Stereoselective LSD-like Activity in a Series of *d*-Lysergic Acid Amides of (*R*)- and (*S*)-2-Aminoalkanes

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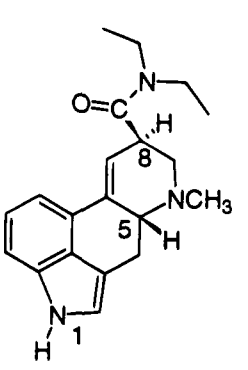
The 3-pentyl-, (*R*)- and (*S*)-2-pentyl-, 2-hexyl-, and 2-heptylamides of *d*-lysergic acid were synthesized and evaluated in biochemical and behavioral assays for LSD-like activity. In radioligand competition studies, the (*R*)-lysergamides were consistently more potent than the (*S*)-amides in displacing [^{3}H]ketanserin from 5-HT$_{2A}$ receptors in rat cortical homogenate and in displacing [^{3}H]-8-OH-DPAT ([^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetralin) from rat hippocampal 5-HT$_{1A}$ receptors. As the amide alkyl was lengthened from pentyl to heptyl, the affinity of the (*R*)-isomers for 5-HT$_{2A}$ sites decreased, while affinity for 5-HT$_{1A}$ sites was maximal for the (*R*)-2-hexyllysergamide. In rats trained to discriminate 0.08 mg/kg LSD tartrate from saline, a similar stereoselective effect was noted in which the (*R*)-alkylamides were more potent than the (*S*)-isomers in producing the LSD-like discriminative stimulus effect. However, as the amide alkyl substituent was increased in length, LSD-like activity decreased, with only partial substitution for training drug being observed for the (*R*)-hexylamide. The (*R*)- and (*S*)-pentyllysergamides were also assayed for their ability to activate intracellular phosphoinositide hydrolysis. Consistent with the binding and behavioral studies, these assays showed that both isomers are potent agonists at the 5-HT$_{2A}$ receptor, but that the (*R*)-pentyllysergamide is approximately 20 times more active than the (*S*)-pentyllysergamide in stimulating phosphoinositide turnover.

Introduction

It has now been more than 50 years since Albert Hofmann's pioneering work with derivatives of lysergic acid and his accidental discovery of the potent psychedelic agent, *d*-lysergic acid *N*,*N*-diethylamide (**1**, LSD).[1] For a period of time following this discovery, several modifications of **1** were carried out in a series of structure–activity relationship (SAR) studies.[2,3] Despite the fact that a large number of compounds had been prepared that possessed a variety of pharmacological effects, **1** has continued to remain one of the most potent hallucinogenic agents known.[4] Therefore, it has been of great interest to determine what molecular properties of **1** lead to its remarkably high potency.

One area of exploration into the SAR of the ergolines has been the replacement of the *N*(6)-methyl of **1** with a variety of alkyl groups. Niwaguchi *et al.*[5] and Hashimoto *et al.*[6,7] originally reported several *N*(6)-alkylnorlysergic acid *N*,*N*-diethylamide derivatives. However, these reports gave no indication of the relative hallucinogenic potential of these derivatives. More recently, work in our laboratory focused on the synthesis and pharmacological evaluation of a series of *N*,*N*-diethyl-*N*(6)-norlysergamides that were similarly substituted at the N(6) position.[8] Of these compounds, the *N*(6)-*n*-propyl derivative was equipotent to **1**, while the *N*(6)-ethyl and *N*(6)-allyl derivatives were more potent than 1 in the two-lever drug discrimination paradigm in rats trained to discriminate LSD from saline.



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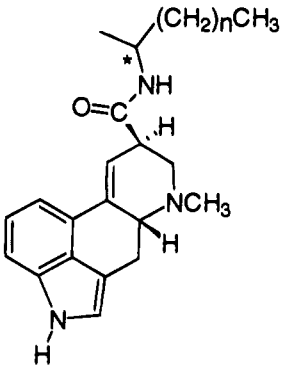

2a (*R*)-, n = 1
2b (*S*)-, n = 1
3a (*R*)-, n = 2
3b (*S*)-, n = 2
4 3-pentylamide
5a (*R*)-, n = 3
5b (*S*)-, n = 3
6a (*R*)-, n = 4
6b (*S*)-, n = 4

