Dopaminergic Benzo[a]phenanthridines: Resolution and Pharmacological Evaluation of the Enantiomers of Dihydrexidine, the Full Efficacy D_1 Dopamine Receptor Agonist

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Racemic *trans*-10,11-dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine (**2**, dihydrexidine) was shown previously to be the first bioavailable full efficacy agonist at the D_1 dopamine receptor. In addition to its full D_1 agonist properties, **2** also is a good ligand for D_2-like dopamine receptors. The profound anti-Parkinsonian actions of this compound make determination of its enantioselectivity at both D_1 and D_2 receptors of particular importance. To accomplish this, the enantiomers were resolved by preparation of diastereomeric (*R*)-*O*-methylmandelic acid amides of racemic *trans*-10,11-dimethoxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine **4** that were then separated by centrifugal chromatography. An X-ray analysis of the (−)-*N*-(*R*)-*O*-methylmandel diastereoamide revealed the absolute configuration to be 6a*S*,12b*R*. Removal of the chiral auxiliary and *O*,*O*-deprotection afforded enantiomeric amines that were then tested for biological activity. In striatal membranes, the (6a*R*,12b*S*)-(+)-enantiomer **2** had about twice the affinity of the racemate and 25-fold greater affinity than the (−)-enantiomer at the D_1 receptor labeled by [^{3}H]SCH23390 ($K_{0.5}$s of 5.6, 11.6, and 149 nM, respectively). Similarly, the (+)-enantiomer **2** had about twice the affinity of the racemate for human D_1 receptors expressed in transfected Ltk$^-$ cells. Functionally, the (+)-enantiomer of **2** was a full agonist, with an EC_{50} of 51 nM in activating striatal dopamine-sensitive adenylate cyclase versus 2.15 μM for the (−)-enantiomer. With respect to D_2-like receptors, (+)-**2** had a $K_{0.5}$ of 87.7 nM in competing with [^{3}H]spiperone at D_2 binding sites in rat striatal membranes versus about 1 μM for the (−)-enantiomer. Together, these data demonstrate that both the D_1 and D_2 activities of dihydrexidine reside principally in the (6a*R*,12b*S*)-(+)-enantiomer. The results are discussed in the context of structure–activity relationships and conceptual models of the D_1 receptor.

Introduction

Dopamine functions as a neurotransmitter in both the central and peripheral nervous systems. Dopamine receptors have been implicated in several neurological disorders such as schizophrenia and Parkinson's disease, as well as in vascular regulation. Additionally, dopamine receptors are the accepted loci of action of many psychotropic drugs, including amphetamine, cocaine, and neuroleptics. Thus, ligands selective for dopamine receptors are important as basic research tools and potential therapeutic agents.

Although the field is in a state of flux, the commonly accepted classification divides dopamine receptors into two general classes called D_1 and D_2,[1] with each group comprising several molecular forms. The D_1-like receptors include at least two gene products (the D_{1a}[2,3] and the D_{1b}[4] or D_5[5]), which are linked functionally to stimulation of cAMP synthesis and preferentially recognize 1-phenyltetrahydrobenzazepines (e.g., SCH23390) versus benzamides (e.g., raclopride or sulpiride). The D_2-like receptors come from at least three genes and include multiple splice variants. The D_2-like receptors (D_{2long},[6] D_{2short},[7] D_3,[8] and D_4[9]) sometimes are linked to inhibition of cAMP synthesis and have the opposite pharmacological specificity from the D_1-like receptors (i.e., having much higher affinity for spiperone or sulpiride versus that for SCH23390).

Because of a prevailing view that D_2-like receptors were responsible for almost all of the clinically important actions of dopamine agonists and antagonists, D_1-like receptors received little attention until the mid-1980s. With the availability of SCH23390 (the first D_1-selective antagonist[10]), it soon became clear that the D_1 receptor had profound psychopharmacological effects and interacted in important ways with D_2 receptors.[11,12] Yet while several excellent antagonists were made from the 1-phenyltetrahydro-3-benzazepine series, the resulting agonists (e.g., SKF38393, **1**) were generally only of partial efficacy relative to dopamine (i.e., in stimulating dopamine-sensitive cAMP synthesis). Despite this potential limitation, SKF38393 and related partial agonists have been used in many studies of D_1 receptor function because they were relatively selective and because no alternatives were available.

We had hypothesized that there would be important pharmacological differences between full and partial D_1 agonists. We therefore directed our efforts toward the characterization of ligand interactions with D_1-like receptors, with the goal of developing D_1 agonists of full

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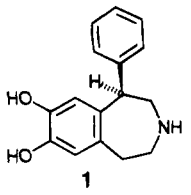
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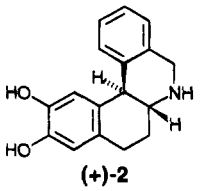
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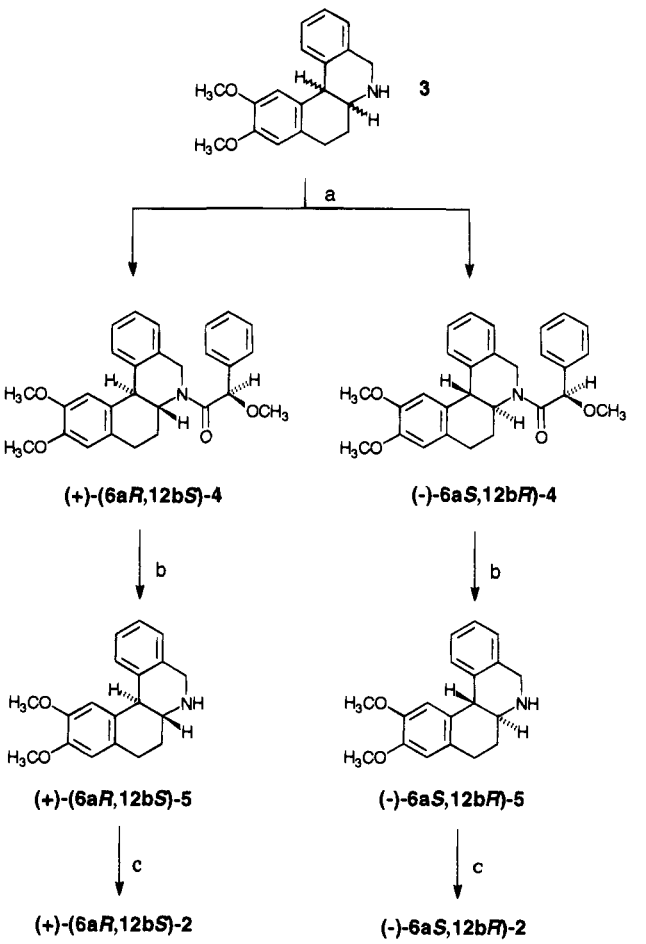
intrinsic activity. On the basis of a conformationally rigid β-phenyldopamine pharmacophore model, these efforts resulted in the synthesis of racemic *trans*-10,-11-dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine (dihydrexidine, **2**[13]), the first potent and bioavailable full efficacy D_1 agonist.[13,14] In addition to its use as a pharmacological probe, **2** has been shown to have impressive anti-Parkinsonian action in the MPTP monkey model.[15] More recently, similar anti-Parkinsonian effects were reported for members of another new class of full D_1 agonists, the phenyl(aminomethyl)-isochromans.[16] Such data underscore the importance of understanding the pharmacophore for the D_1-like receptors.

