# Articles

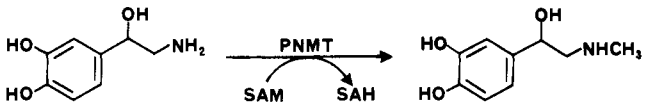
## Importance of the Aromatic Ring in Adrenergic Amines. 5. Nonaromatic Analogues of Phenylethanolamine as Inhibitors of Phenylethanolamine *N*-Methyltransferase: Role of Hydrophobic and Steric Interactions[1a,b]

William C. Vincek, Constance S. Aldrich,[1c] Ronald T. Borchardt, and Gary L. Grunewald*

*Department of Medicinal Chemistry, The University of Kansas, Lawrence, Kansas 66045.* *Received August 19, 1980*

The synthesis of five classes [type A, cycloalkylethanolamines; type B, *n*-alkylethanolamines; type C, 2-(aminomethyl)-2-*n*-alkanols; type D, 1-(aminomethyl)cycloalkanols; type E, *sec*-alkylethanolamines] of nonaromatic analogues of β-phenylethanolamine and an evaluation of their inhibitory potency ($IC_{50}$) for phenylethanolamine *N*-methyltransferase (PNMT) are described. The key intermediates for the synthesis of the ethanolamines were the appropriate aldehydes or ketones. The aldehydes **11a** (cyclobutyl) and **13a** (cycloheptyl) of type A were prepared from the corresponding acids by reduction of the acid to the alcohol with lithium aluminum hydride and oxidation of the alcohol to the aldehyde with pyridinium chlorochromate (PCC). The aldehydes **15a** (cycloundecyl) and **41a** (adamantyl) of type A were prepared by the oxidation of the corresponding alcohols with PCC. The first reported synthesis of cyclononanecarboxaldehyde (type A, **14a**) is described. This aldehyde was prepared via a multistep route beginning with a Favorskii rearrangement of 2-bromocyclodecanone to cyclononanecarboxylic acid. The acid was reduced with lithium aluminum hydride to the corresponding alcohol, which was subsequently oxidized to the aldehyde with PCC. The aldehydes or ketones were converted (with trimethylsilyl cyanide) into their cyanohydrin ethers, which were subsequently reduced to the desired ethanolamine with lithium aluminum hydride. The ethanolamines were tested as inhibitors (LCEC assay) of PNMT. The most potent inhibitors were the type A compounds **8** (cyclooctyl), **13c** (cycloheptyl), **14c** (cyclononyl), and **15c** (cycloundecyl) and the type D compounds **26c** (cyclononyl) and **27c** (cycloundecyl) with $IC_{50}$ values from 6 to 17 μM. It is concluded that the binding site of PNMT accepts hydrophobic groups of an optimal length (~6.4 Å) and width (~2.5 Å) and has a significant height restriction for the hydrophobic group. The ethanolamine side chain prefers to lie away from and in the longitudinal axis of the hydrophobic group. An ethanolamine side chain attached to a cycloalkyl ring of *n* carbon atoms (types A and D) is almost always considerably more potent at inhibiting PNMT than the open-chain compounds of *n* total carbon atoms (types B, C, and E).

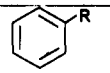
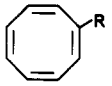
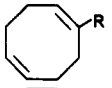
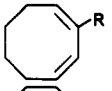
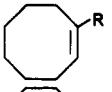
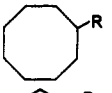
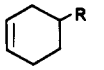
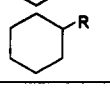
Phenylethanolamine *N*-methyltransferase (PNMT, EC 2.1.1.28) is the enzyme[2] that catalyzes the transfer of a methyl group from *S*-adenosyl-L-methionine (SAM) to norepinephrine (**1**) with the formation of epinephrine (**2**).




**1** **2**

Due to the importance of epinephrine in regulating cellular activity and the recent interest in the central nervous system role of epinephrine in blood-pressure regulation, hypertension,[3-5] and stress,[6] considerable research interest has been directed toward new inhibitors of this enzyme.[7]

Table I. Six- and Eight-Membered Ring Nonaromatic Analogues of Phenylethanolamine and Their $IC_{50}$ Values

| No. | R= —CHCH₂NH₂ (OH) | $IC_{50}$(uM)[a] | $K_m$(uM)±SEM[b] |
|---|---|---|---|
| 3 |  | — | 136±12 |
| 4 |  | 760 | 430±45 |
| 5 |  | 89 | 155±20 |
| 6 |  | 57 | 128±14 |
| 7 |  | 38 | 110±8 |
| 8 |  | 7 | 26±4 |
| 9 |  | 52 | 57±7 |
| 10 |  | 14 | 40±1 |

[a] The correlation coefficients are greater than 0.98. [b] See ref 21.

