Characterization of a Series of 3-Amino-2-phenylpropene Derivatives as Novel Bovine Chromaffin Vesicular Monoamine Transporter Inhibitors

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A series of 3-amino-2-phenylpropene (APP) derivatives have been synthesized and characterized as novel competitive inhibitors, with K_i values in the μM range, for the bovine chromaffin granule membrane monoamine transporter(s) (bVMAT). Although, these inhibitors are structurally similar to the bVMAT substrate tyramine, none of them were measurably transported into the granule. Structure−activity studies have revealed that, while the 3′- or 4′-OH groups on the aromatic ring enhance the inhibition potency, Me or OMe groups in these positions reduce the inhibition potency. Halogen substitution on the 4′-position of the aromatic ring causes gradual increase of the inhibition potency parallel to the electron donor ability of the halogen. Substituents on the NH_2 as well as on the 3-position of the alkyl chain reduce the inhibition potency. Comparative structure−activity analyses of APP derivatives with tyramine and the neurotoxin 1-methyl-4-phenylpyridinium suggest that the flexibility of the side chain and the relative orientation of the NH_2 group may be critical for the efficient transport of the substrate through the bVMAT. Comparable bVMAT affinities of these inhibitors to that of DA and other pharmacologically active amines suggest that they are suitable for the structure−activity and mechanistic studies of monoamine transporters and may also be useful in modeling the mechanism of action of amphetamine-related derivatives.

All monoamine storage vesicles (catecholaminergic, serotonergic, or histaminergic) utilize two, closely related, relatively nonspecific transporters for the vesicular uptake of monoamines.[1−4] These transporters are responsible for maintaining high catecholamine concentration gradients (>10^5) in catecholamine storage vesicles. They are acidic glycoproteins with an apparent molecular weight of 70 000 D_a with 12 transmembrane domains.[5,6] A functional vesicular monoamine transporter from a pheochromocytoma cDNA library (VMAT1) has been cloned and characterized.[6] A second form of the vesicular monoamine transporter (VMAT2) has been cloned from a cDNA library of the brain.[5] The central, peripheral, and enteric neurons express only VMAT2, while neuroendocrine, including chromaffin and enterochromaffin cells exclusively express VMAT1.[7−9] However, VMAT2 appears to be the major transporter in chromaffin granules of the bovine adrenal medulla.[7−10] Catecholamines and histamines are shown to exhibit 3- and 30-fold more affinity, respectively, toward VMAT2 in comparison to VMAT1.[7] Reserpine and ketanserin are slightly more potent inhibitors of VMAT2, while tetrabenazine is a specific inhibitor for VMAT2.[1−4] In addition, amphetamine, methylenedioxyamphetamine, and phenylethylamines are better inhibitors for VMAT2 than VMAT1.[7,11]

Vesicular monoamine transporters play a critical role not only in sorting out, storing, and releasing of neurotransmitters, but also in fine-tuning the neuronal and endocrine informational output.[1−4] In addition, a large number of illicit drugs and neurotoxins are proposed to exert their neuropharmacological and toxic effects, at least partly, through interference with the physiological functions of VMATs.[6−7,10,12−19] Despite the physiological significance, the molecular mechanisms related to the biochemical functions of VMATs are still poorly understood.[20] Therefore, it is a significant goal to develop specific probes, which could be used in the structure−function and mechanistic studies of VMATs. In the present study, we have characterized a series of 3-amino-2-phenylpropene (APP) derivatives as novel reversible competitive inhibitors for the bovine chromaffin granule monoamine transporter(s) (bVMAT) with potencies in the low μM range. Although, the affinities of these inhibitors are significantly less than that of the classical inhibitors such as reserpine, ketanserine, and tetrabenazine,[21−23] they are comparable to the affinities of the most pharamacologically active monoamines, making them highly suitable for pharmacological studies of VMATs. In addition, their high water solubility, competitive kinetics with respect to DA, and structural simplicity make them suitable for structure−activity studies of VMATs as well.

Chemistry. The syntheses of **1**−**3** have been previously reported.[24−26] The parent compound, 3-amino-2-phenylpropene (**1**; APP; previously incorrectly named and abbreviated as PAME; see ref 26), could be conveniently prepared by the allylic bromination of α-methylstyrene, with *N*-bromosuccinimide (NBS), followed by Gabriel phthalimide synthesis. The starting materials for the synthesis of **2** and **3** (the corresponding α-methylstyrene derivatives) could be easily obtained by the treatment of the corresponding ring-hydroxy acetophenones with methylmagnesium bromide followed by simultaneous dehydration/acetylation with acetic anhydride.[24−27] The α-methylstyrene derivatives

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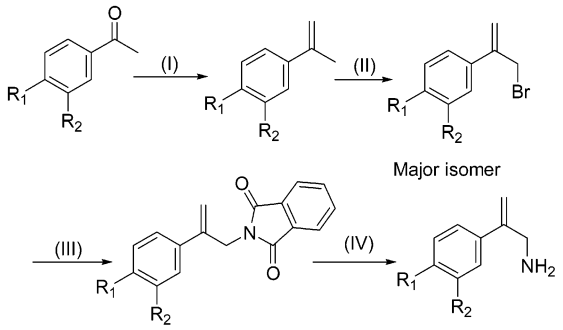
Scheme 1[a]



[a] (I) a. Ph_3PCH_2Br, DMSO; b. n-BuLi; (II) NBS, $CHCl_3$; (III) potassium phthalimide, DMF (IV) a. NH_2NH_2, EtOH; b. NaOH.

for the synthesis of other para-substituted derivatives **5**−**10** were obtained from the corresponding acetophenones using the general Wittig reaction (Scheme 1) which gave a better yield than the two-step Grignard procedure (compounds **4**, **5**, **7**, and **11** have also been previously reported.[28,29]) Since *N*-bromosuccinimide (NBS) bromination of 4-methoxyphenyl α-methylstyrene exclusively produced the vinyllic isomer, **4** was obtained from the corresponding 4-OH phthalimide derivative by methylation of the ring hydroxyl group with CH_3I followed by hydrazinolysis to remove the phthalimide group.

