temperature. The suspension, which turned black within 20 s, was stirred at room temperature for an additional 1.75 h. Diethyl ether (100 mL) was added to the suspension, the solution was filtered through a pad (25 g) of Florisil (Fisher, 100–200 mesh), and the crystal-clear solution was dried (K_2CO_3). The solvent was removed under reduced pressure to yield 1.0 g (100%) of aldehyde **41a**: IR (liquid film) 2720 (CH), 1730 cm^{-1} (C=O); NMR (CCl_4) δ 8.9 (s, CHO), 2.4–1.1 (m, $C_{10}H_{15}$ ring); mp 134–139 °C (lit.[39] 125–128 °C).

Following procedure A, Me_3SiCN (0.66 g, 0.007 mol) was added to aldehyde **41a** (1.0 g, 0.006 mol), yielding 100% of the cyanohydrin ether **41b**: IR (liquid film) 1070 (SiO), 840 cm^{-1} [$(CH_3)_3Si$]. As outlined in procedure B, **41b** in Et_2O (10 mL) was added to $LiAlH_4$ (0.23 g, 0.006 mol) in Et_2O (150 mL) and stirred at reflux for 2.5 h. Workup resulted in 1.0 g (85%) of the ethanolamine **41c**. The ethanolamine was purified as the amine hydrochloride salt. The salt was formed by following procedure C, which yielded a white solid that was recrystallized from EtOH/EtOAc. The recrystallization yielded 0.6 g (52%) of **41c**·HCl (white needles): mp 270 °C dec; IR (free amine) (liquid film) 3370, 3300, 3120, 2920, 2860, 2680, 1585, 1450, 1360, 1340, 1310, 1250, 1175, 1135, 1095, 1070, 1030, 1005, 970, 950 (sh), 920, 880, 860, 830, 800, 740 cm^{-1}; NMR (free amine) (pyridine-d_5) δ 3.7–2.3 (m, 6, $CHOHCH_2NH_2$), 2.2–1.4 (m, 15, $C_{10}H_{15}$ ring); EIMS (70 eV) m/e (relative intensity) 196 ($M^+ + 1$, 0.9), 195 (M^+, 2), 60 (100). Anal. ($C_{12}H_{22}NOCl$) C, H, N.

Biochemistry. The enzyme used was purified from bovine adrenal medulla (Pel-Freez Biologicals) according to the procedure of Connett and Kirshner.[32] The purification was carried through the isoelectric precipitation and dialysis step, which resulted in a sixfold purification of the enzyme. The IC_{50} values were determined on a liquid chromatographic–electrochemical detection system described previously.[24,33] In the assay, the enzyme was solubilized in cold potassium buffer and a normal incubation mixture with water varied so that the final volume was 250 μL: *l*-norepinephrine (9 nmol); *S*-adenosyl-L-methionine (18 nmol); phosphate buffer, pH 8.0 (25 μmol); inhibitor, variable amounts. The reaction mixture was contained in ice prior to initiation of the assay, which was run at 37 °C for 10 min; enzyme was the last constituent added. The reaction was quenched with 250 μL of 0.1 N $HClO_4$ containing a known amount of dihydroxybenzylamine (DHBA) added as an internal standard. All incubations were run in duplicate with two injections per sample. The amount of epinephrine produced was determined by comparing the peak height of the internal standard to an external standard. The external standard was a previously prepared mixture of norepinephrine, epinephrine, DHBA, and dopamine in 0.1 N perchloric acid which had been frozen at −70 °C in 100-μL aliquots; a new aliquot was used each day and was kept on ice during the day's analyses. The external standard was injected at eight sample intervals. Once the external standard to internal standard ratio was determined, it was used to correct the peak height of the epinephrine in the sample. Since peak height was linearly related to the concentration,[24,33] a comparison of the epinephrine peak height in the sample to that in the external standard yielded the concentration of epinephrine in the sample. The percent inhibition was determined by comparing the amount of epinephrine produced at each inhibitory concentration to the amount of epinephrine present in a control incubation which contained no inhibitor. The data were fitted to a least-squares regression analysis, and the point of 50% inhibition was selected. The correlation coefficient indicates the fit of the line to the experimental points.

