Conformational and Steric Aspects of the Inhibition of Phenylethanolamine *N*-Methyltransferase by Benzylamines[1a-c]

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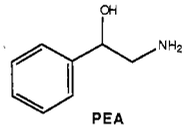
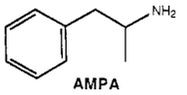
Compounds of the benzylamine (BA) class are potent inhibitors of phenylethanolamine *N*-methyltransferase (PNMT, EC 2.1.1.28). Restriction of the aminomethyl side chain through its incorporation into a cyclic framework as in 1,2,3,4-tetrahydroisoquinoline (THIQ) or 2,3,4,5-tetrahydro-1*H*-2-benzazepine (THBA) results in enhanced potency as an inhibitor, suggesting a conformational effect in the binding of BAs to the active site; however, these ring systems still retain a high degree of flexibility. We have synthesized a series of conformationally defined analogues of benzylamine in order to probe the effect of conformation, as well as the influence of steric bulk, on PNMT inhibition by this class of ligands. In addition, 1-, 3-, and 4-methyl-substituted THIQs were synthesized and evaluated as flexible models for steric bulk tolerance about this ring system. Substitution by a methyl group on either benzylic position of THIQ results in diminished activity as a PNMT inhibitor; however, 3-methyl-THIQ (11) shows enhanced activity as an inhibitor vs THIQ itself. Full conformational restriction of the BA side chain in analogues 4–8 results in a dramatic loss in inhibitor potency. We attribute this effect to a negative steric interaction between the alkyl bridging units above (or below) the heterocyclic ring systems and an active-site amino acid residue. Conformational restriction of THIQ employing a bridging unit that is not located above (or below) the ring system (9) results in only slightly diminished activity compared to THIQ itself. The relative activities of 4–8 were examined in terms of the conformational descriptors τ_1 and τ_2. Although there is no correlation between τ_1 and activity as a PNMT inhibitor, a qualitative relationship between τ_2 (endo or exo) and activity with PNMT is apparent. We believe that the binding of the N–H and/or N–lone pair of electrons may influence the spatial orientation of these molecules at the active site, resulting in positive binding interactions for compounds 4 and 8 and negative interactions for analogues 5–7. The results from the current investigation are compared to those obtained from a similar study involving conformationally defined amphetamines.

The N-methylation of norepinephrine (NE) by *S*-adenosyl-L-methionine (AdoMet) is the final step in epinephrine (Epi) biosynthesis (Figure 1). We have been actively pursuing research directed toward the selective inhibition of the enzyme responsible for catalyzing this process, phenylethanolamine *N*-methyltransferase (PNMT, EC 2.1.1.28), in order to more fully define the function of epinephrine-containing neurons in the mammalian central nervous system. In particular, we are interested in determining the role of brain Epi in the regulation of systemic blood pressure,[2-6] pituitary function,[7-9] and α_2-adrenergic receptors.[10-12]

PNMT is an ideal target for pharmacologic intervention in studies of the function of brain Epi, since its inhibition should affect only Epi levels and not those of its catecholamine precursors, NE and dopamine. For this reason, hundreds of compounds have been examined for their ability to inhibit this enzyme. Predictably, the earliest studied PNMT inhibitors were derived from the 2-phenylethylamine structural unit common to NE and Epi. Hence, two types of PNMT ligands—phenylethanolamines (PEAs), which are always substrates (and thus alternate substrate inhibitors), and α-methylphenylethylamines or amphetamines (AMPAs), which are with few exceptions competitive inhibitors—appeared in the earliest literature accounts in this field.



PEA



AMPA

Structure–activity relationship studies for aromatic ring-substituted derivatives in both of these classes have been performed, revealing a similar optimal substituent type and position for PEAs[13,14] and AMPAs.[15,16] In addition, the optimal position for a bulky phenyl substituent on the aromatic rings of PEA and AMPA is identical.[17] Hence, the data strongly suggest that the aromatic rings in AMPA inhibitors and PEA substrates bind in the same way at the PNMT active site. Since amphetamine ligands do not possess intrinsic activity as substrates, the N-

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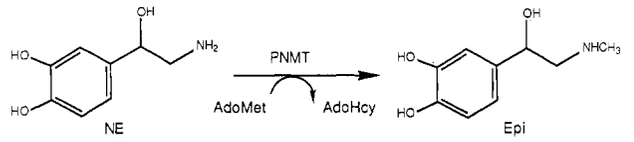
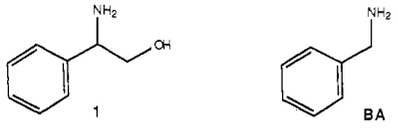


Figure 1. The terminal step in epinephrine (Epi) biosynthesis catalyzed by phenylethanolamine *N*-methyltransferase (PNMT, EC 2.1.1.28), which facilitates the transfer of a methyl group from *S*-adenosyl-L-methionine (AdoMet) to the primary amine in norepinephrine (NE). *S*-Adenosyl-L-homocysteine is also formed through this process.

methylation of phenylethanolamines has been attributed to the presence of the benzylic hydroxyl group.[18]

Our research has focused on determining the bioactive conformation(s) of neurotransmitters at their macromolecular receptors as a means toward delineating active-site and receptor topography. For this reason, several conformationally defined analogues of amphetamine have been prepared and examined for in vitro activity with PNMT.[19] We have described a conformational preference for the binding of amphetamine inhibitors to PNMT and regions of steric intolerance within the active site adjacent to the phenylethylamine binding site.[19] In addition, we have found that phenylethylamines lacking the side chain hydroxyl group found in normal phenylethanolamine substrates can undergo PNMT catalysis provided that the aminoethyl side chain is held in a fully extended conformation and the amino nitrogen resides near the plane of the aromatic ring.[20]

A third class of ligands that bind to the PNMT active site was discovered well after the first two. In an attempt to clarify the features involved in the binding of the ethanolamine side chain in PEAs, the "reverse ethanolamine", 2-amino-2-phenylethanol (**1**), was examined for activity with PNMT. Compound **1** did not undergo PNMT catalysis, but was instead shown to be a competitive inhibitor.[21] The benzylamino portion of this ligand was later identified as the essential structural pharmacophore for activity as a PNMT inhibitor.



As a class of compounds, benzylamines (BAs) represent the most potent inhibitors of PNMT. Structure–activity relationship studies for a number of aromatic ring-substituted benzylamines have revealed that, although the optimal aromatic ring substituent *type* is identical with that for amphetamines and phenylethanolamines, the *position* of substitution is quite different.[22] Furthermore, the optimal position for a phenyl substituent on the aromatic ring portion of BA is different than that for PEA and AMPA.[17] These data appear to be inconsistent with a common mode of binding for BAs and phenylethylamines (PEAs and AMPAs) at the PNMT active site.

Conformational restriction of BAs through the incorporation of the side chain into a 1,2,3,4-tetrahydroisoquinoline (THIQ; **2**)[23] or 2,3,4,5-tetrahydro-1*H*-2-benz-

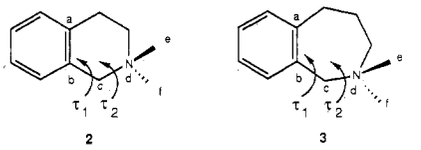
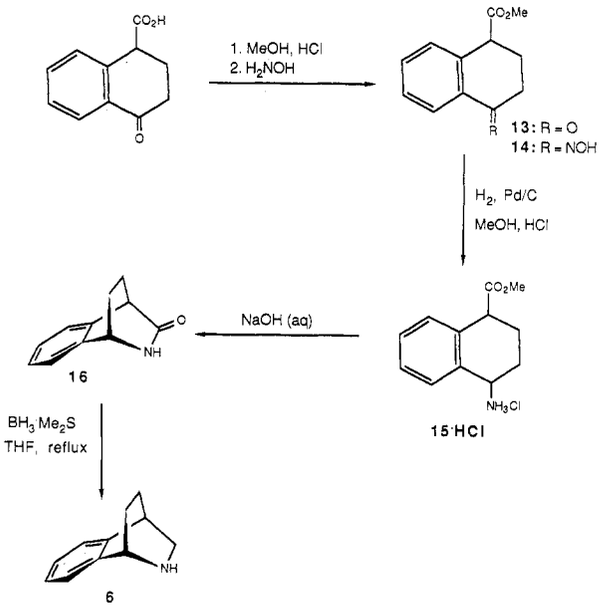


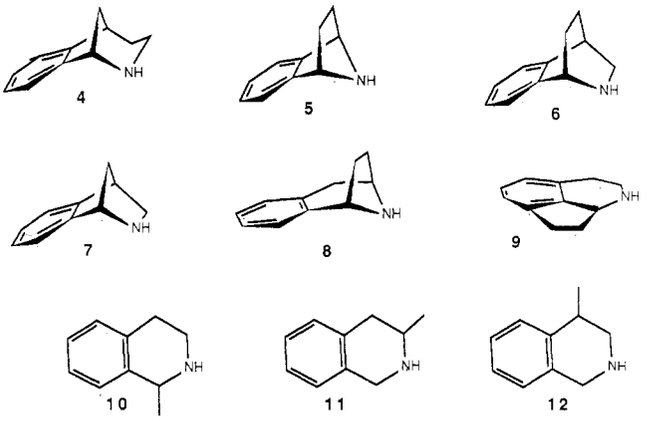
Figure 2. Conformational descriptors τ_1 and τ_2 defined in analogues **2** and **3**. Torsion angle τ_1 is the dihedral angle between the *abc* and the *bcd* planes; torsion angle τ_2 endo is the dihedral angle between the *bcd* and *cdf* planes; and torsion angle τ_2 exo is the dihedral angle between the *bcd* and *cde* planes.

Scheme I



azepine (THBA; **3**)[24] ring system results in enhanced potency of inhibition, suggesting that a particular conformation of the aminomethyl side chain in benzylamine inhibitors is important for activity with PNMT. However, examination of molecular models of **2** and **3** reveals that these ring systems still retain a high degree of conformational flexibility (i.e., $-70° < \tau_1 < 70°$ for **2**; $-90° < \tau_1 < 90°$ for **3**; see Figure 2 for a definition of this conformational descriptor).