Another point of modification of the lysergamide nucleus has been at the amide nitrogen. In the late 1950s, a number of clinical investigations were undertaken to determine the hallucinogenic activity of a series of *N*-monoalkyl- and *N*,*N*-dialkyl amides of lysergic acid.[3,9–12] All of these studies revealed that **1** was unique in its high potency and ability to produce what are now regarded as classical hallucinogenic effects. Even analogs with minor modifications to the alkyl amide moiety had attenuated hallucinogenic activity in humans. Indeed, Hofmann noted that the next higher and lower homologs of LSD (**1**), the dipropylamide and

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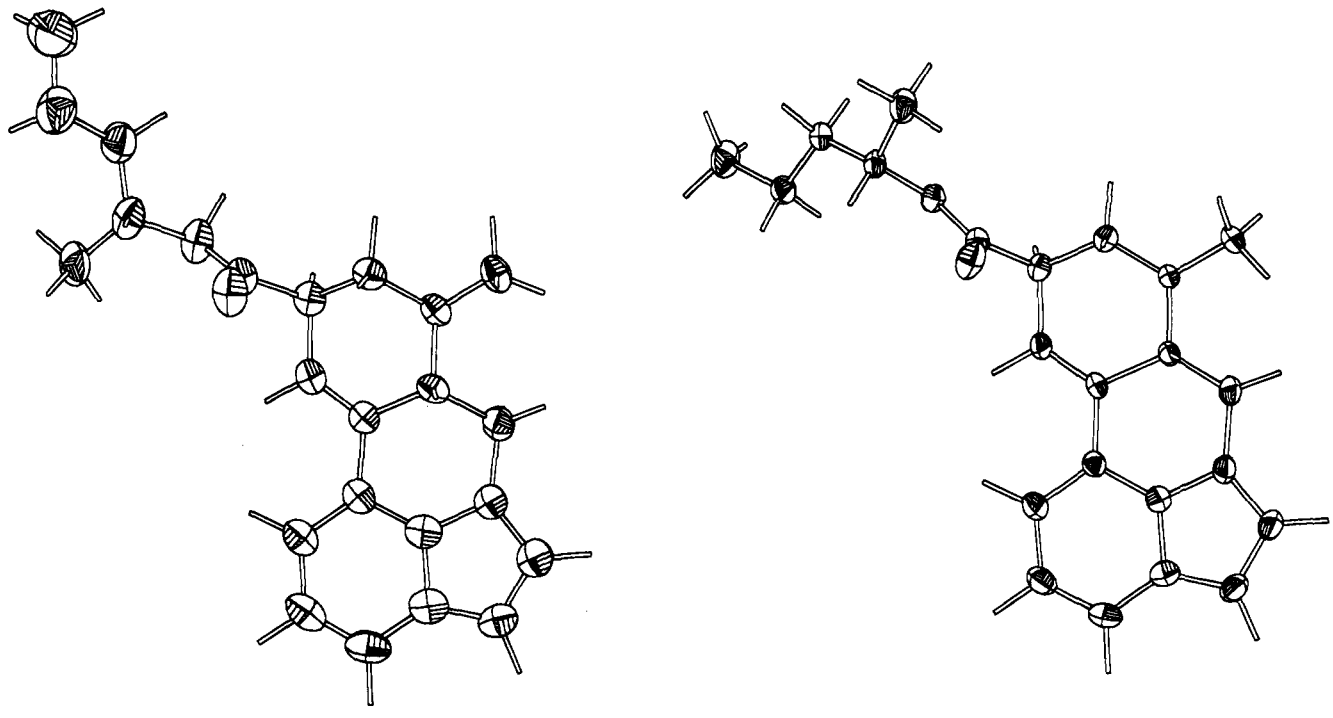
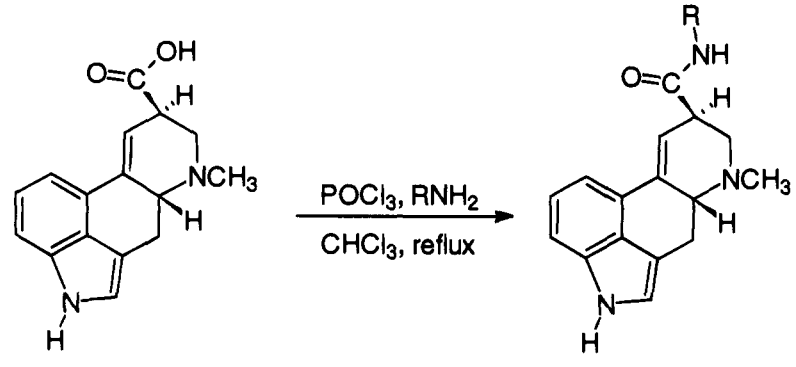


Figure 1. ORTEP representations of (*R*)-2-pentyl *d*-lysergic acid amide **3a** (left) and (*S*)-2-pentyl *d*-lysergic acid amide **3b** (right).