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Importance of the Aromatic Ring in Adrenergic Amines. 6. Nonaromatic Analogues of Phenylethanolamine as Inhibitors of Phenylethanolamine *N*-Methyltransferase: Role of π-Electronic and Steric Interactions[1a]

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To probe the importance of π-electronic and steric interactions of nonaromatic analogues of phenylethanolamine as inhibitors of phenylethanolamine *N*-methyltransferase (PNMT), a series of norbornane and norbornene ethanolamines was prepared and evaluated as inhibitors of PNMT (liquid chromatographic–electrochemical detector assay). Previous studies indicated a major importance of hydrophobic interaction of the ring moiety attached to the ethanolamine side chain, but a possible importance of π-complex formation could have been obscured by conformational differences among the analogues. In this study, norbornane and norbornene substituted with an ethanolamine side chain at positions 1,2-*exo*, and 2-*endo* were prepared from the corresponding aldehydes by addition of trimethylsilyl cyanide (Me_3SiCN) and lithium aluminum hydride reduction. The saturated (norbornane) analogues were two times more potent as inhibitors of the enzyme than were the norbornene analogues, thus suggesting that π-complex formation is not an important contribution to binding and, as previously proposed, a hydrophobic interaction is the significant binding interaction of the ring moiety. The hydrophobic binding area has a critical size that requires the hydrophobic moiety to be of sufficient length (the bridgehead-substituted norbornane and norbornene ethanolamines being too "short" for optimal binding). The 2-*exo* orientation of the ethanolamine side chain was preferred to the 2-*endo* orientation, supporting our earlier hypothesis that the ring moiety prefers to be oriented away from the side chain.

Since the days in 1894 when Oliver and Schafer found that alcoholic extracts of sheep adrenal glands caused a dramatic blood-pressure rise when injected into a cat,[2] the catecholamines have been implicated in the physiology of hypertension. Many of the drugs used for the treatment of hypertension also affect the adrenergic nervous system at a variety of sites. The exact mechanisms of the rise of blood pressure seen in hypertensive individuals is not

(1) (a) Taken, in part, from the Ph.D. Dissertation of D. P. Davis, University of Kansas, 1978. (b) NIH Predoctoral Trainee (Grant GM 1341).

(2) Oliver, G.; Schafer, E. A. *J. Physiol.* **1894**, *16*, i.

0022-2623/81/1824-0012$01.00/0

Table I

no.	yield,[a] %	salt	recrystn solvent	mp, °C	formula	anal.	IC_{50}, μM
9	90	SO_4	95% EtOH	280	$C_9H_{16}NO(SO_4)_{0.5}$	C, H, N	454
10	89	SO_4	95% EtOH	279	$C_9H_{16}NO(SO_4)_{0.5}\cdot 0.5H_2O$	C, H, N	1100
11	81	HCl	EtOAc	238	$C_9H_{16}NOCl$	C, H, N	>1000[b]
12	65	SO_4	95% EtOH	299	$C_9H_{18}NO(SO_4)_{0.5}$	C, H, N	193
13	93	SO_4	95% EtOH/EtOAc	275	$C_9H_{18}NO(SO_4)_{0.5}\cdot 0.5H_2O$	C, H, N	475
14	78	HCl	EtOAc	314	$C_9H_{18}NOCl$	C, H, N	>1000[b]
3		HCl	*c*	*c*	*c*		14
8		HCl	*c*	*c*	*c*		8
21		HCl	c	*c*	*c*		195

[a] Yield of amine base calculated from the starting aldehyde (the cyanohydrin ether was not isolated). [b] Fifty-percent inhibition was not achieved at 10^{-3} M, the highest concentration used. [c] See ref 6.

known. It had been thought to be due to the vasoconstrictor effect of epinephrine on the peripheral resistance vessels, but recent evidence points to a central regulatory role as well.[3a,b]

The final step in the biosynthesis of epinephrine is the N-methylation of norepinephrine catalyzed by the enzyme phenylethanolamine *N*-methyltransferase (PNMT, EC 2.1.1.28).[4] This enzyme has been found predominantly in the adrenal medulla and, more recently, in certain discrete areas of the brain stem.[3a-c] Since PNMT catalyzes the final step in the biosynthesis of epinephrine, one should be able to control epinephrine production without altering norepinephrine levels with a selective inhibitor of PNMT. That such an inhibitor might have useful therapeutic value as an antihypertensive agent is supported by the demonstration that when the PNMT inhibitor SKF 7698 was administered to spontaneously hypertensive rats and to rats made hypertensive by chemical induction (DOCA–salt), their blood pressures could be lowered back to the normal range.[3a,b] These results are encouraging but not definitive, as the PNMT inhibitors evaluated also have other effects such as α-adrenergic blockade. A selective PNMT inhibitor would provide a pharmacological tool for an evaluation of the role of epinephrine and PNMT in the production of hypertension.

The known in vivo inhibitors all have an aromatic ring present in the phenylethylamine-like molecule. The aromatic ring is probably involved in the diverse adrenergic side effects of these compounds. We have found that certain nonaromatic ethanolamines are bound to the catalytic site of PNMT and are methylated.[5,6] This fact led us to further explore the possibilities of finding nonaromatic ethanolamines that would be potent alternate substrate inhibitors of PNMT, but because these ethanolamines lack aromatic nuclei, they might show minimal adrenergic side effects.

