Design and Synthesis of Tricyclic Corticotropin-Releasing Factor-1 Antagonists

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Antagonists of the corticotropin-releasing factor (CRF) neuropeptide should prove to be effective in treating stress and anxiety-related disorders. In an effort to identify antagonists with improved physicochemical properties, new tricyclic CRF_1 antagonists were designed, synthesized, and tested for biological activity. As a result of studies aimed at establishing a relationship between structure and CRF_1 binding affinity, NBI 35965 (**12a**) was identified as a high-affinity antagonist with a pK_i value of 8.5. Compound **12a** proved to be a functional CRF_1 antagonist with pIC_{50} values of 7.1 and 6.9 in the in vitro CRF-stimulated cAMP accumulation and ACTH production assays, respectively, and **12a** also reduced CRF or stress induced ACTH production in vivo.

Introduction

Corticotropin-releasing factor (CRF) is a neuropeptide that has generated a great deal of interest during the past 2 decades, and it is now commonly understood to be the primary mediator of the hypothalamic−pituitary−adrenal (HPA) stress response.[1] Since its purification and molecular identification in the early 1980s, a role for this hormone has been proposed in a variety of endocrine, neuropsychiatric, and neurodegenerative disorders. Primarily CRF is produced in and secreted from parvocellular neurons of the paraventricular hypothalamus and is the primary regulator of the release of adrenocorticotropic hormone (ACTH) as well as other proopiomelanocortin (POMC) derived peptides from the anterior pituitary gland.[2] In addition to its distribution in the hypothalamus, it has been found that CRF is widely expressed extrahypothalamically in many regions of the central nervous system where it mediates the central processes of the stress response including the autonomic, electrophysiological, and behavioral responses to stress.[3−6] It is now well-known that CRF exerts its action through a specific interaction with two distinct transmembrane receptors that belong to the class B subfamily of G-protein-coupled receptors. These receptors, encoded by two distinct genes, are termed CRF_1 and CRF_2 and have been extensively characterized and quantified in a number of species.

A number of clinical investigators have described a clear and significant correlation between hypersecretion of central CRF and the severity of affective or anxiety disorders. Given the primary role of CRF in stimulating pituitary−adrenocortical secretion, it is plausible that hypersecretion of central CRF may play a fundamental role in the etiology of major depression.[7] Hypersecretion of hypothalamic CRF manifests itself in a down-regulation of CRF receptors in the anterior pituitary, as demonstrated by the blunted ACTH response to peripherally administered CRF.[8,9] In depressed patients CRF has also been shown to be elevated extrahypothalamically and is found in high concentrations in the cerebrospinal fluid.[10] This, in turn, has been shown to result in decreased expression of the CRF_1 receptor when measured in the frontal cortex of suicide victims.[11] Furthermore, a significant positive correlation exists between the elevated CRF levels in the cerebrospinal fluid and the degree of insensitivity to dexamethasone suppression of plasma cortisol in depressed individuals.[7] These studies suggest that in these disorders the CRF system is attempting to attenuate the effects of elevated CRF levels by reducing the number of sites for this hormone's action.

Over the past decade, several non-peptide CRF_1 receptor antagonists have been reported and several representative examples are illustrated in Figure 1.[12] Compounds such as antalarmin (**1**),[13] R121919 (**2**),[14,15] compound **3**,[16] and DMP 696 (**4**)[17] have proven to be functional antagonists both in vitro and in vivo with exquisite selectivity for the CRF_1 receptor. The compound R121919 (**2**) has demonstrated preliminary pharmacologic effects in a small open label phase IIA clinical trial in major depressive disorder patients. Compound **2** showed that while effective in reducing the Hamilton depression and anxiety rating scale (HAM-D and HAM-A) scores of depressed individuals, there was little effect on the basal functioning of the HPA axis, suggesting that non-peptide CRF_1 antagonists may prove to be useful therapeutics in the treatment of anxiety and depression.[18,19]

It has been suggested that the clinical development of the early CRF_1 antagonists was limited because of the highly lipophilic nature of compounds such as antalarmin (**1**).[20] The overall objective of this study was to reduce lipophilicity of small-molecule CRF_1 antagonists. It was hoped that restricting the conformational

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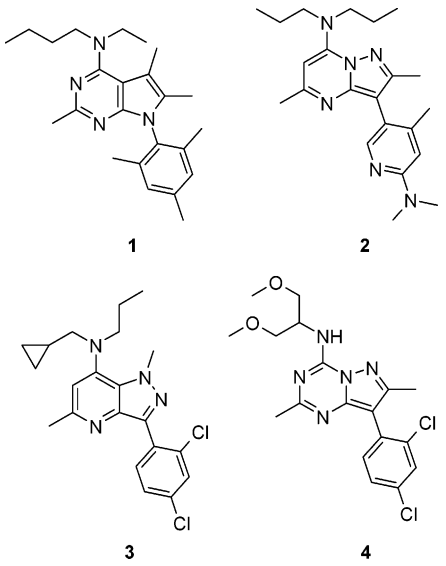


Figure 1. Examples of non-peptide CRF$_1$ antagonists.

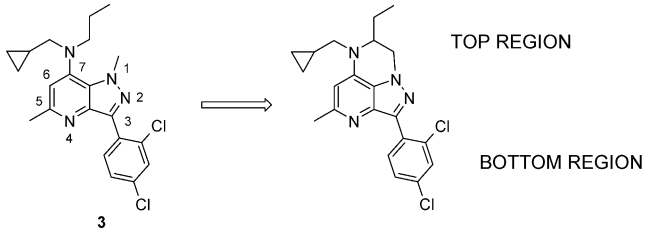


Figure 2. Design of tricyclic CRF$_1$ antagonists.

freedom would result in compounds that would populate a small subset of the biologically relevant conformations of compound **1**, reducing the potential entropic penalty incurred when flexible small-molecule ligands bind to large proteins.[21] Covalent attachment of the N-1 methyl group present in compound **3** to the top region alkyl side chain would restrict the conformational freedom of the flexible "top region" (Figure 2). It was hypothesized that this reduced flexibility would result in reduction of the size of the alkyl chains required for optimal biological activity.

Chemistry

The synthesis of tricyclic analogues of **3** was accomplished by first assembling the pyrazole ring (intermediate **8**) by treating hydrazine hydrochloride with the 1,3-dicarbonyl equivalent **7** (Scheme 1). Intermediate **7** was prepared by condensation of the α-aminoacetophenone **6** with *N*,*N*-dimethylformamide dimethylacetal, and alkylating potassium phthalamide with commercially available chloroketone **5** provided ketone **6**. Formation of an enamine derived from **8** and ethylacetoacetate followed by thermal cyclization in diphenyl ether led to the production of compound **9** in 77% isolated yield over two steps. Conversion of **9** to **10** was carried out in the presence of $POCl_3$. A two-step, one-pot procedure involving a reaction with intermediate **10** and a variety of amino alcohols followed by aqueous HBr-mediated cyclization afforded compounds **11a**−**g**. Final top region side chains were installed via base-mediated alkylation of intermediates **11a**−**g** to yield the final compounds **12a**−**u**. The tritiated analogue of **12a** was prepared bromination of **12a** followed by palladium-catalyzed tritiation.

Attempts to prepare compounds **12v** and **12w** via this route failed and, as a consequence, a modified route illustrated in Scheme 2. The R^1 side chains were installed first by condensing **10** with the amines (cyclohexylamine and 2-amino-1-methoxybutane), and the final cyclization with 1,2-dibromoethane provided **12v** and **12w**.

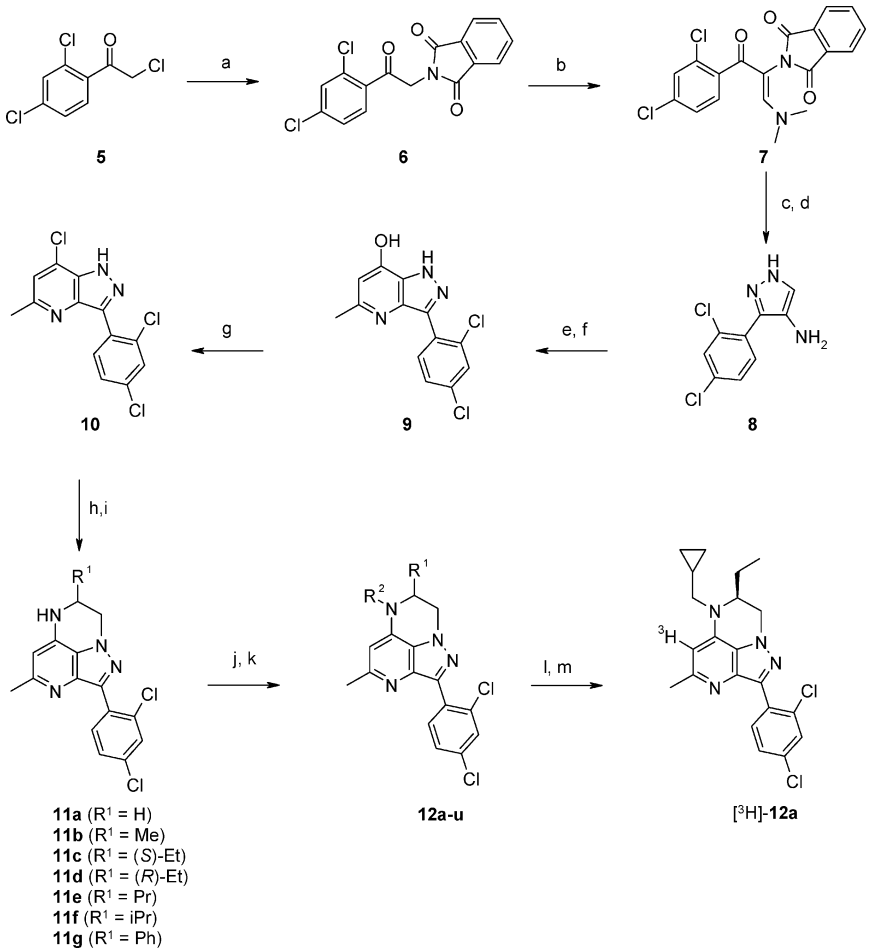
For the bottom region SAR studies, a divergent intermediate strategy was employed. It was presumed that treatment of intermediate **19** with the requisite amino alcohol, cyclization, and alkylation would yield the intermediate **22** that would be then be poised to undergo palladium-mediated Suzuki coupling reactions to rapidly introduce aromatic side chains (Scheme 3). Preparation of **19** first required nitration of pyrazole followed by palladium-catalyzed hydrogenation of the nitro group to afford 4-aminopyrazole **15**. Analogous to the sequence in Scheme 1, **15** was condensed with ethylacetoacetate and subjected to a thermal cyclization to provide **17**. Conversion of **17** to the 7-chloro intermediate **18** was accomplished with $POCl_3$. Owing to the inductively withdrawing nature of the 7-chloro group, **18** was selectively brominated to afford **19** in 13% overall yield over six steps. This synthesis was successfully performed on a 100 g scale, and **19** proved to be a versatile and important intermediate for the subsequent SAR studies. Preparation of the final compounds required the condensation of **19** with (*S*)-2-aminobutanol analogous to the route illustrated in Scheme 1. Cyclization of **20** using 48% HBr at 130 °C followed by alkylation of **21** with cyclopropylmethyl bromide and sodium hydride gave the penultimate intermediate **22**. The final aryl side chains were incorporated by a palladium-catalyzed cross coupling with a variety of aryl boronic acids.

The synthesis of **23n** required a modified route that is outlined in Scheme 4 because the necessary boronic acid was not available commercially. First, the 2-chloro-5-bromobenzotrifluoride (**24**) was acylated in a two-step process that involved cross-coupling with tributyl-(1-ethoxyvinyl)tin to yield **25** after hydrolysis of the enol ether. Intermediate **26** was prepared by a palladium-catalyzed boronation, which was then used as the coupling partner with **22** in a Suzuki cross-coupling reaction to afford **27**. Conversion of the acyl group involved condensation of **27** with methylmagnesium bromide followed by dehydration to provide **28**, which was then hydrogenated to give the final compound **23n**.

Results

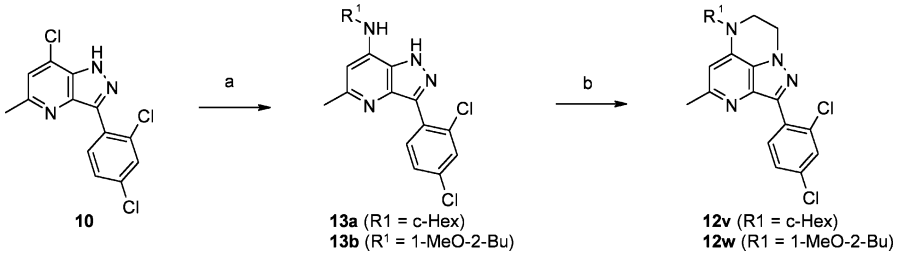
The compounds prepared in this study were screened for their ability to inhibit [^{125}I]sauvagine binding to h-CRF$_1$ LtK transfectant cells, and the pK_i values are reported in Tables 1 and 2.[22] Antalarmin (**1**) and compound **3** were prepared as a positive control, and the pK_i values determined were consistent with previously reported values (Table 1).[13,16] Covalent attachment of the N-1 methyl group to the C-7 branched alkyl side chain of **3** resulted in compound **12a**, which is a high-affinity CRF$_1$ antagonist with a pK_i value of 8.5. The activity of the enantiomer **12b** was significantly reduced (pK_i = 6.9), providing evidence that the pocket within the receptor that interacts with top region side chain has a defined asymmetric shape. Once it was estab-

Scheme 1[a]



[a] (a) Potassium phthalimide, DMF, 25 °C, 71%; (b) DMFDMA, Δ 74%; (c) NH_2NH_2·HCl, aqueous EtOH, Δ; (d) NH_2NH_2·H_2O aqueous EtOH, Δ, 91% (two steps); (e) ethyl acetoacetate, *p*-TsOH, toluene, Δ; (f) Ph_2O, 250 °C, 77% (two steps); (g) $POCl_3$, MeCN, Δ, 95%; (h) amino alcohols, *p*-TsOH, 120 °C; (i) 48% aqueous HBr, 140 °C, 76% (two steps); (j) alkyl halides, NaH, DMF, 25 °C; (l) Br_2, CH_2Cl_2, 25C, 75%; (m) $^3H_2$, Pd/C, $EtNiPr_2$.

Scheme 2[a]



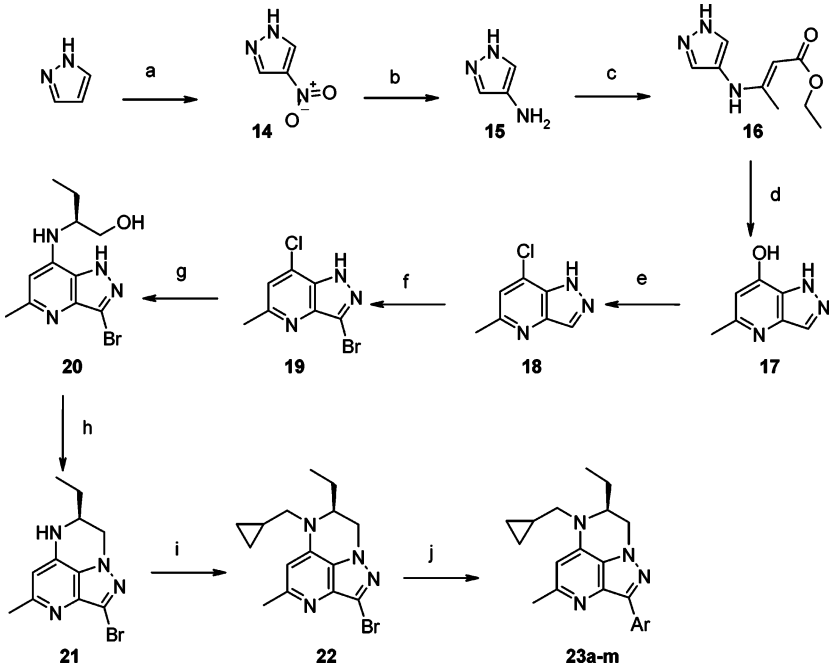
[a] (a) R^1NH_2, TsOH, 130 °C; (b) 1,2-dibromoethane, K_2CO_3, 85 °C.

lished that the tricyclic core structure allows the side chains to adopt biologically relevant conformations, a study was launched to test the hypothesis of whether restricting the conformational freedom of the top region side chains would ultimately result in the introduction of smaller less lipophilic side chains at positions R^1 and R^2.

