five-membered non-aromatic heterocycle, which is substituted with fluoro and (C₁-C₄)alkyl.

[75]. A compound according to any of [1] through [55] above wherein R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a six-membered non-aromatic heterocycle, which is substituted with fluoro and (C₁-C₄)alkyl.

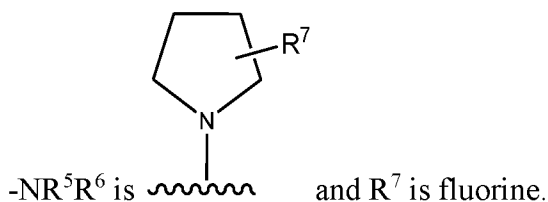
[76]. A compound according to any of [1] through [55] above wherein R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a seven-membered non-aromatic heterocycle, which is substituted with fluoro and (C₁-C₄)alkyl.

[77]. A compound according to any of [1] through [55], [65], [68], [71], and [74] above wherein R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a pyrrolidine ring.

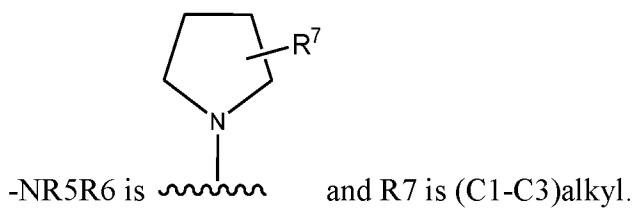
[78]. A compound according to any of [1] through [55], [65], and [77] above wherein -



[79]. A compound according to any of [1] through [55], [65], [68], and [77] above wherein



[80]. A compound according to any of [1] through [55], [65], [71], and [77] above wherein



[81]. A compound according to any of [1] through [80] above wherein R^8 is hydrogen.

[82]. A compound according to any of [1] through [80] above wherein R^8 is halogen.

[83]. A compound according to any of [1] through [80] above wherein R^8 is (C1-C4)alkyl.

[84]. A compound according to any of [1] through [80] above wherein R^8 is fluoro(C1-C4)alkyl.

[85]. A compound according to any of [1] through [80] above wherein R^8 is nitro.

[86]. A compound according to any of [1] through [80] above wherein R^8 is $-\text{SO}_3\text{H}$.

[87]. A compound according to any of [1] through [80] above wherein R^8 is $-\text{N}^+\text{HR}^5\text{R}^6$.

[035] Throughout this specification the terms and substituents retain their definitions.

[036] C₁ to C₁₀ hydrocarbon includes alkyl, cycloalkyl, polycycloalkyl, alkenyl, alkynyl, aryl

and combinations thereof. Examples include benzyl, phenethyl, cyclohexylmethyl, adamantyl, camphoryl and naphthylethyl. Hydrocarbonyl refers to any substituent comprised of hydrogen and carbon as the only elemental constituents. Aliphatic hydrocarbons are hydrocarbons that are not aromatic; they may be saturated or unsaturated, cyclic, linear or branched. Examples of aliphatic hydrocarbons include isopropyl, 2-butenyl, 2-butyryl, cyclopentyl, norbornyl, etc. Aromatic hydrocarbons include benzene (phenyl), naphthalene (naphthyl), anthracene, etc.

[037] Unless otherwise specified, alkyl (or alkylene) is intended to include linear or branched saturated hydrocarbon structures and combinations thereof. Alkyl refers to alkyl groups from 1 to 20 carbon atoms, preferably 1 to 10 carbon atoms, more preferably 1 to 6 carbon atoms. Examples of alkyl groups include methyl, ethyl, propyl, isopropyl, n-butyl, s-butyl, t-butyl and the like.

[038] Cycloalkyl is a subset of hydrocarbon and includes cyclic hydrocarbon groups of from 3 to 8 carbon atoms. Examples of cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, norbornyl and the like.

[039] Unless otherwise specified, the term "carbocycle" is intended to include ring systems in which the ring atoms are all carbon but of any oxidation state. Thus (C₃-C₁₀) carbocycle refers to both non-aromatic and aromatic systems, including such systems as cyclopropane, benzene and cyclohexene; (C₈-C₁₂) carbopolycycle refers to such systems as norbornane, decalin, indane and naphthalene. Carbocycle, if not otherwise limited, refers to monocycles, bicycles and polycycles.

[040] Heterocycle means an aliphatic or aromatic carbocycle residue in which from one to four carbons is replaced by a heteroatom selected from the group consisting of N, O, and S. The nitrogen and sulfur heteroatoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized. Unless otherwise specified, a heterocycle may be non-aromatic (heteroaliphatic) or aromatic (heteroaryl). Examples of heterocycles include pyrrolidine, pyrazole, pyrrole, indole, quinoline, isoquinoline, tetrahydroisoquinoline, benzofuran, benzodioxan, benzodioxole (commonly referred to as methylenedioxyphenyl, when occurring as a substituent), tetrazole, morpholine, thiazole, pyridine, pyridazine, pyrimidine, thiophene, furan, oxazole, oxazoline, isoxazole, dioxane, tetrahydrofuran and the like. Examples of heterocyclyl residues include piperazinyl, piperidinyl, pyrazolidinyl, imidazolyl, imidazolyl, imidazolidinyl,

pyrazinyl, oxazolidinyl, isoxazolidinyl, thiazolidinyl, isothiazolyl, quinuclidinyl, isothiazolidinyl, benzimidazolyl, thiadiazolyl, benzopyranyl, benzothiazolyl, benzoxazolyl, tetrahydrofuryl, tetrahydropyranyl, thienyl (also historically called thiophenyl), benzothienyl, thiamorpholinyl, oxadiazolyl, triazolyl and tetrahydroquinolinyl.

[041] Hydrocarbyloxy refers to groups of from 1 to 20 carbon atoms, preferably 1 to 10 carbon atoms, more preferably 1 to 6 carbon atoms attached to the parent structure through an oxygen. Alkoxy is a subset of hydrocarbyloxy and includes groups of a straight or branched configuration. Examples include methoxy, ethoxy, propoxy, isopropoxy and the like. Lower-alkoxy refers to groups containing one to four carbons. The term "halogen" means fluorine, chlorine, bromine or iodine atoms.

[042] Unless otherwise specified, acyl refers to formyl and to groups of 1, 2, 3, 4, 5, 6, 7 and 8 carbon atoms of a straight, branched, cyclic configuration, saturated, unsaturated and aromatic and combinations thereof, attached to the parent structure through a carbonyl functionality. Examples include acetyl, benzoyl, propionyl, isobutyryl and the like. Lower-acyl refers to groups containing one to four carbons.

[043] As used herein, the term "optionally substituted" may be used interchangeably with "unsubstituted or substituted". The term "substituted" refers to the replacement of one or more hydrogen atoms in a specified group with a specified radical. For example, substituted alkyl, aryl, cycloalkyl, heterocyclyl etc. refer to alkyl, aryl, cycloalkyl, or heterocyclyl wherein one or more H atoms in each residue are replaced with halogen, haloalkyl, alkyl, acyl, alkoxyalkyl, hydroxy lower alkyl, carbonyl, phenyl, heteroaryl, benzenesulfonyl, hydroxy, lower alkoxy, haloalkoxy, oxaalkyl, carboxy, alkoxy carbonyl [-C(=O)O-alkyl], alkoxy carbonylamino [HNC(=O)O-alkyl], aminocarbonyl (also known as carboxamido) [-C(=O)NH₂], alkylaminocarbonyl [-C(=O)NH-alkyl], cyano, acetoxy, nitro, amino, alkylamino, dialkylamino, (alkyl)(aryl)aminoalkyl, alkylaminoalkyl (including cycloalkylaminoalkyl), dialkylaminoalkyl, dialkylaminoalkoxy, heterocyclylalkoxy, mercapto, alkylthio, sulfoxide, sulfone, sulfonylamino, alkylsulfinyl, alkylsulfonyl, acylaminoalkyl, acylaminoalkoxy, acylamino, amidino, aryl, benzyl, heterocyclyl, heterocyclylalkyl, phenoxy, benzyloxy, heteroaryloxy, hydroxyimino, alkoxyimino, oxaalkyl, aminosulfonyl, trityl, amidino, guanidino, ureido, benzyloxyphenyl, and

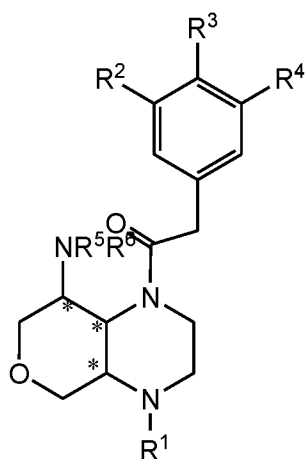
benzyloxy. In one embodiment, 1, 2, or 3 hydrogen atoms are replaced with a specified radical. In the case of alkyl and cycloalkyl, more than three hydrogen atoms can be replaced by fluorine; indeed, all available hydrogen atoms could be replaced by fluorine.

[044] Substituents Rⁿ are generally defined when introduced and retain that definition throughout the specification and in all independent claims.

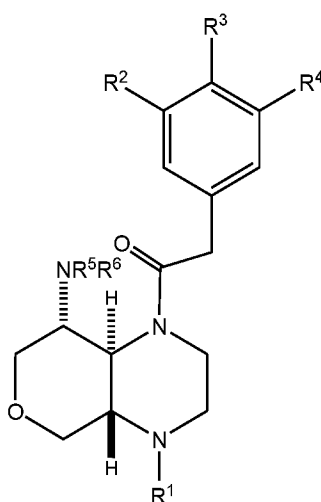
[045] Preparation of compounds can involve the protection and deprotection of various chemical groups. The need for protection and deprotection, and the selection of appropriate protecting groups, can be readily determined by one skilled in the art. Suitable groups for that purpose are discussed in standard textbooks in the field of chemistry, such as *Protective Groups in Organic Synthesis* by T.W.Greene and P.G.M.Wuts [John Wiley & Sons, New York, 1999], in *Protecting Group Chemistry*, 1st Ed., Oxford University Press, 2000; and in *March's Advanced Organic chemistry: Reactions, Mechanisms, and Structure*, 5th Ed., Wiley-Interscience Publication, 2001.

[046] The compounds described herein contain three asymmetric centers and may thus give rise to enantiomers, diastereomers, and other stereoisomeric forms which may be defined in terms of absolute stereochemistry as (*R*)- or (*S*)-. The present invention is meant to include all such possible isomers as racemates, optically pure forms and intermediate mixtures. Optically active isomers may be prepared using homo-chiral synthons or homo-chiral reagents, or optically resolved using conventional techniques such as chiral chromatography. All tautomeric forms are intended to be included. The graphic representations of racemic, ambiscalemic and scalemic or enantiomerically pure compounds used herein are taken from Maehr *J. Chem. Ed.* 62, 114-120 (1985): simple, single bond lines convey connectivity only and no stereochemical implication; solid and broken wedges are used to denote the absolute configuration of a chiral element; wavy lines indicate explicit disavowal of any stereochemical implication which the bond it represents could generate; solid and broken bold lines are geometric descriptors indicating the relative configuration shown but do not denote absolute configurations; and wedge outlines and dotted or broken lines denote enantiomerically pure compounds of indeterminate absolute configuration. Enantiomerically pure means greater than 80 ee, and preferably greater than 90 ee.

[047] For example, the generic structure depicting the compounds of the invention:

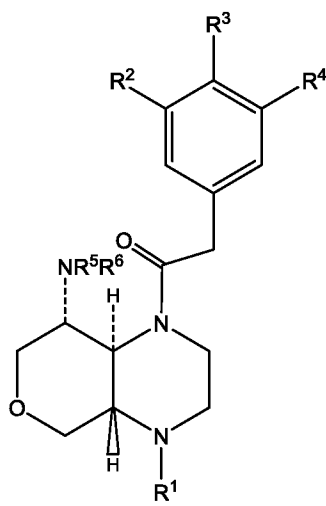


contains three asymmetric centers (labeled with asterisks). In one embodiment, the relative stereochemistry of the diastereomer can be represented as:



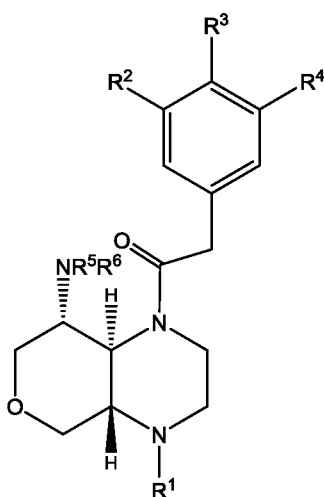
This representation indicates that the material is a mixture of isomers [(4a*S*,8*R*,8a*R*)-8-cyclopentyl-octahydro-1*H*-pyrano[3,4-*b*]pyrazine and (4a*R*,8*S*,8a*S*)-8-cyclopentyl-octahydro-1*H*-pyrano[3,4-*b*]pyrazine] in which the ring junction of the octahydro-1*H*-pyrano[3,4-*b*]pyrazine is *trans* and -NR⁵R⁶ is *cis* to its adjacent hydrogen at the ring junction. The mixture ratio of enantiomers can vary from approximately 1:1 (racemic) to approximately 90:10 (80% e.e.).

[048] The representation:



indicates a single enantiomer of the shown relative stereochemistry but unknown absolute stereochemistry, i.e. it could be either diastereomeric mirror image, as a substantially pure single enantiomer (e.g., approximately 80% e.e. to 99.99% e.e.).

[049] The absolute stereochemistry of an enantiomer is represented as:



This graphical representation indicates that the shown enantiomer predominates over its mirror image, wherein the enantiomeric excess (e.e.) of the shown enantiomer is approximately 80% or higher (e.g., 80% to 99.99%).

[050] As used herein, the terms “treatment” or “treating,” or “palliating” or “ameliorating” refer to an approach for obtaining beneficial or desired results including but not limited to therapeutic

benefit and/or a prophylactic benefit. By therapeutic benefit is meant eradication or amelioration of the underlying disorder being treated. Also, a therapeutic benefit is achieved with the eradication or amelioration of one or more of the physiological systems associated with the underlying disorder such that an improvement is observed in the patient, notwithstanding that the patient may still be afflicted with the underlying disorder

[051] As used herein, and as would be understood by the person of skill in the art, the recitation of “a compound” - unless expressly further limited - is intended to include salts of that compound. In a particular embodiment, the term “compound of formula” refers to the compound or a pharmaceutically acceptable salt thereof.

[052] The term “pharmaceutically acceptable salt” refers to salts prepared from pharmaceutically acceptable non-toxic acids or bases including inorganic acids and bases and organic acids and bases. When the compounds of the present invention are basic – as they are in most cases – salts may be prepared from pharmaceutically acceptable non-toxic acids including inorganic and organic acids. Suitable pharmaceutically acceptable acid addition salts for the compounds of the present invention include acetic, adipic, alginic, ascorbic, aspartic, benzenesulfonic (besylate), benzoic, boric, butyric, camphoric, camphorsulfonic, carbonic, citric, ethanedisulfonic, ethanesulfonic, ethylenediaminetetraacetic, formic, fumaric, glucoheptonic, gluconic, glutamic, hydrobromic, hydrochloric, hydroiodic, hydroxynaphthoic, isethionic, lactic, lactobionic, laurylsulfonic, maleic, malic, mandelic, methanesulfonic, mucic, naphthylenesulfonic, nitric, oleic, pamoic, pantothenic, phosphoric, pivalic, polygalacturonic, salicylic, stearic, succinic, sulfuric, tannic, tartaric acid, teclatic, p-toluenesulfonic, and the like. When the compounds contain an acidic functionality (e.g. $-\text{SO}_3\text{H}$), suitable pharmaceutically acceptable base addition salts for the compounds of the present invention include, but are not limited to, metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from lysine, arginine, N,N'-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium cations and carboxylate, sulfonate and phosphonate anions attached to alkyl having from 1 to 20 carbon atoms.

[053] Also provided herein is a pharmaceutical composition comprising a compound disclosed above, or a pharmaceutically acceptable salt thereof, together with one or more pharmaceutically carriers thereof and optionally one or more other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

[054] The formulations include those suitable for oral, parenteral (including subcutaneous, intradermal, intramuscular, intravenous and intraarticular), rectal and topical (including dermal, buccal, sublingual and intraocular) administration. The most suitable route may depend upon the condition and disorder of the recipient. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association a compound of formula I or a pharmaceutically acceptable salt thereof ("active ingredient") with the 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 carriers or finely divided solid carriers or both and then, if necessary, shaping the product into the desired formulation.

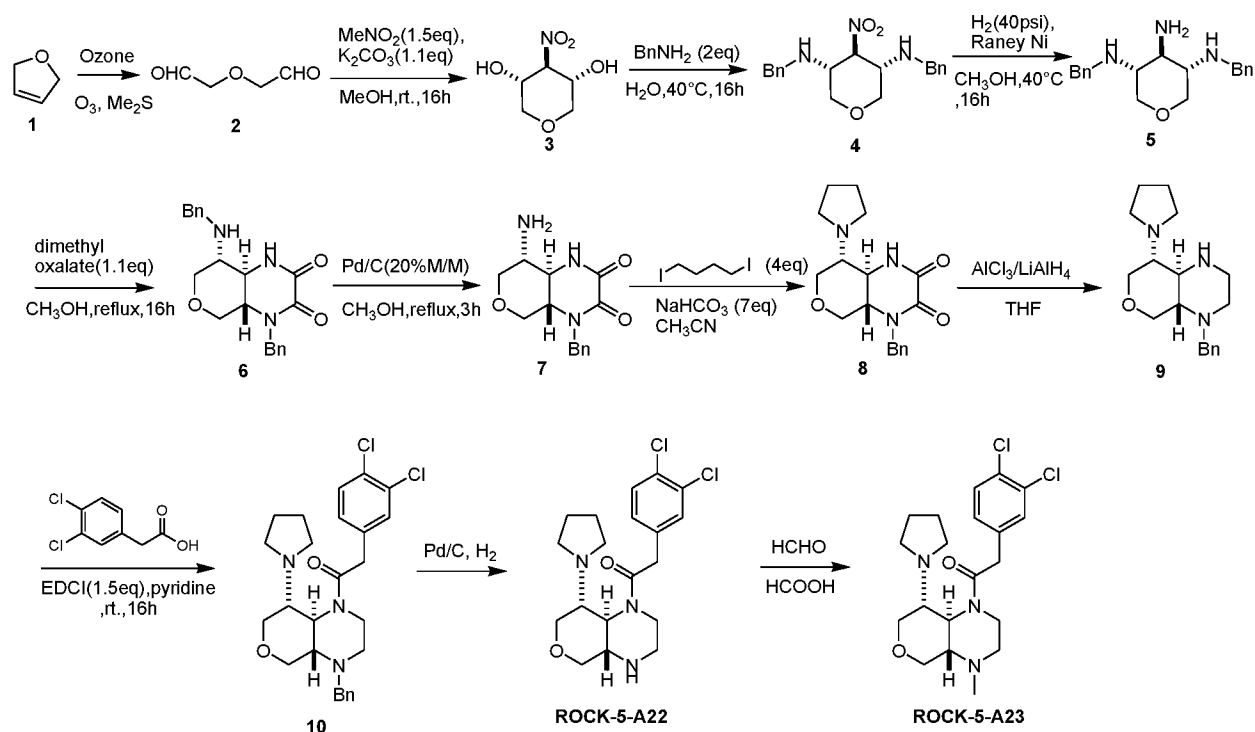
[055] Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

