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Synthesis and Neurotoxic Potential of Racemic and Chiral Dihydroxytetrahydroquinoline Derivatives

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Abstract—The synthesis and preliminary neurotoxic investigation of (±), (+) and (−)-3-amino-6,7-dihydroxy-1,2,3,4-tetrahydroquinoline, (±)-3-amino-6,8-dihydroxy-1,2,3,4-tetrahydroquinoline and (±)-3-aminomethyl-6,7-dihydroxy-1,2,3,4-tetrahydroquinoline are described. While exhibiting relatively no dopamine and only moderate norepinephrine depletions, these compounds elicit serotonin depletions equal to those provided by the well-known serotonergic neurotoxin 5,7-dihydroxytryptamine in whole mouse brain.

Introduction

Neurotoxins, such as 6-hydroxydopamine (6-HDA) and 5,7-dihydroxytryptamine (5,7-DHT), have been employed for more than 25 years to study behavioral, physiological and pharmacological phenomena associated with catecholaminergic and serotonergic neuronal pathways. However, while the chemical lesioning afforded by such agents is highly selective compared with surgical or electrolytic methods, currently employed toxins still exhibit a high degree of non-target tissue destruction. Considering this, it is not surprising that there is an ongoing search for agents which exhibit a higher degree of toxicity toward targeted neuronal populations while eliciting little or no non-target tissue damage. Additionally, the mode(s) of action of 6-HDA,[1] 5,7-DHT[2] and related neurotoxins[3] remains relatively unknown; thus, examination of novel, but similar, neurotoxins will hopefully shed light on this important area as well.

We have accordingly designed fixed side chain analogs of 6-HDA with the intent to (1) provide neurotoxins which are superior to those in use, and (2) provide further information concerning the destructive mode(s) of action of such species. A more complete understanding of how these and other toxins elicit neurodestruction might also provide insight into the underlying mechanisms of neurodegenerative disorders and, ultimately, aid in the postponement of onset and/or alleviation of these disorders. Such an approach has already led to the employment of monoamine oxidase blocking agents to delay onset of Idiopathic Parkinson's Disease following investigation of the mode of action of MPTP.[4,5]

This paper describes the synthesis and a preliminary examination of central nervous system (CNS) toxicity for three putative neurotoxic agents: 3-amino-6,7-dihydroxy-1,2,3,4-tetrahydroquinoline (**9** and its enantiomers), 3-amino-6,8-dihydroxy-1,2,3,4-tetrahydroquinoline (**18**), and 3-aminomethyl-6,7-dihydroxy-1,2,3,4-tetrahydroquinoline (**26**). Synthetic schemes starting from easily available dimethoxybenzaldahydes are presented for each of these agents. Preliminary investigations have shown that these compounds, while exhibiting relatively no dopamine and only moderate norepinephrine depletions, elicit serotonin depletions approximately equivalent to those achieved by the established 5,7-dihydroxytryptamine in mouse whole brain. Additionally, these preliminary experimental results, particularly for neurotoxin **9**, suggest that there is no apparent stereochemical selectivity associated with such agents.

Results and Discussion

Syntheses of 3-amino-6,7-dihydroxy-1,2,3,4-tetrahydroquinoline hydrobromide and 3-amino-6,8-dihydroxy-1,2,3,4-tetrahydroquinoline hydrobromide

The syntheses of 3-amino-6,7-dihydroxy-1,2,3,4-tetrahydroquinoline hydrobromide (**9**) and 3-amino-6,8-dihydroxy-1,2,3,4-tetrahydroquinoline hydrobromide (**18**) followed similar pathways, as depicted in Scheme 1. The first step in the synthesis was the condensation of dimethoxybenzaldehyde **1** or **10** with hippuric acid in the presence of

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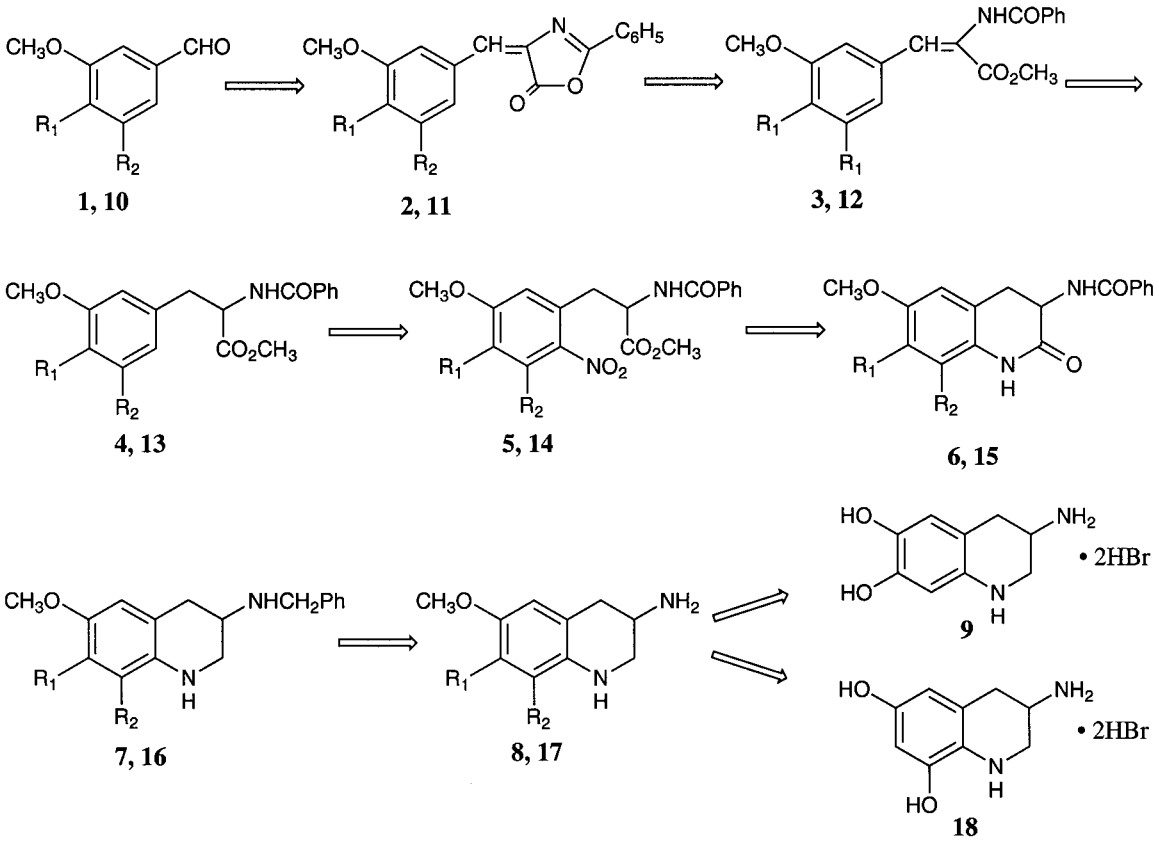


