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Probing the Steric Space at the Floor of the D_1 Dopamine Receptor Orthosteric Binding Domain: 7α-, 7β-, 8α-, and 8β-Methyl Substituted Dihydrexidine Analogues

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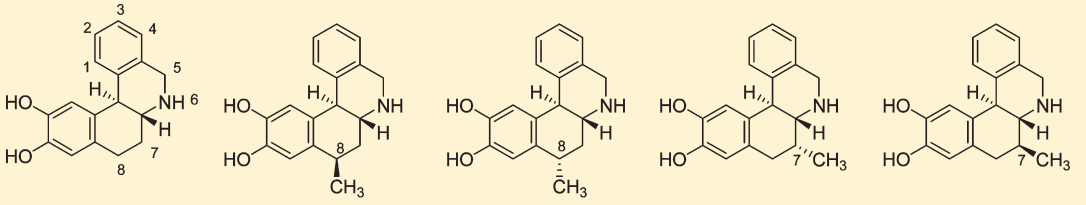
ABSTRACT:



Dihydrexidine, **1** — D_1 Ki 20 nM

8β-Me_{ax}-DHX, **2a** — D_1 Ki 270 nM

8α-Me_{eq}-DHX, **2b** — D_1 Ki 920 nM

7α-Me_{ax}-DHX, **3a** — D_1 Ki >10000 nM

7β-Me_{eq}-DHX, **3b** — D_1 Ki 6540 nM

To probe the space at the floor of the orthosteric ligand binding site in the dopamine D_1 receptor, four methylated analogues of dihydrexidine (DHX) were synthesized with substitutions at the 7 and 8 positions. The 8α-axial, 8β-equatorial, and 7α-equatorial were synthesized by photochemical cyclization of appropriately substituted *N*-benzoyl enamines, and the 7β-axial analogue was prepared by an intramolecular Henry reaction. All of the methylated analogues displayed losses in affinity when compared to DHX (20 nM): 8β-Me_{ax}-DHX (270 nM), 8α-Me_{eq}-DHX (920 nM), 7β-Me_{eq}-DHX (6540 nM), and 7α-Me_{ax}-DHX (>10000 nM). Molecular modeling studies suggest that although the disruption of an aromatic interaction between Phe203$^{5.47}$ and Phe288$^{6.51}$ is the cause for the 14-fold loss in affinity associated with 8β-axial substitution, unfavorable steric interactions with Ser107$^{3.36}$ result in the more dramatic decreases in binding affinity suffered by the rest of the analogues.

INTRODUCTION

The development of the selective dopamine D_1 receptor full agonist dihydrexidine, **1**, has enabled numerous studies aimed at investigating the physiological role of the D_1 dopamine receptor and the potential of this receptor subtype as a therapeutic target. Dihydrexidine has been shown to possess profound antiparkinsonian effects in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesioned monkey model of Parkinson's disease,[1] underscoring the importance of this receptor in the volitional control of motor function (Figure 1). D_1 receptor activation in the prefrontal cortex also has been shown to be essential for the proper performance of cognitive functions such as working memory and attention.[2,3] Recently, a small clinical trial of dihydrexidine demonstrated that a single dose significantly increased prefrontal cortical blood flow in schizophrenia patients.[4] Reward phenomena also are thought to be dependent upon D_1 receptor activation.[5]

Increasing awareness of the physiological roles of the dopamine D_1 receptor has made evident the need to develop ligands with improved pharmacological properties. Modifications of the dihydrexidine template have already led to compounds with increased D_1 selectivity and distinct metabolic profiles.[6] A pharmacophoric moiety has been proposed that provides for binding interactions between polar residues of the binding site and catechol functions, electrostatic interactions between a putative anionic function to engage the protonated ligand amine, and an accessory binding region that accommodates the pendant phenyl ring. In essence, we have successfully employed this "β-phenyldopamine" pharmacophore for the design of several D_1 dopamine-selective full agonists.[7,8] The original conceptual model of the receptor has been applied to understand the molecular structure of the G protein-coupled D_1 receptor itself, with the purpose of identifying the nature of the binding site. Prior mutagenesis studies have identified key polar residues that line the orthosteric binding domain. That is, aspartate 103 in

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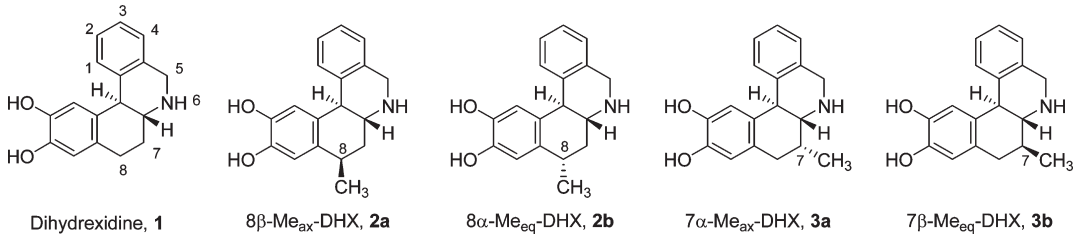
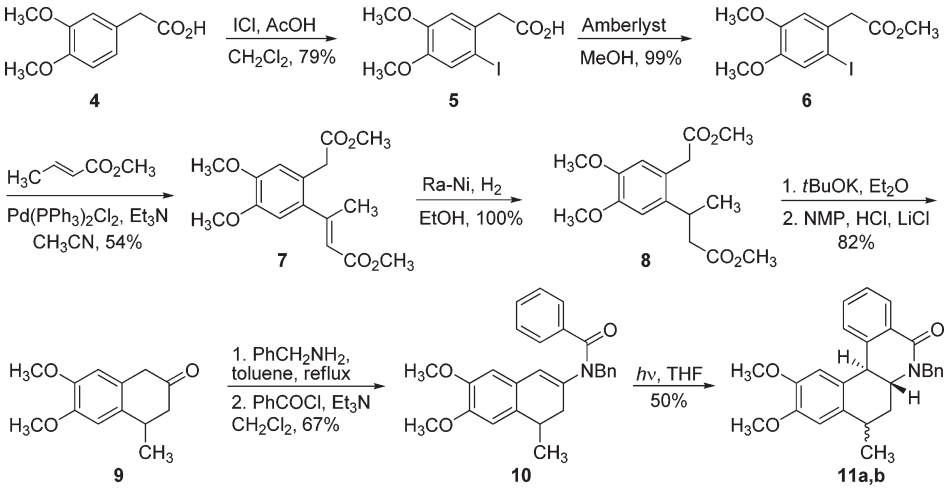


Figure 1. Dihydrexidine and the synthesized 7- and 8-methyl analogues.

Scheme 1. Synthesis of Diastereomeric Amides 11a and 11b



transmembrane helix 3 forms a salt bridge with the protonated amine of the ligand, and serines 198, 199, and 202 in transmembrane helix 5 interact with the catechol moiety. Mutant human dopamine D_1 receptors S198A, S199A, and S202A previously have been examined using the 10- and 11-monohydroxy analogues of **1** to establish that S198 and S199 interact with the 11-OH group, whereas S202 engages the 10-hydroxy group.[9] These results parallel early mutagenesis studies of the β-adrenergic receptors, where it was shown that analogous serine residues in transmembrane domain 5 (TM5) interacted with the catechol moiety of β-adrenergic receptor agonists.[10] These studies clearly identify the area within the receptor where a catechol agonist ligand must bind. Additionally, it has been suggested that phenylalanines 203, 288, and 289 in transmembrane domains 5 and 6 form aromatic interactions with the catechol ring, and one or more hydrophobic and/or aromatic residues in helix 7 (Phe313) and extracellular loop 2 (Leu190) form the binding site for the pendant accessory (e.g., phenyl) ring.[11−15]

In early studies, Grol and Rollema[16] had proposed a region of steric intolerance below the catechol ring, and this feature was incorporated into a working model by McDermed,[17] who demonstrated an area of steric intolerance within "dopamine receptors," specifically located adjacent to the dopamine 5-position, which corresponds to position 9 in **1**. We have assumed (but not tested the hypothesis) that this spatial restriction applies to the D_1 dopamine receptor (e.g., see Brewster et al.[18]), but in fact very little is known of the floor of the orthosteric binding site in the dopamine D_1 receptor. Therefore, we were interested in probing this area of the receptor, with the goal of possibly exploiting this region to develop additional selective agonists.

Thus, to continue developing the structure−activity relationships of these compounds and to obtain a better understanding of the topography of the D_1 dopamine receptor binding site, we now report on the synthesis and receptor affinities of four analogues of dihydrexidine bearing a methyl substituent on the unsaturated B-ring, namely compounds **2a**, **2b**, **3a**, and **3b**. We also report on their affinity at D_1- and D_2-like receptors. Finally, using an in silico activated homology model of the D_1 dopamine receptor based on the first X-ray crystal structure of the β_2-adrenergic receptor,[11] we provide likely explanations for the observed relative affinities of the target compounds. The present study reveals that modifications at the 7- or 8-positions lead to a significant loss of receptor affinity, and molecular modeling suggests that the regional space constraints are sufficient only to accommodate the catechol ring.

CHEMISTRY

Synthesis of 2a and 2b. The synthesis of the 8-Me-DHX analogues **2a** and **2b** is shown in Schemes 1 and 2. Commercially available 3,4-dimethoxyphenylacetic acid was iodinated using iodine monochloride to provide the iodophenylacetic acid **5**, which was esterified prior to Heck olefination with methyl crotonate[19] to afford methyl cinnamate **7**. This compound could be reduced with H_2 over 10% Pd−C, but reduction over Raney nickel in EtOH gave a slightly better yield. Intramolecular Dieckmann condensation gave an intermediate ketoester that was decarboxylated by treatment with lithium chloride and HCl in boiling *N*-methylpyrrolidinone. Treatment of the resulting β-tetralone **9** with benzylamine, followed by benzoyl chloride,

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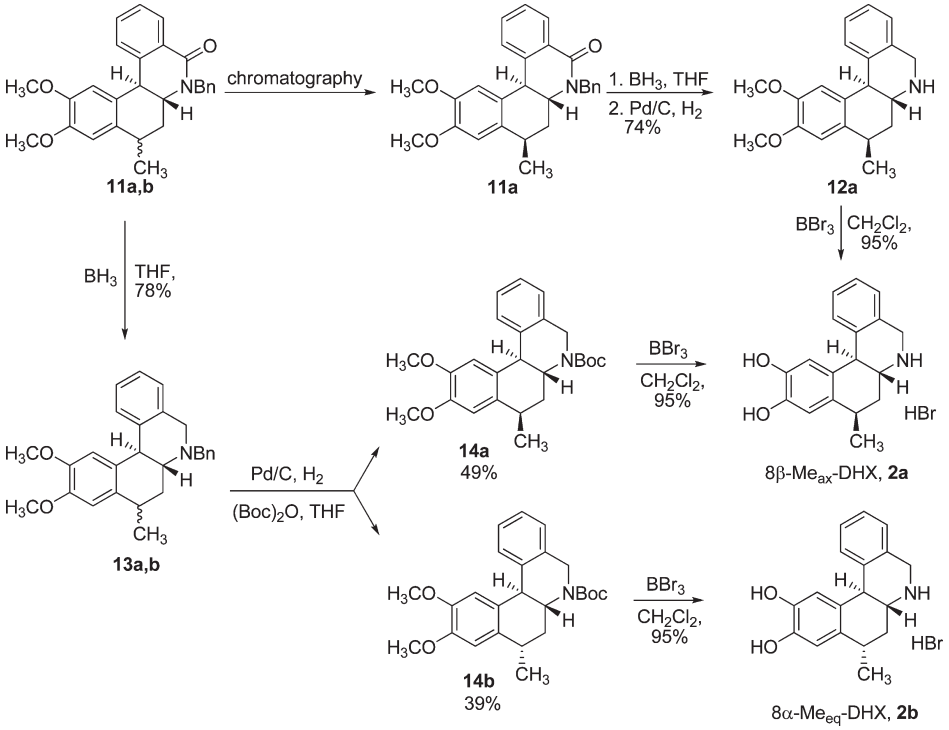
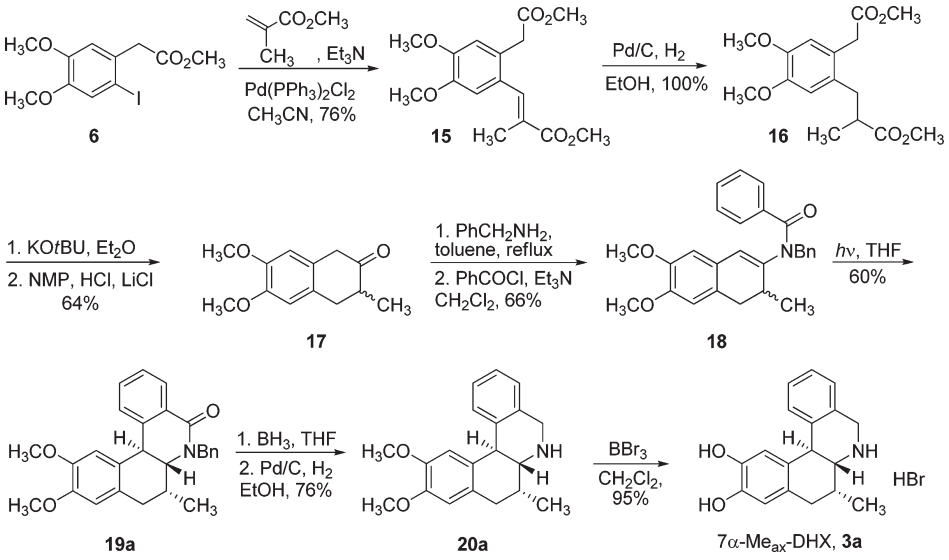
Scheme 2. Final Synthesis of the 8α,β-Methyl Analogues of DHX