A large number of in vitro and a few in vivo classes of inhibitors of PNMT are known. These include (a) dead-

(1) (a) This paper has been presented, in part. See "Abstracts of Papers", 173rd National Meeting of the American Chemical Society, New Orleans, La, Mar 21-25, 1977; American Chemical Society: Washington, D.C., 1977; Abstr MEDI 21. (b) Taken, in part, from the Ph.D. Dissertation submitted to the Graduate School of the University of Kansas by William C. Vincek, 1978. (c) NSF Undergraduate Research Participant, 1975 (Grant 75-04411).
(2) Axelrod, J. *J. Biol. Chem.* **1962**, *237*, 1657.
(3) Grobecker, H.; Saavedra, J. M.; Roizen, M. F.; Weise, V.; Kopin, I. J.; Axelrod, J. *Clin. Sci. Mol. Med.* **1976**, *51*, 377s.
(4) Saavedra, J. M.; Grobecker, H.; Axelrod, J. *Mayo Clinic Proc.* **1977**, *52*, 391.
(5) Sauter, A. M.; Lew, J. Y.; Baba, Y.; Goldstein, M. *Life Sci.* **1977**, *21*, 261.
(6) Sauter, A. M.; Baba, Y.; Stone, E. A.; Goldstein, M. *Brain Res.* **1978**, *144*, 415.

0022-2623/81/1824-0007$01.00/0 

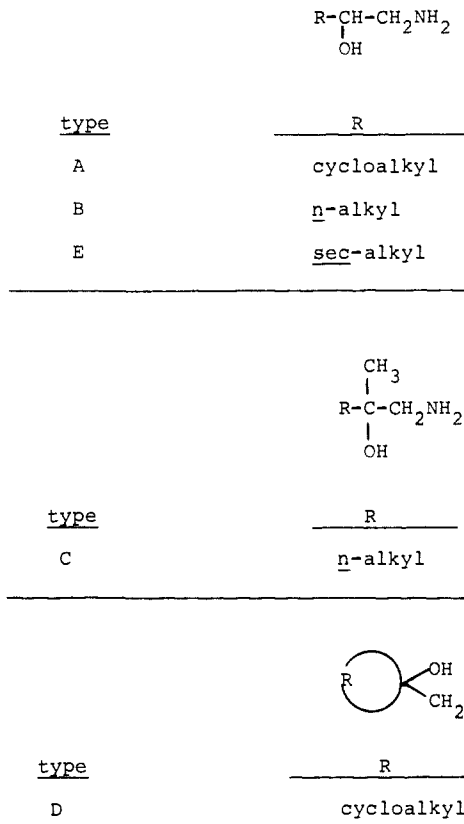
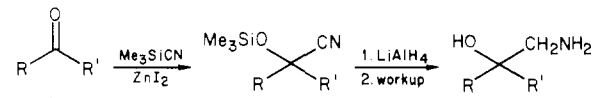



**Figure 1.** Structural types of PNMT inhibitors.

end inhibitors, such as $\alpha$-methylphenylethylamines,[8-10] benzylamines,[11,12] benzamidines,[13] aminobenzimidazoles,[14,15] tetrahydroisoquinolines,[7] benzothieno[3,2-*c*]pyridines,[16] and 2-aminotetralins;[17] (b) inhibitors such as analogues of *S*-adenosylhomocysteine;[18] and (c) alternate substrate-type inhibitors, such as substituted phenylethanolamines.[19] All of these inhibitors contain an aromatic ring which is consistent with the diverse adrenergic side effects reported.[20]

Table II. Synthesis and $IC_{50}$ Values of Nonaromatic Ethanolamines as Inhibitors of PNMT




11-30a, 41a 11-30b, 41b 11-30c, 41c

| type | compd | R | R' | $IC_{50}$, $\mu M$[a] |
|---|---|---|---|---|
| A | 11a-c | cyclobutyl | H | >1000 |
| A | 12a-c | cyclopentyl | H | 446 |
| A | 10 | cyclohexyl | H | 14 |
| A | 13a-c | cycloheptyl | H | 8 |
| A | 8 | cyclooctyl | H | 7 |
| A | 14a-c | cyclononyl | H | 6 |
| A | 15a-c | cycloundecyl | H | 9[b] |
| A | 41a-c | adamantyl | H | 195 |
| B | 16a-c | *n*-butyl | H | 345 |
| B | 17a-c | *n*-pentyl | H | 314 |
| B | 18a-c | *n*-hexyl | H | 109 |
| B | 19a-c | *n*-heptyl | H | 216[c] |
| C | 20a-c | *n*-pentyl | $CH_3$ | 669 |
| C | 21a-c | *n*-hexyl | $CH_3$ | 664 |
| D | 22a-c | $-(CH_2)_4-$ | | >1000 |
| D | 23a-c | $-(CH_2)_5-$ | | >1000 |
| D | 24a-c | $-(CH_2)_6-$ | | 510 |
| D | 25a-c | $-(CH_2)_7-$ | | 72 |
| D | 26a-c | $-(CH_2)_8-$ | | 17 |
| D | 27a-c | $-(CH_2)_{10}-$ | | 8 |
| E | 28a-c | isobutyl | H | 1000 |
| E | 29a-c | 3-pentyl | H | 545 |
| E | 30a-c | 3-methyl-2-pentyl | H | 403 |

[a] The correlation coefficients are greater than 0.970 unless noted otherwise; the $IC_{50}$ values are for the ethanolamine salts of Table III. [b] Correlation coefficient 0.932. [c] Correlation coefficient 0.945.