In this account, we describe the synthesis and in vitro activity of conformationally defined benzylamines **4–8** with PNMT. These analogues have been carefully designed



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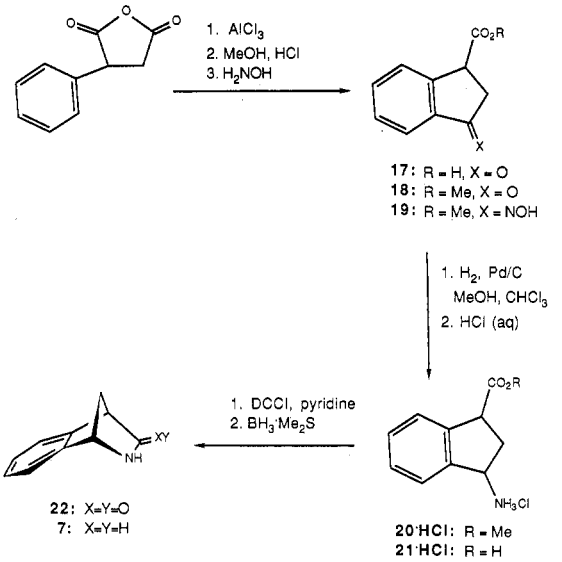
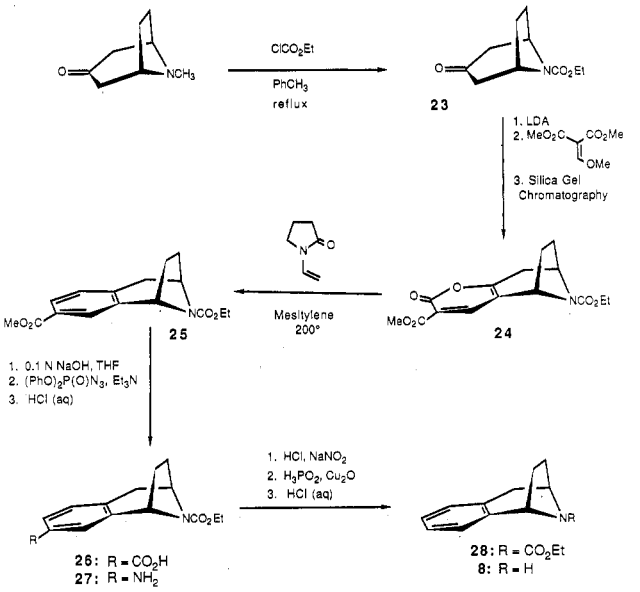
Scheme II



so as to elucidate (1) the optimal geometrical relationship between the aromatic ring and the nitrogen atom (τ_1 angle) as well as (2) the optimal geometrical relationship between the aromatic ring and the N–H and N–lone pair of electrons (τ_2 angle). In addition, the influence of steric bulk about **4**–**8** on their ability to bind to the PNMT active site surface has been evaluated. For this reason, methyl-substituted THIQs **10**–**12** were included as flexible models and 1,8-ethano-THIQ (**9**) as a conformationally restricted (semirigid) one. Finally, since the incorporation of flexible phenylethylamine into conformationally defined bicyclic systems has resulted in unexpected activity as substrates,[20] compounds **4**–**12** were examined for their ability to undergo PNMT-catalyzed methylation.

Chemistry. 7-Azabenzonorbornene (**5**) was prepared by the method of Carpino.[25] Lactam **16** was synthesized by the method of Walker and Alkalay[26] (Scheme I) and subsequently reduced with borane–dimethyl sulfide (BMS)[27] to the desired bicyclic amine **6**. In an analogous fashion, **7** was synthesized (Scheme II) in six steps from the known indanone-3-carboxylic acid (**17**).[28] Thus, Friedel–Crafts cyclization of phenylsuccinic anhydride in the presence of $AlCl_3$ afforded the keto acid **17**, which, without purification, was treated with methanolic hydrogen chloride to afford the corresponding methyl ester **18**. Conversion of **18** to its oxime derivative **19** followed by catalytic hydrogenation gave 3-(methoxycarbonyl)-1-aminoindan hydrochloride (**20**·HCl). The broad band decoupled ^{13}C NMR spectrum of **20** displayed only one set of carbon resonances, implying that up to the detection limits of the instrument (3–5%) a single isomer had been produced. In the case of **14**, similar hydrogenation has been reported to give a preponderance of *cis*-**15**.[26] Examination of the 300-MHz ^1H NMR spectrum of **20** revealed the resonance for each of the protons on C-2 as a triplet of doublets (2.08 ppm, J = 12, 7, and 7 Hz, and 2.75 ppm, J = 12, 6, and 6 Hz). The Karplus equation[29] for vicinal protons bearing a 7-Hz coupling constant fixes the dihedral angle between these protons at 20° (or 135°) while a 6-Hz coupling constant leads to a dihedral angle of 130° (or 30°).

Scheme III



Examination of molecular models of *cis*- and *trans*-**20** reveals that this combination of dihedral angles can only be achieved in the case of *cis*-**20**. Attempted intramolecular cyclization of **20**·HCl, facile in the case of **15**·HCl, was unsuccessful. For this reason, the methyl ester was hydrolyzed, and the resulting amino acid hydrochloride **21**·HCl was treated with dicyclohexylcarbodiimide and pyridine in boiling acetonitrile. Under these conditions, a modest isolated yield (66%) of the bicyclic lactam **22** was obtained. Reduction of **22** with BMS then gave the desired 1,4-methano-THIQ (**7**).

The preparation of the benzo-fused tropane **8** was accomplished by introducing the aromatic ring portion onto a preexisting N-protected tropanone by way of an inverse electron demand Diels–Alder reaction (Scheme III).[30] Commercially available tropan-3-one was converted to *N*-(ethoxycarbonyl)nortropan-3-one (**23**) through the action of ethyl chloroformate in toluene at reflux.[31] Addition of dimethyl (methoxymethylene)malonate to the lithium enolate of **23** in THF at −30 °C afforded, after silica gel chromatography, the 2-pyrone **24**. Inverse electron demand Diels–Alder reaction of **24** with *N*-vinyl-2-pyrrolidone was accomplished at 200 °C in a pressure reaction vessel, yielding benzoate ester **25**. Selective hydrolysis of the methyl ester gave the benzoic acid derivative **26**, which was converted by way of a modified Curtius rearrangement to the aromatic amine **27**.[32] Reductive cleavage of the diazonium salt of **27** with hypophosphorous acid followed by hydrolysis of the crude reaction product **28** afforded the desired 1,3-ethano-THIQ (**8**).

The preparation of the semirigid THIQ derivative **9** proceeded from the known intermediate **29**,[33,34] prepared by acid-catalyzed[35] cyclization of tetrahydroisoquinoline-1-acetic acid. Crude **29** was converted without purification to the corresponding *N*-methoxycarbonyl derivative **30** by reaction with methyl chloroformate (Scheme IV). Hy-

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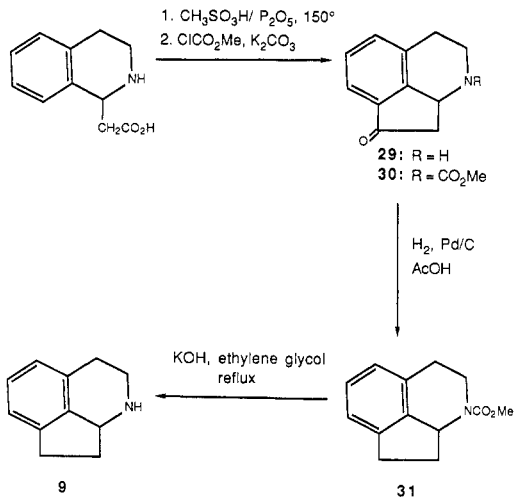
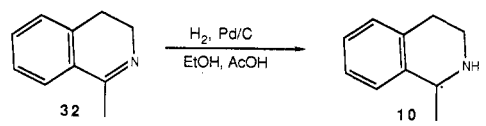
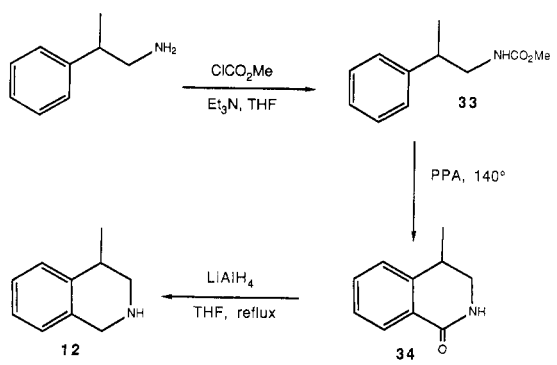
Scheme IV



Scheme V



Scheme VI

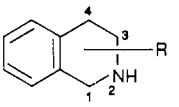


drogenolysis of **30** over a Pd catalyst resulted in the formation of **31**, which underwent alkaline hydrolysis to give the desired 1,8-ethano-THIQ (**9**).

1-Methyl-THIQ (**10**) has been previously prepared by a two-step method involving the dehydration/cyclization of *N*-(chloroacetyl)phenylethylamine followed by catalytic hydrogenation.[36] The commercial availability of 1-methyl-3,4-dihydroisoquinoline (**32**) prompted us to synthesize analogue **10** in a single step by catalytic hydrogenation of **32** (Scheme V). 3-Methyl-THIQ[36] (**11**) was available from a previous study within our group.[19] 4-Methyl-THIQ (**12**) was prepared by the route described in Scheme VI. Methyl carbamate **33**, prepared from commercially available 2-methyl-2-phenylethylamine, underwent polyphosphoric acid (PPA) catalyzed Friedel–Crafts cyclization,[37] affording lactam **34**. Reduction of **34** with $LiAlH_4$ then completed the synthetic sequence.

Biochemistry. Amines **4–12**, as well as THIQ (**2**), were evaluated as their hydrochloride salts for their activity as both substrates and inhibitors of PNMT. Bovine adrenal PNMT,[38] which had been purified according to the method of Connett and Kirshner through the isoelectric precipitation step,[39] was used. In vitro activity with PNMT was assessed by use of a standard radiochemical assay that has been previously described for both substrates[40] and inhibitors.[19] For the determination of the kinetic constants for substrates, at least five concentrations of the variable substrate were employed in the assay. Inhibition constants in this investigation were determined by using at least three different concentrations of the inhibitor with phenylethanolamine as the variable substrate.

Table I. In Vitro Activity of THIQ (**2**) and Methyl-Substituted Analogues **10–12** as Inhibitors of PNMT



R	no.	$K_i \pm$ SEM, μM
H	**2**	10.3 ± 0.86
1-CH_3	**10**	33.1 ± 1.6
3-CH_3	**11**	3.0 ± 0.19
4-CH_3	**12**	70.1 ± 4.1

Results and Discussion

THIQ (**2**), conformationally defined benzylamines **4–8**, semirigid THIQ **9**, and methyl-substituted THIQs **10–12** were examined in vitro both as substrates for PNMT and as inhibitors of the PNMT-catalyzed methylation of phenylethanolamine. The results are presented in Tables I and II. Up to a concentration of 2 mM, no substrate activity was observed for any of these compounds. In vitro PNMT inhibition by **4–12** varied from very good in the case of **11** (K_i = 3.0 μM) to very poor in analogues **5** and **6** (K_i > 2 mM). In each case, the kinetic data support a competitive mode of inhibition, indicating that each inhibitor is binding at or near the site to which the substrate (PEA) is binding.

When only THIQ (**2**) and its monomethyl derivatives **10–12** are considered (Table I), an interesting pattern of activity emerges. Substitution at either of the benzylic positions as in **10** (K_i = 33.1 μM) and **12** (K_i = 70.1 μM) results in a diminished ability, relative to **2** (K_i = 10.3 μM), to successfully compete for the active site with PEA. On the other hand, methyl substitution in the 3-position of the THIQ nucleus results in a threefold enhancement in PNMT inhibition for **11** (K_i = 3.0 μM) with respect to **2**.

We considered the possibility that methyl substitution of **2** might induce conformational changes in the B ring, which could, in turn, affect the ability of these analogues to bind to the PNMT active site. The 300-MHz ^{1}H NMR spectrum for **2** reveals no distinction between individual geminal protons on C-1 (4.0 ppm), C-3 (3.13 ppm), or C-4 (2.79 ppm), suggesting that, at room temperature, **2** exists in a number of low-energy conformations. In the case of **10–12**, however, each geminal proton is distinguishable as an individual resonance, indicating that, at room temperature, a single conformation of these analogues predominates on the NMR time scale. Although the actual conformation of the B ring for **10–12** has not been unambiguously established, molecular models reveal that, in each case, pseudoequatorial positioning of the methyl substituent can lead to a stable half-chair conformation.

