Binding Requirements of Phenolic Phenylethylamines in the Benzonorbornene Skeleton at the Active Site of Phenylethanolamine N-Methyltransferase[1a,b]

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In order to determine the active site binding orientation of norepinephrine, a series of conformationally defined analogues of the tyramines, in which the ethylamine side chain is held fixed by incorporation into a benzonorbornene skeleton, were prepared and evaluated for phenylethanolamine *N*-methyltransferase (PNMT) activity. While *exo*-2-amino-5- and *exo*-2-amino-8-hydroxybenzonorbornene (**7** and **10**, respectively) were prepared from 5-methoxybenzonorbornadiene by azidomercuration/demercuration and reduction, it was necessary to employ both normal (inversion of configuration) and abnormal (retention of configuration) Mitsunobu reactions to prepare, stereoselectively, *exo*-2-amino-6- and *exo*-2-amino-7-hydroxybenzonorbornene (**8** and **9**, respectively) from 6- and 7-methoxybenzonorbornen-2-ol. None of the six analogues were substrates. However, *exo*-2-amino-6-hydroxybenzonorbornene (**8**) and *anti*-9-amino-6-hydroxybenzonorbornene (**12**) displayed significant activity as inhibitors toward PNMT. The greater potency of **8** and **12**, as compared to the parent unsubstituted analogues *exo*-2-amino- and *anti*-9-aminobenzonorbornene (**4** and **5**, respectively), indicates the presence of a spatially compact hydrophilic pocket within the aromatic ring binding region of the active site of the enzyme. Furthermore, the greater activity of **12**, relative to **8**, is consistent with an active site binding preference for molecules in which a more coplanar relationship exists between the aromatic ring and the amine nitrogen. From the findings of this study, it appears that norepinephrine has a different active site binding orientation than most known substrates and competitive inhibitors of PNMT.

The enzyme phenylethanolamine *N*-methyltransferase (PNMT; EC 2.1.1.28), first isolated in 1962 from the monkey adrenal,[2] is known to catalyze the transfer of an active methyl group from *S*-adenosyl-L-methionine (AdoMet) to norepinephrine (NE; **1**) to give the hormone epinephrine (Epi; **2**) (see Figure 1). Although PNMT has subsequently been observed in various regions of the mammalian central nervous system,[3] and central Epi has been postulated to play an important role in such functions as the central regulation of blood pressure,[4-8] the release (and inhibition of release) of pituitary hormones,[9-11] and the regulation of α_2-adrenergic receptors,[12-14] an unambiguous definition of its functional significance has not yet been determined. Perhaps the greatest barrier toward defining the role of central Epi is the lack of a potent yet highly selective PNMT inhibitor.[15,16] Although several potent inhibitors of PNMT are currently available, all suffer from the drawback that they possess significant activity at other biologically relevant sites (i.e., α_2-adrenergic receptors).[17-20]

An approach that we are taking toward the design of a selective PNMT inhibitor is the determination of the overall topography of the active site of the enzyme by defining the various interactions that occur when substrates and competitive inhibitors bind. Increased inhibitor selectivity may then result by taking advantage of those binding features that are exclusively characteristic of the active site of PNMT.

β-Phenylethylamines (including amphetamines) are competitive inhibitors of PNMT. In an examination of the conformation–activity relationships displayed by the conformationally defined amphetamines **3–5**, we found that a fully extended side chain conformation (τ_2 = 180°) is optimal for active site binding.[21] For instance, **4** and **5** (in which the amine and aromatic ring are held in a trans antiperiplanar relationship with τ_2 = 180°) displayed competitive inhibition greater than that observed for amphetamine, while **3** (a gauche side chain conformation with τ_2 = 60°) displayed only weak uncompetitive inhibition kinetics. In addition, the fact that **5** proved to be a better inhibitor than **4** suggested that the enzyme preferentially binds molecules with a more coplanar relationship between the aromatic ring and the amine nitrogen. The greater coplanarity in **5** is the result of a lower torsion angle, τ_1 (the angle which, when τ_2 = 180°, defines the

(1) (a) Paper 9 in our series *Conformationally Defined Adrenergic Agents*; for paper 8 see ref 45. (b) This paper has been presented, in part, at the International Chemical Congress of Pacific Basin Societies (PAC-CHEM), Honolulu, HI, Dec 1984, Abstract 10P47, and at The Biochemistry of *S*-Adenosylmethionine as a Basis for Drug Design: An International Symposium, Bergen, Norway, July 1985, Abstract 31. (c) Summer Undergraduate Research Participant, University of Kansas Dept. of Medicinal Chemistry, 1983, and Sterling Winthrop Undergraduate Research Fellow, University of Kansas Dept. of Medicinal Chemistry, Summer 1984. (d) Postdoctoral Fellow of the American Heart Association, Kansas Affiliate, Inc.; present address: Bennington College, Bennington, VT 05201. (e) NIH Predoctoral Trainee (Grant GM 0075).

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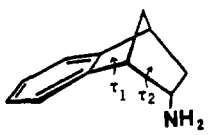

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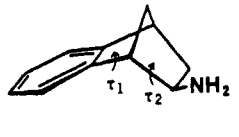

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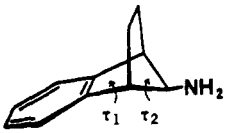


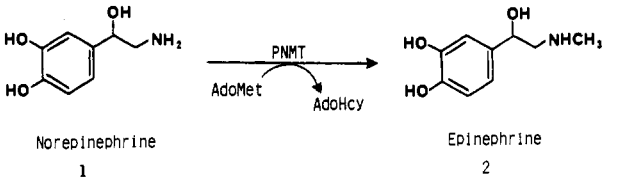
5

6

position of the amine nitrogen with respect to the plane of the aromatic ring). Analogue **5** has a τ_1 of 34° while **4** has a τ_1 of 69°. Another very important result from this study was the fact that **4** and **5** displayed weak but detectable and reproducible activity as substrates.[22,23] This was the first example of activity as a substrate for a β-phenylethylamine that does not possess a hydroxyl (or other heteroatom functionality) at the β-position of the ethylamine side chain. An explanation for this unexpected result is that the rigid framework in **4** and **5** serves the same function as the β-hydroxyl group of an ethanolamine substrate, namely to anchor the primary amino group into a region of the active site in which methylation can occur.

It has been previously shown that electron-withdrawing, lipophilic substituents on the aromatic ring greatly enhance the PNMT binding affinity of phenylethanolamines (substrates),[22,24,25] amphetamines (competitive inhibitors),[22,25,26] and benzylamines (also competitive inhibitors).[25,27] Thus, it has been concluded that the aromatic ring binding region within the active site of the enzyme contains a high degree of lipophilic character. This conclusion, however, is inconsistent with the high activity (K_m = 4.7 ± 1.2 μM[23]) of the natural substrate, norepinephrine (**1**), which contains a hydrophilic catechol functionality. For this reason, it was of interest to determine where, in the aromatic ring binding region of the active site of PNMT, the catechol of NE interacts.

As an initial approach to answering this question, we chose to define the orientation of the catechol hydroxyl groups required for optimal binding of substrates such as **4** and **5**. We thus prepared the catechol analogue **6** and found it to be no better a substrate than **4** itself.[23] This result was clouded, however, by the fact that **6** was unstable under our assay conditions (**6** underwent rapid and irreversible oxidation, and only 31% remained unchanged after a 30-min control incubation without enzyme present). Even when an approximation of the amount of **6** actually present in the assay mixture was made, an unambiguous interpretation of the activity of **6** as a substrate could not be determined.

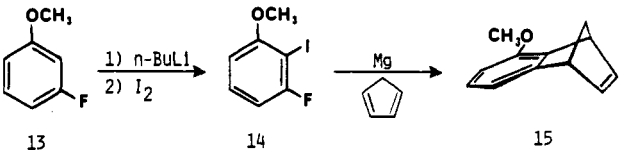


Norepinephrine
1

Epinephrine
2

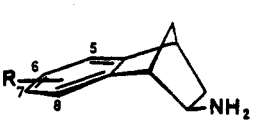
Figure 1. Biosynthetic pathway for epinephrine involving N-methylation of norepinephrine. The enzyme that catalyzes this transformation, phenylethanolamine *N*-methyltransferase (PNMT), utilizes *S*-adenosyl-L-methionine (AdoMet) as the methyl donor. Though the correct name for PNMT is norepinephrine *N*-methyltransferase (EC 2.1.1.28), the abbreviation PNMT is most commonly used.

Scheme I

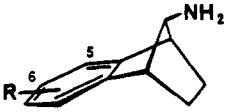


13 14 15

In order to circumvent the instability encountered with catecholamine **6**, we have synthesized and evaluated the tyramine analogues of **6**, compounds **7–10**. Given that a



7, R = 5-OH
8, R = 6-OH
9, R = 7-OH
10, R = 8-OH



11, R = 5-OH
12, R = 6-OH

side chain β-hydroxyl group is not required for activity as a substrate if the ethylamine side chain is locked in a fully extended conformation,[22] amines **7–10** can also be viewed as analogues of the corresponding octopamines. Since *m*- and *p*-octopamine (the phenolic analogues of the catechol norepinephrine) possess only a single aromatic hydroxyl group yet are still good substrates,[28] enhanced activity of **7–10** compared to substrate **4** would be expected for those isomers that contain the most favorable active site binding orientations of the phenolic hydroxyl group. In addition to analogues **7–10**, we have studied the related amines **11** and **12**, which contain a more planar β-phenylethylamine structure (τ_1 = 34°) than that found in **7–10** (τ_1 = 69°). We report here the synthesis and PNMT activity of these six conformationally defined phenolic amines.