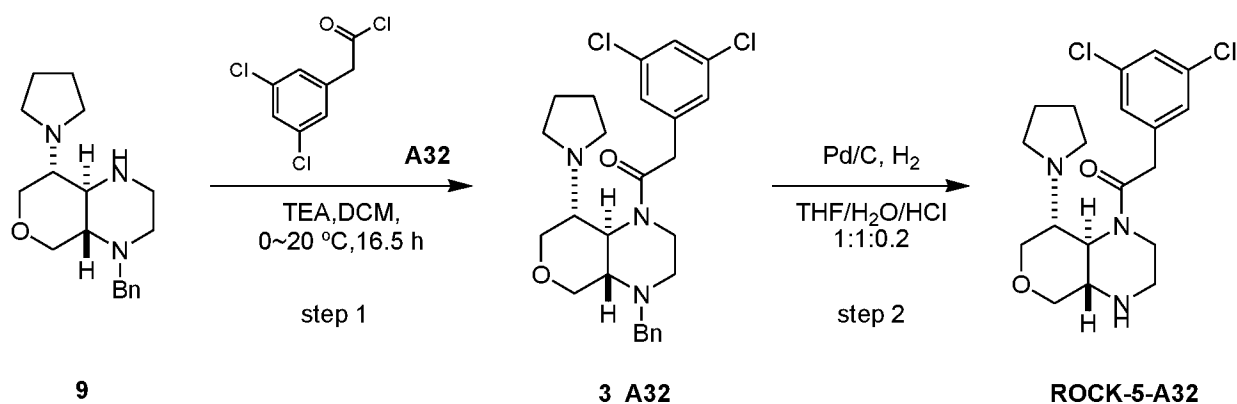
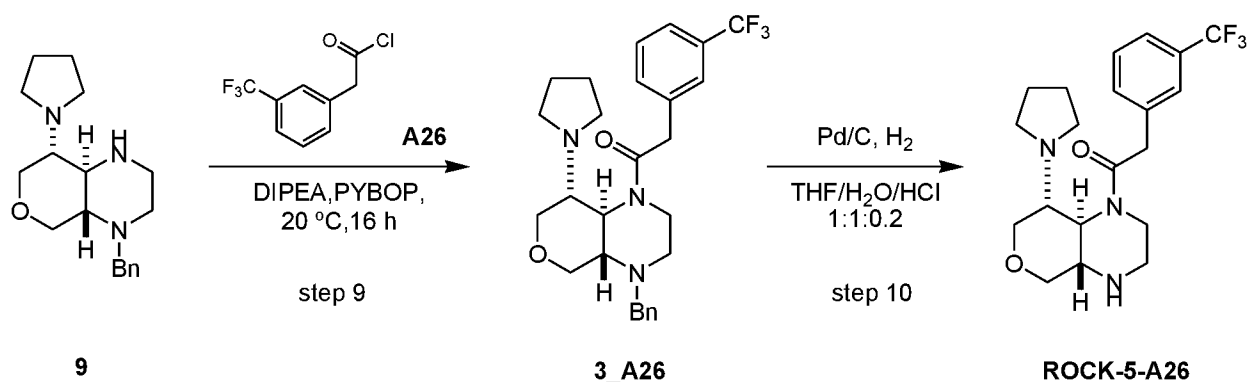
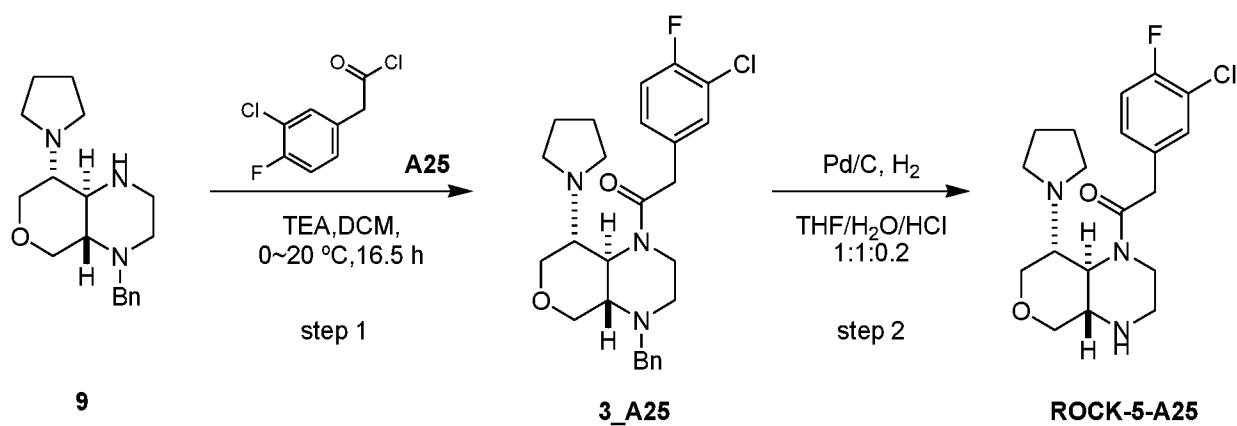
[056] A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide sustained, delayed or controlled release of the active ingredient therein.

[057] Formulations for parenteral administration include aqueous and non-aqueous sterile

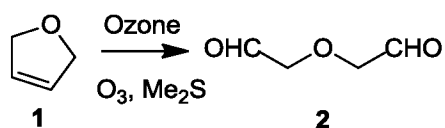
injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient. Formulations for parenteral administration also include aqueous and non-aqueous sterile suspensions, which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of a sterile liquid carrier, for example saline, phosphate-buffered saline (PBS) or the like, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

[058] In general, compounds of formula I can be prepared as described below.





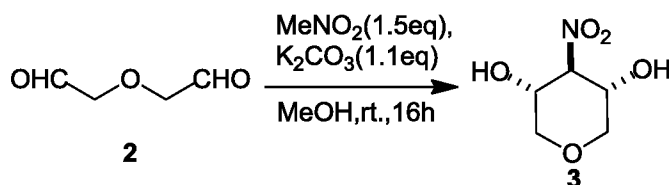
[059] Preparation of intermediate 2



A solution of 2,5-dihydrofuran (50.0 g, 713 mmol, 54 mL, 1 eq) in MeOH (20 mL) and dichloromethane (DCM) (200 mL) was stirred under ozone (15 Psi) at -78 °C for 2 h. To the

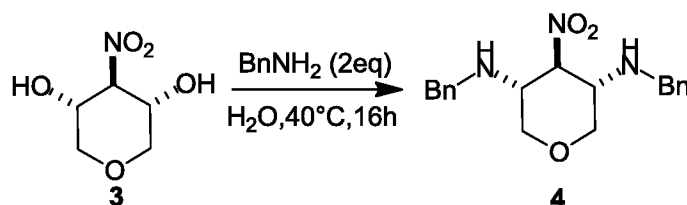
solution was added dimethylsulfide (DMS) (133 g, 2.14 mol, 156 mL, 3 *eq*). The solution was stirred at 25 °C for 14 hrs. The reaction mixture was filtered and concentrated under reduced pressure to furnish 2-(2-oxoethoxy)acetaldehyde. This material was used without further purification.

[060] Preparation of intermediate 3



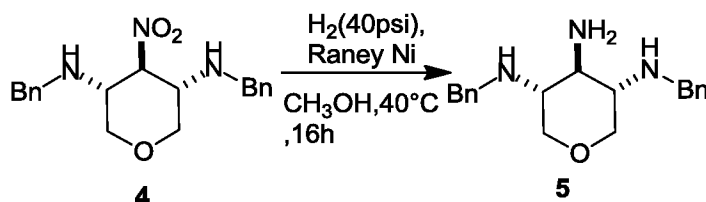
To a solution of 2-(2-oxoethoxy)acetaldehyde (70.0 g, 686 mmol, 1 *eq*) in MeOH (500 mL) was added nitromethane (65.7 g, 1.08 mol, 58.2 mL, 1.57 *eq*) and K₂CO₃ (104 g, 754 mmol, 1.1 *eq*). The resulting solution was then stirred for 3 hr at 0°C. The solution was warmed to 25 oC, and then stirred at 25°C for 13 hrs. TLC(DCM:MeOH=10:1,Rf=0.6) indicated intermediate 2 was completely consumed. The reaction mixture was filtered and concentrated under reduced pressure which provided 4-nitrotetrahydropyran-3,5-diol as a yellow solid.

[061] Preparation of intermediate 4



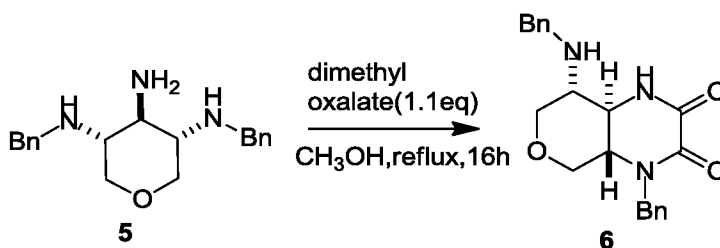
To a solution of 4-nitrotetrahydropyran-3,5-diol (15.0 g, 92 mmol, 1 *eq*) in H₂O (110 mL) was added benzylamine (BnNH₂) (19.7 g, 183.9 mmol, 20.1 mL, 2 *eq*). The resulting solution was stirred at 40 °C for 16 hr. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The residue was purified by preparative HPLC which provided N3,N5-dibenzyl-4-nitro-tetrahydropyran-3,5-diamine as a yellow oil. LC/MS m/z 342.2 (M+H).

[062] Preparation of intermediate 5



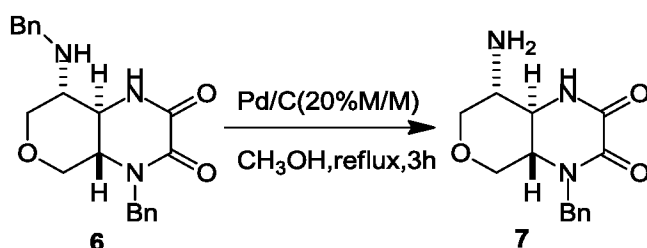
To a solution of N3,N5-dibenzyl-4-nitro-tetrahydropyran-3,5-diamine (5.00 g, 14.7 mmol, 1 *eq*) in MeOH (10 mL) was added Raney-Ni (1.25 g, 14.7 mmol, 1 *eq*). The solution was stirred under H₂ (45 Psi) at 25 °C for 16 hr. The reaction mixture was filtered and concentrated under reduced pressure which provided N3,N5-dibenzyltetrahydropyran-3,4,5-triamine as a yellow oil. ¹H-NMR (400MHz, DMSO-d₆): δ 7.36-7.19 (10H, m), 3.91-3.78 (2H, m), 3.78-3.70 (2H, m), 3.65 (2H, d, *J*=13.7 Hz), 2.88 (2H, t, *J*=10.5 Hz), 2.29-1.90 (6H, m). LC/MS *m/z* 312.3 (M+H).

[063] Preparation of intermediate 6



To a solution of N3,N5-dibenzyltetrahydropyran-3,4,5-triamine (3.10 g, 9.95 mmol, 1 *eq*) in MeOH (30 mL) was added dimethyl oxalate (1.17 g, 9.95 mmol, 1 *eq*). The resulting solution was stirred at 66 °C for 16 hr. The reaction mixture was filtered and dried *in vacuo* which provided 4-benzyl-8-(benzylamino)-1,4a,5,7,8,8a-hexahydropyrano[3,4-b]pyrazine-2,3-dione as a white solid. LC/MS *m/z* = 366.3 (M+H).

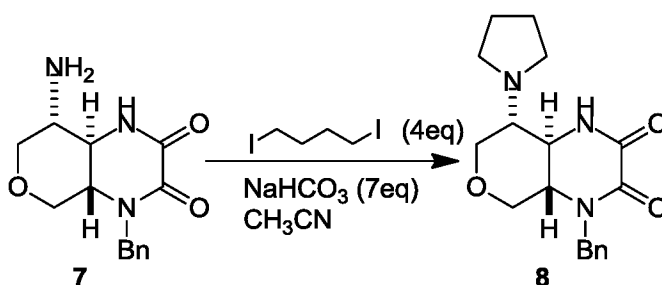
[064] Preparation of intermediate 7



To a solution of 4-benzyl-8-(benzylamino)-1,4a,5,7,8,8a-hexahydropyrano[3,4-b]pyrazine-2,3-dione (2.70 g, 7.39 mmol, 1 *eq*) in MeOH (50 mL) was added Pd/C (1.35 g) and ammonium formate (4.66 g, 73.9 mmol, 10 *eq*). The solution was stirred at 65 °C for 3 hr. The reaction

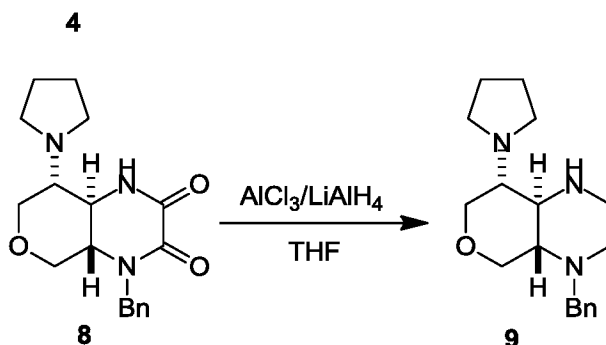
mixture was filtered and concentrated under reduced pressure which provided 8-amino-4-benzyl-1,4a,5,7,8,8a-hexahydropyrano [3,4-b]pyrazine-2,3-dione as a white solid. ¹H-NMR (400MHz, methanol-d₄): δ 7.39-7.33 (2H, m), 7.32-7.24 (3H, m), 4.95-4.89 (2H, m), 4.55 (1H, d, *J*=15.9 Hz), 4.13 (1H, dd, *J*=4.3, 10.9 Hz), 3.87 (1H, dd, *J*=5.0, 11.2 Hz), 3.69 (1H, dt, *J*=4.3, 10.7 Hz), 3.43 (1H, t, *J*=10.4 Hz), 3.29-3.21 (1H, m), 3.05 (1H, t, *J*=11.0 Hz), 2.84-2.75 (1H, m). LC/MS *m/z* 276.3 (M+H).

[065] Preparation of intermediate 8



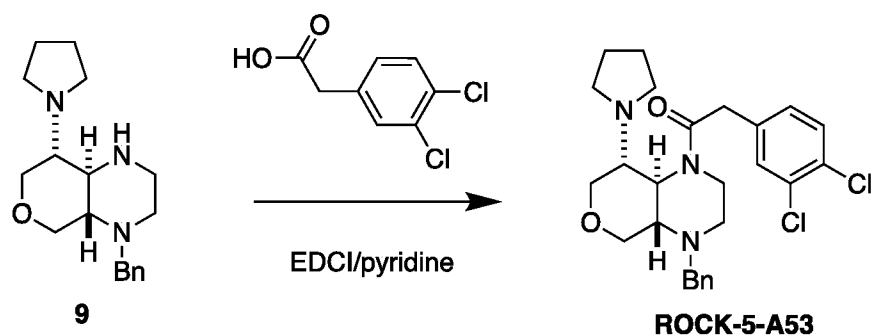
To a solution of 8-amino-4-benzyl-1,4a,5,7,8,8a-hexahydropyrano[3,4-b]pyrazine-2,3-dione (2.00 g, 7.26 mmol, 1 *eq*) in CH₃CN (50 mL) was added NaHCO₃ (4.15 g, 49.4 mmol, 1.92 mL, 6.8 *eq*) and 1,4-diodobutane (9.00 g, 29.0 mmol, 3.81 mL, 4 *eq*). The solution was stirred at 82 °C for 18 hr. The reaction mixture was filtered and concentrated under reduced pressure which provided 4-benzyl-8-pyrrolidin-1-yl-1,4a,5,7,8,8a-hexahydropyrano [3,4-b]pyrazine-2,3-dione as a white solid. ¹H-NMR (400MHz, methanol-d₄): δ 7.39-7.33 (2H, m), 7.32-7.24 (3H, m), 4.93 (1H, d, *J*=15.8 Hz), 4.51 (1H, d, *J*=15.8 Hz), 4.14-4.00 (2H, m), 3.79-3.67 (2H, m), 3.45 (1H, t, *J*=11.0 Hz), 3.28-3.23 (1H, m), 2.97 (1H, dt, *J*=4.4, 10.5 Hz), 2.84-2.76 (2H, m), 2.75-2.67 (2H, m), 1.83-1.71 (4H, m). LC/MS *m/z* 330.0 (M+H).

[066] Preparation of intermediate 9



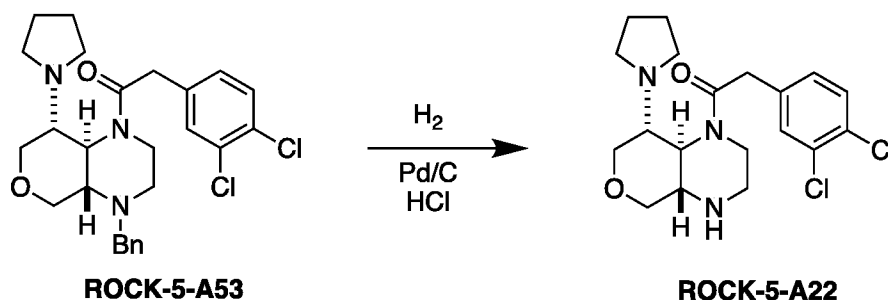
To a solution of AlCl_3 (1.19 g, 8.89 mmol, 486 μL , 1.83 *eq*) in THF (30 mL) was added LiAlH_4 (1.03 g, 27.0 mmol, 5.56 *eq*) at 0 °C. The solution was stirred at 25 °C for 30 min. To this solution was added 4-benzyl-8-pyrrolidin-1-yl-1,4a,5,7,8,8a-hexahydropyrano[3,4-b]pyrazine-2,3-dione (1.60 g, 4.86 mmol, 1 *eq*) at 0°C. The solution was stirred at 0°C for 1 hr and then at 25°C for 30 min. To the solution was added NaOH (2 M) until pH=8. The solution was extracted with ethyl acetate (3 x 30 mL). The organic layer was then dried (Na_2SO_4), filtered, and concentrated *in vacuo* which provided 4-Benzyl -8-pyrrolidin-1-yl-1,2,3,4a,5,7,8,8a-octahydropyrano[3,4-b]pyrazine as a yellow oil. LC/MS m/z 302.3 (M+H).

[067] Preparation of ROCK-5-A53



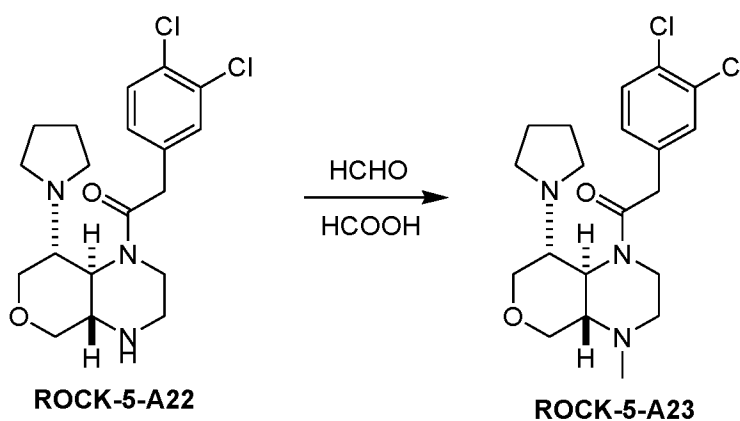
To a solution of 2-(3,4-dichlorophenyl)acetic acid (591 mg, 2.88 mmol, 1.1 *eq*) in pyridine (20 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (754 mg, 3.93 mmol, 1.5 *eq*). The resulting solution was stirred at 25°C for 30 min. To this solution was added 4-benzyl-8-pyrrolidin-1-yl-1,2,3,4a,5,7,8,8a-octahydropyrano[3,4-b]pyrazine (790 mg, 2.62 mmol, 1 *eq*), and the resulting solution was stirred at 25 °C for 15.5 hr. The reaction mixture was quenched by addition of 5 mL of H_2O at 25°C. The mixture was diluted with 40 mL H_2O and extracted with 60 mL ethyl acetate (20 mL x 3). The combined organic layers were dried over Na_2SO_4 , filtered and concentrated under reduced pressure which provided 1-[4-benzyl-8-pyrrolidin-1-yl-3,4a,5,7,8,8a-hexahydro-2H-pyrano(3,4-b)pyrazin-1-yl]-2-(3,4-dichlorophenyl)ethanone as a yellow oil. LC/MS m/z 488.0 (M+H). ^1H NMR (400MHz, methanol- d_4 , TFA salt) δ = 7.50 - 7.28 (m, 7H), 7.21 (dd, J = 2.0, 8.3 Hz, 1H), 4.95 - 4.87 (m, 1H), 4.84 - 4.76 (m, 1H), 4.50 (dd, J = 5.0, 11.2 Hz, 1H), 4.34 (dd, J = 4.4, 11.2 Hz, 1H), 4.12 - 4.02 (m, 2H), 3.92 - 3.76 (m, 3H), 3.67 - 3.44 (m, 6H), 3.40 - 3.33 (m, 2H), 3.16 - 3.01 (m, 1H), 2.85 (td, J = 3.4, 12.1 Hz, 1H), 2.42 - 2.32 (m, 1H), 2.05 (br s, 4H)

[068] Preparation of ROCK-5-A22



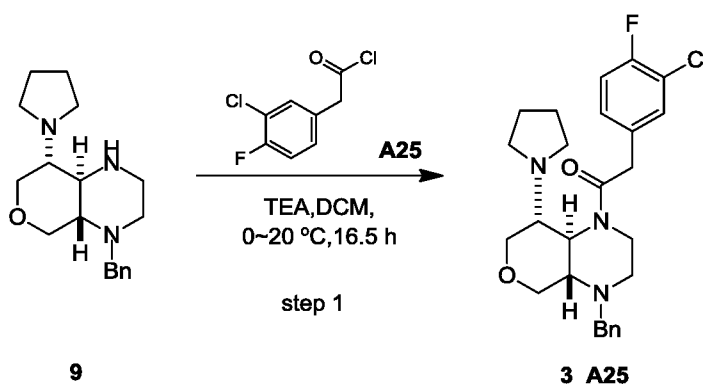
To a solution of palladium on carbon (Pd/C) (1.42 g, 10% wt % Pd) in THF (20 mL) and H₂O (20 mL) was added 1-[4-benzyl-8-pyrrolidin-1-yl-3,4a,5,7,8,8a-hexahydro-2H-pyrano(3,4-b)pyrazin-1-yl]-2-(3,4-dichlorophenyl)ethanone (710 mg, 1.45 mmol, 1 *eq*) and HCl (37%, 14.00 mL). The solution was stirred at 25 °C for 40 min under (1bar) H₂. The reaction mixture was quenched by addition of 40 mL sat. aqueous NaHCO₃ at 25°C. The mixture was filtered. The solution was extracted with DCM 150 mL (50 mL x 3). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure which provided 1-[8-pyrrolidin-1-yl-2,3,4,4a,5,7,8,8a-octahydropyrano(3,4-b) pyrazin-1-yl]-2-(3,4-dichlorophenyl)ethanone as a yellow solid. ¹H-NMR (400MHz, methanol-d₄): δ 7.48-7.44 (2H, m), 7.23 (1H, dd, *J*=2.1, 8.2 Hz), 4.07 (1H, dd, *J*=4.5, 11.2 Hz), 3.97(1H, m, *J*=13.6 Hz), 3.91-3.80 (2H, m), 3.69 (1H, m, *J*=15.6 Hz), 3.35 (1H, s), 3.28-3.18 (2H, m), 3.18-3.09 (1H, m), 3.08-2.90 (4H, m), 2.70 (5H, m), 2.16 (1H, s), 1.72-1.67 (4H, m). LC/MS *m/z* 398.2 (M+H).

