Synthesis and Evaluation of 3-Substituted Analogues of 1,2,3,4-Tetrahydroisoquinoline as Inhibitors of Phenylethanolamine *N*-Methyltransferase[1a]

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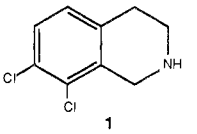
1,2,3,4-Tetrahydroisoquinoline (THIQ) and aryl-substituted derivatives of THIQ are potent inhibitors of the enzyme that catalyzes the formation of epinephrine—phenylethanolamine *N*-methyltransferase (PNMT, E.C. 2.1.1.28). In previous studies, we found that substitution of the 3-position of THIQ with a methyl group resulted in enhanced activity as an inhibitor for 3-methyl-THIQ (**8**) with respect to THIQ itself. To more fully delineate this region of the PNMT active site, we have synthesized and evaluated other 3-substituted THIQ analogues that vary in both steric and electronic character. Extension of the methyl side chain in **8** by a single methylene unit results in diminished potency for 3-ethyl-THIQ (**13**), suggesting that this zone of the active site is spatially compact; furthermore, the region of steric intolerance may be located principally on only "one side" of the 3-position of bound THIQs, since the carbonyl containing (bent) analogues 3-(methoxycarbonyl)-THIQ (**10**) and 3-(aminocarbonyl)-THIQ (**12**) are much less capable of forming a strong enzyme–inhibitor dissociable complex compared to straight-chain derivatives possessing a similar steric component. The good activity of 3-(hydroxymethyl)-THIQ (**11**) as a PNMT inhibitor cannot be explained solely by steric tolerance for this side chain. We believe that an active-site amino acid residue capable of specific (i.e., hydrogen bond) interactions is located in close proximity to the 3-position of bound THIQs and that association of the OH functionality with this active-site residue results in the enhanced in vitro potency of this analogue (K_i = 2.4 μM) compared to that of THIQ (K_i = 10.3 μM). Incorporation of a hydroxymethyl substituent onto the 3-position of the potent PNMT inhibitor 7,8-dichloro-THIQ (SKF 64139, K_i = 0.24 μM) did not result in the same enhancement in inhibitor potency for **17** (K_i = 0.38 μM). This result suggests that simultaneous binding in an optimal orientation of the aromatic halogens, secondary amine, and side-chain hydroxyl functionalities to the PNMT active site is not allowed in this analogue.

Phenylethanolamine *N*-methyltransferase (PNMT, E.C. 2.1.1.28) catalyzes the terminal step in epinephrine (Epi) biosynthesis by facilitating the transfer of the activated methyl group in *S*-adenosyl-L-methionine (AdoMet) to the primary amine in norepinephrine (NE, Figure 1). The discovery of PNMT-containing neurons in the mammalian central nervous system (CNS)[2,3] has led to a number of studies that suggest that epinephrine may function in the central regulation of blood pressure[4-8] and pituitary function.[9-11] However, these results have been based heavily on biochemical and/or pharmacological observations pertaining to the effect of PNMT inhibitors on the particular physiologic process. Unfortunately, the most commonly used PNMT inhibitors, SKF 64139 (**1**), LY 78335 (DCMB, **2**), and LY 134046 (**3**) also interact at other pharmacologically relevant sites in the CNS at or near the concentrations required for PNMT inhibition.[12,13] Thus, no clear definition of the function of CNS epinephrine neurons has been assigned. For this reason, we have undertaken a research program aimed at the design and synthesis of a PNMT-selective inhibitor.



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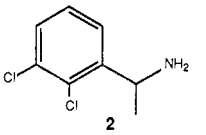

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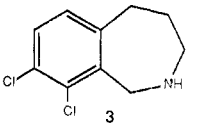

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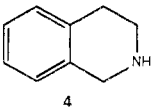
Our approach to this problem has been to identify both the structural and conformational features for the binding of ligands (substrates and competitive inhibitors) to the PNMT active site. Once each of these parameters has been defined, the information can subsequently be applied to the design of a ligand that would be expected to be capable of forming multiple simultaneous binding interactions with the active site.

The optimal aryl substituent type as well as substituent position for the binding of benzylamine ligands to the PNMT active site has been previously described by others.[14] Thus, introduction of a 7,8-dichloro unit into 1,2,3,4-tetrahydroisoquinoline (THIQ, **4**) results in a tremendous enhancement in potency as a PNMT inhibitor for **1**[15] (K_i = 0.24 μM, Table II) as compared to **4** (K_i = 10.3 μM). Even more pronounced is the ability of 2,3-dichloro-α-methylbenzylamine (DCMB, **2**) to competitively inhibit this process (K_i = 0.56 μM) compared to its nonchlorinated parent **5** (K_i = 460 μM).



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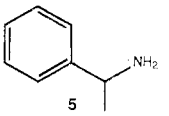

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(1) (a) Taken, in part, from the dissertation submitted by J.A.M. to the Graduate School of the University of Kansas for the degree of Doctor of Philosophy, June, 1987. (b) NIH Predoctoral Trainee (Grant GM07775) and recipient of the Robert Irsay–Norman Dahle Award in Medicinal Chemistry at the University of Kansas (1985, J.A.M.; 1986, D.J.S.).
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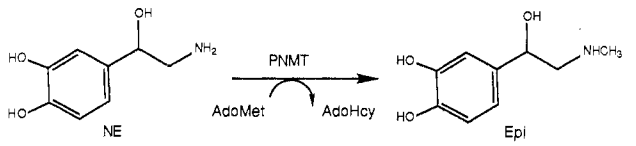
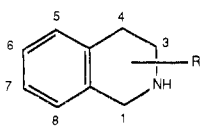


Figure 1. The terminal step in epinephrine (Epi) biosynthesis catalyzed by phenylethanolamine *N*-methyltransferase (PNMT, E.C. 2.1.1.28), which facilitates the transfer of a methyl group from *S*-adenosyl-L-methionine (AdoMet) to the primary amine in norepinephrine (NE). *S*-Adenosyl-L-homocysteine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

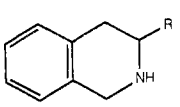
During the course of a study recently performed in our laboratories, we required the in vitro evaluation of **4** and its methyl-substituted derivatives **6–8** as reference compounds for studying the conformational and steric parameters that influence the binding of the side chain of benzylamine inhibitors to the PNMT active site.[16] The



6: R = 1-CH_3
7: R = 4-CH_3
8: R = 3-CH_3

results of this part of the study demonstrated that a region of space within the PNMT active site coinciding with the 3-position of the THIQ nucleus was capable of interacting in a favorable way with a methyl substituent, as manifested in a decrease in the equilibrium dissociation constant of the enzyme–inhibitor complex for **8** (K_i = 3.0 μM) compared to **4** itself (K_i = 10.3 μM). The other methyl-substituted derivatives of **4** (**6** and **7**) did not bind to the active site as effectively (K_i = 33.1 and 70.1 μM, respectively), implying that there exist regions of steric intolerance about both of the benzylic positions of THIQ at the PNMT active site.