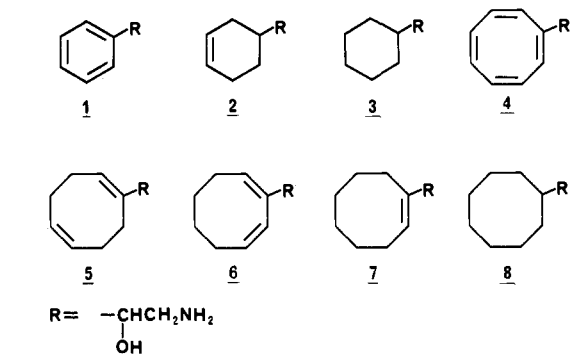


Figure 1. Nonaromatic analogues of phenylethanolamine with varying degrees of π-complexation ability, but with conformational flexibility.

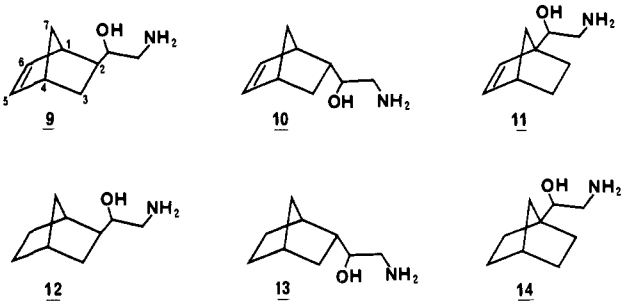


Figure 2. Conformationally defined nonaromatic ethanolamines with varied amounts of π-complexation ability.

In our initial studies to probe the substrate binding requirements of the PNMT active site, a series of cyclohexyl- and cyclooctylethanolamines, having varying degrees of unsaturation, was synthesized (see Figure 1 and Table I).[5a,b] It was supposed that the more unsaturated members [phenylethanolamine (1) and cyclooctatetraenylethanolamine (4)] would be the most active as alternate substrates due to better binding, presumably via a charge-transfer or π-complex interaction, at the hydrophobic area thought to be important for substrate binding. In fact, though, the fully saturated cyclohexylethanolamine (3) and cyclooctylethanolamine (8) were highly active as substrates[5a] and alternate substrate inhibitors[6] in in vitro studies. For a more thorough discussion, see the preceding paper in this series concerning the role of hydrophobic interactions in the binding of ethanolamines to PNMT.[6]

Because of conformational differences among the phenyl, cyclohexenyl, and cyclohexyl rings of the six-membered ring series (1–3) and the cyclooctatetraenyl, cyclooctadienyl, cyclooctenyl, and cyclooctanyl rings of the eight-membered ring series (4–8), the effect of π-electron density on the binding affinity of these series of compounds could have been obscured. Therefore, we synthesized a series of rigid bicyclo[2.2.1]heptylethanolamines.

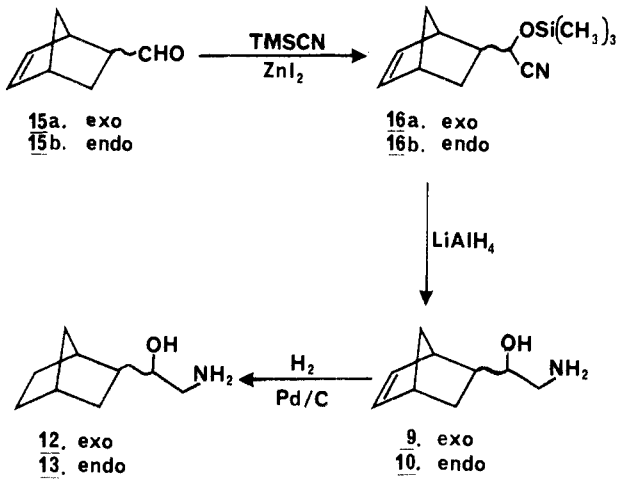
(3) (a) Saavedra, J. M.; Grobecker, H.; Axelrod, J. *Science*, **1976**, *191*, 483. (b) Saavedra, J. M.; Grobecker, H.; Axelrod, J. *Circ. Res.* **1978**, *42*, 529. (c) Sauter, A. M.; Baba, Y.; Stone, E. A.; Goldstein, M. *Brain Res.* **1978**, *144*, 415.

(4) (a) Borchardt, R. T. "Catecholamines and Stress"; Usdin, E.; Kvetnansky, R.; Kopin I. J., Eds.; Pergamon Press: New York, 1976; pp 313–320. (b) Axelrod, J. *J. Biol. Chem.* **1962**, *237*, 1657. (c) Axelrod, J. *Pharmacol. Rev.* **1966**, *18*, 95. (d) Hoffman, A. R.; Ciaranello, R. D.; Axelrod, J. *Biochem. Pharmacol.* **1975**, *24*, 544.

