Lysergamides of Isomeric 2,4-Dimethylazetidines Map the Binding Orientation of the Diethylamide Moiety in the Potent Hallucinogenic Agent *N*,*N*-Diethyllysergamide (LSD)

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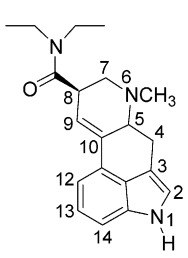
Lysergic acid amides were prepared from (*R*,*R*)-(−)-, (*S*,*S*)-(+)-, and *cis*-2,4-dimethyl azetidine. The dimethylazetidine moiety is considered here to be a rigid analogue of diethylamine, and thus, the target compounds are all conformationally constrained analogues of the potent hallucinogenic agent, *N*,*N*-diethyllysergamide, LSD-25. Pharmacological evaluation showed that (*S*,*S*)-(+)-2,4-dimethylazetidine gave a lysergamide with the highest LSD-like behavioral activity in the rat two lever drug discrimination model that was slightly more potent than LSD itself. This same diastereomer also had the highest affinity and functional potency at the rat serotonin 5-HT_{2A} receptor, the presumed target for hallucinogenic agents, and a receptor affinity profile in a panel of screens that was most similar to that of LSD itself. Both *cis*- and the (*R*,*R*)-*trans*-dimethylazetidines gave lysergamides that were less potent in all relevant assays. The finding that the *S*,*S*-dimethylazetidine gave a lysergamide with pharmacology most similar to LSD indicates that the *N*,*N*-diethyl groups of LSD optimally bind when they are oriented in a conformation distinct from that observed in the solid state by X-ray crystallography. The incorporation of isomeric dialkylazetidines into other biologically active molecules may be a useful strategy to model the active conformations of dialkylamines and dialkylamides.

Introduction

N,*N*-diethyllysergamide (LSD; **1**; Chart 1) is an extremely potent hallucinogenic agent that tolerates very little structural modification without marked loss of potency.[1] Nearly any molecular change attenuates its hallucinogenic potency by an order of magnitude or more. For example, minor alterations in the amide portion of the molecule typically reduce in vivo potency to only 10−30% of that observed with LSD itself.[2] Changes of the in vitro affinities of these amide-modified LSD analogues for selected receptors are often less dramatic[3,4] emphasizing the fact that there is so far no suitable understanding of all of the neurochemical events that underlie the mechanism of action for LSD.

Now that the molecular biology of various serotonin receptor subtypes is being elucidated, a variety of cloned monoamine receptors are available for structure−activity relationship studies. LSD has high affinity for several serotonin receptor subtypes, for α_2 adrenergic receptors,[5] and for dopamine D_1 and D_2 receptors.[6] Continuing efforts in our laboratory to elucidate the structure−activity relationships of hallucinogens, and our more recent focus on the lysergamides, have led to the design of lysergamides containing chirality in the amide function.[2,4,7] These diastereomeric compounds have shown interesting stereoselectivity for several monoamine receptors. We sought, however, a lysergamide that might more closely resemble LSD, where slight changes in the amide might elicit more profound changes in the pharmacology than we had observed in any of the lysergamides thus far examined in our laboratory.

Chart 1



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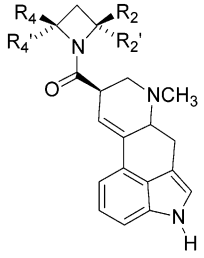

cis-2a	$R_2 = R_4 = CH_3$, $R_2' = R_4' = H$
(2*R*,4*R*)-2b	$R_2 = R_4' = CH_3$, $R_2' = R_4 = H$
(2*S*,4*S*)-2c	$R_2' = R_4 = CH_3$, $R_2 = R_4' = H$

Our goal in this study was primarily to identify the binding conformation of the diethylamide moiety of LSD **1**. In addition, however, we hypothesized that conformational constraint of the amide moiety of **1** might lead to analogues that were targeted only to a subset of the receptors to which **1** binds. In that respect, such molecular probes not only might be useful in sharpening the focus on a few key receptor types that are important for the action of LSD but also might help to elucidate differences in the ligand binding topography of various monoamine receptors. The basic problem we faced was the development of a strategy to constrain the diethylamide in various conformations without the introduction of significant additional steric bulk because of the

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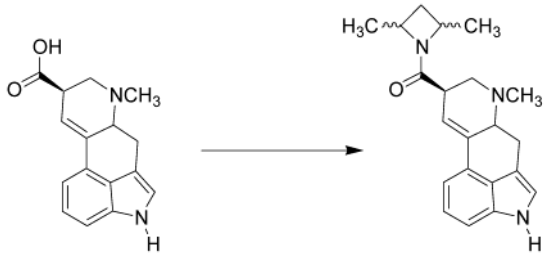
Scheme 1[a]



[a] Reagents: PyBOP, 2,4-dimethylazetidine, CH_2Cl_2, iPr_2EtN.

recognized sensitivity of the ergoline amide binding region of the relevant receptor(s).