These results encouraged us to explore more fully the region of the PNMT active site about the 3-position of THIQ, with the ultimate goal of incorporating an optimized 3-substituent into the dichloro-THIQ skeleton **1** in the hope of gaining both enhanced activity as an inhibitor of PNMT and, more importantly, enhanced bioselectivity. Hence, we now report (1) the synthesis of 3-substituted THIQ derivatives **10–13** and the in vitro evaluation of

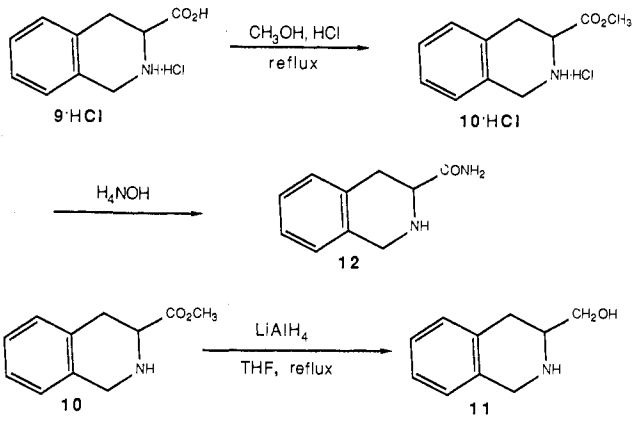


9: R = CO_2H
10: R = CO_2CH_3
11: R = CH_2OH
12: R = $CONH_2$
13: R = CH_2CH_3

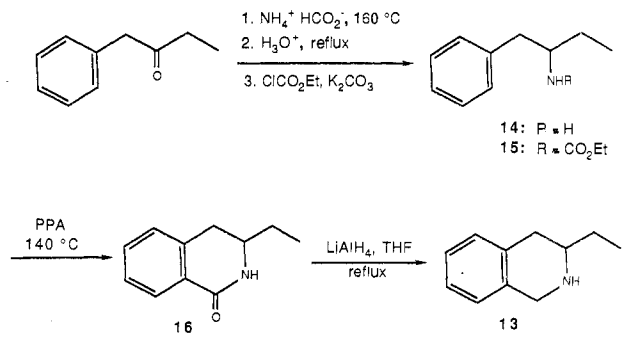
compounds **8–13** as inhibitors of PNMT catalysis, as well as (2) the synthesis and biochemical evaluation of **17**, an analogue of **4** in which both aryl and aliphatic substituents have been optimized for PNMT binding.

Chemistry. 3-Methyl-THIQ (**8**)[17] was available from a previous study in our laboratory; THIQ (**4**) and THIQ-3-carboxylic acid hydrochloride (**9**·HCl) were commercially available (Aldrich Chemical Co., Milwaukee, WI). The preparation of THIQ analogues **10–12** is depicted in

Scheme I



Scheme II



Scheme I. Fischer esterification of **9**·HCl was sluggish, no doubt due to the limited solubility of the amino acid hydrochloride in methanol. Nevertheless, after 48 h at reflux, solution did occur, and after an additional 24 h, a quantitative yield of the desired methyl ester hydrochloride (**10**·HCl)[18] was obtained. Reduction of **10** with $LiAlH_4$ afforded 3-(hydroxymethyl)-THIQ (**11**),[19] while aminolysis of **10**·HCl resulted in the quantitative production of 3-(aminocarbonyl)-THIQ (**12**).

The 3-ethyl derivative **13** was prepared in four steps as depicted in Scheme II. Leuckart reductive amination[20] of 1-phenyl-2-butanone afforded the known 1-phenyl-2-aminobutane (**14**),[21] which, without purification, was treated with ethyl chloroformate to give (in 64% overall yield) the *N*-ethoxycarbonyl derivative **15**. Friedel–Crafts cyclization of **15** was effected with polyphosphoric acid (PPA) at 140 °C.[22] The course of this reaction was conveniently monitored by noting the evolution of ethanol (observed as foaming). Thus, after 10 min at 140 °C, foaming ceased, and lactam **16** was isolated in 89% yield. Reduction of the carbonyl moiety was smoothly accomplished with $LiAlH_4$, affording a 94% yield of the desired 3-ethyl-THIQ (**13**).

3-(Hydroxymethyl)-7,8-dichloro-1,2,3,4-tetrahydroisoquinoline (**17**) was synthesized by the route depicted in Scheme III. Directed aromatic lithiation[23] of 2,3-dichlorobenzamide (**19**, prepared in two steps from commercially available 2,3-dichlorobenzoic acid) with sec-BuLi

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Scheme III

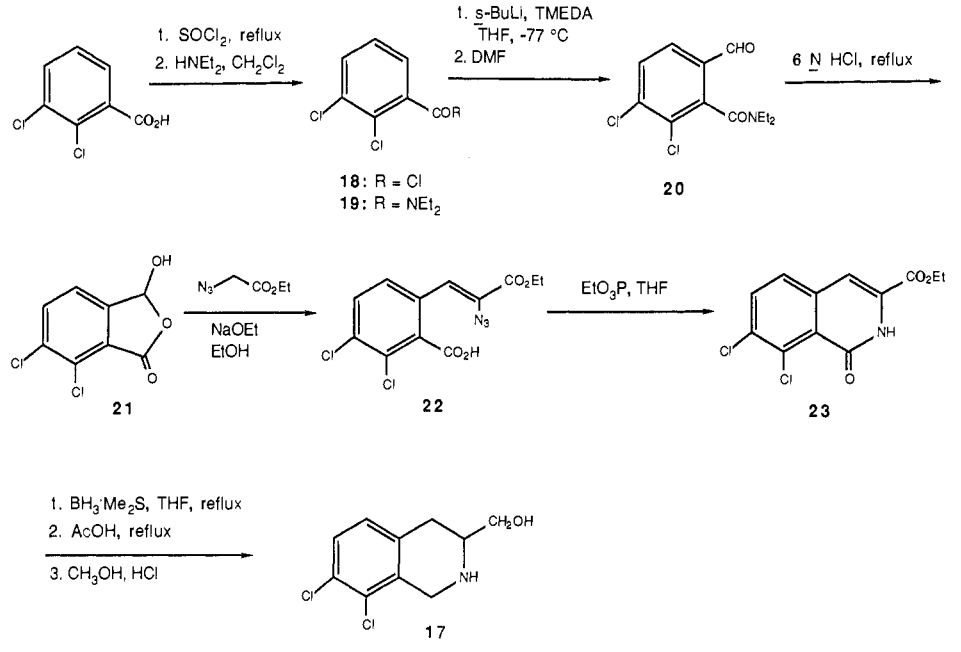
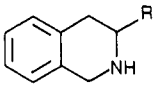


