Synthesis and Structure−Activity Relationships of 3,8-Diazabicyclo[4.2.0]octane Ligands, Potent Nicotinic Acetylcholine Receptor Agonists

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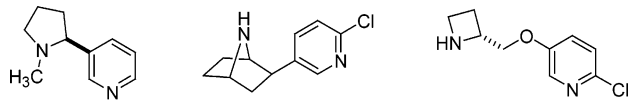
A series of potent neuronal nicotinic acetylcholine receptor (nAChR) ligands based on a 3,8-diazabicyclo-[4.2.0]octane core have been synthesized and evaluated for affinity and agonist efficacy at the human high affinity nicotine recognition site (h$\alpha4\beta2$) and in a rat model of persistent nociceptive pain (formalin model). Numerous analogs in this series exhibit picomolar affinity in radioligand binding assays and nanomolar agonist potency in functional assays, placing them among the most potent nAChR ligands known for the h$\alpha4\beta2$ receptor. Several of the compounds reported in this study (i.e., **24**, **25**, **28**, **30**, **32**, and **47**) exhibit equivalent or greater affinity for the h$\alpha4\beta2$ receptor relative to epibatidine, and like epibatidine, many exhibit robust analgesic efficacy in the rat formalin model of persistent pain.

Introduction

Neuronal nicotinic acetylcholine receptors (nAChRs) have become exciting new targets for medicinal research. One of the most studied areas of interest for this receptor family recently has been that of analgesia, but the nAChRs have also been investigated as potential targets to treat Alzheimer's and Parkinson's diseases, schizophrenia, and depression.[1] The analgesic effects of nicotine (**1**) were first reported in 1932.[2] The much later discovery that the extremely potent antinociceptive activity of epibatidine (**2**), isolated from the skin of a poisonous Ecuadorian tree frog, was the result of the interaction of epibatidine with nAChRs renewed interest in selectively targeting these receptors for the treatment of pain.[3] While epibatidine was found to be an extremely potent analgesic, its poor side effect profile at or near effective analgesic doses (paralysis, seizures, death, etc.) precluded its development for clinical use.[3] These side effects of epibatidine are thought to stem in large part from the activity of epibatidine at the ganglionic and neuromuscular nAChRs.[4] Analogs of epibatidine and nicotine with improved side effect profiles have been sought. One such compound discovered in our labs is ABT-594 (**3**; Figure 1).[5]

Compound **3** is a potent nAChR agonist that is active in a broad range of preclinical models of nociceptive and neuropathic pain.[5,6] Compound **3** is more selective for neuronal nAChRs (vs ganglionic and neuromuscular nAChRs) than is epibatidine and does have an improved therapeutic profile in in vivo models relative to epibatidine. However, **3** exhibits only modest selectivity among the neuronal nAChR subtypes (Table 1).[7a] Generally, nAChR subtype selectivity is thought to be key to improving the therapeutic margin between analgesia and side effects (i.e., gastrointestinal and cardiovascular effects).[8,16] Specifically, it is thought that agonist activity at the $\alpha4\beta2$ receptor subtype, found mainly in the CNS, is responsible for the observed analgesic activity,[9] while activity at the $\alpha3\beta4$ subtype, abundantly expressed in the peripheral nervous system, is the main cause of side effects.[10]



1 (nicotine) **2** (epibatidine) **3** (ABT-594)

Figure 1.

Compound **3** (ABT-594) has been the starting point for a variety of more rigid structural variants making use of an **NCCX** structural motif (bolded in Structure A) where X has been N, O, C, and S (Scheme 1).[11] One such series is the 3,8-diazabicyclo[4.2.0]octane series that includes regioisomers **B** (3-*N* regioisomeric series) and **C** (8-*N* regioisomeric series). The synthesis and pharmacological profile of this series will be discussed herein.

Chemistry

All of the $\alpha4\beta2$ agonists described in this paper were generated by the Buchwald−Hartwig coupling[12] of the appropriate enantiomerically pure diamine with a halopyridine (Scheme 2). The diamine syntheses and the general coupling procedures are discussed below.

The synthesis of the chiral diamines **4** and **5** and their enantiomers (**6** and **7**, respectively) began as shown in Scheme 3. The commercially available ethyl 1-benzyl-3-oxo-4-piperidine carboxylate hydrochloride (**8**) was first converted into the corresponding *tert*-butyl carbamate **9** via removal of the *N*-benzyl protecting group, followed by reaction of the resulting free amine with di-*tert*-butyl dicarbonate. A solution of **9** and (*R*)-α-methylbenzylamine in toluene was refluxed under a Dean−Stark trap to generate the enamine **10**. Reduction of the double bond with sodium triacetoxyborohydride[13] yielded an approximately 1.5:1 mixture of the (3*S*,4*S*)-*cis*-isomer, **11**, and the (3*R*,4*R*)-*cis*-isomer, **12**. At this point, the stereochemistry of the products was assumed based on literature precedence for the predominance of the *cis*-isomer. The *cis*-configuration was confirmed by X-ray analysis of the enantiomer of the reduced product, **14** (**ent-14**, see Experimental Section). This mixture

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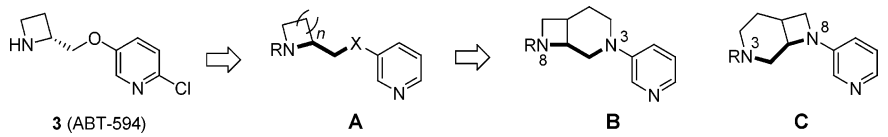
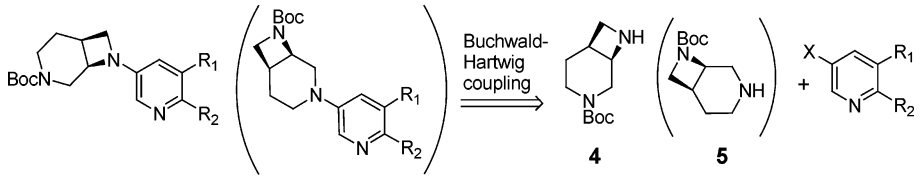
Table 1.

	[^{3}H]-cytisine pK_i ± SEM	[^{3}H]-cytisine K_i (nM)	h$\alpha4\beta2$ EC_{50} (nM) (SEM range)	h$\alpha4\beta2$ max	h$\alpha3\beta4$ EC_{50} (nM) (SEM range)	h$\alpha3\beta4$ max
nicotine (**1**)	9.02 ± 0.07	0.96	6600 (5900−7400)	101 ± 3%	8000 (7400−8600)	89 ± 3%
epibatidine (**2**)	10.38 ± 0.04	0.042	54 (48−61)	133 ± 13%	12 (9.9−14)	103 ± 15%
ABT−594 (**3**)	10.32 ± 0.09	0.048	23 (20−26)	115 ± 3%	188 (160−221)	169 ± 12%

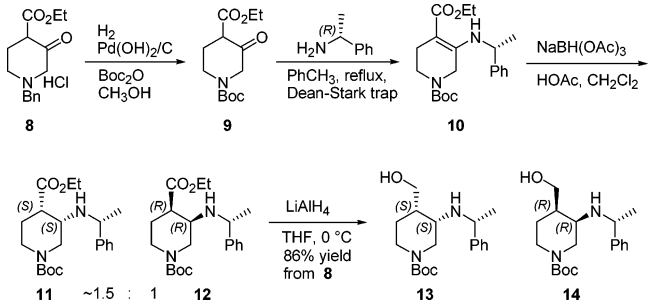
Scheme 1



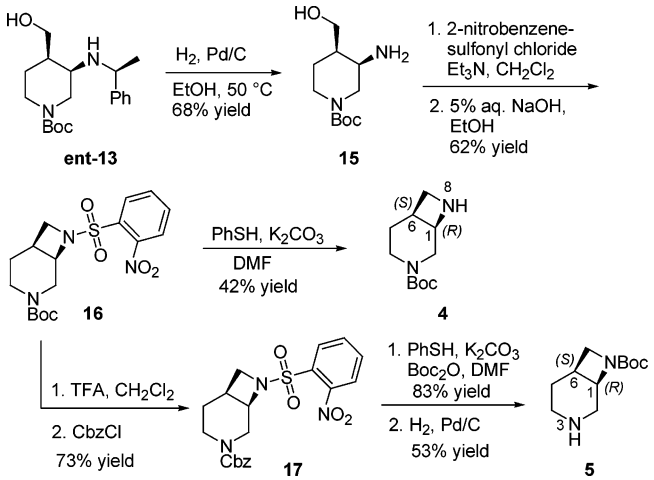
Scheme 2



Scheme 3



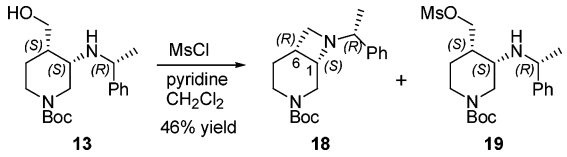
Scheme 4



was then reduced with lithium aluminum hydride to afford the corresponding alcohols, **13** and **14**.

Initially, the two *cis*-isomers, **13** and **14**, were separated by arduous flash column chromatography and were carried on separately. In Scheme 4, this initial route is elaborated using isomer **ent-13**, which was formed as described above, but using (*S*)-α-methylbenzylamine. It should be noted that when (*S*)-α-methylbenzylamine was used, the (3*R*,4*R*)-isomer predominated, whereas use of (*R*)-α-methylbenzylamine resulted in larger amounts of the (3*S*,4*S*)-isomer. As shown in Scheme 4, the α-methylbenzyl group was cleaved via hydrogenolysis to give amine **15**. Reaction with 2-nitrobenzenesulfonyl chloride gave the intermediate bisnosylate, which upon treatment with 5% aqueous NaOH in ethanol, cyclized to sulfonamide **16**.[14] At this point, the nitrobenzenesulfonyl group was removed with thiophenol and K_2CO_3[14] to give diamine core **4**.