Chemistry. Amines **7**, **10**, and **11** were prepared from the key intermediate 5-methoxybenzonorbornadiene (**15**; Scheme I). Although **15** has been prepared by the addition of 3-methoxybenzyne (generated in situ by aprotic diazotization of 6-methoxyanthranilic acid) to cyclopentadiene, the preparation of the requisite anthranilic acid required three steps, which proceeded in only 17% overall yield.[29] After diazotization and cycloaddition, diene **15** was formed in 9% overall yield over the four steps.[29] We have found that **15** can be conveniently prepared in two steps from commercially available 3-fluoroanisole (**13**) as illustrated in Scheme I. Taking advantage of the ortho directing effect of both the methoxy and fluorine substituents,[30] lithiation followed by the addition

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Scheme II

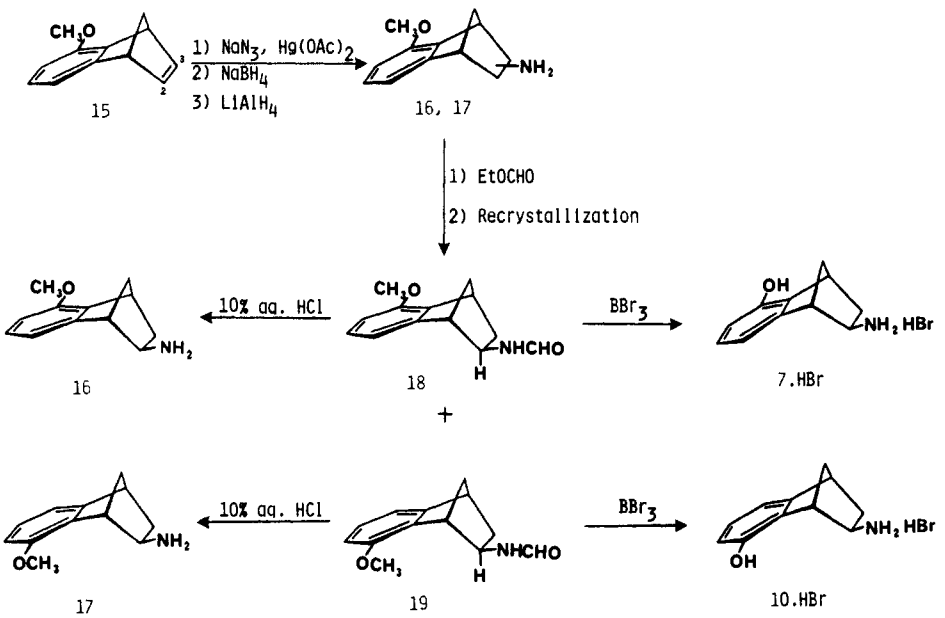
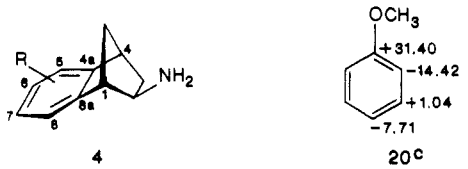


Table I. ^{13}C Chemical Shifts[a,b] of Aromatic Methoxy-Substituted *exo*-2-Aminobenzonorbornenes



4 20[c]

carbon compd. no.	C-1		C-4		C-4a		C-5		C-6		C-7		C-8		C-8a	
	calcd	obsd	calcd	obsd	calcd	obsd	calcd	obsd	calcd	obsd	calcd	obsd	calcd	obsd	calcd	obsd
4, R = H[d]		52.92		43.36		148.53		121.18		125.48		125.68		120.59		146.39
16, R = 5-OMe		53.61		39.38	134.11	135.35	152.58	153.10	111.08	109.15	126.72	126.67	112.88	114.19	147.43	148.66
17, R = 8-OMe		48.61		43.50	149.57	150.73	113.47	113.61	126.52	126.85	111.26	108.38	151.99	153.30	131.97	132.86
25, R = 6-OMe					149.57	150.14	106.76	107.89	156.88	158.22	111.26	109.77	121.63	121.63	138.68	138.63
32, R = 7-OMe					140.82	140.76	122.22	121.05	111.06	109.83	157.08	157.89	106.17	108.48	147.43	148.07

[a] Relative to $CDCl_3$ (77.00 ppm). [b] The calculated chemical shifts were determined by adding the S_x values (S = the SCS relative to benzene and x = the ipso, ortho, meta, or para carbon) of methoxybenzene (**20**) to the observed chemical shift value of the appropriate carbon in **4**. [c] S_x values taken from ref 38. [d] Chemical shift values taken from ref 37.

of iodine afforded **14** in 74% yield; **14** has been previously prepared by the Sandmeyer reaction from 2-amino-3-fluoroanisole in a yield of 50%.[31] Finally, employing the conditions developed by Tanida et al.[32] for similar systems, **14** underwent cycloaddition with cyclopentadiene to give diene **15** in 29% yield over the two chemical steps. The 1,2,3-trisubstitution pattern of **14** was confirmed by spectroscopic methods (IR, ^1H NMR) and by the formation of **15**[29] in the ensuing reaction.

Due to the greater availability of aryl participation at olefinic carbon C-2 (as opposed to C-3), treatment of **15** with sodium azide/mercuric acetate, followed by demercuration and reduction, afforded a 1:2 mixture of exo amines **16** and **17**, respectively (Scheme II). The stereoselectivity of this reaction (exo vs. endo) was determined by ^1H NMR spectrometry and is consistent with previous findings for similar systems.[33-35] Since separation of these amines proved difficult, the amine mixture was converted directly to the formamide derivatives **18** and **19**. These formamides were separated by repeated fractional recrystallization in which the different crystal types were separated manually. The predominant isomer, **19**, crystallized out first as clumps of white needles. Isomer **18** was more soluble and crystallized as fluffy white flakes. Recrystallization of **18** and **19** was continued until a constant melting point was obtained for each. In some runs of this reaction sequence, a small amount of the rearranged *anti*-9-formamide (**21**) was isolated from chromatography of the mother liquors from recrystallization. Its structure was verified by comparing its ^1H NMR to a sample of **21** prepared as shown in Scheme III. Concomitant amine deprotection and methyl ether cleavage of the separated formamides **18** and **19** were accomplished upon treatment with boron tribromide to afford the hydrobromide salts of isomerically pure phenolic amines **7** and **10**.

Structural assignment of the regioisomeric formamides **18** and **19** was accomplished by examination of the ^{13}C NMR spectra of their corresponding free amines (**16** and **17**, respectively; Scheme II), which were available through hydrolysis of the pure formamides with 10% aqueous HCl at reflux. Simple additivity rules[36] (summation of the chemical shifts for *exo*-2-aminobenzonorbornene, **4**,[37] and

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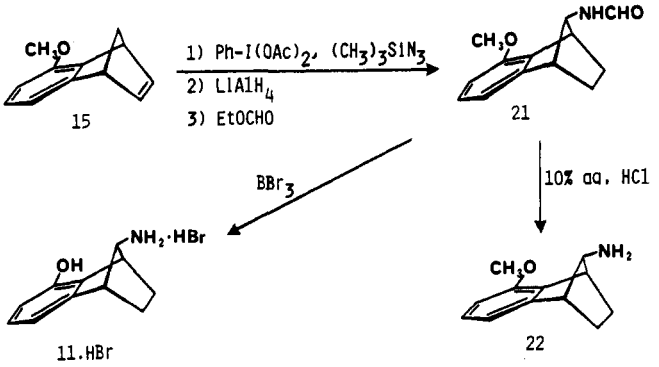
Scheme III

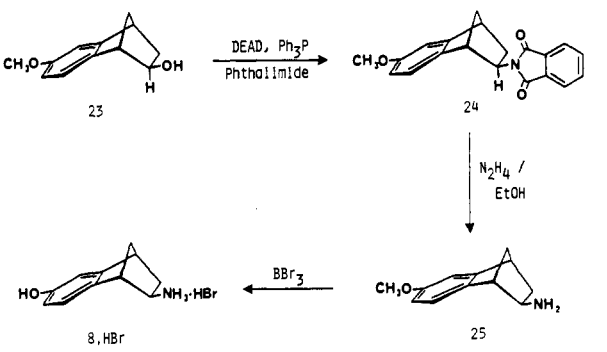


the substituent-induced chemical shift (SCS) values for methoxybenzene, **20**,[38] (Table I)) provided chemical shifts for the aromatic carbons that were in good agreement with the assigned structures. We have previously used this method to assign the aromatic carbons for a number of aryl-substituted benzonorbornen-2-ones.[36] The ^{13}C chemical shifts of carbons C-4 of **16** and C-1 of **17** are also particularly useful for the structural assignment. A methoxy group in the ortho position of a benzofused ring system has been shown to exert a strong shielding effect at the peri carbon of the adjacent ring. For instance, carbon C-8 of 1-methoxynaphthalene is shielded relative to C-8 of naphthalene and occurs 5.80 ppm upfield,[39] and carbon C-4 of 5-methoxy-1,2,3,4-tetrahydronaphthalene is shielded relative to C-1 of 1,2,3,4-tetrahydronaphthalene and occurs 6.40 ppm upfield.[40] Consistent with this, the chemical shift for carbon C-4 of **16** occurs 3.98 ppm upfield of the corresponding carbon in **4**. Likewise, the chemical shift of carbon C-1 of isomer **17** is shifted 4.31 ppm upfield from C-1 of **4**.

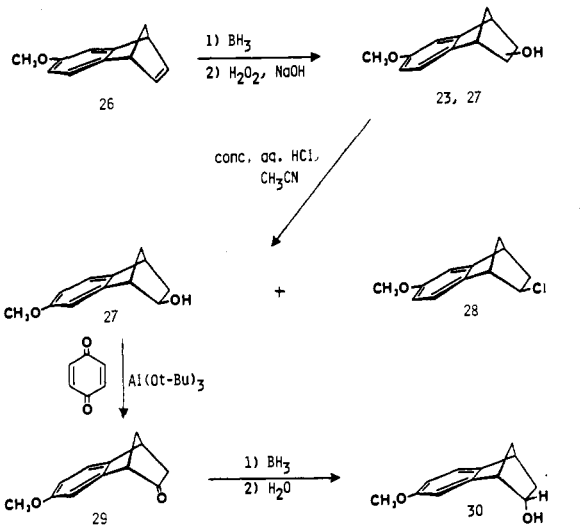
The stereochemical assignment of exo and endo substitution at carbon C-2 of amines **16** and **17**, as well as the exo and endo substitution in the molecules which follow, was based on ^1H NMR spectrometry. The ^1H NMR spectra of 2-substituted benzonorbornenes have been extensively studied. Wilt et al. have shown for the 2-bromo[41] and 2-chloro[42] derivatives, and Grunewald et al. for the 2-amino and 2-(methylamino) derivatives,[34] that the exo hydrogen attached to the substituted carbon of the endo isomer resonates more that 0.5 ppm downfield from the corresponding endo hydrogen of the exo isomer. Also, in the 2-endo derivatives, the endo C-3 hydrogen is distinguishable as a high-field doublet of triplets.

The preparation of phenolic amine **11** is illustrated in Scheme III. Treatment of **15** with iodobenzene diacetate and azidotrimethylsilane according to the method of Ehrenfreund and Zbiral,[43] followed by reduction of the intermediate azide, afforded a mixture of amines that was converted directly to the formamides. Recrystallization of the mixture afforded pure **21**. Hydrolysis and methyl ether cleavage with boron tribromide gave the hydrobromide salt of phenolic amine **11**. A sample of formamide **21** was also hydrolyzed to the methoxy derivative **22** (Scheme III) for spectral characterization.