Our earlier observation[21,23] and later results of Fuller[22] that an aromatic ring is not required for substrate activity suggested that our six- and eight-membered-ring analogues of phenylethanolamine be investigated as alternate substrate inhibitors of PNMT (see Table I). In the series **3–10**, the most active substrates or competitive alternate substrate inhibitors were the fully saturated analogues **8** and **10**. The substrate activity (see $K_m$ values in Table I) of the eight-membered ring series **4–8** suggested that binding improved with increasing lipophilicity of the analogues and that increased $\pi$-electron density was not important. It is possible, however, that charge-transfer complex formation (or less specific $\pi$ complexation) could still be a component to binding, since considerable conformational changes are likely in the series **4–8**. This conformational flexibility might obscure the effects of change in $\pi$-electron density upon binding. This point is addressed in the following paper in this issue.[25]

In this paper, we wish to explore the activity of compounds **4–10** as alternate substrate inhibitors of PNMT and to probe the hydrophobic requirements for binding with several series of saturated analogues of **3**. For convenience, we have designated these new compounds as types A through E (Figure 1). These compounds will allow

(7) Pendleton, R. G.; Kaiser, C.; Gessner, G. *J. Pharmacol. Exp. Ther.* **1976**, *197*, 623. Bondinell, W. E.; Chapin, F. W.; Girard, G. R.; Kaiser, C.; Krog, A. J.; Pavloff, A. M.; Schwartz, M. S.; Silvestri, J. S.; Vaidya, P.; Lam, B. L.; Wellman, G. R.; Pendleton, R. G. *J. Med. Chem.*, **1980**, *23*, 506.
(8) Fuller, R. W.; Hunt, J. M. *Biochem. Pharmacol.*, **1965**, *14*, 1896.
(9) Krakoff, L. F.; Axelrod, J. *Biochem. Pharmacol.* **1967**, *16*, 1384.
(10) Fuller, R. W.; Mills, J.; Marsh, M. M. *J. Med. Chem.* **1971**, *14*, 322.
(11) Fuller, R. W.; Molloy, B. B.; Day, W. A.; Roush, B. W.; Marsh, M. M. *J. Med. Chem.* **1973**, *16*, 101.
(12) Fuller, R. W.; Roush, B. W.; Snoddy, H. D.; Molloy, B. B. *J. Med. Chem.* **1973**, *16*, 106.
(13) Fuller, R. W.; Roush, B. W.; Snoddy, H. D.; Day, W. A.; Molloy, B. B. *J. Med. Chem.* **1975**, *18*, 304.
(14) Pendleton, R. G.; Snow, I. B.; Kaiser, C.; Wang, J.; Green, H. *Biochem. Pharmacol.* **1972**, *21*, 2967.
(15) Mandel, L. R.; Porter, C. C.; Kuehl, Jr., F. A.; Jensen, N. P.; Schmitt, S. M.; Windholz, T. B.; Beattie, T. R.; Carty, J. A.; Christensen, B. G.; Shen, T. Y. *J. Med. Chem.* **1970**, *13*, 1013.
(16) Pendleton, R. G.; Kaiser, C.; Gessner, G.; Finley, E.; Green, H. *J. Pharmacol. Exp. Ther.* **1974**, *190*, 551.
(17) Fuller, R. W.; Molloy, B. B. *Biochem. Pharmacol.* **1977**, *26*, 446.
(18) Borchardt, R. T.; Wu, Y. S.; Huber, J. A.; Wycpalek, A. F. *J. Med. Chem.* **1976**, *19*, 1104, and references cited therein.
(19) Fuller, R. W.; Warren, B. J.; Molloy, B. B. *Biochim. Biophys. Acta* **1970**, *222*, 210.
(20) Grunewald, G. L.; Grindel, J. M.; Patil, P. N.; Salman, K. N. *J. Med. Chem.* **1976**, *19*, 10.
(21) Grunewald, G. L.; Grindel, J. M.; Vincek, W. C.; Borchardt, R. T. *Mol. Pharmacol.* **1975**, *11*, 694.
(22) Fuller, R. W.; Roush, B. W. *Res. Commun. Chem. Pathol. Pharmacol.* **1977**, *17*, 727.
(23) Grunewald, G. L.; Vincek, W. C.; Davis, D. P.; Borchardt, R. T. In "Catecholamines: Basic and Clinical Frontiers"; Usdin, E.; Kopin, I. J.; Barchas, J., Eds.; Pergamon Press: New York, **1979**; Vol. 1, p 189.
(24) Borchardt, R. T.; Vincek, W. C.; Grunewald, G. L. *Anal. Biochem.* **1977**, *82*, 149.
(25) Davis, D. P.; Borchardt, R. T.; Grunewald, G. L. *J. Med. Chem.*, following article in this issue.