Scheme 5



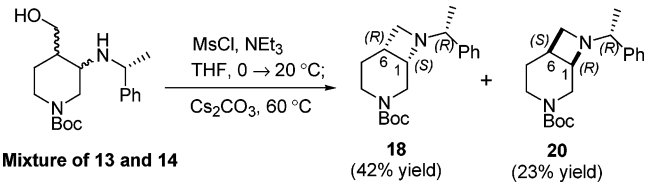
Alternatively, to obtain isomer **5**, which allowed coupling at N3, the *t*-butylcarbamate was cleaved, and the resulting amine was protected as the benzyl carbamate **17**. The *N*-nitrobenzenesulfonyl group was then removed[14] to give the free amine, which was reprotected in situ to give the corresponding *t*-butylcarbamate. Hydrogenation then resulted in the free amine isomer **5**.

While this route was effective in generating initial quantities of core diamines **4** and **5**, as well as their enantiomers (**6** and **7**), the separation of diastereomers **13** and **14** (and **ent-13** and **ent-14**) was difficult, and the protecting group manipulations were cumbersome. It was thought that if alcohols **13** and **14** could be cyclized directly, prior to removal of the chiral auxiliary, the presence of said auxiliary on the rigid, cyclized product might facilitate separation of the two *cis*-isomers. Furthermore, this route would allow for fewer protecting group manipulations.

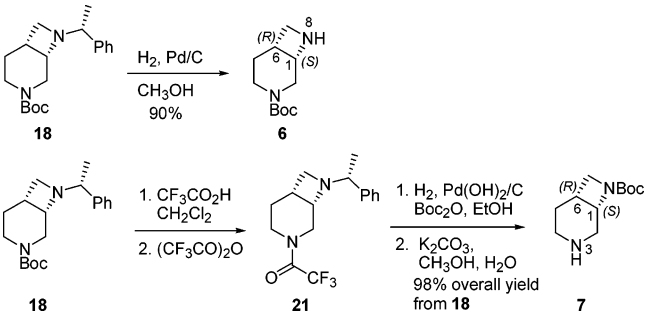
Three primary questions needed to be addressed with the proposed route. First, could the alcohol oxygen be selectively activated in the presence of the basic, acyclic nitrogen? Second, if the selective *O*-activation was possible, would the resulting intermediate cyclize in the presence of the large α-methylbenzyl substituent? Finally, would this route facilitate the separation of the two *cis*-isomers?

To address the first question, single isomer **13** was treated with methanesulfonyl chloride and pyridine in CH_2Cl_2 (Scheme 5). Mesylation occurred exclusively on oxygen, and furthermore, attempted purification of the monomesylate via silica gel chromatography resulted in isolation of both the mesylate **19** and the cyclized product **18**. Ultimately, it was found that the cyclized product **18** could be obtained directly by addition of Cs_2CO_3 to the mesylation reaction mixture.

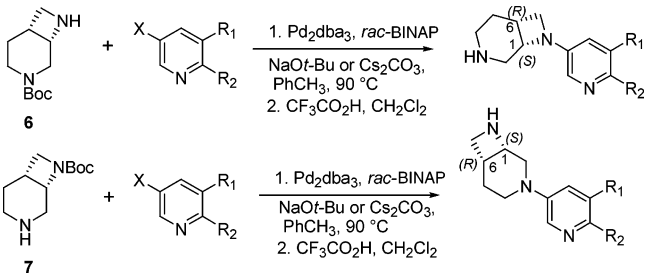
Scheme 6



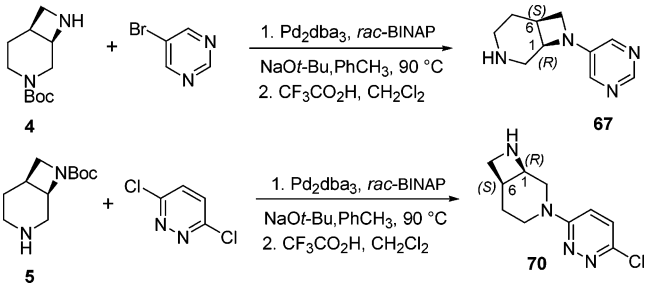
Scheme 7



Scheme 8



Scheme 9



As it had now been demonstrated that both selective activation and the subsequent cyclization were favorable, the only remaining question was that of improving the separation of the diastereomers. To that end, a mixture of isomers **13** and **14** was treated with methanesulfonyl chloride and Et_3N in tetrahydrofuran (Scheme 6). After the mixture had stirred for 1 h at ambient temperature, Cs_2CO_3 was added and the temperature was raised to 60 °C. After stirring at 60 °C for 18 h, it was found that the two products **18** and **20** had readily formed and that they were easily separable via flash column chromatography. The individual isomers such as **18** could then be converted to either the 3-*N* accessible (**7**) or the 8-*N* accessible cores (**6**), as shown in Scheme 7. Diamine **6** was obtained directly by hydrogenolysis of **18**, while **7** was isolated after a protecting group shuffle to give the trifluoroacetamide **21** followed by hydrogenation, protection of the resulting free amine as the *t*-butylcarbamate, and removal of the trifluoroacetyl group. Thus, this new route allowed the more efficient generation of large quantities of the diamine cores (**4**−**7**) than was possible with the initial synthesis.

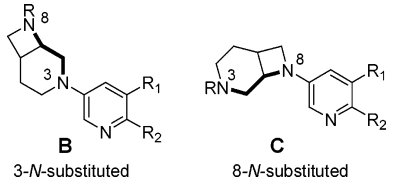


Figure 2.

As discussed above, the diamine cores were coupled to numerous halopyridines via the Buchwald−Hartwig coupling (Scheme 8).[12] Coupling of the diamine core was also carried out using 5-bromopyrimidine and 3,6-dichloropyridazine (Scheme 9).

Results and Discussion

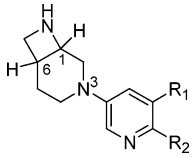
Two main structural series were investigated and are described herein: that in which the pyridine is attached to the diamine at the 8-position (8-*N*-substituted; structure C, Figure 2) and that in which the pyridine is attached to the diamine at the 3-position (3-*N*-substituted; structure B, Figure 2). All compounds shown in Tables 2−4 were evaluated for affinity to the $\alpha4\beta2$ binding site in the cytisine binding assay and for functional activity at the h$\alpha4\beta2$ and h$\alpha3\beta4$ subtypes in the FLIPR cellular assays. Selected compounds were evaluated for analgesic activity in phase 2 of the formalin flinch assay.

Background SAR. The structure−activity relationship (SAR) of the 3,8-diazabicyclo[4.2.0]octane series was primarily explored by varying the small substituents on the 5 and 6 positions of the pyridine ring (R_1 and R_2 in structures B and C, respectively). Previous work by ourselves and others had suggested that small groups at these positions of the pyridine were optimal for $\alpha4\beta2$ ligands.[15,16] Larger groups at the 5-position led to ligands with high affinity but reduced functional activity.[16,17] Ligands with large (i.e., an aromatic group) substitution at the 6-position have reduced cytisine binding and $\alpha4\beta2$ functional activity.[15] Substitutions at the 2 or 4 positions generally resulted in reduced binding and functional activities.[15,16,18] Attachment of the diamine moiety at the 3-pyridyl position has also been found to be optimal.[19] In general, the pyridine ring was preferred, but the 5-pyrimidinyl and 3-pyridazinyl were also investigated in this and other series.[16,20] Finally, in most cases studied, an unsubstituted nitrogen on the diamine moiety (R═H) was required for activity at the $\alpha4\beta2$ receptor.[16,21]

SAR of the 3,8-Diazabicyclo[4.2.0]octane Ligands. As a group, the 3,8-diazabicyclo[4.2.0]octane ligands are among the most potent and efficacious compounds reported for the nAChRs. Several compounds in this series show nanomolar potency in the h$\alpha4\beta2$ functional assay (i.e., **28**, **30**, **32**, and **47**) and most have subnanomolar potency in the cytisine binding assay. Many of these ligands exhibit supramaximal efficacy in the functional assays with responses on the order of 200% that of the maximal nicotine response. Several compounds (i.e., **24**, **25**, **28**, **30**, **32**, and **47**) reported here are as potent or more potent than epibatidine (**2**) in these assays. The specific effects on in vitro and in vivo activity with changes in pyridine substitution as well as diamine regio- and stereoselectivity are discussed below.