It appears that the variation in PNMT inhibition displayed by **10–12** is most probably not due to a conformational effect, but rather to the spatial relationship between

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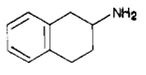
Table II. In Vitro Activities for Inhibition of PNMT, Torsion Angles, Ionization Potentials, and Heats of Formation for Conformationally Defined Benzylamine Analogues 4–9

no.	$K_i \pm$ SEM, μM	τ_1[b]	τ_2[b] exo	τ_2[b] endo	ionization potential[c,e] exo lp	ionization potential[c,e] endo lp	heat of formation[d,e] exo lp	heat of formation[d,e] endo lp
4[a]	174 ± 9.7	90	150	32	9.299 801	9.163 134	29.058 77	30.628 83
5	>2000	32	191	65	9.327 514	9.186 602	41.769 03	43.159 15
6	>2000	58	188	68	9.379 859	9.225 975	24.861 67	25.890 25
7	612 ± 53	70	172	54	9.344 001	9.174 004	45.413 71	46.494 95
8	153 ± 9.8	34	166	42	9.349 802	9.161 295	20.732 77	21.094 59
9	52 ± 2.2	10–35[f]						

[a] Obtained as a generous gift from Prof. Michael Mokotoff, Department of Medicinal Chemistry, University of Pittsburgh; for synthetic details, consult ref 58. [b] In degrees, determined through the use of the SYBYL molecular modeling software[57] ("torsion" command); see Figure 2 for definition. [c] In electron volts, calculated by using the AMPAC semiempirical molecular orbital package (QCPE program 506) in which the MNDO Hamiltonian was employed.[50] [d] In kilocalories, calculated by using the AMPAC semiempirical molecular orbital package (QCPE program 506, MNDO method).[50] [e] Calculations were performed on a VAX 11/750 computer at the Victorian College of Pharmacy, Melbourne, Australia. [f] Maximum range by examination of the molecular model.

Table III. In Vitro PNMT Inhibition by Conformationally Defined Analogues of 2-Aminotetralin[a]

compound	no.	$K_i \pm$ SEM, μM
	35	15 ± 2
	36	479 ± 27
	37	>1000

[a] From ref 19.

the aromatic ring, the secondary amine, and the methyl substituent. That is to say that there exists a degree of steric intolerance at the PNMT active site for methyl-substituted THIQs when the methyl group is located in either benzylic position; however, there is a region of steric bulk tolerance at the active site about the 3-position of the THIQ nucleus which not only accommodates a methyl substituent but does so in such a way as to increase the strength of the enzyme–inhibitor complex.

Turning to the relative activities of conformationally defined analogues 4–8 (Table II), perhaps the most striking feature that appears is the severely diminished ability of these compounds to interact with the active site, as reflected in the large equilibrium dissociation (inhibition) constants. These data are consistent with those obtained for PNMT inhibition studies that we have previously carried out on conformationally defined analogues (**36** and **37**) of 2-aminotetralin (2-AT, **35**), which are presented for reference in Table III. In the case of these analogues, we have attributed the diminished potency of **36** and **37** to steric intolerance above (or below) the 2-AT ring system.[19]

Since **36** and **37** are, like **4–8**, competitive inhibitors of PNMT, it is reasonable to assume that parameters that influence the binding of one type of ligand may influence the other in a similar way. Thus, BA analogues **5** (K_i > 2 mM) and **6** (K_i > 2 mM), each possessing a two-carbon bridge attached to both benzylic positions, are, like AMPA analogue **37** (K_i > 1 mM), incapable of competing effectively with PEA for the active site, while the methylene-bridged derivative **7** (K_i = 612 μM), like **36** (K_i = 479 μM), retains marginal activity. This notion is further supported by the observation that **9** (K_i = 52 μM), a semirigid "flat" structural isomer of **6**, retains most of the intrinsic activity of 1-methyl-THIQ (**10**, K_i = 33.1 μM). Thus, the introduction of bridging units across the B ring of **2** results in dramatically reduced efficacy as an inhibitor of PNMT catalysis, while restraining the B ring by way of an A ring–B ring bridge (as in **9**) does not. Noteworthy in this regard is the fact that conformational restriction of the B ring in THIQ, either partially (such as in the case of **10**) or almost totally (as in **9**), does not in and of itself seriously detract from the intrinsic activity of the parent system. The PNMT binding site for benzylamines apparently does not require conformationally flexible ligands.

If indeed there is a negative steric interaction between a PNMT active site residue and alkyl bridging units above (or below) these ring systems, the relatively good activity of **4** and **8** is, on first inspection, anomalous. Compound **4** (K_i = 174 μM), a conformationally defined analogue of the low-energy chair form of THBA (**3**),[41] possesses, like THIQ derivative **7** (K_i = 612 μM), a single-carbon bridge which joins both of the benzylic positions, but is some three times better than **7** in competing with PEA for the PNMT active site. One possible explanation for this result is a favorable steric interaction of the additional methylene unit (C-3) with the active-site surface which offsets the negative steric interactions of the 1,5-methano bridge. Consistent with this result is the curiously similar threefold enhancement in activity for 3-methyl-THIQ (**11**) with respect to THIQ (**2**) itself. If these compounds are binding in a similar way to the active site, then the additional methylene unit (C-3) in **4** might likely bind favorably to the site at which the 3-methyl group in **11** interacts.

There remains to be explained the good activity of **8** (K_i = 153 μM). This azabicyclic [3.2.1] analogue of THIQ is a close structural isomer of the inactive azabicyclic [2.2.2] analogue **6** (K_i > 2 mM) but is at least an order of magnitude more active. In the case of **8**, the B ring is held fixed by a 1,3-ethano linkage. From the previous discussion, it is perhaps not totally unexpected that 3-substitution results in enhanced potency; however, the magnitude of this effect in **8** is unusually pronounced. Two other apparent differences between **8** and **6** are the angular direction of the alkyl bridging units with respect to the aromatic ring and the conformation in which the B ring of THIQ is held fixed in these systems (a high-energy, full boat form of THIQ in **6** and a low-energy, half-chair conformation of THIQ in **8**).[42]

(41) The assumed low-energy (chair) conformation of THBA is inferred from the known low-energy form of cycloheptene (and benzocycloheptane). For a discussion of the conformation of these carbocyclic systems, see: Riddel, F. G. In *The Conformational Analysis of Heterocyclic Compounds*; Academic: New York, 1980; pp 136–137 and references therein.

(42) The assumed low-energy (half-chair) conformation of THIQ is inferred from the known low-energy form of cyclohexene (and 1,2,3,4-tetrahydronaphthalene). For a discussion of the conformation of these carbocyclic systems, see: Eliel, E. L.; Allinger, N. L.; Angyal, S. J.; Morrison, G. A. In *Conformational Analysis*; American Chemical Society: Washington, DC, 1981; pp 109–110.

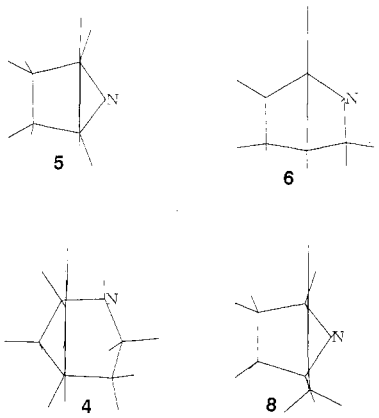


Figure 3. The conformational descriptor τ_1 depicted in SYBYL molecular graphics images of inactive analogues **5** (τ_1 = 32°) and **6** (τ_1 = 58°) as well as active ones **4** (τ_1 = 90°) and **8** (τ_1 = 34°). In these representations, the plane of the aromatic ring is oriented perpendicular to this page. The angular deflection of the C-1–N bond out of the plane of the aromatic ring defines the τ_1 angle. There is no correlation between the τ_1 values for compounds in this series and their PNMT inhibition constants (Table II).

Torsion angles τ_1 and τ_2 (exo and endo) for analogues **4–8** are presented in Table II. In the case of conformationally defined analogues of 2-AT (**35**), we have shown that both of these angles are crucial in the binding of phenylethylamine ligands to PNMT. Because of the similar kinetics of inhibition (competitive) between phenylethylamines and benzylamines, we hoped to observe a correlation between biochemical activity with PNMT and these conformational descriptors. As seen in Table II, the τ_1 value for compounds **4–8** ranges from a low value of 32° in **5**, in which the C-1–N bond and the aromatic ring are nearly coplanar, to a high value of 90° in **4**, in which the C-1–N bond is perpendicular to the plane of the aromatic ring (Figure 3). When the inhibition constants for **4–8** are compared with their τ_1 values (Table II), it is clear that there is no relationship between this conformational descriptor and in vitro activity with PNMT. In fact, the two most potent conformationally defined benzylamines, **4** (K_i = 174 μM) and **8** (K_i = 153 μM), possess τ_1 values that differ by 56°, very nearly the full angular range in this series.

Conversely, it appears that a relationship may exist between the τ_2 angle (exo and endo) and the activity of **4–8** with PNMT. Thus, the two most potent analogues, **4** (K_i = 174 μM) and **8** (K_i = 153 μM), are also the ones characterized by the lowest values of τ_2 (see Table II), while the two inactive compounds, **5** and **6** (K_i > 2000 μM), are defined by the largest values of this conformational descriptor. Hence, in the present series, activity as an inhibitor of PNMT increases as τ_2 (exo and endo) decreases (Figure 4).

The physical basis for this apparent correlation is not entirely clear and raises an issue that is not easily resolved, the directionality of the lone pair (lp) of electrons in these azabicyclic systems. A number of accounts have addressed this issue for a variety of bicyclic amines.[43-49] Included

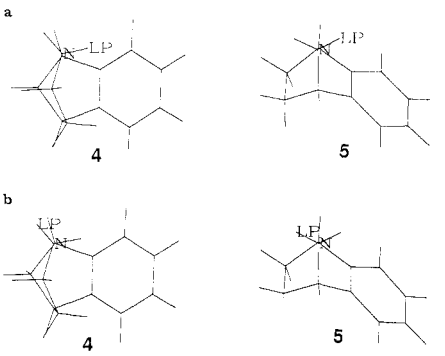
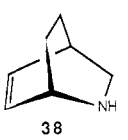


Figure 4. The conformational descriptor τ_2 depicted in SYBYL molecular graphics images of an active analogue (**4**) and an inactive one (**5**). In each case, the molecules are oriented such that the reader is viewing down the C-1 (benzylic bridgehead) carbon–nitrogen bond. (a) The τ_2 endo values for **4** and **5** are 32° and 65°, respectively. (b) The τ_2 exo values for **4** and **5** are 150° and 191°, respectively. Observed PNMT inhibitory constants for these analogues appear to parallel the value of this conformational descriptor such that as τ_2 (endo or exo) decreases, activity as an inhibitor of PNMT increases (Table II).

in a few of these reports are compounds **2** and **5**, as well as 2-azabicyclo[2.2.2]octene (**38**), which may be considered



38

a reasonable model for the azabenzobicyclic [2.2.2] derivative **6**. These studies have focused on determining the magnitude of interaction between a nitrogen lone pair of electrons and an adjacent π system. The methods employed have varied from molecular orbital calculations to determine the energy of n,π homoallylic interacting systems[43] (to which all but compound **5** of this study belong) to experimental techniques involving ^{1}H NMR and ^{13}C NMR contact shift studies,[44-46] photoelectron spectroscopy,[47,48] and iodine–amine charge transfer UV studies.[49] From these reports, it appears that, in the case of **5**, the lone-pair electrons on nitrogen are oriented endo, or toward the aromatic ring, while **38** may exist as an equal mixture of exo and endo forms.

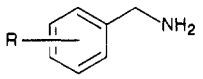
Our own calculations[50] (Table II) predict that the endo lone pair arrangement is slightly (ca. 1 kcal) less stable than the corresponding exo one; however, this difference is too small to confidently predict lone-pair orientation.