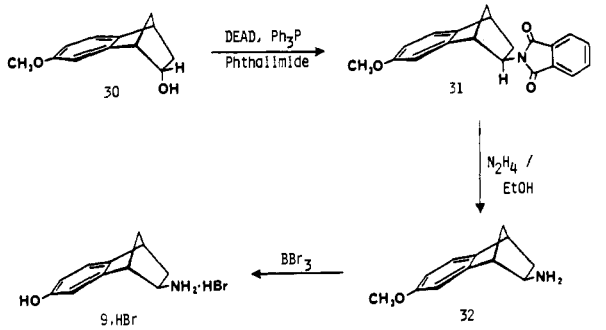
Scheme IV



Scheme V



Scheme VI



Initial efforts to prepare phenolic amines **8** and **9** involved a synthetic strategy similar to that shown in Scheme II for the synthesis of **7** and **10**. Unfortunately, azidomercuration/demercuration of 6-methoxybenzonorbornadiene, prepared according to the method of Tanida et al.,[32] followed by reduction, afforded a mixture of amines that proved inseparable by conventional means. Attempts at separating various derivatized mixtures of these amines also proved fruitless. Amines **8** and **9** were ultimately synthesized by a regioselective route that employed methodology for the stereoselective conversion of alcohols to amines[44] in a manner previously used by us for similar benzonorbornene systems.[45]

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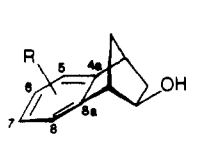
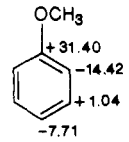
(45) Grunewald, G. L.; Paradkar, V. M.; Pazhenchevsky, B.; Pleiss, M. A.; Sall, D. J.; Seibel, W. L.; Reitz, T. J. *J. Org. Chem.* **1983**, *48*, 2321.

Table II. ^{13}C Chemical Shifts[a,b] of the Aromatic Carbons of 6-Methoxy- and 7-Methoxybenzonorbornen-2(*exo*)-ol



33



20[c]

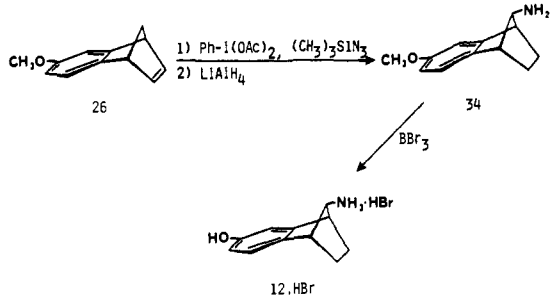
	C-4a		C-5		C-6		C-7		C-8		C-8a	
carbon compd no.	calcd	obsd	calcd	obsd	calcd	obsd	calcd	obsd	calcd	obsd	calcd	obsd
33, R = H[d]		149.42		120.61		126.08		125.50		121.96		144.53
23, R = 6-OMe	150.46	151.08	106.19	107.93	157.48	158.31	111.08	109.75	123.00	122.48	136.82	136.59
27, R = 7-OMe	141.71	141.48	121.65	120.94	111.66	110.04	156.90	157.67	107.54	109.08	145.57	146.01

[a] Relative to $CDCl_3$ (77.00 ppm). [b] The calculated chemical shifts were determined by adding the S_x values (S = the SCS relative to benzene and x = the ipso, ortho, meta, or para carbon) of methoxybenzene (**20**) to the observed chemical shift value of the appropriate carbon in **33**. [c] S_x values taken from ref 38. [d] Chemical shift values taken from ref 37.

Treatment of alcohol **23** (Scheme IV), conveniently prepared through selective solvolysis of a mixture of *exo*-2-chloro-6-methoxy- and *exo*-2-chloro-7-methoxybenzonorbornene according to the method of Tanida et al.,[46] with diethyl azidodicarboxylate (DEAD), triphenylphosphine, and phthalimide (Mitsunobu conditions)[44] afforded *exo*-phthalimide **24**. The aryl participation at carbon C-2 in **23** favors an S_N1 reaction pathway (non-Mitsunobu) in which the phthalimide approaches from the less hindered exo face.[45] Hydrazinolysis of **24** afforded methoxy amine **25**, which upon treatment with boron tribromide gave the hydrobromide salt of phenolic amine **8**.

The synthetic pathway leading to phenolic amine **9** is illustrated in Schemes V and VI. The low yield of requisite alcohol **27** (Scheme V), which was afforded through the solvolysis route employed by Tanida et al.[46] for the synthesis of **23**, necessitated modified methodology for obtaining **27**. Diene **26**[32] was subjected to hydroboration/oxidation conditions to afford a 1:1 mixture (based on ^{13}C NMR) of exo alcohols **23** and **27** (Scheme V). Employing the aryl participation at carbon C-2, as described by Tanida et al.,[46] alcohol **23** was selectively solvolyzed to the chloro derivative **28**, which could be conveniently removed by chromatography. After repeated solvolysis, **27** was obtained in 98% purity (2% of **23**) as determined by ^{13}C NMR spectrometry. Not surprisingly, subjecting **27** to the Mitsunobu conditions described above produced no reaction. The lack of aryl participation at carbon C-2 in **27** (as compared to **23**) favors an S_N2 reaction pathway in which the phthalimide would have to approach from the more hindered endo face. For this reason, **27** was converted to its endo isomer, **30**,[46] by Oppenauer oxidation followed by borane reduction. The resulting stereochemistry of the borane reduction (as determined by ^1H NMR spectrometry) is consistent with previous results in similar systems.[45,47,48] As expected, subjecting **30** to Mitsunobu conditions afforded *exo*-phthalimide **31** (Scheme VI). Subjecting **31** to hydrazinolysis followed by treatment with boron tribromide gave the hydrobromide salt of phenolic amine **9**. The minor amount of alcohol **23** that was present in **27** (Scheme V) was also carried through the above reaction sequence and was ultimately removed from **9**·HBr by recrystallization. In this way, pure **9**·HBr (no **8**·HBr was detectable by ^1H and ^{13}C NMR spectrometry) was obtained for biological testing.

Scheme VII



Structural differentiation of the regioisomeric alcohols **23** and **27** was confirmed by examination of their ^{13}C NMR spectra. Additivity rules[36] employing the ^{13}C chemical shifts of *exo*-2-hydroxybenzonorbornene, **33**,[37] and the SCS values for methoxybenzene, **20**[38] (as described above), for the assignment of the aromatic carbons gave calculated chemical shifts that are in good agreement with the actual values (Table II). Most notably, a significant difference in the chemical shifts of carbons C-4a and C-8a exists between the two regioisomers. Examination of this region of the spectrum allowed the purity of **23** and **27** after solvolysis, as well as the ratio of **23** and **27** from hydroboration/oxidation of diene **26**, to be estimated. Finally, structural verification of the regioisomeric amines **25** and **32** followed from additivity rules as described above and as shown in Table I.

Phenolic amine **12** (Scheme VII) was obtained from diene **26**,[32] through amine **34**, by conditions analogous to those used in the preparation of **11**.

Biochemistry. Amines **7–12** were evaluated as their hydrobromide salts for activity as both substrates and inhibitors. In vitro PNMT activity was assessed by use of a standard radiochemical assay that has been previously described for both substrates[49] and inhibitors.[21] Bovine adrenal PNMT, which had been purified according to Connett and Kirshner[50] through the isoelectric precipitation step, was used. For the determination of the kinetic constants for substrates, at least six concentrations of the variable substrate were assayed. Inhibition constants in this study were determined by using at least three different concentrations of inhibitor, as previously described,[21] with phenylethanolamine as the variable substrate.

Results and Discussion

Conformationally defined analogues **7–12** were examined in vitro for PNMT activity as both substrates and inhib-

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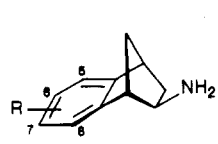
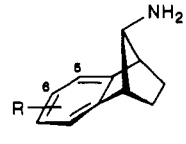
Table III. In Vitro PNMT Activity of the Aromatic-Substituted, Conformationally Defined β-Phenylethylamines 4–12



4, R = H
6, R = 6,7-$(OH)_2$
7, R = 5-OH
8, R = 6-OH
9, R = 7-OH
10, R = 8-OH



5, R = H
11, R = 5-OH
12, R = 6-OH

	(K_i ± SEM), μM	(K_m ± SEM), μM	V_{max}[a]
4	479 ± 27[b]	192 ± 35[c]	0.08[c]
5	258 ± 27[b]	81 ± 20[c]	0.61[c]
6	487 ± 82[c]	686 ± 211[c]	0.44[c]
7	10000		
8	304 ± 15		
9	1114 ± 118		
10	3000		
11	4000		
12	108 ± 5		

[a] Units of V_{max}: nanomoles of product formed per milligram of protein per minute. [b] Taken from ref 21. [c] Taken from ref 23.

itors. The results are shown in Table III. Up to concentrations of 2 mM none of the six analogues demonstrated any detectable activity as a substrate. On the other hand, amines **7–12** displayed varying degrees of competitive inhibition, with the *exo*-2-amino-6-hydroxybenzonorbornene (**8**; K_i = 304 μM) and its *anti*-9-amino-6-hydroxy isomer (**12**; K_i = 108 μM) being the only compounds showing significant potency as inhibitors. The other isomers displayed only weak activity (K_i = 1–10 mM). The fact that this series of analogues displays competitive kinetics denotes binding to the same site to which phenylethanolamine binds.

It is significant that the hydroxy-substituted analogues **8** and **12** show enhanced activity as inhibitors with respect to their parent compounds **4** (K_i = 479 μM) and **5** (K_i = 258 μM). The phenolic hydroxyl group in **8** and **12** thus produces a positive binding contribution to PNMT. This enhanced affinity might be due to the presence of a hydrophilic site within the aromatic ring binding region of the active site of PNMT with which the hydroxyl group can interact in a positive way. Further inspection of the data in Table III also reveals that phenolic amines **7** and **9–11** are weaker inhibitors than the unsubstituted parent analogues **4** and **5**. This decreased activity suggests a negative binding interaction of the aromatic hydroxyl at the active site of PNMT. Taking into account the sharp drop in activity that results upon substitution of the hydroxyl at any carbon other than C-6, the para position with respect to the ethylamine side chain, it appears that the complementary hydrophilic site is spatially compact and is surrounded by larger regions of lipophilic character.