Table I. In Vitro Inhibition of PNMT by 3-Substituted Analogues of 1,2,3,4-Tetrahydroisoquinoline



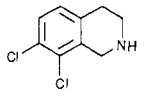
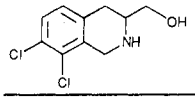
no.	R	$K_i \pm$ SEM, μM	$\Delta G°_{binding}$[a]	MR[b]	π[b]	σ^*[b]
4	H	10.3 ± 0.86	7.07	1.03	0.0	0.49
8	CH_3	3.0 ± 0.19	7.83	5.65	0.56	0.0
9	CO_2H	>2000	>3.83	6.05[c]	−4.36[d]	0.92[e]
10	CO_2CH_3	69.5[f]	5.59	12.87	−0.01	2.00
11	CH_2OH	2.4 ± 0.24	8.00	7.19	−1.0	0.56
12	$CONH_2$	40.5 ± 2.12	6.23	9.81	−1.49	*g*
13	CH_2CH_3	23.9 ± 1.00	6.55	10.3	1.02	−0.10

[a] In kcal/mol, calculated by the equation: $\Delta G = -RT$ (ln K_i), where R = 1.987 cal × mol^{-1} × K^{-1} and T = 310 K. [b] From ref 31. [c] For COO^- (MR for CO_2H = 6.93). [d] For COO^- (π for CO_2H = −0.32). [e] For COO^- (σ^* for CO_2H = 2.90). [f] Extrapolated to zero time from K_i values determined at incubation times of 5, 10, 15, 20, and 30 min; slope 1.53 with r = 0.972. [g] No value available.

and *N,N,N′,N′*-tetramethylethylenediamine (TMEDA) followed by quenching the aryl anion with DMF afforded, after aqueous workup, reproducibly high yields of the 1,2,3,4-tetrasubstituted benzene derivative **20**. Hydrolysis of the diethylamide (6 N HCl, reflux) was accompanied by concomitant lactone formation, resulting in a quantitative yield of the 3-hydroxyphthalide derivative **21**.[24] Treatment of **21** with the anion derived from ethyl azidoacetate afforded (in low isolated yields) azidocinnamate **22**, which, upon treatment with triethyl phosphite, underwent an intramolecular aza-Wittig condensation to yield the isoquinolone **23**.[25] Reduction of **23** to the desired THIQ derivative **17** was then effected with borane–dimethyl sulfide (BMS) in THF at reflux.[26]

Biochemistry. Amines **1**, **4**, **8–13**, and **17** were evaluated as their hydrochloride salts for their activity as both substrates and inhibitors of PNMT. Bovine adrenal PNMT,[27] which had been purified according to the method of Connett and Kirshner through the isoelectric precipitation step,[28] was used. In vitro activity was assessed by use of a standard radiochemical assay that has been previously described for both substrates[29] and inhibitors.[30] Inhibition constants in this investigation were determined

Table II. In Vitro Inhibition of PNMT by Dichloro-Substituted THIQs

compound	no.	$K_i \pm$ SEM, μM	$\Delta G°_{binding}$[a]
	1	0.24 ± 0.06	9.36
	17	0.38 ± 0.05	9.07

[a] In kcal/mol, calculated by the equation: $\Delta G = -RT$ (ln K_i), where R = 1.987 cal × mol^{-1} × K^{-1} and T = 310 K.

(24) Whereas hydrolysis of **20** was facile, hydrolysis of other diethylbenzamides (i.e. **19**) failed even under extremely vigorous conditions (CF_3CO_2H–HCl, 200 °C; KOH, ethylene glycol, 160 °C). Thus, facile hydrolysis of **20** has been attributed to the intramolecular participation of a hydrated aldehyde moiety in the ortho position.

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by using at least three different concentrations of the inhibitor with phenylethanolamine as the variable substrate.

Results and Discussion

As expected, none of the THIQ derivatives prepared in this study demonstrated activity as substrates for PNMT; however, each analogue did display the expected ability to inhibit the PNMT-catalyzed N-methylation of phenylethanolamine (Tables I and II), and did so by a competitive kinetic mechanism, denoting active-site binding. Examination of the results presented in Table I demonstrates the effect of the substituent present in the 3-position of THIQ (**4**) on the observed equilibrium dissociation constant for the enzyme–THIQ complex (K_i).

We attribute the enhanced activity as a PNMT inhibitor for methyl-substituted THIQ **8** (K_i = 3.0 μM) over that of THIQ (**4**) itself to a positive binding interaction between the methyl group (MR = 5.65)[31] and the active site, resulting in a stronger enzyme-inhibitor complex for **8** ($\Delta\Delta G°$ = 0.76 kcal/mol)[32] with respect to that for **4**.[16] From the data in Table I, it now appears that this region of the active site is spatially compact. Thus, extension of the side chain in **8** by a single methylene unit (MR = 10.3 for ethyl) as in **13** results in diminished active-site interactions (K_i = 23.9 μM), corresponding to a loss in binding free energy with respect to either **4** ($\Delta\Delta G°$ = −0.52 kcal/mol) or **8** ($\Delta\Delta G°$ = −1.28 kcal/mol).

Arguments based exclusively on substituent size cannot, however, fully explain the observed enzyme–inhibitor dissociation constant for the hydroxymethyl-substituted THIQ **11** (K_i = 2.4 μM). The side chain in this analogue is intermediate in size (MR = 7.19) with respect to **8** and **13**, yet **11** is as potent as **8** and 10 times more potent than **13** in inhibiting PNMT catalysis. This is represented in a change in binding free energy for **11** compared to **13** of 1.45 kcal/mol, which may reflect a specific (i.e., hydrogen bond) interaction between the OH functionality with an active-site residue. That **8** and **11** display very similar inhibition of PNMT may result from an enhanced affinity of the hydroxymethyl substituent through hydrogen bonding offset by the increased steric bulk of that substituent over that of a methyl group.

The effect of side-chain branching on in vitro PNMT inhibition can be observed in analogue **12**. The carboxamide side chain in **12** is actually less sterically imposing (MR = 9.81) than is the ethyl substituent in **13** (MR = 10.3), but results in a decrease in binding free energy ($\Delta\Delta G°$ = −0.32 kcal/mol) relative to **13**, reflected in an approximately twofold increase in the equilibrium dissociation constant (K_i = 40.5 μM). This suggests that PNMT active-site interactions with 3-substituted THIQs are sensitive not only to the *amount* of steric bulk about the 3-position but also to the spatial *orientation* of the side chain, with linear side chains binding more effectively than similar-sized, carbonyl-containing (bent) chains.