Scheme 3. Synthesis of the 7-Axial Methyl Analogue of DHX



gave access to enamide **10**, which was irradiated using a 450 W medium-pressure quartz mercury-vapor lamp to induce cyclization,[18−20] giving a 50/50 mixture of the α- and β-methyl *trans*-lactams **11** in moderate yield. Chromatographic separation of this mixture allowed isolation of small amounts of pure axial β-methyl lactam **11a** (Scheme 2). The separation process did not, however, allow recovery of a pure sample of the equatorial α-methyl-substituted diastereomer **11b**. Compound **11a** gave crystals suitable for X-ray structure determination, which unambiguously allowed a determination of its stereochemistry. This pure axial-methyl lactam **11a** was then reduced with borane in THF, and *N*-debenzylated by catalytic hydrogenation to yield amine **12a**, which was demethylated with boron tribromide to afford the catecholamine hydrobromide final compound **2a**.

To obtain the desired 8α-Me$_{eq}$-DHX, the mixture **11a,b** obtained by photocyclization was reduced with borane in THF, and the intermediate tertiary amine **13a,b** was *N*-debenzylated and Boc-protected in one step to yield carbamates **14a** and **14b**. This mixture was separable by flash column chromatography, which allowed isolation of both the 8α-axial (**14a**) and 8α-equatorial (**14b**) carbamates. Once purified and separated, the equatorial carbamate **14b** was simultaneously demethylated

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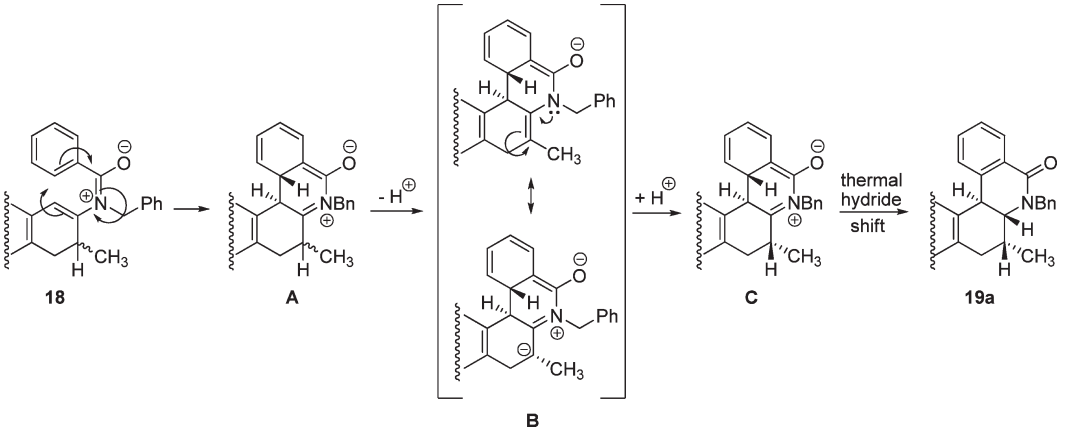
Scheme 4. Proposed Mechanism for the Isomerization and the 7 Position under Photochemical Conditions



and *N*-deprotected using boron tribromide to give **2b** as the hydrobromide salt in excellent yield. It also was possible to obtain **2a** using this route.

Synthesis of Analogues 3a and 3b. Common intermediate **6** provided the starting point for the synthesis of 7α-Me_{ax}-DHX (Scheme 3), which was synthesized using methodology similar to that used to prepare **2a** and **2b**. Heck coupling of **6** with methyl methacrylate gave the methyl cinnamate derivative **15**, which was reduced by catalytic hydrogenation to provide the saturated diester **16**. Dieckmann condensation and decarboxylation allowed access to β-tetralone **17**, which was converted to the enamide **18**.

Unexpectedly, irradiation of **18** as performed previously gave exclusively the 7α-methyl lactam **19a** in 60% yield. The diastereomeric purity of this product suggests that epimerization of the 7β-methyl into the 7α-methyl must have occurred at some point during this transformation. As this photocyclization likely proceeds by an electrocyclic mechanism (Scheme 4) that involves the imidate resonance structure of **18**,[21] it is conceivable that the 7-methine hydrogen, adjacent to the methyl group, is abstracted, giving a resonance stabilized anion **B** that would be free to epimerize. Molecular modeling of this intermediate anion suggests that the methyl group of this species will adopt an α-axial conformation to avoid a steric clash with the *N*-benzyl substituent. Selective protonation from the β side of the moiety, followed by suprafacial [1,5] hydride shift,[22] would give diastereomer **19a** exclusively.

Borane reduction of **19a**, followed by debenzylation yielded the amine **20a**, which gave crystals suitable for X-ray crystal structure determination of its stereochemistry, confirmed the axial orientation of its methyl substituent (Figure 2). Treatment of this amine with boron tribromide furnished the 7α-Me_{ax}-DHX, **3a**, as the hydrobromide salt.

Given the unexpected diastereomeric selectivity of the photocyclization reaction leading to the formation of **3a**, we were unable to produce the 7β-methyl diastereomer **3b** using this approach. To prepare **3b**, we modified our recently reported[23] synthesis of dihydrexidine based on the intramolecular Henry reaction of key nitrobenzophenone **28** (Scheme 5).

To prepare key synthon **28**, 3,4-dimethoxybenzaldehyde was converted to methylcinnamic acid **22** by Knoevenagel condensation with methylmalonic acid.[24] Attempts to reduce this deceptively ordinary olefin using usual catalytic hydrogenation conditions (Pd/C, H_2, EtOH), similar to those employed for

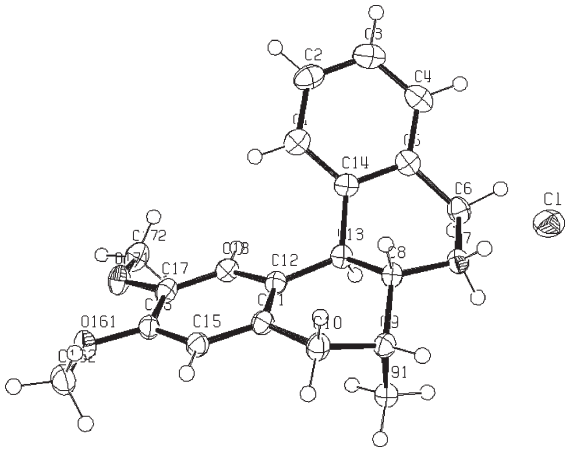


Figure 2. Crystal structure of **20a**.

other cinnamic acids, were unsuccessful. Instead, the conditions reported by Schrecker[25] were employed, which involved vigorous stirring of a highly basic aqueous NaOH solution of the compound with Ni−Al alloy to effect reduction. This procedure provided the saturated acid **23** in quantitative yield. Further reduction with borane in THF gave the alcohol **24**, which was converted to the corresponding bromide using triphenylphosphine and carbon tetrabromide. Alkyl bromide **25** was dissolved in DMF along with $NaNO_2$ to effect nitration,[26] leading only to a 41% yield of the expected nitro compound **26** but with recovery of significant amounts of starting material.

Friedel−Crafts acylation of **26** with phthaloyl dichloride yielded benzophenone **27**. This intermediate was esterified (**28**) to facilitate the subsequent intramolecular Henry cyclization, which was promoted by a catalytic amount of DBU in a protic solvent. The reduction of the resulting nitroalkene **29** (Scheme 6) was considerably slower than previously reported for the desmethyl analogue,[23] taking about four days at reflux in 2-propanol and a very large excess of sodium borohydride for complete reduction. Quenching of the resulting mixture using a concentrated solution of urea in dilute aqueous acetic acid[27] allowed recovery of the inseparable 1,2-*trans* diastereomeric mixture **30a**, **b** but in only a disappointing 21% yield. This mixture was esterified and treated with borane in THF to produce two diastereomeric lactams, **32a** and **32b**, which were separable by chromatography. Inspection of the key methyl signal in the NMR

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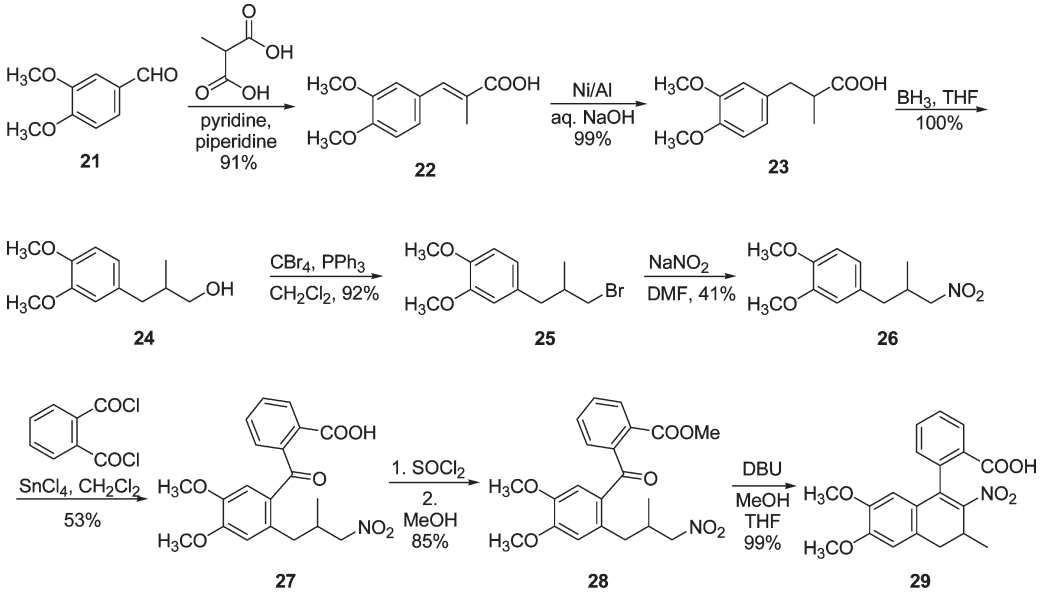
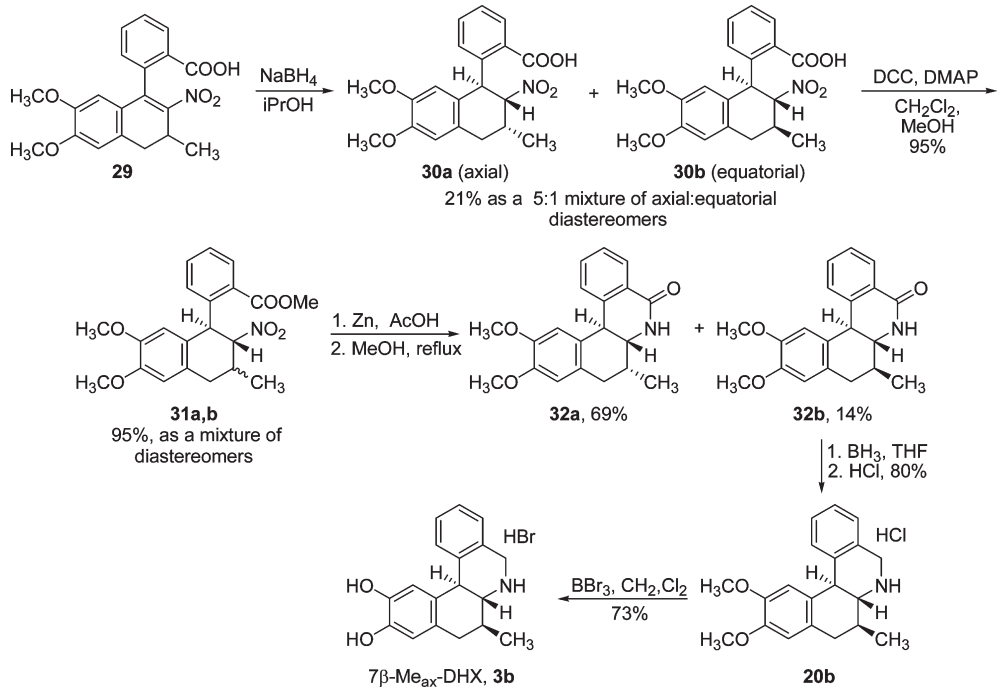
Scheme 5. Synthesis of Nitrodihydrotetralin Intermediate 29



Scheme 6. Final Synthesis of the 7-Equatorial Methyl Analogue of DHX



spectrum of the mixture revealed that these compounds had been carried through the synthesis from the point of diastereomer formation as a 5:1 mixture favoring the axial α-methyl compound **32a** over the desired equatorial-*β*-methyl **32b**. These lactams were separated by flash column chromatography, obtaining amounts that confirmed the 5:1 diastereomeric ratio and allowing a positive determination of the product's stereochemistry by examination of the NMR signal corresponding to the methine hydrogen at the α-position relative to the amide nitrogen. In the case of the 7*β*-equatorial-methyl **32b**, this signal appeared as a triplet with a single coupling constant, in accordance with its double-*trans* relationship with vicinal hydrogens.