The most potent compounds reported in this study are from the 3-*N*-substituted isomeric series (Table 2). For example, the (1*R*,6*S*)-5,6-dibromo analog, **30**, has a K_i of 0.014 nM in the rat cytisine binding assay and EC_{50} values of 7.8 nM (209%) and 8.2 nM (117%) in the h$\alpha4\beta2$ and h$\alpha3\beta4$ functional assays, respectively. As shown in Table 3, substrates in the 8-*N*-

Table 2. In Vitro Biological Activity of 3-*N*-Substituted nAChRs

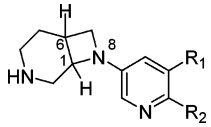


cmpd	stereoisomer	R_1	R_2	[^{3}H]-cytisine pK_i ± SEM	[^{3}H]-cytisine K_i (nM)	hα4β2 EC_{50} (nM) (SEM range)	hα4β2 max	hα3β4 EC_{50} (nM) (SEM range)	hα3β4 max
22	1*R*,6*S*	H	H	10.46 ± 0.08	0.035	71 (61−82)	171 ± 8%	48 (44−52)	121 ± 4%
23	1*S*,6*R*	H	H	10.23 ± 0.07	0.059	330 (300−370)	112 ± 5%	260 (250−280)	118 ± 2%
24	1*R*,6*S*	H	Cl	10.72 ± 0.08	0.019	13 (11−15)	114 ± 2%	29 (26−32)	151 ± 10%
25	1*S*,6*R*	H	Cl	10.50 ± 0.08	0.032	24 (22−26)	130 ± 2%	25 (24−27)	125 ± 2%
26	1*R*,6*S*	Br	H	10.61 ± 0.05	0.024	150 (130−170)	148 ± 7%	76 (70−82)	122 ± 5%
27	1*S*,6*R*	Br	H	10.28 ± 0.03	0.053	1000 (850−1200)	58 ± 2%	950 (910−990)	91 ± 4%
28	1*R*,6*S*	Cl	Cl	10.61 ± 0.06	0.025	12 (9.7−14)	221 ± 15%	9.9 (8.3−12)	132 ± 7%
29	1*S*,6*R*	Cl	Cl	10.57 ± 0.04	0.027	82 (67−100)	79 ± 5%	110 (100−120)	111 ± 6%
30	1*R*,6*S*	Br	Br	10.86 ± 0.03	0.014	7.8 (6.1−9.9)	209 ± 14%	8.2 (7.4−9.1)	117 ± 2%
31	1*S*,6*R*	Br	Br	10.62 ± 0.08	0.024	76 (67−87)	86 ± 3%	110 (100−120)	100 ± 3%
32	1*R*,6*S*	CH_3	Cl	10.51 ± 0.03	0.031	7.2 (5.4−9.6)	207 ± 15%	7.2 (6.5−7.9)	123 ± 2%
33	1*S*,6*R*	CH_3	Cl	10.44 ± 0.07	0.037	47 (38−58)	90 ± 6%	71 (68−74)	103 ± 3%
34	1*R*,6*S*	CN	H	9.92 ± 0.12	0.12	1900 (1200−2900)	112 ± 6%	1000 (590−1700)	127 ± 8%
35	1*S*,6*R*	CN	H	10.19 ± 0.08	0.065	1900 (1500−2400)	76 ± 6%	490 (460−520)	116 ± 2%
36	1*R*,6*S*	OMe	H	10.42 ± 0.11	0.038	130 (120−160)	178 ± 17%	180 (170−190)	103 ± 3%
37	1*S*,6*R*	OMe	H	9.74 ± 0.04	0.18	2700 (2000−3600)	35 ± 4%	4500 (4400−4700)	86 ± 2%
38	1*R*,6*S*	H	OMe	9.01 ± 0.08	0.98	2200 (1900−2600)	96 ± 6%	1100 (1040−1140)	88 ± 4%
39	1*S*,6*R*	H	OMe	8.57 ± 0.058	2.7	4980 (4330−5730)	54 ± 1%	2400 (2300−2450)	93 ± 2%
40	1*R*,6*S*	OEt	H	10.28 ± 0.06	0.052	620 (570−680)	116 ± 3%	124 (117−131)	103 ± 1%
41	1*S*,6*R*	OEt	H	9.80 ± 0.01	0.16	1350 (300−6200)	18 ± 4%	2800 (2700−2900)	86 ± 2%
42	1*S*,6*R*	CH_3	H	10.19 ± 0.07	0.064	650 (500−830)	54 ± 4%	1400 (1300−1500)	87 ± 2%
43	1*R*,6*S*	H	NO_2	8.83 ± 0.07	1.5	100 (94−110)	196 ± 7%	390 (360−410)	119 ± 3%
44	1*S*,6*R*	H	NO_2	8.61 ± 0.08	2.4	110 (94−120)	114 ± 6%	830 (780−880)	98 ± 4%
45	1*R*,6*S*	OMe	Br	10.57 ± 0.02	0.027	21 (18−25)	217 ± 12%	8.0 (7.3−8.8)	120 ± 2%
46	1*S*,6*R*	OMe	Br	10.16 ± 0.04	0.069	360 (260−480)	40 ± 3%	160 (150−180)	108 ± 3%
47	1*R*,6*S*	CN	Br	10.57 ± 0.05	0.027	6.1 (5.0−7.5)	215 ± 19%	4.4 (3.5−5.6)	133 ± 3%
48	1*S*,6*R*		H	10.21 ± 0.07	0.062	180 (160−210)	194 ± 19%	5700 (5300−6100)	99 ± 3%

substituted series are also relatively potent but generally have lower affinities at the tested receptors than the 3-*N*-substituted regioisomers. In all cases where direct comparisons between the two regioisomeric series can be made (i.e., **22** vs **49**, **25** vs **52**, etc.), the 3-*N*-substituted regioisomers are the more potent in hα4β2 binding and functional assays.

Within the 3-*N*-substituted regioisomeric series (Table 3), there is a pronounced stereochemical effect, with the 1*R*,6*S* absolute stereochemistry generally imparting higher binding affinity and greater functional potency than the 1*S*,6*R* enantiomers, as exemplified by compounds **22** and **23**, **30** and **31**, and **36** and **37**. In addition to greater potency, compounds with the 1*R*,6*S* stereochemistry are characterized by significantly greater agonist efficacy in the hα4β2 functional assay, with many analogs exhibiting 200% of the maximal response elicited by nicotine (see **28**, **30**, **32**, **43**, **47**). By contrast, compounds of the 1*S*,6*R* stereochemistry generally exhibit maximal agonist efficacies comparable to nicotine.

Table 3. In Vitro Biological Activity of 8-*N*-Substituted nAChRs



cmpd	stereoisomer	R_1	R_2	[^{3}H]-cytisine pK_i ± SEM	[^{3}H]-cytisine K_i (nM)	h$\alpha4\beta2$ EC_{50} (nM) (SEM range)	h$\alpha4\beta2$ max	h$\alpha3\beta4$ EC_{50} (nM) (SEM range)	h$\alpha3\beta4$ max
49	1*R*,6*S*	H	H	9.91 ± 0.10	0.12	410 (380−440)	105 ± 2%	1700 (1600−1700)	98 ± 4%
50	1*S*,6*R*	H	H	8.95 ± 0.02	1.1	1400 (1300−1500)	84 ± 3%	1540 (1460−1630)	97 ± 3%
51	1*R*,6*S*	H	Cl	9.92 ± 0.08	0.12	37.4 (33.6−41.5)	94 ± 6%	1390 (1270−1530)	129 ± 3%
52	1*S*,6*R*	H	Cl	9.10 ± 0.04	0.80	216 (181−258)	102 ± 6%	1520 (1290−1790)	93 ± 9%
53	1*R*,6*S*	Cl	Cl	9.93 ± 0.08	0.12	271 (252−291)	60 ± 2%	4110 (3930−4290)	69 ± 2%
54	1*S*,6*R*	Cl	Cl	9.52 ± 0.06	0.3	176 (164−190)	127 ± 3%	339 (316−364)	103 ± 2%
55	1*R*,6*S*	CH_3	Cl	10.37 ± 0.08	0.043	76.1 (67.3−86.0)	103 ± 6%	1910 (1790−2030)	91 ± 3%
56	1*S*,6*R*	CH_3	Cl	9.62 ± 0.03	0.24	134 (116−156)	142 ± 9%	298 (276−322)	103 ± 4%
57	1*R*,6*S*	OMe	Br	8.85 ± 0.16	1.4	1690 (1500−1900)	65 ± 4%	2980 (2810−3150)	84 ± 3%
58	1*S*,6*R*	OMe	Br	10.07 ± 0.060	0.085	200 (187−214)	120 ± 2%	202 (184−220)	98 ± 4%
59	1*R*,6*S*	CN	H	9.30 ± 0.05	0.50	2190 (1890−2540)	64 ± 4%	2560 (2180−3000)	91 ± 8%
60	1*S*,6*R*	CN	H	8.58 ± 0.15	2.6	1930 (1590−2350)	83 ± 3%	5560 (5220−5920)	71 ± 4%
61	1*R*,6*S*	CN	Br	10.15 ± 0.01	0.070	109 (96.2−124)	104 ± 4%	794 (757−832)	88 ± 2%
62	1*S*,6*R*	CN	Br	9.52 ± 0.01	0.3	172 (155−192)	157 ± 5%	238 (226−252)	100 ± 3%
63	1*R*,6*S*	$C(O)NH_2$	Br	8.55 ± 0.04	2.8	1490 (1320−1680)	75 ± 4%	23300 (22500−24200)	73 ± 2%
64	1*S*,6*R*	$C(O)NH_2$	Br	8.08 ± 0.02	8.4	340 (317−365)	133 ± 6%	7460 (7170−7770)	90 ± 1%
65	1*R*,6*S*	OMe	H	8.36 ± 0.08	4.4	7690 (6010−9580)	44 ± 5%	63500 (47400−85200)	47 ± 1%
66	1*S*,6*R*	OMe	H	9.25 ± 0.02	0.56	1105 (890−1370)	104 ± 4%	4360 (4140−4580)	88 ± 2%

In the 8-*N*-substituted series (Table 4), many of the compounds investigated showed little difference in affinity between the 1*R*,6*S* and 1*S*,6*R* isomers. However, in the cases of the most potent compounds, which includes the unsubstituted compounds (**49** vs **50**) and many 6-halogenated compounds (**51** vs **52**, **55** vs **56**, and **61** vs **62**), the 1*R*,6*S* isomers exhibited higher affinities than their 1*S*,6*R* counterparts. Of the compounds investigated, only the 1*S*,6*R* isomers with 5-methoxy groups showed increased affinity relative to the corresponding 1*R*,6*S* isomers (**57** vs **58** and **65** vs **66**).