Ester **10** hydrolyzed slowly under the assay conditions (phosphate buffer at pH 8) to amino acid **9**. The K_i value given in Table I (69.5 μM) was obtained by running the radiochemical assay at five time values (5, 10, 15, 20, and 30 min) and extrapolating to zero time. In an NMR experiment (**10** in pD 8.45 phosphate buffer[33] in D_2O without enzyme) in which the appearance of methanol was measured versus time, it is estimated that no more than 15% of the ester is hydrolyzed within the standard 30-min assay. For details, see the Experimental Section.

The enhanced ability for **12** (K_i = 40.5 μM) compared to the THIQ methyl ester derivative **10** (K_i = 69.5 μM) to interact with the active site may be the result of two or more independent factors. First, the more potent (carboxamide) analogue possesses the smaller side chain (M_r = 9.81 for **12** compared to 12.87 for **10**) that might then be expected to interfere with active-site binding to a lesser extent than would the side chain in **10**; and second, the carboxamide NH_2 moiety may be interacting with an active-site residue in a manner similar to that postulated for the binding of the (hydroxymethyl)-THIQ derivative **11** (i.e., H-bonding).

The amino acid **9** (K_i > 2000 μM) is incapable of binding to the PNMT active site. At pH 8.0 (pH of in vitro assay buffer) this analogue is expected to exist as an anion, setting it apart from any of the other THIQ derivatives in this study. This physical property may then form the basis for the lack of PNMT active-site binding of **9** if, for instance, repulsive charge–charge interactions are occurring between the carboxylate anion of **9** with a similarly charged active-site species located in a region of space not far removed from the 3-position of bound THIQ inhibitors. This hypothesis is consistent with the notion that a hydrogen-bond interaction may exist between the hydroxymethyl substituent in **11** (and the carboxamide substituent in **12**) and an active-site residue and suggests that this residue may be a negatively charged species (i.e., CO_2^-). In the case of **11**, the addition of this interaction to those that are fundamental in the binding of a THIQ nucleus to the PNMT active site has resulted in the most potent aryl-unsubstituted THIQ derivative yet described.

With so few 3-substituents it is not possible to rule out other explanations for the relative inhibitor potencies observed and discussed above. However, it does seem clear that neither lipophilicity (as measured by the Hansch π value[31]) nor electronic effects (as measured by the σ^* value[31]) can account for the results. The two most potent analogues (**8** and **11**) are of similar inhibitor potency yet there is more than a 1.5 log difference in π (0.56 for **8** and −1.0 for **11**). Analogues with higher (**13**, 3-ethyl, π = 1.02) or lower (**12**, 3-aminocarbonyl, π = −1.49) values of π are much less potent than either **8** or **11**. Tetrahydroisoquinoline (**4**, σ^* = 0.49) and 3-(hydroxymethyl)-THIQ (**11**, σ^* = 0.56) have similar values of σ^* yet show a fourfold difference in inhibitor potency, with **11** being the more potent and having the larger value of σ^*. Conversely, 3-methyl-THIQ (**8**, σ^* = 0.0) is equiactive with **11** yet the 3-ethyl derivative (**13**, σ^* = −0.10) has a similar σ^* value but is fourfold less active. The steric argument coupled with a hydrogen-bonding interaction, as discussed above, seem best able to account for the observed results.

The presence of aryl halogens in PNMT inhibitors of the benzylamine class has previously been demonstrated to dramatically increase the strength of the enzyme–inhibitor dissociable complex.[14,15] Thus, the chlorine substituents present in THIQ derivative **1** (Table II, K_i = 0.24 μM) increase the free energy of binding to the PNMT active site by a full 2.3 kcal/mol over that of the unsubstituted parent **4** (Table I, K_i = 10.3 μM). However, while

(31) Molecular refractivity (MR) is used as a physical descriptor of steric bulk. For a discussion, see: Hansch, C.; Leo, A. J. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley: New York, 1979; p 44 ff. Values for MR, π and σ^* were taken from this same reference, p 67 ff.

(32) The sign of $\Delta\Delta G°$ reflects a positive or negative contribution to binding energy. In our convention, a negative (−) $\Delta\Delta G°$ denotes a loss in PNMT active-site binding energy with respect to a given standard.

(33) The pH of a solution in D_2O is about 0.45 log unit higher than the corresponding H_2O solution. See *Lange's Handbook of Chemistry*, 13th ed.; Dean, J. A., Ed.; McGraw-Hill: New York, 1985; pp 5–105.

the addition of a polar hydroxymethyl substituent to **4** resulted in enhanced binding ($\Delta\Delta G^\circ$ = 0.92 kcal/mol) for **11** (K_i = 2.4 μM), addition of this unit to **1** does not result in a similar enhancement in PNMT active-site binding for **17** (Table II, K_i = 0.38 μM). If this were so, the calculated range in binding free energy for **17** with respect to **1** (0.92 kcal/mol) would be reflected in a calculated equilibrium dissociation constant of 0.09 μM.

Thus, the contributions to PNMT binding by the aryl halogens and the aliphatic substituent at position 3 of THIQ inhibitors do not appear to be independent of one another, and simultaneous full association of the aryl halogens and the hydroxymethyl group with their respective binding sites within the PNMT active-site cavity is apparently not allowed. Since we have optimized the binding units at opposite ends of the THIQ nucleus, it is conceivable that association of one of these binding groups with the PNMT active site actually pulls the other away from its site of interaction. However, analogue **17** does maintain most of the ability of **1** to inhibit PNMT and possesses an additional (3-hydroxymethyl) substituent, which amounts to a redundancy for PNMT active-site binding.

In summary, we have prepared a series of THIQ derivatives substituted in the 3-position, and have determined that (a) the region of space within the PNMT active site about this position is sterically compact, being capable of accommodating a methyl substituent (as in **8**, K_i = 3.0 μM) to a much greater extent than an ethyl group (as in **13**, K_i = 23.9 μM), (b) this effect appears to be more pronounced for carbonyl containing (bent) derivatives **10** and **12**, suggesting that the region of steric intolerance may exist on only "one side" of the 3-position of the THIQ nucleus, and (c) an active-site residue capable of specific (e.g., H bond) interactions may exist in close proximity to the 3-position of bound THIQs. Association with this residue may then account for the observed enhancement in binding to the PNMT active site for **11** (K_i = 2.4 μM) compared to **13** (K_i = 23.9 μM) and (at least partially) for the enhancement in binding of **12** (K_i = 40.5 μM) compared to **10** (K_i = 69.5 μM). The total lack of activity as an inhibitor of PNMT for the amino acid derivative **9** may then be attributed to unfavorable charge–charge interactions if the active-site residue is a negatively charged species (i.e., carboxylate anion).