Our earliest efforts focused on lysergamides prepared from 2,3-dimethylaziridines, a strategy that would introduce essentially no additional bulk into the amide moiety. Those attempts were abandoned, however, with the discovery of the extreme chemical lability of N-acyl aziridines. Condensation of lysergic acid with either *cis*- or *trans*-2,3-dimethylaziridine, using a variety of very mild methods, led only to isolation of aziridine ring-opened products.[1]

This paper describes the synthesis and pharmacological evaluation of three lysergic acid amides prepared from *cis* (*meso*)-, *trans*-(2*R*,4*R*)-, and *trans*-(2*S*,4*S*)-2,4-dimethylazetidines **2a**−**c**, respectively. Not only did those products prove to be suitably stable but also the azetidine ring is less strained and clearly gives a more appropriate model of a dialkylamide than does an aziridine. The use of dialkylazetidines as constrained amines has been previously recognized.[8]

Compounds **2a**−**c** were compared to **1** for their LSD-like behavioral effects in rats, and selected receptor binding characteristics were assessed. All three compounds were submitted to the NIMH-sponsored Drug Screening Program and compared with LSD **1**, to obtain an affinity profile across a panel of brain receptors. We were thus able to compare the effects of the more flexible parent LSD **1** with the lysergamides prepared from all three possible 2,4-dimethylazetidines (Scheme 1).

Chemistry

Chiral *trans*-2,4-dimethylazetidines were prepared as described recently by Marinetti et al.[9] *cis*-2,4-Dimethylazetidine was prepared, however, by the method of Freeman et al.[10] Although the synthesis described by Marinetti et al.[9] was more straightforward, it provided only poor yields when *cis*-2,4-pentanediol was employed. In view of the difficulty in preparing significant amounts of the chiral dimethylazetidines, as well as an observed reduced chemical reactivity as compared with diethylamine, an improved procedure was also developed for coupling the amines with (+)-lysergic acid. This method employed the peptide condensing agent PyBOP (benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate).[11] Although the workup is not as simple as other methods we have employed,[4,7] this procedure allowed us to obtain good yields of lysergamides from lysergic acid monohydrate using essentially stoichiometric amounts of these scarce amines. Interestingly, because of restricted rotation about the amide CO−N bond, the cis diastereomer **2a** was produced as two distinct products, initially evident by the occurrence of two singlets for H(9) in the ^{1}H NMR at δ 6.14 and 6.24, whereas for both **2b**,**c** this proton appeared as one singlet at δ 6.18. In addition, the methyl groups attached to the azetidine rings of **2b**,**c** appeared as distinct doublets, but in **2a**, there was a multiplicity of signals from the methyl groups in four different environments. We also employed 600 MHz ^{1}H NMR TROESY experiments to confirm that the cis compound **2a** was a mixture of two locked conformers. We were not able to separate these two conformationally locked amides, and biological data are reported for the mixture.

Table 1. DD Data from Substitution Tests in LSD-Trained Rats[a]

	dose					ED_{50} (95% CI)	
drug	mg/kg	μmol/kg	N	% D	% SDL	nmol/kg	mg/kg
LSD **1**	0.01	0.023	12	0	17		
	0.02	0.046	12	0	58	45	0.019
	0.04	0.093	12	0	75	(32−63)	(0.013−0.027)
	0.08	0.186	12	0	100		
cis-**2a**	0.02	0.049	8	12	14		
	0.04	0.097	9	0	33	115	0.047
	0.08	0.194	10	0	70	(72−182)	(0.030−0.075)
	0.16	0.388	10	20	100		
RR-**2b**	0.02	0.049	8	0	25		
	0.04	0.097	10	0	40	134	0.055
	0.08	0.194	10	10	56	(66−271)	(0.027−0.11)
	0.16	0.388	12	17	80		
SS-**2c**	0.01	0.025	18	6	67		
	0.02	0.049	28	7	50	25	0.010
	0.04	0.097	10	0	90	(16−39)	(0.007−0.016)
	0.08	0.194	10	0	100		

[a] The slopes of the dose−response curves for **2b**,**c** and LSD **1** cue are not significantly different (T value = 1.16, table value = 2.776). The slopes of the **2b** and LSD **1** cue as well as the **2a** and LSD **1** cue are not parallel; their slopes are significantly different (T value = 3.31, table value = 2.776 and T value = 3.45, table value = 2.776, respectively).