Scheme 1



the dimethylamide, respectively, were about 10 times less potent in producing the characteristic mental effects of **1**.[9]

Additionally, previous work describing the clinical activity of the four possible isomers of lysergic acid *N*,*N*-diethylamide indicated that the (5*S*,8*S*)-, (5*S*,8*R*)-, and (5*R*,8*S*)-isomers were all inactive as hallucinogens, with only the (5*R*,8*R*)-compound **1** exhibiting hallucinogenic effects in humans.[10,12] This high degree of stereoselectivity, in addition to the profound effects on biological activity by modification of the amide group, led us to examine the effects of introducing a new center of asymmetry to the lysergamides by attaching chiral alkyl groups to the amide nitrogen atom.

In a recent report, we described the synthesis and pharmacological evaluation of the *d*-lysergic acid amides of (*R*)-2-aminobutane **2a** and (*S*)-2-aminobutane **2b**.[13] In that study, both diastereomers were active in the rat behavioral assay, and had high affinity for 5-HT_{2A} and 5-HT_{1A} receptors, but it was the (*R*)-isomer that proved to be more potent in each case. Thus, this was the first report to describe alkylamide derivatives of *d*-lysergic acid that produced hallucinogen-like pharmacology with potencies comparable that of **1**. As an extension of our previous work, we now report on the synthesis and stereoselective pharmacological effects of a series of *d*-lysergamides with extended chiral alkyl substituents on the amide nitrogen.

Chemistry

The condensation of *d*-lysergic acid with the appropriate amines was accomplished efficiently as shown in Scheme 1 using the method of Johnson *et al.*[14] Typically, phosphorus oxychloride and an excess of racemic amine were added simultaneously to a refluxing suspension of *d*-lysergic acid monohydrate in chloroform. After workup, the diastereomeric lysergamides were purified and separated by radial chromatography under an atmosphere of ammonia and nitrogen. The absolute stereochemistry of the pure diastereomers was proven by the X-ray crystal structure analysis of compounds **3a**, **3b**, and **5a**. The ORTEP representations of the (*R*)-2-pentyl- (**3a**) and (*S*)-2-pentyllysergamides (**3b**) are shown in Figure 1. A stereoview drawing of the crystal structure for the (*R*)-2-hexyllysergamide **5b** is shown in Figure 2.

Suitable crystals of **3** and **5a** were obtained for X-ray crystallographic analysis by recrystallizing the maleate salts from 2% aqueous NaCl or KBr solution. Crystallization in this manner resulted in an exchange of counterions, leading to the formation of the less soluble hydrochloride or hydrobromide salts, respectively. These

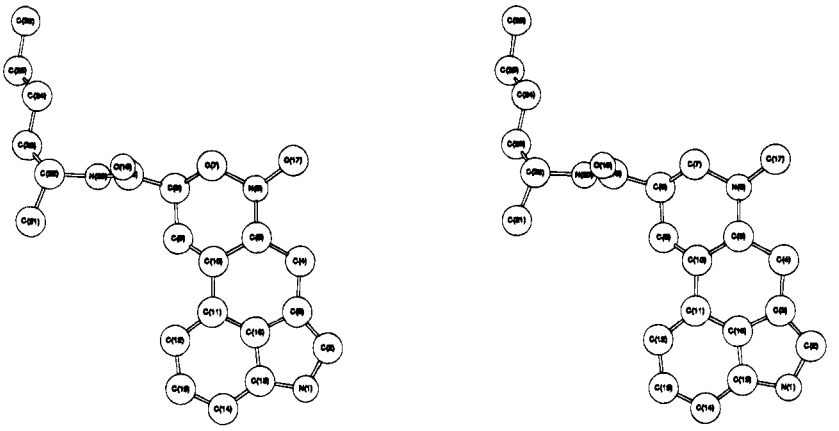


Figure 2. Stereoview representation of the X-ray crystal structure of (*R*)-2-hexyl *d*-lysergic acid amide **5b**. Hydrogens removed for clarity.

crystals were far superior for X-ray structure determination to those obtained by recrystallization of the maleate salts from common organic solvents. Thus, we present here a mild crystallization method that allows for the formation of hydrochloride or hydrobromide salts of lysergamides that would otherwise be extremely difficult to prepare. We were not successful in preparing crystals of **6a** and **6b** suitable for X-ray analysis. Therefore, the stereochemistry of these diastereomers was inferred from their chromatographic mobilities compared to compounds **2**–**5**, where, in each case, the higher R_f isomer possessed the *R* stereochemistry in the amide side chain.