The elucidation of a model of the D_1 pharmacophore requires that the active enantiomer of **2** be known. Previous studies, both *in vivo* and *in vitro*, have used the racemate. Moreover, while (±)-**2** is selective for dopamine receptors, it only has about 10-fold selectivity for D_1 versus D_2 receptors in rat striatum.[13,17] Of particular interest is the unprecedented selectivity of **2** for activating functions mediated by postsynaptic, but not presynaptic, D_2-like receptors.[17-19] It was therefore important to determine in which enantiomer(s) the D_1 and D_2 activities of **2** were contained. The present report describes the successful resolution of the enantiomers of **2** and concludes that both D_1 and D_2 receptor affinities, as well as functional effects, reside in the (6a*R*,12b*S*)-(+)-enantiomer.

Chemistry

Initial attempts to resolve the enantiomers of **2** utilizing classical resolution techniques were ineffective. The tartrate and dibenzoyltartrate diastereomeric salts of the ether-protected amine precursor **3** were inseparable by recrystallization. The successful resolution was accomplished as depicted in Scheme 1. The diastereomeric (*R*)-*O*-methylmandelic acid amides of racemic *trans*-10,11-dimethoxy-5,6,6a,7,8,12b-hexahydrobenzo-[*a*]phenanthridine **4** were prepared according to the procedure of Johansson et al.[20] The secondary amine **3**, prepared as previously described,[13] was coupled with (*R*)-*O*-methylmandeloyl chloride to yield the two diastereomeric amides. The resulting amides were separated by centrifugal chromatography (chromatotron) using 40% ethyl acetate/hexane eluent and a 2 mm silica gel rotor. Crystallization of the individual amides provided the diastereomers in greater than 99% purity, as judged by HPLC analysis. An X-ray quality crystal was obtained for the diastereomer with lower R_f on TLC, and the analysis demonstrated that it corresponded to (6a*S*,12b*R*)-**4** (see Figure 5). Reductive cleavage of the chiral auxiliaries of (6a*R*,12b*S*)-**4** and (6a*S*,12b*R*)-**4** with $LiEt_3BH$ (super hydride) in THF[21] provided the resolved amines (6a*R*,12b*S*)-(+)-**5** and (6a*S*,12b*R*)-(−)-**5**. The enantiomerically pure catecholamines (6a*R*,12b*S*)-(+)-**2** and (6a*S*,12b*R*)-(−)-**2** were prepared from the corresponding methoxy precursors by treatment with BBr_3 and crystallized as their hydrochloride salts.

Scheme 1[a]



[a] (a) i. (*R*)-α-Methoxyphenylacetyl chloride, 20% NaOH (aq), CH_2Cl_2, ii. chromatography; (b) $LiEt_3BH$, THF; (c) BBr_3, CH_2Cl_2.

Pharmacology

The enantiomers of **2** were tested for dopaminergic activity using *in vitro* biochemical techniques; (+)-**2** had about twice the affinity of (±)-**2** for the D_1 receptor in both rat striatal membranes and in transfected Ltk^- cells. In the striatal membranes, the (−)-enantiomer shows significantly less affinity than either the racemate or (+)-enantiomer. Like racemic **2** or dopamine, (+)-**2** causes a doubling of cAMP synthesis in striatal membranes, indicating that (+)-**2** is a full agonist. Conversely, (−)-**2** is nearly 100-fold less potent than (+)-**2** and does not cause a fully maximal response (relative to dopamine) at the highest concentration tested (5 μM). In competition for D_2 receptors, (+)-**2** exhibits about twice the affinity of (±)-**2** and about 15-fold higher affinity than the (−)-enantiomer. These data indicate that the active enantiomer for both D_1 and D_2 receptors is (6a*R*,12b*S*)-(+)-**2**.

Results and Discussion

The primary goal of this work was to test the previously proposed hypothesis[13] that the D_1 affinity and functional efficacy of dihydrexidine (**2**) reside in the (6a*R*,12b*S*)-enantiomer. Figure 1 illustrates that, in both rat striatum and Ltk^- L-hD_1 cells, (6a*R*,12b*S*)-(+)-**2** had approximately twice the affinity (i.e., IC_{50} = 5.6 nM)