The orientation of the lone-pair electrons on nitrogen has a distinct influence on another physical parameter, the ionization potential (IP). As seen in Table II, a positive correlation exists between calculated IPs for endo lp **4–8** and PNMT inhibition constants, while a similar relationship between IP for exo lp and K_i is not evident. This result suggests that it may be the endo lp (exo N–H) form of these bicyclic amines that is important for PNMT active site interactions.

The ionization potentials of both basic[51] and nonbasic[52] amines have been linearly related to basicity. We were interested in determining the pK_a values of the compounds in the present series, but were unable to obtain accurate determinations because sample limitations dictated

(43) Morishisma, I.; Yoshikawa, K. *J. Am. Chem. Soc.* **1975**, *97*, 2950.
(44) Underwood, G. R.; Friedman, H. S. *J. Am. Chem. Soc.* **1974**, *96*, 4989.
(45) Underwood, G. R.; Friedman, H. S. *J. Am. Chem. Soc.* **1977**, *99*, 27.
(46) Yoshikawa, K.; Bekki, K.; Karatsu, M.; Toyoda, K.; Kamio, T.; Morishima, I. *J. Am. Chem. Soc.* **1976**, *98*, 3272.
(47) Morishima, I.; Yoshikawa, K.; Hashimoto, M.; Bekki, K. *J. Am. Chem. Soc.* **1975**, *97*, 4283.
(48) Grutzner, J. B. *J. Am. Chem. Soc.* **1976**, *98*, 6385.
(49) Yoshikawa, K.; Matsui, A.; Morishima, I. *J. Chem. Soc., Perkin Trans. 2* **1977**, 1057.
(50) (a) Dewar, M. J. S.; Thiel, W. *J. Am. Chem. Soc.* **1977**, *99*, 4899. (b) Stewart, J. J. P. *QCPE Bull.* **1986**, *6*, 24a.
(51) Nakajima, T.; Pullman, A. *J. Chim. Phys.* **1958**, *55*, 793.
(52) Nakajima, T.; Pullman, B. *Bull. Soc. Chim. Fr.* **1958**, 1502.

Table IV. Effect of Aromatic Substituents on the Acid Dissociation Constant of, and the Molar pI_{50} Value for, PNMT Inhibition by Benzylamines[a]



R	pK_a[b]	pI_{50},[c] M	R	pK_a[b]	pI_{50},[c] M
4-OMe	9.51	2.78	4-Cl	9.14	4.07
H	9.38	3.12	4-Br	9.13	4.37
4-Me	9.54	3.16	3-Cl	9.01	5.07
4-F	9.30	3.17	3-Br	8.98	5.17
3-Me	9.45	3.82	2,3-Cl_2	7.35[b]	6.23
3-F	9.04	4.00			

[a] A plot of pI_{50} vs pK_a displayed a squared correlation coefficient (r^2) of 0.72. [b] Determined at 25 °C; ref 54. [c] From ref 22.

aqueous concentrations of amine hydrochlorides below the limits of inflection point detection (0.01–0.02 M).[53] However, the predicted range in base strength for endo lp **4–8** (based on the aforementioned correlation data) is less than 0.5 pK_a units; furthermore, the order of base strength for this series would be expected to decrease as **8** > **4** > **7** > **5** > **6**. Thus, from this trend it appears that as BA base strength *increases*, activity as a PNMT inhibitor is enhanced. This, however, is in direct contradiction to the rank order of inhibitor potency for meta- and para-substituted analogues of BA[22] of known base strength[54] (Table IV). The data in Table IV suggest that the *less ionized* the BA ligand is at pH 8 (pH of the in vitro assay buffer solution), the greater its ability to successfully compete with PEA for the active site. If analogues **4–8** are binding to the same amino acid residue at the PNMT active site, then the same trend ought to be observed.

We have considered that the binding of the N–H and/or N–lp electrons of conformationally defined BAs **4–8** at the active site may have a dramatic effect on the orientation of the remainder of the molecule. To visualize this effect, molecular graphics overlays were made of active analogues **4** and **8** with inactive ones **5** and **6** (Figure 5). The four compounds were oriented in the graphics screen (Figure 5a) and aligned with respect to one another by overlaying three points: the center of the aromatic rings, the amino nitrogens, and the endo lp electrons[55] as shown in Figure 5b. The combined van der Waals volume of inactive analogues **5** and **6** was then subtracted from the combined volume of active compound **4** and **8**. The resulting volume display (Figure 5c) represents a region of space occupied by the active analogues but not by the inactive ones.[56] This space is located principally about the 3-position of the THIQ ring system, a region of the PNMT active site that has been shown to interact in a favorable way with a hydrophobic methyl group (Table I). For comparison, the van der Waals volume of 3-methyl-THIQ (**11**) minus that of THIQ (**2**) is presented in Figure 5d.

A slightly different overlay of the same compounds was performed by substituting the C-1 benzylic carbon atoms for the center of the aromatic rings in the least squares fit process. As a result, the aromatic ring portions of **4–6** and **8** are not fixed in space with respect to one another.

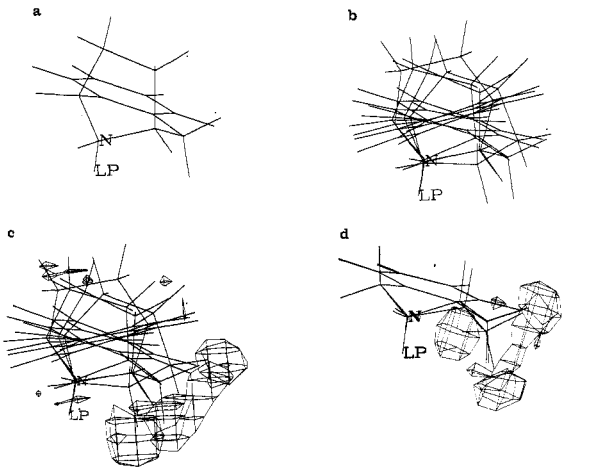


Figure 5. Molecular graphics overlays. The relatively good activity of **4** and **8** as PNMT inhibitors, while closely related systems **5** and **6** are incapable of forming a strong enzyme–inhibitor complex (Table II), may be explained in terms of the spatial orientation of steric bulk in these compounds at the PNMT active site after binding of the N–H and/or N–lp electrons. Each of these compounds was constructed by using the SYBYL molecular modeling software,[57] and energy minimizations were carried out (MAXIMIN command). The molecules are oriented in the screen such that the reader is viewing them from an "end on" perspective (as depicted (a) for compound **8**). A least-squares fit of the center of the aromatic rings, secondary amines, and endo lone-pair electrons (arbitrarily chosen over the exo form)[55] was performed (FIT command), and a composite overlay of **4–6** and **8** was obtained (b). The combined van der Waals volume of the inactive analogues **5** and **6** was then subtracted from the union volume of active ones **4** and **8**, resulting in a volume web display (c) that represents a region of space that the active analogues (but not the inactive ones) occupy.[56] This region is located primarily over the 3-position of THIQ and closely resembles the "extra" van der Waals volume possessed by 3-methyl-THIQ (**11**) over that of THIQ (**2**) itself (d).

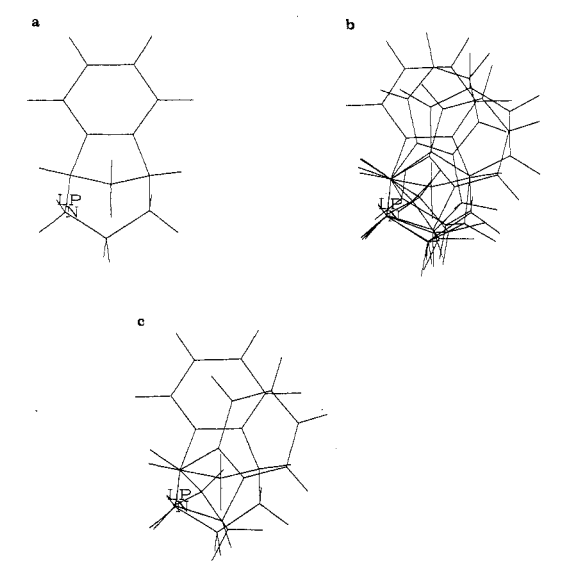


Figure 6. Molecular graphics overlays. When the aromatic ring centers of **4–6** and **8** are replaced in the SYBYL least squares fit process (described in Figure 5) by the C-1 carbon atom and the molecules are oriented as depicted (a) for compound **4**, the resulting composite overlay (b) shows a "fanning" effect of the benzenoid portion of these molecules in the order (clockwise from vertical) **4**, **8**, **6**, and **5**. This order parallels the observed inhibition of PNMT by these analogues. For clarity, this effect is shown (c) with only compounds **4** (oriented vertically, K_i = 174 μM) and **5** (K_i > 2 mM).

Orienting the molecules in the screen as in Figure 6a, we observe that in this case (Figure 6b) the orientation of the

(53) Clarke, F. H. *Calculator Programming for Chemistry and the Life Sciences*; Academic: New York, 1981; p 77.
(54) Blackwell, L. F.; Fischer, A.; Miller, I. J.; Topsom, R. D.; Vaughan, J. *J. Chem. Soc.* **1964**, 3588.
(55) For simplicity, we have considered only endo lp forms in the molecular graphics overlays. Similar overlays of **4–6** and **8** in which the lone-pair geometry was designated as exo results in identical depictions.
(56) Marshall, G. R.; Barry, G. D.; Bosshard, H. E.; Dammkoehler, R. A.; Dunn, D. A. *ACS Symp. Ser.* **1979**, *112*, 205.

aromatic rings in the inactive analogues **5** and **6** is quite different from that of the active ones **4** and **8**. For clarity, this overlay is repeated (Figure 6c) with only compounds **4** (K_i = 174 μM) and **5** (K_i > 2 mM). Hence, the observed equilibrium dissociation constants for **4–8** can be rationalized in terms of the orientation of steric bulk in the alkyl and/or aryl portions of the molecules upon interaction of the N–H and/or N–lp with an active-site residue.

The good activity of **4** and **8** as inhibitors of PNMT may help to explain why conformational restriction of BA by way of a THIQ or THBA ring system results in a dramatic enhancement in potency. As stated earlier, **4** represents the low-energy (chair) form of THBA,[41] and **8** the low-energy (half-chair) conformation of THIQ.[42] From the results of the present study, it appears that when the benzylamine side chain is locked into these conformations, the N–H and N–lp geometry is fixed, and their relationship to the aromatic ring (τ_2 angle) is optimal for binding to the PNMT active site. This suggests that THIQ and THBA themselves bind to the PNMT active site in their respective low-energy conformations.

In summary, we have synthesized a series of conformationally defined analogues of benzylamine in an effort to probe the effect of conformation and steric bulk on their binding to the active site of PNMT. THIQs substituted with a methyl group in the 1-, 3-, or 4-position served as flexible models, and 1,8-ethano-THIQ as a semirigid one for this study. We have determined that a spatially constrained area exists about the 3-position of the THIQ nucleus into which a methyl substituent can fit, and the presence of this 3-methyl group results in a stronger enzyme–ligand complex. This greater activity could result from conformational restriction caused by the presence of the 3-methyl group and/or a positive hydrophobic interaction of the methyl substituent with the PNMT active site. On the other hand, methyl substitution of THIQ in either of the benzylic positions is detrimental to binding. Full conformational restriction of the side chain of BA as in **4–8** results in a diminished ability for these analogues to interact at the PNMT active site. We attribute this to a negative steric interaction between the carbon bridging units in **4–8** and an active-site residue. The relatively good activity of conformationally restricted analogue **9**, in which the carbon–carbon bridge is oriented in a different direction with respect to **4–8**, supports this hypothesis.