Pharmacology

The three diastereomers of **2** were initially tested in the two lever drug discrimination (DD) assay in rats trained to discriminate LSD tartrate (0.08 mg/kg) or the 5-HT_{1A} agonist LY293284 (0.025 mg/kg) from saline. These methods have been described in detail previously.[12,13] The three lysergamides were then assessed for their affinities at the agonist-labeled cloned rat 5-HT_{2A}, 5-HT_{2C}, and human 5-HT_{1A} receptors expressed in NIH3T3 and Chinese hamster ovary (CHO) cells, respectively, and compared with LSD. The ability to stimulate phosphoinositide turnover was also assessed at the rat 5-HT_{2A} receptor, as a measure of functional activity. Finally, the three isomers were submitted to the NIMH-sponsored Drug Screening Program for an analysis of their affinity at a variety of cloned brain receptors and a comparison with LSD **1**.

Results and Discussion

All three of the novel lysergamides completely substituted in LSD-trained rats, and the ED_{50} values are reported in Table 1. The diastereomer **2c** prepared from *S*,*S*-(+)-2,4-dimethylazetidine had in vivo LSD-like behavioral effects and a potency at least comparable to that of LSD itself. Both the *cis*-**2a** and the *R*,*R*-**2b** gave compounds with lower and nearly identical LSD-like activity, and both were significantly less potent than **2c**. In addition, the three isomers were tested for their ability to substitute in rats trained to discriminate the

Table 2. Receptor Affinities for Lysergamides at Serotonin 5-HT_{2A}, 5-HT_{2C}, and 5-HT_{1A} Receptor Subtypes and Functional Potency at the Rat 5-HT_{2A} Receptor

	radioligand binding			5-HT_{2A} function	
CMPD	$5HT_{2A}$[b]	$5HT_{2C}$[b]	$5HT_{1A}$[c]	EC_{50}[d]	PI%[e]
LSD **1**	3.5 ± 0.62	5.5 ± 0.31	1.1 ± 0.01	15 ± 4.7	23
cis-**2a**	7.9 ± 0.85	23 ± 2.9	1.1 ± 0.12	69 ± 8.1	30
R,R-**2b**	21 ± 4.0	130 ± 11	6.8 ± 0.26	102 ± 25	36
S,S-**2c**	8.3 ± 1.7	6.5 ± 0.15	0.45 ± 0.01	19 ± 4.5	43

[a] K_i values ± SEM and EC_{50} values ± SEM are reported in nanomolar and are the means of at least three separate experiments. [b] [^{125}I]DOI was the radioligand. [c] [^{3}H]-8-OH-DPAT was the radioligand. [d] EC_{50} for stimulating phosphoinositide accumulation. [e] Maximum stimulation of IP accumulation, relative to 10 μM 5-HT.

5-HT_{1A} agonist LY293284 ((−)-4*R*-6-acetyl-4-(di-*n*-propylamino)-1,3,4,5-tetrahydrobenz[*c*,*d*]indole).[14,15] Only **2c** fully substituted in these rats, with an ED_{50} of 85 (95% CI: 54−130) nmol/kg, as compared with an ED_{50} of 31 nmol/kg for LY293284 itself. LSD (**1**) itself does not substitute in these animals (unpublished data).

The K_i values determined in our laboratory for the rat 5-$HT_{2A/2C}$ and human 5-HT_{1A} receptors, as well as the ability to stimulate phosphoinositide turnover coupled through the 5-HT_{2A} receptor, are reported in Table 2. These results are consistent with the behavioral effects in the DD studies. Although both **2a** and **2c** have nearly identical affinity at the agonist-labeled 5-HT_{2A} receptor, only **2c** has functional potency comparable to **1**. The EC_{50} values of **2a**,**b** are 4.6- and 5.4-fold higher, respectively. It should also be noted that whereas **2c** has somewhat lower affinity than LSD **1**, it also has higher intrinsic activity, based on the stimulation of phosphoinositide turnover. If the currently accepted hypothesis is correct that hallucinogen action is related to agonist activity at the 5-HT_{2A} receptor,[16] one would conclude from the results in Table 2 that **2c** best fits that description.

The higher affinity of **2c** for the 5-HT_{1A} receptor probably also explains its ability to substitute in the DD assay in rats trained to discriminate the 5-HT_{1A} receptor agonist LY293284. Thus, rats given **2c** can perceive the interoceptive effects both of 5-HT_{2A} and of 5-HT_{1A} receptor activation.

Tables 3 and 4 list affinities for various other receptors reported from the NIHM-sponsored receptor screening panel. The affinities of **2a**,**c** are similar to **1** at the 5-HT_6 and 5-HT_7 receptors, but there is no apparent pattern at the 5-HT_{1E} receptor. Although we had previously reported low nanomolar affinity for LSD at D_2 receptors and 30 nM affinity at D_1 receptors,[6] the affinities at these receptors are reported by the NIMH screen to be much lower. This finding could be attributed to differences in cells, receptor expression levels, protocols, etc. Considered in relation to each other however, at the D_1 receptor, all of the lysergamides had similar low affinity. The *R,R* diastereomer **2b** again had the lowest affinity of all of the analogues tested at the D_2, D_3, and D_4 receptors, indicating that the configuration of the *R,R*-azetidide moiety generally appears to be least complementary to the structures of the monoamine type G-protein-coupled receptor (GPCR) family.