The 5:1 diastereomeric ratio was likely set during reduction of nitroalkene **29**. From the structure of the axial-methyl isomer **30a**, which was the major product of the reduction, it is evident that hydride transfer had occurred preferentially from the same face as the methyl substituent. Molecular modeling of **3a** and **3b** also suggests that the predominant diastereomer is not the thermodynamically favored product, supporting speculation that the actual species that undergoes reduction is probably a high

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Table 1. D_1- and D_2-Like Affinity of Test Compounds

	binding in porcine striatal homogenates[a]		
ligand	D_1-like K_i (nM)	D_2-like K_i (nM)	fold selectivity (D2/D1)
DHX	20 ± 2	240 ± 40	12 ± 2
8β-Me_{ax}-DHX, **2a**	270 ± 34	5530 ± 3180	20 ± 12
8α-Me_{eq}-DHX, **2b**	920 ± 46	3630 ± 550	3.9 ± 0.6
7α-Me_{ax}-DHX, **3a**	>10000	7380 ± 2440	<0.74
7β-Me_{eq}-DHX, **3b**	6540 ± 1120	8670 ± 2290	1.3 ± 0.4
SCH-23390	0.5 ± 0.1	ND	ND
chlorpromazine	ND	8 ± 2	ND

[a] All results shown are the mean ± SEM for four independent experiments.

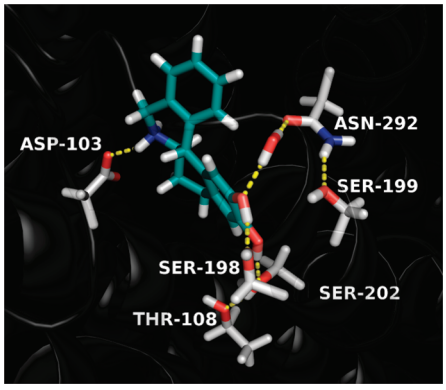


Figure 3. Structure of DHX docked into the activated homology model of the human dopamine D_1 receptor. Key polar residues involved in binding are identified.

molecular weight acyloxyborohydride. If that is the case, perhaps this species undergoes a kinetically favored intramolecular hydride addition that would account for the observed diastereomeric product ratio.

To complete the synthesis, the 7β-equatorial-methyl lactam **32b** was reduced with borane in THF to the amine **20b**, which was then demethylated using boron tribromide to afford the desired **3b**, as the hydrobromide salt, thus completing the series of 7- and 8-methyl substituted dihydrexidine analogues.

RESULTS AND DISCUSSION

Competition binding assays using porcine striatal homogenate preparations revealed that all four methyl-substituted dihydrexidine analogues had lower affinity at D_1- and D_2-like receptors than the parent unsubstituted compound DHX (Table 1). Comparison of the minimum energy models of DHX, **2a**, **2b**, **3a**, and **3b** in vacuum (not shown), and in a model receptor–membrane complex (Figure 3), revealed that all compounds share a near-identical conformation of the tetracyclic ring system. Thus, the dramatic loss of D_1- and D_2-like affinity observed for the methyl substituted analogues can only be attributed to steric effects caused by the presence of the methyl substituents.

The loss of affinity was most dramatic for the 7-methyl substituted compounds **3a** and **3b**. The 7α-axial-methyl ligand **3a** had no appreciable affinity at D_1-like receptors (>10 μM K_i) and had a greater than 30-fold loss of D_2 affinity. The presence of the 7β-equatorial methyl in the DHX template (**3b**) caused a greater than 300-fold decrease in D_1 affinity and more than 35-fold decrease in D_2 affinity.

Substitution at the 8-position with axial and equatorially oriented methyl groups also gave marked decreases in D_1 and D_2 affinity compared to DHX. The 8β-axial-methyl analogue **2a** had the highest affinity of the methylated series but still suffered a more than 13-fold decrease in D_1 affinity compared to DHX. The loss of affinity of the 8α-equatorial-methyl analogue **2b** was more substantial, being about 40-fold lower than the unsubstituted parent ligand.

To gain a better understanding of the potential mode of binding of the parent ligand DHX to the D_1 receptor and to rationalize the relative decrease in affinity observed for the test compounds **2a**,**b** and **3a**,**b**, we constructed a homology model of the D_1 receptor based on an in silico activated model of the first published X-ray crystal structure of the β_2-adrenergic receptor[28] and used an unbiased computer-based docking routine to determine probable binding orientations of DHX to the D_1 binding site. After molecular dynamics (MD) and energy minimization, it was observed that the D_1 receptor binding site accommodates the active (6a*R*,12b*S*)-(+) isomer of DHX within a cavity located between transmembrane helices 3, 5, and 6 that is lined by several residues that have been shown by site-directed mutagenesis experiments to be involved in ligand binding (Figure 3). Aspartate 103, a residue located in the third transmembrane domain that interacts with the protonated amine of the ligand, is highly conserved among monoamine receptors and has been shown by mutagenesis studies to be instrumental in the binding of catecholamines.[11−15]

Mutagenesis studies of the D_1 receptor have identified three serine residues in the fifth transmembrane domain that are of importance in ligand binding: Ser 198, Ser 199, and Ser 202, which are thought to hydrogen-bond to the catechol moiety of DHX.[14] The resulting energy-minimized docked conformation involves a complex hydrogen bonding network between polar residues in TMs 5 (serines 198, 199, and 202) and 6 (asparagine 292) and the catechol hydroxyl groups.

To begin with, the previously known[17] steric restriction in the region of the catechol ring (position 9 in DHX) can be rationalized as an interaction between the ligand aromatic ring and residue Phe203. Our experimental data clearly show that there are strict steric limits that extend outward from the bottom of the catechol ring into the region below the 7,8-ethyl bridge in DHX. Figure 4 illustrates structures of **2a**,**b** and **3a**,**b** in their minimized binding poses, superimposed on the docked structure of DHX (**1**).

According to our model, although all ligands form the critical salt bridge with Asp103, there are differences in the binding orientation and receptor–ligand interactions of the synthesized compounds that might help to explain their differences in binding affinity relative to DHX. In the case of the original template, DHX, the interaction pattern is as follows. Aside from the conserved salt bridge between the amine group and Asp103, a complex hydrogen bonding network is observed around the catechol moiety.[11] The *para*-hydroxyl group serves as a donor in a hydrogen bond with Ser202, which interacts with Thr108 in the same fashion. The *meta*-hydroxyl group, on the other hand, has a more intricate interaction pattern: besides a hydrogen bond accepted by Ser198, it is involved in a water-mediated interaction with Asn292$^{6.55}$ (stable during the time course of the MD

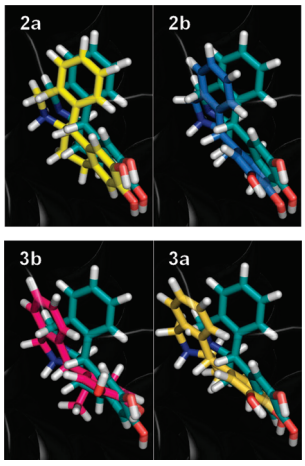


Figure 4. Binding poses for compounds DHX **1** (green), **2a** (yellow), **2b** (blue), **3b** (red), and **3a** (orange), in order of decreasing D_1-like binding affinity. One can gain the general impression from visual inspection that the lower the affinity of the molecule, the more it diverges from the docked pose of DHX.

simulations). We note that a recent report cites the residue at this location in the $D_{2\,L}$ receptor (His6.55) as a major determinant of ligand-based signaling.[15]

The binding profile of the 8β-axially substituted compound **2a**, in which the methyl group points away from TM3, is similar to DHX with respect to the salt bridge and the catechol hydrogen bonding network. The alkyl substituent appears only mildly obtrusive, moderately disrupting an edge−face aromatic interaction between Phe288 and Phe203 by acting as a wedge between the phenyl rings. Considering that these residues are part of a larger cluster of aromatic residues involved in the binding of the catechol ring, it is possible that this disruption could be the cause of the observed nearly 14-fold loss in affinity. This value translates into about 1.6 kcal/mol of free energy of binding, which is in the range of what could be expected from the loss of an aromatic interaction.[29]

In the case of the equatorially substituted compounds, **2b** and **3b**, the methyl groups generally lie in the plane of the catechol ring. The extra bulk of the substituent clashes with TM3 in the vicinity of Ser107. Because of its location on the framework of the ligand, a methyl at the 8-position displays slightly less overlap with the aforementioned residue than when it is at the 7-position, which might explain the observed differences in affinity. In both cases, the steric clash causes a twist of the molecule around the amine−catechol axis so that the methyl group moves away from TM3 and the pendant phenyl ring moves in the opposite direction. This rotation increases the distance between the *meta*-hydroxyl group and Asn292, making it too long for the water-mediated hydrogen bond to occur. In the case of the 7β-equatorially substituted compound, **3b**, another distinct shift can be observed, in this case a translational movement away from TM5, further impairing its binding affinity due to an increase in the distance between the catechol moiety and serines 198, 199, and 202. Notably, for this compound, Ser202 does not form a direct interaction with the ligand and displays a different rotameric state than in the prototypical DHX-bound structure.[11]

Finally, in the case of compound **3a**, with a 7α-axially located methyl group, the alkyl substituent projects directly toward the adjacent TM3, forcing a significant shift in the position of the ligand. To avoid a steric clash between the methyl group and Ser107, the molecule moves slightly toward the extracellular space while keeping the salt bridge intact. This movement happens in such a way that the interaction between the *para*-hydroxyl group and Ser202 is replaced with hydrogen bonds involving Ser198 and Ser199. Consequently, the catechol ring is no longer in its optimal location within the binding pocket (as exemplified by DHX), disrupting the aromatic interactions in this region and resulting in a ligand with poor binding properties.