[069] Preparation of ROCK-5-A23



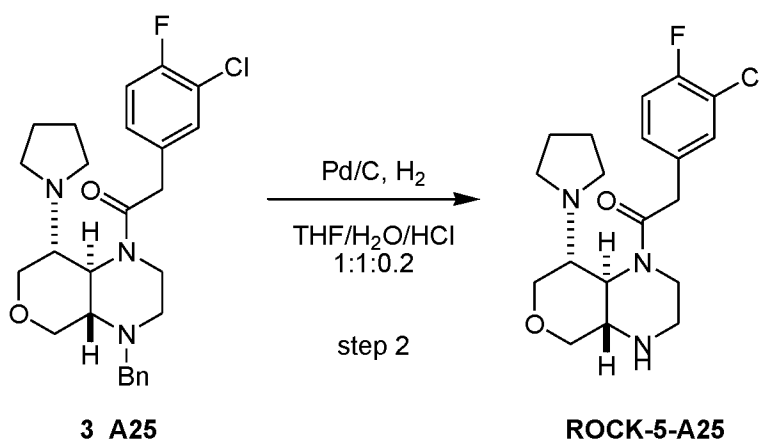
To a solution 1-[8-pyrrolidin-1-yl-2,3,4,4a,5,7,8,8a-octahydropyrano(3,4-b)pyrazin-1-yl]-2-(3,4-dichlorophenyl)ethanone (100 mg, 251 μmol , 1 *eq*) in HCOOH (2 mL) was added HCHO (326 mg, 4.02 mmol, 299 μL , 16 *eq*). The resulting solution was stirred at 80 °C for 2 hrs. The reaction mixture was concentrated under reduced pressure. The residue was diluted with NaHCO_3 (20 mL) and extracted with ethyl acetate 30 mL (10 mL x 3). The combined organic layers were dried over Na_2SO_4 , filtered, and concentrated under reduced pressure to furnish 1-[4-methyl-8-pyrrolidin-1-yl-3,4a,5,7,8,8a-hexahydro-2H- -pyrano(3,4-b)pyrazin-1-yl]-2-(3,4-dichlorophenyl)ethanone as a yellow oil. $^1\text{H-NMR}$ (400MHz, methanol- d_4) δ 8.49 (1H, s), 7.48 (2H, d, $J=8.3$ Hz), 7.23 (1H, dd, $J=2.0, 8.3$ Hz), 4.23 (2H, dt, $J=4.9, 10.9$ Hz), 4.10-4.02 (1H, m), 3.89-3.75 (2H, m), 3.43-3.37 (3H, m), 3.37-3.34 (3H, m), 3.21-3.15 (4H, m), 2.85 (1H, dt, $J=2.7, 11.8$ Hz), 2.45 (1H, dt, $J=5.1, 10.0$ Hz), 2.22 (3H, s), 1.95-1.87 (4H, m). LC/MS m/z 412.1 (M+H).

[070] Preparation of intermediate 3A25



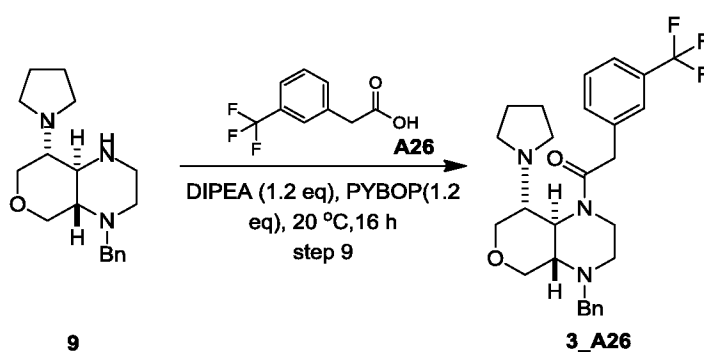
To a mixture of intermediate 9 (23 mg, 76 μmol , 1 *eq*) and triethylamine (TEA) (23 mg, 229 μmol , 32 μL , 3 *eq*) in DCM (1.5 mL) was added a solution of A25 (47 mg, 229 μmol , 3 *eq*) in DCM (0.5 mL) dropwise at 0 °C under N_2 . The mixture was stirred at 0 °C for 30 min and then warmed to 20 °C. The mixture was stirred for 16 hours at 20 °C. The mixture was concentrated under reduced pressure. The residue was purified by flash chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, dichloromethane: methanol = 10/1) to obtain intermediate 3A25 as a yellow solid. LCMS: $m/z = 472.2$ (M+H $^+$).

[071] Preparation of ROCK-5-A25



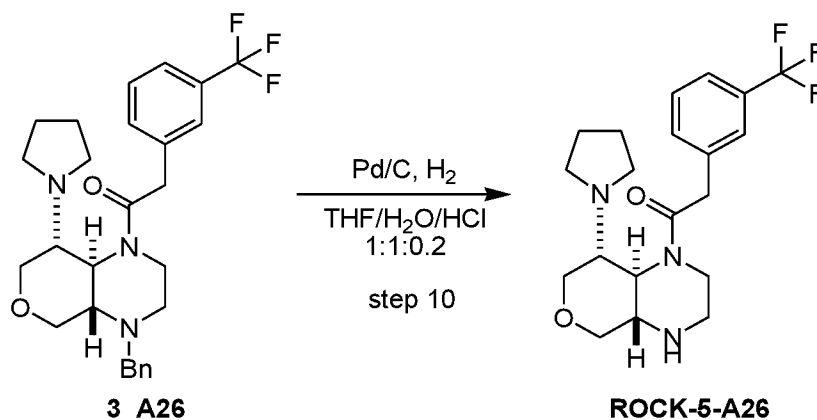
To a solution of intermediate 3A25 (22 mg, 47 μmol , 1 *eq*) in THF (1.5 mL) and H₂O (1.5 mL) was added Pd/C (10 mg, 10 wt% Pd) and concentrated HCl (1.41 mL) under N₂. The suspension was degassed under vacuum and purged with H₂ several times. The mixture was stirred under H₂ (15psi) at 20 °C for 20 mins. The reaction mixture was filtered. The filtrate was diluted with H₂O (50 mL) and neutralized with saturated NaHCO₃ (1 mL). The mixture was extracted with ethyl acetate (20 mL x 3). The combined organic layers were washed with brine (10 mL), dried with anhydrous Na₂SO₄, and concentrated. The residue was purified by prep-HPLC (column: YMC-Actus Triart C18 100x30mmx5 μm ; mobile phase: [water (10mM NH₄HCO₃)-ACN]; B%: 20%-40%,12 min). ROCK-5-A25 was obtained as a light yellow solid. H-NMR: (400MHz, chloroform-d) δ = 7.32 (br d, *J*=6.36 Hz, 1 H) 7.18 - 7.20 (m, 1 H) 7.14 (br s, 1 H) 7.04 - 7.11 (m, 1 H) 7.04 - 7.04 (m, 1 H) 7.01 - 7.01 (m, 1 H) 4.29 (br s, 1 H) 4.12 (br d, *J*=6.97 Hz, 1 H) 3.57 - 3.94 (m, 5 H) 3.18 - 3.39 (m, 2 H) 2.88 - 3.17 (m, 5 H) 2.75 (br s, 5 H) 1.72 (br s, 5 H). LCMS: *m/z* = 382.0 (M+H⁺)

[072] Preparation of intermediate 3A26

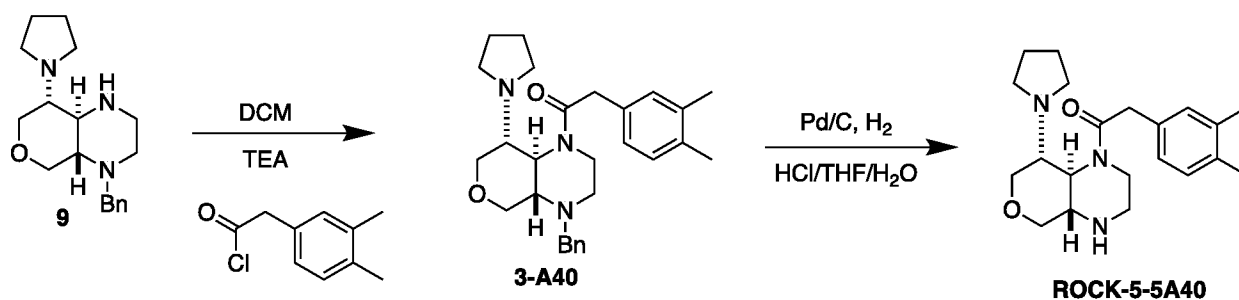


To a mixture of A26 (28 mg, 139 μmol , 1.2 *eq*) and intermediate 9 (35 mg, 116 μmol , 1 *eq*) in DCM (2.5 mL) was added benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) (73 mg, 139 μmol , 1.2 *eq*) and *N,N*-diisopropylethylamine (DIPEA) (18 mg, 139 μmol , 24 μL , 1.2 *eq*) at 20 °C under N_2 . The mixture was stirred at 20 °C for 16 hours. The mixture was concentrated under reduced pressure. The residue was purified by silica gel chromatography (column height: 250 mm, diameter: 100 mm, 100-200 mesh silica gel, petroleum ether : ethyl acetate = 1:1) to obtain intermediate 3_A26 as a light yellow solid. H-NMR: (400MHz, chloroform-*d*) δ = 7.32 - 7.43 (m, 2 H) 7.03 - 7.12 (m, 1 H) 3.59 - 3.75 (m, 6 H) 3.36 - 3.62 (m, 4 H) 3.23 - 3.35 (m, 2 H) 1.84 (br d, $J=13.15$ Hz, 3 H) 1.45 - 1.72 (m, 1 H) 1.45 - 1.72 (m, 4 H) 1.30 - 1.43 (m, 1 H) 1.30 - 1.44 (m, 1 H) 1.29 - 1.44 (m, 1 H) 1.25 - 1.26 (m, 1 H) 0.78 - 0.95 (m, 2 H). LCMS: m/z = 488.2 ($\text{M}+\text{H}^+$).

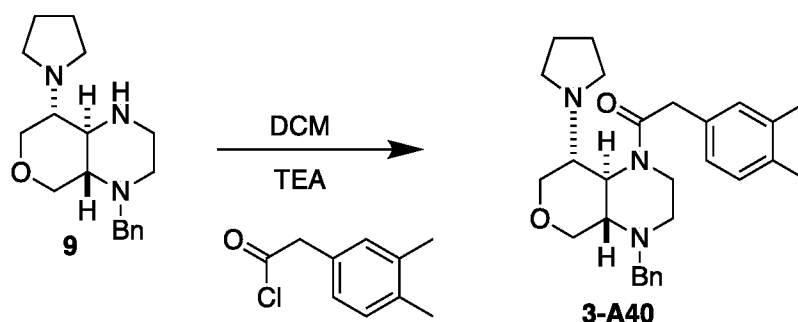
[073] Preparation of ROCK-5-A26



ROCK-5-A40 Scheme

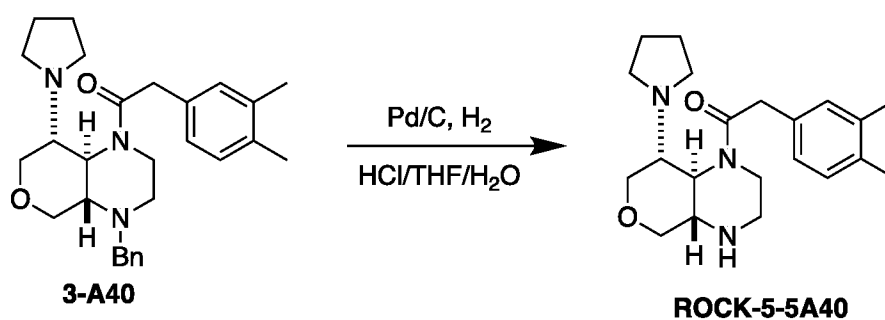


Preparation of 3-A40



Intermediate 9 (50 mg, 166 μmol) and TEA (168 mg, 1.66 mmol, 231 μL) were dissolved in DCM (5 mL) followed by the addition of the acid chloride (90.89 mg, 497.64 μmol). The resulting mixture was stirred at 25 $^{\circ}\text{C}$ under N_2 for 12 hours. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was diluted with EtOAc (10 mL) and adjusted pH 5 with HCl (2 N, 0.5 mL) solution. The aqueous phase was washed with EtOAc (10 mL X 2), and then the pH was adjusted to 8 using an aqueous NaHCO_3 solution. The aqueous phase was extracted with EtOAc (10 mL X 3). The combined organic layers were washed with brine (10 mL), dried over with Na_2SO_4 , filtered and concentrated under reduced pressure. The material was used as is in the next step.

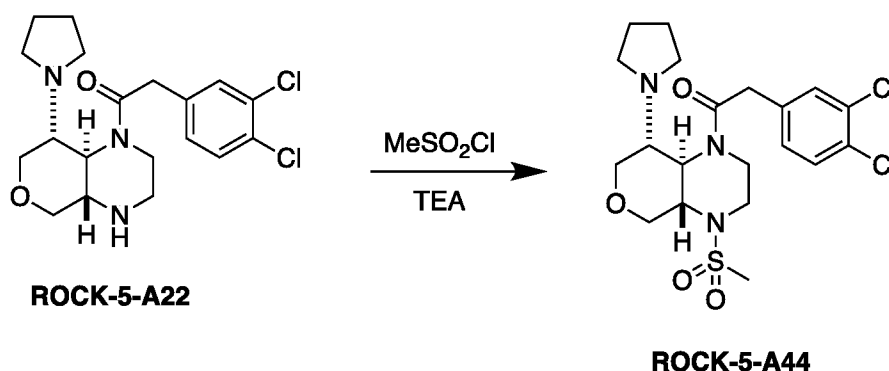
[074] Preparation of ROCK-5-5A40



A solution of 3-A40 (50 mg, 112 μmol), Pd/C (20 mg, 10 wt% Pd), THF (10 mL), conc. HCl (0.3 mL), and H_2O (10 mL) were stirred at 25 $^{\circ}\text{C}$ under H_2 (15 psi) for 12 hrs. The reaction

mixture was filtered through a Celite pad, and the filtrate was concentrated under reduced pressure. The pH was adjusted to 8 with an aqueous NaHCO₃ solution. The mixture was extracted with EtOAc (10 mL X 3). The combined organic layers were washed with brine (10 mL), dried over with Na₂SO₄, filtered, and concentrated under reduced pressure. The residue was purified by prep-HPLC (TFA condition: column: Luna C18 100 x 30 5μ; mobile phase: [water (0.1%TFA)-ACN]; ACN%: 1%-35%, 10min). Compound ROCK-5-A40 was obtained as a white solid. ¹H NMR (400MHz, methanol-d₄) δ = 7.15 - 7.04 (m, 3H), 4.74 (dt, *J* = 4.7, 10.3 Hz, 1H), 4.38 (dd, *J* = 4.4, 11.7 Hz, 1H), 4.29 - 4.08 (m, 3H), 3.92 - 3.91 (m, 1H), 3.92 - 3.83 (m, 1H), 3.84 - 3.74 (m, 1H), 3.84 - 3.73 (m, 1H), 3.91 - 3.71 (m, 3H), 3.70 - 3.60 (m, 3H), 3.58 - 3.47 (m, 2H), 3.33 (br s, 1H), 2.84 (ddd, *J* = 4.1, 9.4, 13.0 Hz, 1H), 2.25 (d, *J* = 7.7 Hz, 6H), 2.02 (br s, 3H). LCMS (MH⁺): 358.2.

[075] Preparation of ROCK-5-A44



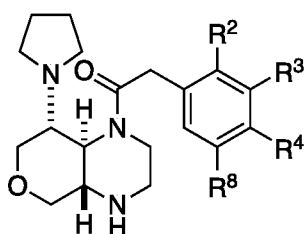
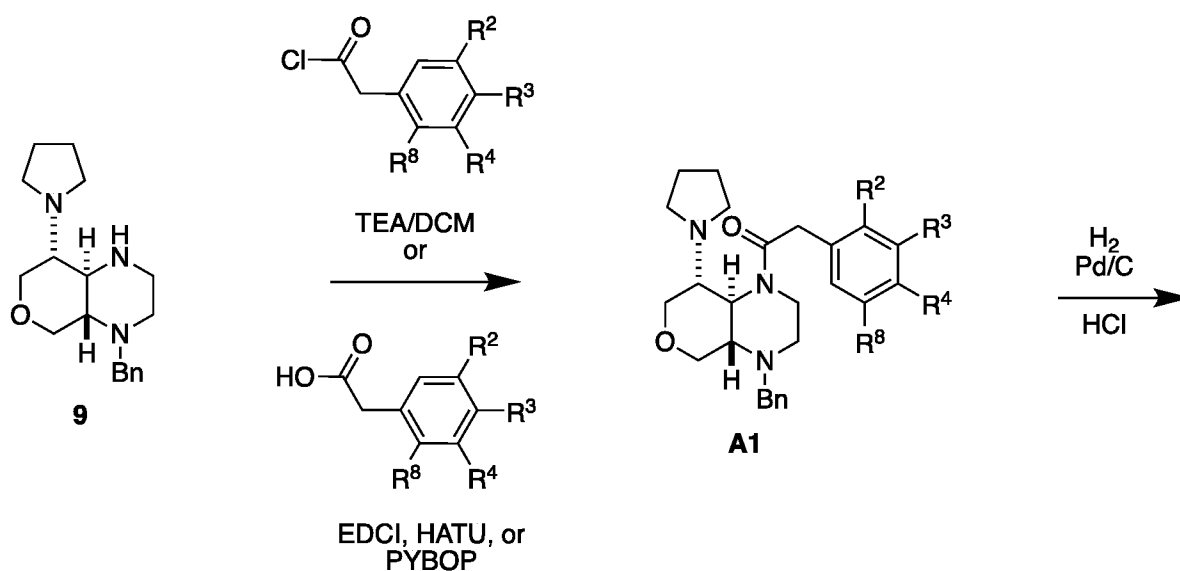
Rock-5-A22 (30 mg, 75 μmol) and methanesulfonyl chloride (17 mg, 151 μmol, 12 μL) were taken up in DCM (3 mL). Triethylamine (38 mg, 377 μmol, 52 uL) was added to the reaction mixture. The reaction vessel was degassed and purged with N₂ (3 times). The mixture was stirred at 20 °C for 2 hours under a N₂ atmosphere. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was purified by prep-HPLC (TFA condition: Column: Nano-micro Kromasil C18 100*30mm 5μm; mobile phase: [water 0.1%TFA)-ACN]; %: ACN: 18%-38%, 10min). ROCK-5-A44 was obtained as a white solid.

¹H NMR (400MHz, methanol-d₄) δ7.51 - 7.44 (m, 2H), 7.22 (dd, *J* = 1.9, 8.3 Hz, 1H), 4.37 - 4.29 (m, 2H), 4.28 - 4.16 (m, 2H), 3.94 - 3.87 (m, 4H), 3.83 - 3.72 (m, 3H), 3.70 - 3.55 (m, 3H),

3.47 (td, $J = 4.2, 12.7$ Hz, 1H), 3.33 (br s, 1H), 2.94 (s, 3H), 2.11 - 1.89 (m, 4H). LCMS (MH⁺) 476.1.