Biological Evaluation. Bovine adrenal chromaffin cells and their granules have been extensively used as models in the study of pharmacologically active amines and related compounds.[1−4] Resealed bovine adrenal chromaffin granule ghosts have also been commonly used as a model for the catecholamine neurotransmitter storage vesicles in the study of the DA uptake and norepinephrine biosynthesis.[1−4,27,30] We have previously developed experimental protocols to examine the kinetics of dopamine (DA) uptake and conversion to norepinephrine (NE) inside resealed bovine chromaffin granule ghosts without using radiolabeled substrates.[27,30] In the present study we have extended these protocols to examine the kinetics of DA uptake into resealed bovine chromaffin granule ghosts through the vesicular monoamine transporter (bVMAT) (see Experimental Section; details of these methods will be published elsewhere). The results presented below clearly demonstrate that the resealed chromaffin granule ghosts are an ideal model system for the detailed kinetic characterization of bVMAT inhibitors and substrates. While the kinetic parameters determined for DA uptake in the present study are in excellent agreement with the previously reported values for intact bovine chromaffin granules and granule ghosts,[15,18,27,30] they are about 6−8 times higher than the kinetic parameters reported for both VMAT1 and VMAT2 expressed in digitonin-permeabilized fibroblast cells.[7]

Results and Discussion

Although DA is the physiological substrate for bVMAT, previous studies have shown that tyramine is also a good substrate for bVMAT with kinetic parameters similar to that of DA.[30] The structural similarity between tyramine and 4′OH APP (**2**) suggests that **2** should also be a good substrate for bVMAT. However,

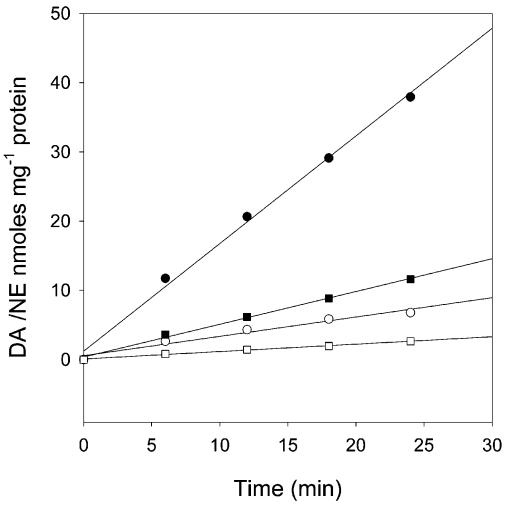


Figure 1. The effect of external 4′OH APP on the time courses of NE production in resealed chromaffin granule ghosts under turnover conditions: Ghosts were resealed to contain 20 mM Tris phosphate, pH 7.0, 100 mM KCl, 150 mM sucrose, 10 mM fumarate, and 100 μg/mL catalase with 20 mM ascorbic acid (pH 7.0) and incubated in a medium (2.5 mL total volume) containing 0.3 M sucrose, 10 mM HEPES, 5 mM Mg-ATP, 5 mM $MgSO_4$, 20 mM ascorbic acid, and 100 μg/mL catalase (pH 7.0) at 30 °C for 10 min. Then, 0.5 mM 4′OH APP was added to one of the mixtures, and both were allowed to incubate further for 2 min. At zero time, the conversion reaction was initiated by the addition of DA to a final concentration of 200 μM, and, at 5 min time intervals, 400 μL aliquots of the incubate were withdrawn and intragranular DA and NE levels were quantified by HPLC-EC as detailed in Experimental Section. (a), ●, NE level in the control, ■, NE level in the presence of 500 μM external 4′OH APP; ○, DA level in the control; □, DA level in the presence of 500 μM external 4′OH APP.

our experiments revealed that externally applied **2** does not accumulate in resealed granule ghosts even at 200−500 μM concentrations, under standard uptake conditions (see Experimental Section). On the other hand, the incubation of resealed chromaffin granule ghosts in a medium containing 500 μM **2** and 200 μM DA under standard turnover conditions resulted in a marked decrease in both DA accumulation and NE production inside the granule ghosts (Figure 1). Similarly, the incubations of resealed granule ghosts with equimolar concentrations of DA + **2** (200 μM) under uptake (nonturnover) conditions revealed that DA uptake is markedly inhibited by **2**, suggesting that **2** could be an efficient inhibitor for bVMAT (data not shown).