Evidence for the overall lipophilic character of the aromatic ring binding region within the active site of PNMT is abundant. Fuller et al.[24] have shown that amphetamine analogues possessing electron-withdrawing, lipophilic substituents in the meta and/or para positions show enhanced activity as inhibitors with respect to amphetamine itself. Similar findings have also been reported for the benzylamine[25,27] and phenylethanolamine[24,25] classes of PNMT ligands. We have further shown, through the use of conformationally defined analogues similar to **7–10**, but with the hydroxyl group replaced by a lipophilic and electron-withdrawing CF_3 group, that a large lipophilic pocket exists in the aromatic ring binding region of PNMT.[22] In this regard, the poor activity as inhibitors of conformationally defined analogues **7** and **9–11** is easily understood. Substitution of a hydroxyl group at any point on the aromatic ring other than at carbon C-6 (as in **7** and **9–11**) would result in an arrangement in which the hydroxyl group cannot fit in or interact with the compact hydrophilic site. Instead, the hydroxyl group would lie close to or in the lipophilic pocket, resulting in a negative binding interaction. This would also explain our previous result that the catechol analogue **6** has the same potency as an inhibitor as does the unsubstituted derivative **4** (Table III). In the case of **6**, the positive interaction of the 6-OH group is cancelled out by the negative interaction of the 7-OH functionality so that the overall contribution to active site binding is negligible.

In addition to showing the presence of a spatially compact hydrophilic pocket within the aromatic ring binding region of the enzyme, the use of conformationally defined systems such as **7–12** also allows the determination of the directionality of this hydrophilic pocket with respect to the point of amine binding. Using the SYBYL molecular graphics package,[51] we have generated Figure 2, which represents that area (webbed), with respect to the aromatic ring binding region and point of amine interaction, in which substitution of a hydroxyl group leads to maximal interaction of the aromatic hydroxyl group, the aromatic ring, and the amino nitrogen of these types of molecules at the active site of the enzyme.

We have previously shown that a fully extended side chain conformation (τ_2 = 180°) of β-phenylethylamines is required for optimal interaction at the active site of PNMT.[21] The fact that conformationally defined analogue **12** is a more potent inhibitor than **8** adds further support to our proposal that greater interaction also results when a relatively coplanar arrangement exists between the aromatic ring and the amine nitrogen (low τ_1 value).

Given the positive interaction of a 6-hydroxy substituent (and the negative interaction of a 7-hydroxy group) and the activity of the catechol **6** as a substrate, it is surprising that phenols **8** and **12** are not also substrates. Apparently, **8** and **12** are not binding in an orientation that allows the methyl donor, AdoMet, to approach close enough to allow transfer of the active methyl group to the amino nitrogen. The difference between the pair (**8**, **12**) and **6** is that **6** has the additional 7-hydroxy substituent. It is possible that the negative interaction of the 7-OH (i.e., with the lipophilic regions in the aromatic ring binding region of the active site) displaces the aromatic ring into a slightly different binding orientation. Since this system is conformationally fixed, this displacement would necessarily translate to the bicyclic portion of the molecule. In this way the amino nitrogen could be shifted into the region of the active site where AdoMet can approach close enough to allow methylation to occur. It would then be a combination of interactions of the catechol in **6** (both positive and negative) that are required to orient the molecule in a manner that results in activity as a substrate. This combination is not possible for **8** or **12**.

The intolerance of an electron-releasing, hydrophilic hydroxyl at any position other than carbon C-6 is surprising in light of the fact that the natural substrate for PNMT, norepinephrine (**1**), contains a hydrophilic catechol

(51) SYBYL Molecular Modeling System Manual (Tripos Associates, Inc., St. Louis, MO), 1985. For a discussion of the use of unions of volumes in the development of the active analogue approach see: Marshall, G. R.; Barry, G. D.; Bosshard, H. E.; Dammkoehler, R. A.; Dunn, D. A. *ACS Symp. Ser.* **1979**, *112*, 205.

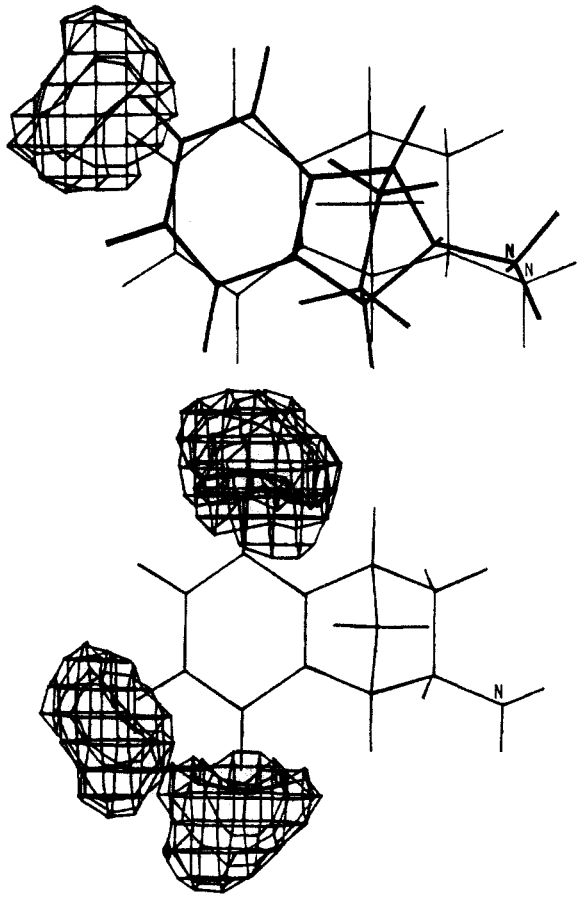


Figure 2. Representation of the hydrophilic binding region surrounding the aromatic ring of bound phenylethylamines at the active site of PNMT. The SYBYL molecular graphics system was used to generate these figures. The six molecules **7–12** were overlayed by a least-squares fit of the center of the aromatic rings, the amino nitrogens, and the carbons to which the nitrogen is attached (using the FIT command of SYBYL). The top figure shows the union of the volumes of **8** and **12** subtracted from the union of the volumes for the four inactive isomers (**7**, **9–11**) using the MVOLUME command. To orient the reader, the structures of *exo*-2-aminobenzonorbornene (**4**, light lines) and *anti*-9-aminobenzonorbornene (**5**, dark lines) are shown. The webbed area represents the volume occupied by the hydroxyl group of the two active phenols **8** and **12**. To prepare this volume representation, the hydroxyl group was allowed to rotate freely to cover all possible space. This volume thus represents the hydrophilic pocket where a positive binding interaction is achieved by the presence of a phenolic hydroxyl group. This would be position C-6 of the active analogues **8** and **12**. Binding in this orientation would result in a combination of three positive interactions: phenolic hydroxyl, aromatic ring, and amino nitrogen. The webbed area (bottom) shows those areas of hydrophilic intolerance in binding. This figure was generated by subtracting the combined volume of the inactive analogues **7** and **9–11** from the combined volume of the active phenols **8** and **12**. Placement of a phenolic hydroxyl group on the aromatic ring so that it lies in one of these regions (positions C-5, C-7, C-8) results in a negative interaction consistent with the low activity (or essential inactivity) as an inhibitor of analogues **7** and **9–11**. For orientation reference, the molecular structure of *exo*-2-aminobenzonorbornene (**4**) is drawn in the figure. The catechol analogue **6** has about the same activity as an inhibitor as does the phenylethylamine parent **4**; this is explained by a positive interaction with the phenolic hydroxyl at C-6 offset by a negative interaction with the phenolic hydroxyl at C-7.

moiety. Phenols **7–12** were synthesized to determine which of the orientations of the aromatic hydroxyl groups in the natural substrate norepinephrine leads to optimal binding. It was expected that substitution of aromatic hydroxyl groups into parent structures **4** and **5** would result in a positive interaction at the aromatic ring binding region used by the catechol of norepinephrine. Increased activity over that observed for substrates **4** and **5** was, therefore, expected. The fact that none of the analogues tested was a substrate indicates that these analogues are not binding in the same region or orientation as the natural substrate, norepinephrine (**1**). This would suggest that NE has a different active site binding orientation than most known PNMT substrates and competitive inhibitors. Two explanations can account for this difference in binding orientation. First, the catechol ring of NE may occupy a unique binding region of the active site different from that of *all* other substrates and competitive inhibitors of PNMT. This would be consistent with the large lipophilic (and minimal hydrophilic) character that has been proposed for the aromatic ring binding region at which substrates, other than norepinephrine, and all competitive inhibitors interact. Alternatively, norepinephrine and the other classes of PNMT ligands may interact at the same aromatic ring binding region, and the lack of activity as a substrate for the phenols of this study may result from conformational differences. The hydroxy-substituted, conformationally defined β-phenylethylamines **7–12** may not, in fact, mimic the binding conformation of norepinephrine, which allows simultaneous interaction of the catechol, β-hydroxy, and amino nitrogen at the active site of the enzyme. Further studies will be needed to determine the correct explanation.

In summary, we have synthesized and evaluated a series of conformationally defined tyramine analogues in order to determine the orientation of the aromatic hydroxyl groups in bound substrates, such as norepinephrine, that leads to optimal binding. We have found that there is relatively little hydrophilic character (only a small hydrophilic pocket as part of, or surrounded by, a larger lipophilic pocket) in the aromatic ring binding region of the active site of PNMT to which most known PNMT ligands bind. In addition, this small hydrophilic pocket has a finite directionality with respect to the point of amine interaction in bound β-phenylethylamines. Further, it appears that this active site preferentially binds molecules that have a more coplanar arrangement between the aromatic ring and the amine nitrogen. From the findings of this study, it does not appear that norepinephrine binds in the same manner as do other known PNMT ligands (phenylethanolamines, amphetamines, and benzylamines). Studies are currently under way to characterize the optimal orientation of norepinephrine for binding to the active site of PNMT.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus calibrated with known compounds and are uncorrected. Proton nuclear magnetic resonance spectra (^{1}H NMR) were obtained on either a Varian EM-360, FT-80A, or XL-300 spectrometer using deuterated chloroform ($CDCl_3$) as the solvent, and chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (Me_4Si; 0.0 ppm). Carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded on either a Varian FT-80A or XL-300 spectrometer using $CDCl_3$ as the solvent, and chemical shifts are reported in parts per million relative to $CDCl_3$ (77.0 ppm). For the hydrobromide salts of the phenolic amines, NMR spectra were taken in deuterated dimethyl sulfoxide (Me_2SO-d_6), in which case chemical shifts are reported relative to Me_2SO (2.50 ppm for ^{1}H and 39.51 ppm for ^{13}C). Infrared spectra (IR) were recorded on either an IBM FT-IR 32 or a Perkin-Elmer IR-727 spectrometer. Electron impact mass spectra (EIMS) were obtained on a Varian Atlas CH-5 mass spectrometer. Combustion analyses were performed on a Hewlett-Packard Model 185B CHN Analyzer at the University of Kansas or by Midwest Microlab Ltd. (Indianapolis, IN) and were

within 0.4% of the calculated values. Preparative centrifugal thin-layer chromatography (PCTLC) was performed on a Harrison Model 7924 Chromatotron using Merck silica gel 60 PF254 containing $CaSO_4 \cdot 0.5H_2O$ binder. Medium-pressure liquid chromatography (MPLC), using an adaptation of the apparatus of Meyers and co-workers,[52] and flash chromatography[53] were performed by using Merck silica gel 60 (230–400 mesh). Bulb-to-bulb distillations were carried out using a Kugelrohr distillation apparatus (Aldrich Chemical Co.).