Thus, although we had initially hypothesized that the individual diastereomers **2a**−**c** might each target different subsets of receptors, in fact, these data show that the stereochemistry of the amide has relatively parallel effects at each receptor examined. This observation would seem to suggest similar structural elements within the ligand binding domains for the amide function of each of the receptors although there are, of course, some exceptions in the data.

The more potent isomer **2c** is illustrated in Figure 1 in its lowest energy-minimized structure, as compared with a minimized structure derived from the reported X-ray crystal structure for LSD.[17] The authors of that study stated, "We believe that the conformation of LSD observed in the crystal is that relevant to its activity as a potent hallucinogen." It is now possible to infer from the present results that upon binding to the 5-HT_{2A} receptor the diethyl groups in the amide of LSD are oriented in a conformation that is more nearly superimposable upon that of the dimethylazetidide group in **2c**. In fact, the least active diastereoisomer **2b** more nearly resembles the conformation in the reported crystal structure of LSD! Furthermore, the ^{1}H NMR chemical shifts of the methyl groups of **1** itself more closely correspond to those of **2c**, indicating perhaps that in solution **1** may also prefer a conformation more similar to **2c** than to **2b**, but additional, more extensive NMR experiments will be necessary to confirm that speculation. In any case, one is led to conclude that crystal packing forces stabilize a conformation of **1** that does not represent the biologically active one.

The data for the cis compound **2a** are somewhat confounded by the fact that it exists as a mixture of

Table 3. Serotonin Receptor Affinities (K_i in nM ± SEM) of Lysergamides Obtained through the NIMH Screening Program[a]

compd	$5HT_{2B}$	$5HT_{1E}$	$5HT_{1D\alpha}$	$5HT_{5a}$	$5HT_6$	$5HT_7$
LSD **1**	30 ± 10	93 ± 10	3.9 ± 0.76	9.0 ± 1.8	6.9 ± 0.54	6.6 ± 1.5
cis-**2a**	44 ± 19	200 ± 25	3.7 ± 0.47	16 ± 3.3	13 ± 1.4	12 ± 2.6
R,R-**2b**	310 ± 100	140 ± 10	2.0 ± 0.26	13 ± 2.5	61 ± 4.0	31 ± 9.6
S,S-**2c**	27 ± 3.0	280 ± 36	2.4 ± 0.17	27 ± 5.5	15 ± 1.1	14 ± 4.6

[a] All values in nanomolar ± SEM. Receptors that were screened for which the lysergamides had no significant affinity include μ, κ, and δ opioid receptors, V1, V2, and V3 receptors, seven types of nicotinic receptor, α_{1A} and α_{1B} adrenergic, the serotonin, norepinephrine, and dopamine reuptake transporters, $GABA_A$, NMDA, mGluR1, mGluR2, mGluR4, mGluR5, mGluR6, and mGluR8 receptors.

Table 4. Other Receptor Affinities (K_i in nM ± SEM) of Lysergamides Obtained through the NIMH Screening Program

compd	D_1	D_2	D_3	D_4	D_5	H_1	β_1	β_2
LSD **1**	180 ± 33	120 ± 37	27 ± 6.0	56 ± 12	340 ± 35	1540 ± 350	140 ± 4.6	740 ± 10
cis-**2a**	230 ± 37	60 ± 14	8.0 ± 3.0	18 ± 2.0	1000 ± 43	500 ± 110	400 ± 29	4610 ± 10
R,R-**2b**	340 ± 13	330 ± 23	150 ± 25	72 ± 5.0	590 ± 46	1400 ± 310	ND	ND
S,S-**2c**	290 ± 35	110 ± 25	6.0 ± 1.0	36 ± 2.0	400 ± 36	2500 ± 550	76 ± 8.2	1100 ± 10

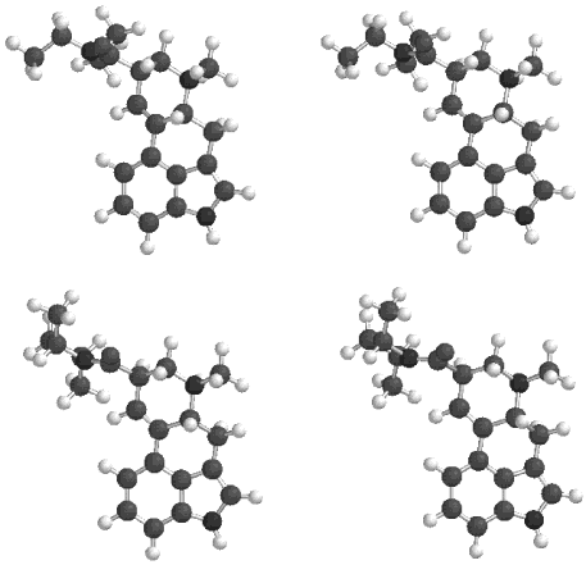


Figure 1. Stereoview (cross-eyed) of the minimized structure of LSD (top), derived from the reported crystal structure,[17] with the more potent lysergamide **2c** shown on the bottom.