Scheme 1. For compounds **1**–**8**, R_1=OCH_3, R_2=H. For compounds **10**–**17**, R_1=H, R_2=OCH_3.

powdered sodium acetate and acetic anhydride. This reaction afforded the azlactones **2** in a 62% yield and **11** in an 86% yield. Basic methanolysis provided opening of the azlactone ring and addition of the desired methoxy group. This reaction provided **3** in a 70% yield and **12** in an 88% yield. The product analyses for both **2** and **3** agreed well with the literature.[6]

Reduction of the vinyl moiety provided the key step by which chirality was able to be conveniently introduced in this synthetic pathway. Conversion of propene derivatives **3** and **12** to their corresponding propane derivatives **4** and **13** was accomplished through catalytic hydrogenation (10% Pd/C) to afford clean products in quantitative yields. Nitration of **4**, employing fuming nitric acid, resulted in the corresponding 6-nitro analog **5** in a 99% yield. Nitration of **13**, however, was more difficult. Using fuming nitric acid (2–6 fold excess), various solvents (chloroform, acetic acid, and a mixture of these), and a wide range of reaction temperatures (−78 to 25°C), the yields of nitrated product **14** varied from 4–16%. These reactions resulted in products which were frequently not readily characterized. Thus, milder nitration reactions were subsequently attempted. These incorporated the use of reagent combinations like ammonium nitrate and trifluoroacetic anhydride, leading to multiple side products but no discernible desired product and, finally, tetranitromethane with pyridine, which resulted in the desired product **14**, but only in an 8% yield. Ultimately, by applying a simplex optimization procedure using 70.4% nitric acid in an acetic acid solvent, a maximal yield of 19% was achieved for the formation of **14**. (A referee has proposed a modified approach to compounds **9** and **26** to avoid some of these nitration problems using commercially available 6-nitropiperonal or 6-nitroveratraldehyde. We believe this approach would be successful, but was not attempted.)

The quinoline derivatives were formed through catalytic hydrogenation, using palladium on carbon. The reduction of the nitro functionality on **5** and **14** resulted in the formation of their corresponding amines, which then underwent intramolecular cyclization to afford the lactam derivatives **6** and **15** in a 100 and 90% yield, respectively. This ring-formation reaction proved to be a very useful and efficient reaction for the production of the tetrahydroquinoline ring structures. A discussion of the use of this general approach to the synthesis of quinonlines can be found in the report of G. Jones;[7] a closely related example is given in Narang et al.[8] Utilizing BH_3/THF, the amide functionalities were converted to the corresponding amine derivatives **7** (89%) and **16** (90%). The benzoyl amide functionality notably experienced reduction at a much slower rate than did the aliphatic amide due to its characteristically low electrophilicity. Catalytic hydrogenation effected *N*-debenzylation providing **8** and **17** in 94 and 83% yields, respectively. To avoid poisoning of the palladium catalyst, these reactions were conducted in the presence of acetic acid. The final step in the synthetic scheme was deprotection of the methyl-ethers. An excess of 48% hydrobromic acid under reflux conditions yielded the desired products **9** (95%) and **18** (86%). The 8-methoxy cleavage required 26 h, which was approximately five times longer than that needed to cleave

Table 1. Optical rotation values for (*R*) and (*S*) **4–9**

Compound	*R*		*S*	
	$[\alpha]_D^{25}$	*c* (g/ml)[a]	$[\alpha]_D^{25}$	*c* (g/ml)[a]
4	−79	1.35	+81	2.5
5	+69	1	−78	1.6
6	−38	1.5	+41	1.3
7	−30	1.3	+36	1.7
8	−26	0.5	+30	0.8
9	+16	0.9	−6	0.9

[a] Compounds **4–8** were in CH_2Cl_2, **9** in H_2O.

the 6-methoxy and/or 7-methoxy functionalities. This excessive reaction time for **18** did not, however, pose a problem since the resultant dihydroxy compound was relatively stable under these reaction conditions. Caution was taken in the final work-up of the hydrobromide salts, **9**, **18** and **26**, to minimize exposure to moisture and oxygen due to their ease of autoxidation.