[076] Some compounds shown in Table A are made in similar fashion via the sequence shown in Scheme A:

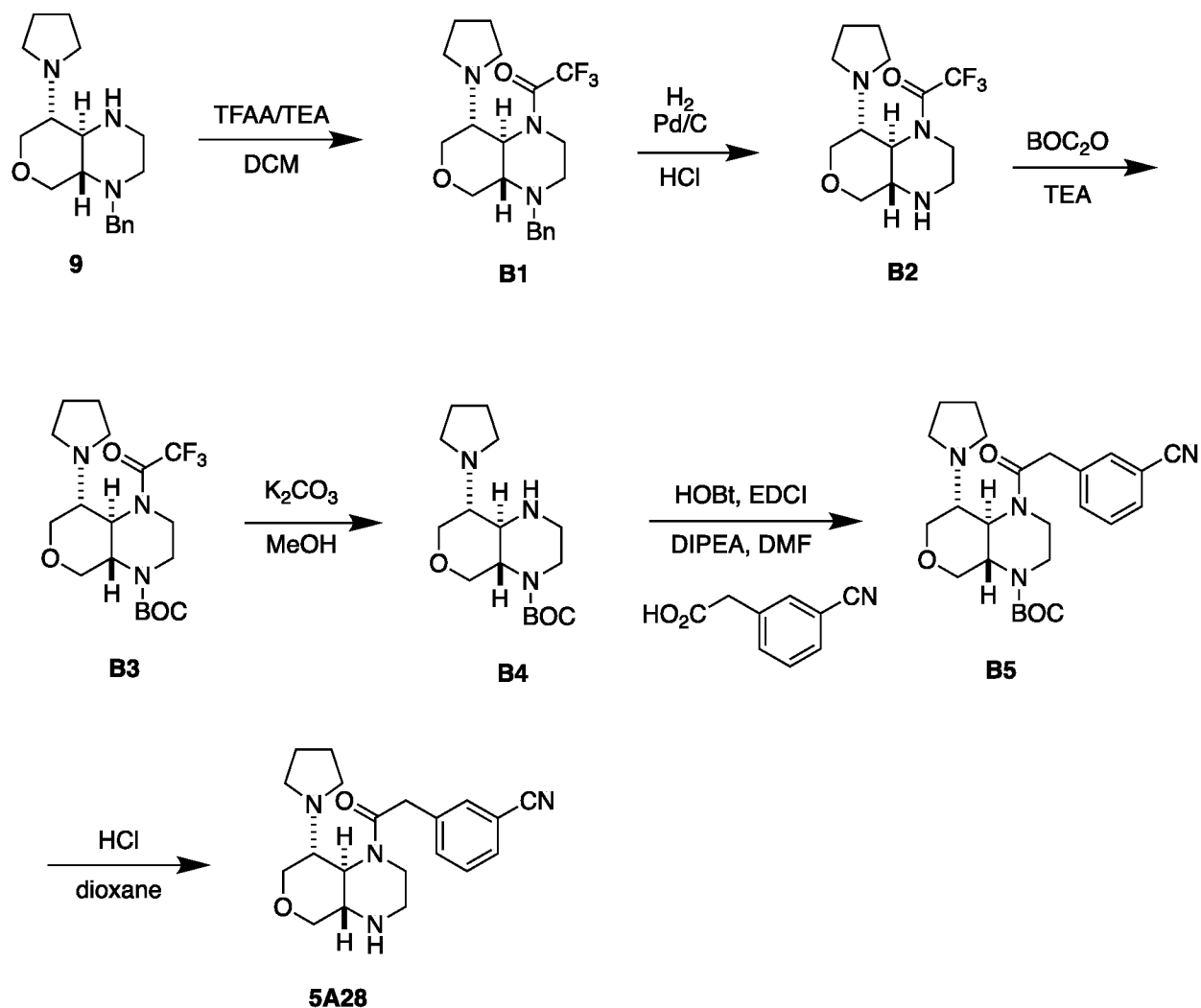
Scheme A



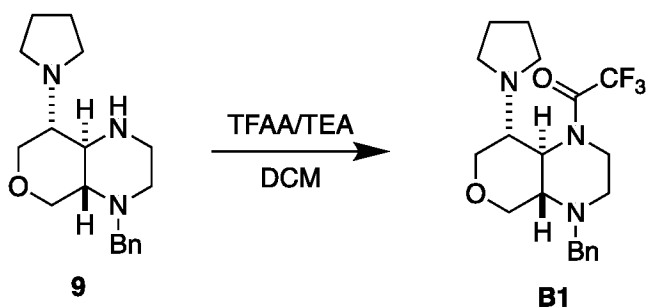
Examples

[077] Some compounds shown in Table A are made via the sequence shown in Scheme B:

Scheme B

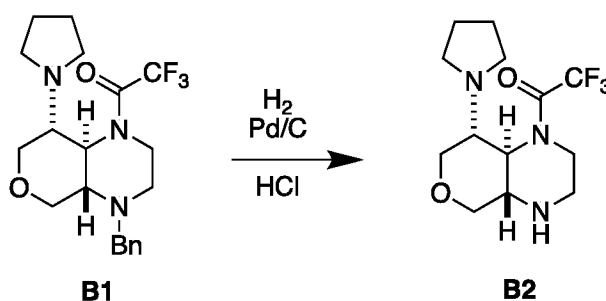


[078] Example experimentals for Scheme B:

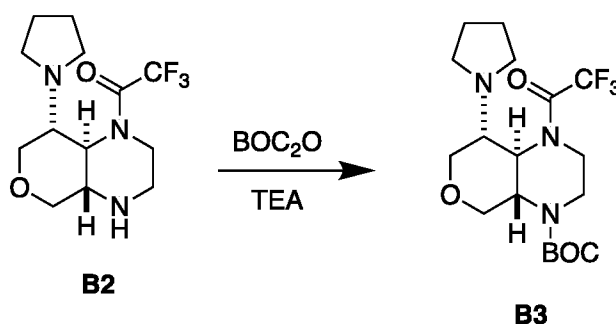


The amine **9** (1 g, 3.3 mmol, 1 *eq*) and TFAA (1.74 g, 8.3 mmol, 1.2 mL, 2.5 *eq*) were taken up in DCM (25 mL). Triethylamine (1.0 g, 10 mmol, 1.4 mL, 3 *eq*) and DMAP (41 mg, 330 μmol ,

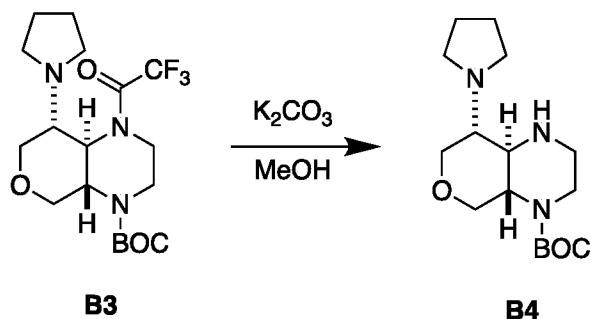
0.1 *eq*) were added to the reaction mixture. The mixture was degassed and purged with N₂ (3 times). The resulting mixture was stirred at 25 °C for 12 hours under a N₂ atmosphere. The reaction mixture was concentrated under reduced pressure. The residue was diluted with EtOAc (10 mL), and the pH was adjusted to 8 with aq. NaHCO₃. The aqueous phase was extracted with EtOAc (10 mL x 2). The combined organic layers were washed with brine (10 mL) and dried over Na₂SO₄. The mixture was filtered and concentrated under reduced pressure. The residue was purified by column chromatography (SiO₂, Petroleum ether/Ethyl acetate = 10/1 to 0:1) which furnished **B1**.



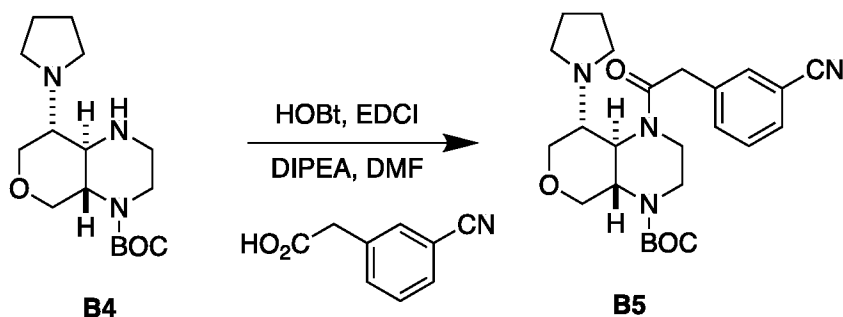
The amide **B1** (950 mg, 2.39 mmol, 1 *eq*) and Pd/C (100 mg, 10 wt% Pd) were taken up in THF (15 mL), H₂O (15 mL) and HCl (0.8 mL). The resulting solution was stirred at 25 °C under H₂ (15psi) for 12 hrs. The reaction mixture was filtered and concentrated under reduced pressure. The residue was basified with aq. NaHCO₃ solution (pH=8). The aqueous solution was extracted with EtOAc 30 mL (10 mL x 3). The combined organic layers were washed with brine (10 mL) and dried over Na₂SO₄. The mixture was filtered and concentrated under reduced pressure. The residue was purified by preparative-HPLC (basic condition: column: Agela Durashell C18 150 x 30 mm 5 μm; mobile phase: [water (0.04%NH₃H₂O)-ACN]; ACN gradient: 5%-35%, 10 min) which provided **B2**.



The amine **B2** (700 mg, 2.28 mmol, 1 *eq*) and TEA (461 mg, 4.56 mmol, 634 μ L, 2 *eq*) were taken up in DCM (20 mL) Di-tert-butyl dicarbonate (497 mg, 2.28 mmol, 523 μ L, 1 *eq*) was added, and the resulting solution was stirred at 25°C under N₂ for 12 hrs. The reaction mixture was concentrated under reduced pressure which provided the Boc protected amine **B3**. The material was used directly in the next step.

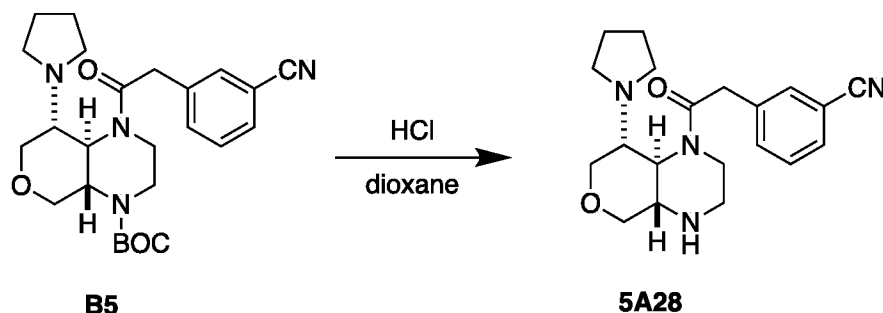


The Boc protected amine **B3** (800 mg, 1.96 mmol, 1 *eq*) and K₂CO₃ (1.36 g, 9.82 mmol, 5 *eq*) were taken up in MeOH (20 mL) and H₂O (20 mL). The resulting mixture was stirred at 25°C under N₂ for 12 hrs. The reaction mixture was concentrated under reduced pressure. The residue was purified by preparative TLC (SiO₂, DCM: MeOH = 10:1 R_f = 0.50) which provided amine **B4**.



The amine **B4** (19 mg, 116 μ mol, 1.2 *eq*), HOBt (20 mg, 144 μ mol, 1.5 *eq*), EDCI (28 mg, 144 μ mol, 1.5 *eq*) and DIPEA (50 mg, 385 μ mol, 67 μ L, 4 *eq*) were dissolved in DMF (10 mL). 2-(3-Cyanophenyl)acetic acid (30 mg, 96 μ mol, 1 *eq*) was added. The mixture was stirred at 25°C under N₂ for 12hrs. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was purified by preparative-HPLC (TFA condition: Column: UniSil 120 x

30 mm, 10 μ m; mobile phase: [water (0.1%TFA)-ACN]; ACN%: 20%-50%, 11 min) which provided amide **B5**.

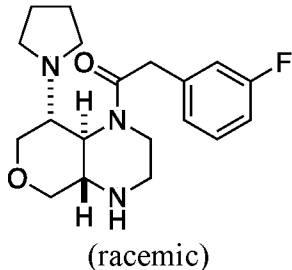
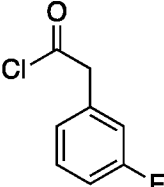
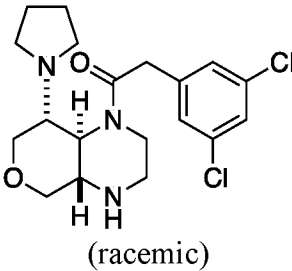
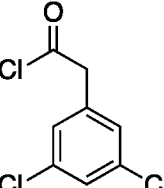
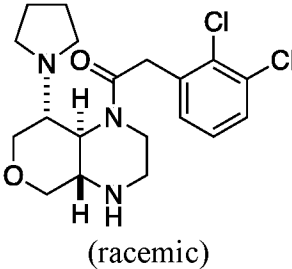
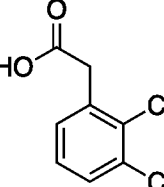
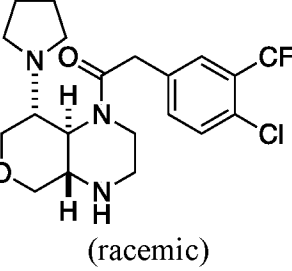
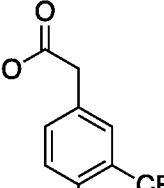


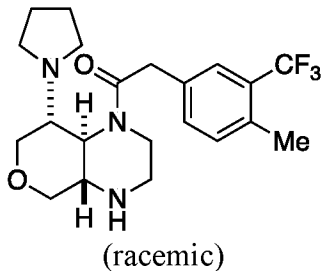
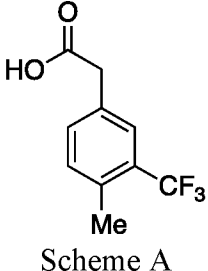
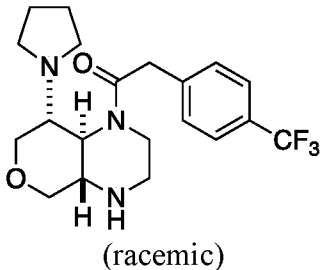
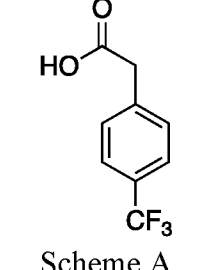
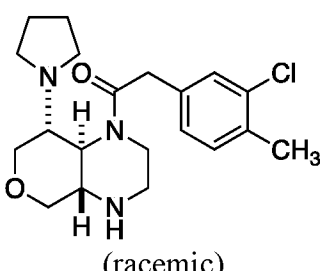
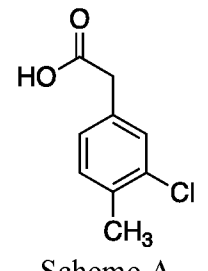
The amide **B5** (17 mg, 37 μ mol, 1 *eq*) was dissolved in dioxane (10 mL). Dioxane/HCl (10 mL, 4.0M) was added. The resulting mixture was stirred at 20°C under N₂ for 6 hrs. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was purified by prep-HPLC (TFA condition: Column: Nano-micro Kromasil C18 100 x 30mm, 5 μ m; mobile phase: [water (0.1%TFA)-ACN]; ACN%: 5%-25%, 10min) which provided **5A28** as the TFA salt. ¹H NMR (400MHz, TFA salt, methanol-d₄) δ 7.72 - 7.61 (m, 3H), 7.56 - 7.51 (m, 1H), 4.70 (dt, *J* = 4.4, 10.4 Hz, 1H), 4.39 (dd, *J* = 4.5, 11.7 Hz, 1H), 4.32 - 4.21 (m, 2H), 4.15 (dd, *J* = 5.2, 10.9 Hz, 1H), 3.98 (s, 2H), 3.90 - 3.64 (m, 4H), 3.60 - 3.50 (m, 3H), 3.38 - 3.34 (m, 2H), 2.03 (br s, 4H). LCMS (MH⁺) 355.1.

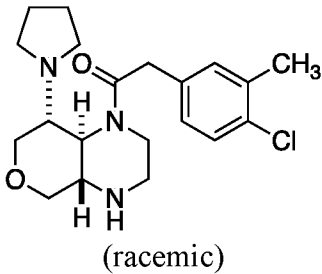
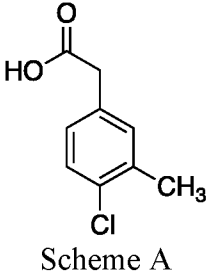
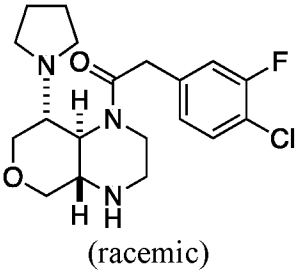
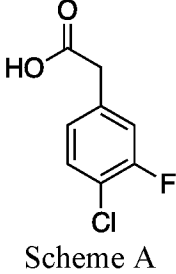
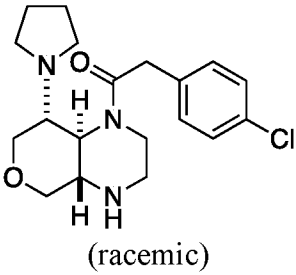
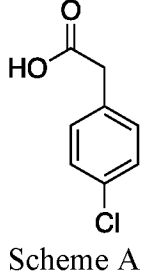
[079] Table A shows spectroscopic data and synthons used to make various compounds via Schemes A and B.

Table A

Example	Structure	Reagent/Scheme	Data	
			¹ H NMR (400 MHz)	LCMS
5A24	 (racemic)	 Scheme A	(methanol-d ₄) δ 7.35 - 7.22 (m, 4H), 4.08 (dd, <i>J</i> = 4.6, 11.2 Hz, 1H), 3.97 (br d, <i>J</i> = 13.2 Hz, 1H), 3.92 - 3.77 (m, 2H), 3.76 - 3.66 (m, 1H), 3.34 (s, 1H), 3.28 - 3.20 (m, 2H), 3.10 - 2.86 (m, 4H), 2.75 (br s, 4H), 2.70 - 2.59 (m, 1H), 1.77 - 1.67 (m, 4H)	(MH ⁺) 364.1

Example	Structure	Reagent/Scheme	Data	
			¹ H NMR (400 MHz)	LCMS
5A27	 <p>(racemic)</p>	 <p>Scheme A</p>	(TFA salt, methanol-d ₄) δ 7.41 - 7.35 (m, 1H), 7.18 - 7.12 (m, 2H), 7.06 (br t, $J=8.6$ Hz, 1H), 4.72 (dt, $J=4.4, 10.3$ Hz, 1H), 4.39 (dd, $J=4.5, 11.6$ Hz, 1H), 4.26 (td, $J=4.3, 15.8$ Hz, 1H), 4.20 - 4.09 (m, 2H), 3.94 (s, 2H), 3.84 - 3.76 (m, 1H), 3.71 - 3.61 (m, 3H), 3.56 - 3.49 (m, 2H), 3.40 - 3.35 (m, 2H), 3.17 - 3.02 (m, 1H), 2.04 (br s, 4H)	(MH ⁺) 348.1
5A32	 <p>(racemic)</p>	 <p>Scheme A</p>	(chloroform-d) δ = 7.19 (s, 2 H) 7.09 (d, $J=1.76$ Hz, 1 H) 4.01 - 4.09 (m, 1 H) 3.74 - 3.82 (m, 1 H) 3.68 - 3.73 (m, 2 H) 3.63 - 3.68 (m, 2 H) 3.51 - 3.61 (m, 2 H) 3.13 - 3.36 (m, 3 H) 2.91 - 3.06 (m, 3 H) 2.89 (s, 1 H) 2.63 - 2.84 (m, 5 H) 1.72 (br s, 5 H)	(MH ⁺) 398.1
5A48	 <p>(racemic)</p>	 <p>Scheme B</p>	(methanol-d ₄) δ = 7.51 (dd, $J=2.1, 7.4$ Hz, 1H), 7.35 - 7.27 (m, 2H), 4.70 (dt, $J=4.5, 10.2$ Hz, 1H), 4.42 - 4.28 (m, 2H), 4.20 - 4.04 (m, 5H), 3.89-3.80 (m, 1H), 3.71 - 3.47 (m, 7H), 3.42 - 3.37 (m, 2H), 2.04 (br s, 4H)	(MH ⁺) 398.1
5A46	 <p>(racemic)</p>	 <p>Scheme B</p>	(TFA salt, methanol-d ₄) δ = 7.73 (s, 1H), 7.61 - 7.48 (m, 2H), 4.71 (dt, J = 4.3, 10.3 Hz, 1H), 4.42 - 4.10 (m, 4H), 4.03 - 3.93 (m, 2H), 3.89 - 3.45 (m, 8H), 3.39 (ddd, $J=$ 4.1, 9.3, 13.0 Hz, 2H),	(MH ⁺) 432.1