Pharmacology

Using a modification of methods described previously,[15] compounds **3**–**6** were evaluated in the two-lever drug discrimination assay in a group of rats trained to discriminate the effects of ip injections of saline from those of **1** (LSD) tartrate (0.08 mg/kg). Potencies were measured using ED_{50} values with 95% confidence intervals (CI) for those compounds that completely substituted for **1**. The ability of the test compounds to compete for radioligand binding to 5-HT_{1A} and 5-HT_{2A} receptor sites was determined using previously established methods.[16,17] Briefly, the ability of **3**–**6** to displace 0.75 nM [^{3}H]-8-OH-DPAT from rat hippocampal homogenate and 0.75 nM [^{3}H]ketanserin from rat frontal cortex homogenate was measured. Two compounds, **3a** and **3b**, were also tested for their effect on the intracellular hydrolysis of phosphoinositide to evaluate the functional properties of this series of compounds. Using previously described methods,[18] cells expressing 5-HT_{2A} receptors[19] were pretreated with [^{3}H]myoinositol for 20 h, followed by exposure to the test drugs in the presence of lithium chloride. Accumulated [^{3}H]inositol monophosphate was then measured.

Results and Discussion

The drug discrimination paradigm has been used extensively to model human hallucinogenic effects by studying the **1**-like discriminative stimulus properties produced in animals.[20,21] In the present series of compounds, those agents possessing the (*R*)-stereochemistry in the amide substituent **2a**–**5a** were consistently more potent in eliciting a **1**-like behavioral response than those with the (*S*)-stereochemistry **2b**–**5b** (Table 1). However, the *in vivo* behavioral response decreased markedly as the length of the *N*-alkyl chain was increased, with the previously reported[13] (*R*)-2-butyllysergamide **2a** being the most potent compound in this series. The nonchiral 3-pentyllysergamide **4** also produced a significant **1**-like discriminative cue with a potency comparable to that of **1** and **2a**, indicating that chirality in the amide substituent is not necessary for high efficacy in this assay.

Table 1. Results of Drug Discrimination Studies in **1**-Trained Rats

drug	dose (nmol/kg)	n^a	% D^b	% SDL[c]	ED_{50} (nmol/kg)	95% CI	potency (rel to LSD)
1, LSD	186	12	0	100	48	32–73	1
2a	12	8	0	13	33	17–66	1.45
	23	8	0	50			
	47	8	0	75			
	93	8	0	75			
	186	8	0	88			
2b	47	9	10	13	124	74–209	0.39
	93	8	0	50			
	186	11	27	75			
	290	10	10	67			
	372	8	0	75			
	465	10	10	100			
3a	55	7	0	14	102	61–169	0.47
	110	9	0	44			
	220	9	30	100			
3b	220	8	0	0	NS		
	440	8	0	0			
	1760	9	11	25			
4	63	12	0	58	52	24–114	0.92
	125	12	0	67			
	250	13	0	85			
	500	14	14	92			
5a	86	10	10	22	PS		
	128	10	0	40			
	171	11	0	55			
	213	11	27	50			
	426	4	0	0			
5b	213	10	30	29	NS		
	426	10	30	0			
	639	8	12.5	0			

[a] Number of animals tested. [b] Number of animals disrupted. [c] Percentage of animals selecting drug lever.