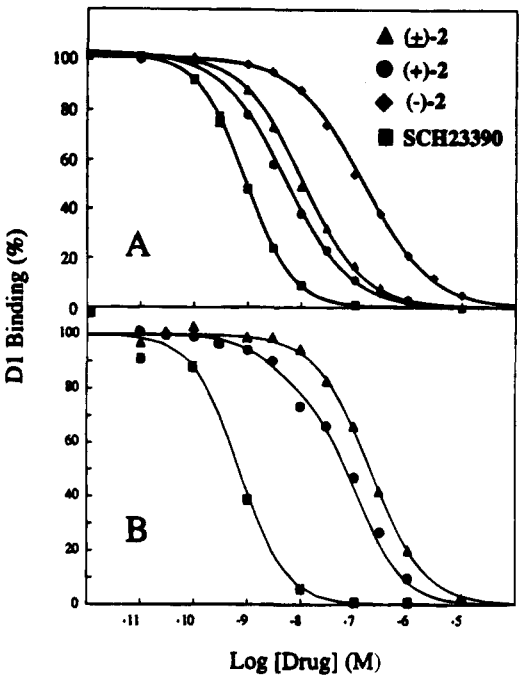


Figure 1. Competition of enantiomers of **2** for D_1 receptors labeled by [^{3}H]SCH23390. (A) Competition in rat striatal membranes. (B) Competition in membranes prepared from Ltk$^-$ cells transfected with human D_1 receptor. (+)-**2** had ca. twice the affinity of racemic **2** in both preparations. In the striatal membranes, (−)-**2** had significantly less affinity than the racemate or the (+)-enantiomer.

Table 1. Comparison of Dihydrexidine and Its Analogues and Certain Other Dopamine Agonists at D_1 and D_2 Receptors in Rat Striatal Membranes

drug	D_1 potency[a] IC_{50} (nM)	D_2 potency IC_{50} (nM)	adenylate cyclase EC_{50} (nM)	maximal stimulation of adenylate cyclase (% versus DA)
(±)-**2**	11.6 ± 1.0	132 ± 19	140	95
(+)-**2**	5.6 ± 1.1	87.7 ± 12.2	60	105
(−)-**2**	149 ± 11	1250 ± 290	>1000	nd
SCH23390	1.01 ± 1.0		NA	NA
domperidone		2.80 ± 1.0	NA	NA

[a] All tests were performed as described in the methods section. Hill coefficients for the agonist binding curves were significantly less than 1, and the data thus are expressed as IC_{50}s since K_i values cannot be determined until the number of binding sites is resolved. To minimize interassay variability, the values in the tables were from assays in which four or more compounds were run on the same day. NA = not applicable (these compounds are antagonists).

of the racemate, which in turn had the high D_1 affinity (IC_{50} = 11.6 nM) expected from earlier studies.[13,17] The (6a*S*,12b*R*)-(−)-enantiomer had significantly lower affinity than either (6a*R*,12b*S*)-(+)-**2** or (±)-**2** at the D_1 receptor.

The data from the dopamine-sensitive adenylate cyclase assay (Table 1 and Figure 2) demonstrate that the (6a*R*,12b*S*)-(+)-enantiomer and the racemate are about 2 orders of magnitude more potent than dopamine, the latter having an EC_{50} of about 5 μM.[13] Furthermore, (6a*R*,12b*S*)-(+)-**2** and the racemate both produce a full agonist response of adenylate cyclase, whereas (6a*S*,12b*R*)-(−)-**2** is significantly less potent (EC_{50} = 2.15 μM) in the adenylate cyclase assay and lacks the full efficacy character of the (+)-antipode. It is of note that prototypical D_1 agonists such as SKF

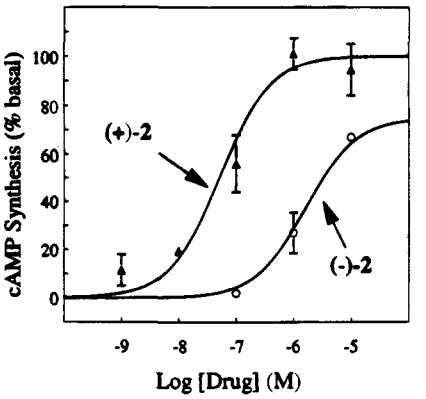


Figure 2. Stimulation of cAMP synthesis by drugs in rat striatal membranes. (+)-**2** was nearly 100-fold more potent than the (−)-enantiomer in stimulating cAMP synthesis in rat striatal membranes.

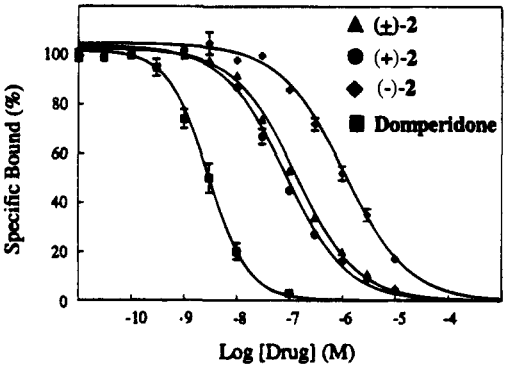


Figure 3. Competition of enantiomers of **2** for D_2 receptors in rat striatal membranes labeled by [^{3}H]spiperone. (+)-**2** had about twice the affinity of racemic **2**, and both the racemate and the (+)-enantiomer had significantly greater affinity than the (−)-enantiomer.

38393 (**1**; EC_{50} = 100 nM)[13] and CY 208−243 (**6**; pD_2 = 6.1)[23] are only partial agonists that cause less than 50% stimulation of adenylate cyclase. A similar pattern was seen in transfected Ltk$^-$ cells (data not shown). This is of importance because there are no D_2-like receptors in these cells. Thus, actions of enantiomers of **2** at D_2 receptors do not influence adenylate cyclase studies in these membrane preparations, consistent with our earlier findings.[24]

While these data demonstrate that the D_1 activity resides in the (6a*R*,12b*S*)-(+)-enantiomer, it was also important to determine in which enantiomer the significant D_2 affinity resided. As shown in Figure 3 and Table 1, here again (6a*R*,12b*S*)-(+)-**2** had about twice the potency of the racemate, with (6a*S*,12b*R*)-(−)-**2** having significantly lower affinity (87.7, 132, and 1250 nM, respectively). Together, our data indicate that both receptor recognition and functional efficacy at D_1 receptors reside principally in the (6a*R*,12b*S*)-(+)-stereoisomer. At D_2 receptors, the binding affinity also is contained in the same isomer. One would reasonably anticipate that functional studies, while not yet performed, would probably follow this same pattern.