Complete removal of the R^2 ethyl group (**12c**) resulted in a 25-fold loss of affinity at the CRF_1 receptor, but much of the lost binding affinity was regained by the introduction of a methyl group at position R^2 (**12d**, pK_i = 8.1). Replacing the (*S*)-ethyl group with a (*S*)-propyl group (**12e**, pK_i = 8.4) or the branched (*S*)-isopropyl group (**12f**, pK_i = 8.3) had no effect on binding affinity within this series, suggesting that this region within the receptor may tolerate larger substituents. The phenyl derivative **12g** was prepared to further probe the shape of this putative binding pocket. Filling the space with the larger phenyl ring did not increase the affinity for this compound; if anything, the activity was slightly reduced (pK_i = 8.0), leaving the original ethyl substituent as the optimal R^2 side chain.

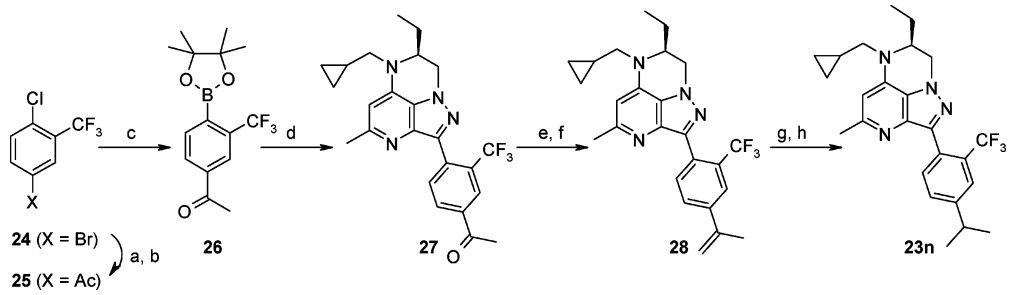
Also contained within Table 1 are the results of a study of the R^1 position in this series of CRF_1 antagonists. Analogous to the R^2 site, the R^1 side chains also appear to be sensitive to the size and shape of the alkyl substituent. Removal of the cyclopropylmethyl side chain resulted in significant loss of biological activity (**11c**, pK_i = 6.1). From the rebuilding of the alkyl chain at R^1, it was concluded that four carbon atoms are

Scheme 3[a]



[a] (a) HNO_3, H_2SO_4, 55 °C, 33%; (b) H_2, Pd/C 95%; (c) ethyl acetoacetate, toluene, Δ; (d) Ph_2O, 200 °C, 46% (two steps); (e) $POCl_3$, MeCN, Δ, 91%; (f) Br_2, MeOH, 98%; (g) (*S*)-(+)-2-amino-1-butanol, *p*-TsOH, 140 °C; (h) 48% aqueous HBr, 130 °C, 43% (two steps); (i) alkyl halides, NaH, DMF, 25 °C, 94%; (j) boronic Acids, $Pd(Ph_3P)_4$, aqueous Na_2CO_3, $Ba(OH)_2$, EtOH, toluene, 60%.

Scheme 4[a]



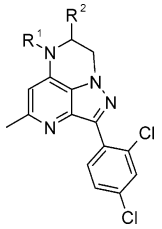
[a] (a) $Bu_3Sn(CHCHOEt)$, $PdCl_2(PP_3)_2$, PhMe, 100 °C; (b) 1 N HCl, THF, 100%; (c) bis(pinacolato)diboron, $PdCl_2(dppf)$, KOAc, PhMe, 100 °C, 62%; (d) **22**, $Pd(Ph_3)_4$, $Na_2CO_{3(aq)}$, $Ba(OH)_{2(aq)}$, PhMe, EtOH, 95%; (e) MeMgBr, THF, −78 °C; (f) TFA, $MgSO_4$, TsOH(cat.), PhMe, 100 °C, 45%; (g) H_2, PtO, EtOH, 40 atm; (h) HCl, Et_2O, 70%.

necessary for high affinity. The methyl (**12h**, pK_i = 5.3), ethyl (**12i**, pK_i = 7.2), propyl (**12j**, pK_i = 7.5), and isopropyl (**12l**, pK_i = 7.1) derivatives were all less active than the cyclopropylmethyl (**12a**, pK_i = 8.5), *n*-butyl (**12k**, pK_i = 8.2), or isobutyl (**12m**, pK_i = 7.8) analogues. Branching of the alkyl substituent results in a moderate loss of affinity when comparing the isobutyl derivative **12m** versus the more compact cyclopropylmethyl analogue **12a**. This observation is further substantiated by the decrease of affinity observed when the ring size is increased to cyclobutyl (**12n,** pK_i = 7.7) or benzyl (**12o**, pK_i = 6.8). Another strategy for reducing lipophilicity in the alkyl side chains involves replacing carbon atoms with oxygen atoms for carbon, and despite the success of this strategy in previous reports,[17] the 2-methoxyethyl derivative **12p** was 17-fold less active in the binding assay.

An alternative top region SAR study was performed on the basis of the observation that when R^2 = H, the trend of branching in the R^1 region and binding was reversed. Unlike the previous example (with R^2 = ethyl), the isopropyl analogue (**12r**, pK_i = 7.1) was more active than the isobutyl derivative **12q** (pK_i = 6.4), suggesting a distinct SAR in the R^2 = H series. As a consequence, additional analogues with increased branching were prepared and evaluated for CRF_1 binding. Addition of two (**12s**, pK_i = 8.4), four (**12t**, pK_i = 8.3), and six (**12u**, pK_i = 8.3) methylene units to the branched alkyl side chain increased the affinity for the receptor. Tying the branched alkyl group back into a cyclohexyl ring (**12v**, pK_i = 6.9) system led to a significant loss of activity, supporting the contention that the hydrophobic pocket that these side chains interact with has a distinct shape that is long (five to seven atoms) and narrow. Analogous to **12p**, exchanging methylene units for oxygen also led to a decrease of binding affinity, as illustrated by compound **12w** (pK_i = 7.2). More polar side chains may adopt a completely different conformation upon receptor binding, and it is possible that adopting the necessary conformation is prevented by the more rigid analogues **12p** and **12w** in contrast to the more flexible DMP 696 (**4**).

Table 2 summarizes the SAR studies completed in regards to the bottom region of the tricyclic series. Goals

Table 1. Top Region SAR



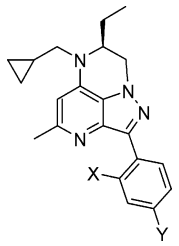
compd	R^1	R^2	pK_i [a]
1			8.6 ± 0.3
3			8.8 ± 0.2
12a	*c*-propylmethyl	(*S*)-ethyl	8.5 ± 0.1
12b	*c*-propylmethyl	(*R*)-ethyl	6.9 ± 0.1
12c	*c*-propylmethyl	H	7.1 ± 0.1
12d	*c*-propylmethyl	(*S*)-methyl	8.1 ± 0.1
12e	*c*-propylmethyl	(*S*)-propyl	8.4 ± 0.3
12f	*c*-propylmethyl	(*S*)-isopropyl	8.3 ± 0.2
12g	*c*-propylmethyl	(*S*)-phenyl	8.0 ± 0.3
11c	H	(*S*)-ethyl	6.1 ± 0.1
12h	methyl	(*S*)-ethyl	5.3 ± 0.2
12i	ethyl	(*S*)-ethyl	7.2 ± 0.1
12j	propyl	(*S*)-ethyl	7.5 ± 0.1
12k	butyl	(*S*)-ethyl	8.2 ± 0.1
12l	isopropyl	(*S*)-ethyl	7.1 ± 0.1
12m	isobutyl	(*S*)-ethyl	7.8 ± 0.2
12n	*c*-butylmethyl	(*S*)-ethyl	7.7 ± 0.1
12o	Bn	(*S*)-ethyl	6.8 ± 0.3
12p	2-methoxyethyl	(*S*)-ethyl	7.3 + 0.1
12q	isobutyl	H	6.4 ± 0.2
12r	isopropyl	H	7.1 ± 0.1
12s	3-pentyl	H	8.4 ± 0.1
12t	4-heptyl	H	8.3 ± 0.1
12u	5-nonyl	H	8.3 ± 0.1
12v	*c*-hexyl	H	6.8 ± 0.1
12w	1-MeO-2-butyl	H	7.2 ± 0.2

[a] Mean pK_i ± SEM, h-CRF_1.

of the study were twofold: (1) to find the optimal aryl substituents that would allow for decreasing carbon content in the top region and (2) to identify polar (less lipophilic) substituents to help offset the contribution of the top region side chains to the overall lipophilicity. Substitution of chlorine at the 2-position of the bottom aromatic (X = Cl) with alternatives led to a decrease in affinity regardless of the substituent. The electron-withdrawing trifluoromethyl (**23a**, pK_i = 7.7) and fluoro (**23d**, pK_i = 7.3) groups as well as electron-donating groups such as methyl (**23b**, pK_i = 8.1) and methoxy (**23c**, pK_i = 7.6) failed to increase the affinity of these analogues for the receptor. In this region, it appears that the shape of the substituent is more important than the electronic nature for high-affinity antagonist binding. This is also consistent with earlier observations that the 2-substituent steers the bottom aromatic ring out of plane relative to the core heterocycle.[23]

In contrast to the 2-position, many of the similar changes at the 4-position of the aromatic side chain offered analogues equipotent to **12a** independent of the electronic nature of the substituents. The trifluoromethyl (**23e**, pK_i = 8.4), methyl (**23f**, pK_i = 8.2), methoxy (**23g**, pK_i = 8.4), and fluoro (**23h**, pK_i = 7.9) substituted analogues bound with comparable affinity as **12a**. Unfortunately the polar, electron-withdrawing carbomethoxy substituent reduced the binding affinity as seen in **23i** (pK_i = 6.7). In an attempt to elucidate a synergistic relationship between the bottom region aromatic substituents, the groups that yielded the highest affinity analogues were combined. The dimethyl (**23j**, pK_i = 7.9), bis-trifluoromethyl (**23l**, pK_i = 8.1), and *p*-methoxy substituted **23k** (pK_i = 8.0) and **23m** (pK_i = 8.1) did not increase CRF_1 binding. The 4-isopropyl derivative **23n** (pK_i = 8.1) also did not increase binding, suggesting that increasing the size of the 4-substituent would not further increase antagonist binding.

Table 2. Bottom Region SAR Summary



compd	X	Y	pK_i [a]
12a	Cl	Cl	8.5 ± 0.1
23a	CF_3	Cl	7.7 ± 0.4
23b	CH_3	Cl	8.1 ± 0.3
23c	CH_3O	Cl	7.6 ± 0.1
23d	F	Cl	7.3 ± 0.1
23e	Cl	CF_3	8.4 ± 0.2
23f	Cl	CH_3	8.2 ± 0.2
23g	Cl	CH_3O	8.4 ± 0.1
23h	Cl	F	7.9 ± 0.1
23i	Cl	COOMe	6.8 ± 0.1
23j	CH_3	CH_3	7.9 ± 0.1
23k	CH_3	CH_3O	8.0 ± 0.1
23l	CF_3	CF_3	8.1 ± 0.4
23m	CF_3	CH_3O	8.1 ± 0.1
23n	CF_3	isopropyl	8.1 ± 0.3

[a] Mean pK_i ± SEM, h-CRF_1.

The tactic of restricting the flexibility of the top region side chains resulted in **12a**, which was the highest affinity CRF_1 antagonist identified in this study. Surprisingly compound **12a** was significantly less lipophilic than compound **3** with experimentally determined log $D_{7.4}$ values of 4.4 and 5.3, respectively. The tricyclic core structure appears to be more basic (pK_a value of 7.93 was measured for the conjugate acid of **12a**) than the starting bicyclic heterocycle (pK_a = 7.5 for the conjugate acid of **3**) presumably because of restriction of rotation of the nitrogen lone pair of electrons at C-7. As a consequence, compound **12a** (NBI 35965) was further evaluated for functional CRF_1 antagonism both in vitro and in vivo.

Measurement of inhibition of CRF-induced cAMP production was performed in the same cell line as that used in the binding studies above but using a live whole-cell preparation to determine intracellular cAMP accumulation. To further elucidate the functional antagonism of these compounds, CRF-stimulated ACTH release was assessed in cultured rat anterior pituitary cells. The IC_{50} values were essentially identical for either the second messenger cAMP (pIC_{50} = 7.1) or the release of ACTH (pIC_{50} = 6.9), demonstrating that this compound acts as a functional antagonist. The equivalent potency determined for compounds in both the human receptor (cAMP) or rat receptor (ACTH) assay demonstrated that these compounds do not exhibit any species selectivity for the CRF_1 receptor. In the absence of agonist in these preparations, there was no cAMP accumulation or ACTH release, demonstrating that **12a** exhibited no intrinsic agonist activity. In addition, in cells expressing

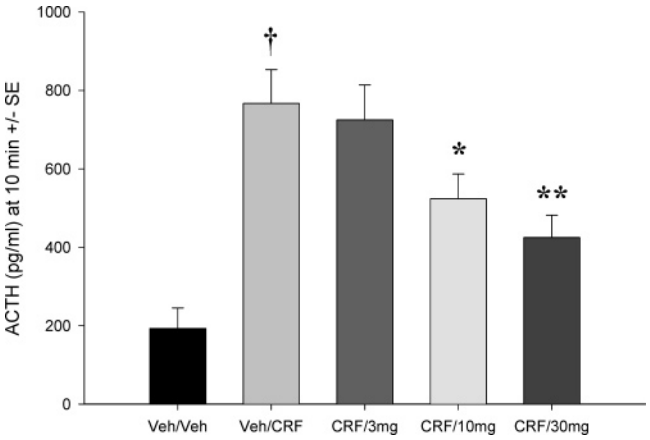


Figure 3. Effects of **12a** administered orally (3, 10, 30 mg/kg) on CRF-induced ACTH release. Dose-dependent effects on CRF-induced ACTH release 10 min following CRF administration: (†) $p < 0.0001$ vs vehicle; (∗) $p < 0.04$ vs CRF; (∗∗) $p < 0.0004$ vs CRF.