To further confirm the above results, the kinetics of the inhibition of bVMAT by **2** was studied using resealed chromaffin granule ghosts under standard uptake conditions. As shown in Figure 2A and B, DA uptake inhibition by **2** was competitive with respect to DA with a K_i of 15.5 ± 0.9 μM. Under similar experimental conditions, K_m for DA was determined to be 23.6 ± 1.4 μM, suggesting that **2** interacts with bVMAT slightly more efficiently than DA ($K_{m,DA}/K_i \sim 1.5$). Furthermore, the parent compound, APP (**1**), was also found to be a weaker competitive inhibitor for bVMAT with a K_i of 40.3 ± 5.3 μM ($K_{m,DA}/K_i \sim 0.6$), demonstrating that the 4′-OH group of **2** plays a significant role in the inhibition of bVMAT which is consistent with the known specificity of bVMAT.[1−4] On the other hand, the transposition of the ring OH group from the 4′- to the 3′-position (**3**) did

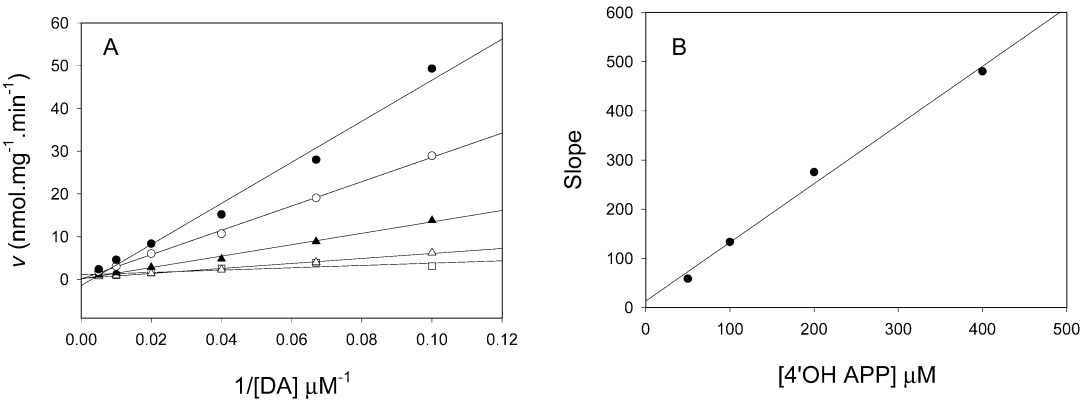
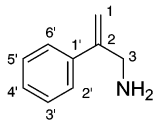


Figure 2. The kinetics of inhibition of DA uptake into resealed chromaffin granule ghosts by 4′OH-APP: Ghosts were resealed to contain 20 mM Tris phosphate, pH 7.0, 100 mM KCl, 150 mM sucrose, 10 mM fumarate, and 100 μg/mL catalase and incubated in a medium containing 0.3 M sucrose, 10 mM HEPES, 5 mM Mg-ATP, 5 mM $MgSO_4$, and 100 μg/mL catalase at 30 °C for 10 min (0.5 mL total volume; final pH 7.0). Then, the appropriate concentration of 4′OH APP (50 μM-400 μM) was added to the incubation mixtures, and they were allowed to incubate further for 2 min. The uptake reaction was initiated by the addition of DA to the respective incubation mixture to give a final DA concentration of 10, 15, 25, 50, 100, and 200 μM. After 6 min, 400 μL aliquots of each incubate were withdrawn, and internal DA levels were quantified by HPLC-EC as detailed in the Experimental Section. (***A***) double reciprocal plot of DA uptake inhibition by (□) 0 μM; (△) 50 μM; (▲) 100 μM; (○) 200 μM, and (●) 400 μM external 4′OH APP; (***B***) re-plot of the slope versus [4′OH APP].

Table 1. Inhibition Kinetic Parameters of APP Derivatives for bVMAT[a]



	APP derivatives	K_i (μM)	$K_{m,DA}/K_i$[b]
1	APP	40.3 ± 5.3	0.57
2	4′OH APP	15.5 ± 0.9	1.52
3	3′OH APP	16.7 ± 1.1	1.40
4	4′OMe APP	102.5 ± 8.1[c]	0.33
5	3′OMe APP	30.2 ± 1.5	0.76
6	4′F APP	42.3 ± 3.1[c]	0.80
7	4′Cl APP	18.0 ± 0.9[c]	1.76
8	4′Br APP	17.7 ± 2.0	1.79
9	4′I APP	12.9 ± 2.3	2.96
10	4′ Me APP	55.9 ± 4.4[c]	0.59
11	*N*-Me 4′OH APP	93.7 ± 11.8	0.39
12	*N*,*N*-diethyl 4′OH APP	202 ± 42[c]	0.15
13	*N*,*N*,*N*-trimethyl APP	*d*	–
14	phenylpyridyl methyl ethane	241 ± 84[c]	0.12
15	3-Me APP	78.4 ± 24.6[c]	0.41
16	*N*,*N*-diethyl 3-Me 4′OH APP	207 ± 37[c]	0.15
17	4′OH 1-Br(*E*) APP	50.0 ± 6.1[c]	0.46
18	4′OMe 1-Br (*E*) APP	*d*	
19	2′-Me, 4′OH APP	*d*	

[a] K_i, inhibition constants for DA uptake were determined in resealed chromaffin granule ghosts under standard uptake conditions as detailed in the Experimental Section. The K_i values were obtained by fitting the experimental data (typically, obtained using four to five different inhibitor, and six different DA concentrations; for further details *see* Figure 2 legend) to the Cleland's COMP program.[33] All inhibitions were competitive with respect to DA. [b] The $K_{m,DA}/K_i$ ratios were calculated from the respective K_i and $K_{m,DA}$ parameters determined for each compound to normalize the variation of K_m for DA for difference ghost preparations. [c] Determined by fitting the experimental data (obtained with 0 and 200 μM inhibitor and six different DA concentrations) to the standard competitive inhibition equation using Sigmaplot Cambridge Software Corp.). [d] Screening experiments show that the inhibition potencies are significantly less than those reported in the table.

not alter the inhibition potency significantly (Table 1), suggesting that both 3′- and 4′- ring hydroxyl groups of the inhibitor may play a similar but distinct role in the interaction with the transporter. In addition, the appropriate control experiments revealed that none of these derivatives were actively taken up into the resealed granule ghosts.