(5) (a) Grunewald, G. L.; Grindel, J. M.; Vincek, W. C.; Borchardt, R. T. *Mol. Pharmacol.* **1975**, *11*, 694. (b) Grunewald, G. L.; Grindel, J. M.; Patil, P. N.; Salman, K. N. *J. Med. Chem.* **1976**, *19*, 10. (c) Grunewald, G. L.; Vincek, W. C.; Davis, D. P.; Borchardt, R. In "Catecholamines: Basic and Clinical Frontiers"; Usdin, E.; Kopin, I. J.; Barchas, J., Eds.; Pergamon Press: New York, **1979**; Vol. 1, p 189.

(6) Vincek, W. C.; Aldrich, C. S.; Borchardt, R. T.; Grunewald, G. L. *J. Med. Chem.*, preceding article in this issue.

Scheme I

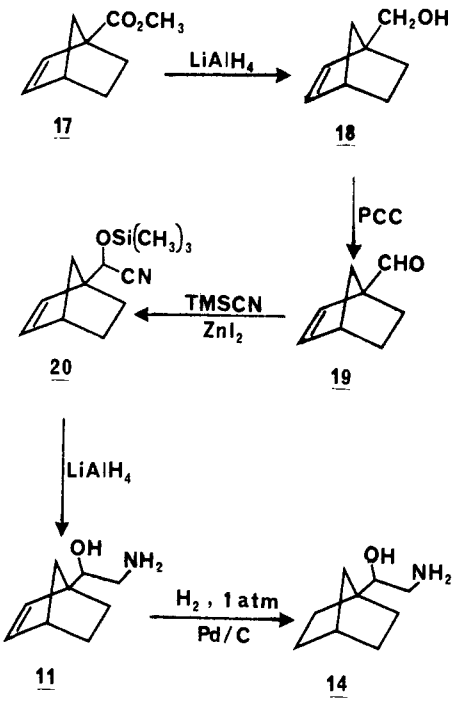


These compounds are shown in Figure 2. In this series, one double bond was introduced without changing the conformation of the bicyclic ring. Because the bicyclic ring does not have the same symmetry as a cyclohexane ring, the ethanolamine side chain was attached at both the C-1 (bridgehead) and C-2 positions. It should also be noted that the orientation of the ethanolamine side chain with respect to the planar side (C_2, C_3, C_5, C_6) of the bicyclic ring differs with the three attachments shown in Figure 2. In compounds **9** and **12** the ethanolamine chain extends at a slight angle to this plane; in compounds **10** and **13** the side chain extends well below this plane; and in compounds **11** and **14** the side chain extends upward at a slight angle with some displacement from the plane. Compounds **9**, **11**, **12**, and **14** should have conformations most like that of phenylethanolamine insofar as the orientation of the side chain to the ring is concerned.

Chemistry. The synthesis of these compounds is shown in Schemes I and II. The *exo*- (**9**) and *endo*-2-(1-hydroxy-2-aminoethyl)bicyclo[2.2.1]hept-5-ene (**10**) were synthesized from *exo*- and *endo*-2-formylbicyclo[2.2.1]-hept-5-ene (**15a** and **15b**, respectively). These aldehydes exist in approximately equal amount in the commercially available Diels–Alder reaction product of cyclopentadiene and acrolein. The two isomers were separated by careful distillation on an autoannular spinning band distillation apparatus. The cyanohydrin ethers **16a** and **16b** were made by the facile addition of trimethylsilyl cyanide (Me_3SiCN) to the aldehyde using ZnI_2 as a catalyst.[7] These cyanohydrin ethers were very water sensitive compounds and were not isolated but immediately reduced to the amino alcohols **9** or **10** using $LiAlH_4$. These amines were isolated as the sulfate salts. The fully saturated amino alcohols **12** and **13** were prepared by catalytic hydrogenation of **9** and **10**, respectively, and were isolated as their sulfate salts.

The synthesis of the bridgehead-substituted ethanolamines **11** and **14** made use of 1-methoxycarbonylbicyclo[2.2.1]hept-2-ene (**17**). Ester **17**, the synthesis of which has been described by us elsewhere,[8] was readily reduced to the hydroxymethyl compound **18** with $LiAlH_4$, and then the alcohol was oxidized to the aldehyde **19** with pyridinium chlorochromate (PCC) in CH_2Cl_2.[9] The synthesis of the cyanohydrin ether **20** and its subsequent reduction to the amino alcohol **11** followed the procedures described above. The saturated ethanolamine **14** was made by the catalytic reduction of **11** and both **11** and **14** were isolated as the hydrochloride salt.