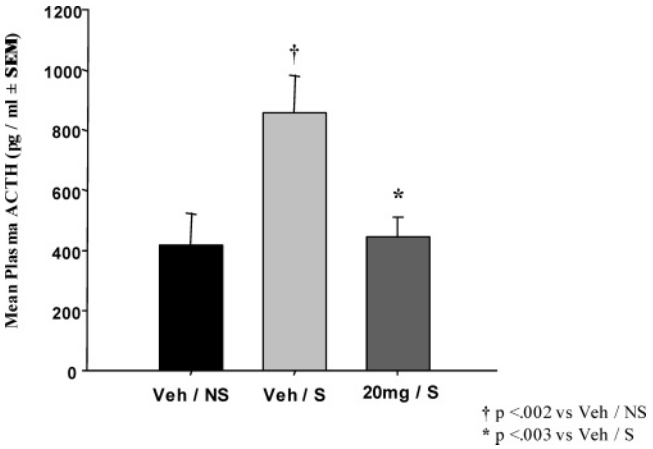


Figure 4. Effect of oral administration of **12a** on increased plasma ACTH levels following 45 min of restraint stress in male CD-1 mice. Animals ($n = 10$/group) were administered either vehicle or 20 mg/kg **12a** 1 h prior to 45 min of restraint stress and assessed for plasma levels of ACTH. The increase in ACTH due to restraint stress was completely attenuated with prior oral administration of **12a**: Veh, vehicle-treated; NS, no Stress; S, stress.

the human or rat CRF_2 receptor **12a** was unable to inhibit [^{125}I]sauvagine binding, clearly establishing the CRF subtype selectivity of this molecule. Recent studies utilizing [^{3}H]-**12a** have detailed the mechanism of the inhibition of CRF-stimulated responses by non-peptide molecules. The data demonstrated that non-peptide antagonists and peptides bind to spatially distinct binding sites with a weak interaction between their binding and that the nature of this inhibition is consistent with allosteric modulation of agonist activity.[24]

Compound **12a** was then subjected to a pharmacokinetic evaluation in rats prior to evaluating in vivo functional antagonism. The estimated oral bioavailability was 34% with a mean maximal plasma concentration at 1 h of 560 ng/mL. Compound **12a** has a volume of distribution 17.8 L/kg, a plasma clearance of 17 mL min^{-1} kg^{-1}, and a half-life of 12 h. The compound also penetrated the blood−brain barrier, resulting in a mean maximal brain concentration of 700 ng/g, and in light of these results compound **12a** was further evaluated in vivo.

The in vivo functional antagonism of compound **12a** was determined using both the CRF-induced ACTH release in normal rats and the restraint stress model of ACTH release in mice. Figure 3 shows that in rats intravenous CRF administration produced a robust increase in the plasma levels of ACTH ($p < 0.0001$). Oral administration of compound **12a** significantly attenuated this effect in a dose-dependent manner with statistically significant reductions observed at the 10 and 30 mg/kg doses ($p < 0.04$−0.004 vs CRF). The magnitude of these attenuations ranged from 43% to 60%. We further characterized the in vivo functional effects of compound **12a** in a mouse restraint stress model. While the end surrogate measure is the same (plasma ACTH concentration), restraint stress is generally considered to be an emotional or psychological stressor.[25,26] For brief restraint-stress in mice, compound **12a** (20 mg/kg, po) was able to completely attenuate the stress-induced increase in plasma ACTH to basal levels, which again demonstrated the functional activity of this molecule as a CRF receptor antagonist (Figure 4).

In addition to the data described above, we have recently examined the effects of CRF_1 receptor antagonism in models of gastric and colonic motility. It is well-documented that stress, mediated through the CRF system, plays a major role in the physiology of the gastrointestinal tract.[27] Preclinical and clinical data have demonstrated that a variety of acute stressors inhibit gastric emptying and increase colonic motility.[28,29] We have shown that CRF and related peptides, injected directly into the central nervous system of mice or rats, inhibit gastric emptying and stimulate distal colonic motor function. Oral administration of the CRF_1 receptor antagonist, NBI-35965 (compound **12a**), reversed the icv CRF-induced colonic motility, demonstrating that the effects of CRF on the lower gut are mediated by the CRF_1 receptor.[30,31]

Conclusions

The objective of this study was to see if the overall lipophilicity of the pyrazolo[4,3-*b*]pyridine class of CRF_1 antagonists could be decreased. Strategically, it was anticipated that restricting the side chain conformational flexibility would ultimately lead to new series of tricyclic antagonists that would possess intrinsically higher affinity for the CRF_1 receptor. The conclusion from these studies is that the new tricyclic series of antagonists are equipotent to their bicyclic counterparts with an experimentally determined log $D_{7.4}$ value of 4.4 due to the surprisingly basic nature of the core heterocycle. Compound **12a** (NBI 35965) proved to be one of the highest affinity compounds for the receptor and was studied further both in vitro and in vivo. These studies confirmed that **12a** is a functional CRF_1 antagonist with improved physicochemical properties and penetrates the central nervous system. Additional studies will be disclosed in due course.

Experimental Section

Synthetic Chemistry. General Methods. Nuclear magnetic resonance (^{1}H, ^{13}C NMR) spectra were obtained with a Varian 300 MHz spectrometer (Mercury) or with a Bruker 500 MHz spectrometer at Numega Labs Inc., San Diego, CA. The chemical shifts are reported in parts per million (δ) downfield

using TMS as the internal standard and $CDCl_3$ as the solvent except where indicated. Matrix-assisted laser desorption/ionization (MALDI) FTMS experiments were performed on an IonSpec FTMS mass spectrometer at The Scripps Research Institute, San Diego, CA. Samples were irradiated with a nitrogen laser operating at 337 nm, and the laser beam was controlled by a variable attenuator and focused on the sample target. Fast atom bombardment (FAB) analysis was carried out on M-Scan's VG Analytical ZAB 2-SE high-field mass spectrometer at M-Scan Inc., West Chester, PA. A cesium ion gun was used to generate ions for the acquired high-resolution mass spectra. Optical activity was measured at 589 nm, 25 °C, using a Perkin-Elmer 341 polarimeter. LC−MS analyses were performed on a Perkin-Elmer Sciex API-100 mass spectrometer using the electron spray ionization technique or on a SpectraSystem P4000 HPLC system coupled with a Finnigan LCD/Deca mass spectrometer using the electrospray ionization technique. Purification of final compounds was conducted on a prep-LCMS Dionex system, using an Alpha C18 30 mm × 75 mm reverse-phase column at a flow rate of 45 mL/min. The mobile phase was a gradient of 95/5 to 5/95 A/B: A= H_2O−0.1%TFA, B = CH_3CN−0.1% TFA. All compounds after purification were reanalyzed on a reverse-phase HPLC−MS system (DIONEX with ESI+ ionization mode, Agilent 1100 or Berger SFC with Agilent 1100). Chiral purity was determined using chiral reverse phase HPLC utilizing a Phenomenex Sumichiral R-VAL & S-NEA, 5 m, 4.6 mm × 150 mm with 87% buffer (10 mM NH_4OAc, pH 4.0)/13% acetonitrile at a flow rate of 1.0 mL/min (total run time of 25 min). The log $D_{7.4}$ and pK_a determinations were performed at Robinson Microlit Laboratories, Madison, NJ, using the GlpKa potentiometric system. All commercially available reagents were used without further purification.

2′-Phthalimide-2,4-dichloroacetophenone (6). To a solution of potassium phthalimide (4.49 kg, 24.2 mol) in anhydrous DMF (8 L) was added **5** (3.56 kg, 15.9 mol) in anhydrous DMF (6 L). The reaction mixture was stirred under room temperature for 2 h, and then the reaction mixture was partitioned between water (15 L) and ethyl acetate (20 L). The aqueous phase was extracted three additional times with ethyl acetate (15 L) each time. The organic extracts were combined and volatiles were evaporated. The resulting orange solid was slurried with 1 L of methyl *tert*-butyl ether (MTBE) and then filtered and washed with MTBE to afford an off-white solid **6** (5.37 kg, 71%) mp 134 °C; ^{1}H NMR ($CDCl_3$) δ 7.85−7.86 (m, 2H), 7.70−7.72 (m, 2H), 7.68 (d, J = 5.0 Hz, 1H), 7.45 (d, J = 0.8 Hz, 1H), 7.33 (dd, J = 5.0, 1.2 Hz, 1H), 5.03 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 192.5, 167.7, 139.1, 134.4, 134.0, 133.3, 132.2, 131.6, 130.9, 127.8, 123.7, 123.6, 47.1; MS (Cl) *m/z* 334.1 (MH^+); GC t_R = 8.25 min; ESI-TOF-HRMS *m/z* calcd for $C_{16}H_9Cl_2NO_3$ (MH^+) 334.0032, found 334.0031. Anal. ($C_{16}H_{19}Cl_2NO_3 \cdot {}^1/_3C_8H_5NO_2$) C, H, N.

1-(2,4-Dichlorophenyl)-3-(dimethylamino)-2-phthalimidepropenone (7). A solution of **6** (5.0 kg, 15 mol) in dimethylformamide dimethylacetal (11.0 kg, 92 mol) was heated at 82 °C for 9 h. The reaction mixture was cooled to ambient temperature, and the resulting solid was filtered and washed with MTBE (2L) to affording **7** as a light-yellow, granular solid (4.3 kg, 74%): mp 219 °C; ^{1}H NMR ($CDCl_3$) δ 7.92 (s, 2H), 7.76 (s, 2H), 7.45 (s, 1H), 7.38 (d, J = 5.0 Hz, 1H), 7.25 (d, J = 5.0 Hz, 1H), 7.03 (s, 1H), 2.97 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 1.86.3, 169.1, 154.4, 137.6, 135.5, 134.4, 132.5, 130.3, 130.0, 127.1, 123.9, 103.7; MS (Cl) *m/z* 389.0 (MH^+). Anal. ($C_{19}H_{14}Cl_2N_2O_3$) C, H, N.

3-Amino-4-(2,4-dichlorophenyl)pyrazole (8). A suspension of **7** (1.42 kg, 3.65 mol) in ethanol (12 L) and water (1.2 L) was heated at 100 °C. Hydrazine monohydrochloride (500 g, 7.3 mol) was added, and the reaction mixture was refluxed for 7 h. Then hydrazine monohydrate (355 mL, 7.3 mol) was added dropwise over 10 min, and the mixture was allowed to reflux for 1 h. The reaction mixture was cooled to room temperature, water (2 L) was added, and the sample was filtered through Celite and concentrated in vacuo. The residue was partitioned between ethyl acetate (10 L) and water (5 L), and the pH of the aqueous phase was adjusted to >7.5 with aqueous NaOH. Then the aqueous phase was extracted with ethyl acetate (2 × 5 L), dried ($MgSO_4$), and evaporated. The crude material was crystallized from dichloromethane (4 L) and hexane (3 L) to yield the desired product **8** as an off-white solid (761 g, 91%): mp 106 °C; ^{1}H NMR ($CDCl_3$) δ 7.43 (d, J = 1.2 Hz, 1H), 7.28 (d, J = 5.3 Hz, 1H), 7.20 (dd, J = 5.0, 1.2 Hz, 1H), 7.13 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 134.895, 133.799, 132.502, 129.808, 129.235, 127.434, 127.127; MS (Cl) *m/z* 228.0 (MH^+). Anal. ($C_9H_7Cl_2N_3$) C, H, N.

3-(2,4-Dichlorophenyl)-5-methyl-7-hydroxypyrazolo-[4,3-*b*]pyridine (9). Ethyl acetoacetate (739 g, 5.68 mol) and *p*-toluenesulfonic acid (10.5 g, 0.055 mol) were added to a solution of **8** (1.296 kg, 5.68 mol) in toluene (12.9 L). The mixture was heated to reflux with a Dean−Stark trap and after 4.5 h concentrated in vacuo. The resulting dark oil was dissolved in diphenyl ether (4.0 L) and slowly added to a flask with diphenyl ether (1 L) preheated to 250 °C. The addition was performed over 2 h while maintaining internal reaction temperature above 230 °C. The reaction mixture was cooled to 70 °C, and heptane (6 L) was added. The resulting precipitate was collected by filtration and the crude material was triturated with THF (2.5 L) to afford **9** (1.28 kg, 77%) as an off-white powder: mp 327 °C; ^{1}H NMR (CD_3OD) δ 7.67 (d, J = 1.2 Hz, 1H), 7.56 (d, J = 5.0 Hz, 1H), 7.47 (dd, J = 5.0, 1.2 Hz, 1H), 6.15 (s, 1H), 2.44 (s, 3H); ^{13}C NMR (CD_3OD) δ 170.91, 151.61, 137.06, 136.46, 134.58, 133.52, 131.00, 130.75, 129.59, 128.71, 119.96, 110.66, 19.57; MS (Cl) *m/z* 294.0 (MH^+). Anal. ($C_{13}H_9Cl_2N_3O$) C, H, N.

7-Chloro-3-(2,4-dichlorophenyl)-5-methylpyrazolo[4,3-*b*]pyridine (10). Dimethylformamide (121 mL, 1.57 mol) was added to a solution of **9** (1.85 kg, 6.29 mol) in anhydrous acetonitrile (9 L), followed by the slow addition of phosphorus oxychloride (2 kg, 13 mol), maintaining the internal temperature below 50 °C. The mixture was heated at reflux for 3 h, and then the mixture was cooled and concentrated in vacuo. Ice (4.5 kg) and water (3 L) were added to the residue, and the solution was carefully neutralized to approximately pH 7.0 with 10 N aqueous NaOH (4 L). The precipitate was collected by filtration and washed with water. The product was dried in vacuo to affording the desired product **10** (1.88 kg, 95%): mp 240 °C; ^{1}H NMR (DMSO) δ 7.82−7.95 (m, 2H), 7.50−7.70 (m, 2H), 2.65 (s, 3H); MS (Cl) *m/z* 312.9 (MH^+). Anal. ($C_{13}H_8Cl_3N_3$) C, H, N.

2-(2,4-Dichlorophenyl)-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (11a). Procedure A. A 1 L round-bottom flask was charged with **10** (32.8 g, 105 mmol), *p*-toluenesulfonic acid (40.0 g, 210 mmol), and ethanolamine (38 g, 630 mmol). The mixture was heated at 140 °C for 3 h. The mixture was then cooled and diluted with aqueous hydrobromic acid (48%, 500 mL) and reheated to 140 °C for 30 h. The mixture was then cooled, poured onto ice, neutralized with 6 N NaOH, and then extracted with ethyl acetate. The organic phase was dried over magnesium sulfate and concentrated in vacuo. The crude material was filtered through a plug of silica gel, eluting with 1:4 EtOH/ethyl acetate and affording **11a** (21.4 g, 64%) of as a light-brown foam. To 1.0 g of product in 10 mL of dichloromethane at 0 °C was charged 1.7 mL (3.4 mmol, 1.1 equiv) of 2.0 M HCl in ether. The solvent was removed in vacuo, affording a light-yellow foam. ^{1}H NMR ($CDCl_3$) δ 7.86 (d, J = 8.8 Hz, 1H), 7.51 (d, J = 1.8 Hz, 1H), 7.32 (dd, J = 1.8, 8.7 Hz), 6.26 (s, 1H), 4.93 (br s, 1H), 4.50 (t, J = 5.3 Hz, 2H), 3.86−3.82 (m, 2H), 2.56 (s, 3H); MS (Cl) *m/z* 319.0 (MH^+); ESI-TOF-HRMS *m/z* calcd for $C_{15}H_{12}Cl_2N_4$ (MH^+) 319.0512, found 319.0511. Anal. ($C_{15}H_{12}Cl_2N_4 \cdot HCl$) C, H, N.