At present, no model exists to explain satisfactorily the significant changes in bioactivity that are associated with subtle structural modifications. A *true* understanding of the dopamine D_1 receptor will not be possible until molecular biologists are able to generate a biochemical form of the receptor that may be studied by X-ray crystallography, perhaps in an agonist-bound

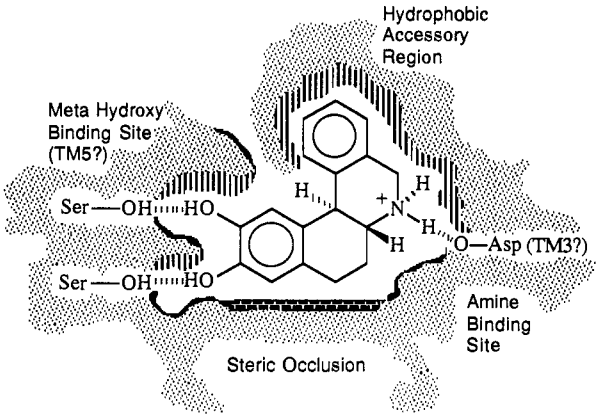
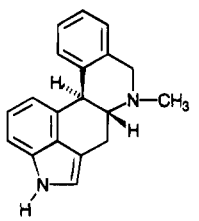


Figure 4. Schematic model of ligand interaction with D_1 receptors.

state. Until then, schematic representations of the receptor via molecular modeling efforts must be based upon the correlation of structural features of available compounds with biological data.

The conceptual dopamine receptor model originally proposed by McDermed et al.[25] contains a stereospecific binding site with regions of association for the amine and meta hydroxy moieties, in addition to a region of steric occlusion. On the basis of the postulated binding orientation of *R*-(+)-6,7-ADTN[25] in the McDermed model, in an earlier paper[13] we illustrated an energy-minimized conformation for the active enantiomer of dihydrexidine and predicted the (6a*R*,12b*S*)-isomer to be the active enantiomer. Furthermore, a recent report of the resolution and biological testing of the enantiomers of CY208–243[26] has demonstrated that the stereochemistry of the more active (−)-isomer **6** corresponds to the 6a*R*,12b*S* configuration of dihydrexidine, leaving little doubt regarding the pharmacophoric orientations of the two different molecular templates at the dopamine D_1 receptor.



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From the results of this work, it is possible to extend concepts addressed in the general McDermed model specifically to encompass the dopamine D_1 receptor (Figure 4). On the basis of the amino acid sequence of the receptor and by analogy to other G-protein-coupled receptors, we speculate that serine residues in transmembrane helix 5 (Ser 199 and 202) may bind to the catechol function.[27] It also seems to be the general consensus that for the monoamine receptors a conserved aspartate in transmembrane helix 3 (Asp 103 in the present instance) binds to the protonated amine through a salt bridge. We propose that for the D_1 receptor this interaction occurs through the N—H that adopts the pseudoequatorial orientation.[28]

In addition to a requirement for both hydroxyls of the catechol and the illustrated absolute configuration, the D_1 receptor must include an accessory binding region

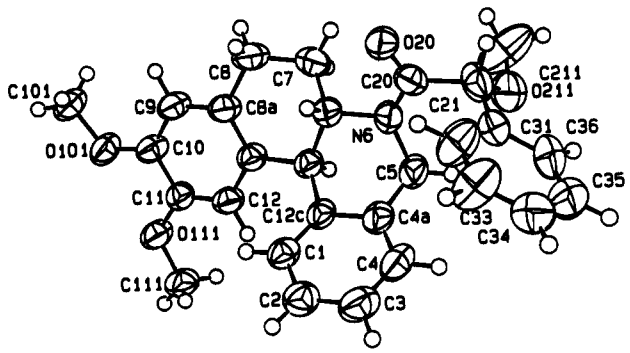

Figure 5. An ORTEP view of (−)-(6a*S*,12b*R*)-*N*-((*R*)-*O*-methylmandeloyl)-10,11-dimethoxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine. Solid state conformation and crystallographic numbering scheme where small circles denote hydrogen atoms.

for the unsubstituted pendent aromatic ring (see Figure 4). We have shown earlier[13] that the addition of this pendent phenyl ring does not confer D_1 selectivity simply by excluding the ligand from D_2-like receptors but actually enhances affinity and efficacy at the D_1 receptor. This structural feature is probably an important contributor to the D_1 selectivity of **6**, which also lacks a catechol function but is not a full agonist. This perception of the D_1 receptor suggests that selectivity of the hexahydrobenzo[*a*]phenanthridines for the D_1 receptor is dependent upon the addition of an aromatic (pendent phenyl) ring. Furthermore, the *R* configuration at C-12b in **2** corresponds to the stereochemistry of the active enantiomer of **1**. The region of steric occlusion shown in Figure 4 is not addressed by the present studies but was a feature of the original McDermed dopamine receptor model; in the absence of any evidence to the contrary, we have included it here. We have previously indicated that the full efficacy properties of dihydrexidine may be due to the presence of the trans extended conformation of the ethylamine moiety or the near coplanarity of the aromatic rings. On the basis of the present work, the *enantioselective* conceptual model of the D_1 receptor appears to be vastly superior to two-dimensional models in accounting for the biological activity of these compounds. Ongoing studies in our laboratories with various structurally constrained β-phenyldopamine molecules will contribute to a more accurate three-dimensional conceptual model of the dopamine D_1 receptor.

One consequence of this work that is yet unaddressed is the ramification of the D_2-like affinity of dihydrexidine and several of its analogues. Existing models of the pharmacophore for various D_2-like receptors do not, at least at first glance, easily accommodate the pendent phenyl ring of the 10,11-dihydroxybenzo[*a*]phenanthridine class of dopamine agonists. Moreover, while dihydrexidine has affinity for D_2-like receptors, recent data indicate that its agonist *functional* properties appear to be limited to only some subpopulations of these receptors (i.e., those located postsynaptically).[17,18] Thus, this drug class may be very useful in understanding ligand interactions with the D_2-like receptor family and may provide important data for incorporation into molecular modeling studies. While outside the present focus on D_1-like receptors, these findings warrant further study.