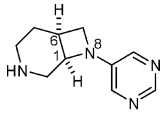
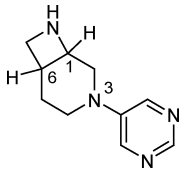
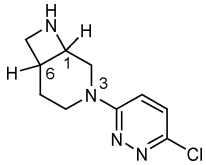
For the sake of clarity, the discussion of the effects of pyridine substitution will be limited to the most potent stereochemical and regiochemical series, specifically, the (1*R*,6*S*)-3-*N*-substituted series. The SAR trends exhibited in this series are generally consistent among the other regio- and stereochemical series.

As discussed above, previous work had suggested that for $\alpha4\beta2$ nAChR activity, small substituents on the 5 and 6 positions of the pyridine ring (R_1 and R_2, respectively) were optimal and larger substitutions at the 2 or 4 positions were detrimental.[15−17] The unsubstituted pyridine, **22**, is a very potent compound at both $\alpha4\beta2$ (K_i = 0.035 nM, EC_{50} = 70.7 nM) and $\alpha3\beta4$ (EC_{50} = 47.9 nM). However, as has been previously reported, halogens in the 6-pyridyl position lead to increased potency at nAChRs,[16] and this trend was found to be true in the case of the 3,8-diazabicyclo[4.2.0]octane ligands as well. The analogs with 6-halogen substitution (**24**, **28**, **30**, **32**, **45**, and **47**) all exhibit subnanomolar binding affinities and low nanomolar potencies in the functional assays. None of these compounds is selective for $\alpha4\beta2$ over $\alpha3\beta4$ nAChRs, with the exception of **24**, which shows only a 2-fold preference for $\alpha4\beta2$ over $\alpha3\beta4$. Other substitutions in the 6-pyridyl position include the methoxy (**38**) and nitro (**43**) moieties, both of which lead to decreased activity in the binding and functional assays relative to **22**.

Ligand **26** is the only case in the stereo- and regiochemical series being discussed that has 5-halo substitution without accompanying 6-halo substitution. Although the binding affinities are comparable between 5-H derivative **22** and the 5-bromo derivative **26**, compound **22** is a more potent agonist in the $\alpha4\beta2$ and $\alpha3\beta4$ nAChR functional assays. A comparison of the 5-H, 6-Cl ligand **24** with **28** and **30**, the 5,6-diCl and 5,6-diBr ligands, respectively, demonstrates that the presence of halogens in the 5 and 6 positions of the pyridine is not detrimental to $\alpha4\beta2$ or $\alpha3\beta4$ activity.

Substitution at the 5-pyridyl position without a concomitant 6-halo group (**34**, **36**, **40**) led to reduced $\alpha4\beta2$ and $\alpha3\beta4$ activities relative to the 5,6-unsubstituted ligand, **22**. However, with 6-halo substitution, several groups were well tolerated in the 5-pyridyl position (**32**, **45**, **47**), with $\alpha4\beta2$ and $\alpha3\beta4$ activities equal to or better than the unsubstituted ligand, **22**. Overall, substitutions on the pyridine ring could have a large

Table 4. In Vitro Biological Activity of Pyrimidine and Pyridazine nAChRs

cmpd	stereoisomer	[^{3}H]-cytisine pK_i ± SEM	[^{3}H]-cytisine K_i (nM)	hα4β2 EC_{50} (nM) (SEM range)	hα4β2 max	hα3β4 EC_{50} (nM) (SEM range)	hα3β4 max
67		8.81 ± 0.07	1.6	8420 (7440−9530)	104 ± 4%	9940 (9360−10600)	93 ± 2%
	(1*S*,6*R*) 						
68	1*R*,6*S*	10.39 ± 0.05	0.040	350 (293−418)	181 ± 10%	108 (102−114)	121 ± 2%
69	1*S*,6*R*	9.71 ± 0.07	0.20	4390 (3620−5330)	79 ± 3%	1020 (984−1070)	106 ± 2%
							
70	1*R*,6*S*	9.83 ± 0.06	0.15	78.3 (65.8−93.1)	222 ± 17%	64.8 (57.6−73.0)	128 ± 6%
71	1*S*,6*R*	8.57 ± 0.18	2.7	359 (311−414)	129 ± 10%	394 (369−420)	113 ± 4%

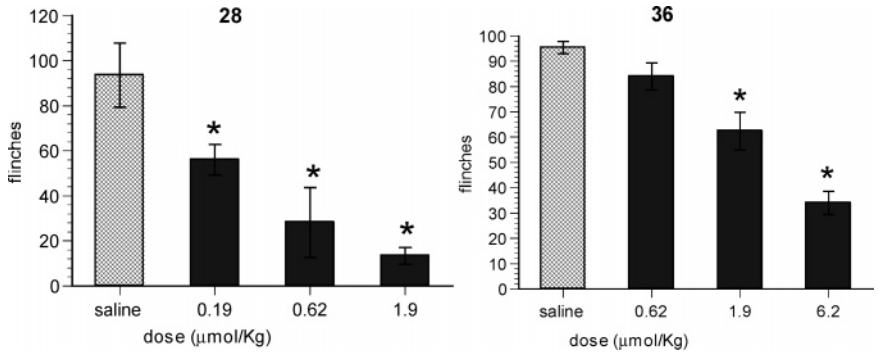


Figure 3. Dose responses of **28** and **36** in the rat formalin model. *The asterisks denote statistical significance.

effect on potencies in the in vitro assays but, in this series, they do not appear to have a substantial effect on selectivity between α4β2 and α3β4 subtypes. These same general trends are also apparent in the (1*S*,6*R*)-3-*N*-substituted series and the 8-*N*-substituted series.

As shown in Table 4, heterocycles other than pyridine were also briefly investigated. The two 3-*N*-substituted isomers (**68** and **69**) and one 8-*N*-substituted isomer (**67**) of the 3,8-diazabicyclo[4.2.0]octane pyrimidines studied had reduced potency in the functional assays relative to their pyridine analogs (**67** vs **50, 68** vs **22**, and **69** vs **23**). These compounds were also not subtype-selective (hα4β2 vs hα3β4) nor were they active in the rat formalin screen. Because of these results, the pyrimidines were not investigated further. The two stereoisomers of the 3-*N*-substituted diazabicyclo[4.2.0]octane-6-chloropyridazine (**70** and **71**) were made. In vitro, these two compounds were less potent than their 6-chloropyridine analogs, **24** and **25**. The (1*R*,6*S*)-isomer, **70**, was still relatively potent and did show some activity in vivo, but overall, offered no advantages over the pyridine analogs.

In Vivo Results. The 3-*N*-substituted series exhibits a good correlation between activity in the in vitro assays and activity in the rat formalin model, although it should be noted that while our functional assays use the human α4β2 and α3β4 receptors, our in vivo studies were performed in rats. For example, the 1*R*,6*S* enantiomer **28** has good activity in the α4β2 FLIPR assay (EC_{50} = 11.8 nM, 221% response) and, as shown in Figure 3, has a good dose−response curve in the formalin model while its enantiomer **29**, which was weaker in the in vitro assays (α4β2 EC_{50} = 82.1 nM, 79% response) exhibited only modest activity in the in vivo model (55% reduction in flinches at 19 μmol/Kg, data not shown). The same is true for ligands **30** (99% reduction in flinches at 6.2 μmol/Kg) and **31** (26% reduction in flinches at 19 μmol/Kg), as well as **34** (80% reduction in flinches at 6.2 μmol/Kg) and **35** (no reduction in flinches at 19 μmol/Kg). The relatively less potent analog **36** (α4β2 EC_{50} = 134 nM, 178% response) also elicited a good dose−response in the formalin model (Figure 3), while its enantiomer **37** (α4β2 EC_{50} = 2670 nM, 35% response) was inactive in the in vivo model.

Overall, in the 8-*N*-substituted series there appears to be less of a correlation between in vitro and in vivo results. The (1*R*,6*S*)-6-chloro analog **51** is more potent in the α4β2 FLIPR assay (EC_{50} = 37.4 nM, 94% response) than its enantiomer **52** (α4β2

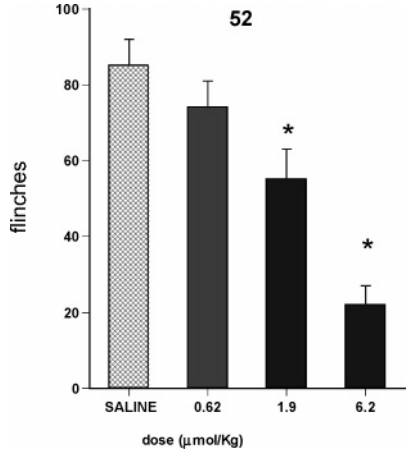


Figure 4. Dose response of **52** in the rat formalin model. *The asterisks denote statistical significance.

EC_{50} = 216 nM, 102% response). Likewise, **51** exhibits 50% reduction in flinches in the rat formalin model at low doses (0.19 μmol/Kg), while **52** is less potent (Figure 4). In contrast, although **55** is more potent at $\alpha4\beta2$ (EC_{50} = 76.1 nM, 103% response) than its enantiomer **56** ($\alpha4\beta2$ EC_{50} = 134 nM, 142% response), **55** is inactive in the rat formalin model, while **56** causes a 75% reduction in the number of flinches at 19 μmol/Kg (data not shown). Overall, the 8-*N*-substituted series is not as active in the in vivo model as the 3-*N*-substituted series, which is consistent with the reduced in vitro activity of the 8-*N*-substituted series relative to the 3-*N*-substituted series.