The kinetic data presented in Table 1 clearly demonstrate that the APP derivatives tested behave as competitive inhibitors for bVMAT with respect to DA. These data (Table 1) also demonstrate that the inhibition potencies of APP derivatives are dependent on the ring, side chain, and N-substituents. As mentioned above, while both 3′ and 4′-OH groups contribute positively toward the inhibition potency, 4′- and 3′-OMe substitutions (**4** and **5**) did not improve the inhibition potency with respect to the parent compound **1** significantly. In fact, while 4′-OMe substitution had a negative effect on the inhibition, relative to the parent compound (**1**), 3′-OMe substitution only slightly improved the inhibition potency, again suggesting the distinct nature of the effects of 3′- and 4′-substituents. More notably, the substitution of halogens on the 4′-position of the phenyl ring exhibited an interesting effect on the inhibition potency. While F substitution on this position had a minimal effect on the inhibition potency, Cl, Br, and I substitutions gradually increased the inhibition potency, yielding 4′-iodo derivative **9** as one of the most potent inhibitors of the series with a K_i of 12.9 ± 2.3 μM ($K_{m,DA}/K_i \sim 3$). Substitution of a Me-group on the 4′-position of the aromatic ring (**10**) had only a small effect on the inhibition potency. Substitution of a Me group on the primary amine functionality of 4′OH APP (**11**) reduced the inhibition potency by a factor of ~6. Similarly, the substitution of the primary amine group with *N*,*N*-diethyl (**12**) or *N*,*N*,*N*-triethyl (**13**) groups decreased the inhibition potency similar to that of *N*-methyl substitution. The weak inhibition potency of the *N*,*N*,*N*-triethyl (**13**) and pyridine (**14**) derivatives suggest that a permanent positive charge at nitrogen does not improve the inhibition potency significantly. In addition, substitution of a Me group in the α-carbon to yield a derivative structurally similar to amphetamines (**15**, **16**) decreased the inhibition potency significantly. Finally, substitution of a bulky Br-group (**17**) on the double bond had only a small effect on the inhibition potency.

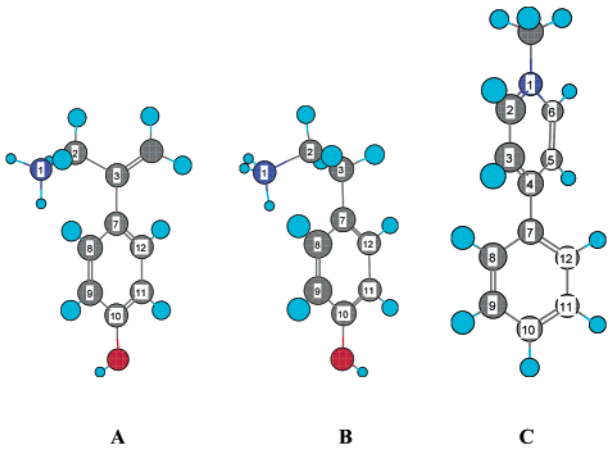


Figure 3. Energy-optimized structures of [4′OH APP], tyramine, MPP^+: ab initio calculations were carried out using the *Gaussian 98-Revision A.9* suite of programs.[31] The molecular geometries were optimized using restricted Hartree−Fock method employing 6-31G* basic set. Energy-optimized structures of (A) 4′OH APP; (B) tyramine; (C) MPP^+. Color code: C: gray; H: light blue; O: red; N: dark blue.

The structure−activity profile of the APP derivatives tested (Table 1) shows that the hydroxyl and large halogens, especially Br and I at the 4′ position of the aromatic ring, enhanced the inhibition potency significantly. Although, the effect of the 4′-OH could be due to H-bonding with a critical residue in the cytosolic phase of the transporter, as previously proposed for bVMAT substrates, the gradual increase of the inhibition potency from F to I in the 4′-halogen-substituted series indicates that the inhibition potency may increase with the electron donor ability of the halogen. Clearly, the effect of halogen substitution could not be due to the increase in the hydrophobicity, since 4′-methyl substitution decreased the inhibition potency noticeably. The experimental data also suggest that the NH_2 group makes an important contribution to the interaction with the transporter probably through H-bonding and/or ionic interactions, and the bulky substituents on nitrogen or at the α-C are not tolerated.