Table III. Physical Properties of Nonaromatic Analogues of Phenylethanolamine

| compd | mp, °C | formula | % yield | anal. |
|---|---|---|---|---|
| 11c·*p*-$CH_3C_6H_4SO_3H$[a] | 161-162 | $C_{13}H_{21}NO_4S$ | 47 | C, H, N |
| 12c·HCl[a] | 207-227 dec | $C_7H_{16}ClNO$ | 30 | C, H, N |
| 13c·$(C_6H_5)_2COHCO_2H$[a] | 222-224 dec | $C_{23}H_{31}NO_4$ | 80 | C, H, N |
| 14c·HCl[a] | 194-196 dec | $C_{11}H_{24}ClNO$ | 50 | C, H, N |
| 15c·HCl[a] | 180-220 dec | $C_{13}H_{28}ClNO$ | 39 | C, H, N |
| 16c·*p*-$CH_3C_6H_4SO_3H$[b] | 170-173 | $C_{13}H_{23}NO_4S$ | 42 | C, H, N |
| 17c·*p*-$CH_3C_6H_4SO_3H$[c] | 205-207 | $C_{14}H_{25}NO_4S$ | 25 | C, H, N |
| 18c·*p*-$CH_3C_6H_4SO_3H$[c] | 213-214 | $C_{15}H_{27}NO_4S$ | 35 | C, H, N |
| 19c·*p*-$CH_3C_6H_4SO_3H$[c] | 208-209 | $C_{16}H_{29}NO_4S$ | 58 | C, H, N |
| 20c·*p*-$CH_3C_6H_4SO_3H$[b] | 124.2-126.2 | $C_{15}H_{27}NO_4S$ | 50 | C, H, N |
| 21c·*p*-$CH_3C_6H_4SO_3H$[b] | 127-128 | $C_{16}H_{29}NO_4S$ | 50 | C, H, N |
| 22c·HCl[a] | 110.5-111 | $C_6H_{14}ClNO$ | 64 | C, H, N |
| 23c·HCl[a] | 211-212 dec | $C_7H_{16}ClNO$ | 45 | C, H, N |
| 24c·HCl[c] | 221-222 dec | $C_8H_{18}ClNO$ | 62 | C, H, N |
| 25c·HCl[a] | 220.5-222 dec | $C_9H_{20}ClNO$ | 75 | C, H, N |
| 26c·HCl[a] | 222-224 dec | $C_{10}H_{22}ClNO$ | 80 | C, H, N |
| 27c·HCl[a] | 193-195 dec | $C_{12}H_{26}ClNO$ | 60 | C, H, N |
| 28c·$CH_3SO_3H$[b] | 152.5-154 | $C_7H_{19}NO_4S$ | 61 | C, H, N |
| 29c·$CH_3SO_3H$[b] | 106.5-107.5 | $C_8H_{21}NO_4S$ | 85 | C, H, N |
| 30c·$CH_3SO_3H$[b] | 91-92.5 | $C_9H_{23}NO_4S$ | 30 | C, H, N |
| 41c·HCl[a] | 270 dec | $C_{12}H_{22}ClNO$ | 52 | C, H, N |

[a] Recrystallized from EtOH/EtOAc. [b] Recrystallized from EtOH/$Et_2O$. [c] Recrystallized from MeOH/$Et_2O$.

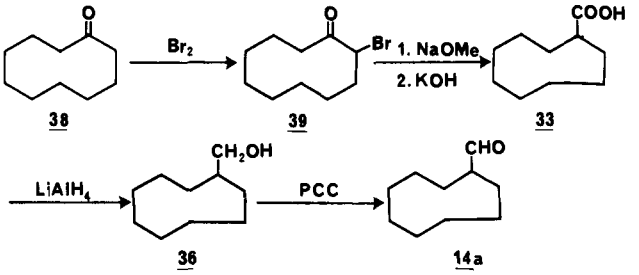
a determination of the optimal hydrophobic requirements for binding, a determination of the requirement for a monocyclic ring, and the ability to tolerate steric bulk near the ethanolamine portion of the molecule.

**Chemistry.** The starting aldehydes (**11a–19a, 28a–30a**) or ketones (**20a–27a**) were readily converted to their cyanohydrin ether intermediates via the addition of trimethylsilyl cyanide[26] ($Me_3SiCN$) as outlined in Table II. The addition of $Me_3SiCN$ to the aldehydes and ketones was complete within 3–10 min, affording yields of 90% or greater. The cyanohydrin ethers were subsequently reduced with lithium aluminum hydride ($LiAlH_4$) in $Et_2O$ to the desired ethanolamines, which were purified as stable salts (see Table III).

Aldehydes **11a**, **13a**, and **14a** were prepared from cyclobutanecarboxylic acid (**31**), cycloheptanecarboxylic acid (**32**), and cyclononanecarboxylic acid (**33**), respectively. Acids **31–33** were reduced to the corresponding alcohols **34–36** with $LiAlH_4$ in ether and were subsequently oxidized to their respective aldehydes **11a**, **13a**, and **14a** with pyridinium chlorochromate (PCC) in $CH_2Cl_2$.[27] Cyclobutanecarboxaldehyde (**11a**) was very unstable; complete removal of the solvent or heating caused rapid decomposition. Removal of the solvent under vacuum was carried out at 20 °C, and $Me_3SiCN$ was added immediately after isolation to avoid unwanted side reactions.

Aldehyde **15a** was prepared by the oxidation of cycloundecylcarbinol (**37**) with PCC. The previously unknown cyclononanecarboxaldehyde **14a** was prepared by way of a Favorskii rearrangement[28] from cyclodecanone (**38**). Ketone **38** was brominated ($Br_2$ in $CCl_4$) to give a high yield of 2-bromocyclodecanone (**39**). Following the addition of NaOMe in MeOH, **39** rearranged to the ester, which was hydrolyzed in situ to yield cyclononanecarboxylic acid (**33**); acid **33** was converted into the aldehyde **14a** via Scheme I.

Scheme I. Synthesis of Cyclononanecarboxaldehyde (14a)




## Biochemistry and Discussion

The $IC_{50}$ values for the alternate substrate inhibitors of types A–E are given in Table II. The importance of a monocyclic ring (types A and D) can be seen in their low $IC_{50}$ values and may be partially explained by first examining the inhibitory activity of the straight-chain analogues (type B), the α-methyl substituted analogues (type C), and the branched-chain analogues (type E).