The relative activities of **4–8** cannot be fully explained by a single physical descriptor; however, it appears that ligand–receptor steric interactions are the primary source of the observed inhibition constants. Furthermore, while it is clear that the angular deflection of the benzylamino nitrogen out of the plane of the aromatic ring (τ_1 angle) does not correlate with activity as an inhibitor of PNMT, the angular relationship between the aromatic ring and the N–H and N–lp electrons (τ_2 angle) appears to have a direct bearing on the strength of the enzyme–ligand complex. This effect may be the result of the orientation of steric bulk in **4–8** upon the interaction of their N–H and/or N–lp electrons with an active-site residue, a hypothesis that is consistent with the observed activity of 3-methyl-THIQ (**11**). Optimization of the τ_2 angle in **4** and **8** may explain the enhanced activity of BAs upon conformational restriction into the THIQ or THBA framework.

The observation that negative steric interactions exist between bridging units in **4–8** and a PNMT active site residue is consistent with our observations for conformationally defined analogues of 2-AT, **36** and **37**, suggesting that these two classes of PNMT inhibitors are binding in the same general region at the active site. We have found, however, that, unlike the 2-AT series, (1) no direct relationship between the conformational descriptor τ_1 and activity as inhibitors of PNMT can be formulated for benzylamines and (2) conformational definition of the aminoalkyl side chain of benzylamine does not result in the ability of these analogues to participate in PNMT catalysis. Taken together with the differences in aromatic ring substitution pattern which exist between ligands of the phenylethylamine type (PEAs, AMPAs) and those of the BA type, these results suggest that, within the PNMT active site cavity, inhibitors of the BA type are binding in a qualitatively different manner (perhaps to a different amino acid residue) compared to substrates and inhibitors of the phenylethylamine type.

Experimental Section

Analogue **4**·HBr[58] was obtained as a generous gift from Prof. Michael Mokotoff (Department of Medicinal Chemistry, School of Pharmacy, University of Pittsburgh). Melting points were determined on a Thomas-Hoover capillary melting point apparatus calibrated with known compounds and are corrected accordingly. Proton nuclear magnetic resonance spectra (^{1}H NMR) and carbon nuclear magnetic resonance spectra (^{13}C NMR) were obtained on either a Varian FT-80A or XL-300 spectrometer using deuteriated chloroform ($CDCl_3$) as the solvent. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (Me_4Si; 0.0 ppm) for ^{1}H NMR, and $CDCl_3$ (77.0 ppm) for ^{13}C NMR. ^{1}H and ^{13}C NMR spectra of HCl salts were obtained by using deuteriated dimethyl sulfoxide (Me_2SO-d_6) as the solvent, in which case chemical shifts are reported relative to Me_2SO (2.50 ppm for ^{1}H and 39.51 for ^{13}C). Coupling constants (J) are reported in hertz (Hz), and s, d, t, q, and m refer to singlet, doublet, triplet, quartet, and multiplet, respectively. Infrared spectra (IR) were recorded on a Perkin-Elmer IR-727 spectrometer and are calibrated relative to polystyrene (1601 cm^{-1}). Electron-impact mass spectra (EIMS) were obtained on a Varian Atlas CH-5 or a Ribermag 10 10 mass spectrometer. Combustion analyses were performed on a Hewlett-Packard Model 185B CHN analyzer at the University of Kansas and were within 0.4% of the calculated values. Preparative centrifugal thin-layer chromatography (PC-TLC) was performed on a Harrison Model 7924 chromatotron using Merck silica gel 60 PF254 containing $CaSO_4$·0.5H_2O binder. Plate thickness and eluent systems employed are reported in parentheses. Bulb-to-bulb distillations were carried out with a Kugelrohr distillation apparatus (Aldrich Chemical Co., Milwaukee, WI), and the boiling range given refers to the internal oven temperature.

S-Adenosyl-L-methionine was obtained from Sigma Chemical Co. (St. Louis, MO). For the radiochemical assays, [*methyl*-^{3}H]-*S*-adenosyl-L-methionine was purchased from New England Nuclear Corp. (Boston, MA). Bovine adrenal glands, required for the purification of the enzyme used in this study, were obtained from Pel-Freez Biologicals (Rogers, AR). Solvents were routinely distilled prior to use; anhydrous tetrahydrofuran (THF) and ether (Et_2O) were distilled from sodium–benzophenone ketyl; dry methylene chloride (CH_2Cl_2) was obtained by distillation over phosphorus pentoxide; dry benzene was obtained by distillation from calcium hydride; anhydrous methanol (MeOH) and ethanol (EtOH) were obtained by distillation from magnesium. Unless otherwise stated, all MeOH and EtOH used was anhydrous. Hexanes refers to a mixture of isomeric hexanes (bp 68–70 °C), petroleum ether refers to low-boiling hydrocarbons (primarily pentanes and hexanes, bp 35–60 °C), and brine refers to saturated aqueous NaCl. All reactions requiring inert conditions were performed in oven-dried or flame-dried glassware under a N_2 or Ar atmosphere.

1,4-Ethano-1,2,3,4-tetrahydroisoquinoline Hydrochloride (6·HCl). A suspension of **16**[26] (0.75 g, 4.3 mmol) in anhydrous THF (10 mL) was treated with borane–dimethyl sulfide[27] (BMS,

(57) *SYBYL Molecular Modeling System Manual* (Tripos Associates, Inc., St. Louis, MO), 1987.
(58) Mokotoff, M.; Jacobson, A. E. *J. Heterocycl. Chem.* **1970**, *7*, 773.

2 M in THF, 4.78 mL, 9.55 mmol) over 10 min at room temperature, and the resulting solution was warmed under reflux for 11 h. To the cooled solution was added MeOH (10 mL) at a rate that maintained a gentle reflux. After an additional warming under reflux for 8 h, HCl(g) was introduced to the system and the pot contents were stirred at room temperature for 16 h. Removal of the volatiles under reduced pressure afforded **6**·HCl as a white solid, which was recrystallized from EtOH–Et_2O as white needles (0.62 g, 3.2 mmol, 73%): mp 123–125 °C; EIMS, m/z (relative intensity) 159 (M^+, 6), 131 (30), 130 (100), 115 (9), 103 (5). Anal. ($C_{11}H_{14}ClN$) C, H, N. A small portion of **6**·HCl was converted to its free base **6** for spectral analysis: IR (film) 3400–3250, 3050, 2950, 740 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.35–2.35 (m, 4 H, H9,10), 2.00 (s, 1 H, exchangeable with D_2O, NH), 2.45–2.75 (m, 1 H, H4), 2.95–3.25 (m, 2 H, H3), 3.85–4.08 (m, 1 H, H1), 7.10–7.25 (m, 4 H, Ar H); ^{13}C NMR (20 MHz, $CDCl_3$) δ 26.2, 28.8, 35.8, 48.6, 52.0, 123.2, 125.3, 127.5, 128.0, 143.2, 145.3.

3-(Methoxycarbonyl)-1-indanone (18). A solution of phenylsuccinic anhydride (9.1 g, 52 mmol) in 1,2-dichloroethane was added dropwise to a rapidly stirred suspension of aluminum trichloride (15.5 g, 117 mmol) in 1,2-dichloroethane at 0 °C.[28] The resulting solution was stirred at room temperature for 30 min and then was treated at 0 °C with H_2O (50 mL). Extraction with Et_2O (5 × 30 mL) followed by drying of the ethereal pool over $MgSO_4$ and evaporation under reduced pressure afforded the crude keto acid **17** as a red oil (9.6 g). This was dissolved in 25 mL of MeOH containing three drops of H_2SO_4, and the resulting solution was warmed under reflux for 16 h. The volatiles were removed under reduced pressure, and crude **18** was distilled (bulb to bulb, 110–115 °C, 0.2 mm) as a pale yellow oil (7.1 g, 37 mmol, 72% from phenylsuccinic anhydride), which crystallized as white needles on standing in the air: mp 43–45 °C; IR (film) 3020, 1734, 1716, 1620, 770 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 2.88–3.06 (m, 2 H, H2), 3.77 (s, 3 H, OCH_3), 4.15–4.35 (m, 1 H, H3), 7.25–7.75 (m, 4 H, Ar H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 40.1, 43.5, 52.6, 123.9, 126.3, 128.8, 134.9, 135.1, 136.1, 150.9, 170.0; EIMS, m/z (relative intensity) 190 (M^+, 48), 158 (9), 132 (19), 131 (100), 130 (65), 103 (67), 77 (61), 51 (23). Anal. ($C_{11}H_{10}O_3$) C, H.

***syn*- and *anti*-3-(Methoxycarbonyl)-1-indanone Oxime (19).** A solution of hydroxylamine hydrochloride (0.85 g, 12 mmol) and sodium acetate (2.22 g, 16.3 mmol) in H_2O (5 mL) was combined with **18** (1.55 g, 8.16 mmol) and enough EtOH (95%) to effect solution. The reaction mixture was warmed under reflux for 1 h, cooled, and extracted with Et_2O (3 × 30 mL). The ethereal pool was dried ($MgSO_4$) and concentrated under reduced pressure to afford **19** as a white solid (1.6 g, 7.6 mmol, 93%): IR ($CHCl_3$) 3650, 3500–3250, 3050, 1740 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, two isomers apparent) δ 3.15–3.60 (m, 2 H, H2), 3.75 and 3.83 (s, 3 H, OCH_3), 4.20–4.40 (m, 1 H, H3), 7.25–7.90 (m, 4 H, Ar H), 9.40 (br s, 1 H, exchangeable with D_2O, NOH); ^{13}C NMR (75 MHz, $CDCl_3$, two isomers apparent) δ 29.66 and 29.71, 46.14 and 46.18, 52.49, 121.70 and 121.74, 125.73 and 125.78, 128.49 and 128.59, 130.69 and 130.73, 135.72 and 135.75, 144.61 and 144.66, 161.44 and 164.48, 172.70 and 172.73; EIMS, m/z (relative intensity) 205 (M^+, 34), 173 (10), 156 (17), 145 (16), 129 (43), 128 (100), 115 (21), 102 (20), 77 (19). The analytical sample was obtained by recrystallization from EtOH (0.97 g, 4.7 mmol, 58%): mp 132–133 °C. Anal. ($C_{11}H_{11}NO_3$) C, H, N.

***cis*-3-(Methoxycarbonyl)-1-aminoindan Hydrochloride (20·HCl).** A solution of **19** (0.87 g, 4.2 mmol) in MeOH (50 mL) containing 3 mL of $CHCl_3$[59] was hydrogenated over 0.2 g of palladium on carbon (10%) at 50 psi of H_2 for 19 h. Removal of the catalyst and concentration under reduced pressure afforded **20**·HCl as a white solid (0.94 g, 4.1 mmol, 98%). An analytical sample of **20**·HCl was obtained by recrystallization from EtOH–Et_2O (0.88 g, 3.9 mmol, 92%): mp 212–213 °C; EIMS, m/z (relative intensity) 191 (M^+, 18), 176 (29), 160 (20), 132 (34), 131 (44), 130 (100), 117 (41), 115 (52). Anal. ($C_{11}H_{14}ClNO_2$) C, H, N. A small sample of **20**·HCl was converted in quantitative yield to its free base **20**: IR (film) 3410 and 3350 (NH_2), 3070, 3000, 1735, 750 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.75 (br s, 2 H, exchangeable with D_2O, NH_2), 2.08 (ddd, 1 H, J = 12, 7, and 7 Hz, H2), 2.75 (ddd, 1 H, J = 12, 6, and 6 Hz, H2), 3.80 (s, 3 H, OCH_3), 3.95 (dd, J = 6 and 7 Hz, H1), 4.35 (dd, 1 H, J = 6 and 7 Hz, H3), 7.20–7.40 (m, 4 H, Ar H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 39.92, 47.70, 52.16, 56.25, 123.85, 124.63, 127.67, 128.01, 139.61, 147.35, 174.13.