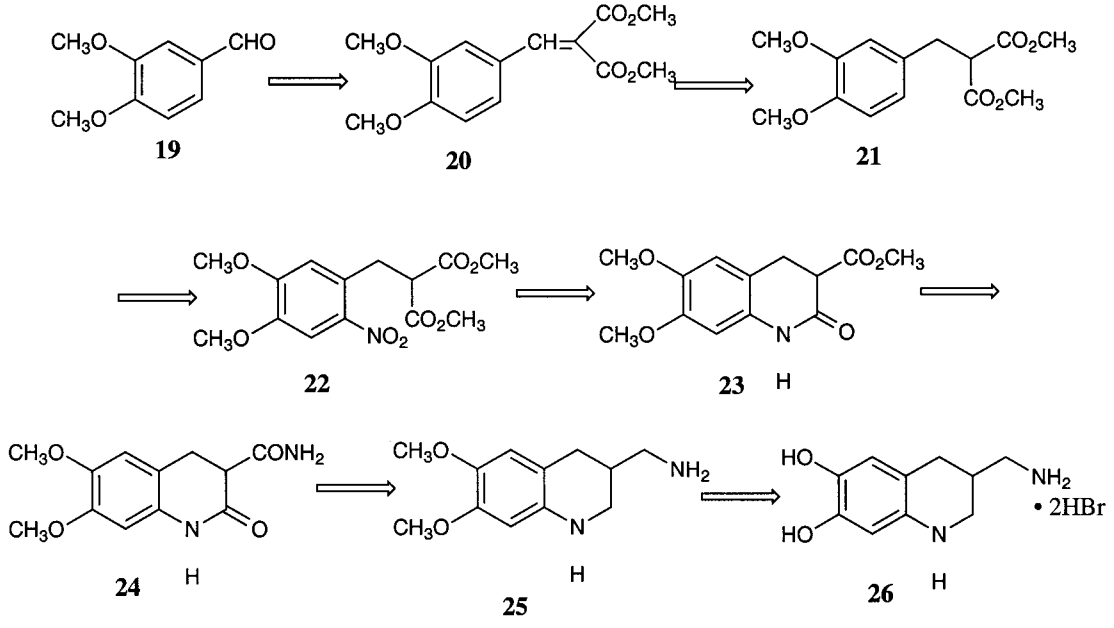
Enantiomeric syntheses

The inclusion of chirality into the 3-amino-6,8-dihydroxy-1,2,3,4-tetrahydroquinoline product was achieved through introduction of asymmetrical hydrogenation of the α-acylamino methylacrylate **3**. This was accomplished utilizing two enantiomeric forms of a rhodium–DUPHOS complex, which were previously used by Burk[9] in the reduction of substituted acetamidoacrylates at advantageously low hydrogen pressures and ambient temperatures. The active catalysts were prepared as reported[10] (*Caution*: handling and storage of the active catalysts needs to be performed in a dry-box). Successful asymmetrical hydrogenation of prochiral olefin **3** yielded (*R*)-**4** and (*S*)-**4** in quantitative amounts. The subsequent synthetic pathways for these enantiomers leading to the desired dihydroxyquinolines followed those described for the racemic compound.

The stereochemistry of the (*R*)-**4** and (*S*)-**4** enantiomers were initially assessed by determining the optical rotation values, as shown in Table 1, and comparing to the reported literature value of $[\alpha]_D^{23}=+83$ (*c* 2.4, $CHCl_3$) for (*S*)-**4**.[11] As can be seen, the results compare reasonably well to the previously reported value for the (*S*)-isomer. The optical rotation values for the enantiomeric forms of **5–9**, also shown in Table 1, could not be employed to assess enantiomeric purity, since optical rotation values have not been reported for these compounds. However, enantiomeric purity, or enantiomeric excess (ee), was determined for **8** using diastereomeric derivatives. Using (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate as a chiral derivatizing agent, (*R*)-**8** and (*S*)-**8** were converted to their diurea derivatives. These derivatives were then independently separated by HPLC, and the diastereomeric ratios were determined from peak areas. The average of the ratios was found to be 96:4 in both cases, which corresponds to a 92% enantiomeric excess. The considerable variability in the optical rotation values for the (*R*)-**9** and (*S*)-**9** enantiomers listed in Table 1 is assumed to be due to the susceptibility of these species to rapid autoxidation in aqueous solution; thus, these are not recommended for use in assessment of optical purity.

Synthesis of 3-aminomethyl-6,7-dihydroxy-1,2,3,4-tetrahydroquinoline dihydrobromide

The synthesis of 3-amino-6,7-dihydroxy-1,2,3,4-tetrahydroquinoline dihydrobromide (**26**) is shown in Scheme 2. The first step in this synthesis was the condensation of 3,4-dimethoxybenzaldehyde (**19**) with dimethylmalonate in the presence of piperidine resulting in product **20** with an 88% yield. Catalytic hydrogenation provided reduction of the benzylidene derivative leading to product **21** in an 86% yield. Spectral analyses showed products **20** and **21** to be in agreement with the literature.[12,13] Nitration of **21** using fuming nitric acid provided product **22** in a quantitative yield. Reduction of the nitro group to the corresponding



Scheme 2.

Table 2. Depletion of whole mouse brain neurotransmitters seven days following toxin administration (significant differences compared to control values (*t* test): $*P<0.05$, $**P<0.01$, $***P<0.001$. Amounts used: (±)- and *R*-(+)-**9**, 83 nmol; *S*-(−)-**9** and **26**, 52 nmol; **18**, 333 nmol; 5,7-DHT, 52 nmol; 6-HDA, 300 nmol)

Compound	*n*	Levels, % controls±SEM		
		NE	DA	5-HT
9	11	54±2***	83±3***	74±3***
R-(+)-**9**	14	52±2***	86±3***	72±2***
S-(−)-**9**	16	55±2***	88±3***	71±3***
18	16	68±1***	91±3*	73±2***
26	17	68±2***	100±1	81±2***
5,7-DHT[a]	13	84±2***		70±2***
6-HDA[b]	17	35±6***	71±6**	88±3*

[a] Data reported by Herman and Bonczek[16] for mouse brain levels seven days after i.c.v. injection of 5,7-DHT. Percent controls reported above are the maximum depletions obtained among the four examined regions, which included cerebral hemispheres, pons/medulla, diencephalon, and striatum.

[b] Data from Ma et al.[14] for whole mouse brain seven days after i.c.v. injection of 6-HDA.

amine, as above, led to intramolecular cyclization of the side chain via nucleophilic substitution. This reaction proceeded rapidly and resulted in product **23** with a yield of 72%.

While appearing straightforward, the production of carboxamide **24** from the methylester **23** proved to be challenging. This conversion was attempted using many different approaches, most of which were not successful. The unsuccessful reagents and conditions attempted included ammonium hydroxide and ammonium chloride, ammonia and ammonium chloride in a pressurized vessel with heat, and a saturated ammonia solution in a pressurized vessel with heat. All these attempts yielded the amide from the methylester, but the major product was the oxidized species, 3-carboxamide-6,7-dimethoxy-2-hydroxyquinoline, and small amounts, if any, of desired **24**. We found that exposure of relatively pure **24**, isolated from these mixtures, to similar reaction conditions exhibited a time dependent conversion to the undesirable oxidized product. Thus, the time for the reaction was optimized to provide maximal formation of **24** with minimal formation of the oxidation product. The optimal approach determined employed an elevated temperature ammonolysis of the methylester with ammonia and ammonium hydroxide in a pressurized vessel. Under these conditions, the major product remained the oxidized form of **24**; however, due to solubility differences in methanol, **24** was isolable in an acceptable yield of 24%. (A referee has suggested the possibility of avoiding this problem through the use of methylmalonate monoamide as a condensing reagent. Although this pathway was not tried, we believe it a worthy approach for future work in this synthesis.) Simultaneous conversion of the two amide groups to the corresponding amines was achieved via diborane reduction, resulting in diamine **25** in an 88% yield. The final step in the synthesis involved cleavage of the methyl ethers to their corresponding hydroxy functionalities using 48% hydrobromic acid under reflux conditions. Compound **26** was obtained in high purity with a yield of 74%.