It should be noted that while many of the tested compounds were shown to be active in the rat formalin model, most active compounds also exhibited side effects, which included prostration, seizures, ataxia, and dyspnea. While the behavioral side effects of some compounds likely impacted the ability of the animals to flinch, they cannot solely account for the observed analgesic effects. For example, multiple compounds showed mild and/or transient side effects that were not apparent at the time of analgesia testing, while others continued to show analgesic efficacy when tested at lower doses that did not induce behavioral side effects (i.e., **30**, **33**, **36**, and **42**). Interestingly, the opposite could also be demonstrated, that is, administration of several of the reported compounds resulted in side effects without any accompanying analgesia at the tested doses (maximum of 19 μmol/Kg; i.e., **44**, **49**, **53**, **55**, **61**, and **71**). Thus, analgesic effects and behavioral side effects were dissociable for these analogs, as has been previously described for epibatidine, which also exhibits significant side effects.[22]

As discussed above, many of the nAChR agonists prepared on this diazabicyclooctane scaffold are extremely potent, and many are active in the rat formalin in vivo screen. Several compounds (i.e., **24**, **25**, **28**, **30**, **32**, and **47**) had equal or higher binding affinity and $\alpha4\beta2$ functional potency than does epibatidine, **2**. It is thought that agonist activity at the $\alpha4\beta2$ receptor subtype is primarily responsible for analgesic activity,[9] while activation of the $\alpha3\beta4$ subtype leads to side effect liabilities.[10] If this is true, the likely conclusions that can be drawn from these results are either that a further improvement of h$\alpha4\beta2$ versus h$\alpha3\beta4$ functional selectivity is required, or that the h$\alpha3\beta4$ subtype is not the sole cause of side effects in the in vivo screen. Also, it should be noted that while our functional assays use the human $\alpha4\beta2$ and $\alpha3\beta4$ receptors, our in vivo studies were performed in rats. Finally, the required level of selectivity necessary to eliminate all in vivo side effects is not currently known.

Experimental Section

Biological Assays. Rat Cytisine Binding Assay. Binding to a desensitized state of nAChRs (predominantly $\alpha4\beta2$) was evaluated by measuring displacement of [^{3}H]-cytisine from rat brain homogenate ($n \geq 3$).[23]

Functional Assays. HEK cell lines expressing the $\alpha4\beta2$ and $\alpha3\beta4$ subunit combinations were used in the determination of functional nAChR agonist activity by measuring intracellular calcium changes using the fluorometric imaging plate reader (FLIPR) system (Molecular Devices, Sunnydale, CA). The cell line was obtained from NeuroSearch (Ballerup, Denmark). Cells were plated at densities of 25000−50000 cells/well in DMEM (GIBCO), supplemented with 10% FBS (GIBCO) in 96-well, clear-bottom, black-walled plates (Corning Costar) manually precoated with poly-D-lysine (Sigma, 75 μL/well of 0.01 g/L solution ≥ 30 min) and allowed to incubate for 24−48 h at 37 °C in 5% CO_2 in a humidified environment. After aspirating off the media, the cell lines were incubated in the dark at room temperature for ∼0.75−1 h with 2−4 μM Fluo-4 AM calcium indicator dye (Molecular Probes, Eugene, OR) dissolved in 0.1 to 0.2% v/v of DMSO (Sigma, U.K.) in NMDG ringer buffer (in mM: 140 NMDG, 5 KCl, 1 $MgCl_2$, 10 HEPES, 10 $CaCl_2$, pH = 7.4). Cells were placed in the FLIPR and 50 μL of 3× stock concentrations of test compounds or buffer only prepared in the same NMDG ringer buffer were added. Raw fluorescence data were corrected by subtracting fluorescence values from wells that received buffer only additions. Peak fluorescent values were determined over the range of drug exposure using FLIPR software and expressed as a percentage of the reference peak response for the positive control of 100 μM nicotine and exported for analysis using Microsoft Excel and GraphPad Prism (San Diego, CA). Data were fitted using a single sigmoidal function in GraphPad determining EC_{50} and maximum responses and expressed as means ± SEM(n), SEM is the standard error of the means and an $n = 6$ constitutes two replicates per plate across three plates.

Rat Formalin Model of Persistent Pain. Following a 30 min habituation period to the testing room and cages, rats were injected i.p. (1 mL/kg) with either the test compound or its vehicle control. Five minutes later, 50 μL of a 5% formalin solution was injected subcutaneously into the dorsal aspect of one of the hind paws. Immediately after formalin injection, the cages were placed on a suspended rack with mirrors positioned below permitting the experimenter to observe the rat from all angles. From 30 to 50 min after the injection of formalin, there is a marked increased in nocifensive behaviors such as flinching, licking, and biting of the injected paw, an increase which has been termed phase 2 or the persistent pain phase of the model. During this 20 min period, rats were observed for the occurrence of nocifensive behaviors. Four rats were run simultaneously, and the experimenter observed each rat for one 15 s observation period during each 1 min interval throughout the 20 min of phase 2. The nocifensive responses were recorded and summed for statistical analyses.[8]

Chemistry. Proton NMR spectra were obtained on a General Electric QE 300 or QZ 300 MHz instrument with chemical shifts (δ) reported relative to tetramethylsilane as an internal standard. Elemental analyses were performed by Robertson Microlit Laboratories. Column chromatography was carried out on silica gel 60 (230−400 mesh). Thin-layer chromatography was performed using 250 mm silica gel 60 glass-backed plates with F_{254} as indicator. The X-ray crystal structures were obtained on a Bruker SMART system. All materials were commercially available and were obtained from Aldrich unless otherwise specified.

3-Oxo-piperidine-1,4-dicarboxylic Acid 1-*tert*-Butyl Ester 4-Ethyl Ester (9). A mixture of commercially available ethyl-*N*-benzyl-3-oxo-4-piperidinecarboxylate hydrochloride (**8**; 75.4 g, 0.25 mol), di-*t*-butyl dicarbonate (58.5 g, 0.27 mol), Et_3N (36 mL, 0.26 mol), and $Pd(OH)_2/C$ (7.5 g, 50% in H_2O) in 660 mL of EtOH was put under 60 psi of H_2 and was shaken for 25 min. The mixture was then filtered, and the filtrate was concentrated under reduced pressure to provide the title compound, which was used in the next

step without further purification. ^{1}H NMR (300 MHz, CD_3OD) δ 1.32 (t, $J = 6.9$ Hz, 3H), 1.48 (s, 9H), 2.29 (m, 3H), 3.5 (t, $J = 5.7$ Hz, 1H), 4.0 (br s, 1H), 4.24 (q, $J = 6.9$ Hz, 2H); MS (DCI/NH_3) m/z 272 $(M + H)^+$, 289 $(M + NH_4)^+$.

5-[(1*R*)-1-Phenyl-ethylamino]-3,6-dihydro-2*H*-pyridine-1,4-dicarboxylic Acid 1-*tert*-Butyl Ester 4-Ethyl Ester (10). Compound **9** (72 g, 0.265 mol), (*R*)-α-methylbenzylamine (35.9 mL, 0.279 mol), and 750 mL of toluene were combined in a 1 L, round-bottom flask equipped with a Dean−Stark trap. The mixture was refluxed for 36 h, with water being removed via the Dean−Stark trap. After cooling to ambient temperature, the solution was concentrated and redissolved in EtOAc. Filtration through silica gel and Celite diatomaceous earth gave the crude title compound (**10**), which was carried on directly to the next reaction. ^{1}H NMR (300 MHz, CD_3OD) δ 1.28 (t, $J = 7.1$ Hz, 3H), 1.47 (m, 9H), 2.29 (m, 1H), 2.32 (s, 3H), 3.03 (m, 1H), 3.25 (m, 1H), 3.47 (m, 1H), 3.63 (m, 1H), 4.02 (m, 1H), 4.16 (q, $J = 7.1$ Hz, 2H), 4.65 (m, 1H), 7.24 (m, 5H); MS (DCI/NH_3) m/z 375 $(M + H)^+$.

(3*S*,4*S*)-1-*t*-Butyl 4-Ethyl (*cis*)-3-{[(1*R*)-1-Phenylethyl]amino}-1,4-piperidinedicarboxylate (11) and (3*R*,4*R*)-1-*t*-Butyl 4-Ethyl (*cis*)-3-{[(1*R*)-1-Phenylethyl]amino}-1,4-piperidinedicarboxylate (12). To a mixture of **10** (0.265 mol), $NaBH(OAc)_3$ (281 g, 1.33 mol), and 200 g of 4 Å powdered molecular sieves in 900 mL of toluene in a 3-neck round-bottom flask equipped with an internal thermometer, mechanical stirrer, and addition funnel at 0 °C was added acetic acid (303 mL, 5.3 mol) dropwise via the addition funnel, with the internal temperature being maintained below 5 °C. After the addition was complete, the mixture was allowed to warm to ambient temperature and stir for 16 h. The reaction was filtered and concentrated under reduced pressure to remove most of the acetic acid. The residue was dissolved in 750 mL of EtOAc, and 500 mL of saturated, aqueous $NaHCO_3$ solution was added slowly to neutralize the residual acid. The layers were separated, and the aqueous layer was extracted with 2 × 100 mL of EtOAc. The combined organics were dried over Na_2SO_4 and concentrated under reduced pressure to give a 1.5:1 mixture of the title compounds (**11** and **12**), which were carried on to the next reaction without separation or further purification. ^{1}H NMR (300 MHz, CD_3OD) δ 1.37 (m, 13H), 1.66 (m, 1H), 1.82 (m, 1H), 1.98 (s, 3H), 2.81 (m, 1H), 3.11 (m, 2H), 3.71 (m, 1H), 3.96 (m, 1H), 4.15 (m, 2H), 7.30 (m, 5H); MS (DCI/NH_3) m/z 377 $(M + H)^+$.