(*S*)-2-(2,4-Dichlorophenyl)-4,7-dimethyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (11b). 11b was prepared as a white powder in 13% yield from **10** and (*S*)-(+)-2-amino-1-propanol according to procedure A. ^{1}H NMR ($CDCl_3$) δ 7.88 (d, J = 8.4 Hz, 1 H), 7.53 (d, J = 1.8 Hz, 1 H), 7.35 (dd, J = 8.3, 2.3 Hz, 1 H), 6.28 (s, 1 H), 4.61−4.51 (m, 2 H), 4.10−4.02 (m, 2 H), 2.58 (s, 3 H), 1.49 (d, J = 6.3 Hz, 3 H); MS (CI) *m/z* 332.9 (MH^+). Anal. ($C_{16}H_{14}Cl_2N_4 \cdot 0.5H_2O$) C, H, N.

(*S*)-2-(2,4-Dichlorophenyl)-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (11c). 11c was prepared as an off-white solid in a 72% yield from **10** according to procedure A and (*S*)-(+)-2-amino-1-butanol. ^{1}H NMR ($CDCl_3$) δ 7.89 (d, J = 8.4 Hz, 1 H), 7.52 (d, J = 2.1 Hz, 1 H), 7.34 (dd, J = 8.4 Hz, 1 H), 6.27 (s, 1 H), 4.63 (br s, 1 H), 4.60 (dd, J = 11.7, 3.3 Hz, 1 H), 4.10 (dd, J = 11.9, 8.9 Hz, 1 H), 3.88−3.78 (m, 1 H), 2.57 (s, 3 H), 1.81 (dq, J = 7.4, 7.4 Hz, 2 H), 1.14 (t, J = 7.5 Hz, 3 H); MS (CI) m/z 347.0 (MH^+). Anal. ($C_{17}H_{16}Cl_2N_4$) C, H, N.

(*R*)-2-(2,4-Dichlorophenyl)-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (11d). 11d was prepared as an off-white solid in a 50% yield from **10** according to procedure A and (*R*)-(−)-2-amino-1-butanol. ^{1}H NMR (CD_3OD) δ 7.62 (dd, J = 1.0, 14.0 Hz, 1H), 7.63 (s, 1 H), 7.44 (dd, J = 14.0, 1.0 Hz, 1H), 6.34 (s, 1 H), 4.54 (dd, J = 12.5, 4.0 Hz, 1H), 4.07 (dd, J = 12.5, 9.0 Hz, 1H), 3.87−3.83 (m, 1H), 2.47 (s, 3H), 1.65−1.95 (m, 2H), 1.38 (t, J = 7.0 Hz, 3 H); MS (Cl) m/z 347.1 (MH^+). ESI-TOF-HRMS m/z calcd for $C_{17}H_{16}Cl_2N_4$ (MH^+) 347.0825, found 347.0821.

(*S*)-2-(2,4-Dichlorophenyl)-4-methyl-7-propyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (11e). 11e was prepared as a yellow powder in 18% yield from **10** and (*S*)-(+)-2-amino-1-pentanol according to procedure A. ^{1}H NMR ($CDCl_3$) δ 7.89 (d, J = 8.4 Hz, 1 H), 7.53 (d, J = 1.8 Hz, 1 H), 7.35 (dd, J = 8.3 Hz, 1 H), 6.27 (s, 1 H), 4.60 (br s, 1 H), 4.59 (dd, J = 12.0, 3.6 Hz, 1 H), 4.10 (dd, J = 12.0, 9.0 Hz, 1 H), 3.96−3.87 (m, 1 H), 2.57 (s, 3 H), 1.78−1.69 (m, 2 H), 1.62−1.52 (m, 2 H), 1.04 (t, J = 7.4 Hz, 3 H); MS (CI) m/z 360.8 (MH^+). Anal. ($C_{18}H_{18}Cl_2N_4$) C, H, N.

(*S*)-2-(2,4-Dichlorophenyl)-7-isopropyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (11f). 11f was prepared as a yellow powder in 17% yield from **10** and (*S*)-(+)-2-amino-3-methyl-1-butanol according to procedure A. ^{1}H NMR: ($CDCl_3$) δ 7.90 (d, J = 8.1 Hz, 1 H), 7.53 (d, J = 1.8 Hz, 1 H), 7.35 (dd, J = 8.3, 2.3 Hz, 1 H), 6.30 (s, 1 H), 4.60 (dd, J = 12.3, 3.9 Hz, 1 H), 4.56 (br s, 1 H), 4.19 (dd, J = 11.9, 9.2 Hz, 1 H), 3.76−3.69 (m, 1 H), 2.58 (s, 3 H), 2.09−2.00 (m, 1 H), 1.15 (d, J = 6.6 Hz, 3 H), 1.14 (d, J = 7.2 Hz, 3 H); MS (CI) m/z 360.9 (MH^+). Anal. ($C_{18}H_{18}Cl_2N_4$) C, H, N.

(*S*)-2-(2,4-Dichlorophenyl)-4-methyl-7-phenyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (11g). 11g was prepared as a yellow solid in a 42% yield from **10** according to procedure A and (*S*)-(−)-2-amino-1-butanol. ^{1}H NMR ($CDCl_3$) δ 7.84 (d, J = 5.0 Hz, 1H), 7.50 (d, J = 1.3 Hz, 1H), 7.44−7.40 (m, 1H), 7.31 (dd, J = 1.3, 5.0 Hz, 1H), 6.33 (s, 1H), 5.05−4.96 (m, 1H), 4.66 (dd, J = 2.4, 7.4 Hz, 1H), 4.28 (dd, J = 5.8, 7.4 Hz, 1H), 2.58 (s, 3H); MS (Cl) m/z 395.1 (MH^+); ESI-TOF-HRMS m/z calcd for $C_{21}H_{16}Cl_2N_4$ (MH^+) 395.0825, found 395.0825.

(*S*)-6-Cyclopropylmethyl-2-(2,4-dichlorophenyl)-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene Mesylate (12a). Procedure B. A solution of **11c** (13.5 g, 38.9 mmol) in anhydrous DMF (60 mL) was cooled to 5 °C with an ice−water bath. NaH (95% suspension in oil, 1.1 g, 46.7 mmol) was added, and the mixture was stirred for 10 min. To the mixture was added bromomethylcyclopropane (6.3 g, 46.7 mmol), and the mixture was stirred at ambient temperature for 1 h. Additional NaH (0.2 g, 7.8 mmol) and bromomethylcyclopropane (1.0 g, 7.8 mmol) were added, and the reaction mixture was stirred for an additional hour. The reaction was quenched with saturated aqueous ammonium chloride, and the mixture was concentrated in vacuo. The residue was diluted with ethyl acetate, washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified via flash chromatography on silica gel, eluting with 1:2 ethyl acetate/hexane to give **12a** (13.1 g, 84%) as an oil.

Procedure C. To a solution of **12a** free-base (11.6 g, mmol) in dichloromethane (50 mL) was added methanesulfonic acid (1.74 mL, 26.8 mmol). The mixture was concentrated in vacuo, and the resulting oil was triturated with 1:10 dichloromethane/ether with stirring overnight. The solid was collected by filtration and washed with ether to afford **12a** methanesulfonate (13.7 g, 100%) as a light-yellow solid: mp 167 °C; ^{1}H NMR ($CDCl_3$) δ 7.56 (d, J = 11.1 Hz, 1H), 7.55 (d, J = 1.7 Hz, 1H), 7.31 (dd, J = 11.1, 1.7 Hz, 1H), 6.28 (s, 1H), 4.70 (d, J = 12.9 Hz, 1H), 4.42 (dd, J = 12.9, 4.7 Hz, 1H), 4.13 (p, J = 4.7 Hz, 1H), 3.75 (dd, J = 14.6, 5.8 Hz, 1H), 3.25 (dd, J = 14.6, 7.0 Hz, 1H), 2.85 (s, 3H), 2.40 (s, 3H), 1.65−1.95 (m, 2H), 1.15 (t, J = 7.0 Hz, 1H), 1.09 (t, J = 7.0 Hz, 3H), 0.65−0.80 (m, 2H), 05.0−0.35 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 153.7, 142.5, 135.9, 134.4, 133.7, 132.2, 128.5, 127.0, 126.3, 124.6, 124.3, 97.2, 58.7, 52.2, 47.2, 38.1, 23.6, 19.7, 9.4, 8.5, 3.1, 2.9; MS (CI) m/z 401.1 (MH^+); $[\alpha]^{250}_D$ −170.0° (c 1.0776 g/100 mL, methanol). Anal. ($C_{21}H_{22}Cl_2N_4 \cdot CH_4O_3S$) C, H, N. Chiral HPLC 99.88% S (AUC), 99.76% ee.

(*R*)-6-Cyclopropylmethyl-2-(2,4-dichlorophenyl)-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene Mesylate (12b). 12b was prepared as a yellow oil in 40% yield from **11d** according to procedures B and C to give **12b** as a mesylate salt. The crude product was purified by flash silica gel chromatography, eluting with 1:2 ethyl acetate/hexane. ^{1}H NMR ($CDCl_3$) δ 7.56 (d, J = 11.1 Hz, 1H), 7.55 (d, J = 1.7 Hz, 1H), 7.31 (dd, J = 11.1, 1.7 Hz, 1H), 6.28 (s, 1H), 4.70 (d, J = 12.9 Hz, 1H), 4.42 (dd, J = 12.9, 4.7 Hz, 1H), 4.13 (p, J = 4.7 Hz, 1H), 3.75 (dd, J = 14.6, 5.8 Hz, 1H), 3.25 (dd, J = 14.6, 7.0 Hz, 1H), 2.85 (s, 3H), 2.40 (s, 3H), 1.65−1.95 (m, 2H), 1.15 (t, J = 7.0 Hz, 1H), 1.09 (t, J = 7.0 Hz, 3H), 0.65−0.80 (m, 2 H), 05.0−0.35 (m, 2H); MS (CI) m/z 401.1 (MH^+); ESI-TOF-HRMS m/z calcd for $C_{21}H_{22}Cl_2N_4$ (MH^+) 401.1289, found 401.1289.

(6)-Cyclopropylmethyl-2-(2,4-dichlorophenyl)-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (12c). 12c was prepared as a yellow oil in 40% yield from **11a** according to procedure B. The crude product was purified by flash silica gel chromatography, eluting with 1:2 ethyl acetate/hexane. ^{1}H NMR ($CDCl_3$) δ 7.89 (d, J = 5.0 Hz, 1H), 7.52 (d, J = 1.2, 1H), 7.33 (dd, J = 5.0, 1.2 Hz, 1H), 6.24 (s, 1H), 4.53 (t, J = 3.3 Hz, 2H), 3.86 (t, J = 3.3 Hz, 2H), 3.30 (d, J = 4.0 Hz, 2H), 2.59 (s, 3H), 1.10 (p, J = 4.1 Hz, 1H), 0.66−0.62 (m, 2H), 0.32−0.29 (m, 2H); MS (CI) m/z 373.1 (MH^+). Anal. ($C_{19}H_{18}Cl_2N_4$) C, H, N.

(*S*)-6-Cyclopropylmethyl-2-(2,4-dichlorophenyl)-4,7-dimethyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (12d). 12d was prepared as a yellow powder in 47% yield from **11b** according to procedure B. ^{1}H NMR ($CDCl_3$) δ 7.89 (d, J = 8.4 Hz, 1H), 7.53 (d, J = 1.8 Hz, 1H), 7.36 (dd, J = 8.4, 2.8 Hz, 1H), 6.21 (s, 1 H), 4.48 (dd, J = 12.3, 2.7 Hz, 1H), 4.37 (dd, J = 12.3, 2.7 Hz, 1H), 4.28−4.19 (m, 1H), 3.50 (dd, J = 14.6, 6.2 Hz, 1H), 3.10 (dd, J = 14.4, 7.2 Hz, 1H), 2.61 (s, 3H), 1.37 (d, J = 6.6 H, 3H), 1.18−1.04 (m, 1H), 0.74−0.60 (m, 2H), 0.42−0.26 (m, 2H); MS (CI) m/z 387.1 (MH^+). Anal. ($C_{20}H_{20}Cl_2N_4$) C, H, N.

(*S*)-6-Cyclopropylmethyl-2-(2,4-dichlorophenyl)-4-methyl-7-propyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (12e). 12e was prepared as a yellow oil in 55% yield from **11e** according to procedure B. ^{1}H NMR ($CDCl_3$) δ 7.90 (d, J = 8.4 Hz, 1H), 7.54 (d, J = 2.4 Hz, 1H), 7.36 (dd, J = 8.4, 2.1 Hz, 1H), 6.19 (s, 1H), 4.56 (dd, J = 12.3, 1.8 Hz, 1H), 4.36 (dd, J = 12.3, 3.3 Hz, 1H), 4.04−3.97 (m, 1H), 3.58 (dd, J = 14.4, 5.7 Hz, 1H), 3.06 (dd, J = 14.4, 6.9 Hz, 1H), 2.61 (s, 3H), 1.73−1.33 (m, 4H), 1.13−1.09 (m, 1H), 0.94 (t, J = 7.2 Hz, 3H), 0.70−0.62 (m, 2H), 0.37−0.28 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 157.9, 141.0, 139.6, 134.6, 134.2, 133.4, 130.4, 130.0, 127.4, 125.3, 98.2, 57.4, 52.1, 48.9, 32.9, 29.9, 25.4, 19.9, 14.1, 9.6, 4.9, 3.4; HRMS (FAB) m/z calcd for $C_{22}H_{24}Cl_2N_4$ (MH^+) 415.1451, found 415.1447.

(*S*)-6-Cyclopropylmethyl-2-(2,4-dichlorophenyl)-7-isopropyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (12f). 12f was prepared as a yellow oil in 37% yield from **11f** according to procedure B. ^{1}H NMR ($CDCl_3$) δ 7.89 (d, J = 8.9 Hz, 1H), 7.53 (d, J = 2.1 Hz, 1H), 7.36 (dd, J = 8.3, 2.3 Hz, 1H), 6.22 (s, 1H), 4.67 (dd, J = 11.4, 1.8 Hz, 1H), 4.31 (dd, J = 12.5, 4.7 Hz, 1H), 3.83−3.73 (m, 2H), 3.01 (dd, J = 14.6, 7.7 Hz, 1H), 2.61 (s, 3H), 2.22−2.10 (m, 1H), 1.18−1.07 (m, 1H), 1.02 (d, J = 6.6 Hz, 3H), 0.85 (d, J = 6.6 Hz, 3H), 0.68−0.57 (m, 2H), 0.37−0.25 (m, 2H); MS (CI) m/z 415.1 (MH^+). Anal. ($C_{22}H_{24}Cl_2N_4$) C, H, N.

(*S*)-6-Methyl-2-(2,4-dichlorophenyl)-4-methyl-7-phenyl-7,8-dihydro-6H-1,3,6,8a-tetraazaacenaphthylene (12g). 12g was prepared as a yellow oil in 40% yield from **11a** according to procedure B. The crude product was purified by flash silica gel chromatography, eluting with 1:1 ethyl acetate/hexane. ^{1}H NMR ($CDCl_3$) δ 7.90 (d, J = 5.0 Hz, 1H), 7.52 (d, J = 1.3 Hz, 1H), 7.36−7.34 (m, 1H), 7.22 (d, J = 5.0 Hz, 1H), 6.38 (s, 1H), 5.17 (t, J = 3.0, 1H), 4.69 (dd, J = 2.8, 7.4 Hz, 1H), 4.50 (dd, J = 3.3, 7.4 Hz, 1H), 3.65 (dd, J = 3.0, 8.9 Hz, 1H), 2.78 (dd, J = 4.8, 8.8 Hz, 1H), 2.65 (s, 3H), 1.06−1.02 (m, 1H), 0.56−0.54 (m, 1H), 0.50−0.47 (m, 1H), 0.15−0.12 (m, 1H), 0.08−0.05 (m, 1H); MS (CI) *m/z* 449 0.1 (MH^+); ESI-TOF-HRMS *m/z* calcd for $C_{25}H_{22}Cl_2N_4$ (MH^+) 449.1294, found 449.1296.