Long-term depletion of endogenous neurotransmitters

Table 2 shows the depletion of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) in whole mouse brains at seven days following intracerebroventricular administration of these agents. The NE depletions produced by **9** and its enantiomers were expected due to their structural similarities to 6-hydroxydopamine (6-HDA) and α-methyl-6-aminodopamine (α-6-ADA).[14] It was surprising, however, that **18** and **26** elicited only moderate NE depletions (30%). Additionally, while 6-HDA provides DA depletions greater than 50%, the racemic and enantiomeric forms of **9** produced only minimal (12–17%) DA alterations. Compound **18** affected DA levels even less, and **26** did not alter DA levels at all, reminiscent of the α-6-ADA results. These modest to minimal NE and minimal to nonexistent DA depletions may be due to hindered neuronal uptake at endogenous neurotransmitter uptake sites. Completely unexpected, however, were the 5-HT depletions elicited by these quinoline species. The racemic and enantiomeric forms of **9** and, separately, compound **18** produced approx. 30% depletions in whole brain 5-HT levels. While these appear on the surface to be relatively modest, they are, in fact, relatively substantial when compared to results obtained from the best known alternative serotonergic agents when employed in mouse brain. These 5-HT depletions are virtually identical to those reported[15,16]for the well known and widely employed 5,6-DHT and 5,7-DHT neurotoxins.

The enantiomers of **9** were investigated to evaluate the effects of chirality in the catecholaminergic side chain on the observed neurotoxicity. Based on the whole mouse brain depletion values, there is no significant difference observed between the individual enantiomers and/or the racemic form. Thus, it would appear that there is no advantage to be gained from using the enantiomers of this compound for neurodegeneration studies.

However, from the preliminary biological investigations, it appears that these dihydroxy-tetrahydroquinoline derivatives, and possibly other such analogs, may prove useful in various neurological and biochemical investigations. Studies are currently being carried out to determine the mode of action(s) of these toxic agents.

Experimental

General methods

Proton and carbon NMR spectra were acquired using a Varian XL-300 MHz spectrometer. Mass spectra were recorded using a VG-ZAB-E instrument. Optical rotation was determined using a Autopol III automatic polarimeter. Melting points were determined using a Mel-Temp II apparatus and were uncorrected. Tetrahydrofuran (THF) was refluxed over calcium hydride and freshly distilled prior to use. Acetic anhydride was distilled from phosphorus pentoxide. Methanol was refluxed over Mg turnings and iodine, distilled, and stored over molecular sieves. Ethyl acetate was refluxed over sodium and benzophenone and

freshly distilled prior to use. All other reagents were employed without further purification.

4-(3,4-Dimethoxybenzylidene)-2-phenyl-5-oxazolone (2). Prepared as previously described.[6]

Methyl-2-benzoylamino-3-(3,4-dimethoxyphenyl)-2-propenoate (3). Prepared as previously described.[6]

(±) Methyl 2-benzoylamino-3-(3,4-dimethoxyphenyl)-propanoate (4). Compound **3** (18.07 g, 53 mmol) was dissolved in hot methanol (100 mL) and to this was added 10% palladium on carbon (1.81 g) and glacial acetic acid (1.81 mL). Hydrogenation was carried out at 45 psi for 12 h in a Parr shaker apparatus. The solution was bubbled with N_2, filtered through a bed of Celite, and the Celite was washed with 10 mL of hot methanol. The solvent was stripped to yield 18 g (99%) of hydrogenated product **4**; mp 105–107°C (Lit.[11] 105–106°C). ^{1}H NMR ($CDCl_3$): d=7.75 (d, *J*=7.2 Hz, 2H), 7.53–7.40 (m, 3H), 6.80 (d, *J*=8.1 Hz, 1H), 6.69 (d, *J*=1.8 Hz, 1H), 6.65 (dd, *J*=4.2 Hz, 1H), 6.7 (d, *J*=7.2 Hz, 1H, exch. CD_3OD), 5.08 (m, 1H), 3.87 (s, 3H), 3.79 (s, 3H), 3.77 (s, 3H), 3.26–3.21 (m, 2H). MS (70 eV-DIP) *m/z* (relative intensity) 343 (M^+, 14), 222 (100), 151 (48), 77 (25).

Methyl-2-benzoylamino-3-(3,4-dimethoxy-6-nitrophenyl)-propanoate (5). Benzylamidomethyl cinnamate (**4**) (2.69 g, 8 mmol) was dissolved in chloroform (26 mL), and the temperature was lowered to 0°C. Fuming nitric acid (1.59 mL) was added dropwise. The reaction was stirred at 0°C for 6 h. The reaction mixture was diluted with chloroform (20 mL) and neutralized with a sodium hydroxide solution to a neutral pH. The layers were separated, and the organic layer was dried (Na_2SO_4) and evaporated to give a yellow solid in a 99% yield. ^{1}H NMR ($CDCl_3$): d=7.74 (d, *J*=6.9 Hz, 2H), 7.57 (s, 1H), 7.53–7.41 (m, 3H), 7.07 (d, *J*=7.5 Hz, 1H, exch. CD_3OD), 6.83 (s, 1H), 5.09 (q, *J*=7.2 Hz, 1H), 3.94 (s, 3H), 3.88 (s, 3H), 3.80 (s, 3H), 3.59 (d, *J*=6.9 Hz, 2H). MS (70 eV-DIP) *m/z* (relative intensity) 388 (M^+, 6), 250 (28), 105 (100).

3-Benzoylamino-6,7-dimethoxy-1,2,3,4-tetrahydro-2-oxoquinoline (6). Compound **5** (403 mg, 1.04 mmol) was dissolved in dry methanol (40 mL) by heating. The solution was cooled to room temperature, and 10% palladium on carbon (49 mg) and glacial acetic acid (1 mL) were added. The reaction mixture was pressurized to 50 psi H_2 in a Parr shaker apparatus. The reaction was complete at 18 h. The solution was bubbled with N_2 and filtered through Celite. The Celite was washed with hot chloroform (10 mL), and the solvent was evaporated from the filtrate to yield a gummy solid. This solid was dissolved in 50 mL $CHCl_3$, washed with saturated sodium bicarbonate solution and water. The organic layer was separated, dried over sodium sulfate, and concentrated to give an off-white solid in quantitative yield. ^{1}H NMR ($CDCl_3$): d=7.90 (d, *J*=6.6 Hz, 2H), 7.8 (br, 1H, exch. CD_3OD), 7.56–7.46 (m, 3H), 7.34 (d, *J*=4.5 Hz, 1H, exch. CD_3OD), 6.78 (s, 1H), 6.40 (s, 1H), 4.76–4.68 (m, 1H), 3.88 (s, 3H), 3.66 (dd, *J*=15.2 and 6.5 Hz, 1H), 2.85 (d, *J*=14.6 Hz, 1H). MS (70 eV-DIP) *m/z* (relative intensity) 326 (M^+, 1), 205 (100).