S-Adenosyl-L-methionine was obtained from Sigma Chemical Co. [*methyl*-^{3}H]-*S*-Adenosyl-L-methionine that was used in the radiochemical assays 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 phosphorous 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. Where appropriate, amine hydrochloride salts were prepared by passing anhydrous HCl gas over a dry ethereal solution of the free base. All reactions requiring anhydrous conditions and/or an inert atmosphere were performed under a positive N_2 or Ar flow, and all glassware was oven-dried and/or flame-dried.

3-Fluoro-2-iodoanisole (14). To a solution of 170 mL of *n*-BuLi in hexane (23.9 mmol) in 400 mL of anhydrous THF at −70 °C was slowly added 30.1 g (23.8 mmol) of 3-fluoroanisole (**13**) in 100 mL of dry THF. The reaction was stirred for 10 min, whereupon a solution of 70.0 g of I_2 (27.5 mmol) in 100 mL of dry THF was added dropwise. The reaction was stirred at −70 °C for an additional 10 min then allowed to warm slowly. At −60 °C, 20 mL of H_2O was carefully added followed by 10 mL of 10% aqueous $Na_2S_2O_3$. After warming to room temperature, the organic and aqueous layers were separated and the latter extracted with hexane (2 × 100 mL). The combined organic layers were washed with 10% aqueous $Na_2S_2O_3$ (100 mL) and H_2O (2 × 100 mL) and dried over Na_2SO_4. Evaporation of the solvents in vacuo afforded 62.9 g of an orange oil, which was fractionally distilled. The fraction that distilled at 65–75 °C (0.1 mm) was recrystallized at 0 °C from hexane to afford 44.7 g (17.7 mmol, 74%) of **14** as a white solid that melted to a clear oil at room temperature: bp 65–67 °C (0.1 mm) [lit.[31] bp 240 °C (760 mm)]; ^{1}H NMR ($CDCl_3$) δ 7.43–7.07 (m, 1 H, ArH), 6.87–6.53 (m, 2 H, ArH), 3.90 (s, 3 H, OCH_3); IR (film) 3110, 3040, 2970, 2850, 1595, 1470, 1435, 1300, 1280, 1240, 1070, 1020, 760 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 164.17, 160.94, 159.69, 130.04, 108.23, 106.33, 56.64; EIMS, *m/e* (relative intensity) 253 (8.0), 252 (100, M$^+$), 237 (26.3), 209 (17.1), 110 (13.6). Anal. (C_7H_6FIO) C, H.

5-Methoxybenzonorbornadiene (15). Diene **15** was prepared by the general procedure used by Tanida et al.[32] for the preparation of related benzonorbornadienes. To 2.43 g (100 mmol) of Mg turnings in a flame-dried flask was added, under N_2, 15 mL of a solution containing 25.2 g (100 mmol) of **14** and 11.2 g (170 mmol) of freshly cracked cyclopentadiene in 200 mL of dry THF. The contents of the flask were gently heated to initiate the reaction, and the remainder of the solution was added at such a rate so as to maintain a gentle reflux. The resulting mixture was heated at reflux for an additional 0.5 h, cooled to room temperature, then treated with 200 mL of saturated aqueous NH_4Cl solution. The reaction was diluted with 400 mL of Et_2O, and the organic and aqueous layers were separated. The aqueous layer was extracted with Et_2O (100 mL). The organic pool was washed with saturated aqueous NH_4Cl solution (100 mL), H_2O (100 mL), 10% aqueous $Na_2S_2O_3$ (2 × 200 mL), and finally H_2O (2 × 100 mL). After drying over Na_2SO_4, evaporation of the solvent in vacuo gave a reddish brown oil, which after bulb-to-bulb distillation (82–85 °C (0.2 mm)) and MPLC using 2% ethyl acetate in hexane as the eluent gave 6.53 g (39%) of **15** as a clear oil. Spectral data (IR, ^{1}H NMR) for **15** were consistent with that reported by Paquette et al.[29]

***exo*-2-Formamido-5-methoxybenzonorbornene (18) and *exo*-2-Formamido-8-methoxybenzonorbornene (19).** To a solution of 5.3 g (80 mmol) of NaN_3 in 50% aqueous THF at 50 °C was added 9.6 g (30 mmol) of mercuric acetate. After complete dissolution, 5.16 g (30 mmol) of olefin **15** in 30 mL of THF was carefully added followed by 30 mL of H_2O. The reaction was stirred at 50 °C for 23 h. After cooling, 30 mL of 15% aqueous KOH was added followed by 0.6 g of $NaBH_4$ in 30 mL of 15% aqueous KOH. After stirring for 10 min, the reaction was cooled to 0 °C and 15.0 g of NaCl was added. The mixture was extracted with Et_2O (3 × 100 mL), and the combined organic pool was washed with water (100 mL) and brine (100 mL). After drying over Na_2SO_4, the azide solution was filtered and used immediately in the next reaction.

The azide–ether solution was carefully dripped into a slurry of 10 g of $LiAlH_4$ in 200 mL of dry Et_2O at 0 °C. The reaction was heated at reflux for 1 h, allowed to stir at room temperature for 18 h, and finally quenched by the sequential addition of 10 mL of H_2O, 10 mL of 15% aqueous NaOH, and 30 mL of H_2O. The reaction was filtered, and the filtrate was washed well with Et_2O and CH_2Cl_2. The organic phase was separated and extracted with 3 N aqueous HCl (4 × 50 mL). The acidic pool was basified with solid KOH and extracted with Et_2O (3 × 100 mL). The combined ether extracts were washed with H_2O (50 mL) and brine (50 mL) and dried over K_2CO_3. Filtration and evaporation of the solvent gave 5.02 g of a yellow oil, which after bulb-to-bulb distillation (92–94 °C (0.2 mm)) afforded 4.12 g (21.8 mmol, 73% from **15**) of a clear oil that was shown by ^{13}C NMR to be a 1:2 mixture of amines **16** and **17**. This amine mixture was not further characterized but was converted directly to the corresponding formamide mixture for separation.

The amine mixture (21.8 mmol) and 17.0 mL of ethyl formate were heated in a Wheaton pressure bottle at 95 °C for 10 h. After cooling, the reaction mixture was evaporated in vacuo to afford 4.94 g of a mixture of formamides **18** and **19**. Separation of the formamides was achieved by fractional recrystallization from hot ethyl acetate/hexane (2:1) to give 1.45 g of **18** and 3.20 g of **19** (98% from the amine mixture). In other runs, up to 5% of the 9-*anti*- isomer **21** was obtained by MPLC of the mother liquors remaining after recrystallization of **18** and **19**.

***exo*-2-Formamido-5-methoxybenzonorbornene (18)** (1.45 g, 6.67 mmol, 22% from **15**) was obtained as hard white needles: mp 133.5–134.5 °C; ^{1}H NMR ($CDCl_3$) δ 8.16 (s, 1 H, CHO), 7.23–6.58 (m, 3 H, ArH), 5.95–5.55 (br, 1 H, NH), 3.80 (s, 3 H, OCH_3), 3.73–3.50 (m, 1 H, bridgehead), 3.38–3.20 (m, 1 H, bridgehead), 2.05–1.30 (m, 5 H, C3 and C9 methylenes and CHN); IR (KBr) 3240, 3042, 2984, 1682, 1651, 1483, 1257, 1070, 741 cm^{-1}; EIMS, *m/e* (relative intensity) 217 (4.5, M$^+$), 172 (3.1), 146 (100), 131 (21.9), 115 (15.9), 103 (20.1). Anal. ($C_{13}H_{15}NO_2$) C, H, N.

***exo*-2-Formamido-8-methoxybenzonorbornene (19)** (3.20 g, 14.7 mmol, 49% from **15**) was obtained as fluffy white flakes: mp 157–157.5 °C; ^{1}H NMR ($CDCl_3$) δ 8.11 (s, 1 H, CHO), 7.24–6.55 (m, 3 H, ArH), 6.35–5.85 (br, 1 H, NH), 3.75 (s, 3 H, OCH_3), 3.60–3.30 (m, 2 H, bridgeheads), 2.10–1.40 (m, 5 H, methylenes and CHN); IR (KBr) 3296, 3052, 2974, 1674, 1651, 1539, 1483, 1263, 1045, 736 cm^{-1}; EIMS, *m/e* (relative intensity) 217 (6.1, M$^+$), 172 (5.7), 146 (100), 131 (24.3), 115 (23.9), 103 (28.2). Anal. ($C_{13}H_{15}NO_2$) C, H, N.

***anti*-9-Formamido-5-methoxybenzonorbornene (21).** Synthesis of the intermediate *anti*-9-amino-5-methoxybenzonorbornene (**22**) was modeled after the procedure described by Ehreundfreud and Zbiral.[43] To a solution of **15** (5.74 g, 33.0 mmol) and iodobenzene diacetate (21.5 g, 67.0 mmol) in 500 mL of dry CH_2Cl_2 at −20 °C was slowly added 15.3 g (134 mmol) of azidotrimethylsilane in 500 mL of dry CH_2Cl_2. The mixture was stirred at −30 to −20 °C for 12 h and was then allowed to warm to room temperature over 14 h. The reaction was poured into 1 L of H_2O, and the layers were separated. The organic layer was washed with H_2O (2 × 200 mL) and saturated aqueous $NaHCO_3$ (3 × 50 mL) and dried over Na_2SO_4. Evaporation of the solvent in vacuo gave 34.0 g of crude azide as a light-yellow oil, which was used immediately in the next reaction.

The crude azide mixture in 200 mL of dry Et_2O was dripped into a slurry of 10.5 g of $LiAlH_4$ in 450 mL of dry Et_2O at 0 °C.