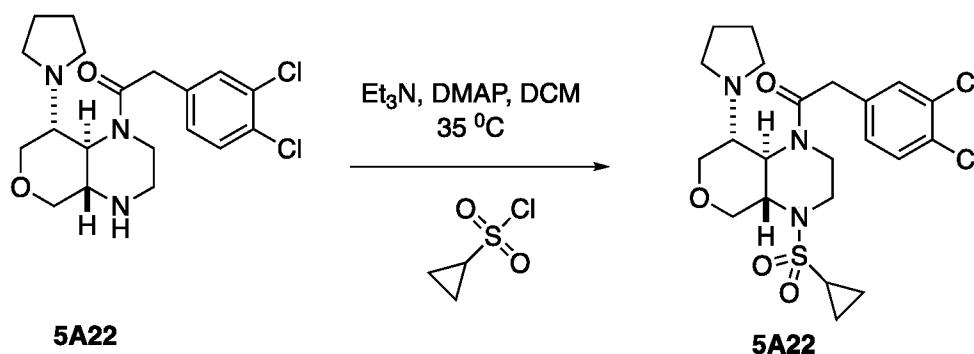
Example	Structure	Reagent/Scheme	Data	
			¹ H NMR (400 MHz)	LCMS
			2.01 (br s, 4H)	
5A47	 <p>(racemic)</p>	 <p>Scheme A</p>	(TFA salt, methanol-d ₄) δ = 7.61 (s, 1H), 7.44 (br d, J = 7.7 Hz, 1H), 7.39 - 7.33 (m, 1H), 4.68 (dt, J = 4.6, 10.4 Hz, 1H), 4.37 (dd, J = 4.3, 11.6 Hz, 1H), 4.26 (br d, J = 16.0 Hz, 1H), 4.16 - 4.08 (m, 2H), 3.94 (s, 2H), 3.84 - 3.75 (m, 1H), 3.69 - 3.61 (m, 3H), 3.54 - 3.47 (m, 2H), 3.44 (br d, J = 12.7 Hz, 2H), 3.26 - 3.17 (m, 2H), 2.47 (s, 3H), 2.02 (br s, 4H)	(MH ⁺) 412.2
5A49	 <p>(racemic)</p>	 <p>Scheme A</p>	(TFA salt, methanol-d ₄) δ = 7.65 (br d, J = 7.9 Hz, 2H), 7.52 (br d, J = 8.1 Hz, 2H), 4.72 (br s, 1H), 4.44 - 4.07 (m, 4H), 4.00 (s, 2H), 3.83 (br s, 1H), 3.68 (br d, J = 10.3 Hz, 3H), 3.55 (br d, J = 10.5 Hz, 2H), 3.44 (br d, J = 10.6 Hz, 2H), 3.25 - 3.13 (m, 2H), 2.02 (br s, 4H)	(MH ⁺) 398.1
5A57	 <p>(racemic)</p>	 <p>Scheme A</p>	(TFA salt, 400MHz, methanol-d ₄) δ = 7.34 - 7.27 (m, 4H), 7.24 (br d, J = 7.6 Hz, 3H), 7.11 (d, J = 7.6 Hz, 1H), 4.78 (br s, 1H), 4.43 (br dd, J = 4.8, 11.4 Hz, 1H), 4.35 - 4.29 (m, 1H), 4.02 (br d, J = 13.8 Hz, 1H), 3.90 (br d, J = 13.4 Hz, 1H), 3.79 (br d, J = 10.0 Hz, 2H), 3.71 (br d, J = 17.6 Hz, 2H), 3.65 - 3.53 (m, 4H), 3.51 - 3.47 (m, 2H),	(MH ⁺) 378.3

Example	Structure	Reagent/Scheme	Data	
			¹ H NMR (400 MHz)	LCMS
			3.25 (br d, <i>J</i> = 13.4 Hz, 1H), 3.14 (t, <i>J</i> = 1.6 Hz, 1H), 2.82 (br s, 1H), 2.66 (br d, <i>J</i> = 12.3 Hz, 1H), 2.36 (s, 3H), 2.06 (br s, 4H)	
5A58	 <p>(racemic)</p>	 <p>Scheme A</p>	(TFA salt, methanol- <i>d</i> ₄) δ = 7.37 - 7.31 (m, 1H), 7.29 - 7.24 (m, 1H), 7.18 - 7.11 (m, 1H), 4.71 (dt, <i>J</i> = 4.5, 10.3 Hz, 1H), 4.37 (dd, <i>J</i> = 4.5, 11.6 Hz, 1H), 4.30 - 4.08 (m, 3H), 3.90 - 3.77 (m, 3H), 3.73 - 3.62 (m, 3H), 3.58 - 3.49 (m, 2H), 3.44 - 3.35 (m, 3H), 3.17 - 3.04 (m, 1H), 2.40 - 2.33 (m, 3H), 2.02 (br s, 4H)	(MH ⁺) 378.1
5A65	 <p>(racemic)</p>	 <p>Scheme A</p>	(TFA salt, methanol- <i>d</i> ₄) δ = 7.44 (t, <i>J</i> = 8.0 Hz, 1H), 7.25 (dd, <i>J</i> = 2.0, 10.3 Hz, 1H), 7.13 (d, <i>J</i> = 8.2 Hz, 1H), 4.70 (dt, <i>J</i> = 4.5, 10.3 Hz, 1H), 4.37 (dd, <i>J</i> = 4.5, 11.6 Hz, 1H), 4.29 - 4.08 (m, 3H), 3.91 (s, 2H), 3.84 - 3.75 (m, 1H), 3.70 - 3.61 (m, 3H), 3.57 - 3.48 (m, 2H), 3.47 - 3.39 (m, 2H), 3.20 (ddd, <i>J</i> = 4.2, 9.3, 13.0 Hz, 1H), 2.03 (br s, 4H)	(MH ⁺) 382.2
5A66	 <p>(racemic)</p>	 <p>Scheme A</p>	(TFA salt, methanol- <i>d</i> ₄) δ = 7.39 - 7.28 (m, 4H), 4.72 (br s, 1H), 4.37 (br d, <i>J</i> = 7.7 Hz, 1H), 4.30 - 4.07 (m, 3H), 3.89 (br s, 2H), 3.79 (br d, <i>J</i> = 11.2 Hz, 1H), 3.66 (br t, <i>J</i> = 10.3 Hz, 3H), 3.59 - 3.49 (m, 2H), 3.38 (br d, <i>J</i> = 12.8 Hz, 3H), 3.08 (br s,	(MH ⁺) 364.1

Example	Structure	Reagent/Scheme	Data	
			¹ H NMR (400 MHz)	LCMS
			1H), 2.02 (br s, 4H)	

[080] ROCK-5A52 is made via the sequence shown in Scheme C:

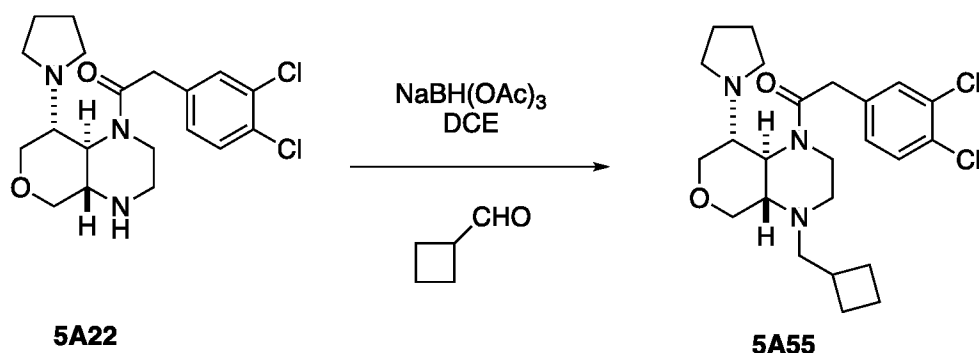
Scheme C



To a solution of **5A22** (100 mg, 250 μ mol, 1 *eq*) in DCM (3 mL) was added Et₃N (127 mg, 1.26 mmol, 175 μ L, 5 *eq*), DMAP (3.1 mg, 25 μ mol, 0.1 *eq*) and cyclopropanesulfonyl chloride (71 mg, 500 μ mol, 2 *eq*). The mixture was stirred at 35°C for 12 hours under N₂. The reaction mixture was concentrated under reduced pressure to give a residue. The residue was purified by preparative-HPLC (column: Waters Xbridge Prep OBD C18 150 x 30 mm, 10 micron; mobile phase: [water(0.1%TFA)-ACN]; ACN%: 10%-70%, 10min). to provide **5A22** as an off-white solid. ¹H NMR (methanol-d₄, TFA salt, 400 MHz) δ 7.48-7.53 (m, 2H), 7.25 (dd, *J*=8.25, 2.02 Hz, 1H), 4.30-4.45 (m, 2H), 4.16-4.29 (m, 2 H), 3.88-4.00 (m, 4H), 3.74-3.87 (m, 3H), 3.47-3.71 (m, 4H), 3.36-3.42 (m, 2H), 2.43-2.55 (m, 1 H), 1.88-2.16 (m, 4H), 0.95-1.16 (m, 4H). LCMS (MH⁺) 502.1.

[081] ROCK-5A55 and compounds shown in Table B are made as shown in Scheme D:

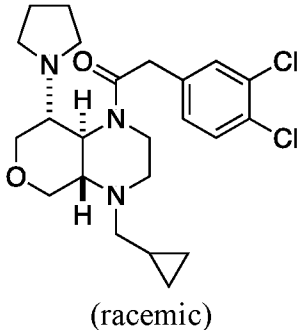
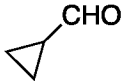
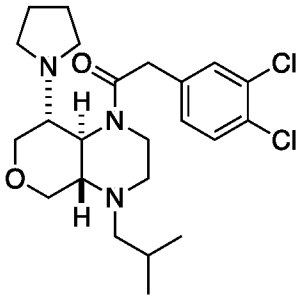
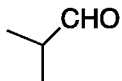
Scheme D

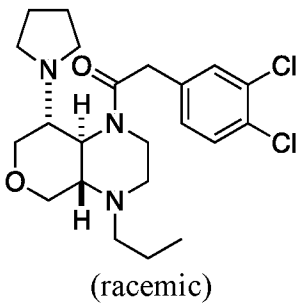
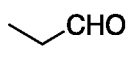


To a solution of **5A22** (10 mg, 25 μmol , 1 *eq*) and cyclobutane carbaldehyde (4.22 mg, 50 μmol , 2 *eq*) in DCE (2 mL) was added $(\text{CH}_3\text{COO})_3\text{BHNa}$ (11 mg, 50 μmol , 2.00 *eq*). The resulting mixture was stirred at 20°C under N_2 for 12 hrs. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was purified by preparative-HPLC (TFA condition: column: Waters Xbridge Prep OBD C18 150 x 30 mm, 10 μm ; mobile phase: [water (0.1%TFA)-ACN]; ACN%: 10%-70%, 10 min) which provided **5A55** as the TFA salt. ^1H NMR (400MHz, TFA salt, methanol- d_4) δ = 7.56 - 7.46 (m, 2H), 7.26 (dd, J = 1.7, 8.2 Hz, 1H), 4.72 (dt, J = 4.8, 10.2 Hz, 1H), 4.42 (dd, J = 4.8, 10.6 Hz, 1H), 4.33 (dd, J = 4.5, 11.7 Hz, 1H), 4.28 - 4.14 (m, 2H), 3.89 (s, 2H), 3.86 - 3.75 (m, 1H), 3.69 - 3.58 (m, 3H), 3.58 - 3.44 (m, 4H), 3.15 (br dd, J = 6.5, 12.8 Hz, 1H), 3.00 - 2.88 (m, 2H), 2.69 (td, J = 7.5, 15.0 Hz, 1H), 2.21 - 1.94 (m, 7H), 1.91 - 1.75 (m, 3H). LCMS (MH^+) 466.1.

Table B

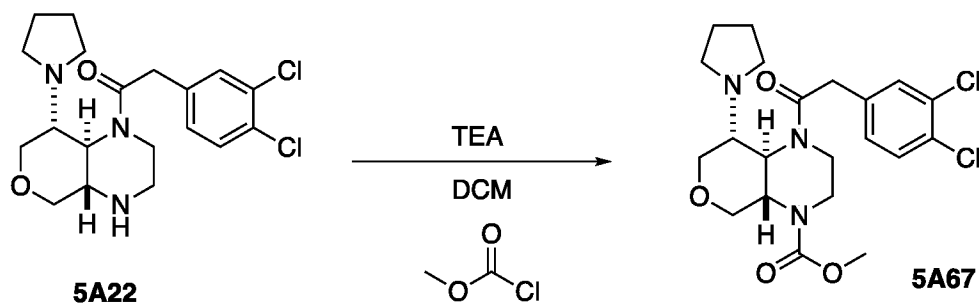
Example	Structure	Reagent Scheme D	Data	
			^1H NMR (400 MHz)	LCMS
5A54	<p style="text-align: center;">(racemic)</p>		(TFA salt, methanol- d_4) δ = 7.55 - 7.46 (m, 2H), 7.26 (dd, J = 1.8, 8.3 Hz, 1H), 4.67 (dt, J = 4.8, 10.1 Hz, 1H), 4.39 - 4.28 (m, 2H), 4.18 - 4.02 (m, 2H), 3.88 (s, 2H), 3.71 (br d, J = 14.5 Hz, 1H), 3.67 - 3.58 (m, 2H), 3.58 - 3.47 (m, 2H), 3.37 (br d, J = 18.2 Hz, 2H), 3.14	(MH^+) 480.2

Example	Structure	Reagent Scheme D	Data	
			¹ H NMR (400 MHz)	LCMS
			(br d, $J = 1.6$ Hz, 1H), 2.84 - 2.64 (m, 3H), 2.16 - 1.94 (m, 5H), 1.81 (br s, 2H), 1.72 - 1.54 (m, 4H), 1.28 - 1.12 (m, 2H)	
5A70	 <p>(racemic)</p>		(400MHz, TFA salt, chloroform-d) $\delta = 7.47 - 7.41$ (m, 2H), 7.19 (br d, $J = 8.1$ Hz, 1H), 4.95 (br s, 1H), 4.51 (br s, 1H), 4.28 - 4.14 (m, 2H), 4.06 (br d, $J = 14.5$ Hz, 1H), 3.91 - 3.75 (m, 4H), 3.73 - 3.63 (m, 3H), 3.60 (br d, $J = 10.3$ Hz, 2H), 3.47 (br s, 1H), 2.83 - 2.75 (m, 1H), 2.75 - 2.56 (m, 2H), 2.02 (br s, 4H), 0.89 (br s, 1H), 0.80 - 0.65 (m, 2H), 0.32 - 0.19 (m, 2H)	(MH ⁺) 452.2
5A71			(TFA salt, chloroform-d) $\delta = 7.52 - 7.46$ (m, 2H), 7.24 (dd, $J = 2.0, 8.2$ Hz, 1H), 4.67 (dt, $J = 4.5, 10.2$ Hz, 1H), 4.32 (ddd, $J = 4.7, 11.4, 19.4$ Hz, 2H), 4.16 - 4.06 (m, 2H), 3.87 (d, $J = 2.2$ Hz, 2H), 3.78 - 3.68 (m, 1H), 3.62 (br dd, $J = 9.9, 11.5$ Hz, 2H), 3.52 (br t, $J = 10.7$ Hz, 2H), 3.39 (br d, $J = 12.3$ Hz, 2H), 3.27 - 3.21 (m, 1H), 2.77 (br s, 1H), 2.69 - 2.60 (m, 1H), 2.51 (br dd, $J = 4.2, 12.1$ Hz, 1H), 2.02 (br s, 4H), 1.94 - 1.83 (m, 1H), 0.95 (dd, $J = 2.9, 6.6$ Hz, 6H)	(MH ⁺) 454.1

Example	Structure	Reagent Scheme D	Data	
			¹ H NMR (400 MHz)	LCMS
5A72	 (racemic)		(chloroform-d) δ = 7.42 - 7.34 (m, 2H), 7.14 (dd, J = 1.9, 8.3 Hz, 1H), 4.18 (dd, J = 4.8, 11.2 Hz, 1H), 4.09 (br s, 1H), 3.76 (br d, J = 15.3 Hz, 2H), 3.68 - 3.58 (m, 1H), 3.34 - 3.03 (m, 3H), 2.90 (br d, J = 9.7 Hz, 1H), 2.71 (br s, 3H), 2.52 - 2.42 (m, 1H), 2.31 - 2.18 (m, 2H), 1.72 (br s, 1H), 1.58 (br s, 6H), 1.48 - 1.33 (m, 2H), 0.85 (t, J = 7.3 Hz, 3H)	(MH ⁺) 440.3

[082] ROCK-5A67 is made as shown in Scheme E:

Scheme E

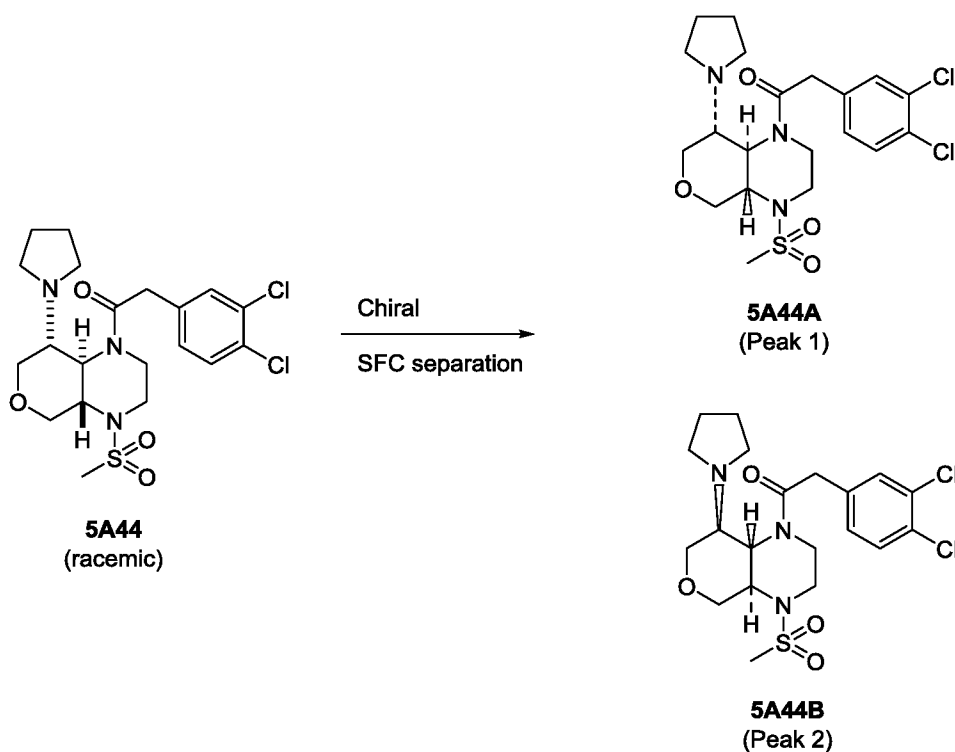


5A22 (5 mg, 12 μ mol, 1 *eq*) and TEA (13 mg, 125 μ mol, 18 μ L, 10 *eq*) were dissolved in DCM (1 mL). Methylchloroformate (2.4 mg, 25 μ mol, 1.94 μ L, 2 *eq*) was added. The resulting mixture was stirred at 20°C under N₂ for 12 hrs. The reaction mixture was concentrated under reduced pressure. The residue was purified by preparative-HPLC (TFA condition: column: Luna C18 100 x 30 mm, 5 micron; mobile phase: [water (0.1%TFA)-ACN]; ACN%: 15%-45%, 10 min) which provided **5A67** as the TFA salt. ¹H NMR (400MHz, TFA salt, chloroform-d) δ = 7.34 (d, J = 8.2 Hz, 1H), 7.25 (d, J = 1.7 Hz, 1H), 6.99 (dd, J = 1.6, 8.2 Hz, 1H), 5.59 (br s, 1H), 4.50 (br s, 1H),

4.20 - 4.04 (m, 3H), 3.92 (br d, $J = 7.6$ Hz, 2H), 3.73 (br d, $J = 5.9$ Hz, 4H), 3.57 (s, 5H), 3.39 (br s, 1H), 3.22 (br s, 1H), 3.02 - 2.79 (m, 2H), 1.93 (br s, 4H). LCMS (MH^+) 456.1.

[083] The preparation of enantiomers ROCK-5A44A and 5A44B via chiral separation is shown in Scheme F. Absolute stereochemistry is undetermined. Enantiomers are characterized by order of elution under the conditions shown.