"locked" conformers, due to the restricted rotation about the C(O)−N bond of the amide. Thus, it seems likely that one of the conformers may have higher activity than the other, or indeed, that only one of them has significant activity at all. Although this observation is important from the point of view of understanding the interaction between the receptor and the ligand, under normal conditions in the more flexible alkylamide of **1**, nonbonded interactions between the two ethyl groups would greatly disfavor a syn orientation of two amide alkyl groups. On the other hand, one could speculate that the receptor is fairly tolerant of the location of one of the alkyl groups on the amide but that there is a tightly restricted space available for the other.

The ability of **2c** to substitute in animals trained to discriminate LY293284 from saline is consistent with its higher affinity at the 5-HT_{1A} receptor than **1**[7] or any of the other diastereomers. Although we have not carried out functional assays at the 5-HT_{1A} receptor, the ability of **2c** to substitute for a 5-HT_{1A} agonist suggests that it is also a potent agonist. Previously, we have shown that certain chiral secondary monoalkylamides of lysergic acid have subnanomolar affinity at the 5-HT_{1A} receptor.[7] Thus, amide modification in lysergamides may be a productive avenue for development of potent 5-HT_{1A} agonists, if substituents are selected that optimize 5-HT_{1A} potency, which at the same time attenuate effects at 5-HT_{2A} receptors that presumably account for the hallucinogenic actions of LSD and pharmacologically similar congeners.

These results have significance in two general areas. First, medicinal chemists are often tempted to assume that the crystal structure of a flexible biologically active molecule may resemble its binding conformation. Whereas this may sometimes be the case, in the present instance, such an assumption would lead to the erroneous prediction that **2b** would be more potent. Indeed, **2b** proved to be the least active of all of the diastereomers!

Second, and perhaps of much greater significance, is the potential application of 2,4-dialkylazetidines as replacements for a variety of *N*,*N*-dialkylamines. To our knowledge, no one thus far has clearly identified this novel rigid analogue strategy as a productive approach to mapping the active conformations of dialkylamines or dialkylamides. The fact that the biological activity of LSD is so uniquely tied to the identity of the diethylamide moiety per se seems to indicate clearly that dimethylazetidines are reasonably good models for diethylamine. If this finding proves to be a general principle, a hypothesis we are presently assessing, there are many drugs containing *N*,*N*-dialkylamine or *N*,*N*-dialkylamide groups that if replaced by a 2,4-dialkylazetidine moiety, might lead to molecules possessing similar or enhanced activity and/or an improved side effect profile.

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 Varian Unity Plus 600 MHz or an Advance DRX 500 MHz instrument. Chemical shifts are reported in δ values (parts per million, ppm) relative to an internal standard of tetramethylsilane in $CDCl_3$. Abbreviations used in NMR analysis are as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br s, broad singlet; dd, doublet of doublets; dt, doublet of triplets. Analytical thin-layer chromatography (TLC) was performed on Baker-flex silica gel 1B2-F plastic plates. High-performance liquid chromatography (HPLC_ analysis for purity of final compounds was performed using a Rainin HPXL pump with a C_{18} reverse phase column (Microsorb MV 3 μm 100A), methanol/0.2 N ammonium nitrate (3:2) as the mobile phase, and a SpectroMonitor 3100 variable wavelength detector set to 254 nm. The electrospray ionization high-resolution mass measurements were obtained on a Finnigan MAT XL95 mass spectrometer (Finnigan MAT Corp., Bremen, Germany). The instrument was calibrated to a resolution of 10 000 with a 10% valley between peaks, using the appropriate polypropylene glycol standard. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter.