Previous drug discrimination studies with **1** have shown that the discriminative behavioral cue seems to be mediated primarily by stimulation of 5-HT_2 receptors.[21–25] Additionally, we have previously shown that *N*-monoalkyllysergamides have high affinity for the 5-HT_{1A} receptor subtype.[13,26] Thus, activation of this receptor may also play a role in the mechanism of action of hallucinogenesis in this class of compounds, particularly if the 5-HT_{1A} effect is synergistic with the activation of 5-HT_{2A} receptors.[20,27] In any event, based on

Table 2. Results of 5-HT_{2A} Receptor Binding Studies for Displacement of [^{3}H]Ketanserin by Lysergic Acid Amides

drug	K_i (nM) ± SEM	Hill coeff ± SEM	$\Delta G°$ (kcal/mol)[a]
1, LSD	4.4 ± 0.2	0.97 ± 0.02	−11.87
2a	8.8 ± 0.3	0.94 ± 0.03	−11.44
2b	34 ± 2	0.91 ± 0.02	−10.61
3a	4.5 ± 0.5	0.90 ± 0.03	−11.86
3b	105 ± 10	0.97 ± 0.01	−9.91
4	8.0 ± 0.2	0.87 ± 0.07	−11.50
5a	16 ± 2	0.86 ± 0.06	−11.07
5b	55 ± 7	0.81 ± 0.04	−10.31
6a	80 ± 9	0.84 ± 0.05	−10.08
6b	357 ± 19	0.77 ± 0.02	−9.16

[a] Estimated free energy of binding at 37 °C, ref 41.

Table 3. Results of 5-HT_{1A} Receptor Binding Studies for Displacement of [^{3}H]-8-OH-DPAT by Lysergic Acid Amides

drug	K_i (nM) ± SEM	Hill coeff ± SEM	$\Delta G°$ (kcal/mol)[a]
1, LSD	5.1 ± 0.4	1.05 ± 0.04	−11.78
2a	2.0 ± 0.2	0.83 ± 0.05	−12.36
2b	4.6 ± 0.3	1.03 ± 0.03	−11.84
3a	0.6 ± 0.1	0.96 ± 0.02	−13.10
3b	8 ± 1	0.92 ± 0.07	−11.50
4	2.1 ± 0.3	0.96 ± 0.02	−12.33
5a	0.32 ± 0	0.90 ± 0.05	−13.49
5b	4.9 ± 0.3	0.91 ± 0.04	−11.80
6a	3.3 ± 0.4	1.06 ± 0.42	−12.06
6b	14 ± 2	0.99 ± 0.05	−11.15

[a] Estimated free energy of binding at 37 °C.

current knowledge, one would expect *in vivo* hallucinogenic activity to parallel the abilities of test compounds to activate these serotonin receptor subtypes.

The results of the radioligand competition studies for compunds **1**−**6** are shown in Tables 2 and 3. As anticipated from our earlier study, the (*R*)-lysergamides **3a**−**6a** had higher affinity for both [^{3}H]ketanserin-labeled 5HT_{2A} sites (Table 2) and [^{3}H]-8-OH-DPAT-labeled 5-HT_{1A} sites (Table 3) than did the (*S*)-lysergamides **3b**−**6b**. Interestingly, maximal affinity for the 5HT_{2A} site was achieved with the (*R*)-2-pentyl compound **3a**, while it was the (*R*)-2-hexyllysergamide **5a** that had the highest affinity for the 5-HT_{1A} binding site. Thus, the behavioral responses obtained in the drug discrimination studies more closely parallel the affinities for ketanserin-labeled 5-HT_{2A} sites. The minor discrepancy observed between affinity for ketanserin-labeled sites and the *in vivo* activity may be partially attributable to pharmacokinetic factors such as differential rates of metabolism.

The results of the radioligand competition studies also indicate that the ligand recognition site on the 5-HT_{1A} receptor is able to tolerate a bulkier alkylamide substituent than is the ligand binding site of the ketanserin-labeled receptors. Indeed, the (*R*)-2-hexyllysergamide **5a** had subnanomolar affinity (K_i = 0.32 nM) for the 5-HT_{1A} site, but at the 5-HT_{2A} site it was less potent than its shorter 2-pentyl analog **3a**. Thus, it appears that there is a slightly larger region within the 5-HT_{1A} ligand binding site, as compared with the 5-HT_{2A} site, that can accommodate the extended chain length of the hexylamide substituent.