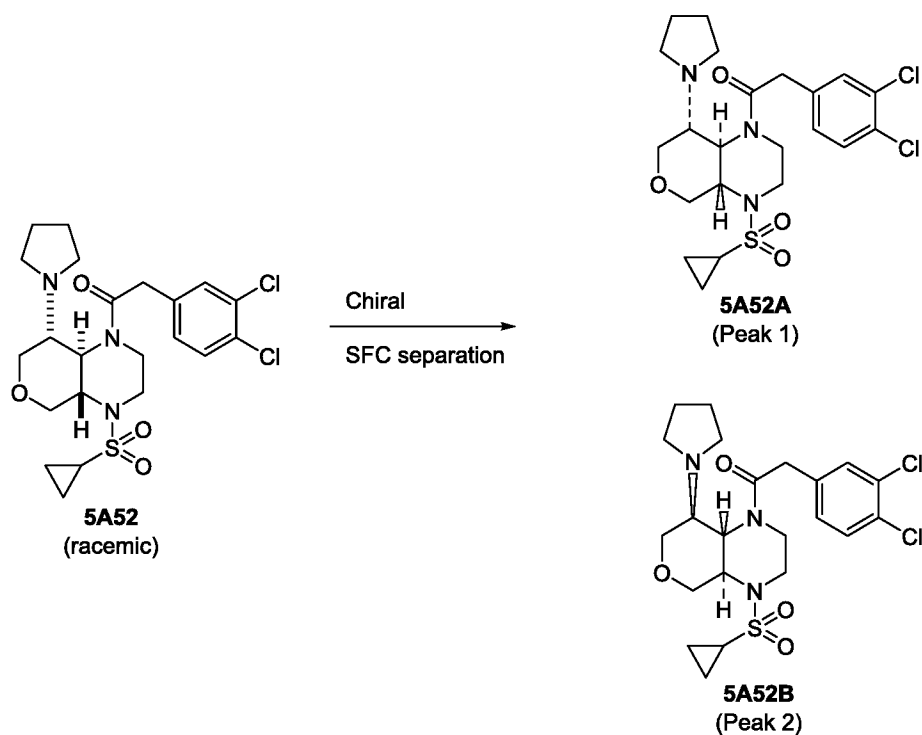
Scheme F



Racemic-**5A44** was separated by chiral SFC (column: DAICEL CHIRALPAK ADH (250 mm x 30 mm, 5 μ m); mobile phase: [Neutral-IPA]; IPA %: 45%-45%, 15min) which provided **5A44A** (Peak 1: retention time – 2.4 minutes) and **5A44B** (Peak 2: retention time – 3.02 minutes).

[084] The preparation of enantiomers ROCK-5A52A and 5A52B via chiral separation is shown in Scheme G. Absolute stereochemistry is undetermined. Enantiomers are characterized by order of elution under the conditions shown.

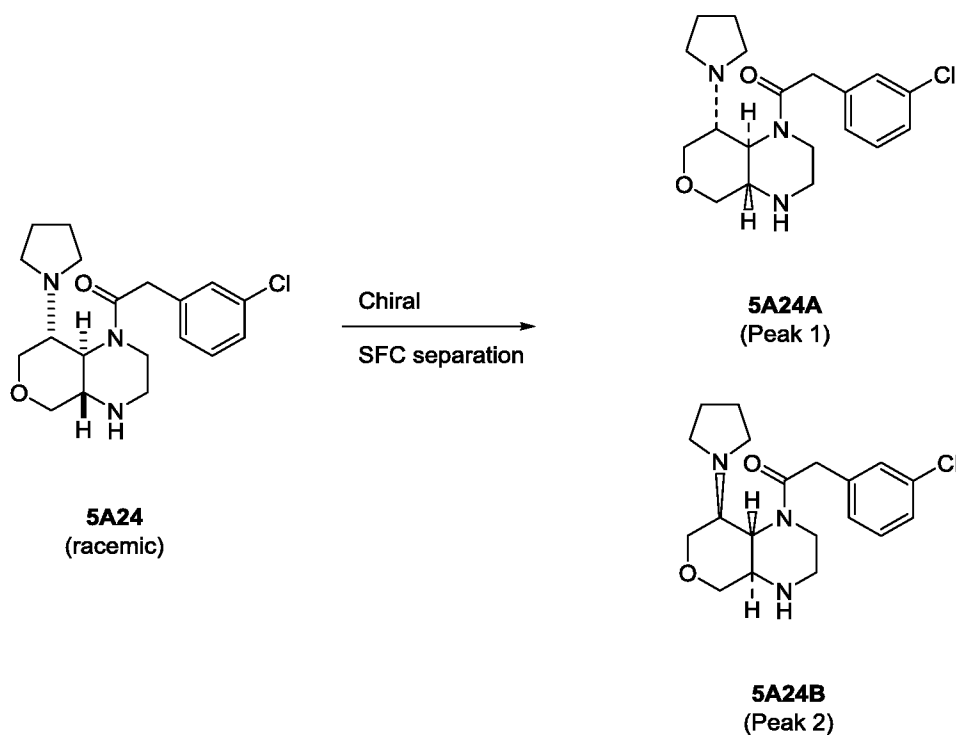
Scheme G



Racemic **5A52** was separated by SFC (column: DAICEL CHIRALCEL OD-H(250 mm x 30 mm, 5 μ m); mobile phase: [0.1%NH₃H₂O EtOH]; EtOH %: 30%-30%, 7min) which provided **5A52A** (Peak 1 – 2.02 minutes) and **5A52B** (Peak 2 – 2.33 minutes).

[085] The preparation of enantiomers ROCK-5A24A and 5A24B via chiral separation is shown in Scheme H. Absolute stereochemistry is undetermined. Enantiomers are characterized by order of elution under the conditions shown.

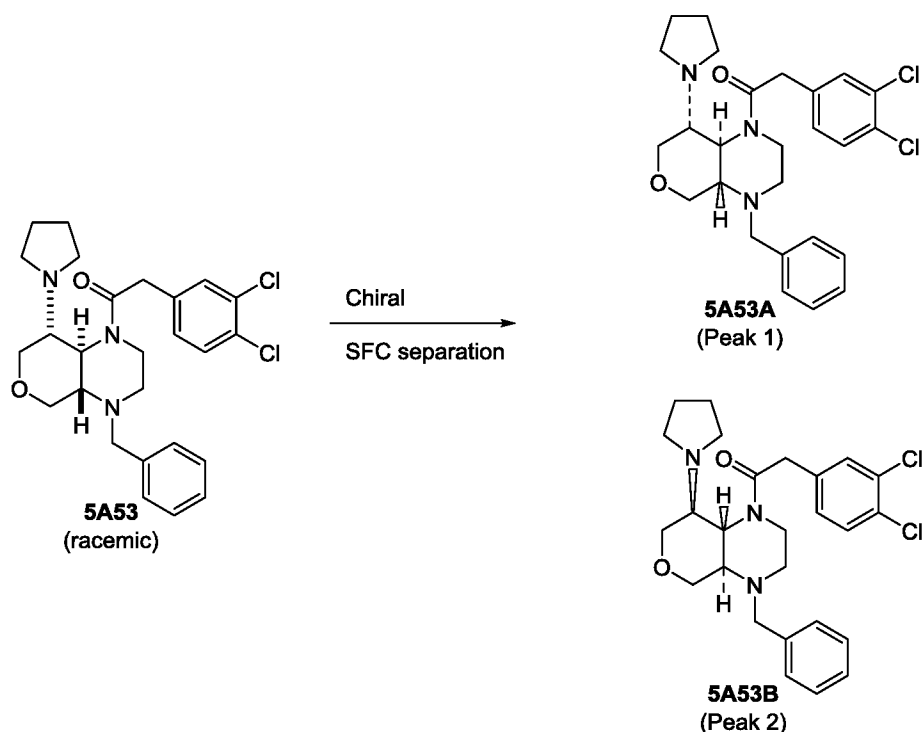
Scheme H



Racemic **5A24** was separated by SFC (column: DAICEL CHIRALPAK AD-H(250 mm x 30 mm, 5 μ m); mobile phase: [Neutral-IPA]; IPA%: 24%-24%, 6 min) which provided **5A24A** (Peak 1 – 1.93 minutes) and **5A24B** (Peak 2 – 2.21 minutes).

[086] The preparation of enantiomers ROCK-5A53A and 5A53B via chiral separation is shown in Scheme I. Absolute stereochemistry is undetermined. Enantiomers are characterized by order of elution under the conditions shown.

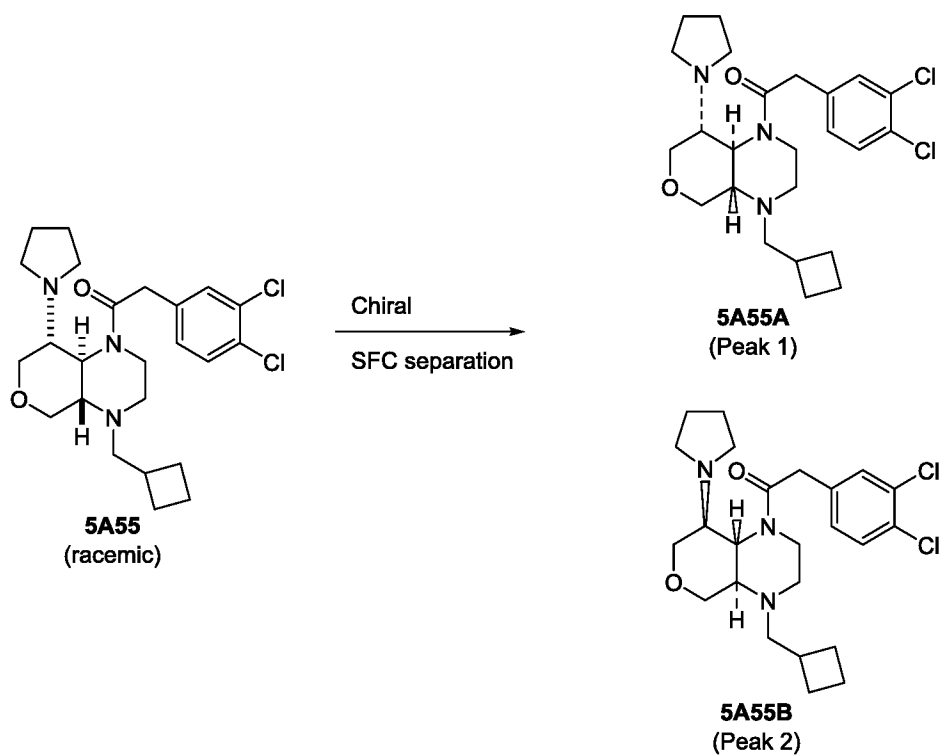
Scheme I



Racemic **5A53** was separated by SFC (column: DAICEL CHIRALCEL OJ (250 mm x 50 mm, 10 μ m); mobile phase: [0.1% $\text{NH}_3\text{H}_2\text{O}$ IPA]; IPA%: 40%-40%, 6.2min) which provided **5A53A** (Peak 1 – 1.55 minutes) and **5A53B** (Peak 2 – 1.73 minutes). Peak 1 (**5A53**) was further purified by prep-HPLC (column: Kromasil 250 x 50mm, 10 μ m; mobile phase: [water (10mM NH_4HCO_3)-ACN]; ACN%: 50%-80%, 10 min).

[087] The preparation of enantiomers ROCK-5A55A and 5A55B via chiral separation is shown in Scheme J. Absolute stereochemistry is undetermined. Enantiomers are characterized by order of elution under the conditions shown.

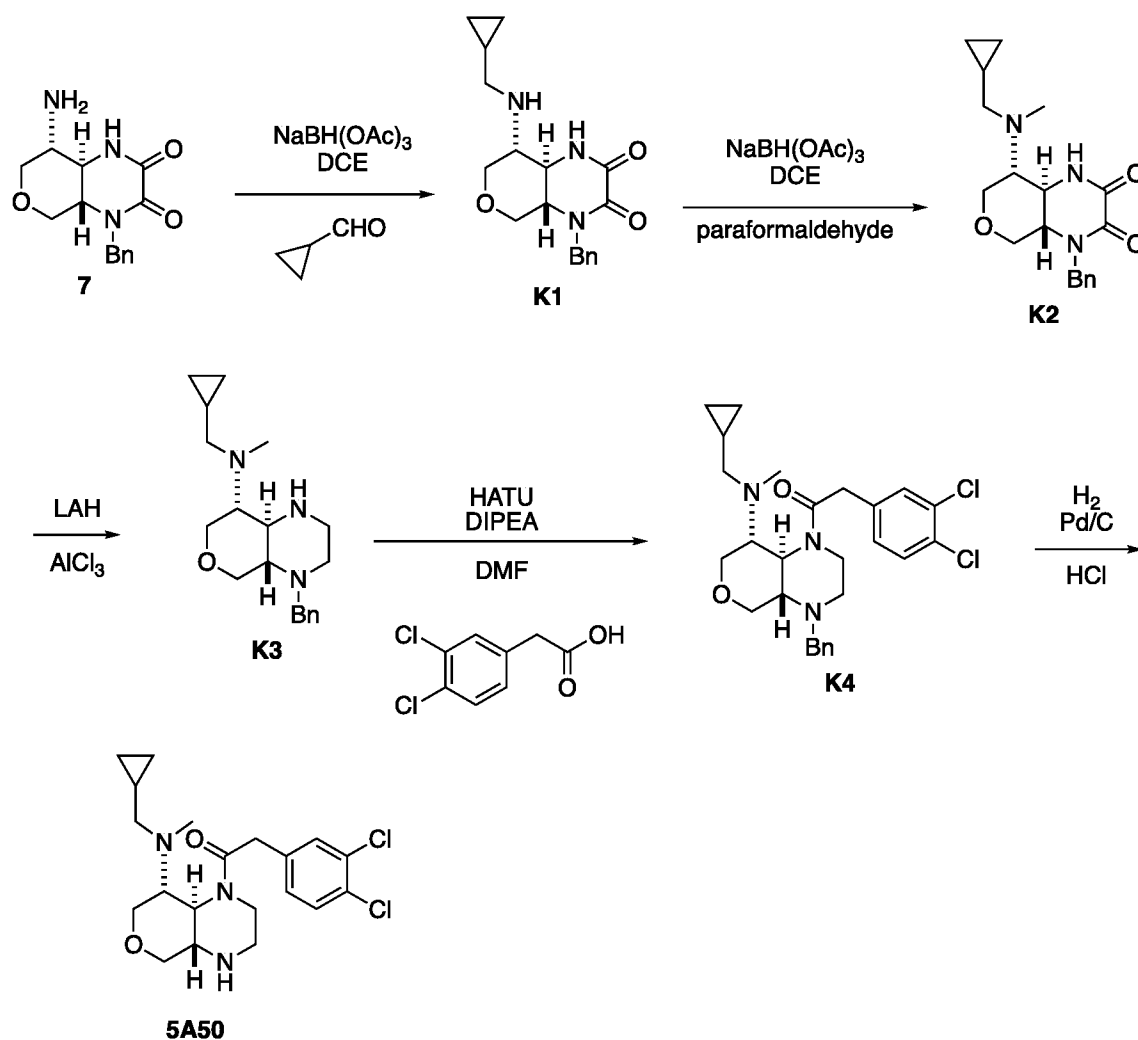
Scheme J



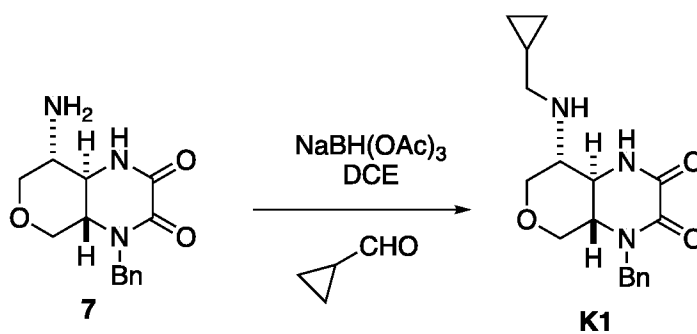
Racemic **5A55** was separated by SFC (column: DAICEL CHIRALPAK AD-H (250 mm x 30 mm, 5 μ m); mobile phase: [0.1% $\text{NH}_3\text{H}_2\text{O}$ EtOH]; EtOH%: 38%-38%, 7 min) which provided **5A55A** (Peak 1 – 1.99 minutes) and **5A55B** (Peak 2 – 2.08 minutes).

[088] ROCK-5A50 is made as shown in Scheme K:

Scheme K

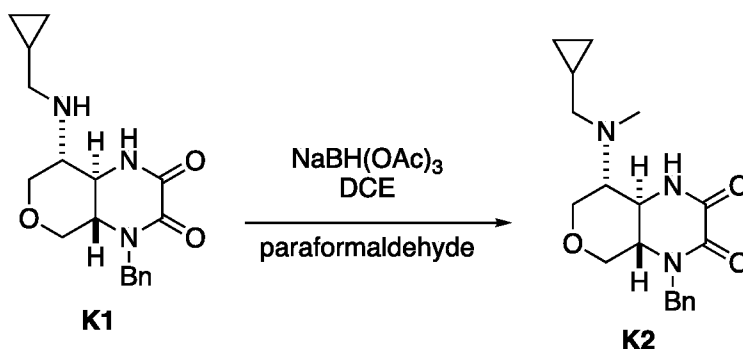


[089] Example experimentals for Scheme K:

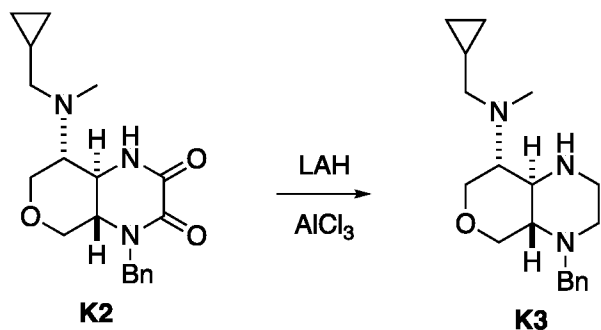


To a solution 7 (500 mg, 1.82 mmol, 1 *eq*) in DCE (20 mL) was added cyclopropanecarbaldehyde (127 mg, 1.82 mmol, 135 μL , 1 *eq*). The mixture was degassed and purged with N_2 (3 times). The mixture was stirred at 20°C for 12hrs under a N_2 atmosphere.

After 12hrs, $\text{NaBH}(\text{OAc})_3$ (770 mg, 3.63 mmol, 2 *eq*) was added. The mixture was stirred at 20°C for 12hrs under a N_2 atmosphere. The reaction mixture was concentrated under reduced pressure. The residue was purified by prep-TLC (SiO_2 , DCM: MeOH = 10:1) which provided **K1**.

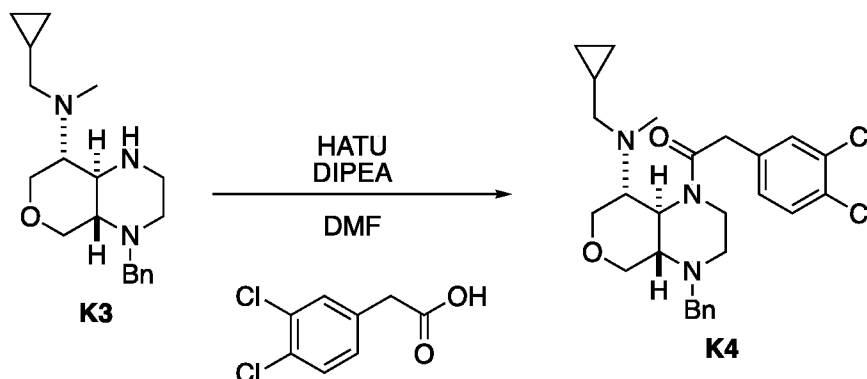


To a solution **K1** (30 mg, 91 μmol , 1 *eq*) in DCE (10 mL) was added $\text{NaBH}(\text{OAc})_3$ (58 mg, 270 μmol , 3 *eq*) and paraformaldehyde (100 mg, 2.91 mmol, 32 *eq*). The mixture was stirred at 20°C for 12hrs under a N_2 atmosphere. The reaction mixture was concentrated under reduced pressure. The residue was purified by preparative-TLC (SiO_2 , DCM: MeOH = 10:1) which provided **K2**.