3-Benzylamino-6,7-dimethoxy-1,2,3,4-tetrahydroquinoline (7). To **6** (2.17 g, 7 mmol) in THF (10 mL) was added BH_3/THF (1 M, 120 mL, 120 mmol) at room temperature under a N_2 atmosphere. This solution was heated at reflux for 8 h, allowed to cool, and slowly hydrolyzed with 6 M HCl until no more hydrogen evolved. This mixture was stirred for an additional 30 min, cooled, basified with a saturated sodium hydroxide solution, and extracted with chloroform (4×50 mL). The combined organic extracts were dried (sodium sulfate), filtered, and concentrated to a residue which was purified by column chromatography (SiO_2, 99:1 EtOAc/Et_3N). The yield of purified *N*-benzylamine derivative **8** was 1.76 g (89%). ^{1}H NMR ($CDCl_3$): d=7.39–7.26 (m, 5H), 6.54 (s, 1H), 6.14 (s, 1H), 3.93 (s, 2H), 3.80 (s, 6H), 3.37–3.32 (m, 1H), 3.16–3.12 (m, 2H), 2.94 (dd, *J*=15.9 and 4.4 Hz, 1H), 2.69 (dd, *J*=15.8 and 7.7 Hz, 1H). MS (70 eV-DIP) *m/z* (relative intensity) 298 (M^+, 100), 192 (43), 91 (44).

3-Amino-6,7-dimethoxy-1,2,3,4-tetrahydroquinoline (8). Compound **7** (1.16 g, 4 mmol) was suspended in glacial acetic acid (34 mL), and 10% palladium on carbon (239 mg) was added. Using a Brown hydrogenation apparatus, the reaction chamber was evacuated, pressurized to ~1 atm H_2, and stirred at room temperature for 42 h. The solution was filtered through a bed of Celite and the solvent evaporated to afford an oil. The oil was dissolved in chloroform (100 mL) and washed with a 10% sodium hydroxide solution followed with water, separated, dried (Na_2SO_4), and concentrated. The crude compound was purified using a column of silica gel (100 g) and 5% MeOH/$CHCl_3$. The purified amine was obtained in 94% yield (755 mg). ^{1}H NMR (DMSO-d^6): d=8.25 (bs, 2H, exch. CD_3OD), 6.56 (s, 1H), 6.22 (s, 1H), 3.65 (s, 3H), 3.63 (s, 3H), 3.50–3.48 (m, 1H), 3.29 (dd, *J*=11.6 and 1.7 Hz, 1H), 3.11 (dd, *J*=11.7 and 5.4 Hz, 1H), 2.96 (dd, *J*=16.4 and 5.3 Hz, 1H), 2.69 (dd, *J*=16.4 and 6.0 Hz, 1H). MS (70 eV-DIP) *m/z* (relative intensity) 208 (M^+, 100), 193 (51), 150 (12).

3-Amino-6,7-dihydroxy-1,2,3,4-tetrahydroquinoline dihydrobromide salt (9). To the dimethoxy derivative **8** (0.35 g, 2 mmol) in a pressure bottle was added 48% hydrobromic acid (1.85 mL), the solution was flushed with argon and heated for 6.5 h at reflux. The solution was lyophilized, and the crude solid obtained was washed with ether (6×2 mL) and acetonitrile (6×2 mL) to obtain 0.55 g (95%) of a tan solid. ^{1}H NMR (D_2O); d=6.76 (s, 1H), 6.68 (s, 0.5H, part. exch.), 3.93–3.90 (m, 1H), 3.76 (d, *J*=12.3 Hz, 1H), 3.40 (t, J_{app}=11.0 Hz, 1H), 3.18 (dd, *J*=16.4 and 5.1 Hz, 1H), 2.86 (dd, *J*=16.4 and 9.0 Hz, 1H). MS (70 eV-DIP) *m/z* (relative intensity) 180 (M^+, 16), 82 (83), 80 (100).

Rhodium complex [(COD)Rh(1,2-Bis((2*S*, 5*S*)-dimethylphospholano)-benzene)]$^+$ OTf$^-$.[10] To bis-(cyclooctadiene)-rhodium triflate (153 mg, 326 mmol) in dry THF (10 mL) was added 1,2-bis-((2*S*, 5*S*)-2,5-dimethylphosphalano)-benzene (100 mg, 326 mmol) in THF (3 mL). The initial yellow solution turned wine-red upon phosphine addition. The solution was stirred for 15 min followed by the slow addition of dry ether (30 mL). A bright orange crystalline compound precipitated; this was filtered, dried, and weighed (130 mg, 60%). ^{1}H NMR (CD_2Cl_2): d=7.75

(m, 4H), 5.62 (br, 2H), 5.05 (br, 2H), 2.75 (m, 2H), 2.65 (m, 2H), 2.20–2.60 (m, 12H), 1.95 (m, 2H), 1.55 (m, 2H), 1.45 (dd, J=18.2 and 7.1 Hz, 6H), 1.01 (dd, J=15.0 and 6.8 Hz, 6H).

The second rhodium complex, [(COD)Rh-(1,2-bis-((2*R*,5*R*)-dimethylphospholano)-benzene)]$^+$ OTf$^-$, was prepared in the same manner as that stated above with the exception that the phosphine ligand employed was 1,2-bis-((2*R*,5*R*)-2,5-dimethylphospholano)-benzene.

General procedure for enantioselective hydrogenations

To the alkene **3** (0.68 g, 2 mmol) dissolved in dry methanol (8 mL) was added the rhodium catalyst (2 mg). All the reagents were handled in a dry box. The reaction was pressurized to 35 psi H_2 and stirred at room temperature for 6 h. The solvent was stripped to afford a crude hydrogenated product which was purified by a short silica gel column (2% MeOH/$CHCl_3$) to yield 0.67 g of pure hydrogenated product. The proton NMR and mass spectral data obtained for the product matched the structural assignments. The proton NMR and mass spectral data obtained for all (*R*) and (*S*) compounds were in agreement with that of their racemic congeners.