It is now widely believed that hallucinogenic activity is mediated primarily through agonist activity at 5-$HT_{2A/2C}$ receptors.[25,28−33] However, some studies have shown that LSD (**1**) acts as a partial agonist,[34,35] or even as an antagonist,[36,37] at 5-HT_{2A} sites. LSD (**1**) has

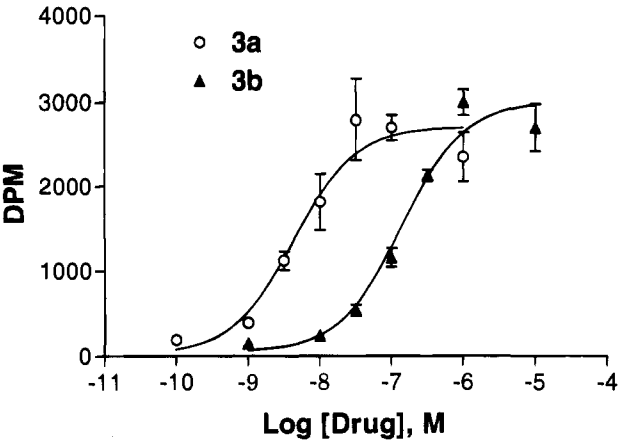


Figure 3. Effect of (*R*)-2-pentyllysergamide **3a** and (*S*)-2-pentyllysergamide **3b** on phosphoinositide hydrolysis in fibroblasts expressing rat 5-HT_{2A} receptors. [^{3}H]inositol-labeled cells were treated with increasing concentrations of **3a** or **3b** for 45 min as described in the Experimental Section. [^{3}H]-Inositol monophosphate formation is plotted on the *y*-axis as disintegrations per minute (dpm). Values are the mean of triplicate determinations and are representative of three individual experiments with each drug. The individual EC_{50} values (in nM) for **3a** were 2.4, 4.3, and 9.4, and the corresponding values for **3b** were 47, 136, and 91 nM.

previously been shown to elicit a prominent phosphoinositide signal after interacting with both 5-HT_{2A} and 5-HT_{2C} receptors.[33,34,38] In order to ascertain the functional nature of the compounds in this study, fibroblasts expressing cloned rat 5-HT_{2A} receptors were utilized to evaluate the agonist properties of the (*R*)- and (*S*)-2-pentyllysergamides **3a** and **3b**, respectively. These cells express a high density of 5-HT_{2A} receptors (~20 000 fmol/mg protein). In our assay, both **3a** and **3b** were found to be highly effective agonists (Figure 3), eliciting maximal responses comparable to those of serotonin, a full agonist. Consistent with the radioligand competition and behavioral assays, the (*R*)-2-pentyllysergamide **3a** was nearly 20 times more potent than was the (*S*)-2-pentyllysergamide **3b**. Furthermore, the phosphoinositide hydrolysis responses were blocked by the 5-HT_{2A} receptor antagonists ketanserin and spiperone, but not by atropine, an M_1-muscarinic antagonist (data not shown). These results confirm that the *N*-alkyllysergamides are potent agonists at 5-HT_{2A} receptors. Preliminary results from our laboratories suggest that **3a** and **3b** also activate phosphoinositide hydrolysis in cells expressing 5-HT_{2C} receptors and that **3a** is much more efficacious than **3b** in eliciting this response.

A comparison of the X-ray structures of diastereomeric compounds **3a** and **3b** (Figure 1) clearly indicates a distinct difference between these two isomers in the positioning of the amide 2-pentyl groups. In both cases, as well as for **5a** (Figure 2), it appears that the amide-containing moiety is extended away from the D ring, with the carbonyl oxygen located on the β-face of the molecule rotated approximately 60-90° above the plane. This is in reasonable agreement with the minimum energy structures obtained for LSD (**1**) and the 2-butylamides **2** in our earlier molecular modeling studies.[13] In that previous work, the minimum energy conformations oriented the carbonyl C=O bond at an angle nearly perpendicular to the plane of the molecule. The crystal structures obtained in the present study indicate that the carbonyl group of the (*R*)-hexylamide **5a** lies es-

sentially perpendicular to the plane of the molecule, while in the 2-pentylamides **3** the carbonyl oxygens are rotated only 30° from this conformation toward C(9) of the ergoline D ring. In addition, the X-ray structures indicate that **3a**, **3b**, and **5** exist in the "flap-up" conformation, an observation in close agreement with other researchers' findings regarding the most stable conformation of **1**, both in solution[39-41] and in the solid state.[42,43]