1-Aminoindan-3-carboxylic Acid Hydrochloride (21·HCl). A solution of **20** (5.00 g, 20.7 mmol) in H_2O (10 mL) was combined with 4 N HCl (10 mL), and the resulting solution was warmed under reflux with stirring for 3.5 h. The solvent was removed by vacuum distillation, leaving **21**·HCl as a gummy white solid, which was recrystallized from acetone, affording the analytical sample (4.02 g, 17.7 mmol, 86%): mp 212–215 °C; EIMS, m/z (relative intensity) 177 (M^+, 49), 176 (65), 160 (20), 132 (60), 131 (63), 130 (100), 117 (59), 116 (55), 115 (75). Anal. ($C_{10}H_{12}ClNO_2$) C, H, N.

1,4-Methano-1,2,3,4-tetrahydroisoquinolin-3-one (22). A solution of **21**·HCl (0.76 g, 3.2 mmol) and pyridine (0.75 g, 9.5 mmol) in acetonitrile (50 mL) was combined with a solution of dicyclohexylcarbodiimide (DCCI, 0.65 g, 3.2 mmol) in acetonitrile (2 mL). The resultant mixture was warmed under reflux for 1 h and then was allowed to cool to room temperature. The insoluble matter (dicyclohexylurea) was filtered through paper, and the filtrate was concentrated under reduced pressure to give crude **22** as a white solid (0.45 g, 90%). Purification of **22** by PCTLC (4 mm, 50% ethyl acetate in hexanes eluent) afforded **22** as a white solid (0.33 g, 2.1 mmol, 66%): mp 144–146 °C; IR (KBr) 3380, 1670 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 2.25–2.45 (m, 1 H, H9), 2.55–2.75 (m, 1 H, H9), 3.61 (br s, 1 H, H4), 4.55–4.65 (m, 1 H, H1), 5.33–5.75 (m, 1 H, exchangeable with D_2O, NH), 7.00–7.45 (m, 4 H, Ar H); ^{13}C NMR (20 MHz, $CDCl_3$) δ 53.5, 57.8, 59.6, 120.7, 123.0, 126.2, 126.5, 143.6, 147.3, 182.5; EIMS, m/z (relative intensity) 159 (M^+, 1), 130 (4), 116 (100), 115 (55). Anal. ($C_{10}H_9NO$) C, H, N.

1,4-Methano-1,2,3,4-tetrahydroisoquinoline (7). A suspension of lithium aluminum hydride (0.21 g, 5.5 mmol) in anhydrous THF (100 mL) was cooled in an ice–water bath and then treated with a solution of **22** (0.40 g, 2.5 mmol) in THF (20 mL) at a rate that did not allow the internal temperature to exceed 10 °C. Upon complete addition, the reaction mixture was allowed to warm slowly to room temperature and then was warmed under reflux for 2 h. The flask was cooled to 5 °C, and excess $LiAlH_4$ was quenched by the Fieser method.[60] The aluminum salts were filtered through paper and washed with several portions of Et_2O. The organic layer was separated, and the aqueous one was extracted with Et_2O (3 × 50 mL). The combined organic pool was dried over K_2CO_3 and concentrated under reduced pressure to give crude **7** as a yellow oil (0.48 g). Distillation (bulb to bulb, 85–90 °C, 0.1 mm) afforded pure **7** (0.32 g, 89% from **22**): IR (film) 3400–3300 (NH), 2980, 1460, 755 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.50–1.95 (m, 3 H, exchangeable NH included), 2.21 (dd, 1 H, J = 1 and 9 Hz, endo H3), 3.15 (dd, 1 H, J = 3 and 9 Hz, exo H3), 3.45–3.55 (m, 1 H, H4), 4.30 (br s, 1 H, H1), 7.05–7.30 (m, 4 H, Ar H); ^{13}C NMR (20 MHz, $CDCl_3$) δ 43.2, 45.3, 47.1, 60.5, 118.5, 120.3, 125.4, 125.9, 144.9, 145.9. The hydrochloride salt **7**·HCl was formed by passing anhydrous HCl(g) through an ethereal solution of **7** (0.32 g). The white precipitate (0.34 g) was recrystallized from EtOH–Et_2O (0.08 g): mp 187–188 °C; EIMS, m/z (relative intensity) 145 (M^+, 25), 130 (11), 117 (24), 116 (100), 115 (70). Anal. ($C_{10}H_{12}ClN$) C, H, N.

***N*-(Ethoxycarbonyl)nortropan-3-one (23).** A solution of tropan-3-one (1.0 g, 7.2 mmol) in toluene (10 mL) was treated with ethyl chloroformate (1.56 g, 14.4 mmol) and a small amount (ca. 10 mg) of potassium carbonate. The mixture was subsequently warmed under reflux for 3 h.[31] At this time, the pot contents were poured onto 50 mL of deionized water. The organic phase was separated, washed with H_2O (3 × 30 mL) and brine (30 mL), and then dried over $MgSO_4$. Evaporation of the solvent under reduced pressure afforded crude **23** as a yellow oil, which was distilled (bulb to bulb, 120–125 °C, 2 mm), yielding pure **23** as a colorless oil (1.2 g, 6.1 mmol, 84%): IR (film) 2990, 1730–1680, 755 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.35 (t, 3 H, J = 6 Hz, CH_3), 1.55–2.15 (m, 4 H), 2.15–2.80 (m, 4 H), 4.18 (q, 2 H, J = 6 Hz, OCH_2), 4.40–4.60 (m, 2 H, bridgehead H); EIMS, m/z (relative intensity)

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197 (M^+, 7.9), 168 (7.1), 152 (8.3), 141 (10.1), 140 (91.9), 139 (100), 124 (11.4), 112 (15.2), 96 (13.8), 82 (32.4), 68 (87.2), 67 (46.1). Anal. ($C_{10}H_{15}NO_3$) C, H, N.

Pyrone Derivative 24. The procedure of Boger and Mullican[30] was employed. A solution of diisopropylamine (3.7 g, 37 mmol) in THF (100 mL) at −70 °C was treated with *n*-butyllithium (26 mL of a 1.4 M solution in hexanes, 37 mmol) over 1 h. The solution was stirred between −77 and −10 °C for 45 min and then was recooled to −77 °C. To the rapidly stirred solution was added a solution of **23** (6.0 g, 30 mmol) in 10 mL of THF. Upon complete addition, the solution was stirred for 1 h between −70 and −10 °C, followed by the dropwise addition of a solution of dimethyl (methoxymethylene)malonate (5.83 g, 33.5 mmol) in THF (20 mL). The pot contents were allowed to warm slowly to room temperature and, after 4 h, were poured onto 200 mL of a 5% HCl solution in brine. The organic phase was separated, and the aqueous one was extracted with Et_2O (3 × 100 mL). The combined organic pool was washed with H_2O (100 mL) and brine (100 mL) and then dried over $MgSO_4$. Evaporation of the solvent under reduced pressure afforded a dark oil, which was purified by column chromatography (23 cm × 6 cm, 500 g of silica gel, 70–230 mesh, 50% hexanes in ethyl acetate eluent). Fractions containing the pyrone were combined and concentrated under reduced pressure, yielding **24** as a pale yellow solid (6.01 g, 19.6 mmol, 64%), which was recrystallized from Et_2O, giving the analytical sample: mp 158–159 °C; IR ($CHCl_3$) 3020, 1760, 1740, 1690, 1540 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.26 (t, 3 H, J = 6 Hz, CH_3), 1.75–1.82 (m, 1 H), 1.95–2.08 (m, 1 H), 2.13–2.28 (m, 1 H), 2.30–2.42 (m, 1 H), 2.47 (d, 1 H, J = 4 Hz), 3.18–3.32 (d, 1 H, J = 4 Hz), 3.87 (s, 3 H, OCH_3), 4.10 (q, 2 H, J = 6 Hz, OCH_2), 4.62 (br s, 1 H, bridgehead H), 4.86 (br s, 1 H, bridgehead H), 8.0 (s, 1 H); EIMS, m/z (relative intensity) 307 (M^+, 36.8), 279 (28), 278 (100), 247 (32.2), 234 (23.6), 206 (86.8). Anal. ($C_{15}H_{17}NO_6$) C, H, N.

1,3-Ethano-2-(ethoxycarbonyl)-7-(methoxycarbonyl)-1,2,3,4-tetrahydroisoquinoline (25). A solution of **24** (1.61 g, 5.24 mmol) and *N*-vinyl-2-pyrrolidone (1.16 g, 10.5 mmol) in distilled mesitylene (10 mL) was warmed to 200 °C in a 15-mL pressure reaction tube for 17 h.[30] The contents were allowed to cool to room temperature and then poured onto 5% HCl in brine (100 mL). The organic layer was separated, and the aqueous pool was extracted with Et_2O (4 × 25 mL). The organic phase was washed with 0.5 N HCl (50 mL) and brine (50 mL) and then dried over $MgSO_4$. Evaporation of the filtrate under reduced pressure afforded crude **25** as a dark red oil (1.57 g), which was purified by PCTLC (4 mm, 50% hexanes in ethyl acetate), affording a light yellow oil (1.29 g, 4.46 mmol, 85% from **24**), which solidified upon trituration with petroleum ether. Recrystallization of **25** from Et_2O gave the analytical sample as white needles: mp 90–92 °C; IR (film) 3020, 2990, 1730, 1690, 745, 720 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.10 (t, 3 H, J = 7 Hz, CH_3), 1.40–2.15 (m, 4 H), 2.50 (d, 1 H, J = 16 Hz, H4), 3.32 (dd, 1 H, J = 17 and 5 Hz, H4), 3.80 (s, 3 H, OCH_3), 3.96 (q, 2 H, J = 7 Hz, OCH_2), 4.48 (m, 1 H, H3 bridgehead), 4.91 (d, 1 H, J = 5 Hz, H1 bridgehead), 6.95–7.05 (m, 1 H, Ar H), 7.65–7.75 (m, 2 H, Ar H); ^{13}C NMR (20 MHz, $CDCl_3$) δ 14.55, 29.03, 35.60, 36.66, 51.82, 52.38, 57.26, 60.99, 126.35, 128.03, 129.68, 129.79, 138.16, 141.35, 154.15, 166.78; EIMS, m/z (relative intensity) 289 (M^+, 35.3), 261 (72.8), 260 (100), 232 (14), 216 (23.2), 188 (86.2). Anal. ($C_{16}H_{19}NO_4$) C, H, N.

1,3-Ethano-2-(ethoxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-7-carboxylic Acid (26). To a solution of **25** (1.17 g, 4.05 mmol) in THF (100 mL) was added 0.1 N NaOH (100 mL, 10 mmol), and the mixture was vigorously stirred for 15 h at room temperature. The resulting solution was poured onto 5% HCl in brine (50 mL), and the organic phase was separated. The aqueous layer was extracted with Et_2O (4 × 30 mL), and the pooled organic layers were washed with H_2O (50 mL) and then dried over $MgSO_4$. Evaporation of the solvent under reduced pressure afforded **26** (1.1 g, 4.0 mmol, 99%) as a colorless oil, which solidified upon treatment with Et_2O: mp 147–147.5 °C; ^1H NMR (80 MHz, $CDCl_3$) δ 1.15 (t, 3 H, J = 7 Hz, CH_3), 1.50–2.30 (m, 4 H, H9,10), 2.60 (d, 1 H, J = 17 Hz, H4), 3.40 (dd, 1 H, J = 16 and 5 Hz, H4), 4.03 (q, 2 H, J = 7 Hz, OCH_2), 4.40–4.60 (m, 1 H, H3 bridgehead), 4.85–5.05 (m, 1 H, H1 bridgehead), 7.00–7.15 (m, 2 H, Ar H), 7.65–7.80 (m, 1 H, Ar H), 11.5 (br s, 1 H, CO_2H); EIMS, m/z (relative intensity) 275 (M^+, 33.1), 247 (64.7), 246 (77), 218 (12.4), 202 (23.9), 173 (100). Anal. ($C_{15}H_{17}NO_4$) C, H, N.