Table 2. D_1-Like Affinity of Test Compounds and Root-Mean-Square Deviation (rmsd) of the Modeling Poses Relative to DHX

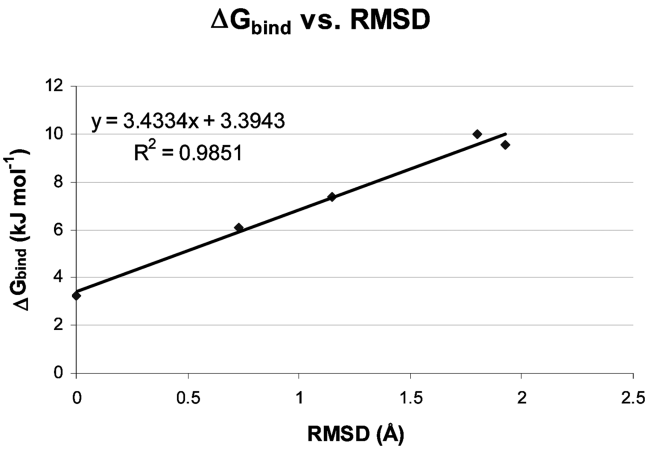
ligand	K_i (nM)	rmsd
DHX	20 ± 2	0.000
8β-Me_{ax}-DHX, **2a**	270 ± 34	0.732
8α-Me_{eq}-DHX, **2b**	920 ± 46	1.146
7α-Me_{ax}-DHX, **3a**	>10000	1.798
7β-Me_{eq}-DHX, **3b**	6540 ± 1120	1.923

One notable aspect of the biological activity of these analogues is the fact that the D_2 receptor was found to be much more sensitive to these substitutions than the D_1 receptor. Even though the compounds displayed a range of affinities from midnanomolar to micromolar at the latter, affinities at the former were all in the micromolar range. It is likely that the larger residue at position 3.36 (a cysteine instead of a serine) expands the region of steric restriction at the bottom of the binding site, therefore causing a more dramatic loss in affinity upon substitution at the 7 and 8 positions.

The differences in affinity for the synthesized compounds also can be rationalized by analyzing the root-mean-square deviation for the heavy carbon atoms of the poses of the analogues with respect to DHX. That is, if one assumes that DHX has a near optimal complementarity to the orthosteric binding site, then the greater the deviation of its congeners from this binding orientation, the greater the decrease in binding energy one ought to expect. Qualitatively, the trends are similar to what is observed with the binding affinities. In the case of the 8-methyl substituted analogues, the axial compound **2a** deviates the least from the position of DHX in the binding pocket, followed by the equatorial compound **2b** (Table 2). Both of these show a smaller rmsd than the 7-substituted compounds. The latter, however, show a slightly different profile in rmsd than they do for affinity, with the lower affinity axial compound **3a** having a smaller deviation. This occurrence might result from the fact that displacement of the 7-axial compound from the position of DHX is more vertical, rather than lateral, which more markedly pushes the catechol moiety out of its binding region. In other words, the receptor may have greater tolerance for pulling the catechol away from TM5 without changing the binding pattern, whereas a vertical movement (toward the extracellular space) of the catechol will more readily force the molecule into a different, less optimal binding pose.

From a quantitative point of view, the rmsd values correlated extremely well to the free energies of binding of the corresponding compounds (R^2 = 0.9851). The previously discussed incongruencies between the rmsd and the biological data for the

7-methylated compounds resulted in only slight deviation from linearity.



CONCLUSION

In conclusion, we have shown that there is little steric tolerance at the floor of the orthosteric binding site in the dopamine D_1 (and D_2) receptor, beyond the ethyl bridge that exists in DHX. There is clearly a region of restricted tolerance at the bottom of the orthosteric binding site that is comprised of Phe203$^{5.47}$, Phe288$^{6.51}$, and Ser107$^{3.36}$, with the latter apparently being responsible for most of the observed losses in affinity in the present series. In the case of 8β-axially substituted compound **2a**, the absence of an unfavorable steric interaction between Ser107 and the ligand allowed the affinity to be only modestly impacted compared to the other analogues. In general, as the overlap of the substituent with the proposed location of TM3 increased, the affinity of the ligand for the receptor decreased, with the effect most evident for 7α-axially methylated compound **3a**, whose affinity was beyond the range of detection.

EXPERIMENTAL SECTION

Chemistry. All reagents were commercially available and were used without further purification unless otherwise indicated. Anhydrous THF was obtained by distillation from benzophenone-sodium under nitrogen immediately before use. Melting points were determined using a Thomas−Hoover apparatus and are uncorrected. ^{1}H NMR spectra were obtained with a Bruker ARX300 (300 MHz) instrument. Chemical shifts are reported in δ values (ppm) relative to an internal reference of TMS. Chemical ionization mass spectra (CIMS), using isobutene as the carrier gas, were obtained with a Finnigan 4000 spectrometer. Elemental analyses were performed by the Purdue University Microanalysis Laboratory and are within ±0.4% of the calculated values. Thin-layer chromatography was performed using J.T. Baker flex silica gel IB2-F, plastic-blacked sheets with fluorescent indicator, visualizing with UV light at 254 nm. Column chromatography was carried out using Silica Gel 60, 230−400 mesh (J.T. Baker). All reactions were carried out under an inert atmosphere of argon unless otherwise indicated.

*(±)-trans-10,11-Dihydroxy-8β-axial-methyl-5,6,6a,7,8,12b-hexahydrobenzo[a]phenanthridine (**2a**).* The free base **12a** (100 mg, 0.32 mmol) was dissolved in 20 mL of CH_2Cl_2, and the solution was cooled to −78 °C. Through a syringe, a 1.0 M solution of BBr_3 in CH_2Cl_2 (3.0 mL, 3.0 mmol) was added slowly to the solution. The reaction was left to warm to room temperature and was stirred overnight. The reaction was then quenched by addition of methanol (15 mL), stirred for 1 h, and the solvents removed by rotary evaporation. The residue was redissolved in 15 mL of MeOH, and the solvents were removed by rotary evaporation. This procedure was repeated twice, and the remaining residue was put under high vacuum for 8 h to afford 110 mg (100%) of the desired hydrobromide salt; mp 199−201 °C. ^{1}H NMR (D_2O): δ 7.53 (m, 4H, Ar_2H), 6.96 (s, 1H, ArH), 6.93 (s, 1H, ArH), 4.48 (bs, 2H, Ar_2CH_2N), 4.35 (d, 1H, Ar_2CHCHN, J = 11.40 Hz), 3.30−3.10 (m, 2H, Ar_2CHCHN and $ArCH(CH_3)CH_2CHN$), 2.20 (m, 1H, $ArCH(CH_3)CH_2CHN$), 2.00 (m, 1H, $ArCH(CH_3)CH_2CHN$), 1.31 (d, 3H, $ArCH(CH_3)CH_2CHN$, J = 6.88 Hz). ESIMS: 282 ($M + H^+$, 100). Anal. ($C_{18}H_{20}BrNO_2 + 2H_2O$) (C, H, N).

*(±)-trans-10,11-Dihydroxy-8α-equatorial-methyl-5,6,6a,7,8,12b-hexahydrobenzo[a] phenanthridine (**2b**).* Compound **14b** (75 mg, 0.18 mmol) was dissolved in 20 mL of CH_2Cl_2, and the solution was cooled to −78 °C. Through a syringe, a 1.0 M solution of BBr_3 in CH_2Cl_2 (3.0 mL, 3.0 mmol) was added slowly. The solution was left to warm to RT, and it was stirred overnight. The reaction was quenched by addition of methanol (5.0 mL). The solvent was removed under vacuum, and the product was recrystallized from 2-propanol. The crystals were collected by filtration and dried under high vacuum to obtain 63 mg (95%) of the desired hydrobromide salt **2b**; mp 200−201 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 7.51 (d, 1H, Ar_2H, J = 7.24 Hz), 7.39 (m, 3H, Ar_2H), 6.85 (s, 1H, ArH), 6.81 (s, 1H, ArH), 4.45 (bs, 2H, Ar_2CH_2N), 4.25 (d, 1H, Ar_2CHCHN, J = 11.14 Hz), 3.13 (m, 1H, Ar_2CHCHN), 3.00 (m, 1H, $ArCH(CH_3)CH_2CHN$), 2.40 (m, 1H, $ArCH(CH_3)CH_2CHN$), 1.75 (m, 1H, $ArCH(CH_3)CH_2CHN$), 1.38 (d, 3H, $ArCH(CH_3)CH_2CHN$, J = 6.96 Hz). ESIMS: 282 ($M + H^+$, 100). Anal. ($C_{18}H_{20}BrNO_2 + 2H_2O$) C, H, N.

*10,11-Dihydroxy-7α-axial-methyl-5,6,6a,7,8,12b-hexahydrobenzo[a]phenanthridine Hydrobromide (**3a**).* Compound **20a** (0.30 g, 0.867 mmol) was dissolved in 20 mL of CH_2Cl_2, and the solution was cooled to −78 °C. A 1.0 M solution of BBr_3 in CH_2Cl_2 (6.0 mL, 6.0 mmol) was added slowly to the solution over 30 min. The solution was allowed to warm up to room temperature and was stirred overnight. The reaction was quenched by addition of dry methanol (10 mL). The solvent was removed under vacuum, and the crude product was recrystallized from 2-propanol. Drying under high vacuum left 260 mg (95%) of the desired hydrobromide salt as a yellowish solid; mp 272−274 °C. ^{1}H NMR (DMSO-d_6): δ 9.13 (bs, 1H, NH), 8.70 (bs, 2H, OH), 7.40 (m, 4H, Ar_2H), 6.68 (s, 1H, ArH), 6.50 (s, 1H, ArH), 4.40 (bs, 2H, Ar_2CH_2N), 4.14 (d, 1H, Ar_2CHCHN, J = 11.4 Hz), 3.48−3.35 (m, 1H, Ar_2CHCHN), 3.10 (m, 1H, $ArCH_2CH(CH_3)CHN$), 2.92 (dd, 1H, $ArCH_2CH(CH_3)CHN$, J = 7.20 Hz, J' = 16.8 Hz), 2.52 (m, 1H, $ArCH_2CH(CH_3)CHN$), 1.09 (d, 3H, $ArCH_2CH(CH_3)CHN$, J = 6.30 Hz). ESIMS: 282 ($M + H^+$, 100). Anal. ($C_{18}H_{20}BrNO_2 + H_2O$) C, H, N.

*(±)-trans-10,11-Dihydroxy-7β-methyl-5,6,6a,7,8,12b-hexahydrobenzo[a]phenanthridine Hydrobromide (**3b**).* The HCl salt **20b** (50 mg, 0.144 mmol) was dissolved in water, and 5 mL of conc $NaHCO_3$ were added. This suspension was extracted into 10 mL of CH_2Cl_2, dried over $MgSO_4$, and filtered. This solution was cooled to −78 °C, and 0.6 mL (0.6 mmol) of a 1 M solution of BBr_3 in CH_2Cl_2 was added. This solution was stirred overnight at RT. It was then quenched by addition of dry methanol and the solvents removed by rotary evaporation. Another 5 mL of dry methanol were added to the residue, and the solution was reduced under reduced pressure to leave a residue containing trace methanol. Addition of EtOAc to this residue induced crystallization of the product, which was collected by filtration to yield 38 mg (72.5% yield) of **3b** as a yellow powder. ^{1}H NMR (DMSO-d_6): δ 9.25 (br, 2H, $^+NH_2$), 8.88 (s, 2H, 2ArOH), 7.46−7.36 (m, 4H, 4ArH), 6.69 (s, 1H, ArH), 6.60 (s, 1H, ArH), 4.40 (s, 2H, CH_2N), 4.18−4.14 (d, 1H, ArCHAr, J_{trans} = 11.1 Hz), 2.80 (dd, 1H, $ArCH_{equat}$, J_{gem} = 15.9 Hz, J_{vic} = 5.4 Hz), 2.59 (t, 1H, CHN, J_{trans} = 11.1 Hz), 2.40 (dd, 1H, $ArCH_{axial}$, J_{gem} = 15.9 Hz, J_{vic} = 7.8 Hz), 2.15 (m, 1H, CCHC), 1.07 (d, 3H, CH_3).

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ESIMS: 282 (M^+, 100). High resolution ESIMS for $C_{18}H_{21}NO_2$ (M^+): calcd 282.1494, found 282.1496.

*(E)-3-(4,5-Dimethoxy-2-methoxycarbonylmethyl-phenyl)-but-2-enoic Acid Methyl Ester (**7**).* 2-Iodo-4,5-dimethoxyphenylacetic acid methyl ester **6**[19] (10.0 g, 30.0 mmol) was dissolved in 50 mL of CH_3CN under an inert atmosphere. Into this solution was added Et_3N (24.0 mL, 0.16 mol), methyl crotonate (26 mL, 0.230 mol), and $Pd(PPh_3)_2Cl_2$ (0.60 g, 0.85 mmol). The reaction mixture was heated at reflux for 4 days and then cooled to RT. The solvent was evaporated, and the residue was dissolved in ether. The undissolved solids were removed by filtration, and the filtrate was washed with 2 M HCl (2 × 100 mL) and H_2O (2 × 100 mL). The organic layer was dried ($MgSO_4$) and filtered, and the solvent was evaporated. The crude mixture was purified by column chromatography on silica, using ethyl acetate:hexanes (10:90) as eluent, to yield the product as a colorless oil 4.95 g (54%). ^{1}H NMR ($CDCl_3$): δ 6.79 (s, 1H, ArH), 6.63 (s, 1H, ArH), 5.78 (d, 1H, ArC(CH_3)-$CHCO_2CH_3$, J = 1.40 Hz), 3.89 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 3.58 (s, 2H, Ar$CH_2CO_2CH_3$), 2.45 (d, 3H, ArC(CH_3)$CHCO_2CH_3$, J = 1.35 Hz). ESIMS: 331 (M + Na^+, 100). Anal. ($C_{16}H_{20}O_6$) C, H, N.