(*S*)-6-Methyl-2-(2,4-dichlorophenyl)-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (12h). Compound **12h** was prepared as a white solid in 39% yield from **11c** according to procedure B. ^{1}H NMR ($CDCl_3$) δ 7.88 (d, J = 8.1 Hz, 1H), 7.59 (d, J = 2.0 Hz, 1H), 7.31 (dd, J = 8.0 Hz, 1H), 6.20 (s, 1H), 4.61(dd, J = 11.9, 3.0 Hz, 1H), 4.31 (dd, J = 12.5, 3.0 Hz, 1H), 4.00−3.82 (m, 1H), 3.44 (s, 3H), 2.59 (s, 3 H), 1.81 (dq, J = 7.2, 7.3 Hz, 2H), 1.10 (t, J = 7.6 Hz, 3H); MS (CI) *m/z* 361.0 (MH^+). Anal. ($C_{18}H_{18}Cl_2N_4$) C, H, N.

(*S*)-6-Ethyl-2-(2,4-dichlorophenyl)-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (12i). 12i was prepared as a white solid in 42% yield from **11c** according to procedure B. ^{1}H NMR ($CDCl_3$) δ 7.90 (d, J = 8.3 Hz, 1H), 7.51 (d, J = 2.1 Hz, 1H), 7.29 (dd, J = 8.3 Hz, 1H), 6.15 (s, 1H), 4.51 (dd, J = 11.8, 3.2 Hz, 1H), 4.38 (dd, J = 11.8, 2.9 Hz, 1H), 3.98 (m, 1H), 3.58 (m, 2H), 2.65 (s, 3H), 1.73−1.32 (m, 5H), 0.91 (t, J = 7.3 Hz, 3H); MS (CI) *m/z* 375.1 (MH^+). Anal. ($C_{19}H_{20}Cl_2N_4$) C, H, N.

(*S*)-6-Propyl-2-(2,4-dichlorophenyl)-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (12j). 12j was prepared as a white solid in 48% yield from **11c** according to procedure B. ^{1}H NMR ($CDCl_3$) δ 7.89 (d, J = 8.1 Hz, 1H), 7.51 (d, J = 2.0 Hz, 1H), 7.31 (dd, J = 8.3 Hz, 1H), 6.27 (s, 1H), 4.55 (dd, J = 11.7, 3.3 Hz, 1H), 4.30 (dd, J = 11.9, 3.2 Hz, 1H), 4.00 (m, 1H), 3.51 (m, 2H), 2.57 (s, 3H), 1.92 (dq, J = 7.1, 7.2 Hz, 2H), 1.73 (m, 2H), 1.14−1.01 (m, 6H); MS (CI) *m/z* 389.1 (MH^+). Anal. ($C_{20}H_{22}Cl_2N_4$) C, H, N.

(*S*)-2-(2,4-Dichlorophenyl)-6-butyl-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene TFA (12k). 12k was prepared from **11c** according to procedure B and then purified by preparative TLC to obtain the desired product as a pale-yellow oil (52 mg, 65%). ^{1}H NMR ($CDCl_3$) δ 7.91 (d, J = 8.1 Hz, 1H), 7.53 (d, J = 2.1 Hz, 1H), 7.35 (dd, J = 8.1, 2.1 Hz, 1H), 6.14 (s, 1H), 4.54 (dd, J = 12.5, 2.1 Hz, 1H), 4.33 (dd, J = 12.5, 3.3 Hz, 1H), 3.70 (m, 1H), 3.62 (dt, J = 14.6, 6.8 Hz, 1H), 3.21 (dt, J = 14.6, 7.4 Hz, 1H), 2.59 (s, 3H), 1.58−1.80 (m, 4H), 1.42 (m, 2H), 1.03 (t, J = 3.6 Hz, 3H), 0.99 (t, J = 2.4 Hz, 3H); MS (CI) *m/z* 403.0 (MH^+). Anal. ($C_{21}H_{24}Cl_2N_4$) C, H, N.

(*S*)-2-(2,4-Dichlorophenyl)-7-ethyl-6-isopropyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene TFA (12l). 12l was prepared from **11c** according to procedure B and then purified by preparative LC−MS to obtain the desired product as a TFA salt (14 mg, 23%). ^{1}H NMR ($CDCl_3$) δ 7.55 (d, J = 6.6 Hz, 1H), 7.54 (d, J = 1.6 Hz, 1 H), 7.37 (dd, J = 6.6, 1.6 Hz, 1 H), 6.31 (s, 1 H), 4.74 (dd, J = 10.3 Hz, 1 H), 4.27 (m, 1 H), 4.20 (dd, J = 10.3, 3.1 Hz, 1H), 4.08 (m, 1H), 3.65 (m, 1H), 2.76 (s, 3H), 1.79 (pent, J = 5.5 Hz, 1H), 1.53 (d, J = 5.5 Hz, 3H), 1.47 (d, J = 5.5 Hz, 3H), 1.08 (t, J = 6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 155.2, 142.9, 138.7, 136.7, 134.6, 133.1, 130.1, 127.7, 127.2, 126.9, 125.6, 98.3, 55.8, 51.2, 47.9, 31.2, 26.4, 21.0, 20.7, 10.9; HRMS (FAB) *m/z* calcd for $C_{20}H_{22}Cl_2N_4$ (MH^+) 389.1294, found 389.1299.

(*S*)-2-(2,4-Dichlorophenyl)-7-ethyl-6-isobutyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene Trifluoroacetate (12m). 12m was prepared from **11c** according to procedure B and then purified by preparative LC−MS to obtain the desired product as a TFA salt (12 mg, 19%). ^{1}H NMR: ($CDCl_3$) δ 7.56 (d, J = 6.7 Hz, H), 7.54 (d, J = 1.6 Hz, 1H), 7.38 (dd, J = 6.7, 1.6 Hz, 1H), 6.21 (s, 1H), 4.68 (dd, J = 10.3, 0.8 Hz, 1H), 4.41 (dd, J = 10.3, 3.5 Hz, 1H), 3.88 (m, 1H), 3.66 (dd, J = 11.4, 5.2 Hz, 1H), 3.13 (dd, J = 11.4, 6.8 Hz, 1H), 2.76 (s, 3H), 2.15 (m, 1H), 1.85 (m, 1H), 1.75 (m, 1H), 1.04−1.10 (m, 9H); ^{13}C NMR: ($CDCl_3$) δ 155.4.0, 143.4, 138.9, 136.7, 134.6, 133.1, 130.1, 127.7, 127.2, 126.9, 125.6, 97.9, 61.9, 56.9, 47.9, 31.2, 27.7, 24.5, 21.0, 20.3, 11.0; HRMS (FAB) *m/z* calcd for $C_{21}H_{24}Cl_2N_4$ (MH^+) 403.1451, found 403.1456.

(*S*)-6-Cyclobutylmethyl-2-(2,4-dichlorophenyl)-7-ethyl-4-methyl-7,8-dihydro-6H-1,3,6,8a-tetraazaacenaphthylene Trifluoroacetate (12n). 12n was prepared from **11c** according to procedure B and then purified by preparative LC−MS to obtain the desired product as a TFA salt (21 mg, 22%). ^{1}H NMR ($CDCl_3$) δ 7.54 (d, J = 6.6 Hz, 1H), 7.54 (d, J = 1.6 Hz, 1H), 7.37 (dd, J = 6.6, 1.6 Hz, 1H), 6.25 (s, 1H), 4.66 (dd, J = 10.3, 1.0 Hz, 1H), 4.35 (dd, J = 10.3, 3.3 Hz, 1H), 3.90 (m, 1H), 3.82 (dd, J = 11.5, 5.4 Hz, 1H), 3.60 (m, 1H), 3.44 (dd, J = 11.5, 6.4 Hz, 1H), 2.78 (m, 1H), 2.74 (s, 3H), 2.18 (m, 1H), 1.74−1.87 (m, 4H), 1.08 (t, J = 6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 155.2, 143.5, 138.7, 136.7, 134.6, 133.1, 130.1, 127.7, 127.2, 126.7, 125.6, 97.8, 61.3, 54.2, 48.0, 33.9, 26.8, 26.5, 24.6, 21.0, 18.7, 10.9; HRMS (FAB) *m/z* calcd for $C_{22}H_{24}Cl_2N_4$ (MH^+) 4415.1451, found 415.1445.

(*S*)-6-Benzyl-2-(2,4-dichlorophenyl)-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (12o). 12o was prepared as a white solid in 59% yield from **11c** according to procedure B. ^{1}H NMR ($CDCl_3$) δ 7.89 (d, J = 8.3 Hz, 1H), 7.54−7.49 (m, 4H), 7.31−7.29 (m, 3H), 6.20 (s, 1H), 4.62 (dd, J = 11.9, 3.6 Hz, 1H), 4.37 (dd, J = 12.1, 2.9 Hz, 1H), 3.99 (m, 1H), 3.58 (m, 2H), 2.64 (s, 3H), 1.74−1.53 (m, 2H), 1.01 (t, J = 7.0 Hz, 3 H); MS (CI) *m/z* 437.1 (MH^+). Anal. ($C_{24}H_{22}Cl_2N_4$) C, H, N.

(*S*)-2-(2,4-Dichlorophenyl)-7-ethyl-6-(2-methoxy-ethyl)-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene Trifluoroacetate (12p). 12p was prepared from **11c** according to procedure B and then purified by preparative LC−MS to obtain the desired product as a TFA salt (25 mg, 40%). ^{1}H NMR ($CDCl_3$) δ 7.54 (d, J = 6.6 Hz, 1H), 7.54 (d, J = 1.6 Hz, 1H), 7.37 (dd, J = 6.6, 1.6 Hz, 1H), 6.27 (s, 1H), 4.64 (dd, J = 10.3, 1.1 Hz, 1H), 4.39 (dd, J = 10.3, 3.4 Hz, 1H), 4.10 (m, 1H), 3.94 (m, 1H), 3.64−3.68 (m, 3H), 3.38 (s, 3H), 2.73 (s, 3H), 2.17 (s, 3H), 1.87 (m, 1H), 1.77 (m, 1H), 1.08 (t, J = 6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 155.1, 143.4, 138.7, 136.7, 134.6, 133.1, 130.1, 127.7, 127.3, 126.7, 125.8, 97.8, 70.3, 62.0, 59.6, 49.2, 48.1, 24.7, 20.9, 10.8; HRMS (FAB) *m/z* calcd for $C_{20}H_{22}Cl_2N_4O$ (MH^+) 405.1243, found 405.1238.

1-(2,4-Dichlorophenyl)-5-isobutyl-7-methyl-4,5-dihydro-3*H*-1,2a,5,8-tetraazaacenaphthylene Trifluoroacetate (12q). 12q was prepared from **11a** according to procedure B and then purified by preparative LC−MS to obtain the desired product as a TFA salt (41 mg, 17%). ^{1}H NMR (CD_3OD) δ 7.71 (d, J = 4.2 Hz, 1H), 7.63 (d, J = 5.1 Hz, 1H), 7.52 (dd, J = 4.2, 1.2 Hz, 1H), 6.77 (s, 1H), 4.63 (t, J = 3.6 Hz, 2H), 4.15 (t, J = 3.6 Hz, 2H), 3.56 (d, J = 4.5 Hz, 2H), 2.66 (s, 3H), 2.15−2.95 (m, 1H), 1.06 (d, J = 3.9 Hz); ESI-HRMS *m/z* calcd for $C_{19}H_{20}Cl_2N_4$ (MH^+) 375.1138, found 375.1152; LC−MS *m/z* 375 ($M + H^+$).

2-(2,4-Dichlorophenyl)-6-isopropyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (12r). 12r was prepared in 17% yield from **11a** according to procedure B, and the crude product was purified by flash silica gel chromatography, eluting with 1:2 ethyl acetate/hexane. ^{1}H NMR ($CDCl_3$) δ 7.87 (d, J = 5.0 Hz, 1H), 7.51 (d, J = 1.2 Hz, 1H), 7.33 (dd, J = 5.0, 1.2 Hz, 1H), 6.24 (s, 1H), 4.49 (t, J = 3.2 Hz, 2H), 4.15 (p, J = 4.0 Hz, 1H), 2.59 (s, 3H), 1.32 (d, J = 4.0 Hz, 6H); MS (CI) *m/z* 361.1 (MH^+). Anal. ($C_{18}H_{18}Cl_2N_4$) C, H, N.

1-(2,4-Dichlorophenyl)-5-(1-ethylpropyl)-7-methyl-4,5-dihydro-3*H*-1,2a,5,8-tetraazaacenaphthylene Trifluoroacetate (12s). 12s was prepared from **11a** according to procedure B and then purified by preparative LC−MS to obtain the desired product as a TFA salt (39 mg, 16%). ^{1}H NMR (CD_3OD) δ 7.72 (d, J = 1.2 Hz, 1H), 7.64 (d, J = 4.8 Hz, 1H), 7.52 (dd, J = 1.2, 4.8 Hz), 6.89 (s, 1H), 4.62 (t, J = 3.3 Hz, 2H), 4.152 (m, 1H), 4.03 (t, J = 3.3 Hz, 2H), 2.67 (s, 3H),

1.85−1.79 (m, 4H), 0.97 (t, 3H); ESI-HRMS *m/z* calcd for $C_{20}H_{22}Cl_2N_4$ (MH^+) 389.1294, found 389.1294; LC−MS *m/z* 389 ($M + H^+$).

2-(2,4-Dichlorophenyl)-4-methyl-6-(1-propylbutyl)-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (12t). 12t was prepared as a yellow oil in 58% yield from **11a** according to procedure B, and the crude product was purified by flash silica gel chromatography, eluting with 1:2 ethyl acetate/hexane. ^{1}H NMR ($CDCl_3$) δ 7.60 (d, J = 8.8 Hz, 1H), 7.46 (d, J = 2.3 Hz, 1H), 7.31 (dd, J = 2.3, 8.8 Hz, 1H), 6.34 (s, 1H), 4.57 (t, J = 5.3 Hz, 2H), 4.03 (p, J = 7.0 Hz, 1H), 3.91 (t, J = 5.3 Hz, 1H), 2.90 (s, 3H), 1.67−1.76 (m, 4H), 1.32 (sextet, J = 7.0 Hz, 4H), 0.98 (t, J = 7.0 Hz, 6H); MS (CI) *m/z* 417.1 (MH^+). Anal. ($C_{22}H_{26}Cl_2N_4$·HCl) C, H, N.

5-(1-Butylpentyl)-1-(2,4-dichlorophenyl)-7-methyl-4,5-dihydro-3*H*-1,2a,5,8-tetraazaacenaphthylene Trifluoroacetate (12u). 12u was prepared from **11a** according to procedure B and then purified by preparative LC−MS to obtain the desired product as a TFA salt (21 mg, 15%). ^{1}H NMR (CD_3OD) δ 7.72 (d, J = 1.2 Hz, 1H), 7.64 (d, J = 5.1 Hz, 1H), 7.52 (dd, J = 1.2, 5.1 Hz), 6.89 (s, 1H), 4.60 (t, J = 3.6 Hz, 2H), 4.28 (m, 1H), 4.03 (t, J = 3.6 Hz, 2H), 2.67 (s, 3H), 1.80−1.75 (m, 4H), 1.41−1.31 (m, 8H), 0.91 (t, J = 3.3 Hz, 6H); ESI-HRMS *m/z* calcd for $C_{24}H_{30}Cl_2N_4$ (MH^+) 445.1920, found 445.1915; LC−MS *m/z* 445 ($M + H^+$).