Synthesis of Lysergamides. General Method. Lysergic acid monohydrate (obtained from NIDA) (200 mg, 0.75 mmol), PyBOP[11] (426 mg, 0.82 mmol), and the appropriate 2,4-dimethylazetidine (109 mg, 0.9 mmol) were suspended in 20 mL of CH_2Cl_2. Diisopropylethylamine (193 mg, 1.5 mmol) was added, and the reaction was stirred for 3 h. The reaction was then quenched by the addition of 20 mL of 7.5 M concentrated NH_4OH, the CH_2Cl_2 layer was separated, and the aqueous phase was extracted with 10 mL of CH_2Cl_2. The organic layers were combined and washed with H_2O (2 × 30 mL) and brine (15 mL) and then dried ($MgSO_4$). Filtration and solvent removal by rotary evaporation under reduced lighting, followed by drying under high vacuum, produced a light golden foam. This crude product was then subjected to purification by centrifugal thin layer preparative chromatography (Chromatotron, Harrison Research) over a silica rotor and elution with 4:1 CH_2Cl_2/hexane under an N_2−ammonia atmosphere. The faster-moving blue fluorescent band was collected, and the solvent was removed under vacuum in the dark. The normal lysergamides were then dissolved in *tert*-BuOH and combined with 0.5 equiv of (+)-tartaric acid dissolved in a minimum amount of ^{i}PrOH. The solutions were stored at 0 °C overnight. The crystalline products were collected by filtration and dried overnight at 60 °C under high vacuum. In the absence of more extensive heating, which caused gradual decomposition, these conditions were not sufficient to remove all traces of solvent, evident in the NMR spectra, and satisfactory elemental analyses could not be obtained. Thus, purity was assured by the fact that all of the compounds had first of all been purified using the Chromatotron and were isolated as a single spot on TLC analysis in two different systems with visualization using Van Urk's indole spray reagent. Subsequently, HPLC analysis showed essentially a single eluting peak representing 99+%

of the sample for all final compounds. There were no contaminant peaks in the ^{1}H NMR other than traces of solvents (*tert*-BuOH, *i*PrOH), and the HR mass spectra were consistent with the expected structures.

(2′*RS*,4′*SR*)-(+)-9,10-Didehydro-6-methylergoline-8*β*-(*cis*-2,4-dimethylazetidide)-(+)-tartrate 2a. Compound **2a** was obtained using *cis*-2,4-dimethylazetidine prepared as described by Freeman et al.[10] Condensation with lysergic acid monohydrate as described above afforded the amide **2a** in 85% yield, as its (+)-tartrate salt, mp 155−157 °C (dec). ^{1}H NMR (d_6-DMSO): δ 1.28−1.41 (m, 6H), 2.45 (s, 3H), 2.48−2.61 (m, 2H), 2.95 (m, 1H), 3.05 (m, 1H), 3.39 (m, 1H), 3.44 (dd, J = 14.7, 5.6, 1H), 4.13 (s, 1H), 4.17 (m, 1H), 4.46 (m, 1H), 6.14, 6.24 (2s, 1H), 6.99 (m, 3H), 7.13 (d, J = 7.2, 1H), 10.66 (s, 1H). ms (m/z 336 (m + 1); $[\alpha]_D$ = +90° (c = 0.1, EtOH). HRMS for $C_{21}H_{26}N_3O$ 336.2076 (M + H)$^+$; measured 336.2075.

(2′*R*,4′*R*)-(+)-9,10-Didehydro-6-methylergoline-8*β*-(*trans*-2,4-dimethylazetidide)-(+)-tartrate 2b. This product was obtained using *R*,*R*-*trans*-2,4-dimethylazetidine prepared as described by Marinetti et al.[9] and isolated as the HCl salt, mp 121−122 °C, with $[\alpha]_D$ = −20.58° (c = 2.5, EtOH); lit[9] $[\alpha]_D$ = −8° (acetate salt; c = 1, $CHCl_3$). Condensation with lysergic acid monohydrate as described above afforded the amide **2b** in 78% yield, as its (+)-tartrate salt, mp 155−157 °C (dec). ^{1}H NMR (d_6-DMSO): δ 1.35 (d, J = 6.2, 3H), 1.38 (d, J = 6.2 Hz, 3H), 1.86−1.91 (m, 1H), 1.93−1.99 (m, 1H), 2.44 (s, 3H), 2.61 (t, J = 11.0 Hz, 1H), 2.89 (dd, J = 11.1, 4.9, 1H), 3.04 (m, 1H), 3.35 (m, 1H), 3.43 (dd, J = 14.6, 5.5, 1H), 4.11 (s, 1H), 4.27 (m, 1H), 4.60 (m, 1H), 6.18 (s, 1H), 6.96 (s, 1H), 7.00 (m, 2H), 7.13 (h, 1H), 10.66 (s, 1H). ms (m/z 336 (m + 1); $[\alpha]_D$ = +15° (c = 0.1, EtOH). HRMS for $C_{21}H_{26}N_3O$ 336.2076 (M + H)$^+$; measured 336.2075.