Experimental Section

Chemistry. Materials and Methods. Melting points were determined on a Thomas-Hoover Meltemp apparatus and are uncorrected, except where indicated. ^{1}H NMR spectra were recorded on a Chemagnetics 200 MHz or a Varian VXR-500S 500 MHz instrument. Chemical shifts are reported in δ values (parts per million, ppm) relative to an internal standard of tetramethylsilane in $CDCl_3$, except where noted. Abbreviations used in NMR analysis are as follows: s, singlet; d, doublet; t, triplet; m, multiplet; dt, doublet of triplets. Analytical thin-layer chromatography (TLC) was performed on Baker-flex silica gel 1B2-F plastic plates. Microanalyses were obtained from the Purdue Microanalytical Laboratory and Galbraith Laboratories, Inc. The chemical ionization mass spectra (CIMS) were determined on a Finnigan 4000 quadrupole spectrometer using ammonia or isobutane as the reagent gas, as noted, and are reported as *m/e* (relative intensity). Optical rotations were obtained on a Perkin-Elmer 241 polarimeter. Solvents and reagents were used as purchased, except as noted. THF was distilled from sodium metal/benzophenone ketyl.

(6a*R*,12b*S*)-(+)-6-((*R*)-α-Methoxyphenylacetyl)-10,11-dimethoxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine, (+)-4, and (6a*S*,12b*R*)-(−)-6-((*R*)-α-Methoxyphenylacetyl)-10,11-dimethoxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine, (−)-4. *R*-(−)-α-Methoxyphenylacetic acid (31 mg, 0.19 mmol; $[\alpha]^{25}$ −154°; Aldrich Chemical Co.) was added to a 10 mL round bottom flask containing thionyl chloride (0.5 mL). After stirring for 2 h at 25°, the volatiles were removed. Residual thionyl chloride was removed azeotropically by the codistillation of benzene to provide (*R*)-(−)-*O*-methylmandeloyl chloride. This residue was dissolved in 1 mL of dichloromethane. Racemic 10,11-dimethoxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine hydrochloride[13] (50 mg, 0.15 mmol) was dissolved in water (1 mL) and dichloromethane (1 mL). After the salt had dissolved, 1N NaOH (0.5 mL) was added followed by addition of the dichloromethane solution of the mandeloyl chloride. When TLC analysis (5% methanol/dichloromethane, NH_3 atmosphere) indicated that all of the amine had been consumed, the layers were separated, and the aqueous layer was extracted with dichloromethane (3 × 5 mL). The pooled dichloromethane fractions were washed sequentially with saturated aqueous sodium carbonate solution (10 mL), 5% HCl (2 × 5 mL), and brine. After drying over $MgSO_4$, the dichloromethane solution was filtered and the solvent was removed by rotary evaporation. The residual oil was then separated into its components using a chromatotron (Harrison Research, Palo Alto, CA) by elution on a 1 mm silica gel rotor with 40% ethyl acetate/hexane. The two major fractions were collected and concentrated by rotary evaporation. The faster moving component was crystallized from hexane to provide 16 mg (24%): mp 147–149 °C; $[\alpha]_D$ −97.45° (*c* 0.25, EtOH); CIMS (isobutane) (M + 1) 444; ^{1}H NMR δ 7.45 (d, 1, ArH, J = 7.6 Hz), 7.38 (m, 2, ArH), 7.33 (m, 3, ArH), 7.21 (m, 1, ArH), 7.00 (s, 1, ArH), 6.97 (t, 1, ArH, J = 7.4 Hz), 6.72 (s, 1, ArH), 6.21 (d, 1, ArCH, J = 7.41 Hz), 5.15 (s, 1, ArCHO), 4.90 (d, 1, ArCH, J = 14.7 Hz), 4.28 (d, 1, ArCH, J = 14.7 Hz), 4.20 (d, 1, Ar_2CH, J = 12.6 Hz), 3.91 (s, 3, OCH_3), 3.83 (s, 3, OCH_3), 3.65 (s, 3, OCH_3), 3.61 (m, 1, CHN), 3.11 (m, 1, CHAr), 2.90 (m, 1, CHAr), 2.78 (m, 1, CHCN), 1.66 (m, 1, CHCN). Anal. ($C_{25}H_{23}NO_2$) C, H, N.

The slower running component was isolated and crystallized from ethyl acetate to provide the other diastereomer (20 mg, 30%): mp 171–172 °C; $[\alpha]_D$ +155.91° (*c* 0.25, EtOH); CIMS (isobutane) (M + 1) 444; ^{1}H NMR δ 7.49 (d, 1, ArH, J = 7.69 Hz), 7.44–7.35 (m, 6, ArH), 7.09 (t, 1, ArH), 6.72 (s, 1, ArH), 7.01 (s, 1, ArH), 6.69 (d, 1, ArCH, J = 6.78 Hz), 5.04 (s, 1, ArCHO), 4.86 (d, 1, ArCH, J = 14.4 Hz), 4.17 (d, 2, Ar_2CH, ArCH), 3.90 (s, 3, OCH_3), 3.83 (s, 3, OCH_3), 3.60 (m, 1, CHN), 3.41 (s, 3, OCH_3), 3.11 (m, 1, CHAr), 2.92 (m, 1, CHAr), 2.78 (m, 1, CHCN), 1.66 (m, 1, CHCN). Anal. ($C_{25}H_{23}NO_2$) C, H, N.