Optimization of the fundamental THIQ structure in both the aromatic ring portion (by the inclusion of a 7,8-dichloro unit) and in the aliphatic region (by the inclusion of a 3-hydroxymethyl substituent) has been performed. The resulting THIQ analogue **17** (K_i = 0.38 μM) did not exhibit an equilibrium dissociation constant consistent with the simultaneous interaction of each individual binding unit (Cl, NH, OH) present in **17** with the PNMT active site. However, very little binding energy is lost upon the introduction of the 3-hydroxymethyl side chain into **1** ($\Delta\Delta G^\circ$ = −0.29 kcal/mol), so potency for PNMT inhibition has not been seriously compromised. Studies for determining the in vitro activity of **17** at other pharmacologically important sites in the CNS are currently under way.

Experimental Section

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 with 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. 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) and chemical-ionization mass spectra (CIMS) were obtained on a Varian Atlas CH-5 or a Ribermag R 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 (PCTLC) was performed on a Harrison Model 7924 Chromatotron with use of Merck silica gel 60 PF254 containing $CaSO_4 \cdot 0.5H_2O$ 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.), 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 the 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.

3-(Methoxycarbonyl)-1,2,3,4-tetrahydroisoquinoline Hydrochloride (10·HCl).[18] A solution of tetrahydroisoquinoline-3-carboxylic acid hydrochloride (**9**·HCl; 10.0 g, 46.8 mmol) in MeOH (200 mL) saturated with HCl(g) was warmed under reflux for 72 h. The volatiles were removed under reduced pressure, affording **10**·HCl as a white solid (10.7 g, 46.8 mmol) that was recrystallized from MeOH–Et_2O: mp 302–303 °C dec; EIMS, m/z (relative intensity) 191 (M^+, 2), 176 (1), 132 (100), 130 (58). Anal. ($C_{11}H_{14}ClNO_2$) C, H, N. For spectral analysis, a portion of the hydrochloride salt was converted to the free base, which was distilled (bulb-to-bulb, bp 95–98 °C, 0.1 mm), affording **10** as a colorless liquid: IR (film) 3370 (NH), 3050, 2970, 1740 (CO), 750 cm^{-1}; ^{1}H NMR (300 MHz) δ 2.08 (br s, 1 H, exchangeable with D_2O, NH), 2.90 (dd, 1 H, J = 13 and 9 Hz, H4), 3.05 (dd, 1 H, J = 13 and 5 Hz, H4), 3.65–3.75 (m, 1 H, H3), 3.80 (s, 3 H, CH_3), 4.00 (d, 1 H, J = 13 Hz, H1), 4.07 (d, 1 H, J = 13 Hz, H1), 7.00–7.15 (m, 4 H, Ar H); ^{13}C NMR (75 MHz) δ 31.26, 47.00, 51.72, 55.49, 125.71, 125.97, 125.88, 128.79, 132.79, 134.57, 173.14.

3-(Hydroxymethyl)-1,2,3,4-tetrahydroisoquinoline (11).[19] To a rapidly stirred suspension of $LiAlH_4$ (0.36 g, 9.40 mmol) in THF (25 mL) was added a solution of **10** (1.2 g, 6.3 mmol) in THF (10 mL) over 10 min. Upon complete addition, the system was warmed under reflux for 16 h. Excess $LiAlH_4$ was destroyed by the Fieser method,[34] and the liquid was decanted from the aluminum salts and then treated with Et_2O (100 mL) and H_2O (100 mL). The organic part was separated, and the aqueous one was extracted with Et_2O (3 × 50 mL). The organic pool was dried (K_2CO_3) and concentrated under reduced pressure, affording crude **11** as a yellow oil (1.09 g) that was distilled (bulb-to-bulb, bp 83–85 °C, 0.3 mm) to a colorless liquid (0.91 g, 5.6 mmol, 88%): IR (film) 3600–3200 (NH, OH), 2950, 1060, 750 cm^{-1}; ^{1}H NMR (300 MHz) δ 2.58 (dd, 1 H, J = 16 and 10 Hz, H4), 2.67 (dd, 1 H, J = 16 and 4 Hz, H4), 2.95–3.05 (m, 1 H, H3), 3.24 (br s, 2 H, exchangeable with D_2O, NH, OH), 3.48 (dd, 1 H, J = 11 and 7 Hz, OCH_2), 3.74 (dd, 1 H, J = 11 and 4 Hz, OCH_2), 3.98 (d, 1 H, J = 16 Hz, H1), 4.08 (d, 1 H, J = 16 Hz, H1), 7.05–7.20 (m, 4 H, Ar H); ^{13}C NMR

(34) Fieser, L. F.; Fieser, M. *Reagents for Organic Synthesis*; Wiley: New York, 1967; p 583.

(75 MHz) δ 30.84, 47.81, 55.02, 65.36, 125.76, 125.99, 126.16, 129.21, 133.92, 135.22. The hydrochloride salt **11**·HCl was formed by passing a stream of anhydrous HCl(g) over a solution of **11** in Et_2O and then recrystallized from EtOH–Et_2O, affording the analytical sample: mp 195–196 °C (lit.[19] mp 185–188 °C); EIMS, *m/z* (relative intensity) 163 (M^+, 1), 144 (3), 132 (100), 130 (51), 117 (11), 115 (11). Anal. ($C_{10}H_{14}ClNO \cdot {}^1/_2H_2O$) C, H, N.

3-(Aminocarbonyl)-1,2,3,4-tetrahydroisoquinoline (12). A mixture of **10**·HCl (2.30 g, 10.1 mmol) and H_4NOH (100 mL) was allowed to stir at room temperature for 16 h. The white solid that formed was filtered, washed with several portions of H_2O (150 mL total) and petroleum ether (50 mL), and then allowed to dry in the air (1.76 g, 10.0 mmol, 99%): mp 162–163 °C; IR (KBr) 3350 and 3200 (NH_2, NH), 1660, 730 cm^{-1}; ^1H NMR (300 MHz) δ 1.78 (br s, 2 H, $CONH_2$), 2.90 (dd, 1 H, *J* = 13 and 8 Hz, H4), 3.25 (dd, 1 H, *J* = 13 and 4 Hz, H4), 3.50–3.60 (m, 1 H, H3), 3.97 (d, 1 H, *J* = 13 Hz, H1), 4.08 (d, 1 H, *J* = 13 Hz, H1), 5.75 (br s, 1 H, exchangeable with D_2O, NH), 7.05–7.20 (m, 4 H, Ar H); ^{13}C NMR (75 MHz) δ 30.78, 47.74, 56.52, 125.60, 126.25, 126.60, 129.26, 134.08, 135.76, 175.95. A solution of **12** (0.41 g) in Et_2O (30 mL) was treated with Et_2O saturated with HCl(g). The white solid **12**·HCl (0.42 g) was filtered and recrystallized from MeOH–Et_2O, affording the analytical sample: mp 294–295 °C dec; CIMS (NH_3), *m/z* (relative intensity) 177 ($M^+ + 1$, 98), 132 (100), 130 (25). Anal. ($C_{10}H_{13}ClN_2O$) C, H, N.