*3,(4,5-Dimethoxy-2-methoxycarbonylmethyl-phenyl)-butyric Acid Methyl Ester (**8**).* The olefin **7** (9.0 g, 29.2 mmol) was dissolved in EtOH (200 mL) and shaken in a Parr hydrogenator at 60 psi over 1.0 g of Ra-Ni until H_2 uptake ceased. The suspension was filtered over a pad of Celite, and the filtrate was evaporated. Bulb to bulb distillation (140−145 °C, 0.2 Torr) gave the pure product as a colorless oil (8.9 g, quant). ^{1}H NMR ($CDCl_3$): δ 6.72 (s, 1H, ArH), 6.68 (s, 1H, ArH), 3.87 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.78 (s, 1H, Ar$CH_2CO_2CH_3$), 3.70 (s, 3H, OCH_3), 3.63 (s, 1H, Ar$CH_2CO_2CH_3$), 3.61 (s, 3H, OCH_3), 3.51−3.40 (m, 1H, ArCH(CH_3)$CH_2CO_2CH_3$), 2.57 (m, 2H, ArCH-(CH_3)$CH_2CO_2CH_3$), 1.25 (d, 3H, ArCH(CH_3) $CH_2CO_2CH_3$, J = 6.83 Hz). ESIMS: 333 (M + Na^+, 100). Anal. ($C_{16}H_{22}O_6$) C, H, N.

*6,7-Dimethoxy-4-methyl-3,4-dihydro-1H-naphthalen-2-one (**9**).* To a mixture of potassium *tert*-butoxide (3.90 g, 35.0 mmol) in dry Et_2O (150 mL), under inert atmosphere and protected from light, a solution of **8** (10.5 g, 33.83 mmol) in Et_2O (150 mL) was added dropwise through a dropping funnel. The reaction was left to stir for 45 min at RT. The solids were separated by filtration, washed on the filter with Et_2O, and dried under high vacuum and protected from light to afford a quantitative yield of the potassium enolate as an off-white powder. This potassium salt (10.5 g, 33.19 mmol) and LiCl (1.70 g, 40.0 mmol) were dissolved in NMP (100 mL). Concentrated HCl (4.0 mL, 40.0 mmol) was added, turning the color of the solution from dark-red to yellow. The flask was then placed in an oil bath preheated to 125 °C and heated at reflux overnight under and inert atmosphere. The mixture was allowed to cool to RT (protected from light). Then, the mixture was diluted with EtOAc (50 mL) and the organic layer was washed with H_2O (3 × 15 mL) and brine (3 × 10 mL). The organic layer was dried ($MgSO_4$) and simultaneously decolorized with charcoal. After filtration over Celite, the solvent was evaporated. Bulb to bulb distillation (125−130 °C, 0.1 Torr) gave a yellowish oil (6.0 g, 82.1%). ^{1}H NMR ($CDCl_3$): δ 6.78 (s, 1H, ArH), 6.62 (s, 1H, ArH), 3.91 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.55 (d, 2H, ArCH_2CO, J = 3.52 Hz), 3.22 (m, 1H, ArCH(CH_3)CH_2CO), 2.71 (dd, 1H, ArCH(CH_3)CH_2CO, J = 5.33 Hz, J' = 16.0 Hz), 2.37 (dd, 1H, ArCH(CH_3)CH_2CO, J = 6.50, J' = 16.0 Hz), 1.30 (d, 3H, ArCH(CH_3CH_2)CO, J = 7.0 Hz). CIMS: 221 (M^+ + H, 100). Anal. ($C_{13}H_{16}O_3$) C, H, N.

*N-Benzyl-N-(6,7-dimethoxy-4-methyl-3,4-dihydro-naphthalen-2-yl)-benzamide (**10**).* In a flask equipped with a condenser and a Dean−Stark trap, a solution of **9** (5.0 g, 22.7 mmol) in toluene (80 mL) was added benzylamine (2.60 g, 2.70 mL, 24.0 mmol). The solution was heated at reflux overnight with continuous azeotropic removal of water. The reaction was allowed to cool to RT, and the solvent was evaporated. The residue was dissolved in CH_2Cl_2, and triethylamine (2.50 g, 3.50 mL, 25.0 mmol) was added. The mixture was cooled in an ice bath. Benzoyl chloride (3.50 g, 3.0 mL, 25.0 mmol) in CH_2Cl_2 (3.0 mL) was added dropwise to the cold solution. After complete addition, the mixture was allowed to warm to RT and was left to stir overnight. The mixture was the diluted in CH_2Cl_2, and the organic layer was washed with 2 M HCl (2 × 15 mL), 1 M NaOH (2 × 10 mL), and brine (2 × 10 mL). The mixture was dried with $MgSO_4$ and filtered, and the solvent was evaporated. The product was then purified by column chromatography on silica, using EtOAc:hexanes (10:90) as the eluent to yield a yellowish oil, 6.29 g (67%). ^{1}H NMR ($CDCl_3$): δ 7.60 (d, 2H, Ar_2H, J = 7.41 Hz), 7.43−7.22 (m, 8H, Ar_2H and Ar_3H), 6.58 (s, 1H, ArH), 6.38 (s, 1H, ArH), 6.02 (s, 1H, ArCHCN), 4.98 (d, 2H, Ar_3CH_2N, J = 4.30 Hz), 3.84 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 2.69 (m, 1H, ArCH-(CH_3)CH_2CN), 2.26 (dd, 1H, ArCH(CH_3)CH_2CN, J = 6.80, J' = 16.50 Hz), 1.92 (dd, 1H, ArCH(CH_3)CH_2CN, J = 6.80, J' = 16.50 Hz), 0.89 (d, 3H, ArCH(CH_3)CH_2CN, J = 6.89 Hz). CIMS: 414 (M + H^+, 100). Anal. ($C_{27}H_{27}NO_3$) C, H, N.

*N-Benzyl-10,11-dimethoxy-8-methyl-6a,7,8,12b-tetrahydro-6H-benzo[a]-phenanthridin-5-one (**11a,b**).* A solution of **10** (1.0 g, 2.40 mmol) in dry THF (250 mL) was introduced into a 250 mL photochemical reactor. The solution was stirred under argon and irradiated for 1 h with a 450 W Hanovia medium-pressure quartz mercury-vapor lamp seated in a water-cooled quartz immersion well. The solution was concentrated in vacuo, and the product mixture (50/50% *cis*/*trans* by ^{1}H NMR) was purified by column chromatography on silica, using EtOAc:hexanes (20:80) to yield the mixture of stereoisomers that crystallized as white needles 0.50 g (50%).

*N-Benzyl-10,11-dimethoxy-8β-axial-methyl-6a,7,8,12b-tetrahydro-6H-benzo[a]-phenanthridin-5-one (**11a**).* The mixture **11a,b** (3 g) was subjected to rotary chromatography eluting with EtOAc:hexanes (1:99) as obtaining a trace amount of crystalline **11a**, which was used to seed its selective crystallization from the mixture **11a,b**, obtaining 1.1 g of pure **11a** as white crystals. ^{1}H NMR ($CDCl_3$): δ 8.21 (dd, 1H, Ar_2H, J = 1.70 Hz, J' = 7.20 Hz), 7.59 (d, 1H, Ar_2H,J = 7.30 Hz), 7.52−7.40 (m, 2H, Ar_2H), 7.34−7.18 (m, 5H, Ar_3H), 6.93 (s, 1H, ArH), 6.65 (s, 1H, ArH), 5.36 (d, 1H, Ar_3CH_2N, J = 16.0 Hz), 4.75 (d, 1H, Ar_3CH_2N, J = 16 Hz), 4.30 (d, 1H, Ar_2CHCHN, J = 11.30 Hz), 4.00 (m, 1H, Ar_2CHCHN), 3.89 (s, 6H, $2OCH_3$), 3.00 (m, 1H, ArCH(CH_3)CH_2CHN), 2.09 (m, 1H, ArCH(CH_3)CH_2CHN), 1.95 (m, 1H, ArCH(CH_3)CH_2CHN), 1.10 (d, 3H, ArCH(CH_3)CH_2CHN, J = 7.27 Hz); mp 215−217 °C. CIMS: 414 (M + H^+, 100). Anal. ($C_{27}H_{27}NO_3$) C, H, N.

*(±)-trans-10,11-Dimethoxy-8β-axial-methyl-5,6,6a,7,8,12b-hexahydrobenzo[a]phenanthridine (**12a**).* A solution of **11a** (1.1 g, 2.66 mmol) in dry THF (100 mL) was cooled in an ice−salt bath, and 6.0 mL of a 1 M solution of BH_3 in THF was added through a syringe and the reaction was heated at reflux overnight. Carefully, 10 mL of a 1 M solution of HCl in MeOH was added to the solution and the reaction was stirred at reflux for 1 h. The solvents were removed by rotary evaporation, and the product was dissolved in 15 mL of dry EtOH and rotary evaporated. The residue was dissolved in 15 mL of dry EtOH and rotary evaporated three more times. The remaining material was then crystallized from EtOH/Et_2O to afford 0.9 g (78%) of yellowish crystals of 6-benzyl-10,11-dimethoxy-8β-axial-methyl-5,6,6a,7,8,12b-hexahydro benzo[*a*]phenanthridine hydrochloride; mp (free amine) 190−192 °C. ^{1}H NMR ($CDCl_3$, free amine): δ 7.48 (d, 1H, Ar_2H, J = 7.50 Hz), 7.42−7.20 (m, 7H, Ar_2H and Ar_3H), 7.08 (d, 1H, Ar_2H, J = 7.32 Hz), 6.92 (s, 1H, ArH), 6.87 (s, 1H, ArH), 4.14 (d, 1H, Ar_2CH_2N, J = 10.73 Hz), 4.00 (d, 1H, Ar_2CH_2N, J = 13.21 Hz), 3.95 (s, 3H, OCH_3), 3.85 (m, 1H, Ar_2CHCHN), 3.79 (s, 3H, OCH_3), 3.50 (d, 1H, Ar_3CH_2N, J = 15.21 Hz), 3.30 (d, 1H, Ar_3CH_2N, J = 13.23 Hz), 3.10 (m, 1H, Ar_2CHCHN), 2.50 (m, 1H, ArCH(CH_3)CH_2CHN), 2.22 (m, 1H, ArCH(CH_3)CH_2CHN), 1.82 (m, 1H, ArCH(CH_3)CH_2CHN), 1.45 (d, 3H, ArCH(CH_3)CH_2CHN, J = 6.84 Hz). EIMS: 400 (M + H^+). Anal. ($C_{27}H_{29}NO_2$ + $^1/_3H_2O$) C, H, N. To a solution of this

hydrochloride salt (0.8 g, 1.83 mmol) in 95% ethanol (250 mL) was added 100 mg of 10% Pd−C catalyst and shaken at room temperature under 60 psi of H_2 for 6 h. After removal of the catalyst by filtration, the solution was concentrated to dryness under vacuum. The remaining residue was crystallized from EtOH/Et_2O to afford 0.6 g (95.2%) of the title compound product; mp (free amine) 131−133 °C. ^{1}H NMR (300 MHz, $CDCl_3$, free amine): δ 7.41 (d, 1H, Ar_2H, J = 7.37 Hz), 7.02 (m, 2H, Ar_2H), 7.10 (d, 1H, Ar_2H, J = 7.12 Hz), 6.86 (s, 1H, ArH), 6.79 (s, 1H, ArH), 4.00 (m, 2H, Ar_2CH_2N), 3.84 (s, 3H, OCH_3), 3.80 (d, 1H, Ar_2CHCHN, J = 11.0 Hz), 3.69 (s, 3H, OCH_3), 3.00 (m, 1H, Ar_2CHCHN), 2.60 (m, 1H, $ArCH(CH_3)CH_2CHN$), 1.85−1.65 (m, 2H, $ArCH(CH_3)CH_2CHN$), 1.35 (d, 3H, $ArCH(CH_3)CH_2CHN$, J = 6.82 Hz). CIMS: 310 ($M + H^+$).