(2′*S*,4′*S*)-(+)-9,10-Didehydro-6-methylergoline-8*β*-(*trans*-2,4-dimethylazetidide)-(+)-tartrate 2c. Compound **2c** was obtained using *S*,*S*-*trans*-2,4-dimethylazetidine as described by Marinetti et al.[9] and isolated as the HCl salt, mp 120−122 °C, with $[\alpha]_D$ = +18.04° (c = 2.5, EtOH); no lit[9] rotation reported. Condensation with lysergic acid monohydrate afforded the amide **2c** in 70% yield, as its (+)-tartrate salt, mp 148−150 °C (dec). ^{1}H NMR (d_6-DMSO): δ 1.29 (d, J = 6.2, 3H), 1.38 (d, J = 6.2 Hz, 3H), 1.93 (m, 2H), 2.45 (s, 3H), 2.56 (t, J = 11.2 Hz, 1H), 2.97 (dd, J = 11.2 4.9, 1H), 3.04 (m, 1H), 3.37 (m, 1H), 3.44 (dd, J = 14.6, 5.5, 1H), 4.15 (s, 1H), 4.29 (m, 1H), 4.59 (m, 1H), 6.18 (s, 1H), 6.97−7.01 (m, 3H), 7.13 (d, J = 7.3, 1H), 10.66 (s, 1H). ms (m/z 336 (m + 1); $[\alpha]_D$ = +101° (c = 0.1, EtOH). HRMS for $C_{21}H_{26}N_3O$ 336.2076 (M + H)$^+$; measured 336.2075.

Pharmacology Methods. DD Studies. Male Sprague−Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 200−220 g at the beginning of the DD study were divided into groups and trained to discriminate LSD tartrate (n = 18) or the 5-HT$_{1A}$ agonist LY293284[14] (n = 14) from saline. None of the rats had previously received drugs or behavioral training. Water was freely available in the individual home cages, and a rationed amount of supplemental feed (Purina Lab Blox) was made available after experimental sessions so as to maintain approximately 80% of free-feeding weight. Lights were on from 7:00 to 19:00. The laboratory and animal facility temperature was 22−24 °C, and the relative humidity was 40−50%. Experiments were performed between 9:00 and 17:00 each day, Monday−Friday. The animal protocols used in the present experiments were consistent with current NIH principles of animal care and were approved by the Purdue University Animal Care and Use Committee.

Six standard operant chambers (model E10-10RF, Coulbourn Instruments, Lehigh Valley, PA) consisted of modular test cages enclosed within sound-attenuated cubicles with fans for ventilation and background white noise. A white house light was centered near the top of the front panel of the cage, which was also equipped with two response levers, separated by a food hopper (combination dipper pellet trough, model E14-06, module size 1/2), all positioned 2.5 cm above the floor. Solid state logic in an adjacent room, interfaced through a Med Associates (Lafayette, IN) interface to a PC microcomputer, controlled reinforcement and data acquisition with a locally written program.

A fixed ratio (FR) 50 schedule of food reinforcement (Noyes 45 mg of dustless pellets) in a two lever paradigm was used. The DD procedure details have been described elsewhere.[7,15,18−21] Training sessions lasted 15 min and were conducted at the same time each day. Levers were cleaned between animals with 10% ethanol solution to avoid olfactory cues.[22] Animals were trained to an FR$_{50}$ until an accuracy of at least 85% (number of correct presses × 100/number of total presses) was attained for eight of 10 consecutive sessions (approximately 40−60 sessions). Once criterion performance was attained, test sessions were interspersed between training sessions, either one or two times per week. At least one drug and one saline session separated each test session. Rats were required to maintain the 85% correct responding criterion on training days in order to be tested. In addition, test data were discarded when the accuracy criterion of 85% was not achieved on the two training sessions following a test session. Test drugs were administered ip 30 min prior to the sessions; test sessions were run under conditions of extinction, with rats removed from the operant chamber when 50 presses were emitted on one lever. A minimum of three different doses was used for each tested compound, and at least eight rats were tested at each dose. If 50 presses on one lever were not completed within 5 min, the session was ended and scored as a disruption. Treatments were randomized at the beginning of the study.

The training drugs, dosages, and sources used in the DD studies were as follows: (+)-lysergic acid diethylamide tartrate (LSD; 0.08 mg/kg, 186 nmol/kg, NIDA), and LY293284 (0.025 mg/kg, 75 nmol/kg) a generous gift of the Eli Lilly Laboratories (Indianapolis, IN). All training drugs were dissolved in 0.9% saline and were injected intraperitoneally in a volume of 1 mL/kg, 30 min before the session.

Data Analysis. Data from the DD study were scored in a quantal fashion, with the lever on which the rat first emitted 50 presses in a test session scored as the "selected" lever. The percentage of rats selecting the drug lever (%SDL) for each dose of test compound was determined. The degree of substitution was determined by the maximum %SDL for all doses of the test drug. "No substitution" is defined as 59% SDL or less, and "partial" substitution is 60−79% SDL. If the drug was one that completely substituted for the training drug (at least one dose resulted in a %SDL = 80% or higher) the method of ref 23 was used to determine the ED$_{50}$ and 95% confidence interval (95% CI). If the percentage of rats disrupted (%D) was 50% or higher, the ED$_{50}$ value was not determined, even if the %SDL of nondisrupted animals was higher than 80%.