If one assumes hydrophobic interactions to be important at the active site of the enzyme, the straight-chain analogues (type B) probably would interact with the enzyme in a fully extended or minimum energy conformation. If one further assumes that the active site is of finite length and width, then maximum enzyme inhibitor interactions would occur when (1) the length of the inhibitor is the same as that of the substrate cavity in the enzyme and (2) the width of the inhibitor is optimal for fitting into the substrate cavity of the enzyme. Therefore, at least three parameters may be factored out as important for inhibitory activity in this enzyme system. The first two parameters, the length and width, are frequently combined under the term "steric bulk". The third parameter is the lipophilicity, and, for in vitro enzyme systems, it is generally considered a measure of the hydrophobic interactions between the inhibitor and the enzyme. Although the lipophilicity of these analogues appears to be important,[21] simply increasing the lipophilicity should not increase inhibitory activity unless it is accompanied with the appropriate steric requirements.

If one assumes the inhibitory activity of **18c** (*n*-hexyl) to be due to its chain length[29] (~6.4 Å), then decreasing

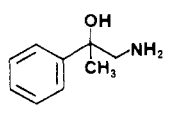
(26) Evans, D. A.; Carroll, G. L.; Truesdale, L. K. *J. Org. Chem.* **1974**, *39*, 914.
(27) Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, 2647.
(28) Traynham, J. G.; Dehn, J. S. *J. Am. Chem. Soc.*, **1967**, *89*, 2139.
(29) The length of the carbon chain was determined by measuring from the center of the first carbon to the center of the last carbon in the R group using Dreiding models. The ring width was measured as the distance between the carbons adjacent to the carbon bearing the R group using Dreiding models.

the length would decrease steric–hydrophobic interactions and decrease activity. Increasing the chain length beyond the optimum (~6.4 Å) would cause less effective steric–hydrophobic interactions and also result in decreased inhibition.

Cyclization of straight-chain type B compound **18c** (*n*-hexyl) into type A compound **10** (cyclohexyl) results in an 8-fold increase in inhibitory activity (**18c**, $IC_{50}$ = 109 μM; **10**, $IC_{50}$ = 14 μM). Since the six-membered ring compound **10** is not the same length as **18c** (**10**, 2.9 Å; **18c**, 6.4 Å), the increase in activity may be due to ring width (**10**, 2.5 Å). The importance of ring width can also be shown by comparing type B compound **19c** (*n*-heptyl) to its cyclized, type A analogue **13c** (cycloheptyl): **19c**, $IC_{50}$ = 216 μM; **13c**, $IC_{50}$ = 8 μM. It is important to note that the ring width of all of the monocyclic ring compounds (types A and D) is approximately 2.5 Å. Yet, the cyclization of type B compound **17c** (*n*-pentyl) into type A compound **12c** (cyclopentyl) does not result in increased inhibitory activity. On the contrary, there is little difference in the $IC_{50}$ values (**17c**, $IC_{50}$ = 314 μM; **12c**, $IC_{50}$ = 446 μM). Comparing **12c** (cyclopentyl) to other type A or D series compounds, it appears that the orientation of the ethanolamine side chain to the ring is important. For compound **12c** (cyclopentyl), the five-membered ring is almost planar and the ethanolamine side chain must lie above or below the plane of the ring. In the larger rings in the type A series, compounds **8**, **10**, and **11c–15c**, the side chain may lie away from and on the longitudinal axis of the ring. In the aromatic series of inhibitors,[8] the carbon bearing the hydroxyl group is bonded to an aromatic ($sp^2$-hybridized) carbon and must lie in the plane of the ring. As mentioned earlier, maximum enzyme–inhibitor interactions should occur with optimal steric–hydrophobic parameters. Since type B compound **18c** (*n*-hexyl) is of optimal length (~6.4 Å), then the 11-membered ring **15c** (length ~6 Å, width 2.5 Å) should be one of the best inhibitors, and, to date, no more potent inhibitor in the type A series is known.

In the branched-chain type E series, the effects of inhibitor length and width on the $IC_{50}$ value again appear to be important. Compound **28c** (isobutyl) and **16c** (*n*-butyl) have similar lipophilicities but the length of **28c** is only ~2.3 Å compared to ~4.0 Å for **16c**. This decrease in length is consistent with a higher $IC_{50}$ value (**28c**, $IC_{50}$ > 1000 μM; **16c**, $IC_{50}$ = 345 μM). Compound **29c** (3-pentyl) is an open-chain analogue of type A compound **12c** (cyclopentyl). Earlier it was postulated that **12c** was a poor inhibitor because the ethanolamine side chain must lie above or below the plane of the ring; this is not true for the open-chain analogue **29c**. Compound **29c** is not planar and the side chain may be oriented freely in space, but due to the steric crowding of the ethanolamine side chain by the two adjacent ethyl groups proper enzyme fit for inhibition is not achieved. This same argument may be applied to explain the high $IC_{50}$ value for **30c** (3-methyl-2-pentyl), since the ethanolamine side chain would be hindered by the adjacent methyl groups.