*(±)-trans-10,11-Dimethoxy-8α-equatorial-methyl-6a,7,8,12b-tetrahydro-5H-benzo[a]phenanthridine-6-carboxylic Acid tert-Butyl Ester (**14b**).* A solution of **11a,b** (1.1 g, 2.66 mmol) in dry THF (100 mL) was cooled in an ice−salt bath and 6.0 mL (6.0 mmol) of a 1 M solution of BH_3 in THF was added through a syringe. The reaction was heated at reflux overnight. Slowly, 10 mL of a 1 M solution of HCl in MeOH was added to the solution and the reaction was stirred at reflux for 1 h. The solvents were removed by rotary evaporation, and the crude product was dissolved in 15 mL of dry EtOH and rotary evaporated. The residue was dissolved in 15 mL of dry EtOH and rotary evaporated three more times. The diastereomeric product mixture **13a,b** was crystallized from EtOH/Et_2O to afford 0.9 g (78%) that was used without further purification for the next step.

A solution of the free base of **11a,b** (3.0 g, 7.50 mmol), *t*-Boc anhydride (3.0 g, 14.0 mmol), and Pd (10% on carbon) in dry THF (10.0 mL) was hydrogenated under 1 atm H_2 for 2 days. The suspension was filtered through a pad of Celite and the filtrate concentrated to afford 2.70 g (88%) of product as a mixture of diastereomers. Silica-gel flash column chromatography eluting with EtOAc:hexanes 20:80 allowed separation of this mixture to afford 1.2 g (39%) of **14b** and 1.5 g of **14a** (49%). For **14b**: mp 133−134 °C. ^{1}H NMR ($CDCl_3$): δ 7.50 (d, 1H, Ar_2H, J = 7.60 Hz), 7.25 (m, 3H, Ar_2H), 6.97 (s, 1H, ArH), 6.66 (s, 1H, ArH), 5.05 (d, 1H, Ar_2CHCHN, J = 14.64 Hz), 4.15 (d, 1H, Ar_2CH_2N, J = 14.60 Hz), 4.02 (d, 1H, Ar_2CH_2N, J = 11.70 Hz), 3.85 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.44 (m, 1H, Ar_2CHCHN), 2.99 (m, 1H, $ArCH(CH_3)CH_2CHN$), 2.50 (d, 1H, $ArCH(CH_3)CH_2CHN$*J*), 1.84 (m, 1H, $ArCH(CH_3)CH_2CHN$), 1.39 (s, 9H, NBoc), 1.30 (d, 3H, $ArCH(CH_3)CH_2CHN$, J = 10.84 Hz). CIMS: 410 ($M + H^+$). Anal. ($C_{25}H_{31}NO_4$) C, H, N.

*3,(4,5-Dimethoxy-2-methoxycarbonylmethyl-phenyl)-2-methyl-acrylic Acid Methyl Ester (**15**).* Under an inert, dry atmosphere, 2-iodo-4,5-dimethoxyphenylacetic acid methyl ester **6**[19] (10.0 g, 30.0 mmol) was dissolved in 150 mL of CH_3CN. To the solution was added Et_3N (12.0 mL, 89.0 mmol), methyl methacrylate (13.0 mL, 0.120 mol), and $Pd(PPh_3)_2Cl_2$ (0.42 g, 0.60 mmol). The reaction mixture was heated at reflux overnight. The mixture was left to cool to RT, and the solvent was removed under reduced pressure. The remaining material was dissolved in 50 mL of CH_2Cl_2, and the solution was washed with H_2O (2 × 50 mL), 2 M HCl (2 × 50 mL), and H_2O (2 × 30 mL). The organic layer was dried ($MgSO_4$) and filtered, and the solvent was evaporated. The crude product was purified by silica gel flash column chromatography eluting with EtOAc:hexanes 90:10 to afford 7.0 g (76%) of a white solid; mp 31−32 °C. ^{1}H NMR ($CDCl_3$): δ 7.70 (d, 1H, ArCH, J = 1.2 Hz), 6.82 (s, 1H, ArH), 6.75 (s, 1H, ArH), 3.87 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 3.55 (s, 2H, $ArCH_2CO_2CH_3$), 1.96 (d, 3H, $ArCHC(CH_3)CO_2CH_3$, J = 1.2 Hz). ESIMS: 331 ($M + Na^+$, 100). Anal. ($C_{16}H_{20}O_6$) C, H, N.

*3,(4,5-Dimethoxy-2-methoxycarbonylmethyl-phenyl)-2-methyl-propionic Acid Methyl Ester (**16**).* The olefin **15** (9.0 g, 29.2 mmol) was dissolved in hot EtOH (200 mL), and to this solution was added 10% palladium on carbon (1.0 g). The reaction mixture was shaken in a Parr hydrogenator with H_2 at 60 psi until H_2 uptake had ceased. The suspension was filtered over Celite, and the solvent was evaporated. Bulb to bulb distillation (145−150 °C/0.2 Torr) gave, quantitatively, a colorless oil that solidified; mp 33−35 °C. ^{1}H NMR ($CDCl_3$): δ 6.67 (s, 1H, ArH), 6.63 (s, 1H, ArH), 3.86 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 3.63 (s, 3H, OCH_3), 3.59 (s, 2H, $ArCH_2CO_2CH_3$), 3.00 (m, 1H, $ArCH_2CH(CH_3)CO_2CH_3$), 2.68 (m, 2H, $ArCH_2CH(CH_3)CO_2CH_3$ and $ArCH_2CH(CH_3)CO_2CH_3$), 1.19 (d, 3H, $ArCH_2CH(CH_3)CO_2CH_3$, J = 6.6 Hz. ESI-MS (m/z) 333 ($M^+ + Na^+$, 100). Anal. ($C_{16}H_{22}O_6$) C, H, N.

*6,7-Dimethoxy-3-methyl-3,4-dihydro-1H-naphthalen-2-one (**17**).* To a suspension of potassium *tert*-butoxide (2.3 g, 20.0 mmol) in dry Et_2O (30 mL) under an inert atmosphere and shielded from light, a solution of **16** (6.0 g, 19.33 mmol) in Et_2O (50 mL) was added dropwise through a dropping funnel. The reaction was stirred for 45 min at RT. The solids were collected by filtration, washed on the filter with Et_2O, and dried under high vacuum, shielded from light, affording quantitative yield of the potassium enolate as an off-white powder. This potassium salt (2.0 g, 6.32 mmol) and LiCl (0.42 g, 10.0 mmol) were dissolved under an inert atmosphere in NMP (20 mL). Concentrated HCl (1.0 mL, 10.0 mmol) was added, turning the color of the solution from dark-red to yellow. The flask was then placed in an oil bath preheated to 125 °C and heated at reflux overnight under argon. While shielded from light, the mixture was allowed to cool to RT and it was diluted with ethyl acetate (50 mL). This solution was washed with H_2O (3 × 30 mL) and brine (3 × 10 mL). It was then dried over $MgSO_4$ and simultaneously decolorized with charcoal. After filtration over Celite, the solvent was evaporated to obtain 0.90 g of an off-white oil that rapidly crystallized (64%); mp 85−87 °C. ^{1}H NMR ($CDCl_3$): δ 6.68 (s, 1H, ArH), 6.58 (s, 1H, ArH), 3.86 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.51 (d, 2H, $ArCH_2CO$, J = 2.40 Hz), 3.00 (dd, 1H, $ArCH_2CH(CH_3)CO$, J = 5.70 Hz, J' = 15.3 Hz), 2.75 (dd, 1H, $ArCH_2CH(CH_3)CO$, J = 10.8 Hz, J' = 15.3 Hz), 2.61−2.51 (m, 1H, $ArCH_2CH(CH_3)CO$), 1.17 (d, 3H, $ArCH_2CH(CH_3)CO$, J = 6.6 Hz). CI-MS 221 ($M^+ + H^+$, 100). Anal. ($C_{13}H_{16}O_3$) C, H, N.

*N-Benzyl-N-(6,7-dimethoxy-3-methyl-3,4-dihydro-naphthalen-2-yl)-benzamide (**18**).* In a flask equipped with a condenser and a Dean−Stark trap containing a solution of **17** (5.0 g, 22.7 mmol) in toluene (80.0 mL), benzylamine (2.60 g, 2.70 mL, 24.0 mmol) was added. The solution was heated at reflux overnight with continuous azeotropic removal of water. The reaction was allowed to cool to RT, and the solvent was evaporated. The remaining brown oil was dissolved in CH_2Cl_2 (20 mL) and triethylamine (2.50 g, 3.50 mL, 25.0 mmol) and cooled in an ice bath. Benzoyl chloride (3.50 g, 3.0 mL, 25.0 mmol) in CH_2Cl_2 (3.0 mL) was added dropwise to the cold solution. After complete addition, the mixture was allowed to warm to room temperature and was left to stir overnight. The mixture was the diluted in CH_2Cl_2, and the organic layer was washed with 2 M HCl (2 × 15 mL), 1 M NaOH (2 × 10 mL), and brine (2 × 10 mL). The mixture was dried with $MgSO_4$ and filtered, and the solvent was evaporated. The residue was dissolved in Et_2O, and the solution was filtered over a Celite. After evaporating the solvent, the product (6.2 g, 66%) was crystallized from Et_2O; mp: 118−120 °C. ^{1}H NMR ($CDCl_3$): δ 7.70 (d, 2H, Ar_2H, J = 7.8 Hz), 7.45−7.27 (m, 8H, Ar_2H and Ar_3H), 6.51 (s, 1H, ArH), 6.41 (s, 1H, ArH), 6.13 (s, 1H, ArCHCN), 5.31 (d, 1H, Ar_3CH_2N, J = 15.0 Hz), 4.71 (d, 1H, Ar_3CH_2N, J = 15.0 Hz), 3.81 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 2.52 (dd, 1H, $ArCH_2CH(CH_3)CHN$, J = 6.4, J' = 15.1 Hz), 2.25 − 2.11 (m, 2H, $ArCH_2CH(CH_3)CN$ and $ArCH_2CH(CH_3)CN$), 0.75 (d, 3H, $ArCH_2CH(CH_3)CN$, J = 6.6 Hz). ESIMS: 414 ($M + H^+$, 100). Anal. ($C_{27}H_{27}NO_3$) C, H, N.

*(±)-trans-N-Benzyl-10,11-dimethoxy-7α-methyl-6a,7,8,12b-tetrahydro-6H-benzo[a]phenanthridin-5-one (**19a**).* A solution of **18** (1.0 g, 2.40 mmol) in dry THF (250 mL) was placed into a 250 mL photochemical reactor. The solution was stirred under argon and irradiated for

1 h with a 450 W Hanovia medium-pressure quartz mercury-vapor lamp seated in a water-cooled quartz immersion well. The solution was concentrated in vacuo and crystallized from Et_2O to provide yellowish crystals (600 mg, 60%); mp 214−216 °C. ^{1}H NMR ($CDCl_3$): δ 8.20 (dd, 1H, Ar_2H, J = 1.60, J' = 7.36 Hz), 7.51 (d, 1H, Ar_2H, J = 7.46 Hz), 7.45 (m, 2H, Ar_2H), 7.30−7.10 (m, 5H, Ar_3H), 6.95 (s, 1H, ArH), 6.60 (s, 1H, ArH), 5.59 (d, 1H, Ar_3CH_2N, J = 16.0 Hz), 4.30 (d, 2H, Ar_3CH_2N and Ar_2CHCHN, J = 16.0 Hz and J = 12.8 Hz), 3.90 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.76 (dd, 1H, $ArCH_2CH(CH_3)CHN$, J = 3.1, J' = 15.1 Hz), 2.81 (dd, 1H, $ArCH_2CH(CH_3)CHN$, J = 3.70, J' = 16 Hz), 2.55−2.43 (m, 2H, $ArCH_2CH(CH_3)CHN$ and $ArCH_2CH(CH_3)CHN$), 1.00 (d, 3H, $ArCH_2CH(CH_3)CHN$, J = 6.6 Hz). ESIMS: 436 (M + Na^+, 100). Anal. ($C_{27}H_{27}NO_3$) C, H, N.