Apart from the monoamines, the only other known substrate for VMAT is the neurotoxin MPP^+.[18,19] Since MPP^+ is structurally rigid and significantly different from the common monoamines, several key structural parameters that are critical for bVMAT substrates could be derived from its structure (Figure 3C). For example, (a) the permanent positive charge on the nitrogen of MPP^+ indicates that the protonated form of the monoamine may be the substrate for bVMAT; (b) the relatively bulky pyridine ring in place of the ethylamine side chain of the monoamines suggests that the steric bulk at the benzylic carbon does not affect the substrate activity significantly; and (c) since 3-phenyl derivative of MPP^+ is not a substrate for bVMAT [Wimalasena, D. S.; Perera, R. P., Wimalasena, K. unpublished results (manuscript in preparation)], the planer arrangement of the C10, C7 of the aromatic ring with the N1 nitrogen atom of the pyridine ring (Figure 3C) may be critical for the transport through bVMAT. On the basis of this evidence, we propose that the conformationally restricted mobility of the side chain of APP derivatives, in comparison to the corresponding phenyethylamines such as tyramine, may play a critical role in the inhibition of bVMAT. As shown in Figure 3, the dihedral angles C7−C3−C2−N1 of the optimized structures (RHF/6-31G*) of 4′OH APP (note that numbering of atoms is different from that of Table 1) and tyramine, in protonated forms, are similar and are 54.17° and 54.62°, respectively. However, the close inspection of the torsional barriers calculated for the dihedral angle C7−C3−C2−N1 of these two compounds show that the in plane configuration of C10, C7, and N1 (i.e., C7−C3−C2−N1 dihedral angle is 180°) in 4′OH APP is about 1.9 kcal/mol more unfavorable in comparison to tyramine. Therefore, if such a planer conformation is critical at a high energy state of the transport process then, APP may not be efficiently transported due to the relatively high rotational barrier. Further studies are currently underway in our laboratory to test the above model for the transport of biogenic amines through bVMAT.

Conclusions

The above results demonstrate that although the bVMAT inhibition potencies of the APP derivatives are weaker than the classical inhibitors such as reserpine, tetrabenazine, and ketanserin, they are kinetically fully characterizable and possess affinities similar to that of DA and most other pharmacologically active monoamines. Therefore, they are good candidates for the structure−activity, mechanistic, and pharmacological studies of monoamine transporters. Based on the structure−activity relationships of a number of APP derivatives, a testable molecular model for the transport vs inhibition of the transporter is proposed. In addition, due to the remarkable structural similarities between APP and amphetamine derivatives, we believe that they may also possess interesting pharmacological properties and may be useful in modeling the mechanism of action of amphetamine-related derivatives.

Experimental Section

VMAT Inhibition Kinetics. All inhibition kinetic experiments were carried out using properly characterized resealed bovine chromaffin granule ghosts as previously described under standard uptake conditions[30] (further details of these procedures will be published elsewhere). Briefly, the washed granule membranes were resealed to contain 20 mM Tris phosphate, 100 mM KCl, 150 mM sucrose, 10 mM fumarate, and 100 μg/mL catalase, and with no ascorbate (pH 7.0). Resealed granule ghosts were purified by a discontinued Ficoll/sucrose density gradient. The resealed granule ghosts were incubated in a medium (0.5 mL total volume) containing 0.3 M sucrose, 10 mM HEPES, 5 mM Mg-ATP, 5 mM $MgSO_4$, and 100 μg/mL catalase, (pH 7.0) at 30 °C for 10 min (uptake conditions). Then, the desired concentrations of the inhibitor was added to the mixture and allowed to incubate further for 2 min. Following the incubation period, the uptake reaction was initiated by the addition of the desired concentration of DA and, at 6 min time intervals, 400 μL aliquots of the incubate were withdrawn and diluted into 5.0 mL of ice-cold 0.4 M sucrose, 10 mM HEPES, pH 7.0, and stored at 0 °C until the incubation period was completed. These samples were then centrifuged at 36 000*g* for 25 min at 4 °C, the supernatants removed, the pellets gently washed three times with 0.4 M sucrose, 10 mM HEPES, pH 7.0, and the tubes swabbed dry. Then, 500 μL of 0.1 M $HClO_4$ was added, the pellets were homogenized, and the extraction was allowed to proceed for 20 min at room temperature. After low speed centrifugation to remove coagulated protein, 20 μL of the acidic extracts were analyzed for catecholamines by reversed phase HPLC-EC. In

standard turnover experiments, the granules were resealed to contain 20 mM ascorbic acid and incubated as above except that 20 mM ascorbic acid was included in the external incubation medium.

Synthesis. All ^{1}H and ^{13}C NMR spectra were recorded with a Varian Inova 400 MHz spectrometer (Varian), and mass spectra were obtained from a Finnigan LCQ-Deca ion trap mass spectrometer (Thermoquest Corporation, San Jose, CA). All reagents and solvents were obtained from various commercial sources with the highest purity available and used without further purification. Trimethylsilane (for organic solvents) or 3-(trimethylsilyl)propionic acid sodium salt (for D_2O) were used as internal standards for NMR.

Computational Calculations. Ab initio calculations were carried out using the *Gaussian 98-Revision A.9* suite of programs.[31] The rotational barriers were calculated by using the built-in scan mode. The dihedral angles C7−C3−C2−N1 (see Figure 3A,B) were changed by 5° increments, the geometries were completely optimized, and the energies were calculated.

3-Amino-2-phenylpropene·HCl (1). Mp 178−179 °C (lit. 178−179 °C);[26] ^{1}H NMR (D_2O) δ 7.47−6.95 (m, 5 H), 5.73 (s, 1 H), 5.54 (s, 1 H), 4.13 (s, 2 H); ^{13}C NMR (D_2O) δ 44.8, 119.5, 128.4, 131.1, 131.2, 139.2, 142.6; MS (ESI) *m*/*z* 134.2 (M^+).

3-Amino-2-(4′-hydroxyphenyl)propene·HCl (2). This was synthesized by the method of Padgette *et al.*[26] Yield 17.9%; mp 173−175 °C (lit. 175 °C);[26] ^{1}H NMR (D_2O) δ 7.3 (d, 2 H), 6.81 (d, 2 H), 5.41 (s, 1 H), 5.18 (s, 1 H), 3.86 (s, 2 H); ^{13}C NMR (D_2O) δ 45.4, 118.3, 118.4, 130.5, 132.0, 142.5, 158.8; MS (ESI) *m*/*z* 149 (M^+).