Scheme II

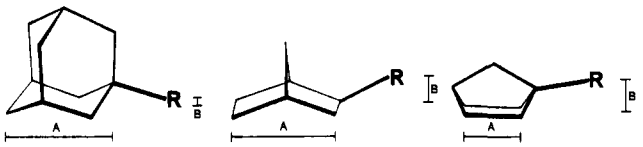


Biological Results and Discussion

The compounds reported here were evaluated as alternate substrate inhibitors of PNMT using the methods described in the previous paper in this series.[6] In our previous studies[5,6] it appeared that hydrophobic and steric interactions were the dominant ones in the binding of these alternate substrate inhibitors to the active site of PNMT. It was not clear, however, whether conformational changes might have obscured more specific electronic interactions of the π-complex or charge-transfer type. The data shown in Table I appear to rule out any importance of such π-electronic interactions. In the exo- or endo-substituted compounds (**9** vs. **12** or **10** vs. **13**), it is seen that the fully saturated member of each pair is the more potent. Conformational differences between members of each pair are not present because of the rigid bicyclic rings. The increased inhibitory activity of the saturated members of each pair with respect to its unsaturated counterpart is likely due to the increased lipophilicity of the saturated compounds. The activity of the two bridgehead ethanolamines **11** and **14** was too low for a comparison to be meaningful.

In comparing compounds **9** and **12** it can be seen that there is a twofold difference in the IC_{50} values, with the saturated **12** being the more potent member of the series. It was not unexpected that **9** and **12** would be more potent than **10** and **13**, respectively, because in **9** and **12** the conformation of the ethanolamine side chain is exo to the ring; models of these compounds closely resemble those of the potent cyclohexyl- and cyclooctylethanolamines, **3** and **8** of Figure 1, insofar as the orientation of the side chain to the ring. In compounds **10** and **13**, the ethanolamine side chain is endo to the ring; the angle that the side chain makes with the ring may prevent the molecule from lying "flat" in the binding area of the enzyme and would make it less tightly bound as evidenced by the higher IC_{50}

(7) Evans, D. A.; Carroll, G. L.; Truesdale, L. K. *J. Org. Chem.* **1974**, *39*, 914.
(8) Grunewald, G. L.; Davis, D. P. *J. Org. Chem.* **1978**, *43*, 3074.
(9) Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, 2647.
(10) Borchardt, R. T.; Vincek, W. C.; Grunewald, G. L. *Anal. Biochem.* **1977**, *82*, 149.



21 12 14

Figure 3. Comparison of orientation of the ethanolamine side chain with respect to the ring. Distance A compares the length of the ring in contact with the hydrophobic binding area and B is the distance that the side chain is held above this proposed binding surface.

values. Since the only difference between **12** and **13** is the orientation of the side chain to the ring, this lack of planarity likely accounts for the twofold difference in inhibitory activity. The same reasoning holds true for the unsaturated members **9** and **10**; they also show a twofold difference in inhibition which must be due solely to the difference in orientation of the ethanolamine side chain.

In the case of the bridgehead-substituted ethanolamines **11** and **14**, their very low inhibitory activity may be due to the shorter distance between the point of attachment of the ethanolamine side chain and the furthest carbon of the hydrocarbon ring, as compared to **12**. As such, **12** would likely have a greater surface area in contact with the enzyme. This idea is given further credence when one looks at the IC_{50} for 1-adamantylethanolamine (**21**), which has an IC_{50} value of 195 μM in the same test system.[6] While the ring size for adamantyl (six carbons) is the same as the ring size of the larger ring of the bicycloheptyl system **14**, the adamantyl ring is fixed in the chair conformation and as such has a much larger area in contact with the enzyme surface as compared to **14** where the cyclohexane ring is locked into a boat conformation. This shortens the effective length of the hydrocarbon ring which could come in contact with the receptor surface. This same trend of potency vs. "length" of the hydrocarbon portion of the molecule was the subject of the previous paper of this series.[6] When one looks[6] at the differences between the IC_{50} values of cyclobutylethanolamine (IC_{50} = 1000 μM), cyclopentylethanolamine (IC_{50} = 446 μM), and cyclohexylethanolamine (IC_{50} = 14 μM), it becomes apparent that there is a tremendous difference in IC_{50} values with only a small change of ring size or conformation when one is dealing with these small rings. Apparently the bridgehead compounds **11** and **14** are just at the threshhold of the size necessary for significant inhibitory activity, and the exo compounds **9** and **12** have enough of an increase in effective ring surface bound to make them moderately active.

A comparison of the surface in contact with the binding site is depicted graphically in Figure 3 for ethanolamines **12**, **14**, and **21** (compare distance A). It should also be noted that the angle of the ethanolamine side chain with respect to the hydrocarbon ring is not identical in all three cases. The larger value of distance B in the bridgehead-substituted compound **14** may contribute to its significantly low inhibitory activity—once the ethanolamine side chain binds, the hydrocarbon ring may need to be pushed further "into" the hydrophobic binding site than can be sterically tolerated.