(*R*)-(−)-Methyl-2-benzoylamino-3-(3,4-dimethoxyphenyl)-propanoate [(*R*)-(−)-4]. Compound **(*R*)-(−)-4** was prepared following the procedure described above for enantioselective hydrogenations. The rhodium catalyst used was [(COD)Rh-(1,2-bis-((2*R*, 5*R*)-dimethylphospholano)-benezene)]$^+$ OTf$^-$. The measured optical rotation was $[\alpha]_D^{25}$=−79(c 1.35, CH_2Cl_2).

(*R*)-(+)-Methyl-2-benzoylamino-3-(3,4-dimethoxy-6-nitrophenyl)-propanoate [(*R*)-(+)-5]. The experimental procedure followed was the same as that described for **5**; $[\alpha]_D^{25}$=+69 (c 1.0, CH_2Cl_2).

(*R*)-(−)-3-Benzoylamino-6,7-dimethoxy-1,2,3,4-tetrahydro-2-oxoquinoline [(*R*)-(−)-6]. The experimental procedure followed was the same as that described for **6**; $[\alpha]_D^{25}$=−38 (c 1.5, CH_2Cl_2).

(*R*)-(−)-3-Benzoylamino-6,7-dimethoxy-1,2,3,4-tetrahydroquinoline [(*R*)-(−)-7]. The experimental procedure followed was the same as that described for **7**; $[\alpha]_D^{25}$=−30 (c 1.3, CH_2Cl_2).

(*R*)-(−)-3-Amino-6,7-dimethoxy-1,2,3,4-tetrahydroquinoline [(*R*)-(−)-8]. The experimental procedure followed was the same as that described for **8**; $[\alpha]_D^{25}$=−26 (c 0.5, CH_2Cl_2).

(*R*)-(+)-3-Amino-6,7-dihydroxy-1,2,3,4-tetrahydroquinoline [(*R*)-(+)-9]. The experimental procedure followed was the same as that described for **9**; $[\alpha]_D^{25}$=+16 (c 0.9, H_2O).

(*S*)-(+)-Methyl-2-benzoylamino-3-(3,4-dimethoxyphenyl)-propanoate [(*S*)-(+)-4]. **(*S*)-(+)-4** was prepared following the procedure described above for enantioselective hydrogenations. The rhodium catalyst used for this conversion was [(COD)Rh-(1,2-bis-((2*S*, 5*S*)-dimethylphospholano)-benzene)]$^+$OTf$^-$. The optical rotation was measured to be $[\alpha]_D^{25}$=+81 (c 2.5, CH_2Cl_2).

(*S*)-(−)-Methyl-2-benzoylamino-3-(3,4-dimethoxy-6-nitrophenyl)-propanoate [(*S*)-(−)-5]. The experimental procedure followed was the same as that described for **5**; $[\alpha]_D^{25}$=−78 (c 1.6, CH_2Cl_2).

(*S*)-(+)-3-Benzoylamino-6,7-dimethoxy-1,2,3,4-tetrahydro-2-oxoquinoline [(*S*)-(+)-6]. The experimental procedure followed was the same as that described for **6**; $[\alpha]_D^{25}$=+41 (c 1.3, CH_2Cl_2).

(*S*)-(+)-3-Benzoylamino-6,7-dimethoxy-1,2,3,4-tetrahydroquinoline [(*S*)-(+)-7]. The experimental procedure followed was the same as that described for **7**; $[\alpha]_D^{25}$=+36 (c 1.7, CH_2Cl_2).

(*S*)-(+)-3-Amino-6,7-dimethoxy-1,2,3,4-tetrahydroquinoline [(*S*)-(+)-8]. The experimental procedure followed the same was the same as that described for **8**; $[\alpha]_D^{25}$=+30 (c 0.8, CH_2Cl_2).

(*S*)-(−)-3-Amino-6,7-dihydroxy-1,2,3,4-tetrahydroquinoline [(*S*)-(−)-9]. The experimental procedure followed was the same as that described for **9**; $[\alpha]_D^{25}$=−6 (c 0.9, H_2O).

4-(3,5-Dimethoxybenzylidene)-2-phenyl-5-oxazolone (11). 3,5-dimethoxybenzaldehyde (17 g, 102 mmol), hippuric acid (19.25 g, 107 mmol), anhydrous sodium acetate (8.39 g, 102 mmol) and acetic anhydride (50 mL, 530 mmol) were combined and heated at 85°C with continuous stirring for 2.5 h. During the reaction, the color of the mixture turned from white to a bright yellow. The reaction mixture was cooled to room temperature, cold ethanol (65 mL) was added, and the resultant yellow crystalline product was further cooled, then filtered with suction. The product was washed with cold ethanol (2×25 mL), boiling water (3×20 mL) and ice-cold ether (5 mL). The yield was 86%. ^{1}H NMR ($CDCl_3$): d=8.14 (bd, J_{app}=6.9 Hz, 2H), 7.63–7.49 (m, 3H), 7.40 (d, J=2.4 Hz, 2H), 7.15 (s, 1H), 6.65 (t, J=2.4 Hz, 1H), 3.87 (s, 6H).

Methyl-2-benzoylamino-3-(3,5-dimethoxyphenyl)-2-propenoate (12). Compound **11** (26.3 g, 85 mmol), anhydrous sodium carbonate (26.82 g, 255 mmol), and dry methanol (263 mL) were refluxed for 45 min, during which time the yellow color of the reaction mixture disappeared. The reaction mixture was filtered hot, and the solid was washed with hot methanol (2×15 mL). The collected filtrate was cooled in the refrigerator for at least 3 h. The white crystalline product which formed was collected by filtration and washed with 1:1 methanol/water (2×10 mL). The yield was 88%. ^{1}H NMR ($CDCl_3$): d=7.85 (bd, J_{app}=7.2 Hz, 2H), 7.7 (bs, 1H), 7.56–7.43 (m,3H), 7.36 (s, 1H), 6.64 (d, J=2.1 Hz, 2H), 6.40 (t, J=2.1 Hz, 1H), 3.85 (s,3H), 3.65 (s, 6H).