(52) Meyers, A. I.; Slade, J.; Smith, R. K.; Mihelich, E. D.; Hershenson, F. M.; Liang, C. D. *J. Org. Chem.* **1979**, *44*, 2247.
(53) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

The reaction was stirred at room temperature for 18 h then quenched by the sequential addition of 12 mL of H_2O, 12 mL of 20% aqueous NaOH, and 45 mL of H_2O. The reaction was filtered, and the residue was washed well with Et_2O. The organic and aqueous layers were separated, and the latter was extracted with ether (3 × 50 mL). The organic pool was extracted with 3 N HCl (5 × 40 mL), and the combined acidic phases were basified with KOH pellets and extracted with Et_2O (5 × 40 mL). The ether layer was dried over K_2CO_3 and after filtration was evaporated in vacuo to give 2.8 g of a brown oil, which was distilled bulb-to-bulb (102 °C (0.5 mm)) to afford 1.82 g of a clear oil that was primarily amine **22** as shown by thin-layer chromatography (95% $CHCl_3$: 5% MeOH saturated with NH_3; R_f = 0.3 on 1- × 3-in. Whatman plates coated with a 200-μm thickness of silica gel). For purification, the crude amine was converted directly to the formamide according to the procedure described for **18** and **19**. Recrystallization from ethyl acetate/hexane afforded 1.05 g (6.9 mmol, 72%) of pure **21** as white needles: mp 100–101 °C; ^1H NMR ($CDCl_3$) δ 8.10 (s, 1 H, CHO), 7.20–6.58 (m, 3 H, ArH), 6.05–5.65 (br, 1 H, NH), 3.90–3.63 (m, 4 H, CHN and OCH_3), 3.58–3.35 (m, 1 H, bridgehead), 3.33–3.10 (m, 1 H, bridgehead), 2.15–1.70 (m, 2 H, methylene), 1.30–1.20 (m, 2 H, methylene); IR (KBr) 3270, 3059, 2968, 1670, 1589, 1485, 1265, 1091, 750 cm^{-1}; EIMS, *m/e* (relative intensity) 217 (12.1, M^+), 172 (44.5), 159 (100), 157 (44.7), 144 (29.3), 129 (18.5), 115 (21.9). Anal. ($C_{13}H_{15}NO_2$) C, H, N.

General Procedure for the Hydrolysis of Formamides 18, 19, and 21 to Produce Amines 16, 17, and 22. A mixture of the formamide (300 mg, 1.38 mmol) and 30 mL of 10% aqueous HCl was heated at reflux for 4 h. The reaction was evaporated under reduced pressure. Anhydrous EtOH was added, and any remaining water was removed by azeotropic distillation. The resulting amine hydrochloride was obtained quantitatively as a white solid, a fraction of which was converted to the free base for spectral characterization.

***exo*-2-Amino-5-methoxybenzonorbornene (16)**: ^1H NMR ($CDCl_3$) δ 7.12–6.45 (m, 3 H, ArH), 3.70 (s, 3 H, OCH_3), 3.50–3.30 (m, 1 H, bridgehead), 2.98–2.75 (m, 2 H, CHN and bridgehead), 1.90–1.10 (m, 4 H, C3 and C9 methylenes), 1.32 (s, 2 H, exchangeable in D_2O, NH_2); IR (film) 3362, 3293, 3063, 2959, 1610, 1589, 1481, 1464, 1296, 1263, 1066, 980, 787, 750 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 153.10 (C-5), 148.66 (C-8a), 135.35 (C-4a), 126.67 (C-7), 114.19 (C-8), 109.15 (C-6), 55.39, 53.61, 53.32, 45.21, 39.38 (C-4), 38.99. **16**·HCl (recrystallized from MeOH/Et_2O): mp 254 °C dec; EIMS, *m/e* (relative intensity) 189 (10.0, M^+), 172 (1.6), 147 (12.5), 146 (100), 131 (27.8), 115 (19.8), 103 (24.8). Anal. ($C_{12}H_{15}NO$·HCl) C, H, N.

***exo*-2-Amino-8-methoxybenzonorbornene (17)**: ^1H NMR ($CDCl_3$) δ 7.17–6.53 (m, 3 H, ArH), 3.80 (s, 3 H, OCH_3), 3.37–3.13 (m, 2 H, bridgeheads), 3.10–2.90 (m, 1 H, CHN), 2.10–1.13 (m, 4 H, C3 and C9 methylenes), 1.41 (s, 2 H, exchangeable in D_2O, NH_2); IR (film) 3364, 3297, 3063, 2967, 1610, 1589, 1481, 1464, 1377, 1304, 1263, 1061, 789, 744 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 153.30 (C-8), 150.73 (C-4a), 132.86 (C-8a), 126.85 (C-6), 113.61 (C-5), 108.38 (C-7), 55.13, 52.72, 48.61 (C-1), 45.00, 43.50, 39.11. **17**·HCl (recrystallized from 95% EtOH/Et_2O): mp 236–237 °C; EIMS, *m/e* (relative intensity) 189 (14.2, M^+), 147 (19.2), 146 (100), 131 (57.3), 115 (27.0), 103 (34.1). Anal. ($C_{12}H_{15}NO$·HCl) C, H, N.

***anti*-9-Amino-5-methoxybenzonorbornene (22)**: ^1H NMR ($CDCl_3$) 7.00–6.45 (m, 3 H, ArH), 3.68 (s, 3 H, OCH_3), 3.23–3.00 (m, 1 H, bridgehead), 2.97–2.75 (m, 2 H, bridgehead and CHN), 2.09–1.75 (m, 2 H, methylene), 1.31–0.95 (m, 2 H, methylene), 1.21 (s, 2 H, exchangeable in D_2O, NH_2); IR (film) 3380, 3307, 2963, 1587, 1483, 1464, 1288, 1263, 1088, 752, 677 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 152.95 (C-5), 148.82, 133.87, 126.42, 113.80, 108.50, 67.59, 55.22, 48.38, 44.07, 23.29, 22.62. **22**·HCl (recrystallized from MeOH/Et_2O): mp 275 °C dec; EIMS, *m/e* (relative intensity) 189 (18.6, M^+), 172 (39.0), 160 (50.3), 159 (100), 158 (87.3), 144 (48.4), 115 (41). Anal. ($C_{12}H_{15}NO$·HCl) C, H, N.

***exo*-2-Phthalimido-6-methoxybenzonorbornene (24)**. Phthalimide **24** was prepared by using conditions analogous to those previously described.[44,45] To a solution of **23**[46] (1.1 g, 5.8 mmol), phthalimide (1.05 g, 7.14 mmol), and triphenylphosphine (1.82 g, 6.94 mmol) in 25 mL of anhydrous THF was added 1.1 mL (7.0 mmol) of diethyl azidodicarboxylate. The reaction was stirred at room temperature under N_2 for 24 h. Ten milliliters of brine was added to quench the reaction, and the organic and aqueous layers were separated. The aqueous layer was extracted with Et_2O (2 × 20 mL). The organic pool was washed with H_2O (10 mL) and brine (10 mL) and dried over Na_2SO_4. Filtration and evaporation of the solvent in vacuo gave 5.84 g of a viscous yellow oil, which was purified by PCTLC with methylene chloride as the eluent. Recrystallization from EtOH/Et_2O afforded 1.07 g (3.35 mmol, 58%) of pure **24**: mp 139.5–140.5 °C; ^1H NMR ($CDCl_3$) δ 7.81–7.55 (m, 4 H, phthalimide ArH), 7.16 (d, 1 H, *J* = 7.9 Hz, H8), 6.81 (d, 1 H, *J* = 2.3 Hz, H5), 6.61 (dd, 1 H, *J* = 7.9 and 2.3 Hz, H7), 4.19–4.00 (m, 1 H, CHN), 3.75 (s, 3 H, OCH_3), 3.53–3.30 (m, 2 H, bridgeheads), 2.85–2.49 (m, 2 H, methylene), 1.95–1.55 (m, 2 H, methylene); IR (KBr) 3015, 2976, 1772, 1711, 1589, 1478, 1466, 1443, 1412, 1373, 1360, 1308, 1267, 1230, 1128, 1097, 1072, 871, 720 cm^{-1}; EIMS, *m/e* (relative intensity) 319 (2.8, M^+), 172 (1.5), 159 (5.0), 146 (100), 131 (17.3), 115 (6.3), 103 (15.5). Anal. ($C_{20}H_{17}NO_3$) C, H, N.

***exo*-2-Amino-6-methoxybenzonorbornene (25)**. A mixture of phthalimide **24** (900 mg, 2.81 mmol) and hydrazine hydrate (600 mg, 11.9 mmol) in 40 mL of 95% EtOH was heated at reflux for 7 h. The reaction was evaporated in vacuo to an oily white solid, which was transferred to a separatory funnel with the aid of Et_2O (30 mL) and 3 N aqueous NaOH (30 mL). The two layers were separated and the aqueous layer extracted with Et_2O (5 × 20 mL). The combined organic pool was washed with H_2O (10 mL) and was extracted with 1 N aqueous HCl (5 × 20 mL). The acidic extracts were basified with solid KOH and extracted with Et_2O (5 × 20 mL). The combined organic extracts were dried over K_2CO_3, filtered, and evaporated in vacuo to yield 580 mg of an oil, which was distilled bulb-to-bulb (85 °C (0.35 mm)) to give 430 mg (2.27 mmol, 81%) of **25** as a clear oil: ^1H NMR ($CDCl_3$) δ 7.02 (d, 1 H, *J* = 7.9 Hz, H8), 6.72 (d, 1 H, *J* = 1.9 Hz, H5), 6.53 (dd, 1 H, *J* = 7.9 and 1.9 Hz, H7), 3.74 (s, 3 H, OCH_3), 3.30–2.71 (m, 3 H, bridgeheads and CHN), 2.05–1.10 (m, 4 H, C3 and C9 methylenes), 1.36 (br s, 2 H, exchangeable in D_2O, NH_2); IR (film) 3374, 3284, 2963, 1624, 1610, 1589, 1478, 1441, 1342, 1300, 1282, 1259, 1228, 1174, 1128, 1067, 1030, 970, 866, 804 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 158.2 (C-6), 150.14 (C-4a), 138.63 (C-8a), 121.63 (C-8), 109.77 (C-7), 107.89 (C-5), 55.20, 53.76, 52.10, 45.18, 43.76, 39.59. **25**·HCl (recrystallized from EtOH/Et_2O): mp 254 °C dec; EIMS, *m/e* (relative intensity) 189 (8.4, M^+), 146 (100), 131 (25.9), 115 (9.1), 103 (20.7). Anal. ($C_{12}H_{15}NO$·HCl) C, H, N.