1,3-Ethano-2-(ethoxycarbonyl)-7-amino-1,2,3,4-tetrahydroisoquinoline (27). A solution of **26** (380 mg, 1.4 mmol), diphenyl phosphorazidate[32] (760 mg, 2.8 mmol), and triethylamine (280 mg, 2.8 mmol) in anhydrous benzene (150 mL) was warmed under reflux for 12 h and then was alowed to cool to room temperature, and 10 mL of 1 N HCl was added to hydrolyze the intermediate isocyanate. The mixture was warmed under reflux for an additional 1 h and then recooled to room temperature. The organic layer was separated, and the aqueous phase was made alkaline by the addition of 4 N NaOH and then extracted with Et_2O (5 × 30 mL). The organic pool was dried over $MgSO_4$, filtered, and concentrated under reduced pressure, affording **27** as a yellow oil (110 mg, 0.45 mmol, 32%), which solidified upon standing in the air: mp 107–108 °C; IR (film) 3456, 3360, 2980, 1686, 733 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.12 (t, 3 H, J = 7 Hz, CH_3), 1.35–2.15 (m, 4 H, H9,10), 2.33 (d, 1 H, J = 16 Hz, H4), 3.15 (dd, 1 H, J = 17 and 5 Hz, H4), 3.47 (br s, 2 H, exchangeable with D_2O, NH_2), 3.98 (q, 2 H, J = 7 Hz, OCH_2), 4.30–4.52 (m, 1 H, H3 bridgehead), 4.65–4.75 (m, 1 H, H1 bridgehead), 6.25–6.43 (m, 2 H, Ar H), 6.60–6.80 (m, 1 H, Ar H). Compound **27**·HCl was prepared in quantitative yield by passing anhydrous HCl (g) over a rapidly stirred solution of **27** in Et_2O. The analytical sample of **27**·HCl was obtained by recrystallization from EtOH–Et_2O: mp 175–177 °C dec; EIMS, m/z (relative intensity) 246 (M^+, 62), 218 (22), 217 (51), 189 (30), 173 (17), 158 (26), 157 (100), 145 (59). Anal. ($C_{14}H_{19}ClN_2O_2$) C, H, N.

1,3-Ethano-1,2,3,4-tetrahydroisoquinoline Hydrochloride (8·HCl). A solution of **27** (0.24 g, 0.98 mmol) in concentrated HCl (2 mL) at −5 °C was treated with a solution of $NaNO_2$ (0.10 g, 0.14 mmol) in H_2O (0.5 mL). After 20 min, H_3PO_2 (2 mL of a 50% aqueous solution) was added, followed by approximately 5 mg of Cu_2O. The reaction was allowed to proceed at room temperature for 20 h, then the reaction mixture was made alkaline with 4 N NaOH, and the crude product was extracted into Et_2O (5 × 50 mL). The organic layers were pooled, washed with H_2O (50 mL) and brine (50 mL), and then dried ($MgSO_4$). Evaporation of the solvent under reduced pressure gave impure **28** as a dark oil (0.16 g). This was dissolved in 6 N HCl (10 mL) and warmed under reflux for 21 h. The solution was allowed to cool to room temperature and then was washed with Et_2O (4 × 20 mL) to remove nonalkaline impurities. The pH of the aqueous part was adjusted to 14 with NaOH pellets, and the aqueous part was extracted with Et_2O (4 × 50 mL). The combined organic layers were washed with H_2O (50 mL) and brine (50 mL) and then dried over K_2CO_3. Concentration under reduced pressure gave crude **8** (70 mg), which was purified by sublimation (50–60 °C, 0.1 mm), affording **8** as a crystalline white solid (50 mg, 0.31 mmol, 32% from **27**): mp 67–69 °C; ^1H NMR (80 MHz, $CDCl_3$) δ 1.50–2.20 (m, 5 H, 1 H exchangeable with D_2O, NH, H9, and H10), 2.50 (dd, 1 H, J = 1 and 17 Hz, H4), 3.13 (dd, 1 H, J = 16 and 5 Hz, H4), 3.70–3.90 (m, 1 H, H3 bridgehead), 4.05–4.20 (m, 1 H, H1 bridgehead), 6.90–7.10 (m, 4 H, Ar H). A solution of **8** (50 mg) in Et_2O was treated with anhydrous HCl(g) to give a quantitative yield (60 mg) of **8**·HCl (mp 226–228 °C). Recrystallization from EtOH–Et_2O gave the analytical sample: mp 223–224 °C dec; IR (KBr) 3100–2600 (NH·HCl), 1600, 1500, 1460, 1420, 1370, 780, 750 cm^{-1}; ^{13}C NMR (75 MHz, Me_2SO-d_6) δ 26.53, 33.89, 34.58, 52.88, 56.40, 125.95, 126.46, 128.19, 129.27, 130.29, 136.12; EIMS, m/z (relative intensity) 159 (M^+, 6), 144 (3), 143 (2), 131 (34), 130 (100), 115 (9). Anal. ($C_{11}H_{14}ClN$) C, H, N.

***N*-(Methoxycarbonyl)-1,2,3,8a-tetrahydrocyclopent[*ij*]isoquinolin-7(8*H*)-one (30).** Compound **29** was prepared by a modification[35] of the procedure employed by Smissman[33] and by Pelletier.[34] Tetrahydroisoquinoline-1-acetic acid (1.0 g, 5.2 mmol) was added slowly to a mixture of phosphorus pentoxide (1.0 g) in methanesulfonic acid (10 g) which had been preheated to 150 °C. After complete addition, the reaction was allowed to continue with vigorous stirring for 30 min at this temperature, and then the mixture was added dropwise to 150 mL of 1 N NaOH. The product was partitioned between H_2O and CH_2Cl_2, the organic part was separated, and the aqueous one was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic pool was washed with H_2O (100 mL) and dried (K_2CO_3). This solution of the crude amino ketone **29** was cooled to 5 °C in an ice–water bath and then treated with methyl chloroformate (0.98 g, 10.4 mmol). After 1 h, the drying agent was filtered, and the volatiles were removed

under reduced pressure, affording crude **30** as a dark solid (1.27 g). Purification by PCTLC (4 mm, 50% hexanes in ethyl acetate eluent) afforded **30** as a white solid (0.97 g, 4.2 mmol, 69% from the amino acid): mp 151–153 °C; IR (film) 1705, 1680 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 2.60 (dd, 1 H, J = 17 and 6 Hz), 2.94 (br s, 2 H), 3.43 (dd, 1 H, J = 17 and 6 Hz), 3.78 (s, 3 H, OCH_3), 4.25–4.40 (m, 2 H), 4.90 (t, 1 H, J = 6 Hz, H1), 7.25–7.65 (m, 3 H, Ar H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.01, 42.63, 49.32, 50.79, 52.69, 121.04, 128.81, 131.56, 135.03, 136.54, 150.31, 156.80, 201.86; EIMS, m/z (relative intensity) 231 (M^+, 31), 216 (100), 172 (43), 144 (50), 116 (89), 115 (92), 102 (11), 89 (20), 77 (21). Anal. ($C_{13}H_{13}NO_3$) C, H, N.

***N*-(Methoxycarbonyl)-1,8-ethano-1,2,3,4-tetrahydroisoquinoline (31).** Compound **30** (1.4 g, 6.1 mmol) in glacial acetic acid (50 mL) was subjected to hydrogenolysis (50 psi of H_2) in the presence of 10% palladium on carbon for 31 h. The catalyst was filtered, and the volatiles were removed under reduced pressure, affording a yellow oil (1.4 g), which was purified by PCTLC (4 mm, 25% ethyl acetate in hexanes eluent). Concentration of homogeneous fractions under reduced pressure gave **31** as a colorless oil (1.1 g, 4.9 mmol, 81%): IR (film) 2988, 1699, 770 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.45–2.05 (m, 2 H), 2.6–3.05 (m, 4 H), 3.80 (s, 3 H, OCH_3), 4.20–4.45 (m, 2 H), 4.55–4.85 (m, 1 H), 6.85–7.25 (m, 3 H, Ar H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.08, 29.95, 37.18, 42.43, 52.49, 57.55, 122.43, 123.80, 127.33, 134.50, 139.85, 141.65, 153.36; EIMS, m/z (relative intensity) 217 (M^+, 15), 202 (100), 158 (43), 130 (67), 115 (57), 59 (40). Anal. ($C_{13}H_{15}NO_2$) C, H, N.

1,8-Ethano-1,2,3,4-tetrahydroisoquinoline (9). Methyl carbamate **31** (0.21 g, 0.96 mmol) was combined with a solution of 10% aqueous KOH (10 mL) in ethylene glycol (10 mL), and the resulting solution was warmed to 100 °C for 14 h. The cooled solution was extracted with Et_2O (10 × 50 mL), and the combined organic extracts were washed with H_2O (2 × 50 mL) and dried (Na_2CO_3). Evaporation of the solvent under reduced pressure afforded crude **9** as a yellow oil (160 mg), which was distilled (bulb to bulb, 90–95 °C, 0.15 mm) to a colorless liquid (0.15 g, 0.94 mmol, 98%): IR (film) 3370–3250, 3040, 764 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.40–1.85 (m, 2 H), 2.25–3.50 (m, 7 H), 4.05 (m, 1 H, H1), 6.75–7.30 (m, 3 H, Ar H); ^{13}C NMR (20 MHz, $CDCl_3$) δ 26.29, 30.22, 35.69, 45.06, 60.11, 121.47, 125.03, 127.17, 132.05, 141.29, 142.99. The hydrochloride salt **9**·HCl was formed by treating an ethereal solution of **9** (0.15 g) with a saturated solution of HCl(g) in Et_2O. Removal of the volatiles under reduced pressure afforded a white solid (0.16 g), which was recrystallized from MeOH–Et_2O: mp 250 °C dec; EIMS, m/z (relative intensity) 159 (M^+, 27), 158 (100), 130 (97), 115 (31). Anal. ($C_{11}H_{14}ClN$) C, H, N.