5-Cyclohexyl-1-(2,4-dichlorophenyl)-7-methyl-4,5-dihydro-3*H*-1,2a,5,8-tetraazaacenaphthylene Trifluoroacetate (12v). A mixture of compound **10** (1.0 g, 3.2 mmol), cyclohexylamine (2.9 g, 32 mmol), and *p*-toluenesulfonic acid (973 mg, 5.1 mmol) was heated at 130 °C for 24 h. The reaction mixture was cooled to room temperature, dissolved in EtOAc, extracted with saturated $NaHCO_3$, washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel chromatography using 70% EtOAc in hexanes to yield **13a**. Intermediate **13a** (300 mg, 0.8 mmol) was treated with dibromoethane (180 mg, 0.096 mmol) and K_2CO_3 (332 mg, 2.4 mmol) in 2-butanone (10 mL). The reaction mixture was heated at 85 °C for 24 h, then concentrated in vacuo, dissolved in EtOAc, extracted with water, dried ($MgSO_4$), and concentrated again in vacuo. The product was purified by purified by preparative LC−MS to obtain the desired product **12v** as a TFA salt (35 mg, 11%). ^{1}H NMR (CD_3OD) δ 7.72 (d, J = 1.2 Hz, 1H), 7.62 (d, J = 5.1 Hz, 1H), 7.52 (dd, J = 1.2, 5.1 Hz), 6.84 (s, 1H), 4.10 (t, J = 3.6 Hz, 2H), 4.10 (m, 3H), 2.69 (s, 3H), 1.97−1.94 (m, 4H), 1.81−1.78 (m, 3H), 1.31−1.29 (m, 1H); ESI-HRMS *m/z* calcd for $C_{21}H_{22}Cl_2N_4$ (MH^+) 401.1294, found 401.1294; LC−MS *m/z* 401.

[3-(2,4-Dichlorophenyl)-5-methyl-1*H*-pyrazolo[4,3-*b*]-pyridin-7-yl]-(1-methoxymethylpropyl)amine (13b). A mixture of compound **10** (0.50 g, 1.6 mmol), 2-amino-1-methoxybutane (1.6 g, 16 mmol), and *p*-toluenesulfonic acid monohydrate (0.5 g, 2.6 mmol) was heated at 130 °C in a sealed tube overnight. The reaction mixture was diluted with ethyl acetate, extracted with saturated aqueous $NaHCO_3$, washed with brine, dried ($NaSO_4$), and concentrated in vacuo. The residue was purified with flash chromatography on silica gel, eluted with 50−100% ethyl acetate/hexane to yield **13b** as a light-tan solid (0.33 g, 53%). ^{1}H NMR ($CDCl_3$) δ 8.03 (br s, 1H), 7.43 (d, J = 2.1 Hz, 1H), 7.23−7.26 (m, 2H), 6.18 (s, 1H), 3.60−3.70 (m, 1H), 3.45 (d, J = 3.6 Hz, 2H), 3.30 (s, 3H), 2.56 (s, 3H), 1.66−1.74 (m, 1H), 1.53−1.61 (m, 1H), 0.95 (t, 3H); MS (CI) *m/z* 378.9, 380.9 (MH^+). Anal. ($C_{18}H_{20}Cl_2N_4O$·0.25H_2O) C, H, N.

2-(2,4-Dichlorophenyl)-6-(1-methoxymethylpropyl)-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene Trifluoroacetate (12w). A mixture of compound **13b** (0.20 g, 0.53 mmol), 1,2-dibromoethane (0.12 g, 0.63 mmol), and potassium carbonate (0.22 g, 1.6 mmol) in 2-butanone (5 mL) was heated at 85 °C in a sealed tube for 24 h. Additional 1,2-dibromoethane (0.12 g, 0.63 mmol) and potassium carbonate (0.22 g, 1.6 mmol) were added and heated overnight. The reaction mixture was concentrated and purified by preparative LC−MS to obtain the desired product **12w** as a TFA salt (46 mg, 14%). ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 7.58 (d, J = 2.1 Hz, 1H), 7.54 (d, J = 8.1 Hz, 1H), 7.41 (m, 1H), 6.52 (s, 1H), 4.50−4.66 (m, 2H), 4.14−4.23 (m, 1H), 3.86−4.06 (m, 2H), 3.67 (d, J = 6.0 Hz, 2H), 3.36 (s, 3H), 2.71 (s, 3H), 1.80−1.85 (m, 2H), 1.04 (t, 3H); MS (CI) *m/z* 405.0, 407.1 (MH^+). Anal. ($C_{20}H_{22}Cl_2N_4O$·2TFA·H_2O) C, H, N.

4-Nitro-1*H*-pyrazole (14). Pyrazole (425 g, 6.24 mol) was added in portions to concentrated sulfuric acid (3 L) while keeping the reaction temperature below 40 °C. To the solution was then added 70% nitric acid (430 mL, 6.86 mol) dropwise while maintaining the temperature below 55 °C. The mixture was heated at 55 °C for 5 h, and then the solution was cooled to 0 °C and 6 kg of ice was added. The mixture was neutralized with 50% aqueous NaOH, and the resulting slurry was diluted with 6 L of EtOAc with the precipitate being removed by filtration. The filtrate was separated, and the organic phase was washed with water (4 L) and brine (4 L), dried (Na_2SO_4), and concentrated in vacuo. The crude product was crystallized from ethanol to afford **14** (629 g, 89%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.85−7.86 (m, 2H), 7.70−7.72 (m, 2H), 7.68, d, J = 5.0 Hz, 1H), 7.45 (d, J = 0.8 Hz, 1H), 7.33 (dd, J = 5.0, 1.2 Hz, 1H), 5.03 (s, 2H); MS (Cl) *m/z* 114.0 (MH^+). Anal. ($C_3H_3N_3O_2$) C, H, N.

3-(1*H*-Pyrazol-4-ylamino)but-2-enoic Acid Ethyl Ester (16). To a solution of **14** (50 g, 442 mmol) in ethanol (250 mL) was added 10% Pd/C (2.5 g), and the mixture was hydrogenated at 20−30 psi on a Parr shaking apparatus for 4 h. The reaction mixture was filtered through Celite, washed with ethanol, and concentrated in vacuo to yield 3-aminopyrazole (**15**) as an oil: GC−MS *m/z* 84 (MH^+). The crude product of **15** was dissolved in anhydrous toluene (450 mL). Then ethyl acetoacetate (53.5 mL, 420 mol) and *p*-toluenesulfonic acid (250 mg, 1.3 mmol) were added, and the reaction mixture was refluxed using a Dean−Stark trap for 2 h. The mixture was then cooled to ambient temperature and concentrated in vacuo. The residue was dissolved in ethyl acetate (250 mL) and methanol (25 mL) and filtered through a silica gel plug using ethyl acetate as the eluent. The fractions collected were combined and concentrated to afford **16 (**75.5 g, 88%) as an off-white solid in two steps from **14**. ^{1}H NMR ($CDCl_3$) δ 9.72 (s, 1H), 7.30 (s, 2H), 7.25 (s, 1H), 4.55 (s, 1H), 4.02 (q, J = 5.4 Hz, 2H), 1.79 (s, 3H), 1.18 (t, J = 5.4 Hz, 3H); MS (CI) *m/z* 196.0 (MH^+). Anal. ($C_9H_{13}N_3O_2$) C, H, N.

5-Methyl-1*H*-pyrazolo[4,3-*b*]pyridine-7-ol (17). Enamine **16 (**50 g, 256 mmol) was added portionwise to a flask with diphenyl ether (100 mL) preheated at 250°C while maintaining the solution temperature above 220 °C. The solution was heated at 220 °C for an additional 10−15 min and then slowly cooled to room temperature. The mixture was diluted with MTBE (100 mL) and then stirred at room temperature for 1 h. The precipitate was collected by filtration and washed with MTBE to afford the desired product **17** (35.7 g, 90%) as an off-white solid. ^{1}H NMR (CD_3OD) δ 8.80 (s, 1H), 6.12 (s, 1H), 2.41 (s, 3H); MS (CI) *m/z* 150.0 (MH^+). Anal. ($C_7H_7N_3O$) C, H, N.

7-Chloro-5-methyl-1*H*-pyrazolo[4,3-*b*]pyridine (18). To a solution of **17** (131 g, 878 mmol) in anhydrous acetonitrile (600 mL) was added phosphorus oxychloride (164 mL, 1.76 mol). After being heated at reflux for 3 h, the reaction mixture was cooled to room temperature and then poured over 1.5 kg of ice. The resulting mixture was neutralized to pH 6−7 with approximately 50% NaOH (210 mL, 5.3 mol), resulting in a precipitate. The solid was collected by filtration to yield 116 g of the desired product after drying. The mother liquor was extracted with ethyl acetate, affording an additional 24 g of product. The combined product **18** (140 g, 95%) was a tan solid. ^{1}H NMR ($CDCl_3$) δ 8.20 (s, 1H), 7.20 (s, 1H), 2.71 (s, 3H); MS (CI) *m/z* 168.0 (MH^+). Anal. ($C_7H_6ClN_3$) C, H, N.

3-Bromo-7-chloro-5-methyl-1*H*-pyrazolo[4,3-*b*]pyridine (19). Bromine (15 mL, 296 mmol) in 50% aqueous methanol (30 mL) was added dropwise at 0 °C to a solution of **18 (**41 g, 247 mmol) in 50% aqueous methanol (500 mL). The mixture was then stirred for 30 min, and the resulting precipitate was collected by vacuum filtration. The resulting filter cake was neutralized, first by resuspending in water and

slowly adding saturated sodium bicarbonate while stirring. The neutralized precipitate was filtered, washed with water, and dried in vacuo at 45 °C, affording **19** (60 g, 98%) as a light-yellow powder. ^{1}H NMR ($CDCl_3$) δ 10.82 (s, 1H), 7.32 (s, 1H), 2.73 (s, 3H); MS (CI) *m/z* 247.0, 249.0 (MH^+). Anal. ($C_7H_5BrClN_3$) C, H, N.

(*S*)-2-Bromo-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (21). A mixture of **19** (20.0 g, 81.1 mmol), *p*-toluenesulfonic acid (17.0 g, 89.2 mmol), and (*S*)-(+)-2-amino-1-butanol (21.7 g, 243 mmol) was heated at 150 °C for 16 h. Aqueous hydrobromic acid (48%, 125 mL) was added to the mixture and then heated at 120 °C for 3 days. The mixture was cooled to room temperature and poured onto crushed ice. The resulting mixture was neutralized with 4 N KOH and extracted with EtOAc. The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The crude product was purified by flash silica gel chromatography (3:2 ethyl acetate/hexanes) to afford **21** (9.8 g, 43% yield) over two steps. ^{1}H NMR (CD_3OD) δ 6.25 (s, 1 H), 4.62 (s, 1 H), 4.50 (dd, J = 2.0, 4.0 Hz, 1 H), 4.05 (dd, J = 4.0, 12.5 Hz, 1 H), 3.85−3.75 (m, 1 H), 2.47 (s, 3 H), 1.75 (q, J = 7.0 Hz, 2 H), 1.15 (t, J = 7.0 Hz, 3 H); MS (CI) *m/z* 282.0, 284.0 (MH^+); ESI-TOF-HRMS *m/z* calcd for $C_{11}H_{13}BrN_4$ (MH^+) 281.0396, found 281.0397.

(*S*)-6-Cyclopropylmethyl-2-bromo-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (22). A solution of **21** (2.81 g, 10 mmol) in 50 mL of anhydrous DMF was added to NaH (60% in mineral oil, 600 mg, 15 mmol) and stirred at room temperature for 15 min. Bromomethylcyclopropane (2.99 g, 22 mmol) was then added slowly, and the mixture was stirred at room temperature for 1 h. The reaction was quenched with water, and the sample layer was extracted with ethyl acetate, washed with brine, dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (9:1 DCM/methanol) provided **22** (2.13 g, 64%). ^{1}H NMR ($CDCl_3$) δ 6.17 (s, 1H), 4.48 (dd, J = 8, 1.8 Hz, 1H), 4.22 (dd, J = 12.3, 3.9 Hz, 1H), 3.90−3.84 (m, 1H), 3.54 (dd, J = 14.5, 6 Hz, 1H), 3.03 (dd, J = 14.5, 7.5 Hz, 1H), 2.60 (s, 3H), 1.80−1.68 (m, 1H), 1.64−1.51 (m, 1H), 1.12−1.02 (m, 1H), 0.99 (t, J = 7.5 Hz, 3H), 0.71−0.54 (m, 2H), 0.36−0.24 (m, 2H); MS (CI) *m/z* 334.9, 337.0 (MH^+); HRMS *m/z* calcd for $C_{15}H_{19}BrN_4$ (MH^+) 335.0866, found 335.0877.

(*S*)-2-(4-Chloro-2-trifluoromethylphenyl)-6-cyclopropylmethyl-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (23a). Procedure D. To a solution of compound **22** (60 mg, 0.18 mmol) in dioxane/water (2 mL/1 mL) was added sodium carbonate (106 mg, 1 mmol) and 4-chloro-2-trifluoromethyphenylboronic acid (58 mg, 0.26 mmol). The reaction mixture was degassed with nitrogen for 10 min, tetrakis(triphenylphosine)palladium(0) (21 mg, 0.02 mmol) was added, and the reaction mixture was heated at 100 °C overnight. The reaction mixture was partitioned between brine and EtOAc. The organic layer was dried (sodium sulfate), evaporated, and purified by flash chromatography (silica, 5% MeOH/DCM with 1% TEA) to give compound **23a** (32 mg, 49%). ^{1}H NMR ($CDCl_3$) δ 7.78 (d, J = 8.7 Hz, 1H), 7.70−7.64 (m, 1H), 7.56−7.45 (m, 1H), 6.26 (s, 1H), 4.69 (d, J = 12.9 Hz, 1H), 4.43 (dd, J = 12.9, 2.1 Hz, 1H), 4.24 (br s, 1H), 3.75 (dd, J = 14.7, 6 Hz, 1H), 3.26 (dd, J = 14.7, 7.8 Hz, 1H), 2.82 (s, 3H), 1.86−1.76 (m, 1H), 1.73−1.62 (m, 1H), 1.26 (s, 3H), 1.08−1.01 (m, 1H), 0.88−0.77 (m, 2H), 0.43−0.36 (m, 2H); HRMS (FAB) *m/z* calcd for $C_{22}H_{22}ClF_3N_4$ (MH^+) 435.1563, found 435.1557.

(*S*)-2-(4-Chloro-2-methylphenyl)-6-cyclopropylmethyl-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (23b). 23b was prepared according to procedure D with 4-chloro-2-(trifluoromethyl)phenylboronic acid. The final product was purified by preparative LC−MS to give compound **23b** (112 mg, 49%). ^{1}H NMR ($CDCl_3$) δ 7.51 (d, J = 7.5 Hz, 1H), 7.28 (d, J = 8.7 Hz, 2H), 6.29 (s, 1H), 4.67 (d, J = 12.9, 1H), 4.40 (dd, J = 12.3, 4.2 Hz, 1H), 4.15−4.12 (m, 1H), 3.74 (dd, J = 14.7, 6.3 Hz, 1H), 3.26 (dd, J = 14.7, 6.9 Hz, 1H), 2.75 (s, 3H), 2.37 (s, 3H), 1.94−1.82 (m, 1H), 1.79−1.69 (m, 1H), 1.18−1.15 (m, 1H), 1.07 (t, J = 7.4 Hz, 3H), 0.83−0.70 (m, 2H), 0.48−0.34 (m, 2H); ^{13}C NMR: ($CDCl_3$) δ 161.4, 155.4, 143.2, 141.1, 139.3, 135.4, 132.3, 130.8, 127.3, 126.9, 126.4, 125.6, 97.9, 77.5, 77.2, 77.0, 60.1, 53.4, 48.0, 24.7, 20.8, 20.6, 10.8, 9.5, 4.9, 3.8; HRMS (FAB) *m/z* calcd for $C_{22}H_{25}ClN_4$ (MH^+) 381.1846, found 381.1836. Anal. ($C_{22}H_{25}N_4$) C, H, N.