***exo*-2-Hydroxy-7-methoxybenzonorbornene (27)**. A solution of 6-methoxybenzonorbornadiene[32] (**26**; 3.50 g, 20.3 mmol) in 75 mL of anhydrous THF was treated with 40 mL of 1 M borane in THF (40 mmol) and stirred at room temperature for 10 h. After cooling with an ice bath, 10 mL of H_2O was added followed by 30 mL of 3 N aqueous NaOH and 80 mL of 30% H_2O_2. The reaction was allowed to stir an additional 2 h, at which time it was extracted with Et_2O (5 × 40 mL). The combined ether extracts were washed with H_2O (2 × 20 mL) and brine (20 mL) and dried over Na_2SO_4. Filtration and evaporation of the solvent in vacuo afforded 4.6 g of an oil, which was purified by MPLC with hexane/ethyl acetate (4:1) as the eluent to afford 2.9 g (15.2 mmol, 75%) of a mixture of **23** and **27**. The mixture of alcohols was added to a solution of 20 mL of acetonitrile and 5.0 mL of concentrated HCl, and the resultant solution was heated at 50 °C for 12 h. After cooling, the reaction mixture was extracted with Et_2O (5 × 20 mL). The organic pool was dried over Na_2SO_4, filtered, and evaporated in vacuo to give 3.05 g of a yellow oil. Purification by MPLC with hexane/ethyl acetate (4:1) as the eluent afforded pure **28** and a mixture of alcohols enriched in isomer **27**. Recycling of the alcohol mixture through this solvolysis step an additional four times produced 825 mg of an oil, which by ^{13}C NMR was 98% enriched in **27**: bp 98 °C (0.1 mm) [lit.[46] bp 120 °C, (1.0 mm)]; IR (film) 3360 cm^{-1}.

2-Oxo-7-methoxybenzonorbornene (29). Ketone **29** was prepared by conditions analogous to those employed for benzonorbornene-2-one.[47] In this manner 200 mg (1.05 mmol) of alcohol **27** afforded 195 mg (1.04 mmol, 98%) of ketone **29**: bp 95 °C (0.5 mm) [lit.[46] bp 110 °C (1.0 mm)]; IR (film) 1746 cm^{-1}.

***endo*-2-Hydroxy-7-methoxybenzonorbornene (30)**. Reduction of ketone **29** to alcohol **30** was carried out following conditions similar to those used for the reduction of benzonorbornen-2-one[47] except that borane was used as the hydride source. Under these conditions 200 mg (1.06 mmol) of ketone **29** yielded 180 mg (0.95 mmol, 89%) of **30** as a white solid: mp 54.5–56 °C

[lit.[46] mp 63.0–63.5 °C]; IR (KBr) 3412 cm^{-1}.

***exo*-2-Phthalimido-7-methoxybenzonorbornene (31).** A solution of alcohol **30** (600 mg, 3.15 mmol), phthalimide (640 mg, 4.3 mmol), and triphenylphosphine (1.1 g, 4.2 mmol) in 25 mL of anhydrous THF was treated with 0.65 mL (4.12 mmol) of diethyl azidodicarboxylate.[44] The reaction was stirred at room temperature under Ar for 72 h. Thirty milliliters of brine was added, and the two layers were separated. The aqueous layer was washed with ether (4 × 20 mL). The combined organic pool was dried over Na_2SO_4, filtered, and evaporated in vacuo to give 2.55 g of a viscous yellow oil, which was purified by flash chromatography with CH_2Cl_2 as the eluent to give 850 mg (2.66 mmol, 84%) of **31** as a white solid: mp 133–134 °C; ^1H NMR ($CDCl_3$) δ 7.85–7.55 (m, 4 H, phthalimide ArH), 7.08 (d, 1 H, J = 8.0 Hz, H5), 6.88 (d, 1 H, J = 2.3 Hz, H8), 6.66 (dd, 1 H, J = 8.0 and 2.3 Hz, H6), 4.33–3.97 (m, 1 H, CHN), 3.77 (s, 3 H, OCH_3), 3.55–3.40 (m, 2 H, bridgeheads), 2.88–2.45 (m, 2 H, methylene), 2.10–1.45 (m, 2 H, methylene); IR (KBr) 2970, 1709, 1612, 1478, 1466, 1370, 1354, 1331, 1289, 1246, 1233, 1128, 1089, 1030, 719, 662 cm^{-1}; EIMS, m/e (relative intensity) 319 (3.8, M^+), 146 (100), 131 (20.4), 115 (7.0), 103 (16.5), 77 (8.6). Anal. ($C_{20}H_{17}NO_3$) C, H, N.

***exo*-2-Amino-7-methoxybenzonorbornadiene (32).** By use of analogous conditions to those employed in the conversion of phthalimide **24** to amine **25**, 850 mg (2.67 mmol) of phthalimide **31** gave 440 mg (2.32 mmol, 87%) of **32** as a clear oil: bp 86 °C (0.05 mm); ^1H NMR ($CDCl_3$) δ 7.00 (d, 1 H, J = 7.9 Hz, H5), 6.78 (d, 1 H, J = 2.4 Hz, H8), 6.55 (dd, 1 H, J = 7.9 and 2.4 Hz, H6), 3.75 (s, 3 H, OCH_3), 3.28–3.10 (m, 1 H, CHN), 3.05–2.85 (m, 2 H, bridgeheads), 1.95–1.20 (m, 4 H, methylenes), 1.40 (br s, 2 H, exchangeable in D_2O, NH_2); IR (film) 3370, 3298, 2959, 1610, 1589, 1477, 1433, 1344, 1286, 1246, 1230, 1172, 1126, 1086, 1028, 970, 868, 827, 770, 664 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 157.89 (C-7), 148.07 (C-8a), 140.76 (C-4a), 121.05 (C-5), 109.83 (C-6), 108.48 (C-8), 55.33, 53.52, 53.40, 45.36, 42.61, 38.99. **32**·HCl (recrystallized from $EtOH/Et_2O$): mp 249–250 °C dec; EIMS, m/e (relative intensity) 189 (10.8, M^+), 172 (2.2), 146 (100), 131 (30.7), 115 (11.2), 103 (23.3). Anal. ($C_{12}H_{15}NO$·HCl) C, H, N.

***anti*-9-Amino-6-methoxybenzonorbornene (34).** By employing conditions analogous to those used for the synthesis of **22**, diene **26** (4.1 g, 23.8 mmol) was converted to 657 mg (3.47 mmol, 16% from **26**) of amine **34**: ^1H NMR ($CDCl_3$) δ 7.03 (d, 1 H, J = 7.9 Hz, H8), 6.75 (d, 1 H, J = 2.2 Hz, H5), 6.60 (dd, 1 H, J = 7.9 and 2.2 Hz, H7), 3.75 (s, 3 H, OCH_3), 3.05–2.85 (m, 3 H, CHN and bridgeheads), 2.15–1.90 (m, 2 H, methylene), 1.38 (s, 2 H, exchangeable with D_2O, NH_2), 1.23–1.05 (m, 2 H, methylene); IR (film) 3380, 3314, 2959, 1612, 1586, 1479, 1292, 1261, 1242, 1109, 1036, 821, 603 cm^{-1}; ^{13}C NMR ($CDCl_3$) δ 157.91, 148.24, 139.20, 121.46, 110.19, 107.69, 67.65, 55.21, 48.71, 47.40, 23.67, 23.33. **34**·HCl (recrystallized from $EtOH/Et_2O$): mp 264–265 °C dec; EIMS, m/e (relative intensity) 189 (24.4, M^+), 172 (47.9), 160 (80.6), 159 (100), 158 (74.7), 144 (48.8), 129 (34.6), 128 (33.5), 115 (42.1), 91 (21.8). Anal. ($C_{12}H_{15}NO$·HCl) C, H, N.

General Procedure for the Preparation of Phenolic Amines 7–12. To a stirred solution of the respective formamide or free amine in dry CH_2Cl_2 (12.5 mL/mmol of starting methyl ether) under N_2 at −78 °C was added 2.5 equiv of BBr_3 (1 M in CH_2Cl_2). The mixture was allowed to gradually warm to room temperature and was then heated at reflux for 1 h. The reaction was cooled to −70 °C, and MeOH (5 mL/mmol of starting material) was carefully added. The cooling bath was removed and N_2 rapidly bubbled through the reaction for 0.5 h. The solution was concentrated in vacuo to $^1/_4$ volume and the residual liquid heated at reflux with MeOH (5 mL/mmol of starting methyl ether) for 15 min, then concentrated again to $^1/_4$ volume. The MeOH reflux procedure was repeated, and the solution was evaporated to a residue, which was dried under vacuum at 60–80 °C. The HBr salts of the phenolic amines were isolated as tan solids.

***exo*-2-Amino-5-hydroxybenzonorbornene Hydrobromide (7·HBr).** Formamide 18 (217 mg, 1.0 mmol) yielded 181 mg (0.71 mmol, 71%) of the amine hydrobromide **7**·HBr: mp 165 °C dec; ^1H NMR (Me_2SO-d_6) δ 9.26 (s, 1 H, OH), 8.07 (br s, 3 H, NH_3^+), 6.95–6.50 (m, 3 H, ArH), 3.70–3.25 (m, 2 H, bridgeheads), 3.23–2.75 (m, 1 H, CHN), 2.25–1.35 (m, 4 H, C3 and C9 methylene); IR (KBr) 3082 (br), 1974, 1594, 1480, 1454, 1381, 1298, 1279, 1034, 787, 754 cm^{-1}; ^{13}C NMR (Me_2SO-d_6) δ 150.51, 146.10, 132.44, 126.93, 114.42, 112.69, 51.84, 47.63, 44.92, 38.76, 34.18; EIMS, m/e (relative intensity) 175 (6.2, M^+), 158 (2.3), 132 (100), 131 (41.1), 115 (7.4), 103 (10.8). Anal. ($C_{11}H_{13}NO$·HBr) C, H, N.

***exo*-2-Amino-6-hydroxybenzonorbornene Hydrobromide (8·HBr).** Amine **25** (410 mg, 2.17 mmol) yielded 461 mg (1.80 mmol, 83%) of **8**·HBr (recrystallized from $EtOH/Et_2O$): mp 221–223 °C; ^1H NMR (Me_2SO-d_6) δ 9.16 (br s, 1 H, OH), 8.13 (br s, 3 H, NH_3^+), 7.05–6.39 (m, 3 H, ArH), 3.55–2.92 (m, 3 H, bridgeheads and CHN), 2.06–1.07 (m, 4 H, methylenes); IR (KBr) 3087 (br), 1990, 1595, 1495, 1356, 1218, 1129, 1031, 930, 814, 782 cm^{-1}; ^{13}C NMR (Me_2SO-d_6) δ 156.28, 149.24, 134.13, 122.25, 111.81, 109.00, 52.13, 46.42, 45.06, 42.96, 34.57; EIMS, m/e (relative intensity) 175 (6.0, M^+), 158 (14), 132 (100), 115 (8.4), 103 (13.3). Anal. ($C_{11}H_{13}NO$·HBr) C, H, N.