(3*S*,4*S*)-4-Hydroxymethyl-3-[(1*R*)-phenyl-ethylamino]-piperidine-1-carboxylic Acid *tert*-Butyl Ester (13) and (3*R*,4*R*)-4-Hydroxymethyl-3-[(1*R*)-1-phenyl-ethylamino]-piperidine-1-carboxylic Acid *tert*-Butyl Ester (14). To a slurry of $LiAlH_4$ (0.292 mol) in 1 L tetrahydrofuran at 0 °C was added a mixture of **11** and **12** (0.265 mol) dropwise via addition funnel. The ice bath was removed after the addition was complete, and the mixture was stirred at ambient temperature for 1 h. The reaction was quenched by the slow addition of approximately 100 g $Na_2SO_4 \cdot 10H_2O$ (excess). The mixture was stirred for 16 h and then was filtered, concentrated under reduced pressure, and purified via column chromatography (SiO_2, 33% hexanes−EtOAc) to give 76.5 g of a ∼1.5:1 mixture of *cis*-isomers (**13** and **14**; 0.23 mol, 86%). Note that the isomers (**13** and **14**) could be separated via silica gel chromatography at this stage or more readily at a later stage (see the experimental procedures for **18** and **20**). Data for **13**: ^{1}H NMR (300 MHz, CD_3OD) δ 1.35 (s, 9H), 1.45 (s, 3H), 1.58 (m, 2H), 1.78 (m, 1H), 2.91 (m, 3H), 3.73 (m, 2H), 3.90 (m, 3H), 7.27 (m, 5H); MS (DCI/NH_3) m/z 335 $(M + H)^+$. Data for **14**: ^{1}H NMR (300 MHz, CD_3OD) δ 1.55 (m, 15H), 2.72 (m, 3H), 3.55 (m, 2H), 3.95 (m, 1H), 4.11 (m, 1H), 4.28 (d, $J = 13.6$ Hz, 1H), 7.29 (m, 5H); MS (DCI/NH_3) m/z 335 $(M + H)^+$.

5-[(1*S*)-1-Phenyl-ethylamino]-3,6-dihydro-2*H*-pyridine-1,4-dicarboxylic Acid 1-*tert*-Butyl Ester 4-Ethyl Ester (ent-10). Compound **9** (90.4 g, 0.333 mol) in toluene (250 mL) was treated with (*S*)-α-methylbenzylamine (42.4 g, 0.350 mol). The mixture was warmed to reflux with a Dean−Stark trap until the distillate was clear (7 h) and ∼7 mL of H_2O had been collected. The mixture was concentrated under reduced pressure to provide the title compound (**ent-10**), which was carried on directly to the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (t, $J = 7.1$ Hz, 3H), 1.47 (m, 10H), 2.32 (m, 1H), 2.35 (s, 3H), 3.13 (m, 1H), 3.55 (m, 2H), 3.90 (m, 1H), 4.17 (q, $J = 7.1$ Hz, 2H), 4.55 (m, 1H), 7.27 (m, 5H), 9.17 (s, 1H); MS (DCI/NH_3) m/z 375 $(M + H)^+$.

(3*S*,4*S*)-1-*t*-Butyl 4-Ethyl (*cis*)-3-{[(1*S*)-1-Phenylethyl]amino}-1,4-piperidinedicarboxylate (ent-12) and (3*R*,4*R*)-1-*t*-Butyl 4-Ethyl (*cis*)-3-{[(1*S*)-1-Phenylethyl]amino}-1,4-piperidinedicarboxylate (ent-11). Compound **ent-10** (62.3 g, 0.167 mol), $NaBH(OAc)_3$ (150 g, 0.708 mol), and powdered 4 Å molecular sieves (133 g) in toluene (730 mL) in a 3-neck round-bottom flask equipped with a mechanical stirrer, thermometer, and addition funnel at 0 °C was treated with acetic acid (191 mL, 3.30 mol) dropwise with the internal temperature being maintained below 5 °C. After the addition was complete, the ice bath was removed, and the mixture was stirred for 20 h and filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (1 L) and quenched by slow addition of saturated, aqueous $NaHCO_3$. The layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organics were dried over Na_2SO_4 and filtered, and the filtrate was concentrated under reduced pressure to provide the product as an ∼1:1.5 mixture of the two *cis*-isomers, **ent-12** and **ent-11** (60.0 g, 0.159 mol, 94% yield). ^{1}H NMR (300 MHz, CD_3OD) δ 1.30 (m, 12H), 1.49 (m, 3H), 1.61 (m, 1H), 1.83 (m, 1H), 2.69 (m, 2H), 3.04 (m, 2H), 3.86 (m, 3H), 4.18 (m, 2H), 7.27 (m, 5H); MS (DCI/NH_3) m/z 377 $(M + H)^+$.

(3*S*,4*S*)-4-Hydroxymethyl-3-[(1*S*)-phenyl-ethylamino]-piperidine-1-carboxylic Acid *tert*-Butyl Ester (ent-14) and (3*R*,4*R*)-4-Hydroxymethyl-3-[(1*S*)-1-phenyl-ethylamino]-piperidine-1-carboxylic Acid *tert*-Butyl Ester (ent-13). A mixture of compounds **ent-11** and **ent-12** (60.0 g, 0.159 mol) in tetrahydrofuran (200 mL) was added dropwise to a mixture of lithium aluminum hydride (7.00 g, 0.175 mol, 95%) in tetrahydrofuran (300 mL) at 0 °C. After the addition was complete, the mixture was allowed to warm to ambient temperature and was quenched by slow addition of $Na_2SO_4 \cdot 10H_2O$ (excess). The mixture was stirred for 16 h and filtered, and the filtrate was concentrated under reduced pressure. The two isomers, **ent-13** and **ent-14**, were separated via flash column chromatography (50% ethyl acetate/hexanes) to provide two diastereomers, a more mobile diastereomer determined to be the (3*S*,4*S*) diastereomer (**ent-14**; $R_f = 0.27$ in 75% ethyl acetate/hexanes, 15.0 g, 44.8 mmol, 28% yield) and a less mobile diastereomer determined to be the (3*R*,4*R*) diastereomer (**ent-13**; $R_f = 0.20$ in 75% ethyl acetate/hexanes, 22.5 g, 67.3 mmol, 42% yield). Data for **ent-13**: ^{1}H NMR (300 MHz, CD_3OD) δ 1.35 (s, 9H), 1.54 (m, 4H), 1.78 (m, 1H), 2.89 (m, 2H), 2.95 (m, 1H), 3.73 (m, 2H), 3.90 (m, 3H), 7.22 (m, 1H), 7.33 (m, 5H); MS (DCI/NH_3) m/z 335 $(M + H)^+$. Data for **ent-14**: ^{1}H NMR (300 MHz, CD_3OD) δ 1.28 (m, 3H), 1.60 (m, 3H), 1.51 (s, 9H), 2.60 (m, 2H), 2.77 (m, 1H), 3.55 (m, 2H), 3.95 (q, $J = 6.7$ Hz, 1H), 4.11 (m, 1H), 4.28 (m, 1H), 7.22 (m, 1H), 7.33 (m, 4H); MS (DCI/NH_3) m/z 335 $(M + H)^+$.

The more mobile diastereomer (**ent-14**) was subjected to X-ray analysis. Single crystals suitable for X-ray diffraction were grown by slow evaporation from an ethyl acetate solution. Crystal data: MW = 334.46, $C_{19}H_{30}N_2O_3$, crystal dimensions 0.40 × 0.20 × 0.05 mm, orthorhombic, $P2_12_12_1$ (#19), $a = 6.5230(13)$, $b = 12.469(3)$, $c = 23.107(5)$ Å, $V = 1879.4(6)$ Å^3, $Z = 4$, $D_{calc} = 1.18$ g/cm^{-3}. Crystallographic data were collected using Mo Kα radiation ($\lambda = 0.71069$ Å). Refinement of the structure using full matrix least-squares refinement of 229 parameters on 4615 reflections with $I > 2.00\sigma(I)$ gave $R = 0.062$, $R_w = 0.138$.

(3*R*,4*R*)-3-Amino-4-hydroxymethyl-piperidine-1-carboxylic Acid *tert*-Butyl Ester (15). A mixture of **ent-13** (30.5 g, 0.13 mol) and 6.16 g of $Pd(OH)_2$/C (20 wt %, 50% wet) in 300 mL of CH_3OH was shaken under 4 atm of H_2 for 4 h at 50 °C. The mixture was cooled to ambient temperature, filtered, and concentrated to give 20.5 g of the title compound **15** (89 mmol, 68% yield). ^{1}H NMR (300 MHz, CD_3OD) δ 1.46 (s, 10H), 1.55 (m, 1H), 1.75 (m, 1H), 2.77 (m, 1H), 2.96 (m, 1H), 3.10 (m, 1H), 3.54 (m, 2H), 3.99 (ddd, $J = 13.3$, 3.1, 1.9 Hz, 1H), 4.07 (m, 1H); MS (DCI/NH_3) m/z 231 $(M + H)^+$.

(1*R*,6*S*)-8-(2-Nitrobenzenesulfonyl)-3,8-diaza-bicyclo[4.2.0]-octane-3-carboxylic Acid *tert*-Butyl Ester (16). To compound **15** (20.5 g, 89 mmol) in 500 mL of CH_2Cl_2 at 0 °C was added Et_3N (37.3 mL, 26.8 mmol) and 2-nitrobenzenesulfonyl chloride. The ice bath was removed, and the mixture was stirred at ambient temperature for 48 h. The reaction mixture was then concentrated under reduced pressure and was redissolved in 200 mL of EtOH and 100 mL of 5% aqueous NaOH solution. This mixture was stirred at ambient temperature for 1 h, concentrated under reduced pressure, and redissolved in EtOAc (300 mL). The layers were separated, and the aqueous layer was extracted with 3 × 50 mL of EtOAc. The combined organics were dried over Na_2SO_4, filtered, concentrated under reduced pressure, and purified via flash column chromatography (50% ethyl acetate/hexanes) to give 22.1 g of the title compound, **16** (55.6 mmol, 62% yield). ^{1}H NMR (300 MHz, CD_3OD) δ 1.41 (m, 9H), 1.82 (m, 1H), 2.01 (m, 1H), 2.60 (m, 1H), 3.19 (m, 1H), 3.40 (m, 1H), 3.72 (m, 2H), 4.06 (m, 2H), 4.59 (m, 1H), 7.81 (m, 3H), 8.03 (m, 1H); MS (DCI/NH_3) *m*/*z* 398 (M + H)$^+$.