(6a*R*,12b*S*)-(+)-10,11-Dimethoxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine Hydrochloride, (+)-5. The (+)-(6a*R*,12b*S*)-*O*-methylmandelamide **4** (435 mg, 0.982 mmol) was dissolved in 25 mL of dry THF under nitrogen. The solution was cooled to 0 °C, and then, 6 mL of a 1.0 M solution of $LiEt_3BH$ in THF was added slowly via syringe. The solution was stirred at 0 °C for 12 h. The solution was poured into 25 mL of ice-cooled 1.0 M HCl. The aqueous layer was washed with 2 × 20 mL of ether and then made alkaline with NH_3. The resulting free base was isolated by extraction with 3 × 30 mL of dichloromethane. The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The HCl salt was formed with acidic ethanol, and the product was crystallized from acetonitrile to afford 217 mg (67%): mp 232–234 °C; $[\alpha]_D$ +106° (*c* 0.75, EtOH); CIMS (isobutane) (M + 1) 296; ^{1}H NMR (DMSO-d_6) δ 9.65 (s, 2, NH_2^+), 7.44–7.32 (m, 4, ArH), 6.86 (s, 2, ArH), 4.37 (d, 2, $ArCH_2N$, J = 4.12 Hz), 4.23 (d, 1, Ar_2CH, J = 11.07 Hz), 3.75 (s, 3, OCH_3), 3.68 (s, 3, OCH_3), 3.00–2.94 (ddd, 1, CHN), 2.86–2.79 (m, 2, $ArCH_2$), 2.22–2.15 (m, 1, CHCN), 1.98-1.89 (m, 1, CHCN). Anal. ($C_{19}H_{22}ClNO_2$) C, H, N.

(6a*S*,12b*R*)-(−)-10,11-Dimethoxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine Hydrochloride, (−)-5. Following an indentical procedure to that for (+)-**5**, the (−)-(6a*S*,12b*R*)-*O*-methylmandelamide **4** (344 mg, 0.777 mmol) gave 173 mg (66%) of the hydrochloride following crystallization from acetonitrile: mp 230–232 °C; $[\alpha]_D$ −107° (*c* 0.75, EtOH); CIMS (isobutane) (M + 1) 296; ^{1}H NMR (DMSO-d_6) δ 9.62 (s, 2, NH_2^+), 7.42–7.32 (m, 4, ArH), 6.86 (s, 2, ArH), 4.37 (d, 2, $ArCH_2N$, J = 4.12 Hz), 4.23 (d, 1, Ar_2CH, J = 11.08 Hz), 3.75 (s, 3, OCH_3), 3.68 (s, 3, OCH_3), 3.00–2.93 (dt, 1, CHN), 2.88–2.76 (m, 2, $ArCH_2$), 2.22–2.15 (m, 1, CHCN), 1.98–1.89 (m, 1, CHCN). Anal. ($C_{19}H_{22}ClNO_2$) C, H, N.

(6a*R*,12b*S*)-(+)-10,11-Dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine Hydrochloride, (+)-2. The hydrochloride salt of (+)-**5** (217 mg, 0.655 mmol) was dissolved in water and converted to the free base with concentrated NH_4OH. The free base was extracted into 3 × 25 mL of dichloromethane, the organic solution was dried over $MgSO_4$, filtered, and concentrated to a clear oil using rotary evaporation. The residue was dissolved in 20 mL of dichloromethane, and the solution was cooled to −78 °C. A 1.0 M solution of BBr_3 in CH_2Cl_2 (3 mL, 3 mmol) was added slowly to the solution of the free base via syringe under nitrogen over 30 min. The solution was left to warm to room temperature and was stirred overnight. The reaction was quenched by the addition of 5 mL of methanol. The solvent was removed via rotary evaporation, and the flask was left under high vacuum for 8 h. The residue was dissolved in water, and the pH was adjusted to 9–10 with a saturated solution of sodium bicarbonate under nitrogen. The precipitated free base was isolated by suction filtration, washed on the filter with cold water, and dried under vacuum. The filtrate was extracted with 3 × 25 mL of dichloromethane. The dichloromethane extracts were dried ($MgSO_4$), filtered, and concentrated on the rotary evaporator. This residue and the solid product were combined and dissolved in ethanol. The solution was acidified with ethanolic HCl, and the volatiles were removed *in vacuo*. The HCl salt was isolated following crystallization from EtOAc/isopropyl alcohol to yield 158 mg (79%): mp > 120 °C dec; $[\alpha]_D$ +83° (*c* 0.25, EtOH); CIMS (isobutane) (M + 1) 268; ^{1}H NMR (DMSO-d_6) δ 9.62 (s, 2, NH^+), 8.87, 8.85 (2s, 2, OH), 7.43–7.37 (m, 3, ArH), 7.34 (m, 1, ArH), 6.70 (s, 1, ArH), 6.61 (s, 1, ArH), 4.35 (d, 2, $ArCH_2N$, J = 3.94 Hz), 4.13 (d, 1, Ar_2CH, J = 11.26 Hz), 2.97–2.90 (m, 1, CHN), 2.78–2.72 (m, 1, $ArCH_2$), 2.72–2.64 (m, 1, $ArCH_2$), 2.18–2.11 (m, 1, CHCN), 1.93–1.84 (m, 1, CHCN); HRMS (CI, isobutane) calculated for $C_{17}H_{18}ClNO_2$, 268.1338; found, 268.1332.

(6a*S*,12b*R*)-(−)-10,11-Dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine Hydrochloride, (−)-2. Following a procedure identical to that for (+)-**2**, 404 mg (1.219 mmol) of (−)-**5** hydrochloride was converted to 351 mg (94%) of the HCl salt following crystallization from EtOAc/isopropyl alcohol; mp > 120 °C dec; $[\alpha]_D$ −86° (*c* 0.25, EtOH); CIMS (isobutane) (M + 1) 268; ^{1}H NMR (DMSO-d_6) δ 9.61 (s, 2, NH_2^+), 8.87, 8.85 (2s, 2, OH), 7.43–7.37 (m, 3, ArH), 7.34 (m, 1, ArH), 6.70 (s, 1, ArH), 6.61 (s, 1, ArH), 4.35 (d, 2, $ArCH_2N$, J = 3.11 Hz), 4.13 (d, 1, Ar_2CH, J = 10.98 Hz), 2.97–2.90 (m,

1, CHN), 2.78–2.72 (m, 1, $ArCH_2$), 2.72–2.64 (m, 1, $ArCH_2$), 2.18–2.11 (m, 1, CHCN), 1.93–1.84 (m, 1, CHCN); HRMS (CI, isobutane) calculated for $C_{17}H_{18}ClNO_2$, 268.1338; found, 268.1332.