***exo*-2-Amino-7-hydroxybenzonorbornene Hydrobromide (9·HBr).** Amine **32** (210 mg, 1.11 mmol) afforded 275 mg (1.07 mmol, 97%) of **9**·HBr (recrystallized from $EtOH/Et_2O$): mp 204.5–206 °C; ^1H NMR (Me_2SO-d_6) δ 9.16 (br s, 1 H, OH), 8.16 (br s, 3 H, NH_3^+), 7.42–6.71 (m, 3 H, ArH), 3.56–3.20 (m, 2 H, bridgeheads), 3.18–3.00 (m, 1 H, CHN), 2.25–1.59 (m, 4 H, C3 and C9 methylenes); IR (KBr) 3362, 3026, 2978, 1988, 1595, 1489, 1448, 1348, 1197, 1124, 829 cm^{-1}; ^{13}C NMR (Me_2SO-d_6) δ 155.67, 145.47, 137.86, 121.49, 112.46, 109.62, 51.81, 47.58, 45.17, 41.93, 35.18; EIMS, m/e (relative intensity) 175 (7.5, M^+), 158 (2.3), 132 (100), 115 (4.8), 103 (6.2), 80 (7.4). Anal. ($C_{11}H_{13}NO$·HBr) C, H, N.

***exo*-2-Amino-8-hydroxybenzonorbornene Hydrobromide (10·HBr).** Formamide 19 (217 mg, 1.0 mmol) afforded 214 mg (0.84 mmol, 84%) of the amine hydrobromide **10**·HBr: mp starts to decompose slowly at 125 °C; ^1H NMR (Me_2SO-d_6) δ 9.33 (s, 1 H, OH), 8.05 (br s, 3 H, NH_3^+), 6.91–6.52 (m, 3 H, ArH), 3.65–3.50 (m, 1 H, bridgehead), 3.39–3.17 (m, 1 H, bridgehead), 3.04 (m, 1 H, CHN), 2.20–1.55 (m, 4 H, methylenes); IR (KBr) 3365, 3028, 2965, 1957, 1592, 1480, 1380, 1278, 1039, 745 cm^{-1}; ^{13}C NMR (Me_2SO-d_6) δ 151.06, 149.84, 128.67, 127.60, 113.64, 112.06, 51.28, 44.77, 43.23, 42.95, 34.60; EIMS, m/e (relative intensity) 175 (8.5, M^+), 158 (4.1), 132 (100), 131 (45.6), 115 (8.6), 103 (14.8). Anal. ($C_{11}H_{13}NO$·HBr) C, H, N.

***anti*-9-Amino-5-hydroxybenzonorbornene Hydrobromide (11·HBr).** Formamide 21 (217 mg, 1.0 mmol) yielded 219 mg (0.86 mmol, 86%) of the amine hydrobromide **11**·HBr: mp 221–224 °C dec; ^1H NMR (Me_2SO-d_6) δ 9.33 (br s, 1 H, OH), 8.06 (br s, 3 H, NH_3^+), 6.95–6.54 (m, 3 H, ArH), 3.60–2.95 (m, 3 H, bridgeheads and CHN), 2.25–1.80 (m, 2 H, methylene), 1.30–0.95 (m, 2 H, methylene); IR (KBr) 3356, 3026, 2976, 1591, 1483, 1458, 1298, 1207, 1153, 1115, 1095, 758 cm^{-1}; ^{13}C NMR (Me_2SO-d_6) δ 150.46, 146.12, 129.19, 127.46, 114.07, 112.16, 61.74, 44.85, 40.63, 22.84, 22.43; EIMS, m/e (relative intensity) 175 (26.1, M^+), 158 (54.6), 146 (62.5), 145 (83.6), 144 (100), 132 (49.7), 131 (41.1), 115 (45.3), 103 (14.4). Anal. ($C_{11}H_{13}NO$·HBr) C, H, N.

***anti*-9-Amino-6-hydroxybenzonorbornene Hydrobromide (12·HBr).** Amine **34** (420 mg, 2.22 mmol) yielded 462 mg (1.80 mmol, 81%) of **12**·HBr (recrystallized from $EtOH/Et_2O$): mp 270–272 °C; ^1H NMR (Me_2SO-d_6) δ 9.18 (br s, 1 H, OH), 8.07 (br s, 3 H, NH_3^+), 6.98–6.50 (m, 3 H, ArH), 3.67–3.00 (m, 3 H, bridgeheads and CHN), 2.23–1.94 (m, 2 H, methylene), 1.29–1.00 (m, 2 H, methylene); IR (KBr) 3324 (br), 2970, 1919, 1601, 1489, 1466, 1379, 1250, 1219, 1107, 1076, 833 cm^{-1}; ^{13}C NMR (Me_2SO-d_6) δ 156.18, 145.44, 134.47, 121.85, 112.69, 108.87, 61.77, 44.88, 43.75, 23.42, 22.91; EIMS, m/e (relative intensity) 175 (9.2, M^+), 158 (33.4), 147 (26.5), 146 (61.2), 145 (63.1), 144 (70.3), 115 (37.5), 91 (21.2), 80 (100). Anal. ($C_{11}H_{13}NO$·HBr) C, H, N.

Radiochemical Assay for PNMT Activity. The assay employed in this investigation has been described elsewhere.[21,49] Briefly, a typical assay mixture consisted of 50 μL of 0.5 M phosphate buffer (pH 8.0), 25 μL of a 10 μM solution of unlabeled AdoMet, 5 μL of [*methyl*-^{3}H]AdoMet, containing approximately 2 × 10^6 dpm (specific activity 15 mCi/mmol), 25 μL of substrate solution, 25 μL of inhibitor solution (if added), 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 quenched by the addition of 250 μL of 0.5 M borate (pH 10) buffer and extracted with toluene/isoamyl alcohol (7:3). The organic layer was removed and transferred to a scintillation vial and diluted with cocktail for counting. The mode of inhibition was ascertained by inspection of the $1/V$ vs. $1/S$ plot of the data.

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Synthesis and Adrenergic Activity of Ring-Fluorinated Phenylephrines

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2-Fluoro-, 4-fluoro-, and 6-fluorophenylephrine (6-FPE) were synthesized from the corresponding fluorinated 3-hydroxybenzaldehydes. New routes to 2-fluoro- and 6-fluoro-3-hydroxybenzaldehydes were developed based on regioselective lithiation of 2- and 4-[(dimethyl-*tert*-butylsilyl)oxy]fluorobenzene ortho to fluorine. As with norepinephrine and isoproterenol analogues, the adrenergic properties of phenylephrine were markedly altered by ring fluorination. The order of potency of the fluoro analogues as α_1-adrenergic agonists in the stimulation of contraction of aortic strips and of phosphatidylinositol turnover and potentiation of cyclic AMP accumulation in guinea pig synaptoneurosomes was 6-FPE > PE > 4-FPE > 2-FPE. The same pattern was observed for the displacement of radioligands specific for α_1- and α_2-adrenergic receptors on brain membranes. The order of potency for the displacement of [^{3}H]dihydroalprenolol, a β-specific adrenergic ligand from brain membranes, was 2-FPE > 4-FPE = PE ≫ 6-FPE. 6-FPE was much more selective for α-adrenergic receptors compared to β-receptors than was phenylephrine. A rationale for the observed fluorine-induced alterations in potency and selectivity of the FPEs for α- and β-adrenergic systems is presented based on fluorine-induced conformations due to electrostatic repulsion of fluorine and the benzyl hydroxyl group.

In 1979, we reported that ring-fluorinated analogues of norepinephrine display striking adrenergic agonist specificities that are dependent on the site of the fluorine substituent.[1] Thus, 2-fluoronorepinephrine (2-FNE) exhibits nearly pure β-adrenergic agonist properties, while 6-fluoronorepinephrine (6-FNE) exhibits predominantly α-adrenergic properties in several systems.[1-4] The adrenergic agonist properties of 5-fluoronorepinephrine (5-FNE) are quite similar to those of norepinephrine (NE), although a 2–7-fold greater potency relative to NE has been observed in some β-adrenergic systems. Several studies in both peripheral and central systems, in vivo and in vitro, have demonstrated the usefulness of these analogues as pharmacological tools.[3]

This remarkable and unexpected "fluorine effect" provides potential information regarding the structural and electronic requirements of α- vs. β-adrenergic receptor interactions with NE. Extension of investigation of the fluorine effect to isoproterenol (ISO), a specific and potent β-adrenergic agonist, provided further insights.[5] The 6-fluoro substituent in 6-FISO reduced β-adrenergic potency compared to ISO, while 2-FISO and 5-FISO were essentially identical in activity as β-adrenergic agonists to ISO itself. None of the FISOs exhibited significant α-adrenergic potency, suggesting that the effect of 6-fluorine substitution was to inhibit interaction with the β-adrenergic receptor, i.e., that the effect is "negative". Such an interpretation could also be used to explain the activity of the FNEs in that the substitution of fluorine in the 6-position in this series does ablate interaction with the β-adrenergic receptor. Conversely, a 2-fluoro substituent has a "negative" effect on interactions of NE with the α-adrenergic receptor.

We have now extended this study to the classic α-adrenergic agonist phenylephrine (PE).[3,6] This also represents the first noncatechol system we have investigated. While our primary interest concerns the adrenergic agonist properties of 2-FPE and 6-FPE (numbering such that the phenolic group is assigned the 3-position), the absence of the 4-hydroxyl group permits a new pattern of fluorine substitution, i.e., with fluorine in the 4-position. In this paper, we describe the synthesis of 2-FPE (**2a**), 4-FPE (**2b**), and 6-FPE (**2c**) and their α-adrenergic agonist properties in the aortic strip and brain preparations. We have compared the agonist properties with binding efficacies to α_1-, α_2-, and β-adrenergic receptors.

Table I. Ultraviolet Spectral Data[a]

compd (solvent)[b]	Δ_{max}, nm	ϵ	compd (solvent)[b]	Δ_{max}, nm	ϵ
2a (A)	270.5	1200	**2c** (A)	279.5	2830
2a (B)	286.5	2100	**2c** (B)	300.5	3600
2b (A)	270.5	1750	1 (A)	272.5	1700
2b (B)	286.5	3460	1 (B)	291.0	3100

[a] No attempts were made to account for possible effects of side-chain amine protonation. [b] Solvents: A, H_2O; B, 0.1 N NaOH.

Chemistry. The synthetic approach to **2a–c** (UV spectral data in Table I) was based on the corresponding benzyloxyfluorobenzaldehydes (**3a-c**) as key intermediates. Of these, **3b** and **3c** were prepared by introduction of the hydroxyl group into commercially available fluorobenz-

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