(*S*)-2-(4-Chloro-2-methoxyphenyl)-6-cyclopropylmethyl-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (23c). Procedure E. To a solution of **22** (66 mg, 0.18 mmol) in toluene (3.5 mL) and ethanol (0.5 mL) was added 2-methoxy-4-chlorophenylboronate ester (59 mg, 0.22 mmol), aqueous sodium carbonate (3 M, 2 mL), and saturated aqueous barium hydroxide (0.1 mL). The reaction mixture was degassed with nitrogen for 10 min, and $Pd(PPh_3)_4$ (21 mg, 0.02 mmol) was added. The mixture was heated under nitrogen at 100 °C, and once the starting materials were consumed, the mixture was cooled, poured into water, and extracted with ethyl acetate. The organic layer was washed with brine, dried, and concentrated in vacuo.

The crude product was purified by flash chromatography (silica, 5% MeOH/DCM with 1% TEA) to obtain **23c** as a free base (26 mg, 37%). ^{1}H NMR ($CDCl_3$) δ 7.98 (d, J = 8.3 Hz, 1H), 7.06 (dd, J = 8.3, 1.8 Hz, 1H), 7.02 (d, J = 1.8 Hz, 1H), 6.17 (s, 1H), 4.58 (dd, J = 12.3, 1.6 Hz, 1H), 4.33 (dd, J =12.3, 4 Hz, 1H), 3.92 (s, 3H), 3.89−3.93 (m, 1H), 3.60 (dd, J = 14.4, 5.9 Hz, 1H), 3.07 (dd, J = 14.4, 7.3 Hz, 1H), 2.63 (s, 3H), 1.76 (m, 1H), 1.65 (m, 1H), 1.10 (m, 1H), 1.02 (t, J = 7.4 Hz, 3H), 0.59−0.72 (m, 2H), 0.27−0.38 (m, 2H); MALDI-HRMS *m/z* calcd for $C_{22}H_{25}ClN_4O$ (MH^+) 397.1790, found 397.1784.

(*S*)-2-(4-Chloro-2-fluorophenyl)-6-cyclopropylmethyl-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (23d). 23d was prepared according to procedure E with 2-fluoro-4-chlorophenylboronate ester (56 mg, 0.22 mmol). The final product was purified by flash chromatography (silica, 5% MeOH/DCM with 1% TEA) to obtain **23d** as a free base (34 mg, 49%). ^{1}H NMR ($CDCl_3$) δ 8.43 (t, J = 8.1 Hz, 1H), 7.28 (dd, J = 8.4, 2 Hz, 1H), 7.24 (dd, J = 10.5, 2 Hz, 1H), 6.20 (s, 1H), 4.60 (dd, J = 12.4, 1.6 Hz, 1H), 5.35 (dd, J = 12.3, 3.9 Hz, 1H), 3.90−3.96 (m, 1H), 3.59 (dd, J = 14.4, 5.9 Hz, 1H), 3.07 (dd, J = 14.4, 7.3 Hz, 1H), 2.65 (s, 3H), 1.71−1.82 (m, 1H), 1.53−1.67 (m, 1H), 1.05−1.16 (m, 1H), 1.01 (t, J = 7.5 Hz, 3H), 0.60−0.72 (m, 2H), 0.26−0.38 (m, 2H); MALDI-HRMS *m/z* calcd for $C_{21}H_{22}ClFN_4$ (MH^+) 385.1590, found 385.1592.

(*S*)-2-(2-Chloro-4-trifluoromethylphenyl)-6-cyclopropylmethyl-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene Trifluoroacetate (23e). 23e was prepared according to procedure D with 4-chloro-2-(trifluoromethyl)phenylboronic acid. The final product was purified by preparative LC−MS to give compound **23e** as a TFA salt (53 mg, 37%). ^{1}H NMR ($CDCl_3$) δ 7.77 (d, J = 9.0 Hz, 1H), 7.65 (d, J = 9.0 Hz, 1H), 6.30 (s, 1H), 4.71 (d, J = 12.9, 1H), 4.45 (dd, J = 12.9, 3.9 Hz, 1H), 4.15−4.11 (m, 1H), 3.74 (dd, J = 14.7, 6 Hz, 1H), 3.67−3.60 (m, 1H), 3.26 (dd, J = 14.7, 7.8 Hz, 1H), 2.78 (s, 3H), 1.94−1.84 (m, 1H), 1.81−1.68 (m, 1H), 1.41 (dd, J = 13.5, 6.3 Hz, 1H), 1.09 (t, J = 7.4 Hz, 3H), 0.82−0.71 (m, 2H), 0.48−0.36 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 155.7, 143.2, 138.6, 134.7, 133.0, 132.4, 127.3, 125.7, 124.0, 97.9, 77.5, 77.2, 77.0, 60.2, 53.4, 48.2, 24.6, 21.0, 10.9, 9.5, 4.9, 3.8; HRMS (FAB) *m/z* calcd for $C_{22}H_{22}ClF_3N_4$ (MH^+) 435.1563, found 435.1564.

(*S*)-2-(2-Chloro-4-methylphenyl)-6-cyclopropylmethyl-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene Trifluoroacetate (23f). 23f was prepared according to procedure D with 4-chloro-2-(trifluoromethyl)phenylboronic acid. The final product was purified by preparative LC−MS to give compound **23f** as a TFA salt (49 mg, 43%). ^{1}H NMR ($CDCl_3$) δ 7.48 (d, J = 7.8 Hz, 1H), 7.34 (s, 1H), 7.19 (d, J = 8.4 Hz, 1H), 6.29 (s, 1H), 4.69 (d, J = 12.9, 1H), 4.41 (dd, J = 12.9, 3.9 Hz, 1H), 4.15−4.10 (m, 1H), 3.74 (dd, J = 14.7, 6.3 Hz, 1H), 3.65 (s, 3H), 3.26 (dd, J = 14.7, 7.8 Hz, 1H), 2.74 (s, 3H), 2.39 (s, 1H), 1.94−1.82 (m, 1H), 1.80−1.70 (m, 1H), 1.07 (t, J = 7.4 Hz, 3H), 0.82−0.69 (m, 2H), 0.47−0.33 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 155.1, 143.2, 141.8, 140.1, 133.5, 132.0, 130.7, 128.1, 127.1, 125.6, 125.5, 97.8, 77.5, 77.2, 77.0, 60.2, 53.4, 48.0, 24.6, 21.3, 20.9, 10.9, 9.5, 4.9, 3.8; HRMS (FAB) *m/z* calcd for $C_{22}H_{25}ClN_4$ (MH^+) 381.1846, found 381.1832.

(*S*)-2-(2-Chloro-4-methoxyphenyl)-6-cyclopropylmethyl-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene Trifluoroacetate (23g). 23g was prepared according to procedure D with 4-chloro-2-trifluoromethyphenylboronic acid. The final product was purified by preparative LC−MS. The desired product (**23g**) was obtained as a TFA salt (28 mg, 30%). ^{1}H NMR ($CDCl_3$ + CD_3OD) δ 7.51 (d, J = 8.7 Hz, 1H), 7.10 (d, J = 2.4 Hz, 1H), 6.96 (m, 1H), 6.41 (s, 1H), 4.71 (d, J = 12.9 Hz, 1H), 4.43 (m, 1H), 4.16−4.22 (m, 1H), 3.87 (s, 3H), 3.79 (m, 1H), 3.31 (m, 1H), 2.71 (s, 3H), 1.87−1.94 (m, 1H), 1.71−1.81 (m, 1H), 1.14−1.20 (m, 1H), 1.09 (t, 3H), 0.73−0.78 (m, 2H), 0.37−0.48 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 161.43, 154.87, 143.35, 139.66, 134.56, 132.99, 126.81, 125.56, 120.73, 115.76, 113.31, 97.70, 60.20, 55.81, 53.37, 47.91, 24.54, 20.83, 10.85, 9.44, 4.83, 3.75; MALDI-HRMS *m/z* calcd for $C_{22}H_{25}ClN_4O$ (MH^+) 397.1790, found 397.1781; LC−MS *m/z* 397.1 (MH^+).

(*S*)-2-(2-Chloro-4-fluorophenyl)-6-cyclopropylmethyl-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (23h). 23h was prepared according to procedure E with 2-chloro-4-fluorophenylboronate ester (56 mg, 0.22 mmol). The final product was purified by flash chromatography (silica, 5% MeOH/DCM with 1% TEA) to obtain **23h** as a free base (18.7 mg, 27%). ^{1}H NMR ($CDCl_3$) δ 7.80−7−92 (m, 1H), 7.27 (dd, J = 8.5, 2.6 Hz, 1H), 7.10 (dt, J = 2.5, 8.3 Hz, 1H), 6.19 (s, 1H), 4.59 (dd, J = 12.3, 1.4 Hz, 1H), 4.36 (dd, J = 12.3, 4 Hz, 1H), 3.91−3.98 (m, 1H), 3.61 (dd, J = 14.4, 5.8 Hz, 1H), 3.08 (dd, J = 14.4, 7.3 Hz, 1H), 2.63 (s, 3H), 1.72−1.82 (m, 1H), 1.56−1.70 (m, 1H), 1.07−1.18 (m, 1H), 1.03 (t, J = 7.4 Hz, 3H), 0.57−0.73 (m, 2H), 0.26−0.39 (m, 2H); MALDI-HRMS *m/z* calcd for $C_{21}H_{22}ClFN_4$ (MH^+) 385.1590, found 385.1594.

(*S*)-2-(2-Chloro-4-methoxycarbonylphenyl)-6-cyclopropylmethyl-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (23i). 23i was prepared according to procedure D with 2-chloro-4-carbomethoxyphenylboronic acid. The final product was purified by flash chromatography (silica, 5% MeOH/DCM with 1% TEA) to obtain **23i** as a free base (29 mg, 37%). ^{1}H NMR ($CDCl_3$) δ 8.19 (d, J = 1.2 Hz, 1H), 8.11 (d, J = 8.1 Hz, 1H), 8.03 (m, 1H), 6.20 (s, 1H), 4.61 (d, J = 12.3 Hz, 1H), 4.38 (m, 1H), 3.95 (s, 3H), 3.90−3.95 (m, 1H), 3.59 (m, 1H), 3.06 (m, 1H), 2.60 (s, 3H), 1.71−1.82 (m, 1H), 1.57−1.67 (m, 1H), 1.09−1.14 (m, 1H), 1.02 (t, 3H), 0.61−0.70 (m, 2H), 0.30−0.37 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 166.24, 158.49, 141.30, 139.04, 137.62, 136.14, 133.37, 132.56, 131.84, 130.62, 127.93, 125.22, 98.31, 58.74, 52.55, 51.94, 48.49, 26.06, 23.61, 11.11, 9.53, 4.78, 3.32; MALDI-HRMS *m/z* calcd for $C_{23}H_{25}ClN_4O_2$ (MH^+) 425.1739, found 425.1730.

(*S*)-6-Cyclopropylmethyl-2-(2,4-dimethylphenyl)-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (23j). 23j was prepared according to procedure D with 2,4-dimethylphenylboronic acid. The final product was purified by flash chromatography (silica, 5% MeOH/DCM with 1% TEA) to give compound **23j** (32 mg, 49%). ^{1}H NMR ($CDCl_3$) δ 7.71 (d, J = 7.5 Hz, 1H), 7.08−7.12 (m, 2H), 6.16 (s, 1H), 4.54 (dd, J = 1.8, 12.3 Hz, 1H), 4.31 (dd, J = 3.9, 12.3 Hz, 1H), 3.88−3.93 (m, 1H), 3.59 (dd, J = 5.7, 14.5 Hz, 1H), 3.05 (dd, J = 7.2, 14.0 Hz, 1H), 2.59 (s, 3H), 2.49 (s, 3H), 2.25 (s, 3H), 1.71−1.82 (m, 1H), 1.60−1.69 (m, 1H), 1.09−1.14 (m, 1H), 1.02 (t, J = 7.5 Hz, 3H), 0.61−0.69 (m, 2H), 0.30−0.37 (m, 2H); MS (CI) *m/z* 361.2 (MH^+). Anal. ($C_{23}H_{28}N_4$) C, H, N.

(*S*)-6-Cyclopropylmethyl-7-ethyl-2-(4-methoxy-2-methylphenyl)-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (23k). 23k was prepared according to procedure D with 2-methyl-4-methoxyphenylboronic acid (36.5 mg, 0.22 mmol). The final product was purified by flash chromatography (silica, 5% MeOH/DCM with 1% TEA) to obtain **23k** as a free base (35 mg, 52%). ^{1}H NMR ($CDCl_3$) δ 7.76 (d, J = 9.2 Hz, 1H), 6.82−6.87 (m, 2H), 6.17 (s, 1H), 4.54 (dd, J = 12.2, 1.7 Hz, 1H), 4.30 (dd, J = 12.3, 3.8 Hz, 1H), 3.88−3.95 (m, 1H), 3.83 (s, 3H), 3.59 (dd, J = 14.4, 5.9 Hz, 1H), 3.07 (dd, J = 14.5, 7.3 Hz, 1H), 2.62 (s, 3H), 2.51 (s, 3H), 1.73−1.83 (m, 1H), 1.58−1.70 (m, 1H), 1.06−1.17 (m, 1H), 1.02 (t, J = 7.4 Hz, 3H), 0.58−0.72 (m, 2H), 0.27−0.39 (m, 2H); MALDI-HRMS *m/z* calcd for $C_{23}H_{28}N_4O$ (MH^+) 377.2336, found 377.2334.

(*S*)-2-(2,4-Bis-trifluoromethyl-phenyl)-6-cyclopropylmethyl-7-ethyl-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (23l). 23l was prepared according to procedure D with 2,4-bistrifluoromethylphenylboronic acid (57 mg, 0.22 mmol). The final product was purified by flash chromatography (silica, 5% MeOH/DCM with 1% TEA) to obtain **23l** as a free base (24 mg, 23%). ^{1}H NMR ($CDCl_3$) δ 8.15 (d, J = 8.1 Hz, 1H), 8.06 (s, 1H), 7.91 (d, J = 7.9 Hz, 1H), 6.22 (s, 1H), 4.58 (dd, J = 12.4, 1.6 Hz, 1H), 4.38 (dd, J = 12.2, 4.1 Hz, 1H), 3.92−3.98 (m, 1H), 3.60 (dd, J = 14.5, 5.9 Hz, 1H), 3.08 (dd, J = 14.4, 7.3 Hz, 1H), 2.60 (s, 3H), 1.73−1.83 (m, 1H), 1.54−1.68 (m, 1H), 1.07−1.17 (m, 1H), 1.02 (t, J = 3.9 Hz, 3H), 0.58−0.73 (m, 2H), 0.28−0.40 (m, 2H); MALDI-HRMS *m/z* calcd for $C_{23}H_{22}F_6N_4$ (MH^+) 469.1821, found 469.1828.