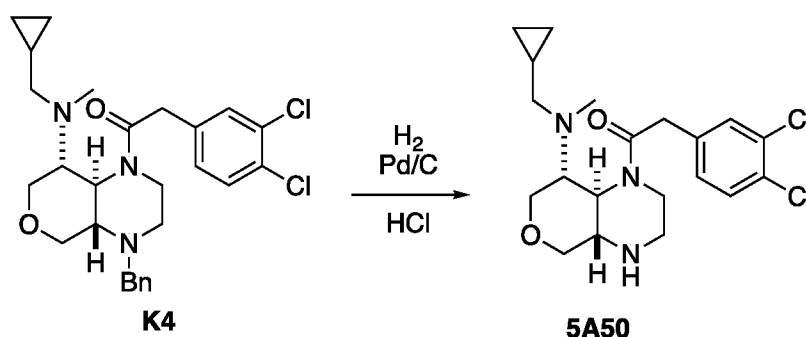


To a solution AlCl_3 (105 mg, 786 μmol , 43 μL , 2.7 *eq*) in THF (20 mL) was added LiAlH_4 (90 mg, 2.4 mmol, 8.1 *eq*) at 0°C. The resulting mixture was stirred at 20°C for 0.5 hr. A solution of **K2** (100 mg, 291 μmol , 1 *eq*) in THF (20 mL) was added to the reaction mixture at 0°C. The resulting mixture was stirred at 0°C (1 hr) and then allowed to warm to 20°C (6 hrs). The reaction mixture was quenched with 2 N $\text{NaOH}_{(\text{aq})}$ (30mL) at 0°C. The mixture was filtered, and the filtrate was concentrated to give a residue. The residue was diluted with EtOAc (50 mL), dried over sodium sulfate, and concentrated. The residue was purified by preparative-HPLC

(TFA condition: column: Nano-micro Kromasil C18 100 x 30mm, 5 μ m; mobile phase: [water (0.1%TFA)-ACN]; ACN%: 8%-28%, 10min) which provided **K3**.



The amine **K3** (39 mg, 190 μ mol, 1.2 *eq*), DIPEA (82 mg, 634 μ mol, 110 μ L, 4 *eq*) and HATU (72 mg, 190 μ mol, 1.2 *eq*) were dissolved in DMF (3 mL). 2-(3,4-Dichlorophenyl)acetic acid (50 mg, 159 μ mol, 1 *eq*) was added. The resulting mixture was stirred at 20 $^{\circ}$ C for 12 hrs. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was purified by preparative-HPLC (TFA condition: column: Nano-Micro UniSil 5-100 C18 ULTRA 100 x 250 mm, 5 μ m; mobile phase: [water (0.1%TFA)-ACN]; ACN%: 25%-55%, 11 min) which provided **K4**.

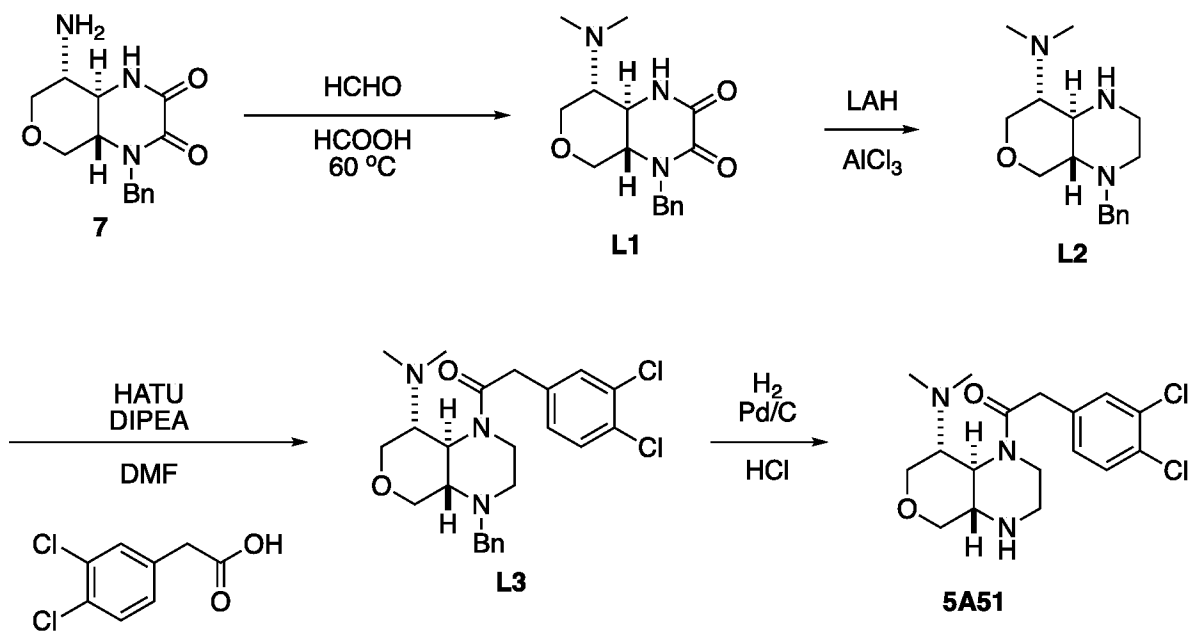


A solution of **K4** (40 mg, 80 μ mol, 1 *eq*), Pd/C (5.5 mg, 80 μ mol, 10 wt% Pd, 1 *eq*) in THF/H₂O/HCl (2 mL, 2 mL, 0.4 mL) was stirred at 20 $^{\circ}$ C under H₂ (15psi) for 6 hrs. The mixture was filtered through a pad of Celite, and the filtrate was concentrated. The residue was purified by preparative-HPLC (TFA condition: column: Waters Xbridge 150 x 25 mm, 5 micron; mobile phase: [water (0.1%TFA)-ACN]; ACN%: 10%-40%, 12 min) which provided **5A50**. ¹H NMR (400MHz, TFA salt, methanol-d₄) δ = 7.54 - 7.47 (m, 2H), 7.24 (dd, *J* = 2.0, 8.2 Hz, 1H),

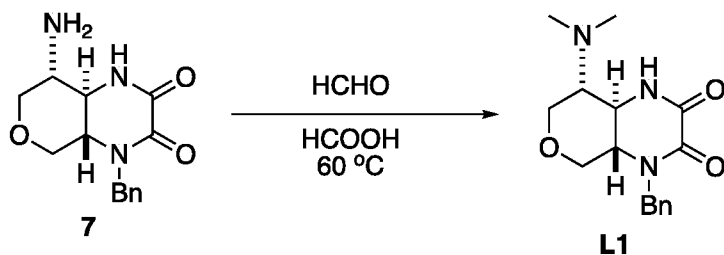
4.71 (br s, 1H), 4.36 (br dd, $J = 3.9, 11.6$ Hz, 2H), 4.31 - 4.16 (m, 2H), 4.10 (br dd, $J = 4.8, 11.1$ Hz, 1H), 3.90 (s, 2H), 3.68 (br s, 3H), 3.59 - 3.42 (m, 2H), 3.36 - 3.33 (m, 1H), 3.27 - 3.19 (m, 1H), 3.17 - 3.06 (m, 1H), 2.97 (br s, 2H), 1.12 (br s, 1H), 0.74 (br d, $J = 6.5$ Hz, 2H), 0.41 (br s, 2H). LCMS (MH^+) 412.1.

[090] ROCK-5A51 is made as shown in Scheme L:

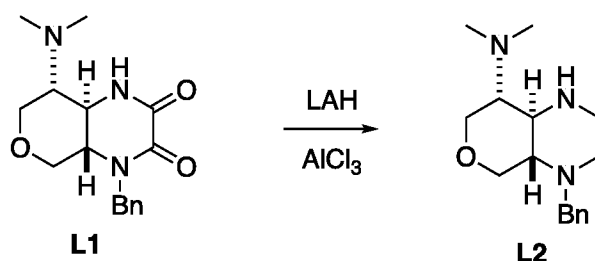
Scheme L



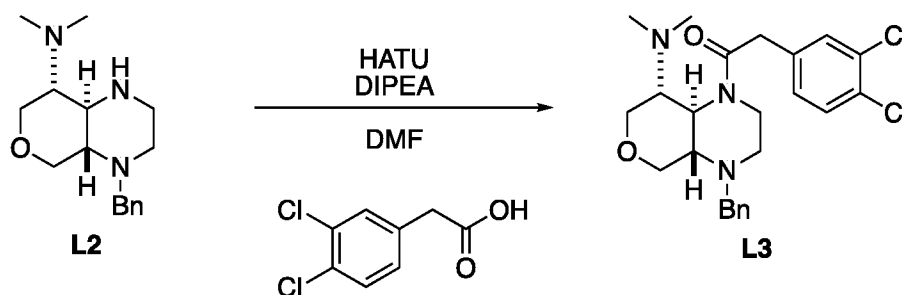
[091] Example experimentals for Scheme L:



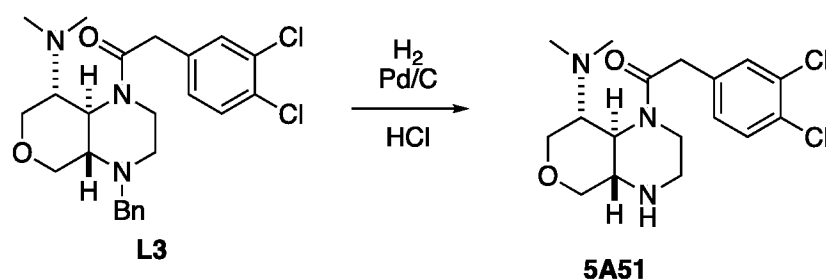
To a solution **7** (200 mg, 726 μ mol, 1 *eq*) in HCOOH (20 mL) was added HCHO (1.89 g, 23 mmol, 1.73 mL, 37% purity, 32 *eq*). The mixture was stirred at 60 °C for 12 hours under a N₂ atmosphere. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was purified by prep-TLC (SiO₂, DCM: MeOH = 10:1) which furnished **L1**.



To a solution AlCl_3 (154 mg, 1.16 mmol, 63 μL , 2.7 *eq*) in THF (20 mL) was added LiAlH_4 (132 mg, 3.47 mmol, 8.1 *eq*) at 0°C. The resulting mixture was stirred at 20°C for 0.5 hr. A solution of **L1** in THF (20 mL) was added to the mixture at 0 °C. The mixture was stirred at 0 °C for 1hr and then at 20°C for 6 hours. The reaction mixture was quenched with 2N $\text{NaOH}_{(\text{aq})}$ (30 mL) at 0°C. The mixture was filtered, and the filtrate was concentrated. The residue was diluted with EtOAc (50 mL), dried over sodium sulfate, filtered, and concentrated. The residue was purified by preparative-HPLC (TFA condition: column: Nano-micro Kromasil C18 100 x 30mm, 5 μm ; mobile phase: [water (0.1%TFA)-ACN]; ACN%: 5%-25%, 10 min) which provided **L2**.



The amine **L2** (107 mg, 523 μmol , 1.2 *eq*), DIPEA (225 mg, 1.74 mmol, 304 μL , 4 *eq*) and HATU (199 mg, 523 μmol , 1.2 *eq*) were dissolved in DMF (3 mL). 2-(3,4-Dichlorophenyl)acetic acid (120 mg, 436 μmol , 1 *eq*) was added. The mixture was stirred at 20 °C for 12 hrs. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was purified by preparative-HPLC (basic condition: column: Waters Xbridge 150 x 25 mm, 5 μm ; mobile phase: [water (0.04% $\text{NH}_3\text{H}_2\text{O}$ +10mM NH_4HCO_3)-ACN]; ACN%: 45%-75%, 10 min). which provided **L3**.



A solution of **L3** (15 mg, 32 μmol , 1 *eq*) and Pd/C (2 mg, 10 wt% Pd) in THF/HCl/H₂O (2 mL/0.4 mL/2 mL) was stirred at 20°C under H₂ (15 psi) for 6 hours. The mixture was filtered through a plug of Celite, and the filtrate was concentrated under reduced pressure to remove solvent. The residue was purified by preparative-HPLC (TFA condition: column: Luna C18 100 x 30 mm, 5 μm ; mobile phase: [water (0.1%TFA)-ACN]; ACN%: 1%-30%, 10 min) which provided **5A50**. ¹H NMR (400MHz, TFA salt, methanol-d₄) δ = 7.53 - 7.47 (m, 2H), 7.24 (dd, *J* = 2.0, 8.2 Hz, 1H), 4.64 (dt, *J* = 4.2, 10.8 Hz, 1H), 4.42 (dd, *J* = 4.2, 11.5 Hz, 1H), 4.33 - 4.18 (m, 2H), 4.11 (dd, *J* = 5.0, 11.0 Hz, 1H), 3.94 - 3.83 (m, 2H), 3.79 - 3.60 (m, 3H), 3.58 - 3.44 (m, 2H), 3.29 - 3.21 (m, 1H), 2.95 (br s, 6H). LCMS (MH⁺) 372.1.

[092] Compounds of the invention may be tested in the following screens

Tritiated U69,593 binding for KOP-R membranes; tritiated DAMGO binding for MOP-R membranes; and tritiated DPDPE binding for DOP-R membranes:

Membranes from cells stably expressing kappa, mu or delta opioid receptor constructs (PathHunter U2OS hOPRK1, CHO-K1 rOPRM1 and CHO-K1 OPRD1 β -arrestin cell line, DiscoverX, Fremont, CA, USA) were used. Cells were scraped from tissue culture plates, homogenized with a tissue tearor homogenizer in membrane buffer (10mM Tris, 100mM NaCl, and 1mM EDTA; pH 7.4), and centrifuged at 20,000 g for 30 minutes at 4°C and frozen at -80°C until use. Prior to use, the pellets were resuspended in binding buffer (50mm Tris, 100mm NaCl, pH 7.4), homogenized with a dounce homogenizer and 50 μg incubated with 1.0 nM of the appropriate tritiated ligand ([³H]U69,593, [³H]DAMGO or [³H]DPDPE for kappa, mu or delta binding, respectively) and the appropriate concentration of compound for 60 minutes at 30°C. Membranes with bound tritiated ligand were collected on Whatman GF/B filter paper (Brandel, Gaithersburg, MD, USA) utilizing a Brandel harvester. Bound tritiated ligand was quantified

using a TriCarb-2900TR scintillation counter (Packard, Downers Grove, IL, USA) following addition of 4 ml ReadySafe scintillation fluid (Beckman Coulter, Indianapolis, IN, USA). Compiled data for each compound was normalized to the maximal binding in the absence of compound, with non-specific binding subtracted. Curve fitting was done in Microcal Origin 17.0 software using logistic sigmoidal fit, to determine IC₅₀.

GTP γ S

Membranes from U2OS cells stably expressing human kappa opioid receptors were used. Cells were scraped from tissue culture plates, homogenized with a tissue tearor homogenizer in membrane buffer (10mM Tris, 100mM NaCl, and 1mM EDTA; pH 7.4), and centrifuged at 20,000 g for 30 minutes at 4°C and frozen at -80°C until use. Prior to use, the pellets were resuspended in assay buffer (50mM Tris, 100mM NaCl, 5mM MgCl₂, and 1mM EDTA; pH 7.4) and homogenized with a dounce homogenizer and 50 μ g incubated with 0.1 nM [³⁵S]GTP γ S, 10 nM GDP, and the appropriate concentration of agonist for 20 minutes at 30°C. To test inhibition, all samples were incubated with 100nM U69,593 as well as the appropriate concentration of compound. Membranes with bound [³⁵S]GTP γ S were collected on Whatman GF/B filter paper (Brandel, Gaithersburg, MD, USA) utilizing a Brandel harvester. Bound [³⁵S]GTP γ S was quantified using a TriCarb-2900TR scintillation counter (Packard, Downers Grove, IL, USA) following addition of 4 mL ReadySafe scintillation fluid (Beckman Coulter, Indianapolis, IN, USA). “No Stim” indicates that there was no stimulation in this assay, at the highest dose tested, 10,000 nM. NA indicates not applicable. For each compound, curve fitting was done in Microcal Origin 17.0 software using logistic sigmoidal fit, to determine EC₅₀ and maximal percent stimulation, which in turn was normalized to the maximal percent stimulation of the test full agonist compound U69,593 to determine % efficacy.

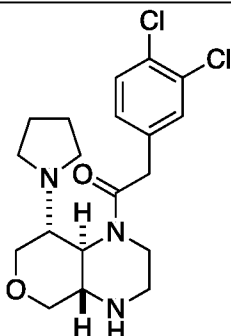
β_2 -Arrestin

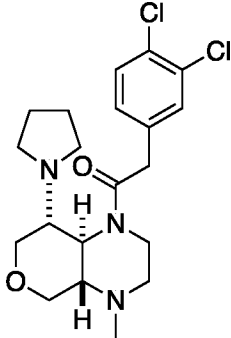
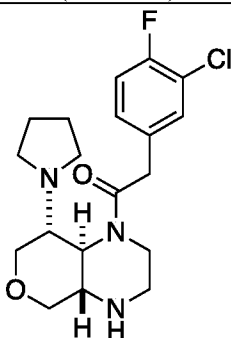
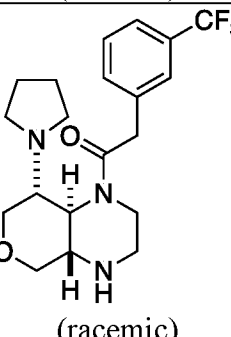
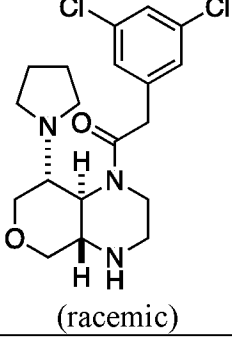
Experiments were conducted using the PathHunter Detection Kit obtained from DiscoverX. Cells stably expressing kappa, mu or delta opioid receptor constructs (PathHunter U2OS hOPRK1, CHO-K1 rOPRM1 and CHO-K1 OPRD1 β -arrestin cell line, DiscoverX, Fremont, CA, USA) were plated in 96- or 384-well plates. Cells were stimulated with the compounds for 90 minutes

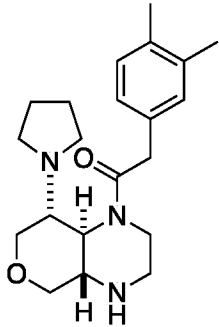
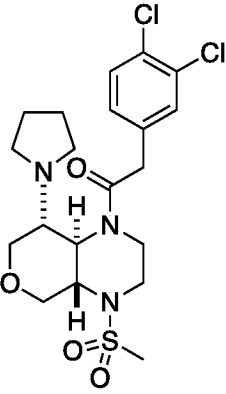
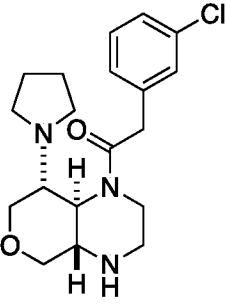
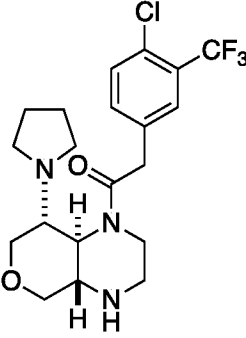
at 37°C. To test inhibition, all samples were incubated with 100nM U69,593 as well as the appropriate concentration of compound. Cells were then incubated for 60 minutes in the presence of galactosidase substrate, yielding chemiluminescent product. Chemiluminescence was measured using a Synergy Neo microplate reader (BioTek, Winooski, VT, USA). Antagonism assays were done in the same manner, in the presence of 300nM U69,593, 1µM DAMGO or 1µM DPDPE for KOP-R, MOP-R or DOP-R assays, respectively. “No Stim” indicates that there was no stimulation in this assay, at the highest dose tested, 10,000 nM. NA indicates not applicable. For each compound, curve fitting was done in Microcal Origin 17.0 software using logistic sigmoidal fit, to determine EC₅₀ and maximal percent stimulation, which in turn was normalized to the maximal percent stimulation of the test full agonist compound U69,593 to determine % efficacy.