***N*-(Ethoxycarbonyl)-1-phenyl-2-aminobutane (15).** A mixture of 1-phenyl-2-butanone (10.0 g, 67.5 mmol) and ammonium formate (50 g, 790 mmol) was warmed to 160 °C for 48 h, at which time 6 N HCl (100 mL) was added, and the system was warmed under reflux for an additional 12 h. The resulting solution was made alkaline by the cautious addition of KOH pellets and extracted with Et_2O (5 × 100 mL). The organic pool was dried (K_2CO_3) and concentrated under reduced pressure, affording crude **14**[21] as a yellow oil (7.40 g). To a rapidly stirred mixture of the crude **14** (7.40 g) and K_2CO_3 (20 g) in THF (100 mL) was added ethyl chloroformate (6.50 g, 59.5 mmol), and the resulting solution was allowed to stir at room temperature for 2 h. The solid was filtered and washed with Et_2O, and the filtrate was concentrated under reduced pressure, affording an orange oil (10.03 g) that was distilled (bulb-to-bulb, bp 86–88 °C, 0.07 mm), yielding **15** as a colorless oil (9.40 g, 42.5 mmol, 63% from 1-phenyl-2-butanone) that solidified on standing in the air to a white solid: mp 36–37 °C; IR (film) 3370 (NH), 3060, 3010, 2980, 1700 (CO), 1630, 750, 700 cm^{-1}; ^1H NMR (300 MHz) δ 0.91 (t, 3 H, *J* = 7 Hz, CH_3), 1.2 (t, 3 H, *J* = 6 Hz, CH_3), 1.25–1.38 (m, 1 H), 1.45–1.60 (m, 1 H), 2.65–2.85 (m, 2 H, Ar CH_2), 3.8 (br s, 1 H, exchangeable with D_2O, NH), 4.04 (q, 2 H, *J* = 6 Hz, OCH_2), 4.92 (m, 1 H, H2), 7.1–7.3 (m, 5 H, Ar H); ^{13}C NMR (75 MHz) δ 10.0, 14.3, 26.6, 40.7, 53.2, 60.0, 125.9, 127.9, 129.1, 138.1, 156.0; EIMS, *m/z* (relative intensity) 222 ($M^+ + 1$, 1), 192 (2), 130 (100), 91 (69), 58 (100). Anal. ($C_{13}H_{19}NO_2$) C, H, N.

1-Oxo-3-ethyl-1,2,3,4-tetrahydroisoquinoline (16). The preceding ethyl carbamate (**15**; 1.0 g, 4.5 mmol) was added to polyphosphoric acid[22] (PPA, 20.0 g) that had been preheated to 140 °C, and the mixture was stirred with a glass rod for 10 min, at which time the vigorous foaming had subsided. The pot contents were cautiously poured onto crushed ice, and the resulting aqueous mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure, affording crude **16** as an off-white solid (0.8 g) that was purified by PCTLC (4 mm, 30% ethyl acetate in hexanes eluent), yielding **16** as a white solid (0.7 g, 4.0 mmol, 89%): mp 107.3–108.8 °C; IR (KBr) 3300 (NH), 1680 (CO), 750 cm^{-1}; ^1H NMR (300 MHz) δ 1.03 (t, 3 H, *J* = 7 Hz, CH_3), 1.52–2.80 (m, 2 H), 2.75 (dd, 1 H, *J* = 16 and 11 Hz, H4), 2.90 (dd, 1 H, *J* = 16 and 5 Hz, H4), 3.50–3.65 (m, 1 H, H3), 7.10–7.45 (m, 3 H, Ar H), 7.80 (br s, 1 H, exchangeable with D_2O, NH), 7.98–8.12 (m, 1 H, Ar H); ^{13}C NMR (75 MHz) δ 9.55, 27.75, 33.18, 52.23, 126.47, 127.11, 127.32, 128.53, 131.66, 137.85, 166.26; EIMS, *m/z* (relative intensity) 175 (M^+, 3), 145 (100), 128 (37), 118 (17), 90 (15). Anal. ($C_{11}H_{13}NO$) C, H, N.

3-Ethyl-1,2,3,4-tetrahydroisoquinoline (13). A solution of **16** (2.00 g, 11.4 mmol) in THF (50 mL) at 5 °C was treated with a solution of $LiAlH_4$ in THF (22.8 mL, 22.8 mmol) over 10 min. The resulting solution was allowed to warm to room temperature and then warmed under reflux over 18 h. After cooling, the excess $LiAlH_4$ was destroyed by the Fieser method,[34] the liquid was decanted, and the gummy aluminum salts were washed with acetone (20 mL), affording a granular white solid that was filtered and washed with Et_2O (100 mL). The filtrate was combined with the decanted liquid and extracted with 1 N HCl (3 × 100 mL). The aqueous pool was made alkaline by the addition of KOH pellets and then extracted with Et_2O (3 × 100 mL). The combined organic layers were dried over K_2CO_3 and then concentrated under reduced pressure, affording crude **13** as a yellow oil (2.20 g) that was distilled (bulb-to-bulb, bp 60–65 °C, 0.1 mm) as a colorless liquid (1.73 g, 10.7 mmol, 94%): IR (film) 3330 (NH), 3050, 3000, 740 cm^{-1}; ^1H NMR (300 MHz) δ 0.95 (t, 3 H, *J* = 7 Hz, CH_3), 1.30 (br s, 1 H, exchangeable with D_2O, NH), 1.38–1.50 (m, 2 H), 2.38 (dd, 1 H, *J* = 15 and 9 Hz, H4), 2.55–2.70 (m, 2 H, H3, H4), 3.85 (d, 1 H, *J* = 13 Hz, H1), 3.95 (d, 1 H, *J* = 13 Hz, H1), 6.95–7.15 (m, 4 H, Ar H); ^{13}C NMR (75 MHz) δ 9.78, 28.95, 34.41, 47.97, 54.45, 124.97, 125.22, 125.49, 128.49, 134.23, 135.19. A solution of **13** (1.73 g) in Et_2O (20 mL) was treated with Et_2O saturated with HCl(g), and the white hydrochloride salt **13**·HCl was filtered and recrystallized from CH_2Cl_2–hexanes, affording the analytical sample: mp 217–217.5 °C; EIMS, *m/z* (relative intensity) 161 (M^+, 3), 160 (3), 132 (100), 130 (29), 104 (27). Anal. ($C_{11}H_{16}ClN$) C, H, N.