(*S*)-6-Cyclopropylmethyl-7-ethyl-2-(4-methoxy-2-trifluoromethylphenyl)-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (23m). 23m was prepared according to procedure D with 4-methoxy-2-trifluoromethylphenylboronic acid. The final product was purified by flash chromatography (silica, 5% MeOH/DCM with 1% TEA) to obtain **23m** as a free base (29 mg, 37%). ^{1}H NMR ($CDCl_3$) δ 7.78 (d, J = 8.3 Hz, 1H), 7.32 (d, J = 2.6 Hz, 1H), 7.18 (dd, J = 2.6, 8.3 Hz, 1H), 6.19 (s, 1H), 4.57 (dd, J = 1.8, 12.3 Hz, 1H), 4.35 (dd, J = 3.9, 12.3 Hz, 1H), 3.92−4.00 (m, 1H), 3.89 (s, 3H), 3.61 (dd, J = 6.1, 14.5 Hz, 1H), 3.06−3.14 (m, 1H), 2.64 (s, 3H), 1.75−1.81 (m, 1H), 1.59−1.69 (m, 1H), 1.10−1.15 (m, 1H), 1.02 (t, J = 7.5 Hz, 3H), 0.64−0.73 (m, 2H), 0.32−0.38 (m, 2H); MS (CI) *m/z* 431.2 (MH^+). Anal. ($C_{23}H_{25}F_3N_4O$) C, H, N.

(*S*)-7-Ethyl-6-isobutyl-2-(4-isopropyl-2-trifluoromethylphenyl)-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene Hydrochloride (23n). A mixture of 2-chloro-5-bromobenzotrifluoride (24) (4.5 g, 17.5 mmol), tributyl(1-ethoxyvinyl)tin (7 g, 19.4 mmol), and $PdCl_2(PPh_3)_2$ (0.61 g, 0.875 mmol) in toluene (30 mL) was refluxed for 2 h. The mixture was then cooled and filtered through a pad of silica gel with EtOAc. The organic layer was washed with 1 N HCl and saturated aqueous potassium fluoride solution, dried, filtered, and concentrated in vacuo. The residue was stirred for 3 h in a mixture of THF and 1 N HCl and then partitioned between EtOAc and water. The organic layer was separated, dried, filtered, and evaporated. Silica gel chromatography of the residue (10% EtOAc/hexanes) afforded compound **25** (5.16 g, 100%).

A mixture of the **25** (5.15 g, 17.5 mmol), bis(pinacolato)-diboron (5.3 g, 21 mmol), $PdCl_2$(dppf) (1.3 g, 1.75 mmol), and KOAc (2.0 g, 21 mmol) in toluene (60 mL) was refluxed for 48 h with an additional 0.65 g of $PdCl_2$(dppf) being added after 24 h. The mixture was partitioned between EtOAc and water, and the organic layer was separated, dried ($MgSO_4$), filtered, and concentrated in vacuo. Silica gel chromatography of the residue (15−20% EtOAc/hexanes) afforded the boronate ester **26** (4.74 g, 62%), which was shown to be 70% pure by GC−MS, with the impurity being bis(pinacolato)diborane. ^{1}H NMR ($CDCl_3$) δ 8.22 (s, 1H), 8.06−8.09 (m, 1H), 7.82 (d, J = 8 Hz, 1H), 2.65 (s, 3H), 1.27 (s, 12H).

To a solution of the 2-trifluoromethyl-4-acetylphenylboronic acid pinacol ester **26** (5.52 g, 12.5 mmol) and **22** (3.0 g, 8.96 mmol) in toluene (35 mL) and ethanol (10 mL) was added 2 M aqueous sodium carbonate solution (12.5 mL), saturated aqueous barium hydroxide solution (8 mL), and $Pd(PPh_3)_4$ (1.0 g, 0.9 mmol). The mixture was heated under nitrogen at 100 °C, and after disappearance of the starting material the mixture was cooled, poured into water, and extracted with ethyl acetate. The organic layer was washed with brine, dried, and evaporated. Silica gel chromatography of the residue (40% EtOAc/hexanes) afforded **27** (3.78 g, 95%). MS (CI) *m/z* 443.2 (MH^+).

A solution of methylmagnesium bromide (1.4 M in toluene/THF, 75:25, 12.2 mL, 17.1 mmol) was added dropwise to a solution of **27** (3.78 g, 8.55 mmol) in THF (60 mL) at −78 °C. The mixture was then warmed to room temperature and allowed to stir for 2 h. An additional 3 mL (4.2 mmol) of methylmagnesium bromide was added, and the mixture was

heated to 60 °C for 30 min to consume all of the starting material. After cooling, the mixture was poured onto water, extracted with ethyl acetate, dried ($MgSO_4$), and conctrated in vacuo. This material was dissolved in toluene (50 mL) and TFA (1.25 mL). Then $MgSO_4$ (1.1 g. 9.1 mmol) and *p*-toluenesulfonic acid (50 mg, 0.26 mmol) were added, and the mixture was heated for 16 h. The mixture was filtered, and the solvent was evaporated. Silica gel chromatography of the residue (50% EtOAc/hexanes + 2% Et_3N) afforded **28** (1.68 g, 45%) as an oil. ^{1}H NMR ($CDCl_3$) δ 7.87 (dd, J = 4.5, 3 Hz, 2 H), 7.71 (dd, J = 8.1, 1.8 Hz), 6.19 (s, 1H), 5.47 (s, 1H), 5.18−5.19 (m, 1 H), 4.55 (dd, J = 12.3, 2.4 Hz, 1H), 2.35 (dd, J = 12.3, 4.2 Hz, 1H), 3.88−3.95 (m, 1 H), 3.59 (dd, J = 14.1, 6 Hz, 1 H), 3.05 (dd, J = 14.1, 7.5 Hz, 1 H), 2.20 (s, 3H), 2.58 (s, 3H), 1.70−1.80 (m, 1 H), 1.56−1.67 (m, 1 H), 1.07−1.14 (m, 1 H), 1.01 (t, J = 7.5 Hz, 3 H), 0.86−0.90 (m, 2H), 0.58−0.73 (m, 2H), 0.28−0.38 (m, 2H); MS (CI) *m/z* 441.2 (MH^+).

A solution of **28** (1.65 g, 3.75 mmol) and PtO (0.10 g) in ethanol (30 mL) was shaken under an atmosphere of hydrogen at 40 psi for 1 h. The mixture was filtered through a plug of silica gel and Celite, and the filtrate was evaporated to dryness. The residue was dissolved in diethyl ether (50 mL) and treated with a 1.0 M solution of HCl in ether (3.56 mL, 3.56 mmol). After trituration, the organic solvent was decanted off and the remaining solid was washed three times with ether and dried under vacuum to afford the hydrochloride salt of the compound as a pale-yellow powder (**23n**, 1.25 g, 70%). HPLC showed the compound to be 96.3% pure. ^{1}H NMR ($CDCl_3$) δ 7.76 (d, J = 8.1 Hz, 1 H), 7.63 (s, 1H), 7.48 (d, J = 8.4 Hz, 1 H), 6.18 (s, 1H), 4.53 (dd, J = 12.3, 1.8 Hz, 1H), 4.34 (dd, J = 12.3, 4.2 Hz, 1H), 3.88−3.96 (m, 1 H), 3.58 (dd, J = 14.1, 6 Hz, 1H), 2.96−3.08 (m, 2H), 2.57 (s, 3H), 1.70−1.81 (m, 1H), 1.57−1.67 (m, 1H), 1.30 (d, J = 7.2 Hz, 6H), 1.07−1.17 (m, 1 H), 1.01 (t, J = 7.5 Hz, 3 H), 0.58−0.73 (m, 2H), 0.26−0.39 (m, 2H); MS (CI) *m/z* 443.2 (MH^+). Anal. ($C_{25}H_{29}N_4F_3$·HCl) C, H, N.

Biology. In Vitro Binding and Functional Studies. Radioligand binding assays and functional inhibition of CRF-induced cAMP production were performed essentially as previously described for the cloned CRF_1 receptor in L-CRF_1 cell membranes.[22] Equilibrium binding of unlabeled ligands was measured in duplicate by inhibition of radioligand binding ([^{125}I]sauvagine) to LtK^- cells expressing the human CRF_1 receptor. Assay buffer (30 μL of DPBS, 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 2.7 mM KCl, 138 mM NaCl) supplemented with 10 mM $MgCl_2$, 2 mM ethylene glycol bis[β-aminoethyl]-*N*,*N*,*N*′,*N*′-tetraacetic acid (pH 7.4), 20 μL of unlabeled ligand, 50 μL of radioligand, and 100 μL of L-CRF_1 cell membranes were sequentially added to low protein-binding 96-well plates (Corning no. 3605). The final concentration of radioligand was approximately 90 pM for [^{125}I]sauvagine with a total of 5 μg of membrane. Unlabeled compounds were serially diluted for final concentrations of 10 pM to 1 μM. Following a 2 h incubation at room temperature, bound and free radioligands were separated by rapid vacuum filtration. In all assays the total radioligand bound to the filter (total binding) was less than 20% of the total amount of radioligand added. Nonspecific binding was determined in the presence of an excess of the unlabeled analogue of the radioligand. Bound and nonspecific radioactivity was monitored using a Packard Cobra II γ counter (78% efficiency) and analyzed using the nonlinear curve-fitting algorithm software Prism (GraphPad Inc., CA).

CRF-Stimulated cAMP Production in Cells Expressing Human CRF_1 Receptors. For the inhibition of CRF-stimulated cAMP formation, the assays were again performed as described.[22] One day prior to assay, L-hCRF_1 cells were transferred to 96-well tissue culture plates (100 000 cells/well in 200 μL of medium). On the day of assay, the medium was removed and the cells were washed with 200 μL of DPBS. Following aspiration of DPBS, 75 μL of cAMP assay buffer was added to each well (DMEM without phenol red, supplemented with 2 mM glutamine, 1 mM sodium pyruvate, 10 mM HEPES, 50 IU/mL penicillin, 50 μg/mL streptomycin, and 1 mM IBMX). Corticotropin-releasing factor and non-peptides were then added in a volume of 25 μL of cAMP assay buffer at various concentrations for inhibition, and the cells were incubated for 30 min at 37 °C in 5% CO_2. Total cAMP produced was measured by chemiluminescent immunoassay (Tropix, Bedford, MA) and measured on an Analyst (LJL Biosystems Inc., CA). All IC_{50} values were calculated using the nonlinear curve-fitting algorithm software Prism as above.

CRF-Stimulated ACTH Release from Cultured Rat Anterior Pituitary Cells. For the inhibition of ACTH release from primary rat pituitary cell cultures, five whole pituitaries are collected from 7-week-old female Sprague Dawley rats. Pituitaries are washed six times with HEPES buffer (2.5 g/L BSA, 10 mg/L deoxyribonuclease I, 8.0 g/L NaCl, 0.37 g/L KCl, 100 mg/L sodium phosphate dibasic, 6.0 g/L HEPES, 2.0 g/L glucose) and minced. The tissue is then digested with 10 mL of collagenase for 1.5 h at 37 °C with trituration every 30 min. The digest is then transferred to a 50 mL conical centrifuge tube and centrifuged at 1000 rpm for 4 min. The supernatant is discarded, and the pellet is resuspended in 10 mL of neuraminidase solution and incubated for 9 min at 37 °C. The suspension is centrifuged at 1000 rpm for 5 min, and the pellet was washed once with 10 mL of BBM-T medium (11.49 g/L Custom Media Mixture, Irvine Scientific, CA; 1.83 g of $NaCO_3$/L; 2.4 g of HEPES/L; 2.0 g/L BSA; 10.0 mg/L transferrin; 50 000 IU/L penicillin and streptomycin; 1 μg/L insulin; 0.1 μg/L EGF; 0.4 μg/L T3; 0.7 μg/L PTH; 10 μg/L glucagon). The resulting pellet is finally resuspended in 3% FCS/BBM-T medium and cultured in 96-well tissue culture plates for 2−3 days at a density of 40 000 cells/well in a final volume of 200 μL of medium.

For the assay of antagonists, cells are washed once with BBM-T and test samples are added in various concentrations (1 μM to 1 pM) with 0.5 nM r/hCRF in 200 mL BBM-T and incubated for 4 h at 37 °C. The medium is then aspirated and assessed for ACTH release using a standard RIA kit (MP Biomedicals, NY). Again, all data were analyzed using the nonlinear curve-fitting algorithm software Prism as above.

In Vivo CRF-Induced ACTH Release in Rats. Three days prior to testing with compound, rats were anesthetized (n = 6 per group) with isoflurane and implanted with a femoral vein catheter (IITC no. 26A; PE 10 silastic) in the right groin area. The catheter was secured in place with 4-0 suture. A gastric catheter was placed in the stomach and sutured with a purse string suture (4-0 suture) to secure the cannula in place. The cannulae were fed subcutaneously to the dorsal section of the rat (behind the ears), where they exited and were sutured in place. All external incisions were closed using standard wound clips. On the day of testing, fed rats were weighed and then connected to PE50 tubing via manosil tubing. They were then placed in opaque collection containers, with the PE50 tubing drawn through the top of the container, and habituated to the containers for 1 h. Following a baseline blood draw, Compound **12a** (3, 10, or 30 mg/kg) or vehicle (2 mL/kg) was infused via the intragastric tube. Sixty minutes later, CRF (0.3 nmol/kg) or vehicle (0.5 mL/kg) was injected iv. The CRF vehicle was a 0.1% BSA and 10 mM acetic acid solution. Blood was drawn at 2, 10, and 30 min following the CRF injection, collected in EDTA-coated tubes, and centrifuged at 2500 rpm (4 °C) for 20 min. Plasma was frozen (−80 °C) until the time of assay. The ACTH levels were determined in these samples using a standard ACTH RIA kit (MP Biomedicals, NY), with sample ACTH values calculated from a log-transformed standard curve. ACTH values over time were analyzed using repeated measures and mixed-design ANOVA. Peak (10 min time point) ACTH values were analyzed using one-way ANOVA, with Fischer's PLSD as the post hoc method of testing dose group differences.

Restraint Stress-Induced ACTH Release in Mice. Male CD-1 mice (24−26 g, 10/group) were weighed and handled once a day prior to the restraint stress. On the day of testing, animals were dosed by oral gavage with **12a** (20 mg/kg) or vehicle (10 mL/kg 5% mannitol-*d* (w/v) in water) 60 min prior to the initiation of the stressor. Restraint stress was evoked by allowing the mice to enter a 50 mL plastic conical tube (tip

removed to allow free flow of air) and held in place for 45 min. The animals cannot alter their position once inside the tube. Following the 45 min of restraint stress, the animals are removed and immediately placed in an isoflurane chamber for a maximum of 2 min and blood is collected via cardiac puncture into 0.5 M EDTA-coated tubes. The blood is kept on ice, and plasma is separated by centrifugation (6000*g* at 4 °C for 15 min). Mice in the nonstress control group remained in their home cages for the duration of the experiment following oral gavage with the vehicle. Plasma ACTH concentration was measured using a standard ACTH RIA kit (MP Biomedicals, NY). The ACTH values over time were analyzed using repeated measures and mixed-design ANOVA with peak ACTH values analyzed using a one-way ANOVA with post hoc Fischer's test for group differences.

Rat Pharmacokinetics. Compound **12a** was dosed as a mesylate salt in aqueous solution. The pharmacokinetics profile was determined in male Sprague Dawley rats (N = 3/time points at a dose of 10 mg/kg). The dosing solution was prepared in purified water and filtered through a 0.2 μm nylon filter prior to administration (2 mL/kg) via the tail vein (iv) or oral gavage (po).

Supporting Information Available: Tables of HPLC purity and elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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