Pharmacology Methods. Radioreceptor Competition Assays. Radioligands [^{125}I]DOI (2200 Ci/mmol) and [^{3}H]8-OH-DPAT (124 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Mianserin was purchased from Sigma Chemical Corp. (St. Louis, MO), and serotonin creatinine sulfate and cinanserin were purchased from Research Biochemicals Incorporated (Natick, MA).

NIH3T3 fibroblast cells expressing either the rat 5-HT$_{2A}$ or the 5-HT$_{2C}$ receptor[24] were a generous gift of Dr. David Julius. CHO cells stably transfected with the human 5-HT$_{1A}$ receptor were a gift from Pharmacia & Upjohn, Inc., Kalamazoo, MI.

Cells were maintained in minimum essential medium containing 10% dialyzed fetal bovine serum (Gibco-BRL, Grand Island, NY) and supplemented with L-glutamine (1%), Pen/Strep (1%), and either Geneticin (157 units; NIH3T3 cells) or Hygromycin B (45 units; CHO cells). The cells were cultured at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Confluent cell monolayers were washed with sterile filtered phosphate-buffered saline and incubated in serum free Opti-MEM (Gibco-BRL, Grand Island, NY) for 5 h. After they were incubated, the cells were harvested by centrifugation (15 000g, 20 min) and placed immediately in a −80 °C freezer until the assays were performed.

For saturation binding assays, 0.25−12 nM [^{3}H]8-OH-DPAT (for 5-HT$_{1A}$) or 0.125−5.0 nM [^{125}I]DOI (for 5-HT$_{2A/2C}$) were used. The total volume of the assay was 250 μL. Nonspecific binding was defined as the binding measured in the presence of 10 μM cinanserin (5-HT$_{2A}$ cells), 10 μM mianserin (5-HT$_{2C}$ cells), or 10 μM serotonin (5-HT$_{1A}$ cells). Competition experiments were carried out in a total volume of 500 μL with either

0.80 nM [^{3}H]8-OH-DPAT or 0.20 nM [^{125}I]DOI. Previously harvested cells were resuspended and added to each well containing assay buffer (50 mM Tris, 0.5 mM EDTA, 10 mM $MgCl_2$; pH 7.4), radioligand, and test compound (or in the case of the saturation isotherm assays, cinanserin, mianserin, or serotonin). The incubation was carried out at 25 °C for 60 min and then terminated by rapid filtration through GF/B Unifilters that had been pretreated with 0.3% polyethylenimine for 30 min, using a prechilled Packard 96 well harvester (Packard Instrument Corp.). The filters were washed using chilled buffer (10 mM Tris, 154 mM NaCl; pH 7.4) and dried overnight. The following day, Microscint-O (Packard Instrument Corp.) was added, and radioactivity was determined using a TopCount (Packard) scintillation counter. Data analysis was performed by nonlinear regression using the program GraphPad Prism (GraphPad Software, San Diego, CA) to analyze the saturation and competition binding curves. K_i values were calculated using the Cheng−Prusoff equation. Each assay was run in duplicate with 10 drug concentrations, and assays were repeated 3−5 times ($n = 3-5$).

Phosphoinositide Hydrolysis Studies. Functional studies were carried out in NIH3T3 cells expressing the rat 5-HT_{2A} receptor. Accumulation of inositol phosphates was determined using a modified version of a previously published protocol.[25] Briefly, cells expressing the rat 5-HT_{2A} receptor were labeled for 18−20 h in CRML-1066 medium containing 1.0 μCi/mL [^{3}H]*myo*-inositol. After the cells were pretreated with 10 μM pargyline/10mM LiCl for 15 min, agonist ligands were added to each well for 30 min at 37 °C, under an atmosphere of 95% O_2 and 5% CO_2. The assay was terminated by aspirating the medium and adding 10 mM formic acid. After they were incubated for 16 h at 4 °C, the [^{3}H]inositol phosphates were separated from the cellular debris on Dowex-1 ion-exchange columns and eluted with 1.0 M ammonium formate and 0.10 M formic acid. The vials were counted for tritium using a TriCarb scintillation counter (Packard Instrument Corp.).

NIMH-Sponsored Screening. Screening of lysergamide analogues at a large number of cloned GPCRs was performed using the resources of the NIMH Psychoactive Drug Screening Program essentially as previously detailed.[26] In brief, initial screening assays were performed using 10 μM test compounds at a large number of cloned GPCRs. For compounds that induced >50% inhibition at 10 μM, K_i determinations were performed (1−10 000 nM test compound) with each K_i value replicated 2−3 times. The K_i values reported here (mean ± SEM) were calculated using GraphPad Prizm. Complete details of assay conditions are available on-line at http://pdsp.cwru.edu/nimh/binding.htm.

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