3-Amino-2-(3′-hydroxyphenyl)propene·HCl (3). This was also synthesized by using the same procedure as (**2**). Yield 31%; mp 144−145 °C (lit. 143−145 °C);[26] ^{1}H NMR (D_2O) δ 6.86−7.61 (m, 4 H), 5.73 (s, 1 H), 5.54 (s, 1 H), 4.13 (s, 2 H); ^{13}C NMR (D_2O) δ 45.3, 115.8, 118.5, 120.2, 121.1, 133.1, 141.5, 142.8, 158.6; MS (ESI) *m*/*z* 149 (M^+).

3-Amino-2-(4′-methoxyphenyl)propene·HCl (4). An ethanol solution of *N*-[2-(4′-acetoxyphenyl)-2-propenyl]phthalimide was treated with 1 M NaOH until the pH of the solution was about 10 and stirred for 15 min. The resultant solution was acidified to pH 8 using concentrated HCl, and ethanol was removed under reduced pressure. The resultant product was extracted into $CHCl_3$ and concentrated under reduced pressure to yield a white solid which was dissolved in DMF and treated with CH_3I in the presence of K_2CO_3 to give *N*-[2-(4′-methoxy)-2-propenyl]phthalimide. This was purified by column chromatography using 10% ethyl acetate/hexane, and the phthalimide group was removed as described by McDonald et al.[24] to yield 3-amino-2-(4′-methoxy)propene. Yield: 18.6%; mp 162−164 °C; 1H NMR (D_2O) δ 7.31 (d, 2H), 7.01 (d, 2H), 5.68 (s, 1H), 5.45 (s, 1H), 4.13 (s, 2H), 3.86 (s, 3H); ^{13}C NMR (D_2O) δ 45.3, 58.1, 114.8, 117.4, 131.4, 131.7, 140.0, 162.7; MS (ESI) *m*/*z* 149 (M^+). Anal. ($C_{10}H_{14}ClNO$) C, H, N.

2-(3′-Methoxyphenyl)propene. A solution of n-BuLi (18 mL, 0.05 mol in hexane) was added dropwise to a solution of triphenylphosphonium bromide (14.3 g, 0.04 mol) in 25 mL of DMSO. The solution was stirred for 1 h at RT, and 3-methoxyacetophenone (6 g, 0.04 mol) in 10 mL of DMSO was added dropwise. The mixture was stirred overnight and quenched with water, and the product was extracted with hexane. The concentrated hexane extract was purified by column chromatography using hexane as a solvent. Yield 89%; ^{1}H NMR ($CDCl_3$) δ 7.60 (m, 2 H), 7.27(d, d 1 H), 6.98 (d, 1 H) 5.35(s, 1 H), 5.09 (s, 1 H), 2.21 (s, 3 H), 2.12 (s, 3 H).

The following compounds **5**−**10** were synthesized from the corresponding α-methylstyrene derivatives which were obtained by the general Wittig reaction as described for 2-(3′-methoxyphenyl)propene using the standard procedure.

3-Amino-2-(3′-methoxyphenyl)propene·HCl (5). Mp 158−160 °C; ^{1}H NMR (D_2O) δ 6.86−7.61 (m, 4 H), 5.73 (s, 1 H), 5.54 (s, 1 H), 4.13 (s, 2 H); ^{13}C NMR (D_2O) δ 45.3, 58.1, 114.8, 117, 120.6, 121.8, 133.0, 141.5, 142.9, 161.9; MS (ESI) *m*/*z* 149 (M^+). Anal. ($C_{10}H_{14}ClNO$) C, H, N.

3-Amino-2-(4′-fluorophenyl)propene·HCl (6). Mp 168−169 °C; ^{1}H NMR (D_2O) δ 3.97 (s, 2H), 5.32 (s, 1H), 5.52 (s, 1H), 7.07 (t, 2H), 7.40 (d, d, 2H); ^{13}C NMR (D_2O) δ 42.9, 115.7, 116.0, 117.5, 128.3, 128.4, 133.4, 139.7, 161.3, 164.5; MS (ESI), *m*/*z* 152.3 (M^+). Anal. ($C_9H_{11}ClFN$) C, H, N.

3-Amino-2-(4′-chlorophenyl)propene·HCl (7). Mp 178−178 °C; ^{1}H NMR (D_2O) δ 3.98 (s, 2H) 5.36 (s, 1H), 5.58 (s, 1H), 7.37 (s, 4H); ^{13}C NMR (D_2O) d 45.2, 120.5, 130.4, 131.6, 136.7, 138.30, 142.1; MS (ESI), *m*/*z* 168.3 (M^+). Anal. ($C_9H_{11}Cl_2N$) C, H, N.

3-Amino-2-(4′-bromophenyl)propene·HCl (8). Mp 183−185 °C; ^{1}H NMR (D_2O) δ3.98 (s, 2H), 5.37 (s, 1H), 5.60 (s, 1H), 7.30 (d, 2H), 7.52 (d, 2H); ^{13}C NMR (D_2O) d 45.0, 120.4, 124.7, 130.5, 134.4, 138.7, 142.0; MS (ESI), *m*/*z* 214.2 (M^+). Anal. ($C_9H_{11}BrClN$) C, H, N.