*(±)-trans-10,11-Dimethoxy-7α-methyl-5,6,6a,7,8,12b-hexahydrobenzo-[a]phenanthridine (**20a**).* A solution of **19a** (2.0 g, 4.80 mmol) in dry THF (100 mL) was cooled in an ice−salt bath, and 10.0 mL of a 1 M solution of BH_3 in THF was added via a syringe. The reaction was heated at reflux overnight. Carefully, 15 mL of a 1 M solution of HCl in MeOH was added to the solution and the reaction was stirred at reflux for 1 h. The solvents were removed under reduced pressure, and the remaining solids were again dissolved in 20 mL of dry EtOH. The solvents were removed under reduced pressure. The remaining material was crystallized from EtOH/Et_2O to afford 1.72 g (85%) of (±)-*trans-N*-benzyl-10,11-dimethoxy-7α-methyl-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine as colorless crystals; mp: 208−211 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.68 (dd, 2H, Ar_2H, J = 1.80, J = 6.90 Hz), 7.50 (m, 2H, Ar_2H), 7.43−7.35 (m, 5H, Ar_3H), 6.88 (s, 1H, ArH), 6.55 (s, 1H, ArH), 4.83 (dd, 1H, Ar_2CH_2N, J = 4.51, J' = 14.7 Hz), 4.70 (dd, 1H, Ar_2CH_2N, J = 2.75, J' = 12.30 Hz), 4.36 (d, 1H, Ar_3CH_2N, J = 14.87 Hz), 4.28 (d, 1H, Ar_3CH_2N, J = 11.76 Hz),), 3.88 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.50 (m, 1H, Ar_2CHCHN), 2.87 (m, 1H, Ar_2CHCHN), 2.55 (dd, 1H, $ArCH_2CH(CH_3)CHN$, J = 4.30, J' = 16.20 Hz), 2.50 (dd, 1H, $ArCH_2CH(CH_3)CHN$, J = 2.0 Hz, J' = 17.0 Hz), 1.72 (m, 1H, $ArCH_2CH(CH_3)CHN$), 1.30 (d, 3H, $ArCH_2CH(CH_3)CHN$, J = 6.79 Hz. CIMS: 400.20 (M + H^+, 100). To a solution of this hydrochloride salt (1.0 g, 2.30 mmol) in 95% ethanol (250 mL) was added 0.1 g of 10% Pd/C, and the solution was shaken at room temperature under 60 psi of H_2 for 8 h. After removal of the catalyst by filtration over Celite, the solution was concentrated to dryness under vacuum and the residue crystallized from EtOH/Et_2O to afford 0.86 mg (89.5%) of crystalline salt; mp 149−152 °C. ^{1}H NMR (DMSO-d_6): δ 7.46−7.31 (m, 4H, Ar_2H), 6.86 (s, 1H, ArH), 6.84 (s, 1H, ArH), 4.41 (s, 2H, Ar_2CH_2N), 4.24 (d, 1H, Ar_2CHCHN), 3.77 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 3.36 (bs, 1H, Ar_2CHCHN), 3.15 (dd, 1H, $ArCH_2CH(CH_3)CHN$, J = 4.57 Hz, J' = 11.35 Hz), 3.03 (dd, 1H, $ArCH_2CH(CH_3)CHN$, J = 4.90 Hz, J' = 15.48 Hz), 2.56 (m, 1H, $ArCH_2CH(CH_3)CHN$), 1.10 (d, 3H, $ArCH_2CH(CH_3)CHN$, J = 6.62 Hz). CIMS: 310 (M + H^+, 100). Anal. ($C_{20}H_{23}NO_2$) C, H, N).

*(±)-trans-10,11-Dimethoxy-7β-methyl-5,6,6a,7,8,12b-hexahydrobenzo-[a]phenanthridine Hydrochloride (**20b**).* The 7β-methyl lactam **32b** (70 mg, 0.216 mmol) was dissolved in 10 mL of dry THF, and 1 mL (1 mmol) of a 1 M solution of BH_3 in THF was added. This solution was stirred at reflux for 14 h, and water was added to quench the reaction. The mixture was extracted three times with 5 mL of CH_2Cl_2, and the organic solvents were pooled and washed once with water. The solution was dried over $MgSO_4$, filtered, and the solvent removed to leave a clear residue. This residue was treated with 5 mL of a 2 M solution of HCl in EtOH and heated at reflux for 10 min. EtOAc was added to this solution and, after cooling to RT overnight, produced a precipitate, which was collected by filtration. A second crystallization gave a total of 60 mg (80% yield) of the HCl salt **20b** as a white powder; mp 246−252 °C dec. ^{1}H NMR (D_2O): δ 7.31−7.18 (m, 4H, 4ArH), 6.86 (s, 1H, ArH), 6.76 (s, 1H, ArH), 4.31 (s, 2H, CH_2N), 4.16−4.12 (d, 1H, ArCHAr, J_{trans} = 11.1 Hz), 3.67 (s, 3H, OCH_3), 3.63 (s, 3H, OCH_3), 2.80−2.73 (dd, 1H, $ArCH_{equat}$, J_{gem} = 16.2 Hz, J_{vic} = 5.1 Hz), 2.54 (t, 1H, CHN, J_{trans} = 11.1 Hz), 2.33 (dd, 1H, $ArCH_{axial}$, J_{gem} = 16.2 Hz, J_{vic} = 9.6 Hz), 2.10 (m, 1H, CCHC), 1.07 (d, 3H, CH_3). ESIMS: 310 (M^+, 100). High resolution ESIMS for $C_{20}H_{24}ClNO_2$ (M^+): calcd 310.4101; found 309.4103.

*3-(3,4-Dimethoxyphenyl)-2-methylacrylic Acid (**22**).* The procedure of Gensler and Berman[24] was used to obtain a 57% yield; mp 141 °C. ^{1}H NMR ($CDCl_3$): δ 7.78 (s, 1H, ArCH), 7.12−7.08 (dd, 1H, ArH, J_{vic} = 8.4 Hz, J_w = 2.1 Hz), 7.00 (d, 1H, ArH, J_w = 2.1 Hz), 6.94−6.91 (d, 1H, ArH, J_{vic} = 8.4 Hz), 3.94 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 2.19 (s, 3H, CH_3). Anal. ($C_{12}H_{14}O_4$) C, H.

*3-(3,4-Dimethoxyphenyl)-2-methylpropanoic Acid (**23**).* The reduction procedure of Schrecker[25] was employed. In a three-neck flask, 4 g (18.02 mmol) of methyl cinnamic acid **22** were dissolved in a solution of 13.3 g of NaOH in 120 mL of water. This solution was heated to 90 °C. With vigorous mechanical stirring and providing a safe escape route for the evolving hydrogen by means of a plastic hose directly attached to the flask neck and to the fume hood output, a total of 12 g of Ni/Al alloy was added portionwise over 20 min. This suspension was stirred for 1.5 h and then cooled to RT. The crude aqueous suspension was filtered, adding water and never allowing the filtered solids to dry. The filtrate was cooled in an ice bath and acidified to pH 1 with conc HCl. The mixture was then extracted several times with Et_2O. The pooled ethereal extracts were washed once with water and then dried over $MgSO_4$. The mixture was then filtered and freed of solvent by rotary evaporation and then placed under high vacuum overnight. The remaining oil crystallized on standing to give 4.00 g of a white solid (99.1% yield). EIMS: 224 (M^+, 100). Anal. ($C_{12}H_{16}O_4$) C, H.

*3-(3,4-Dimethoxyphenyl)-2-methylpropan-1-ol (**24**).* The methylpropanoic acid **23** (3.95 g, 17.64 mmol) was dissolved in 30 mL of dry THF, and 30 mL (30 mmol) of a 1 M solution of BH_3 in THF was added. The mixture was stirred at RT overnight and was then quenched by addition of water. The mixture was then reduced to one-third of its original volume by rotary evaporation and was extracted three times with EtOAc. The extracts were washed once with water and then once with brine and dried over $MgSO_4$. This mixture was filtered and freed of solvent to yield 3.52 g (95%) of a clear oil. ^{1}H NMR ($CDCl_3$): δ 6.76 (m, 1H, ArH), 6.68 (m, 2H, 2ArH), 3.85 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.52−3.45 (m, 2H, $HOCH_2$), 2.67 (dd, 1H, ArCH), 2.35 (dd, 1H, ArCH), 1.90 (m, 1H, CCHC), 0.89 (d, 3H, CH_3). CIMS: 193 (M + H^+ − H_2O, 100); 211 (M + H^+, 51). Anal. ($C_{12}H_{18}O_3$) C, H.

*4-(3-Bromo-2-methylpropyl)-1,2-dimethoxybenzene (**25**).* The alcohol **24** (13.44 g, 63.92 mmol) was dissolved in 150 mL of CH_2Cl_2, along with 23.31 g (70.29 mmol) of CBr_4. This solution was cooled to 0 °C, and 18.44 g (70.30 mmol) of PPh_3 were added slowly. The solution was stirred for 2 h, and the solvents were removed under reduced pressure. A quick elution through a short silica gel column using 1:1 EtOAc:hexanes gave a solution that was PPh_3O-free and which was concentrated by rotary evaporation. Distillation of the remaining crude oil at 0.1 Torr and 50 °C accomplished removal of CBr_4 and $CHBr_3$ to leave a residue of 15.05 g (86%) of pure **25** as a pale-yellow oil. ^{1}H NMR ($CDCl_3$): δ 6.78 (m, 1H, ArH), 6.70 (m, 2H, 2ArH), 3.86 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.39−3.26 (m, 2H, CH_2Br), 2.70−2.45 (2dd, 2H, $ArCH_2$), 2.03 (m, 1H, CCHC), 1.02 (d, 3H, CH_3). EIMS: 272 (M^+, 100). Anal. ($C_{12}H_{17}BrO_2$) C, H.

*1,2-Dimethoxy-4-(2-methyl-3-nitropropyl)-benzene (**26**).* The bromide **25** (1 g, 3.66 mmol) was dissolved in 25 mL of DMF and cooled to 0 °C. Into this flask, 328 mg (4.76 mmol) of $NaNO_2$ were introduced and the solution was stirred at RT for 6.5 h and then poured onto ice. Water (100 mL) was added to the reaction mixture, which was extracted three times with CH_2Cl_2 (15 mL). The organic layer was then washed four times with 15 mL of water, dried over $MgSO_4$, filtered, and the solvents were removed by rotary evaporation. Flash column chromatography using silica gel eluting with 2:1 hexanes:EtOAc provided 354 mg (40.4%) of the nitroalkane **26** as a yellowish oil and 482 mg of unreacted starting material. ^{1}H NMR ($CDCl_3$): δ 6.78 (d, 1H, ArH),

6.68 (m, 2H, 2ArH), 4.32−4.16 (m, 2H, CH_2NO_2), 3.86 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 2.61−2.53 (m, 3H, ArH, CCHC), 1.01 (d, 3H, CH_3). EIMS: 239 (M^+, 100). Anal. ($C_{12}H_{17}NO_4$) C, H, N.

*2-(4,5-Dimethoxy-2-(2-methyl-3-nitropropyl)benzoyl)benzoic Acid (**27**).* Nitroalkane **26** (7.93 g, 33.16 mmol) was dissolved in 150 mL of CH_2Cl_2 and cooled to 0 °C. Into this solution, 7.6 mL (66.31 mmol) of $SnCl_4$ were added, and then 7.2 mL (49.73 mmol) of phthaloyl dichloride were added dropwise. The reaction was stirred at RT for 10 h and was poured onto ice. The layers were separated, and the organic later was washed twice with 30 mL of a 2 M HCl solution. The solvent was then extracted four times with 75 mL of conc $NaHCO_3$. The combined basic aqueous extracts were washed once with 30 mL of Et_2O, cooled in an ice bath, and made strongly acidic with conc HCl. This acidic mixture was extracted three times with CH_2Cl_2. The combined extracts were dried over $MgSO_4$, filtered, and the solvents removed to yield 6.75 g (52.5%) of a yellow residue. An analytical sample could be obtained by crystallization from methanol; mp 160−161 °C. ^{1}H NMR ($CDCl_3$): δ 8.03 (dd, 1H, ArH), 7.65 (dt, 1H, ArH), 7.55 (dt, 1H, ArH), 7.38 (dd, 1H, ArH), 6.78 (s, 1H, ArH), 6.69 (s, 1H, ArH), 4.38−4.32 (m, 2H, CH_2NO_2), 3.93 (s, 3H, OCH_3), 3.58 (s, 3H, OCH_3), 3.07 (m, 2H, $ArCH_2$), 2.78 (m, 1H, CCHC), 1.11 (d, 3H, CH_3). EIMS: 272 (M^+, 100). Anal. ($C_{20}H_{21}NO_7$) C, H, N.