These data support three conclusions: (1) the inhibitory activity of these compound is lowered by a factor of two with the introduction of a double bond, which suggests that the binding of the molecule to the enzyme is lessened by the presence of the double bond (since all the other factors are the same when comparing **9** and **12** or **10** and **13**); (2) a certain critical size of the hydrocarbon portion of the molecule is required for binding to the PNMT catalytic site, **11** and **14** being too "short" to cause effective binding while **9**, **10**, **12**, and **13** are all "long enough" to have effective binding; and (3) the orientation of the ethanolamine side chain with respect to the ring has a considerable effect on binding, the exo orientation being about twice as effective as the endo orientation.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed on an F&M Model 185, CHN Analyzer at the University of Kansas and were within 0.4% of the calculated values. Infrared spectra were recorded on a Beckman IR-33 spectrophotometer. Proton magnetic resonance spectra were recorded on a Varian EM-360 spectrometer, using tetramethylsilane as an internal standard. Mass spectra were determined on a Varian-Atlas CH-5 electron-impact mass spectrometer at 70 eV. Solvents and reagents were routinely distilled or recrystallized prior to use. Reactions were routinely performed in a dry nitrogen atmosphere.

General Synthesis for Cyanohydrin Ethers 16a, 16b, and 20. The general method for preparation of trimethylsilyl cyanohydrin ethers is described in the preceding paper in this issue.[6]

***exo*-2-[(Trimethylsiloxy)cyanomethyl]bicyclo[2.2.1]-hept-5-ene (16a).** To 10.50 g (86.1 mmol, 13.2 mL) of aldehyde **15a** was added 9.41 g (95.0 mmol, 12.0 mL) of Me_3SiCN: IR (liquid film) 3080 (olefin), 2200 cm^{-1} (CN).

***endo*-2-[(Trimethylsiloxy)cyanomethyl]bicyclo[2.2.1]-hept-5-ene (16b).** To 6.49 g (53 mmol) of aldehyde **15b** was added 7.43 g (75 mmol, 9.5 mL) of Me_3SiCN: IR (liquid film) 3080 (olefin), 2280, 2190 cm^{-1} (CN).

1-[(Trimethylsiloxy)cyanomethyl]bicyclo[2.2.1]hept-2-ene (20). To 2.00 g (16.4 mmol) of **19** was added 2.48 g (25 mmol, 3.17 mL) of Me_3SiCN: IR (liquid film) 3080 (olefin), 2200 cm^{-1} (CN).

General Synthesis of Ethanolamines 9–11. This reduction was based on the synthesis of Evans et al.[7] To a three-neck flask fitted with a reflux condenser, rubber addition septum, and N_2 bubbler was added the $LiAlH_4$ and anhydrous Et_2O. This slurry was stirred under N_2 and the trimethylsiloxycyanohydrin was added dropwise via syringe at a rate that maintained a gentle reflux of the solvent. After the addition was complete, the stirred mixture was heated at reflux for 1 h. The mixture was cooled in an ice bath and the excess $LiAlH_4$ destroyed by the method described by Fieser.[11] The mixture of salts was stirred for 2 h to completely hydrolyze the silyl ether and aggregate the solids. The white slurry was filtered and the filter cake washed three times with Et_2O. The clear filtrates were dried (Na_2CO_3) and the Et_2O was removed in vacuo to yield the amine as a clear oil.

The sulfate salt was made by dissolving the amine in EtOH–EtOAc (1:1) or EtOAc and adding concentrated H_2SO_4 dropwise to the rapidly stirred solution under N_2. After a small portion of H_2SO_4 was added the salt was filtered. This procedure was repeated until no more precipitate was formed. The salt was filtered in a N_2 atmosphere and recrystallized from EtOH–EtOAc.

***exo*-2-(1-Hydroxy-2-aminoethyl)bicyclo[2.2.1]hept-5-ene (9).** To 5.70 g (150 mmol) of $LiAlH_4$ in 200 mL of anhydrous Et_2O was added 19.0 g (86 mmol) of **16a**. The amine was recovered as a clear oil (11.9 g, 90.4% from the aldehyde). The sulfate salt was precipitated from EtOH–EtOAc (1:1) and recrystallized from 95% EtOH to yield 3.96 g (49% from 6.12 g of amine used) of a white nonhygroscopic salt: mp 280 °C dec; IR (liquid film, amine base) 3390, 3320 cm^{-1} (NH, OH); NMR ($CDCl_3$, amine base) δ 6.02 (m, 2, C=CH), 3.22 (m, 1, C*H*CH), 2.92 (s, 3, OH, NH_2, exchangeable), 2.96–2.53 (m, 4, H-1, H-4, C*H*NH_2); mass spectrum, m/e 153 (M^+). Anal. [$C_9H_{16}NO(SO_4)_{0.5}$] C, H, N.