The placement of a methyl group, in addition to the hydroxyl group, on the β carbon of the aromatic series of inhibitors has been shown to abolish all substrate activity.[19] Compounds **20c** and **21c** were designed to test the effect of a β-methyl group on nonaromatic analogues of **40**. The




**40**

type C compounds **20c** and **21c** do show inhibitory activity (**20c**, $IC_{50}$ = 669 μM; **21c**, $IC_{50}$ = 664 μM). Cyclization of **21c** (β-methyl, *n*-hexyl) into type D compound **24c** (cycloheptyl) only slightly increased inhibitory activity (**24c**, $IC_{50}$ = 510 μM). As the monocyclic ring size is increased in the type D series, however, the $IC_{50}$ values decreased. The major difference between the type A and the type D series is the ethanolamine side chain. In the type A series, the ethanolamine chain is not a part of the ring itself. The side chain is free to move in space independent of the monocyclic ring. The ring may assume many conformations without drastically affecting the binding of the ethanolamine side chain. This freedom of movement for the ethanolamine portion of the molecule apparently permits the side chain to easily assume the proper conformation for good inhibitory activity. In the type D series, the hydroxyl-bearing carbon in the ethanolamine side chain is part of the monocyclic ring. It was noted above that the orientation of the side chain appears to be important for enzyme inhibition for the type A compounds. The best orientation of the side chain appears to be on the longitudinal axis of the ring. In the smaller rings of the type D series this orientation is not possible (**22c**, $IC_{50}$ > 1000 μM; **23c**, $IC_{50}$ > 1000 μM; type A, **10**, $IC_{50}$ = 14 μM). As the ring is enlarged in the type D series, it becomes easier for the ethanolamine portion of the molecule to assume the proper orientation or conformation for good enzyme fit. Therefore, in the type D series, one sees a more selective inhibition due to the restricted freedom of the ethanolamine portion of the molecule [**24c** (cycloheptyl), $IC_{50}$ = 510 μM; **25c** (cyclooctyl), $IC_{50}$ = 72 μM; **27c** (cycloundecyl), $IC_{50}$ = 8 μM].

In addition to a reasonable tolerance to length (~6.4 Å) and width (~2.5 Å), some tolerance for height at the hydrophobic binding site is also noted in the moderate activity of the adamantyl type A compound **41c** ($IC_{50}$ = 195 μM). The symmetrical 10-carbon atom containing adamantyl ring analogue is, however, not nearly as active as an inhibitor as the type A monocyclic analogues **14c** and **15c** (with 9 or 11 carbon atoms in the ring). Thus, the height of the adamantyl ring appears to be too large. It is also reasonable that the orientation of the adamantyl ring with respect to the side chain[25] may not allow the carbon atoms near the side chain to occupy the hydrophobic binding site, thus "shortening" the hydrophobic interactions possible. This point will be discussed in the following paper in this issue.[25]

In summary, it appears that hydrophobic interactions play a major role in determining inhibitory activity of nonaromatic analogues of phenylethanolamines. The relationship between molecular length and width and ethanolamine side-chain orientation with respect to the plane of the ring also are important. These results support our earlier hypothesis[20,21,23] that hydrophobic interactions are of primary importance for inhibition at the active site of PNMT.

## Experimental Section

Melting points were taken on a Thomas Hoover capillary melting-point apparatus and are uncorrected. Microanalyses were performed on a Hewlett-Packard 185B CHN analyzer and were within ±0.4% of theoretical values. Infrared spectra were recorded on either a Beckman IR-33 or a Perkin-Elmer 727 spectrophotometer. NMR data were recorded on either a Varian T-60 or a Varian EM-360 spectrometer with $Me_4Si$ as the internal standard. Deuterium exchanges were performed on all compounds possessing labile hydrogens. Mass spectra were obtained on a Varian Atlas CH5 EI mass spectrometer.

Pyridinium chlorochromate (PCC), dihydroxybenzylamine hydrobromide, and all of the starting compounds were purchased from Aldrich Chemical Co. *l*-Norepinephrine, *S*-adenosyl-L-

methionine, and tris(hydroxymethyl)aminomethane hydrochloride (Tris) were purchased from Sigma Chemical Co. Lithium aluminum hydride ($LiAlH_4$) was purchased from Alfa Products. Solvents were routinely distilled prior to use. Trimethylsilyl cyanide ($Me_3SiCN$) was prepared by the method of Zubrick et al.[30] and was stored in 10-mL vials under argon with Teflon stoppers.

**General Procedures for the Preparation of Ethanolamines. A. Trimethylsilyl Cyanohydrin Ethers.**[26] A flame-dried one-neck flask containing 1 equiv of aldehyde or ketone and a catalytic amount (1–10 mg) of zinc iodide was equipped with a serum cap, a static head for pressure equilibrium, and a magnetic stirring bar. To the flask was slowly added 1.1 equiv of $Me_3SiCN$, via syringe, over a period of 5–10 min. For most of the aldehydes and ketones, the reaction was exothermic and cooling with an ice bath is recommended. The addition of $Me_3SiCN$ to the carbonyl moiety can be monitored by the disappearance of the C=O stretch in the IR. In most cases, the yields of the intermediate cyanohydrin ether were greater than 90% as based on the loss of the C=O stretch in the IR. The crude cyanohydrin ether, usually a light yellow to orange oil, was used in the subsequent step without further purification.