1-Methyl-1,2,3,4-tetrahydroisoquinoline (10). A solution of 1-methyl-3,4-dihydroisoquinoline (**32**, 0.90 g, 6.2 mmol) in a mixture of EtOH (10 mL) and acetic acid (10 mL) containing 5% Pd/C (0.20 g) was hydrogenated at 3 atm of H_2 for 3 h. The catalyst was filtered, and the volatiles were removed under reduced pressure. The yellow residue was partitioned between Et_2O (50 mL) and 1 N NaOH (50 mL). The layers were separated, and the aqueous one was extracted with Et_2O (3 × 50 mL). The organic pool was dried (K_2CO_3) and then concentrated under reduced pressure, yielding crude **10** as a yellow liquid (0.82 g). Distillation (bulb to bulb, 78–80 °C, 0.06 mm) afforded pure **10** as a colorless oil (0.75 g, 5.1 mmol, 82%): IR (film) 3300–3260 (NH), 3080, 3040, 2980, 1490, 1450, 750 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.45 (d, 3 H, J = 7 Hz, CH_3), 2.70 (dt, 1 H, J = 16 and 4 Hz, H4), 2.79–2.90 (m, 1 H, H4), 2.94–3.03 (m, 1 H, H3), 3.23 (dt, 1 H, J = 15 and 4 Hz, H3), 4.07 (q, 1 H, J = 7 Hz, H1), 7.00–7.17 (m, 4 H, Ar H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.57, 29.92, 41.68, 51.42, 125.64, 125.67, 127.69, 129.45, 134.63, 140.43. A solution of **10** in Et_2O was treated with anhydrous HCl(g), affording **10**·HCl in quantitative yield (0.93 g): mp 178–180 °C. The analytical sample was obtained by recrystallization from EtOH–Et_2O as colorless needles: mp 178–178.5 °C (lit.[36] mp 178 °C); EIMS, m/z (relative intensity) 147 (M^+, 3), 146 (13), 132 (100), 130 (10), 117 (24), 115 (12). Anal. ($C_{10}H_{14}ClN$) C, H, N.

***N*-(Methoxycarbonyl)-2-phenylpropylamine (33).** Methyl chloroformate (17.5 g, 185 mmol) was added dropwise to an ice-cooled, rapidly stirred solution of 2-methyl-2-phenylethylamine (5.0 g, 37 mmol) and triethylamine (3.7 g, 37 mmol) in THF (200 mL). The resulting white suspension was allowed to warm slowly to room temperature and then to remain there for 24 h. Water (100 mL) was added, the layers were separated, and the aqueous one was extracted with ether (3 × 100 mL). The combined organic layers were washed with water (100 mL), dried ($MgSO_4$), and concentrated under reduced pressure, yielding a yellow oil (7.2 g). Distillation (bulb to bulb, 75–80 °C, 0.10 mm) afforded pure **33** as a colorless liquid (6.6 g, 34 mmol, 92%): IR (film) 3380 (NH), 1720 (C=O), 690, 740 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.25 (d, 3 H, J = 7 Hz, CH_3), 2.88–2.95 (m, 1 H, H2), 3.17–3.29 (m, 1 H, H1), 3.38–3.48 (m, 1 H, H1), 3.60 (s, 3 H, OCH_3), 4.42 (br s, 1 H, exchangeable with D_2O, NH), 7.2–7.4 (m, 5 H, Ar H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.91, 39.86, 47.58, 51.77, 126.47, 127.01, 128.45, 143.89, 156.89; EIMS, m/z (relative intensity) 193 (M^+, 4), 164 (2), 118 (40), 105 (57), 88 (100), 59 (15), 44 (49). Anal. ($C_{11}H_{15}NO_2$) C, H, N.

1-Oxo-4-methyl-1,2,3,4-tetrahydroisoquinoline (34). Methyl carbamate **33** (3.00 g, 15.5 mmol) was added slowly to polyphosphoric acid,[37] which had been warmed to 140 °C. The resulting mixture was manually stirred at this temperature for 20 min and then was poured onto crushed ice (ca. 200 g). The aqueous suspension was extracted with CH_2Cl_2 (10 × 50 mL), and the organic pool was dried over $MgSO_4$. Concentration under reduced pressure afforded crude **34** as a yellow oil (2.39 g), which was distilled (bulb to bulb, 115–120 °C, 0.10 mm), yielding pure **34** as a colorless liquid (1.60 g, 9.94 mmol, 64%): IR (film) 3400–3200 (br, NH), 3000, 1670, 1610, 760 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.34 (d, 3 H, J = 7 Hz, CH_3), 3.05–3.15 (m, 1 H, H4), 3.25–3.35 (m, 1 H, H3), 3.60–3.70 (m, 1 H, H3) 7.20–7.50 (m, 3 H, Ar H), 7.78 (br s, 1 H, exchangeable with D_2O, NH), 8.03–8.10 (m, 1 H, H8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 18.23, 32.08, 46.20, 125.66, 126.71, 127.72, 128.03, 132.15, 143.79, 166.53; EIMS, m/z (relative intensity) 161 (M^+, 70), 132 (100), 131 (25), 104 (70), 103 (26), 78 (27), 77 (27), 51 (17). Anal. ($C_{10}H_{11}NO$) C, H, N.

4-Methyl-1,2,3,4-tetrahydroisoquinoline (12). A solution of $LiAlH_4$ in THF (15 mL, 15 mmol) was added dropwise to a rapidly stirred solution of **34** (1.60 g, 9.94 mmol) in THF (100 mL) under argon. Upon complete addition, the reaction mixture was warmed under reflux for 24 h. Excess $LiAlH_4$ was destroyed by the Fieser method,[60] the liquid was decanted, and the aluminum salts were washed with Et_2O (100 mL). The filtrate was washed with H_2O (3 × 100 mL) and brine (100 mL) and then dried over K_2CO_3. Evaporation of the solvent under reduced pressure resulted in a pale yellow oil (1.37 g), which was distilled (bulb to bulb, 55–60 °C, 0.10 mm) to yield **12** as a colorless oil (1.21 g, 8.23 mmol, 83%): IR (film) 3300 (NH), 3050, 3000, 1490, 1460, 1440, 750 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.24 (d, 3 H, J = 7 Hz, CH_3), 1.75 (br s, 1 H, exchangeable with D_2O, NH), 2.79 (dd, 1 H, J = 13 and 5 Hz, H3), 2.84–2.92 (m, 1 H, H4), 3.19 (dd, 1 H, J = 13 and 5 Hz, H3), 3.95 (d, 1 H, J = 16 Hz, H1), 4.05 (d, 1 H, J = 16 Hz, H1), 7.00–7.18 (m, 4 H, Ar H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.42, 31.89, 48.52, 50.90, 125.57, 125.86, 126.12, 128.03, 135.41, 139.95. A solution of **12** (1.21 g) in Et_2O was treated with anhydrous HCl(g) to form the salt **12**·HCl (1.4 g, mp 126–128 °C), which was recrystallized from EtOH–Et_2O as white needles: mp 129–130 °C; EIMS, m/z (relative intensity) 147 (M^+, 39), 146 (42), 132 (7), 118 (79), 117 (100), 115 (25), 91 (19). Anal. ($C_{10}H_{14}ClN$) C, H, N.

Radiochemical PNMT Assay. The assay employed in this investigation has been described elsewhere.[19,40] Briefly, a typical assay mixture consisted of 50 μL of 0.5 M phosphate buffer (pH 8.0), 25 μL of a 10 mM solution of unlabeled AdoMet, 5 μL of [*methyl*-^{3}H]AdoMet, containing approximately 2 × 10^6 dpm (specific activity approximately 15 Ci/mmol), 25 μL of substrate solution, 25 μL of inhibitor solution, 25 μL of the enzyme preparation, and sufficient water to achieve a final volume of 250 μL. After incubation for 30 min at 37 °C, the reaction was terminated by the addition of 250 μL of 0.5 M borate buffer (pH 10) and the mixture was extracted with 2 mL of toluene–isoamyl alcohol (7:3). The organic layer (1.0 mL) was removed, transferred to a scintillation vial, and diluted with cocktail for counting. The mode of inhibition was ascertained by inspection of the $1/V$ vs $1/S$ plot of the data.

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Registry No. 4·HBr, 28446-49-3; 5·HCl, 5176-31-8; 6, 111634-87-8; 6·HCl, 111634-86-7; 7, 111634-88-9; 7·HCl, 111635-04-2; 8, 111634-89-0; 8·HCl, 111635-06-4; 9, 53921-73-6; 9·HCl, 111635-07-5; 10, 4965-09-7; 10·HCl, 111635-08-6; 11, 29726-60-1; 12, 110841-71-9; 12·HCl, 111661-47-3; 16, 3118-16-9; 17, 29427-69-8; 18, 29427-70-1; 19 (isomer 1), 111634-92-5; 19 (isomer 2), 111634-90-3; 20, 111634-91-4; 20·HCl, 111634-93-6; 21, 111634-94-7; 22, 111634-95-8; 23, 32499-64-2; 24, 111634-96-9; 25, 111634-97-0; 26, 111634-98-1; 27, 111634-99-2; 27·HCl, 111635-05-3; 28, 111635-00-8; 29, 53921-72-5; 30, 111635-01-9; 31, 111635-02-0; 32, 2412-58-0; 33, 111635-03-1; 34, 70079-42-4; PNMT, 9037-68-7; phenylsuccinic anhydride, 1131-15-3; tropinone, 532-24-1; dimethyl (methoxymethylene)malonate, 22398-14-7; *N*-vinyl-2-pyrrolidone, 88-12-0; 1,2,3,4-tetrahydroisoquinoline-1-acetic acid, 105400-81-5; 2-methyl-2-phenylethylamine, 582-22-9.

(8β)-Ergoline-8-carboxylic Acid Cycloalkyl Esters as Serotonin Antagonists: Structure–Activity Study

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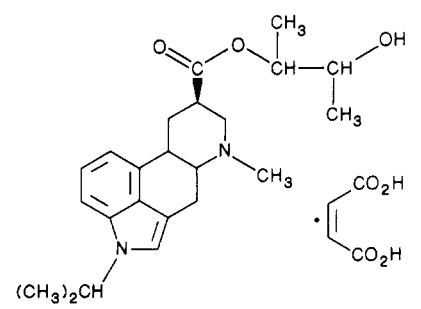
A series of (8β)-6-methyl-1-(1-methylethyl)ergoline-8-carboxylic acid cycloalkyl esters were prepared and examined for blockade of vascular $5HT_2$ receptors. The antagonist in this series that had the highest $5HT_2$ receptor affinity was (8β)-6-methyl-1-(1-methylethyl)ergoline-8-carboxylic acid cyclohexyl ester (**3**). This compound was therefore chosen as the basic backbone of a structure–activity study to determine what effect different N^1-substituents, N^6-substituents, and ester ring substituents had on $5HT_2$ receptor affinity. Maximal $5HT_2$ receptor affinity was obtained when the N^1-substituent was isopropyl, the N^6-substituent was methyl, and there was a hydroxy or keto substituent in the 4-position of the ester cyclohexyl ring.

Esters of (8β)-6-methylergoline-8-carboxylic acids such as LY53857 (**1**) are potent and selective antagonists of $5HT_2$ receptors.[1,2] Compound **1** has been widely used to study $5HT_2$–receptor interactions[3-7] because of its high potency and greater selectivity for $5HT_2$ receptors (relative to α_1 receptors) than other potent $5HT_2$ receptor antagonists.[8] However, **1** is a mixture of isomers[1] which may possess different pharmacokinetic profiles complicating in vivo estimates of efficacy. With **1** as a prototype, it was our goal to search for even more potent and/or selective antagonists that were composed of a single isomer.

Affinities of these compounds for $5HT_2$ receptors were determined by their ability to antagonize serotonin-induced contractions in the rat jugular vein, a tissue known to possess $5HT_2$ receptors that are responsible for serotonin-induced contractions.[9]



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Previously, we established that maximal $5HT_2$ receptor affinity was obtained when the indole nitrogen of the ergoline (N^1) was alkylated with an isopropyl group.[2] We also determined that the stereochemical orientation of the ester side chain had only minimal influence on the $5HT_2$ receptor affinity.[10] It was important to identify further the effects of structural changes in the molecule on $5HT_2$ receptor affinity, in particular, to determine the influence of the N^6-substituent on $5HT_2$ receptor affinity and define what ester moiety gave the highest affinity for $5HT_2$ re-

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