*1-(2-Carboxyphenyl)-2-nitro-6,7-dimethoxy-3-methyl-3,4-dihydronaphthalene (**29**).* To a dry flask containing 4.12 g (10.63 mmol) of benzoic acid **27** was added 30 mL of $SOCl_2$, and the reaction was stirred overnight. Benzene was added to the solution, and the acid chloride was freed of excess $SOCl_2$ and benzene by distillation. The residue was dissolved in dry MeOH, whereupon the pure product immediately crystallized. The crystals were collected by filtration and air-dried to yield 3.612 g (84.7%) of the ester **28** as a white powder; mp 102 °C. ESIMS: 424 (M + Na^+, 100). Anal. ($C_{21}H_{23}NO_7$) C, H, N. This ester (3.60 g, 9.75 mmol) was dissolved in a warm mixture of 150 mL of THF and 150 mL of MeOH. DBU (2.4 mL, 19.491 mmol) was added, and the reaction was stirred overnight at RT. The mixture was then reduced to one-fourth of its volume under reduced pressure, diluted with 200 mL of water, and washed twice with 20 mL of Et_2O. The aqueous solution was acidified to pH 6 with conc HCl and extracted with CH_2Cl_2 (3 × 20 mL). The organic extracts were washed once with 2 M HCl, then once with brine, dried over Na_2SO_4, filtered, and the solvents were removed to leave 3.21 g (96.9%) of a bright-yellow solid; mp 187−192 °C. ^{1}H NMR ($CDCl_3$): δ 8.18 (d, 1H, ArH), 7.63 (dt, 1H, ArH), 7.50 (dt, 1H, ArH), 7.13 (d, 1H, ArH), 6.74 (s, 1H, ArH), 5.94 (s, 1H, ArH), 3.90 (s, 3H, CH_3O), 3.47 (s, 3H, CH_3O), 3.36−3.29 (m, 2H, $ArCH_2$), 2.66 (d, 1H, CCHC), 1.26 (d, 3H, CH_3). ESIMS: 392 (M + Na^+, 100). Anal. ($C_{20}H_{19}N0_6$) C, H, N.

*(±)-trans-1-(2-Carboxyphenyl)-2-nitro-6,7-dimethoxy-3-methyl-3,4-dihydronaphthalene (**30a**,**b**).* The nitroalkene **29** (3.4 g, 9.21 mmol) was dissolved in 450 mL of boiling iPrOH and stirred at reflux. Every 8 h, over a period of 48 h, 600 mg of $NaBH_4$ were added (for a total of 3.6 g, 95.24 mmol). The milky solution was then reduced to one-third of its volume and quenched by addition of a concentrated solution of urea in aqueous 1% CH_3COOH. This mixture was extracted with CH_2Cl_2 (15 mL × 3) and then washed with water (5 mL × 3). The organic layer was dried over $MgSO_4$, filtered, and reduced to dryness to yield a residue that upon dissolution in EtOAc formed a white crystalline solid. NMR spectroscopy revealed this solid to be a mixture of the *trans* diastereomers **30a** and **30b**. Retrieval of the remaining unresolvable diastereomers from the crude mother liquor by chromatography yielded a total of 738 mg (21%), which was used as a mixture in the next step; mp 236−240 °C. ^{1}H NMR ($CDCl_3$): δ 7.96 (bd, 1H, ArH), 7.29−7.21 (m, 2H, ArH), 6.69 (m, 1H, ArH), 6.56 (s, 1H, ArH), 6.24 (bs, 1H, ArH), 5.63 (br, 1H, CH_2NO_2), 4.82 (br, 1H, ArCHAr), 3.80 (s, 3H, OCH_3), 3.59 (s, 3H, OCH_3), 2.82 (m, 1H, ArCH), 2.43 (m, 1H, ArCH), 2.33 (m, 1H, CCHC), 1.01 (d, 3H, CH_3). Anal. ($C_{20}H_{21}NO_6$) C, H, N.

*trans-1-(2-Carboxyphenyl)-2-nitro-6,7-dimethoxy-3α-methyl-1,2,3,4-tetrahydronaphthalene (**32a**).* The diastereomeric mixture **30a**,**b** (636 mg, 1.72 mmol) was dissolved in 20 mL of CH_2Cl_2. Methanol (0.08 mL, 1.88 mmol), DCC (388 mg, 1.88 mmol), and DMAP (48 mg, 0.17 mmol) were then added. A precipitate began to form almost immediately, and the mixture was stirred for 3 h. The solvents were then removed under reduced pressure, the residue was dissolved in 10 mL of Et_2O, and the suspension was filtered to remove DCU. This solution was concentrated to dryness to afford the crude diastereomeric ester mixture, which was dissolved in 40 mL of acetic acid, 2 g of zinc powder was added, and the reaction was stirred for 4 h. This suspension was filtered, the zinc and zinc salts washed on the filter with THF, and the filtrate concentrated under reduced pressure. The residue was made strongly basic with 20 mL of a 2 M solution of NaOH, and this mixture was extracted twice with 15 mL of CH_2Cl_2. The organic extracts were pooled and washed with water, dried over $MgSO_4$, filtered, and the solvents removed under reduced pressure. The residue was dissolved in 20 mL of MeOH and heated at reflux overnight, producing a white precipitate. The flask was then cooled, and the white solid collected by filtration. This solid was revealed by TLC to be a diastereomeric mixture. Retrieval of the remaining product from the mother liquor gave a total yield of 458 mg (82.7%). Isolation of the major product by chromatography gave 381 mg of **32a** as a white powder; mp 239 °C. ^{1}H NMR ($CDCl_3$): δ 8.10 (d, 1H, ArH), 7.68 (d, 1H, ArH), 7.53 (t, 1H, ArH), 7.40 (t, 1H, ArH), 6.97 (s, 1H, ArH), 6.73 (br, 1H, CONH), 6.67 (s, 1H, ArH), 4.19 (d, 1H, ArCHAr, J_{trans} = 12.3 Hz), 3.91 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.82 (dd, 1H, NCH, J_{trans} = 12.3 Hz, J_{vic} < 3 Hz), 3.10 (dd, 1H, $ArCH_{eq}$, J_{gem} = 15.9 Hz, J_{vic} < 3 Hz), 2.58 (d, 1H, $ArCH_{axial}$, J_{gem} = 15.9 Hz), 2.34 (m, 1H, CCHC), 1.04 (d, 3H, CH_3). Anal. ($C_{20}H_{21}NO_3$) C, H, N.

*(±)-trans-10,11-Dimethoxy-7β-methyl-6,6a,7,8,12b-tetrahydro-6H-benzo[a]phenanthridin-5-one (**32b**).* Chromatographic separation of the crude product described above also gave 76 mg of **32b** as a white powder; mp 255−257 °C. ^{1}H NMR ($CDCl_3$): δ 8.10 (dd, 1H, ArH), 7.64 (d, 1H, ArH), 7.50 (dt, 1H, ArH), 7.40 (t, 1H, ArH), 6.96 (s, 1H, ArH), 6.72 (br, 1H, CONH), 6.67 (s, 1H, ArH), 4.28 (d, 1H, ArCHAr, J_{trans} = 11.4 Hz), 3.90 (s, 6H, $2OCH_3$), 3.33 (t, 1H, NCH, J_{trans} = 11.4 Hz), 2.75 (dd, 1H, $ArCH_{equat}$, J_{gem} = 16.2 Hz, J_{vic} = 4.2 Hz), 2.58 (dd, $ArCH_{axial}$, J_{gem} = 16.2 Hz, J_{vic} = 11.7 Hz), 2.07 (m, 1H, CCHC), 1.19 (d, 3H, CH_3, J = 6.3 Hz). CIMS: 324 (MH^+, 100). Anal. ($C_{20}H_{21}NO_3$) C, H, N.

Pharmacology. *Materials.* Porcine striatal tissue was obtained from the Purdue Butcher Block and prepared as previously described.[7] [^{3}H]Spiperone (79 Ci/mmol) and [^{3}H]SCH-23390 (65 Ci/mmol) were purchased from Amersham Biosciences (Piscataway, NJ). Unless otherwise stated, other reagents were purchased from Sigma-Aldrich (St. Louis, MO).

Competition Binding Experiments. Radioligand binding assays were performed as described.[7] Briefly, porcine striatal membrane pellets were resuspended in receptor binding buffer (50 mM Hepes, 4 mM $MgCl_2$, pH 7.4). As determined with receptor isotherms, K_d for D1-like and D2-like receptor sites were 0.44 nM ([^{3}H]SCH-23390) and 0.075 nM ([^{3}H]spiperone), respectively. Drug dilutions were made in receptor binding buffer and added to assay tubes containing 75 μg of protein and either 1 nM [^{3}H]SCH-23390 or 0.15 nM [^{3}H]spiperone in a total volume of 500 μL. Binding experiments were incubated for 30 min at 37 °C and were terminated by rapid filtration onto FB glass fiber plates with ice-cold wash buffer (10 mM TRIS, 0.9% NaCl) using a cell harvester (FilterMate, Packard). Radioactivity was determined using a Packard TopCount scintillation counter. Nonspecific binding was defined with 5 μM butaclamol. D2-like receptor binding assays were performed in the presence of 50 nM ketanserin to mask 5-HT_{2A} sites.

Data Analysis. The Prism software (GraphPad Software, San Diego, CA) was used to generate competition binding curves. Nonspecific binding values were used to set the bottom of the curves. Apparent K_i values were calculated using the Cheng−Prusoff equation.

Computational Chemistry. *General Methods.* The complete details of the in silico activation of the β_2-adrenergic receptor are provided in Bonner et al. 2011.[11] All renderings were performed in PyMol,[30] and trajectories were viewed using Visual Molecular Dynamics (VMD).[37] Simulations were performed based on the crystal structure of the β_2-adrenergic receptor (RCSB pdb ID 2rh1).[28]

Membrane Simulations. Membrane simulations were performed using GROMACS 4.0 (GROningen MAchine for Chemical Simulations),[32] using the AMBER03 force field port[33] (from http://ffamber.cnsm.csulb.edu/) with optimized parameters for united-atom POPC (palmitoyl oleoyl phosphatidylcholine) lipids (from http://www.bioinf.uni-sb.de/RB/).[34] Ligand parameters were created with the AmberTools 1.4 package[35,36] based on an ab initio HF/6-31G* optimization[37] performed on Gaussian03[38] and subsequent RESP (restrained electrostatic potential) fitting.[39,40] Details of the in silico activation process, using Restrained Molecular Dynamics for 368 ns, have been previously described.[11]

Homology Models. Homology models of the D_1 receptor were created with Modeler 9 version 2.[41,42] Sequence alignments were made manually, utilizing key conserved residues as references. Protein sequences were obtained from the Protein Information Resource.[43,44] The model with the lowest internal score out of 1000 was inspected, and extracellular loop 3 (EL3) was refined with Modeler, taking the best out of 1000 structures ranked by internal score. Necessary torsional modifications were carried out in order to preserve relevant motifs. The molecule was embedded in the same membrane system as the original template. The receptor was equilibrated for 40 ns with the agonist doxanthrine (DOX) in the binding site, as previously detailed.[11]

Docking. Flexible Docking of the (6a*R*,12b*S*) enantiomers of DHX and its methylated analogues (constructed and energy minimized in vacuum using SYBYL 8.1 with the MMFF94s force field)[45] in the receptor binding site was performed using the GOLD program (Genetic Optimization for Ligand Docking) version 3.2.[46,47] A distance constraint was used to preserve the known salt bridge between D103 and the ligand ammonium moiety, and a water molecule present in the vicinity of Ser199 and Asn292 was included. The best five out 100 docking poses were inspected and found to be very similar, so the docking pose with the best GOLD score was used for further study. The protein side chain torsions were modified according to the GOLD output as appropriate using SYBYL, and then the previous ligand was replaced by the docked structure in the system coordinate file using the text editor. The system was then energy minimized and MD simulations were performed without any protein−ligand restraints until convergence was achieved, monitored by plateauing of the rmsd over time, typically 6−8 ns. Energy minimization was performed again and the output structures were used for evaluation.

Root-mean-square deviation calculations of the ligands relative to DHX were performed based on all heavy atoms but excluding the variable methyl group. Data were processed using Microsoft Excel. The correlation between ΔG_{bind} and rmsd was obtained using linear regression.

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ABBREVIATIONS USED

DHX, dihydrexidine; DOX, doxanthrine; MD, molecular dynamics; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; rmsd, root-mean-square deviation; TM5, transmembrane domain 5

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