***endo*-2-(1-Hydroxy-2-aminoethyl)bicyclo[2.2.1]hept-5-ene (10).** To 4.56 g (120 mmol) of $LiAlH_4$ in 200 mL of anhydrous Et_2O was added 11.76 g (53.2 mmol) of **16b**. The amine was recovered as a pale yellow oil (7.27 g, 89% from the aldehyde). A 4.26-g portion was precipitated as the sulfate salt from EtOAc and recrystallized from 95% EtOH to yield 2.90 g (30%) of a

(11) Fieser, L. F.; Fieser, M. "Reagents for Organic Synthesis"; Wiley-Interscience: New York, 1967; Vol. 1; p 584.

nonhygroscopic salt: mp 279 °C dec; IR (liquid film, amine base) 3380, 3310 cm^{-1} (NH, OH); NMR ($CDCl_3$, amine base) δ 6.02 (m, 2, C=CH), 2.87 (m, 3, OH, NH_2, exchangeable), 2.72 (m, 4, H-1, H-2, H-4, C*H*OH), 1.97 (m, 2, CH_2NH_2); mass spectrum, m/e 153 (M^+). Anal. [$C_9H_{16}NO(SO_4)_{0.5}\cdot 0.5\ H_2O$] C, H, N.

1-(1-Hydroxy-2-aminoethyl)bicyclo[2.2.1]hept-2-ene (11). To 1.30 g (34 mmol) of $LiAlH_4$ in 100 mL of anhydrous Et_2O was added 3.60 g (13.3 mmol) of **20**. The amine was recovered as a yellowish oil (2.02 g, 81% from **19**). This oil was precipitated as the hydrochloride salt from Et_2O and recrystallized from EtOAc to yield 1.26 g (59%) of a nonhygroscopic salt: mp 238 °C dec; IR (liquid film, amine base) 3380, 3300 cm^{-1} (NH, OH); NMR ($CDCl_3$, amine base) δ 5.98 (m, 2, C=CH), 3.80 (m, 1, C*H*OH), 3.37 (m, 3, OH, NH_2, exchangeable), 2.80 (m, 3, H-4, CH_2NH_2); mass spectrum, m/e 153 (M^+). Anal. ($C_9H_{16}NOCl$) C, H, N.

General Procedure for Synthesis of 12–14. The method of Augustine was used.[12] The catalyst (5% Pd on carbon, 0.1 g per 1 g of olefin) was suspended in 10% HOAc–90% EtOH in a N_2-flushed flask. The olefin was dissolved in the same solvent and added to the flask. Hydrogenation was accomplished on either the Parr low pressure or atmospheric hydrogenation apparatus. After 1 equiv of H_2 was consumed, the catalyst was filtered and the solvent removed in vacuo. The residue was dissolved in water and extracted with Et_2O, then the pH was adjusted to 12, and the residue was again extracted with Et_2O. The second Et_2O extract was washed with saturated brine and dried (Na_2CO_3). The solvent was removed in vacuo to yield the amine as a clear oil. The amine was stored as either the sulfate or hydrochloride salt.

***exo*-2-(1-Hydroxy-2-aminoethyl)bicyclo[2.2.1]heptane (12).** To a Parr reaction vessel was added 5.81 g (37.9 mmol) of **9** in 200 mL of solvent. The hydrogenation was accomplished at 46 psi for 16 h. The clear oily product (3.79 g, 65%) was precipitated as the sulfate salt from absolute EtOH and recrystallized from 95% EtOH to yield 3.95 g (51%) of a nonhygroscopic salt: mp 299 °C dec; IR (liquid film, amine base) 3380, 3310, 3190 cm^{-1} (NH, OH); NMR ($CDCl_3$, amine base) δ 3.10 (m, 1, C*H*OH), 2.40 (m, 1, H-2), 2.25 (m, 3, NH_2, OH, exchangeable), 2.23 (m, 1, H-1), 2.00 (m, 1, H-4); mass spectrum, m/e 155 (M^+). Anal. [$C_9H_{18}NO(SO_4)_{0.5}$] C, H, N.

***endo*-2-(1-Hydroxy-2-aminoethyl)bicyclo[2.2.1]heptane (13).** To a Parr reaction vessel was added 3.01 g (20 mmol) of **10** in 200 mL of solvent. The hydrogenation was accomplished at 36 psi. The clear oily product (2.84 g, 93%) was precipitated as the sulfate salt from EtOAc and recrystallized from 95% EtOH–EtOAc to yield 2.27 g (57%) of a nonhygroscopic salt: mp 275 °C dec; IR (liquid film, amine base) 3380, 3310 cm^{-1} (OH, NH); NMR ($CDCl_3$, amine base) δ 3.32 (m, 1, C*H*OH), 2.7–2.4 (m, 2, CH_2NH_2), 2.55 (s, 3, OH, NH_2, exchangeable), 2.17 (m, 2, H-1, H-4); mass spectrum, m/e 155 (M^+). Anal. [$C_9H_{18}NO(SO_4)_{0.5}\cdot 0.5H_2O$] C, H, N.