***N*,*N*-Diethyl-2,3-dichlorobenzamide (19).** A solution of 2,3-dichlorobenzoic acid (19.1 g, 100 mmol) in thionyl chloride (22 mL, 300 mmol) was warmed under reflux for 2 h. Excess $SOCl_2$ was removed by distillation (bp 73–74 °C), and the dark residue that remained was distilled (bulb-to-bulb, bp 55–58 °C, 0.32 mm), yielding **18** as a colorless oil (20.0 g, 95.7 mmol) that was dissolved in CH_2Cl_2 (50 mL) and added dropwise to a rapidly stirred solution of diethylamine (14.6 g, 200 mmol) in CH_2Cl_2 (200 mL) at 5 °C. Upon complete addition, the solution was allowed to warm to room temperature and then was concentrated under reduced pressure. Et_2O (300 mL) was added, the solid diethylamine hydrochloride was removed by filtration, and the filtrate was concentrated under reduced pressure to afford crude **19** as a yellow oil (22.5 g) that was distilled (bulb-to-bulb, bp 110–115 °C, 0.35 mm) to a pale yellow liquid (21.9 g, 89.6 mmol, 90% from the benzoic acid): IR (film) 3030, 2990, 1650, 1440, 800, 750 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.08 (t, 3 H, *J* = 7 Hz, CH_3), 1.29 (t, 3 H, *J* = 7 Hz, CH_3), 3.05–3.21 (m, 2 H), 3.30–3.47 (m, 1 H), 3.68–3.85 (m, 1 H), 7.10–7.56 (m, 3 H, Ar H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.34, 13.69, 38.79, 42.50, 125.27, 127.74, 128.47, 130.26, 133.20, 138.45, 166.56; EIMS, *m/z* (relative intensity) 246 and 244 (M^+, 19, 25), 210 (22), 175 (66), 173 (100), 147 (16), 145 (25), 109 (15), 111 (9). Upon standing in the air, the oil crystallized to colorless plates: mp 36.5–37.5 °C. Anal. ($C_{11}H_{13}Cl_2NO$) C, H, N.

3,4-Dichloro-2-[(*N*,*N*-diethylamino)carbonyl]benzaldehyde (20). The procedure of Beak and Snieckus was employed.[23] To a rapidly stirred solution of TMEDA (5.2 g, 45 mmol) and sec-BuLi (32 mL, 45 mmol) in THF (400 mL) at −77 °C was added a solution of diethylamide **19** (5.0 g, 20 mmol) in THF (40 mL) at a rate that did not allow the internal temperature to exceed −70 °C. Upon complete addition, the solution was allowed to stir at −77 °C for an additional 60 min, treated with DMF (7.5 g, 102 mmol), and allowed to warm to room temperature. H_2O (200 mL) was added, the layers were separated, and the aqueous one was extracted with Et_2O (3 × 100 mL). The organic pool was washed with H_2O (100 mL) and brine (100 mL), dried ($MgSO_4$), and concentrated under reduced pressure, affording crude **20** as a red oil (6.0 g) that was distilled (bulb-to-bulb, bp 140–145 °C, 0.35 mm) to a pale yellow liquid (4.8 g, 18 mmol, 90%): IR (film) 3030, 2980, 1710, 1650, 875, 820, 800 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.10 (t, 3 H, *J* = 7 Hz, CH_3), 1.36 (t, 3 H, *J* = 7 Hz, CH_3), 3.09–3.22 (m, 2 H), 3.55–3.67 (m, 1 H), 3.70–3.83 (m, 1 H), 7.61 (d, 1 H, *J* = 8 Hz, Ar H), 7.81 (d, 1 H, *J* = 8 Hz, Ar H), 9.93 (s, 1 H, CHO); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.13, 13.47, 38.97, 42.58, 128.47, 130.09, 130.57, 131.91, 139.40, 139.93, 163.97, 188.35; EIMS, *m/z* (relative intensity) 276 and 274 (M^+, 1.5 and 2.4), 246 (24), 244 (36), 203 (38), 201 (56), 175 (17), 173 (26), 147 (10), 145 (16), 111 (11), 109 (26), 72 (100). The oil crystallized as white plates on standing in the air, mp 69–70 °C. Anal. ($C_{12}H_{13}Cl_2NO_2$) C, H, N.

6,7-Dichloro-3-hydroxyphthalide (21). Compound **20** (9.0 g, 33 mmol) was suspended in 6 N HCl (500 mL), and the system was warmed under reflux. After 1 h, complete solution occurred,

and the reaction was allowed to proceed at reflux for an additional 13 h. The solution was cooled to 5 °C, and the precipitated **21** was collected (6.3 g, 29 mmol, 88%): mp 156–157 °C; IR (KBr) 3500–3200 (OH), 1740, 840, 750 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 6.48 (s, 1 H, H3), 7.20 (s, 1 H, OH), 7.43 (d, 1 H, J = 8 Hz, Ar H), 7.75 (d, 1 H, J = 8 Hz, Ar H); EIMS, m/z (relative intensity) 220 and 218 (M^+, 12 and 19), 203 (4), 201 (7), 192 (11), 190 (17), 175 (68), 173 (100), 147 (15), 146 (15), 145 (24), 144 (17), 111 (18), 109 (32), 84 (18), 75 (44), 74 (68), 73 (30). Anal. ($C_8H_4Cl_2O_3$) C, H.

3-(Ethoxycarbonyl)-7,8-dichloro-1(2*H*)-isoquinolone (23). To a rapidly stirred solution of NaOEt (25.1 mmol) in EtOH (20 mL) at −15 °C was added a solution of **21** (1.1 g, 5.0 mmol) and ethyl azidoacetate (2.6 g, 20 mmol) in EtOH (5 mL) over 5 min. The yellow suspension was stirred at −15 °C for 30 min and then allowed to warm slowly to room temperature. After 2 h at room temperature, the mixture was poured onto 0.1 N HCl (200 mL), and the precipitated azidocinnamate **22** was collected (0.4 g, 1.2 mmol, 24%): mp 130–135 °C dec. Without further purification, the azidocinnamate (0.3 g, 1.0 mmol) in THF (50 mL) was treated with triethyl phosphite (0.2 g, 1.1 mmol), and the solution was allowed to stir at room temperature under argon for 62 h.[25] Evaporation of the volatiles under reduced pressure afforded a yellow solid that was triturated with Et_2O, affording pure **23** as a white solid (0.1 g, 0.4 mmol), 40% from **21**): mp 194–195 °C; IR (KBr) 3250, 3120, 1730, 1660, 870, 820 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.40 (t, 3 H, J = 7 Hz, CH_3), 4.39 (q, 2 H, J = 7 Hz, OCH_2), 7.18 (s, 1 H, H4), 7.45 (d, 1 H, J = 8 Hz, Ar H), 7.72 (d, 1 H, J = 8 Hz, Ar H), 9.00 (br s, 1 H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.20, 62.86, 109.78, 126.08, 127.35, 128.96, 134.05, 135.83, 137.55, 159.33, 160.43, 160.96; EIMS, m/z (relative intensity) 287 and 285 (M^+, 18 and 28), 259 (6), 257 (10), 215 (48), 213 (100), 211 (63), 185 (21), 183 (20), 159 (49), 157 (74), 150 (18), 148 (46). Anal. ($C_{12}H_9Cl_2NO_3$) C, H, N.