X-ray Crystallography of (6aS,12bR)-(−)-N-((R)-α-Methoxyphenylacetyl)-10,11-dimethoxy-5,6,7,8,6a,12b-hexahydrobenzo[a]phenanthridine, (−)-4. The absolute configuration of this compound was clearly established on the basis of the known *R*-stereochemistry of the *O*-methylmandelic acid used to prepare (−)-**4**.

Crystal Data: $C_{28}H_{29}NO_4$; formula weight = 443.55; orthorhombic; space group $P2_12_12_1$ (No. 19); $Z = 4$; $a = 8.8868$ (6) Å, $b = 12.027$ (4) Å, $c = 21.940$ (2) Å, $V = 2344$ (1) Å^3; calculated density = 1.26 g/cm^3; absorption coefficient $\mu = 0.78$ cm^{-1}. Intensity data were collected at 20 °C with Mo Kα radiation ($\lambda = 0.71073$ Å) on an Enraf-Nonius CAD4 computer-controlled kappa axis diffractometer equipped with a graphite crystal, incident beam monochromator. Data were collected using the $\omega - 2\theta$ scan technique. The scan rate varied from 1×b0°/min to 20°/min (in ω). The variable scan rate allows rapid data collection for intense reflections where a fast scan rate is used and assures good counting statistics for weak reflections where a slow scan rate is used. Data were collected to a maximum 2θ of 55.0°. The scan range (in degrees) was determined as a function of θ to correct for the separation of the Kα doublet;[29] the scan width was calculated as follows: ω scan width = 0.56 + 0.350 tan θ. Moving-crystal moving-counterbackground counts were made by scanning an additional 25% above and below this range. Thus, the ratio of peak counting time to background counting time was 2:1. The counteraperature was also adjusted as a function of θ. The horizontal aperature width ranged from 1.9 to 2.4 mm; the vertical aperture was set at 4.0 mm. The diameter of the incident beam collimator was 0.7 mm, and the crystal to detector distance was 21 cm. For intense reflections, an attenuator was automatically inserted in front of the detector; the attenuator factor was 12.9.

A total of 3076 reflections were collected, of which 3076 were unique and not systematically absent. Lorentz and polarization corrections were applied to the data. The linear absorption coefficient is 0.8/cm for Mo Kα radiation. No absorption correction was made. The structure was solved using SHELX-86 (G. M. Sheldrick, Institut fur Anorganische Chemie der Universitat Gottingen, F.R.G.). The remaining atoms were located in succeeding difference Fourier syntheses. Hydrogen atoms were located and added to the structure factor calculations, but their positions were not refined. The structure was refined in full-matrix least squares where the function minimized was $\Sigma w(|F_o| - |F_c|)^2$ and the weight w is defined as per the Killean and Lawrence method with terms of 0.020 and 1.0.[30]

Scattering factors were taken from Cromer and Waber.[31] Anomalous dispersion effects were included in F_c;[32] the values for $\delta f'$ and $\delta f''$ were those of Cromer.[33] Only the 1609 reflections having intensities greater than 3.0 times their standard deviation were used in the refinements. The final cycle of refinement included 298 variable parameters and converged (largest parameter shift was 0.14 times its esd) with unweighted and weighted agreement factors of:

$$R1 = \sum|F_o - F_c|/\sum F_o = 0.056;\ R2 = \mathrm{SQRT}(\sum w(F_o - F_c)^2/\sum F_o^2) = 0.065$$

The standard deviation of an observation unit weight was 1.59. There were no correlation coefficients greater than 0.50. The highest peak in the final difference Fourier had a height of 0.21 e/Å^3 with an estimated error based on δF[34] of 0.04. Plots of $\Sigma w(|F_o| - |F_c|)^2$ versus $|F_o|$, reflection order in data collection, $\sin\theta/\lambda$, and various classes of indices showed no unusual trends. All calculations were performed on a VAX computer using SDP/VAS.[35]

Pharmacology Methods. [^{3}H]SCH23390 was synthesized as described by Wyrick et al.[36] (±)-DHX was synthesized as previously described.[13] [^{3}H]Spiperone was purchased from Amersham Corp. (Arlington Heights, IL). $Na^{125}I$ was supplied by New England Nuclear (Boston, MA), and HEPES buffer was purchased from Research Organics, Inc. (Cleveland, OH). SCH23390 was a gift from Schering Corp. (Bloomfield, NJ) or was purchased from Research Biochemicals Inc. (Natick, MA). Domperidone and ketanserin were gifts of Janssen Pharmaceutica (New Brunswick, NJ). Dopamine, cAMP, isobutylmethylxanthine (IBMX), and chlorpromazine were obtained from Sigma Chemical Co. (St. Louis, MO). cAMP primary antibody was obtained from Dr. Gary Brooker (George Washington University, Washington, DC), and secondary antibody (rabbit antigoat IgG) covalently attached to magnetic beads was purchased from Advanced Magnetics, Inc. (Cambridge, MA).

D_1 and D_2 Radioreceptor Assays. Radioligand binding followed the method of Schulz et al.[37] with minor modifications. For the rat studies, male Sprague–Dawley rats (Charles River, Raleigh, NC) weighing 200–400 g were decapitated, and the brains were quickly removed and placed into ice cold saline. After a brief chilling period, brains were sliced into 1.2 mm coronal slices with the aid of a dissecting block similar to that described by Heffner et al.[38] The striatum was dissected from two slices containing the majority of this region, and the tissue was either used immediately or stored at −70 °C until the day of the assay. After dissection, rat striata were homogenized by seven manual strokes in a Wheaton Teflon-glass homogenizer with ice cold 50 mM HEPES buffer with 4.0 mM $MgCl_2$, pH 7.4 (25 °C). Tissue was centrifuged at 27000*g* (Sorvall RC-5B/SS-34 rotor; DuPont, Wilmington, DE) for 10 min, and the supernatant was discarded. The pellet was homogenized (five strokes), resuspended in ice cold buffer, and centrifuged again. The final pellet was suspended at a concentration of approximately 2.0 mg wet wt/mL. Assay tubes (1 mL final volume) were incubated at 37 °C for 15 min. Nonspecific binding of [^{3}H]SCH23390 (ca. 0.25 nM) was defined by adding unlabeled SCH23390 (1 μM). Binding was terminated by filtering with 15 mL of ice cold buffer on a Skatron or Brandel cell harvester (Skatron Inc., Sterling, VA; Brandel Inc., Gaithersburg, MD) using glass fiber filter mats (Skatron No. 7034; Brandel GF/B). Filters were allowed to dry, and 2–4 mL of Scintiverse E (Fischer Scientific Co., Fair Lawn, NJ) was added. After shaking for 30 min, radioactivity was determined on an LKB-1219 Betarack liquid scintillation counter. Tissue protein levels were estimated using the folin reagent method of Lowry et al.[39] adapted to a Technicon autoanalyzer I (Tarrytown, NY).