(12) Augustine, R. L. "Catalytic Hydrogenation"; Marcel Dekker: New York, 1965; p 57.

1-(1-Hydroxy-2-aminoethyl)bicyclo[2.2.1]heptane (14). To a round-bottom flask was added 1.00 g (6.5 mmol) of **11** in 25 mL of solvent. Hydrogenation was accomplished at atmospheric pressure for 70 min. The clear oily product (0.79 g, 78%) was precipitated as the hydrochloride salt from Et_2O and recrystallized from EtOAc to yield 0.73 g (75%) of a nonhygroscopic salt: mp 314 °C dec; IR (liquid film, amine base) 3370, 3300 cm^{-1} (OH, NH); NMR ($CDCl_3$, amine base) δ 3.62 (d of d, 1, C*H*OH), 2.57 (m, 2, CH_2NH_2), 2.43 (s, 3, OH, NH_2, exchangeable), 2.13 (m, 1, H-4); mass spectrum, m/e 155 (M^+). Anal. ($C_9H_{18}NOCl$) C, H, N.

1-(Hydroxymethyl)bicyclo[2.2.1]hept-2-ene (18). To a 500-mL three-neck flask fitted with a reflux condenser and N_2 bubbler was added 2.08 g (55 mmol) of $LiAlH_4$ and 200 mL of anhydrous Et_2O. This suspension was stirred for 20 min under N_2, and then 8.22 g (54.7 mmol) of **17** was added dropwise at a rate that maintained a gentle reflux. After the addition was complete (15 min), the mixture was heated at reflux for 1 h. The mixture was cooled in an ice bath, and the excess $LiAlH_4$ was destroyed by the method described by Fieser.[11] The resultant slurry was filtered under vacuum, and the filter cake was washed (3 × 30 mL) with Et_2O. The combined filtrates were dried (Na_2SO_4). The solvent was removed in vacuo to yield 6.39 g (94%) of a clear oil: IR (liquid film) 3370 (OH), 3080 cm^{-1} (olefin); NMR ($CDCl_3$) δ 6.02 (m, 2, C=CH), 3.87 (s, 2, CH_2OH), 3.38 (s, 1, OH), 2.83 (m, 1, H-4); mass spectrum, m/e 124 (M^+).

1-Formylbicyclo[2.2.1]hept-2-ene (19). To a 240-mL round-bottom flask fitted with a dropping funnel and N_2 bubbler was added 16.59 g (77 mmol) of dry pyridinium chlorochromate (PCC) and 150 mL of CH_2Cl_2. This slurry was stirred under N_2 for 10 min, and then 6.38 g (51.5 mmol) of **18** dissolved in 50 mL of CH_2Cl_2 was added dropwise via the addition funnel over a period of 10 min. The black solution was stirred 2 h at 25 °C and then poured into 200 mL of Et_2O containing Celite (filter aid). The resultant suspension was filtered, and the filter cake was washed with Et_2O (3 × 20 mL). The solvent volume was reduced to 25% of the original volume in vacuo and filtered again to remove chromium salts. The greenish-brown filtrate was washed with 1% aqueous EDTA (4 × 50 mL). The clear Et_2O solution was washed with saturated brine and dried (Na_2SO_4).

The solvent was removed in vacuo to yield 4.40 g (71%) of a clear oil with a strong odor resembling heptanal: IR (liquid film) 3080 (olefin), 2830, 2730 (CHO), 1725 cm^{-1} (C=O); NMR ($CDCl_3$) δ 9.95 (d, 1, C*H*O), 6.18 (m, 2, C=CH), 2.97 (m, 1, H-4).

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α-Ethynyl and α-Vinyl Analogues of Ornithine as Enzyme-Activated Inhibitors of Mammalian Ornithine Decarboxylase

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α-Ethynyl- and α-vinylornithine were designed and synthesized as potential enzyme-activated inhibitors of mammalian ornithine decarboxylase. These two new inhibitors produce both immediate and time-dependent inhibition of rat liver ornithine decarboxylase in vitro. The inhibitions exhibit saturation kinetics. The apparent dissociation constants (K_I) are 10 and 810 μM, and the times of half-inactivation at infinite concentration of inhibitor ($t_{1/2}$) are 8.5 and 27 min, respectively, for α-ethynyl- and α-vinylornithine. In rats, α-ethynylornithine causes a rapid dose-dependent decrease of ornithine decarboxylase activity in prostate and, to a lesser extent, in thymus and testis.

The pyridoxal phosphate (PLP) dependent enzyme L-ornithine carboxy-lyase (EC 4.1.1.17, ODC), which catalyzes the decarboxylation of ornithine to putrescine, can be rate limiting in the biosynthesis of the polyamines

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