3-(Hydroxymethyl)-7,8-dichloro-1,2,3,4-tetrahydroisoquinoline (17). A solution of **23** (0.32 g, 1.13 mmol) in THF (100 mL) was treated at room temperature with borane–dimethyl sulfide (BMS)[26] in THF (5.65 mL, 11.3 mmol) over 5 min. The resulting solution was warmed under reflux for 22 h. Acetic acid (glacial, 2 mL) was cautiously added, and the resultant solution was again warmed under reflux for 1 h. A saturated solution of HCl(g) in MeOH (50 mL) was added, and the volatiles were removed under reduced pressure. The oily residue that remained was partitioned between H_2O (20 mL) and Et_2O (20 mL), the layers were separated, the aqueous one was extracted with Et_2O (3 × 20 mL), and the organic pool was discarded. The pH of the aqueous pool was adjusted to pH 13 by the addition of solid KOH pellets, and the alkaline products were extracted into Et_2O (4 × 50 mL). The organic pool was dried (K_2CO_3) and then concentrated under reduced pressure, affording crude **17** as a white solid (0.26 g) that was purified by PCTLC (2 mm, CH_2Cl_2–CH_3OH–H_4NOH, 250:25:1, eluent) as a white solid (0.13 g, 0.56 mmol, 50%) that was further purified by distillation (bulb-to-bulb, 170–173 °C, 0.75 mm) affording 0.11 g of a colorless liquid that solidified on standing in the air: IR (film) 3600–3000 (NH, OH) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.40 (br s, 2 H, exchangeable with D_2O, NH, OH), 2.57 (dd, 1 H, J = 16 and 11 Hz, H4), 2.66 (dd, 1 H, J = 16 and 4 Hz, H4), 2.92–3.94 (m, 1 H, H3), 3.53 (dd, 1 H, J = 8 and 11 Hz, CH_2O), 3.79 (dd, 1 H, J = 4 and 11 Hz, CH_2O), 3.90 (d, 1 H, J = 17 Hz, H1), 4.22 (d, 1 H, J = 17 Hz, H1), 6.96 (d, 1 H, J = 8 Hz, Ar H), 7.25 (d, 1 H, J = 8 Hz, Ar H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.66, 46.71, 53.92, 65.20, 127.70, 128.31, 130.11, 130.18, 134.66, 135.28; CIMS (NH_3), m/z (relative intensity) 234 and 232 (M^+ + 1, 64 and 100), 202 (18), 200 (33), 198 (19). A solution of **17** (0.11 g, 0.47 mmol) in Et_2O was treated with anhydrous HCl(g) to give the hydrochloride salt **17**·HCl (0.12 g) that was recrystallized from EtOH as white plates, mp 265–267 °C dec. Anal. ($C_{10}H_{12}Cl_3NO$) C, H, N.

Radiochemical PNMT Assay. The assay employed in this investigation has been described elsewhere.[29,30] 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 ca. 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 extracted with 2 mL of toluene–isoamyl alcohol (7:3). The organic layer (1.0 mL) was removed and transferred to a scintillation vial and diluted with cocktail for counting. The mode of inhibition was ascertained by inspection of the $1/V$ versus $1/S$ plot of the data.

Because of the alkaline hydrolysis observed for ester **10**, the assay was repeated as above for this compound at incubation times of 5, 10, 15, 20, and 30 min (with a reduction in unlabeled AdoMet concentration from 10 to 1 mM for the 5-min incubation). The K_i values determined were 78.9 ± 8.6 μM (5 min); 84.5 ± 29.0 and 83.6 ± 9.8 μM (10 min); 92.6 ± 10.0 and 89.0 ± 8.8 μM (15 min); 95.0 ± 15.9, 105.7 ± 7.8, and 102.4 ± 8.9 μM (20 min); 114.0 ± 6.3 and 115.9 ± 7.2 μM (30 min). These values were plotted versus time (slope 1.53, r = 0.972) and extrapolated to zero time for the K_i = 69.5 μM value reported in Table I. The amount of hydrolysis versus time was estimated from an NMR experiment. A solution of 2.46 mg of sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4, 3.43 mg of monobasic potassium phosphate (anhydrous), and 83.2 mg of dibasic potassium phosphate (anhydrous) in 1.00 mL of deuterium oxide had a "pH" (pD) value of 8.45 as measured with a glass electrode pH meter.[33] To this solution was added 10.07 mg of **10** (0.044 35 mmol). The ^1H NMR spectrum (300 MHz) was recorded approximately 5 min after initial mixing and at 10, 20, 30, and 40 min later at a probe temperature of 37 °C. A new singlet appeared in the spectrum at 3.37 for methanol; the relative area of this methanol singlet was compared with that of the methyl singlet (3.82) from ester **10** by integration. The percent of methanol formed (and hence the percent of hydrolysis of **10**) was 6.1%, 12.5%, 15.6%, and 19.7% at 15, 25, 35, and 45 min, respectively. That the methanol peak was correctly identified was confirmed by the addition of 5 μL of methanol at the end of the experiment; the peak monitored at 3.37 was enhanced. The calculated amount of ester remaining after 15 min was 93.9%. When this value was used to calculate the K_i determinations, a value of K_i = 90.1 μM was obtained as compared to the observed values of 92.6 ± 10 μM and 89.0 ± 8.8 μM.

Acknowledgment. We are grateful for the assistance of Kevin Criscione in the determination of the in vitro activity of some of the compounds involved in this study. We thank Moorthy S. S. Palanki for the NMR stability study on compound **10**. This research was supported by USPHS Grant HL 34193 and a grant from the University of Kansas General Research Fund.

Registry No. **1**·HCl, 57987-77-6; **4**·HCl, 14099-81-1; **8**·HCl, 112794-28-2; **9**·HCl, 41994-51-8; **10**, 57060-86-3; **10**·HCl, 57060-88-5; **11**, 63006-93-9; **11**·HCl, 99074-98-3; **12**, 112794-29-3; **12**·HCl, 112794-30-6; **13**, 111422-13-0; **13**·HCl, 41565-84-8; **14**, 53309-89-0; **15**, 112794-31-7; **16**, 112794-32-8; **17**, 112794-33-9; **17**·HCl, 112794-34-0; **18**, 2905-60-4; **19**, 59472-22-9; **20**, 112794-35-1; **21**, 112794-36-2; **22**, 112794-37-3; **23**, 112794-38-4; PNMT, 9037-68-7; 1-phenyl-2-butanone, 1007-32-5; 2,3-dichlorobenzoic acid, 50-45-3.