For D_2-like receptors, the procedure was as described for D_1-like receptors with the following changes. [^{3}H]Spiperone was used as the radioligand, and nonspecific binding of [^{3}H]-spiperone was defined by adding unlabeled 1 μM chlorpromazine. Ketanserin (50 nM) was added to mask binding of [^{3}H]spiperone to serotonin receptors.

Adenylate Cyclase Measurements: Striatum. The automated HPLC method of Schulz and Mailman[22] was used to measure adenylate cyclase activity by separating cAMP from other labeled nucleotides. Briefly, rat striatal tissue was removed and homogenized at 50 mL/g of tissue in 5 mM HEPES buffer (pH 7.5) containing 2 mM EGTA. After homogenization of the tissue with a Teflon-glass homogenizer, 50 mL/g of 100 mM HEPES–2 mM EGTA was added and mixed with one additional stroke. A 20 μL aliquot of this tissue homogenate was added to a prepared reaction mixture, yielding a final volume of 100 μL containing 0.5 mM ATP, 0.5 mM IBMX, [α-^{32}P]ATP (0.5 μCi), 1 mM cAMP, 2 mM $MgCl_2$, 100 mM HEPES buffer, 2 μM GTP, 0 or 100 μM dopamine and/or drug, 10 mM phosphocreatine, and 5 units of creatine phosphokinase. The reaction was initiated by transferring the samples from an ice bath to a water bath at 30 °C and terminated 16 min later by the addition of 100 μL of 3% sodium dodecyl sulfate. Proteins and much of the noncyclic nucleotides were precipitated by the addition of 300 μL each of 4.5% $ZnSO_4$ and 10% $Ba(OH)_2$ to each incubation tube. The samples were centrifuged at 10000*g* for 8 min and the supernatants removed and loaded onto an ISIS autoinjector.

The HPLC separations were carried out using a Waters RCM 8 × 10 module equipped with a C18, 10 μM cartridge, using a mobile phase of 150 mM sodium acetate, 24% meth-

anol, pH 5.0. A flow rate of 1.3 mL/min was used for separation. A UV detector equipped for 254 nM was used to measure the unlabeled cAMP added to the sample tubes, to serve as an internal standard and a marker for the labeled cAMP. Sample recovery was based on UV measurement of total unlabeled cAMP peak areas. The radioactivity in each fraction was determined by an on-line HPLC radiation detector (Inus Systems, Tampa, FL).

Radioreceptor Assays in Transfected D_1 Receptors. The present studies were conducted with Ltk$^-$ cells (mouse fibroblasts) that expressed the human D_{1A} receptor, L-hD_1.[40] The cells were grown in DMEM-H medium containing 4500 mg/L glucose, L-glutamine, 10% fetal bovine serum, and 700 ng/mL G418. In these studies, D_1 receptor levels were ca. 5000 fmol/mg of protein. All cells were maintained in a humidified incubator at 37 °C with 5% CO_2. Cells were grown in 75 cm^2 flasks until confluent. The cells were rinsed and lysed with 10 mL of ice cold hypoosmotic buffer (HOB) (5 mM HEPES, 2.5 mM $MgCl_2$, 1 mM EDTA; pH 7.4) for 10 min at 4 °C. Cells were then scraped from the flasks using a sterile cell scraper from Baxter (McGaw Park, IL). Flasks received a final rinse with 5 mL of HOB. The final volume of the cell suspension recovered from each flask was ca. 14 mL. Scraped membranes from several flasks were then combined. The combined cell suspension was homogenized (10 strokes), 14 mL at a time, using a 15 mL Wheaton Teflon-glass homogenizer. The cell homogenates were combined and spun at 43000*g* (Sorvall RC-5B/SS-34 rotor; DuPont, Wilmington, DE) at 4 °C for 20 min. The supernatant was removed, and the pellet was resuspended (10 strokes) in 1 mL of ice cold HOB for each original flask of cells homogenized. This homogenate was then spun again at 43000*g* at 4 °C for 20 min. The supernatant was removed, and the final pellet was resuspended (10 strokes) in ice cold storage buffer (50 mM HEPES, 6 mM $MgCl_2$, 1 mM EDTA; pH 7.4) to yield a final concentration of ca. 2.0 mg of protein/mL. Aliquots of the final homogenate were stored in microcentrifuge tubes at −80 °C. Prior to their use for radioligand binding or adenylate cyclase assays, protein levels for each membrane preparation were quantified using the BCA protein assay reagent (Pierce, Rockford, IL) adapted for use with a microplate reader (Molecular Devices, Menlo Park, CA).

Frozen membranes were thawed and resuspended in assay buffer (50 mM HEPES with 6 mM $MgCl_2$ and 1 mM EDTA; pH 7.4) containing a fixed concentration of [^{3}H]SCH23390 (0.2 nM) in a final assay volume of 500 μL. Triplicate determinations were performed at data point. Assay tubes were incubated at 37 °C for 15 min. Tubes were filtered rapidly through Skatron glass fiber filter mats and the filters rinsed with 5 mL of ice cold assay buffer using a Skatron micro cell harvester (Skatron Instruments Inc., Sterling, VA). Filters were allowed to dry and then punched into scintillation vials (Skatron Instruments Inc., Sterling, VA). OptiPhase HiSafe II scintillation cocktail (2 mL) was added to each vial. After the vials shook for 30 min, radioactivity in each sample was determined on an LKB Wallac 1219 Rackbeta liquid scintillation counter (Wallac Inc., Gaithersburg, MD).

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