Methyl-2-benzoylamino-3-(3,5-dimethoxyphenyl)propanoate (13). Compound **12** (9.89 g, 29 mmol) was dissolved in hot THF (55 mL, dry). To this solution was added dry methanol (55 mL), 10% palladium on carbon

(1.50 g) and glacial acetic acid (0.75 mL). The hydrogenation was carried out at 60 psi H_2 for 10 h in a Parr shaker apparatus. The solution was filtered through Celite. The Celite was washed with hot methanol/chloroform (1:3, 3×10 mL), and the solvent was evaporated, providing a white solid in quantitative yield. ^{1}H NMR ($CDCl_3$): d=7.73 (d, *J*=7.8 Hz, 2H), 7.52–7.38 (m, 3H), 6.59 (d, *J*=7.2 Hz, 1H), 6.33 (d, *J*=2.4 Hz, 1H), 6.26 (d, *J*=2.4 Hz, 2H), 5.06 (dd, *J*=12.9 and 5.4 Hz, 1H), 3.77 (s, 3H), 3.69 (s, 6H), 3.25–3.12 (m, 2H).

Methyl-2-benzoylamino-3-(3,5-dimethoxy-6-nitrophenyl)-propanoate (14). Compound **13** (3.41 g, 9.9 mmol) was dissolved in glacial acetic acid (136 mL), and purged with N_2. To this solution was added, dropwise, 70.4% nitric acid (2.25 mL, 39.6 mmol) mixed with glacial acetic acid (4 mL). The reaction was stirred at 15°C for 24 h. The reaction mixture was neutralized with a sodium hydroxide solution to a neutral pH. Chloroform (50 mL) was added, the layers separated, the chloroform layer dried (Na_2SO_4), and the solvent evaporated to give a blackish product which was purified by column chromatography (SiO_2, 75:25 $CHCl_3$/EtOAc, 40 g SiO_2/g crude). Yield of the pure nitrated product, which was off-white, was 19%. ^{1}H NMR ($CDCl_3$): d=7.78 (bd, J_{app}=6.9 Hz, 2H), 7.51–7.38 (m, 3H), 7.1 (d, *J*=7.0 Hz, 1H), 6.43 (d, *J*=2.4 Hz, 1H), 6.39 (d, *J*=2.4 Hz, 1H), 4.98 (ddd, *J*=9.3 and 7.1 and 5.1 Hz, 1H), 3.83 (s, 3H), 3.77 (s, 3H), 3.76 (s, 3H), 3.23 (dd, *J*=14.4 and 5.1 Hz, 1H), 3.03 (dd, *J*=14.4 and 9.3 Hz, 1H).

3-Benzoylamino-6,8-dimethoxy-1,2,3,4-tetrahydro-2-oxo-quinoline (15). The experimental procedure for **6** was followed with the exception of the solvent and the reaction time. The nitro compound **14** (4.25 g, 10.9 mmol) was dissolved in a hot mixture of dry methanol (140 mL), dry THF (6 mL), 1,4-dioxane (120 mL) and reacted for 48 h. This resulted in a yield of 90%. ^{1}H NMR ($CDCl_3$): d=7.86 (bd, J_{app}=6.9 Hz, 2H), 7.71 (bs,1H), 7.54–7.41 (m, 3H), 7.33 (d, *J*=4.3 Hz, 1H), 6.39 (d, *J*=2.7 Hz, 1H), 6.37 (d, *J*=2.1 Hz, 1H), 4.66 (ddd, *J*=14.1 and 6.3 and 4.4 Hz, 1H), 3.84 (s, 3H), 3.78 (s, 3H), 3.69 (dd, *J*=15.3 and 6.3 Hz, 1H), 2.83 (dd, *J*=15.3 and 14.1 Hz, 1H).

3-Benzylamino-6,8-dimethoxy-1,2,3,4-tetrahydroquino-line (16). The experimental procedure followed was the same as that described for **7**. Reaction time was 3 h. The yield of **16** obtained was 90%. ^{1}H NMR ($CDCl_3$): d=7.38–7.22 (m, 5H), 6.28 (d, *J*=2.4 Hz, 1H), 6.17 (d, *J*=2.4 Hz, 1H), 3.89 (s, 2H), 3.78 (s, 3H), 3.72 (s, 3H), 3.36 (bt, J_{app}=7.2 Hz, 1H), 3.15–3.08 (m, 2H), 2.95 (dd, *J*=16.5 and 3.9 Hz, 1H), 2.69 (dd, *J*=16.5 and 7.5 Hz, 1H). MS (70 eV-DIP) *m/z* (relative intensity) 298 (M^+, 100), 192 (88).

3-Amino-6,8-dimethoxy-1,2,3,4-tetrahydroquinoline (17). The experimental procedure followed was the same as described for **6** with slight modification. A reaction time of 98 h in a Parr shaker apparatus (65 psi H_2) was employed. The product was obtained in 83% yield. ^{1}H NMR ($CDCl_3$): d=6.28 (d, *J*=2.4 Hz, 1H), 6.16 (d, *J*=2.7 Hz, 1H), 3.78 (s, 3H), 3.71 (s, 3H), 3.39–3.32 (m, 1H), 3.30 (bd, J_{app}=11.7 Hz, 1H), 3.06–2.95 (m, 2H), 2.46 (dd, *J*=16.2 and 6.3 Hz, 1H). MS (70 eV-DIP) *m/z* (relative intensity) 208 (M^+, 100), 193 (34), 192 (23).

3-Amino-6,8-dihydroxy-1,2,3,4-tetrahydroquinoline hydrobromide (18). The experimental procedure followed was the same as described for **9**. Compound **17** was refluxed for 26 h, providing a tan solid with a yield of 86%. ^{1}H NMR (D_2O): d=6.38 (d, *J*=2.7 Hz, 0.5H, part, exch.), 6.3 (d, *J*=2.4 Hz, 1H), 3.98–3.88 (m, 1H) [alternatively, this multiplet could be reported as 3.93 (dddd, *J*=9.9, 9.6, 5.3 and 3.5 Hz, 1H)], 3.83 (ddd, *J*=12.3, 3.5 and 1.7 Hz, 1H) 3.42 (dd, *J*=12.3 and 10.2 Hz, 1H), 3.23(bdd, J_{app}=16.8 and 5.4 Hz, 1H), 2.94 (dd, *J*=16.8 and 9.3 Hz, 1H). MS (70 eV-DIP) *m/z* (relative intensity) 180 (M^+, 100), 164 (22), 82 (53), 80 (55).