(1*R*,6*S*)-3,8-Diaza-bicyclo[4.2.0]octane-3-carboxylic Acid *tert*-Butyl Ester (4). To **16** (22.1 g, 55.6 mmol) in 100 mL of DMF was added PhSH (7.4 mL, 72.3 mmol) and K_2CO_3 (23.8 g, 0.172 mol). This mixture was stirred at ambient temperature for 50 h and then was filtered and concentrated under reduced pressure. Purification via column chromatography (9:1:0.1 CH_2Cl_2/CH_3OH/NH_4OH) gave 5 g of the title compound (**4**; 23.6 mmol, 42% yield). ^{1}H NMR (300 MHz, CD_3OD) δ 1.47 (s, 9H), 1.77 (m, 1H), 1.95 (m, 1H), 2.81 (m, 1H), 2.99 (m, 1H), 3.29 (m, 2H), 3.38 (m, 1H), 3.73 (m, 2H), 4.15 (m, 1H); MS (DCI/NH_3) *m*/*z* 213 (M + H)$^+$.

(1*R*,6*S*)-8-(2-Nitro-benzenesulfonyl)-3,8-diaza-bicyclo[4.2.0]-octane-3-carboxylic Acid Benzyl Ester (17). To **16** (2.82 g, 7.1 mmol) in CH_2Cl_2 (20 mL) was treated with trifluoroacetic acid (20 mL), and the solution was stirred at room temperature for 1 h. The mixture was concentrated under reduced pressure, and the residue was dissolved in CH_2Cl_2 (30 mL) and treated with triethylamine (1.29 mL, 9.2 mmol) and benzyl chloroformate (1.21 mL, 8.5 mmol) at 0 °C. The mixture was stirred at room temperature for 16 h and then washed successively with 1 N HCl (10 mL), 1 N NaOH (10 mL), and brine (10 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to provide the title compound **17** (2.23 g, 5.2 mmol, 73% yield). ^{1}H NMR (300 MHz, CD_3OD) δ 1.82 (m, 1H), 2.03 (m, 1H), 2.66 (m, 1H), 3.21 (dd, J = 14.7, 1.9 Hz, 1H), 3.46 (m, 1H), 3.76 (m, 2H), 4.13 (m, 2H), 4.68 (m, 1H), 5.05 (m, 2H), 7.31 (m, 6H), 7.74 (m, 2H), 8.00 (m, 1H); MS (DCI/NH_3) *m*/*z* 449 (M + NH_4)$^+$.

(1*R*,6*S*)-3,8-Diaza-bicyclo[4.2.0]octane-8-carboxylic Acid *tert*-Butyl Ester (5). Compound **17** (2.23 g, 5.2 mmol) in DMF (20 mL) was treated with K_2CO_3 (2.37 g, 17.2 mmol) and thiophenol (0.80 mL, 7.8 mmol) and allowed to stir at room temperature for 16 h. The mixture was further treated with di-*t*-butyl dicarbonate (2.27 g, 10.4 mmol) and was allowed to stir for an additional 16 h at room temperature. The mixture was diluted with diethyl ether (150 mL), washed with 5 × 20 mL of 1:1 brine and H_2O, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (3% CH_3OH/CH_2Cl_2) to provide the intermediate (1*R*,6*S*)-3,8-diaza-bicyclo[4.2.0]octane-3,8-dicarboxylic acid 3-benzyl ester 8-*tert*-butyl ester (1.5 g, 43 mmol, 83% yield, MS (DCI/NH_3) *m*/*z* 347 (M + H)$^+$).

Intermediate (1*R*,6*S*)-3,8-diaza-bicyclo[4.2.0]octane-3,8-dicarboxylic acid 3-benzyl ester 8-*tert*-butyl ester (1.5 g, 4.3 mmol) in methanol (20 mL) was treated with 10% Pd/C (0.10 g), and the mixture was stirred under H_2 (1 atm) at ambient temperature for 2 h. The mixture was filtered, concentrated under reduced pressure, and purified via column chromatography (9:1:0.1 CH_2Cl_2/CH_3OH/NH_4OH) to provide the title compound, **5** (0.49 g, 2.3 mmol, 53% yield). ^{1}H NMR (300 MHz, CD_3OD) δ 1.44 (s, 9H), 1.66 (m, 1H), 2.00 (m, 1H), 2.57 (m, 2H), 2.86 (m, 1H), 3.02 (m, 1H), 3.20 (dd, J = 14.2, 2.4 Hz, 1H), 3.48 (dd, J = 8.0, 3.6 Hz, 1H), 3.96 (t, J = 7.8 Hz, 1H), 4.15 (ddd, J = 7.5, 4.4, 2.4 Hz, 1H); MS (DCI/NH_3) *m*/*z* 213 (M + H)$^+$.

(1*S*,6*R*)-8-[(1*R*)-1-Phenyl-ethyl]-3,8-diaza-bicyclo[4.2.0]octane-3-carboxylic Acid *tert*-Butyl Ester (18) and (1*R*,6*S*)-8-[(1*R*)-1-Phenyl-ethyl]-3,8-diaza-bicyclo[4.2.0]octane-3-carboxylic Acid *tert*-Butyl Ester (20). To the mixture of compounds **13** and **14** (76.5 g, 0.23 mol) in 1.1 L of tetrahydrofuran at 0 °C was added Et_3N (95.8 mL, 0.687 mol), followed by methanesulfonyl chloride (23 mL, 0.30 mol). The ice bath was removed after the additions were complete, and the reaction was allowed to warm to ambient temperature and stirred for 1 h. Cs_2CO_3 (~100 g) was added, and the mixture was warmed to 60 °C and stirred for 16 h. The reaction was cooled to ambient temperature and filtered, and the filtrate was washed with 2 × 100 mL of H_2O. The layers were separated, and the aqueous layer was extracted 2 × 100 mL of EtOAc. The combined organics were dried over Na_2SO_4 and concentrated under reduced pressure. The material was purified, and the isomers were separated via column chromatography (50% hexanes/EtOAc) to give 30.7 g of the major isomer (less mobile), (1*S*,6*R*)-3,8-diaza-bicyclo-[4.2.0]octane-3-carboxylic acid *tert*-butyl ester, **18** (97 mmol, 42%), and 16.5 g of the minor isomer (more mobile), (1*R*,6*S*)-3,8-diaza-bicyclo[4.2.0]-octane-3-carboxylic acid *tert*-butyl ester, **20** (52 mmol, 23%). Data for **18**: ^{1}H NMR (300 MHz, CD_3OD) δ 1.19 (d, J = 6.4 Hz, 3H), 1.40 (m, 9H), 1.84 (m, 2H), 2.44 (m, 1H), 2.62 (m, 1H), 2.78 (m, 1H), 3.16 (m, 2H), 3.29 (m, 2H), 3.37 (m, 1H), 3.83 (ddd, J = 12.6, 8.7, 4.1 Hz, 1H), 7.27 (m, 5H); MS (DCI/NH_3) *m*/*z* 317 (M + H)$^+$. Data for **20**: ^{1}H NMR (300 MHz, CD_3OD) δ 1.21 (m, 3H), 1.48 (m, 9H), 1.78 (m, 1H), 1.91 (m, 1H), 2.40 (m, 1H), 2.84 (m, 2H), 3.12 (m, 2H), 3.42 (m, 2H), 3.91 (m, 2H), 7.17 (m, 1H), 7.26 (m, 4H); MS (DCI/NH_3) *m*/*z* 317 (M + H)$^+$.

(1*S*,6*R*)-3,8-Diaza-bicyclo[4.2.0]octane-3-carboxylic Acid *tert*-Butyl Ester (6). A mixture of **18** (12.6 g, 39.8 mmol) and wet $Pd(OH)_2$/C (20 wt %, 3.25 g) was shaken under a 60 psi atmosphere of H_2 for 2.1 days at 50 °C. The mixture was then filtered and concentrated to give 7.6 g of the title compound, **6** (35.8 mmol, 90% yield). ^{1}H NMR (300 MHz, CD_3OD) δ 1.47 (s, 9H), 1.76 (m, 1H), 1.93 (m, 1H), 2.78 (m, 1H), 3.19 (dd, J = 8.5, 4.1 Hz, 1H), 3.24 (m, 1H), 3.38 (m, 1H), 3.64 (dd, J = 14.4, 2.9 Hz, 1H), 3.74 (t, J = 8.3 Hz, 1H), 3.82 (m, 1H), 4.10 (m, 1H); MS (DCI/NH_3) *m*/*z* 213 (M + H)$^+$.

(1*S*,6*R*)-2,2,2-Trifluoro-1-[8-((1*R*)-1-phenyl-ethyl)-3,8-diaza-bicyclo[4.2.0]oct-3-yl]-ethanone (21). To a solution of **18** (10 g, 31.6 mmol) in 35 mL of CH_2Cl_2 at 0 °C was added 20 mL of trifluoroacetic acid. The ice bath was removed, and the mixture was stirred at ambient temperature for 1 h. The mixture was then concentrated and filtered through a plug of Celite and silica gel with 9:1:0.1 CH_2Cl_2/CH_3OH/NH_4OH. The complete removal of the *t*-butylcarbamate group was confirmed by mass spectroscopy (MS (DCI/NH_3) *m*/*z* 217 (M + H)$^+$), and the material was carried on.