**B. Reduction of Trimethylsilyl Cyanohydrin Ethers to Ethanolamines.** A nitrogen-purged three-necked flask equipped with a condenser, an addition funnel with a pressure-equalizing side arm, and a magnetic stirring bar was charged with $LiAlH_4$ (3 mol-equiv) in anhydrous $Et_2O$ under an atmosphere of $N_2$ or Ar. An ethereal solution of the crude cyanohydrin ether (1 equiv) obtained from procedure A was added to the stirred $LiAlH_4$ suspension at a rate sufficient to maintain a gentle reflux (usually addition was complete within 3–10 min). The suspension was heated at reflux for an additional length of time, usually 1.0 h and cooled to room temperature, and the excess $LiAlH_4$ was destroyed by the successive "**cautious**" addition of $n$ mL of water, $n$ mL of 15% NaOH solution, and $3n$ mL of water for $n$ grams of $LiAlH_4$.[31] The resulting white granular precipitate was filtered and washed. The ethereal ethanolamine solution was dried over pellets of KOH. After drying, the ethereal solution was carefully decanted from the drying agent and the $Et_2O$ was removed under reduced pressure to yield the desired ethanolamine.

**C. Preparation of Ethanolamine Salts.** The ethanolamine from procedure B was converted into a stable salt by reacting it with the appropriate acid (see Table III). The hydrochloride salt was formed by bubbling dry HCl into a stirred $Et_2O$–ethanolamine solution (1.0 g of ethanolamine/200 mL of $Et_2O$). The methanesulfonate, $p$-toluenesulfonate, and benzilate salts were formed by the addition of the acid (1 equiv) in $Et_2O$ to a stirred solution of the ethanolamine (1 equiv) in $Et_2O$. The crude salts were filtered through a sintered glass funnel under a positive pressure of dry nitrogen and were recrystallized from appropriate solvents (see Table III).

**Cyclobutanecarboxaldehyde (11a).** A flask was charged with $LiAlH_4$ (11.0 g, 0.289 mol) in $Et_2O$ (300 mL) and placed under an atmosphere of argon. Cyclobutanecarboxylic acid (**31**; 10.0 g, 0.1 mol) in $Et_2O$ (50 mL) was slowly added to the suspension over a period of 1 h. The suspension was stirred at room temperature for an additional 1.5 h, after which the excess $LiAlH_4$ was destroyed by the method described in procedure B. The solvent was removed under reduced pressure to yield 8.3 g (96%) of a clear, very pale-yellow oil of the crude alcohol **34**: bp 141–142 °C (743 mm) [lit.[34] 141–143 °C (742 mm)]; $n^{22}_D$ 1.4438.

Alcohol **34** (4.0 g, 0.031 mol) in $CH_2Cl_2$ (60 mL) was added in one portion to PCC[27] (14.9 g, 0.069 mol) in $CH_2Cl_2$ (150 mL) at room temperature. The suspension, which turned black within 2 min, was stirred at room temperature for an additional 1 h. At the end of this time, $Et_2O$ (500 mL) was added to the reaction mixture, which was then filtered through a small pad (50 g) of Florisil (Fisher, 100–200 mesh). The solvents were immediately removed under reduced pressure to yield 2.9 g (99%) of a greenish-yellow oil of **11a**:[35] IR (liquid film) 1720 $cm^{-1}$; NMR ($CCl_4$) $\delta$ 9.6 (d, 1, CHO).

**Cycloheptanecarboxaldehyde (13a).** Following the procedure for **11a**, cycloheptanecarboxylic acid[28] (**32**; 10.0 g, 0.07 mol) in $Et_2O$ (50 mL) was slowly added to $LiAlH_4$ (8.0 g, 0.21 mol) in $Et_2O$ (300 mL). The suspension was stirred at room temperature for an additional 1.5 h. Following workup, the ethereal alcohol solution was dried ($K_2CO_3$), and the $Et_2O$ was removed under reduced pressure to yield 8.98 g (99%) of alcohol **35** (clear colorless oil): $n^{20}_D$ 1.4735 (lit.[36] $n^{20}_D$ 1.4750–1.4762).

Alcohol **35** (4.0 g, 0.031 mol) in $CH_2Cl_2$ (60 mL) was added in one portion to PCC (10.1 g, 0.047 mol) in $CH_2Cl_2$ (150 mL) at room temperature. Following the same procedure as for alcohol **34**, the solvents were removed under reduced pressure to yield 3.5 g (89%) of aldehyde **13a** (yellow oil): $n^{25}_D$ 1.4675 (lit.[37] $n^{21}_D$ 1.4635); IR (liquid film) 2700, 1715 $cm^{-1}$; NMR ($CCl_4$) $\delta$ 9.15 (s, 1, CHO).

**Cyclononanecarboxaldehyde (14a).**[38] To a mixture of cyclodecanone (**38**; 3.0 g, 0.019 mol), $CCl_4$ (6 mL), and $H_2O$ (9 mL) heated at reflux was added bromine (3.11 g, 0.019 mol) in 3 mL of $CCl_4$. The bromine–$CCl_4$ solution was added over a period of 15 min with heating to maintain the mixture at reflux; during this addition period, the mixture was irradiated with a 100-W light bulb. The solution was then cooled to room temperature and poured into a separatory funnel with additional $CCl_4$ (40 mL). The mixture was washed with brine until the brine wash was neutral to litmus and the $CCl_4$ layer was dried ($K_2CO_3$). Removal of the $CCl_4$ under reduced pressure yielded 4.22 g (95%) of 2-bromocyclodecanone (**39**, pale yellow oil): IR (liquid film) 1715 (C=O), 770, 755 $cm^{-1}$ (CBr). The $\alpha$-bromo ketone **39** was used in the next step without further purification.