Dimethyl-3,4-dimethoxybenzylidenemalonate (20). Prepared as previously described.[12,13]

Dimethyl-3,4-dimethoxybenzylmalonate (21). Prepared as previously described.[12,13]

Dimethyl-3,4-dimethoxy-6-nitrobenzylmalonate (22). Experimental procedures employed to synthesize **5** were followed. Fuming nitric acid (16.3 mL, 354 mmol) was added dropwise to a solution of dimethyl-3,4-dimethoxybenzylmalonate **21** (16.6 g, 58.9 mmol) dissolved in chloroform (160 mL) at 0°C with a nitrogen atmosphere. The reaction was stirred at 0°C for 4 h. The reaction mixture was neutralized and washed with a saturated sodium hydroxide solution followed by water. The organic layer was dried (Na_2SO_4), and solvent stripped to give a yellow solid in quantitative yield. ^{1}H NMR ($CDCl_3$): d=7.63 (d, *J*=2.1 Hz, 1H), 6.78 (d, *J*=2.1 Hz, 1H), 3.96–3.93 (m, 1H), 3.92 (s, 6H), 3.69 (s, 6H), 3.49 (dd, *J*=7.5 and 2.1 Hz, 2H).

3-Methylester-6,7-dimethoxy-1,2,3,4-tetrahydro-2-oxo-quinoline (23). The experimental procedure followed was the same as described for **6** with slight modification. The nitrophenyl derivative **22** (1.2 g, 3.67 mmol) was dissolved in a mixture of warm THF (15 mL, dry) and methanol (60 mL, dry), then 10% Pd/C and glacial acetic acid (0.6 mL) were added. The white precipitate, collected by suction filtration, was pure product and was obtained in a 72% yield. ^{1}H NMR ($CDCl_3$): d=7.69 (bs, 1H), 6.68 (s, 1H), 6.31 (s, 1H), 3.83 (s, 6H), 3.74 (s, 3H), 3.60 (dd, *J*=8.4 and 6.3 Hz, 1H), 3.30 (dd, *J*=15.9 and 8.4 Hz, 1H), 3.05 (dd, *J*=15.9 and 6.3 Hz, 1H). MS (70 eV-DIP) *m/z* (relative intensity) 265 (M^+, 21), 206 (100). MS (FAB) *m/z* (relative intensity) 266 (M+1, 100), 265 (98), 206 (54).

3-Carboxamide-6,7-dimethoxy-1,2,3,4-tetrahydro-2-oxo-quinoline (24). To a pressure bottle was added the methyl-ester derivative **23** (200 mg, 0.755 mmol), methanol (8 mL) and THF (8 mL). This mixture was heated to dissolve the ester, then cooled to 0°C and degassed with argon. To this 0°C solution was added ammonium hydroxide (4 mL), then saturated with ammonia gas, and the pressure bottle sealed and heated to 65°C for 22 h. A white precipitate began to form as the reaction proceeded. The solution was cooled to room temperature and the precipitate was collected by suction filtration. Any further purification is not recommended due to the instability of the product. A yield of 24% was obtained. ^{1}H NMR (DMSO-d^6): d=7.43 (s, 1H), 7.07 (s, 1H), 6.81 (s, 1H), 6.49 (s, 1H), 3.69 (s, 3H), 3.67 (s, 3H), 3.28 (dd, *J*=9.9 and 6.6 Hz, 1H), 3.08 (dd, *J*=15.9 and

9.9 Hz, 1H), 2.88 (dd, J=15.9 and 6.6 Hz, 1H). MS (70 eV-DIP) m/z (relative intensity) 250 (M^+, 32), 206 (100). MS (12 eV-DIP) m/z (relative intensity) 250 (M^+, 30), 206 (100). MS (FAB) m/z (relative intensity) 251 (M+1, 100), 250 (63), 206 (81).

3-Aminomethyl-6,7-dimethoxy-1,2,3,4-tetrahydroquinoline (25). The experimental procedure followed was the same as described for **7** with slight modification. The carboxamide compound **24** (100 mg, 0.4 mmol) and BH_3/THF (1 M, 9.6 mL, 9.6 mmol) was heated at reflux for 3.5 h with continuous stirring. This reaction gave a pure product with a yield of 88%. ^{1}H NMR ($CDCl_3$): d=6.50 (s, 1H), 6.09 (s, 1H), 3.76 (s, 6H), 3.33 (dd, J=10.8 and 3.3 Hz, 1H), 2.94 (dd, J=10.8 and 8.7 Hz, 1H), 2.76 (dd, J=15.9 and 5.1 Hz, 1H), 2.69 (dd, J=7.2 and 2.4 Hz, 2H), 2.39 (dd, J=15.9 and 8.7 Hz, 1H), 2.00–1.86 (m, 1H). MS (70 eV-DIP) m/z (relative intensity) 222 (M^+, 100), 192 (12), 190 (70).

3-Aminomethyl-6,7-dihydroxy-1,2,3,4-tetrahydroquinoline hydrobromide (26). The experimental procedure followed was the same as that described for **9**. A light tan solid was isolated for this reaction in a 74% yield. ^{1}H NMR (D_2O); d=6.79 (s, 1H), 6.75 (s, 0.5H, part. exch.), 3.67 (bd, J_{app}=11.1, 1H), 3.19–3.03 (m, 3H), 2.94 (dd, J=15.6 and 3.9 Hz, 1H), 2.59 (dd, J=15.6 and 10.8 Hz, 1H), 2.54–2.44 (m, 1H). MS (12 eV-DIP) m/z (relative intensity) 194 (M^+, 76), 176 (26).

Mouse brain neurotransmiter levels

ICR:Hsd male mice, weighing approximately 30 g at the time of experiment, from Harlan Sprague-Dawley (Madison, WI) were employed. Animals had access to food and water ad libitum, and a minimum of one week was allowed between receipt of animals and commencement of the depletion studies. To observe the maximal depletion capabilities for each tested toxin, we employed dosages which provided approximately 50% survival rates for the tested animals. Bilateral intracerebroventricular injections (3.5 μL total volume, isotonic saline containing the test compound) were performed under a light ether anesthesia.[17] Control animals were injected with the same volume of isotonic saline. Animals were sacrificed seven days following injection by microwave irradiation (7 kW, 150 ms) using a NJE model 2603–10 kW (New Japan Radio, Tokyo, Japan).[18,19] Brains were removed and prepared for analysis as previously described.[20] Brains weights ranged from 450–525 mg. Transmitter levels were determined utilizing liquid chromatography with electrochemical detection.[20,21] Typical control values for the neurotransmitters determined were (nmol/g, mean±SEM): NE, 2.98±0.08; DA, 8.19±0.2; and 5-HT, 4.5±0.06.

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