To the intermediate free-amine (31.6 mmol) in 220 mL of tetrahydrofuran at −30 °C was added Et_3N (5.43 mL, 38.8 mmol), followed by trifluoroacetic anhydride (4.6 mL, 32.6 mmol). This mixture was stirred for 1.5 h as it warmed from −30 to −10 °C. The mixture was quenched with 50 mL of saturated, aqueous $NaHCO_3$ and was allowed to warm to ambient temperature. The layers were separated, and the aqueous layer was extracted with 3 × 20 mL of EtOAc. The combined organics were washed with 1 × 10 mL of brine and then dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude material was dissolved in 100 mL of EtOAc and filtered through a plug of Celite and silica gel with EtOAc to give the title compound (**21**; 9.9 g, >100% yield). ^{1}H NMR (300 MHz, CD_3OD) δ 1.95 (m, 2H), 2.54 (m, 1H), 2.67 (dd, J = 14.9, 2.4 Hz, 1H), 2.82 (ddd, J = 13.9, 3.4, 1.7 Hz, 1H), 3.05 (m, 1H), 3.20 (m, 1H), 3.27 (m, 1H), 3.41 (m, 3H), 3.57 (ddd, J = 13.3, 6.5, 4.6 Hz, 1H), 3.92 (ddd, J = 13.5, 9.1, 4.6 Hz, 1H), 4.14 (m, 1H), 7.27 (m, 5H); MS (DCI/NH_3) *m*/*z* 313 (M + H)$^+$.

(1*S*,6*R*)-3,8-Diaza-bicyclo[4.2.0]octane-8-carboxylic Acid *tert*-Butyl Ester (7). A mixture of **21** (~31.6 mmol), di-*t*-butyldicarbonate (7.7 g, 35.3 mmol), and wet $Pd(OH)_2$/C (10 wt %, 2.1 g) in 125 mL of EtOAc was shaken under a 60 psi atmosphere of H_2 for 16.5 h at 50 °C. The material was filtered and concentrated under

reduced pressure to give a quantitative amount of the intermediate (1*S*,6*R*)-3-(2,2,2-trifluoro-acetyl)-3,8-diaza-bicyclo[4.2.0]octane-8-carboxylic acid *tert*-butyl ester, which was used directly below (MS (DCI/NH_3) *m*/*z* 326 $(M + NH_4)^+$).

To a solution of the (1*S*,6*R*)-3-(2,2,2-trifluoro-acetyl)-3,8-diaza-bicyclo[4.2.0]octane-8-carboxylic acid *tert*-butyl ester (31.6 mmol) in 150 mL of CH_3OH and 30 mL of H_2O was added K_2CO_3 (5.1 g, 37.2 mmol). This mixture was stirred at ambient temperature for 16 h and then concentrated under reduced pressure. The crude material was filtered through a plug of Celite and silica gel with 9:1:0.1 $CH_2Cl_2/CH_3OH/NH_4OH$. The still crude material was purified via column chromatography (9:1:0.1 $CH_2Cl_2/CH_3OH/NH_4OH$) to give 6.6 g of the title compound **7** (31 mmol, 98% yield). ^{1}H NMR (300 MHz, CD_3OD) δ 1.45 (s, 9H), 1.81 (m, 1H), 2.15 (m, 1H), 2.65 (m, 1H), 2.91 (ddd, J = 13.0, 8.6, 4.2 Hz, 1H), 3.09 (dd, J = 14.2, 3.4 Hz, 1H), 3.28 (m, 1H), 3.39 (dd, J = 14.2, 2.0 Hz, 1H), 3.49 (dd, J = 8.1, 3.1 Hz, 1H), 4.01 (t, J = 7.8 Hz, 1H), 4.33 (m, 1H); MS (DCI/NH_3) *m*/*z* 213 $(M + H)^+$.

Representative Procedures: (1*S*,6*R*)-3-(5-Bromo-pyridin-3-yl)-3,8-diaza-bicyclo[4.2.0]octane Fumarate (27). Buchwald−Hartwig Coupling: A mixture of tris(dibenzylideneacetone)-dipalladium ($Pd_2(dba)_3$, 65 mg, 0.071 mmol; Strem) and 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP, 73 mg, 0.12 mmol; Strem) in 5 mL of $PhCH_3$ was warmed to 85 °C for 15 min. This mixture was added via cannula to a solution of **7** (0.50 g, 2.4 mmol) and 3,5-dibromopyridine (0.73 g, 3.1 mmol) in 30 mL of $PhCH_3$ at ambient temperature. NaO*t*-Bu (0.34 g, 3.53 mmol) was added, and the mixture was warmed to 80 °C and allowed to stir for 16 h. The reaction mixture was then concentrated under reduced pressure and purified via column chromatography (50% hexanes/EtOAc) to give (1*S*,6*R*)-3-(5-bromo-pyridin-3-yl)-3,8-diaza-bicyclo[4.2.0]-octane-8-carboxylic acid *tert*-butyl ester (0.55 g, 1.5 mmol, 63% yield). Data for intermediate: ^{1}H NMR (300 MHz, CD_3OD) δ 1.31 (s, 9H), 1.95 (m, 1H), 2.21 (m, 1H), 2.83 (m, 1H), 3.39 (m, 2H), 3.71 (dt, J = 12.9, 4.4 Hz, 1H), 3.76 (m, 1H), 3.95 (t, J = 8.5 Hz, 1H), 4.03 (m, 1H), 4.52 (m, 1H), 7.34 (s, 1H), 7.85 (s, 1H), 8.04 (s, 1H); MS (DCI/NH_3) *m*/*z* 190 $(M + H)^+$.

Removal of *t*-Butylcarbamate Group: To the coupled product (1*S*,6*R*)-3-(5-bromo-pyridin-3-yl)-3,8-diaza-bicyclo[4.2.0]octane-8-carboxylic acid *tert*-butyl ester (0.26 g, 0.71 mmol) in 6 mL of CH_2Cl_2 at 0 °C was added 4 mL of trifluoroacetic acid. The mixture was allowed to warm to ambient temperature. After stirring for 2 h, the mixture was concentrated under reduced pressure and was purified via column chromatography (9:1:0.1 $CH_2Cl_2/CH_3OH/NH_4OH$) to give the free amine, (1*S*,6*R*)-3-(5-bromo-pyridin-3-yl)-3,8-diaza-bicyclo[4.2.0]octane, which was directly converted to the corresponding salt.

Salt Formation: To the free amine (0.71 mmol) in 5 mL of 10% CH_3OH in Et_2O was added fumaric acid (82 mg, 0.71 mmol) in 2 mL of 10% CH_3OH in Et_2O. The resulting precipitate was isolated via filtration to give **27** (0.12 g, 0.31 mmol, 44% yield). ^{1}H NMR (300 MHz, CD_3OD) δ 2.02 (m, 1H), 2.21 (m, 1H), 3.16 (m, 1H), 3.35 (m, 1H), 3.55 (dd, J = 14.7, 3.2 Hz, 1H), 3.91 (m, 3H), 4.17 (dd, J = 11.0, 9.0 Hz, 1H), 4.80 (dt, J = 9.2, 3.1 Hz, 1H), 6.64 (s, 2H), 7.49 (dd, J = 2.7 Hz, 1H), 7.99 (d, J = 1.7 Hz, 1H), 8.15 (d, J = 2.7 Hz, 1H); MS (DCI/NH_3) *m*/*z* 268, 270 $(M + H)^+$; Anal. ($C_{11}H_{14}BrN_3 \cdot C_4H_4O_4 \cdot 0.25H_2O$) C, H, N.

(1*S*,6*R*)-3-(5,6-Dibromo-pyridin-3-yl)-3,8-diaza-bicyclo[4.2.0]-octane Fumarate (31). The intermediate from the coupling of **7** with dibromopyridine, (1*S*,6*R*)-3-(5-bromo-pyridin-3-yl)-3,8-diaza-bicyclo[4.2.0]octane-8-carboxylic acid *tert*-butyl ester (0.20 g, 0.54 mmol) in 20 mL of CH_3CN at −40 °C was treated with *N*-bromosuccinimide (NBS, 96 mg, 0.54 mmol). This mixture was stirred at −40 °C for 1.5 h, then quenched with 5 mL of H_2O, and allowed to warm to ambient temperature. The layers were separated, and the aqueous layer was extracted with 3 × 5 mL of CH_2Cl_2. The combined organics were dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, and purified via column chromatography (50% hexanes/EtOAc) to give 0.23 g (1*S*,6*R*)-3-(5,6-dibromo-pyridin-3-yl)-3,8-diaza-bicyclo[4.2.0]octane-8-carboxylic acid *tert*-butyl ester (0.51 mmol, 95% yield, MS (DCI/NH_3) *m*/*z* 448 $(M + H)^+$). This material was deprotected and its fumarate salt formed as described for **25** to give **31** (0.107 g, 0.22 mmol, 43% yield). ^{1}H NMR (300 MHz, CD_3OD) δ 2.02 (m, 1H), 2.22 (m, 1H), 3.18 (m, 1H), 3.33 (m, 1H), 3.54 (dd, J = 14.6, 3.1 Hz, 1H), 3.88 (m, 3H), 4.17 (dd, J = 11.0, 9.0 Hz, 1H), 4.79 (dt, J = 9.1, 2.7 Hz, 1H), 6.68 (s, 2H), 7.60 (d, J = 3.1 Hz, 1H), 7.98 (d, J = 3.1 Hz, 1H); MS (DCI/NH_3) *m*/*z* 348 $(M + H)^+$; Anal. ($C_{11}H_{13}Br_2N_3 \cdot C_4H_4O_4 \cdot H_2O$) C, H, N.

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Supporting Information Available: Elemental analysis for all final compounds, experimental information and data for compounds **22**−**26**, **28**−**30**, and **32**−**71**, X-ray data for **ent-14**, and ^{1}H NMR spectra for representative compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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