3-Amino-2-(4′-iodophenyl)propene·HCl (9). Mp 208−210 °C; ^{1}H NMR (D_2O) δ 4.08 (s, 2H), 5.46 (s, 1H), 5.70 (s, 1H), 7.28 (d, 2H), 7.82 (d, 2H);^{13}C NMR (D_2O) d 45.5, 97.2, 121.0, 131.2, 139.8, 141.1, 142.8; MS (ESI), *m*/*z* 260.0 (M^+). Anal. ($C_9H_{11}ClIN$) C, H, N.

3-Amino-2-(4′-methylphenyl)propene·HCl (10). Mp 170−173 °C (lit. 170−173 °C);[28] ^{1}H NMR (D_2O) δ 2.25 (s, 3H) 3.97 (s, 2H), 5.28 (s, 1H), 5.53 (s, 1H), 7.18 (d, 2H), 7.29 (d, 2H); ^{13}C NMR (D_2O) d 22.9, 45.3, 119.1, 128.8, 132.3, 136.8, 142.1, 142.9; MS (ESI), *m*/*z* 148.1 (M^+). Anal. ($C_{10}H_{14}NCl$) C, H, N.

3-(*N*-Methylamine)-2-(4′-hydroxyphenyl)propene·HCl (11). A solution of 4′-acetoxy-phenyl α- (bromomethyl)-styrene (1 g, 3.9 mmol) in THF (10 mL) was added dropwise to a solution of methylamine (2 M solution, 5 mL, 0.001 mol) in THF in the presence of $NaHCO_3$ (0.2 g) and stirred for 5 h under nitrogen. The resulting solution was filtered and concentrated under reduced pressure. The HCl salt of the product was recrystalized from ethanol/ether. Yield 35.9%; mp 149−150 °C; ^{1}H NMR (D_2O) δ 7.3 (d, 2 H), 6.81 (d, 2 H), 5.41 (s, 1 H), 5.18 (s, 1 H), 3.86 (s, 2 H); ^{13}C NMR (D_2O) δ 48.4, 52.1, 116.7, 121.2, 122.4, 130.5, 132.2, 143.1, 158.2; MS (ESI) *m*/*z* 164.4 (M^+). Anal. ($C_{10}H_{14}ClNO$) C, H, N.

3-(*N*,*N*-Diethylamino)-2-(4′-hydroxyphenyl)propene·HCl (12). This was synthesized by the same procedure described for **11,** except that diethylamine was used in place of methylamine. Yield 32.4%; mp 182−185 °C; ^{1}H NMR (D_2O) δ 7.41 (d, 2H), 6.95(d, 2H), 5.66 (s, 1H), 5.58 (s, 1H), 4.68 s, 2H), 3.27 (q, 4H), 1.21 (t, 6H); ^{13}C NMR (D_2O) δ 15.0, 59.2, 66.9, 119.1, 123.7, 131.6, 133.8, 146.1, 159. 7; MS (ESI) *m*/*z* 206.3 (M^+). Anal. ($C_{13}H_{20}ClNO$) C, H, N.

3-(*N*,*N*,*N*-Trimethylamino)-2-phenyl-propene·HBr(13). This was synthesized by the same procedure used for **11,** except that triethylamine was used in place of methylamine. Yield 60%; mp 123−124 °C; ^{1}H NMR (D_2O) δ 8.46−8.48 (m, 2 H), 8.37−8.39 (m, 3 H), 6.85 (s, 1 H), 6.67 (s, 1 H), 5.37 (s, 2 H), 3.90 (s, 9H); ^{13}C NMR (D_2O) δ 56.0, 71.42, 129.2, 131.5, 131.9, 139.9, 141.6; MS (ESI) *m*/*z* 176.1 (M^+). Anal. ($C_{12}H_{18}BrN$) C, H, N.

1-(2-Phenyl-2-propenyl)pyridinium Bromide (14). This was synthesized by the same procedure used for **11,** except that pyridine was used in place of methylamine. Mp 115−116 °C (lit. 115−116);[28] ^{1}H NMR (D_2O) δ 5.68 (s, 1H), 5.81 (s, 2H), 5.86 (s, 1H), 7.42−7.48 (m, 3H), 7.54−7.58 (m, 2H), 8.04 (t, 2H), 8.51 (t, 1H), 8.95 (d, 2H); ^{13}C NMR (D_2O) δ 66.1, 123.9, 128.9, 130.7, 131.6, 131.6, 138.3, 143.0, 146.6, 148.9; MS (ESI), *m*/*z* 196.1(M^+).

2-Bromo-1-phenyl-1-propanone. This was prepared by the method of King and Ostrum[29] in 82% yield. ^{1}H NMR ($CDCl_3$) δ 1.88 (d, 2H), 5.29 (q, 1H), 7.44 (m, 3H), 8.11 (d, 2H).

2-Phthalimido-1-phenylpropanone. A solution of 2-bromo-1-phenyl-1-propanone (3 g, 0.01 mol) in 10 mL of DMF, potassium phthalimide (1.85 g, 0.01 mol) and K_2CO_3 (0.3 g) were mixed and heated to 90 °C under N_2 for 1 h and an additional 1 h at RT and filtered. The filtrate was concentrated under vacuum. The solid product was recrystalized from $CHCl_3$/hexane. Yield 56%; ^{1}H NMR ($CDCl_3$) δ 1.74 (d, 3H), 5.62 (q, 1H), 7.41 (m, 3H), 7.71 (dd, 2H), 7.96 (m, 4H).

3-Phthalimido 2-phenylbutene. This was synthesized using the same procedure as for 1-(3′-methoxyphenyl)propene. Yield 75%; ^{1}H NMR ($CDCl_3$) δ 1.24 (d, 3H), 5.01 (s, 1H), 5.05 (q, 1H), 5.12 (s, 1H), 7.25 (m, 3H), 7.5 (dd, 2H), 7.61−7.72 (m, 4H).