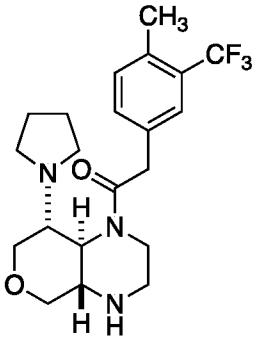
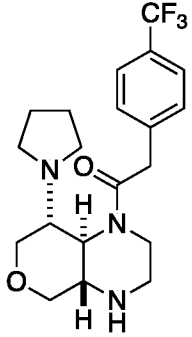
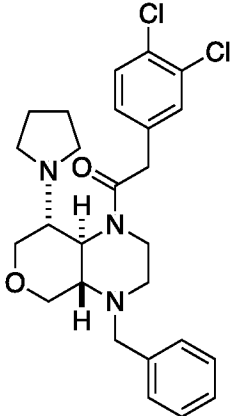
[093] Representative compounds of the invention were tested in the foregoing screens with the following results:

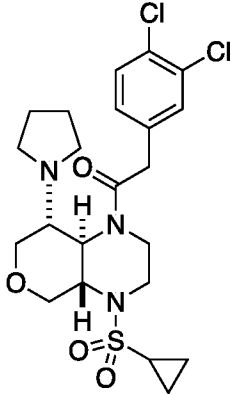
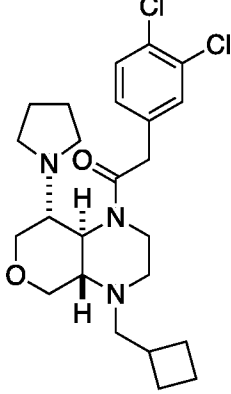
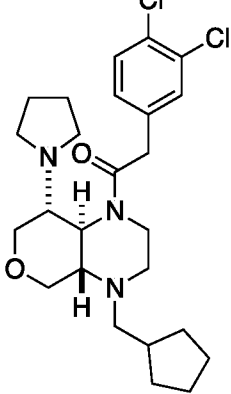
Table A. Stimulation data

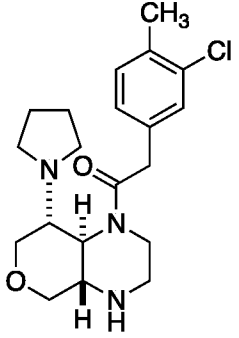
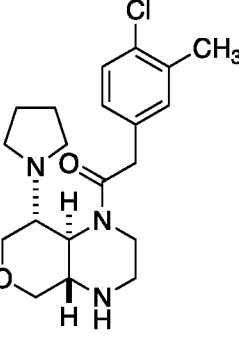
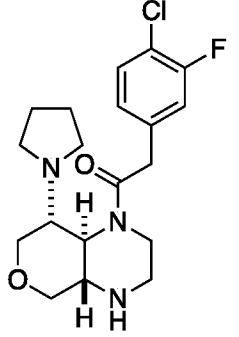
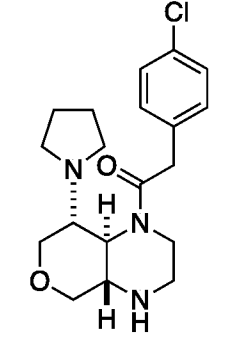
Example	Structure	Binding IC ₅₀ (nM)	GTPγS stimulation		β-arrestin coupling	
			EC ₅₀ (nM)	% efficacy	EC ₅₀ (nM)	% efficacy
5A22	 <p>(racemic)</p>	65	63	96	1132	67

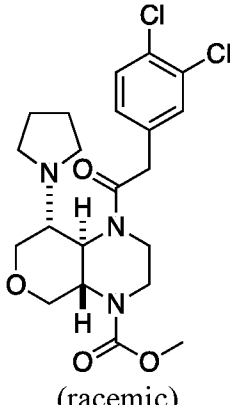
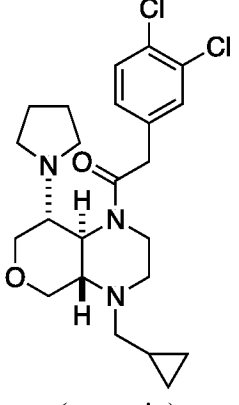
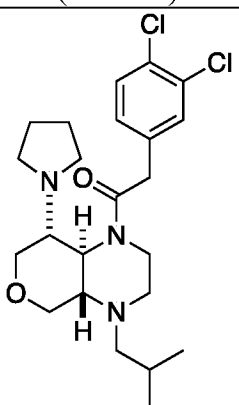
Example	Structure	Binding IC ₅₀ (nM)	GTPγS stimulation		β-arrestin coupling	
			EC ₅₀ (nM)	% efficacy	EC ₅₀ (nM)	% efficacy
5A23	 (racemic)	103	46	76	5540	44
5A25	 (racemic)	249	571	93	13600	29
5A26	 (racemic)	112	130	108	3260	42
5A32	 (racemic)	410	464	66.5	No stim	No stim

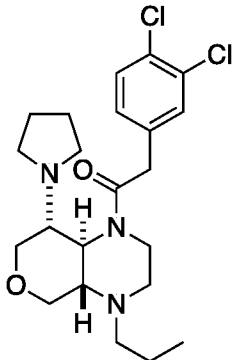
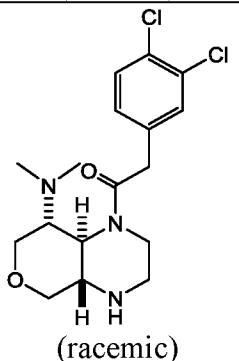
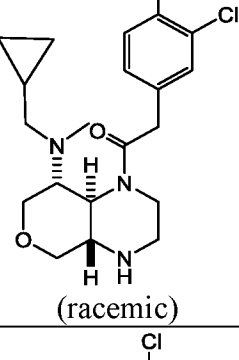
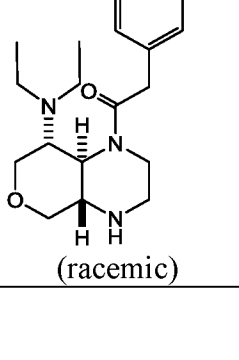
Example	Structure	Binding IC ₅₀ (nM)	GTPγS stimulation		β-arrestin coupling	
			EC ₅₀ (nM)	% efficacy	EC ₅₀ (nM)	% efficacy
5A40	 (racemic)	719	1703	111	No stim	No stim
5A44	 (racemic)	19	6.7	88	6200	55
5A24	 (racemic)	251	213	64	NA	No stim
5A46	 (racemic)	15	6.3	131	NA	100% at 10 μM

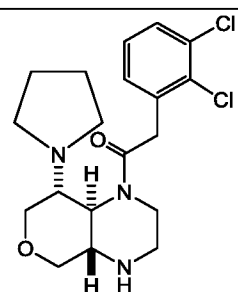
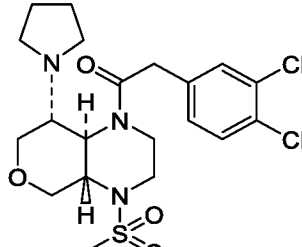
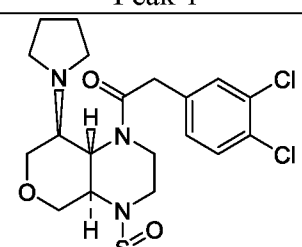
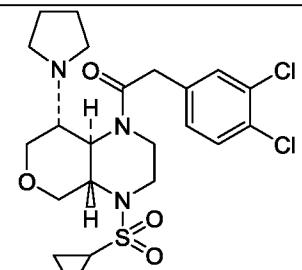
Example	Structure	Binding IC ₅₀ (nM)	GTP γ S stimulation		β -arrestin coupling	
			EC ₅₀ (nM)	% efficacy	EC ₅₀ (nM)	% efficacy
5A47	 (racemic)	31	9.0	84	4900	78
5A49	 (racemic)	102	109	69	11000	36
5A53	 (racemic)	40	12	82	NA	80% at 10 μ M

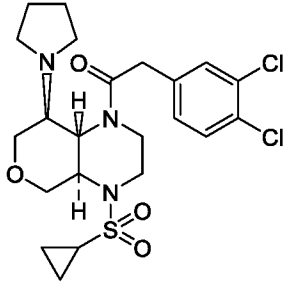
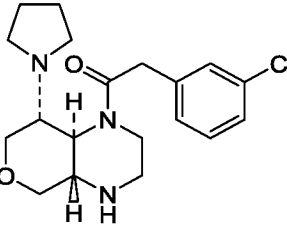
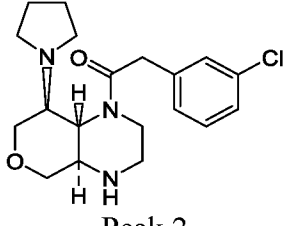
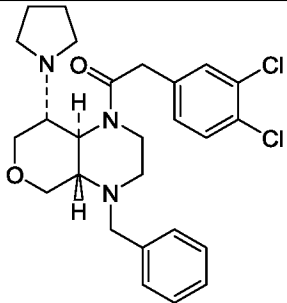
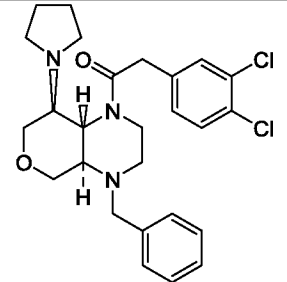
Example	Structure	Binding IC ₅₀ (nM)	GTPγS stimulation		β-arrestin coupling	
			EC ₅₀ (nM)	% efficacy	EC ₅₀ (nM)	% efficacy
5A52	 (racemic)	5.1	3.0	101	3300	79
5A55	 (racemic)	57	21	67	NA	40% at 10 μM
5A54	 (racemic)	73	31	73	NA	20% at 10 μM

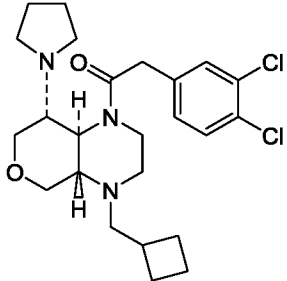
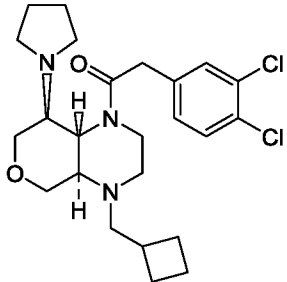
Example	Structure	Binding IC ₅₀ (nM)	GTPγS stimulation		β-arrestin coupling	
			EC ₅₀ (nM)	% efficacy	EC ₅₀ (nM)	% efficacy
5A57	 (racemic)	42	62	98	NA	20% at 10 μM
5A58	 (racemic)	116	261	161	NA	20% at 10 μM
5A65	 (racemic)	68	136	121	NA	20 % at 10 μM
5A66	 (racemic)	456	566	140	NA	20% at 10 μM

Example	Structure	Binding IC ₅₀ (nM)	GTPγS stimulation		β-arrestin coupling	
			EC ₅₀ (nM)	% efficacy	EC ₅₀ (nM)	% efficacy
5A67	 (racemic)	41	12	85	5200	85
5A70	 (racemic)	71	19	90	NA	50% at 10 μM
5A71	 (racemic)	27	14	93	NA	40% at 10 μM

Example	Structure	Binding IC ₅₀ (nM)	GTP γ S stimulation		β -arrestin coupling	
			EC ₅₀ (nM)	% efficacy	EC ₅₀ (nM)	% efficacy
5A72	 (racemic)	26	12	111	NA	50% at 10 μ M
5A51	 (racemic)	120	108	71	NA	No stim
5A50	 (racemic)	297	93	79	NA	No stim
5A64	 (racemic)	1917	2125	68	NA	No stim

Example	Structure	Binding IC ₅₀ (nM)	GTPγS stimulation		β-arrestin coupling	
			EC ₅₀ (nM)	% efficacy	EC ₅₀ (nM)	% efficacy
5A48	 <p>(racemic)</p>	1043	21788	181	NA	No stim
5A44A	 <p>Peak 1</p>	5.4	3.4	119	NA	80 % at 10 μM
5A44B	 <p>Peak 2</p>	18	4.6	105	NA	70 % at 10 μM
5A52A	 <p>Peak 1</p>	3.6	1.7	119	NA	60 % at 10 μM

Example	Structure	Binding IC ₅₀ (nM)	GTPγS stimulation		β-arrestin coupling	
			EC ₅₀ (nM)	% efficacy	EC ₅₀ (nM)	% efficacy
5A52B	 <p>Peak 2</p>	27	18	96	NA	25 % at 10 μM
5A24A	 <p>Peak 1</p>	463	384	115	NA	0 % at 10 μM
5A24B	 <p>Peak 2</p>	1125	1370	102	NA	0 % at 10 μM
5A53A	 <p>Peak 1</p>	108	18	80	NA	80 % at 10 μM
5A53B	 <p>Peak 2</p>	44	4.8	58	NA	80 % at 10 μM

Example	Structure	Binding IC ₅₀ (nM)	GTPγS stimulation		β-arrestin coupling	
			EC ₅₀ (nM)	% efficacy	EC ₅₀ (nM)	% efficacy
5A55A	 Peak 1	94	30	84	NA	No stim
5A55B	 Peak 2	20	13	62	NA	80% at 10 μM

It has been demonstrated that prolactin release from the pituitary is a reliable biomarker of KOP-r agonism across species. Thus, demonstration of the release of prolactin by a compound which is predicted from *in vitro* GTPγS assays in cell lines expressing KOP-r, in a manner blocked by a selective kappa antagonist, indicates an *in vivo* KOP-r agonistic effect. The demonstration of differential maximal efficacy in prolactin release compared to the full unbiased agonist U50488-induced release, coupled with submaximal kappa opioid receptor mediated GTPγS, indicates that the compound has *in vivo* partial agonist KOP-r activity.

[094] In the case of rotarod incoordination, kappa agonist effects in this assay reflect kappa-opioid receptor arrestin mediated signaling. This assay is thought to be a sensitive measure of the sedative properties of kappa opioid receptor agonists. Generally, a compound which has reduced efficacy in the coupling of arrestin with the kappa opioid receptor is thought to have a lowered potential for the sedative side effects of kappa opioid receptor ligands. Rotarod assays *in vivo* are employed to confirm this possibility.

Prolactin

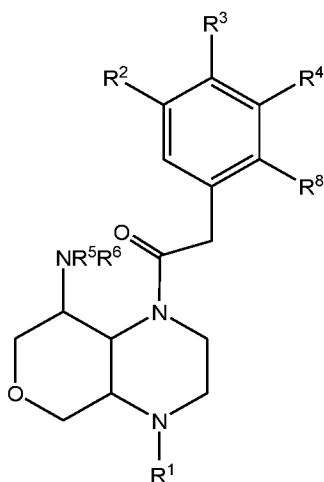
[095] Mice were injected intraperitoneally with the compound to be tested 30 minutes prior to sampling. Trunk blood was collected by rapid decapitation, followed within 2 hours by preparation of serum. Serum prolactin levels were determined using a commercially available enzyme-linked immunoassay (AbCam, Cambridge, UK) following dilution of serum 5-fold in assay buffer. In this screen, compound 5A22 exhibited significant prolactin release at 5 mg/kg, and compound 5A23 exhibited significant prolactin release at 30 mg/kg.

Rotarod

Rotarod experiments were conducted with mice using a dedicated rodent rotarod apparatus, with up to five animals tested concurrently (IITC Life Science, Woodland Hills, CA, USA). Rotarod rotation rate begins at 3 rotations per minute, and ramps to 30 rotations per minute over the course of 300 s, at which time the assay is terminated and animals removed to their home cage. Animals were acclimated to the rotarod on at least two occasions prior to the day of the test. On the day of the test, baseline times for each animal to fall off the rotarod were recorded. Mice were then injected intraperitoneally with vehicle or compound, and rotarod measurements conducted, beginning 0-2 minutes after injection, and then subsequently at select time points thereafter. Animals which failed to remain on the rotarod for at least 150 seconds during baseline testing were removed from the analysis. In this screen, compound 5A22 exhibited no significant incoordination at 10mg/kg, but significant incoordination at 30 and 90 mg/kg, and compound 5A23 also exhibited no significant incoordination at 10 mg/kg but statistically significant incoordination at 30 and 90 mg/kg.

CLAIMS

1. A compound of Formula I



wherein

R^1 is chosen from hydrogen, (C_1-C_{10}) hydrocarbyl, $-C(=O)(C_1-C_{10})$ hydrocarbyl, $-C(=O)O(C_1-C_{10})$ hydrocarbyl and $-SO_2(C_1-C_{10})$ hydrocarbyl;

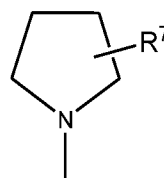
R^2 , R^3 , R^4 , and R^8 are chosen independently from hydrogen, halogen, (C_1-C_4) alkyl, fluoro (C_1-C_4) alkyl, nitro, $-SO_3H$ and $-N^+HR^5R^6$; with the provisos that (1) at least one of R^2 , R^3 , R^4 , and R^8 must be other than hydrogen; and (2) when R^2 or R^4 is fluorine, at least one of the remaining substituents on phenyl must be other than hydrogen; and


R^5 and R^6 are chosen from (C_1-C_{10}) hydrocarbyl, optionally substituted with fluoro, or, taken together with the nitrogen to which they are attached, R^5 and R^6 form a five-, six- or seven-membered non-aromatic heterocycle, which may be optionally substituted with fluoro or (C_1-C_4) alkyl.

2. A compound according to claim 1 wherein R^8 is hydrogen and one of R^2 , R^3 , and R^4 is hydrogen and the remaining two are chosen from hydrogen, halogen, fluoro (C_1-C_4) alkyl, and (C_1-C_3) alkyl.

3. A compound according to claim 2 wherein two of R^2 , R^3 , and R^4 are hydrogen and the remaining one is chosen from chloro, fluoro, trifluoromethyl, and methyl.

4. A compound according to claim 2 wherein R^1 is hydrogen.
5. A compound according to claim 2 wherein R^1 is chosen from (C₁-C₇)hydrocarbyl and -SO₂(C₁-C₈)hydrocarbyl.
6. A compound according to claim 5 wherein R^1 is chosen from (C₁-C₇)alkyl, benzyl, and -SO₂(C₁-C₃)alkyl.
7. A compound according to claim 6 wherein R^1 is -SO₂(C₁-C₃)alkyl.
8. A compound according to claim 5 wherein R^1 is (C₁-C₇)hydrocarbyl.
9. A compound according to claim 1 wherein the ring junction of the octahydro-1H-pyrano[3,4-*b*]pyrazine is *trans* and -NR⁵R⁶ is *cis* to its adjacent hydrogen at the ring junction.



10. A compound according to claim 1 wherein -NR⁵R⁶ is , wherein R⁷ is chosen from hydrogen, fluoro and (C₁-C₃)alkyl.
11. A compound according to claim 1 wherein:
 - R¹ is chosen from hydrogen, (C₁-C₇)hydrocarbyl, -C(=O)O(C₁-C₃)hydrocarbyl and -SO₂(C₁-C₈)hydrocarbyl;
 - R², R³, and R⁴ are chosen independently from hydrogen, halogen, (C₁-C₃)alkyl, and fluoro(C₁-C₃)alkyl;
 - R⁵ and R⁶ are chosen from (C₁-C₆)hydrocarbyl, or, taken together with the nitrogen to which they are attached, R⁵ and R⁶ form a five-, six- or seven-membered non-aromatic heterocycle; and
 - R⁸ is hydrogen.
12. A compound according to claim 11 wherein:

- R¹ is chosen from hydrogen, (C₃-C₄)alkyl, -C(=O)O(C₁-C₃)hydrocarbyl, and -SO₂(C₁-C₈)hydrocarbyl;
- R² is hydrogen;
- R³ and R⁴ are chosen independently from hydrogen, halogen, (C₁-C₃)alkyl, and fluoro(C₁-C₃)alkyl; and
- R⁵ and R⁶, taken together with the nitrogen to which they are attached, form a five-, six- or seven-membered non-aromatic heterocycle.
13. A compound according to claim 11 wherein:
- R¹ is methyl or (C₅-C₇)hydrocarbyl.
14. A compound according to claim 11 wherein R¹ is -CH₂R¹⁰ and R¹⁰ is hydrogen or (C₄-C₆)carbocycle.
15. A compound according to claim 11 wherein R¹ is H, R⁵ is methyl and R⁶ is methyl or cyclopropylmethyl.
16. A compound according to claim 11 wherein:
- R³ is fluoro(C₁-C₃)alkyl when R² and R⁴ are hydrogen.
17. A compound according to claim 11 wherein:
- R⁵ and R⁶ together with nitrogen form a pyrrolidine ring.
18. A method for activating a kappa opioid receptor, comprising contacting a kappa opioid receptor with a compound according to any of claims 1 to 17.
19. A method for treating addiction, comprising administering to a patient a compound according to any of claims 1 to 17.
20. A method according to claim 19 wherein said addiction is an addiction to cocaine.
21. A method according to claim 19 wherein said compound is a compound according to any of claims 14-16.

22. A method for treating a mood disorder, comprising administering to a patient a compound according to any of claims 1 to 17.
23. A method for treating pruritus, comprising administering to a patient a compound according to any of claims 1 to 17.
24. A method for treating pain, comprising administering to a patient a compound according to any of claims 1 to 17.
25. A method for treating inflammation, comprising administering to a patient a compound according to any of claims 1 to 17.
26. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a compound according to any of claims 1 to 17.

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.: 21
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/064422

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/35; A61K 31/495; A61K 31/4985; C07D 241/36; C07D 241/44; C07D 487/04 (2019.01)
 CPC - A61K 31/35; A61K 31/495; A61K 31/4985; C07D 241/36; C07D 241/44; C07D 487/04 (2019.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 514/248; 514/249; 514/253.12 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 8,211,908 B2 (KIYOTO et al) 03 July 2012 (03.07.2012) entire document	1-20, 22-26
A	US 2013/0150340 A1 (SANOFI) 13 June 2013 (13.06.2013) entire document	1-20, 22-26
A	US 2015/0133432 A1 (SANOFI) 14 May 2015 (14.05.2015) entire document	1-20, 22-26

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

10 January 2019

Date of mailing of the international search report

07 FEB 2019

Name and mailing address of the ISA/US

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