A solution of **39** (3.5 g, 0.015 mol) in MeOH (5 mL) was added, over a period of 30 min, to a solution of sodium methoxide in MeOH [1.2 g, 0.05 g-atom of Na in MeOH (20 mL) at −20 °C]. The mixture was stirred at −20 °C for an additional 0.5 h, then allowed to warm to room temperature over a period of 2 h, and finally heated at reflux for 1 h. The mixture was diluted with aqueous KOH [4.0 g, 0.071 mol of KOH in $H_2O$ (10 mL)] and heated at reflux for 4 h, cooled to room temperature, and extracted with ether (3 × 50 mL). The aqueous layer was acidified with concentrated HCl and extracted with $Et_2O$ (2 × 100 mL). The $Et_2O$ layer was dried ($MgSO_4$) and removed under reduced pressure to yield 1.88 g (74%) of acid **33**.[28]

Acid **33** (1.85 g, 0.011 mol) in $Et_2O$ (25 mL) was slowly added to $LiAlH_4$ (1.5 g, 0.039 mol) in $Et_2O$ (100 mL). The mixture was heated at reflux for 2 h and the excess $LiAlH_4$ was destroyed as described in procedure B, yielding 1.7 g (100%) of alcohol **36** (clear, colorless oil).[28] Anal. ($C_{10}H_{18}O_2$) C, H, N.

Alcohol **36** was oxidized to **14a** by the same procedure as alcohol **34**. Alcohol **36** (0.14 g, 0.90 mmol) in $CH_2Cl_2$ (5 mL) was added to PCC (0.29 g, 1.3 mmol) in $CH_2Cl_2$ (20 mL) to yield 0.11 g (80%) of **14a**: IR (liquid film) 2720 (CH), 1740 $cm^{-1}$ (C=O); NMR ($CCl_4$) $\delta$ 9.5 (s, 1, CHO); EIMS (70 eV) $m/e$ (relative intensity) 154 ($M^+$, 1), 69 (100).

**Cycloundecanecarboxaldehyde (15a)** was prepared from cycloundecanemethanol (**37**) using the same oxidation procedure as described for alcohol **34**. Alcohol **37** (2.0 g, 0.011 mol) in $CH_2Cl_2$ (10 mL) was added to PCC (3.49 g, 0.016 mol) in $CH_2Cl_2$ (60 mL) to yield 1.83 g (93%) of **15a** (colorless oil): IR (liquid film) 2710 (CH), 1730 $cm^{-1}$ (C=O); NMR ($CCl_4$) $\delta$ 9.5 (d, 1, CHO).

**2-Adamantyl-2-hydroxyethylamine (41c).** 1-Adamantanemethanol (1.0 g, 0.006 mol) in $CH_2Cl_2$ (15 mL) was added in one portion to PCC[27] (1.94 g, 0.009 mol) in $CH_2Cl_2$ (50 mL) at room

(30) Zubrick, J. W.; Dunbar, B. I.; Durst, H. D. *Tetrahedron Lett.* **1975**, 71.
(31) Micovic, V. M.; Mihailovic, M. L. J. *J. Org. Chem.* **1953**, *18*, 1190.
(32) Connett, R. J.; Kirshner, N. *J. Biol. Chem.* **1970**, *245*, 329.
(33) Keller, R.; Oke, A.; Mefford, I.; Adams, R. N. *Life Sci.* **1976**, *19*, 995.
(34) Wilt, J. W.; Roberts, D. D. *J. Org. Chem.* **1962**, *27*, 3434.
(35) Colman, H. G.; Perkin, W. H. *J. Chem. Soc.* **1887**, *51*, 228.
(36) Royals, E. E.; Neal, A. H. *J. Org. Chem.* **1956**, *21*, 1448.
(37) de Botton, M. M. *C. R. Hebd. Seances Acad. Sci., Ser. C* **1968**, *226*, 1101.
(38) Another route considered for the synthesis of **14a** was the procedure (Taguchi, H.; Tanaka, S.; Yamamoto, H.; Nozaki, H. *Tetrahedron Lett.* **1973**, 2465) for the synthesis of $\alpha,\beta$-unsaturated aldehydes from ketones. Using cyclohexanone as a model compound, attempts to distill [~100 °C (20 Torr)] cyclohex-1-enecarboxaldehyde from the reaction mixture resulted in a violent explosion.

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 ($CCl_4$) $\delta$ 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-$d_5$) $\delta$ 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 $\mu$L: *l*-norepinephrine (9 nmol); *S*-adenosyl-L-methionine (18 nmol); phosphate buffer, pH 8.0 (25 $\mu$mol); 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 $\mu$L 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-$\mu$L 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.

**Acknowledgment.** This research was supported by a grant-in-aid from the American Heart Association (Kansas Affiliate, Inc.), by the University of Kansas General Research Fund, and the National Institutes of Health (Research Grant HL 21887). The technical assistance of Richard Lampe (PNMT assays) and assistance in the preparation of this manuscript by Michael F. Rafferty are gratefully acknowledged.

(39) Stepanov, F. N.; Dovgan, N. L. *Zh. Org. Khim.* **1968**, *4*, 277.

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

Dwight P. Davis,[1b] Ronald T. Borchardt, and Gary L. Grunewald*

*Department of Medicinal Chemistry, The University of Kansas, Lawrence, Kansas 66045.* *Received August 19, 1980*

To probe the importance of $\pi$-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 $\pi$-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 $\pi$-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 