3-Amino-2-phenylbutene·HCl (15). Hydrazinolysis of 3-phthalimido-2-phenylbutene gave the desired product. Yield 59%; mp 177−179 °C; ^{1}H NMR (D_2O) δ 1.45 (d, 3H), 4.548 (q, 1H), 5.39 (s, 1H), 5.54 (s, 1H), 7.48 (s, 5H); ^{13}C NMR (D_2O) δ 20. 7, 52.4, 117.3, 129.8, 131.6, 131.8, 141.3, 149.4; MS (ESI) *m*/*z* (%) 148.1 (M^+). Anal. ($C_{10}H_{14}ClN$) H, N, C: calc, 65.39; found, 64.03.

2-Bromo-1-(4′-hydroxyphenyl)propanone. This was prepared by the method of King and Ostrum.[32] Yield 66%; ^{1}H NMR (DMSO-d_6) δ 10.12 (broad, 1H), 7.89 (d, 2H), 6.85 (d, 2H), 5.65 (q, 2H), 1.66 d, 3H).

2-(*N*,*N*-Diethylamine)-1-(4′-hydroxyphenyl)propanone. 2-Bromo-1-(4-hydroxyphenyl)propanone (2 g, 8.6 mmol) was dissolved in 20 mL of THF and diethylamine (1.2 mL, 8.6 mmol) was added dropwise. The solution was stirred for 2 h, and the unreacted amine was removed under reduced pressure to yield a light brown liquid. Yield 86%; ^{1}H NMR (DMSO-d_6) δ 10.32 (broad, 1H), 7.88 (d, 2H), 6.88 (d, 2H), 5.69 (q, 1H), 2.94 (q, 4H), 1.79 (d, 2H) 1.08 (t, 6H).

3-(*N*,*N*-Diethylamine)(4′-hydroxyphenyl)butene·HCl (16). This was synthesized from 2-(*N*,*N*-diethylamine)-1-(4-hydroxyphenyl)propanone using the general Wittig reaction as described for 1-(3-methoxyphenyl)propene. Yield 16.8%; mp 186 °C; ^{1}H NMR (D_2O) δ 7.28 (d, 2H), 6.82 (d, 2H), 5.49 (s, 1H), 5.41 (s, 1H), 4.52 (q, 1H), 3.06 (q, 4H), 1.41 (d, 3H), 1.07 (t, 6H); ^{13}C NMR (D_2O) δ 57.2, 62.1, 118.9, 122.7, 131.5, 133.7, 146.1, 159.2; MS ESI *m*/*z* 220.2 (M^+). Anal. ($C_{14}H_{22}NOCl$) C, H, N.

(*E*)-2-(4′-Hydroxyphenyl)-3-bromoallylamine·HCl (17). This was prepared by the method of McDonald et al.[24] starting from 2-(4′-acetoxyphenyl)propene. Yield 16.3%; mp 187−189 °C; ^{1}H NMR (D_2O) δ 7.41 (d, 2H), 6.95 (d, 2H), 6.82 (s, 1H), 4.08 (s, 2H); ^{13}C NMR (D_2O) δ 45.39, 113.72, 119.21, 119.43, 130.54, 132.23, 143.12, 159.15; MS (ESI) *m*/*z* 228 (M^+). Anal. ($C_9H_{11}BrClNO$) C, H,N.

(*E*)-2-(4-Methoxyphenyl)-3-bromoallylamine·HCl (18). The NBS bromination of 4-methoxy-α-methylstyrene exclusively produced the corresponding dibromide in 81% yield. This was converted to the corresponding phthalimide, and the desired amine was obtained by usual hydrazinolysis. Yield 67%; mp 190−192; °C ^{1}H NMR (D_2O) δ 7.42 (d, 2H), 7.03 (d, 2H), 6.82 (s, 1H), 4.28 (s, 2H), 3.86 (s, 3H); ^{13}C NMR (D_2O) δ 43.2, 58.1, 113.7, 117.3, 131.0, 131.5, 139.9, 162.30; MS (ESI) *m*/*z* 242.1 (M^+).

3-Amino-2-(2′-methyl-4′-hydroxyphenyl)propene·HCl (19). This was synthesized by the same procedure as (**3**) starting from 4′-hydroxy-2′-methyl acetophenone. Yield 32%; mp 190−192 °C ^{1}H NMR (D_2O) δ 7.13 (d, 2H), 6.83−6.78 (m, 3H), 5.58 (s, 1H), 5.24 (s, 1H), 3.89 (s, 2H), 2.27 (s, 3H); ^{13}C NMR (D_2O) δ 21.7, 47.1, 115.5, 119.9, 121.6, 132.8, 133.1, 140.5, 143.2, 158.0; MS (ESI) *m*/*z* 164.2 (M^+).

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Appendix

Abbreviations: APP, 3-amino-2-phenylpropene; ATP, adenosine triphosphate; bVMAT, bovine chromaffin granule vesicular monoamine transporter(s); DA, dopamine; HEPES, 4-(2-hydroxyethyl)-1-piperazinesulfonic acid; m-DβM, membranous dopamine β-monooxygenase; MPP^+, 1-methyl-4-phenylpyridinium; NBS, *N*-bromosuccinimide; NE, norepinephrine; s-DβM, soluble dopamine β-monooxygenase; VMAT1, human vesicular monoamine transporter-1; VMAT2, human vesicular monoamine transporter-2.

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