As noted above, the primary structural difference between diastereomers, however, seems to be in the position of the amide alkyl side chains. In the crystal structure of both (*R*)-isomers **3a** and **5a**, the amide alkyl moiety remains in the plane of the molecule but the longer branch is extended away from the indole rings in a "northern" direction as evident in Figures 1 and 2. By contrast, in the (*S*)-pentyl isomer **3b**, the longer alkyl branch is pointed away from the N(6) nitrogen in a "western" direction. These clear differences in conformation presumably play an important role in the differential binding of these drugs to the 5-HT receptors. If the carbonyl oxygen of these lysergamides must interact in some way with a hydrogen bond donor in the active site, it seems possible that receptor affinity may be attenuated in the (*S*)-series due to the alkyl side chain's interference in some way with this hydrogen bond formation. Conversely, we postulate that there is a properly positioned hydrophobic pocket within the receptor that can accommodate the extended portions only of the (*R*)-alkyl groups. This pocket is of limited size, however, since activity markedly drops off for the longer (*R*)-alkyllysergamides of this series.

In general, our findings show that the chirality and size of the amide alkyl substituent of *d*-lysergic acid amides are important determinants of binding to serotonin receptors and in eliciting a discriminative stimulus. Although receptor activation may be dependent upon direct insertion of the amide alkyl group into a hydrophobic binding pocket, it is also possible that a secondary positioning of the carbonyl oxygen atom due to a receptor−alkyl group interaction may be important. However, since the carbonyl oxygen atom is a likely hydrogen bond acceptor, we have hypothesized that this interaction is crucial for receptor activation. Thus, the chirality of the amide substituent may directly or indirectly influence the nature of this interaction, with the (*R*)-isomers allowing for the "best fit" to the receptor sites examined here. In addition, alteration of the size of the amide alkyl group lends some degree of site selectivity, with the (*R*)-2-pentyllysergamide binding most tightly to [^{3}H]ketanserin-labeled sites and the (*R*)-2-hexyllysergamide binding most tightly to [^{3}H]-8-OH-DPAT-labeled sites.

In summary, we have defined the optimum stereochemistry and the steric limits for chiral 2-alkylamide substituents in a series of *d*-lysergic acid amides for producing LSD-like behavioral effects in rats and for binding to serotonin 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor subtypes. Also, we have shown that the two 2-pentyllysergamides (**3a** and **3b**) in this series are full agonists at the 5-HT$_{2A}$ receptor subtype and that their potencies here parallel other measures of biological activity. This work has led to an increased understanding of the topography of these serotonin receptor subtype binding sites. Further work is currently underway in our laboratory using other sets of chiral, rigid analogs of **1** to determine the active binding orientation of the *N*-alkyl groups of *d*-lysergic acid diethylamide.

Experimental Section

Chemistry. Melting points were determined using a Thomas-Hoover apparatus and are uncorrected. ^{1}H-NMR spectra (500 MHz) were recorded for the lysergamide free amines in $CDCl_3$ using a Varian VXR-500S NMR spectrometer. Chemical shifts are reported in δ values (ppm) relative to tetramethylsilane (TMS) as an internal reference (0.03% v/v). Abbreviations used in NMR analyses are as follows: s = singlet, d = doublet, t = triplet, dd = doublet of doublets, q = quartet, p = pentet, m = multiplet, b = broad. Chemical ionization mass spectra (methane as carrier gas) were obtained with a Finnegan 4000 spectrometer. IR measurements were taken using a Perkin-Elmer 1600 Series FTIR spectrophotometer. Optical rotations were measured at 27 °C using a Perkin-Elmer Model 241 polarimeter. Elemental analyses were performed by the Purdue University Microanalysis Laboratory and were within ±0.4% of the calculated values unless otherwise noted. Thin-layer chromatography was typically performed using Baker-flex silica gel IB2-F, plastic-backed plates with fluorescent indicator (2.5 × 7.5 cm, J. T. Baker), eluting with ethyl acetate, and visualizing with UV light at 254 nm. All reactions were carried out in the dark under an inert atmosphere of dry nitrogen using glassware that had been meticulously cleaned and dried overnight in a 120 °C oven.

General Method for the Preparation of Chiral Alkylamides of *d*-Lysergic Acid. *N*-((*R*)-2-Pentyl)-9,10-didehydro-6-methylergoline-8β-carboxamide (3a) and *N*-((*S*)-2-Pentyl)-9,10-didehydro-6-methylergoline-8β-carboxamide (3b). *d*-Lysergic acid monohydrate (300 mg, 1.05 mmol) and 25 mL of dry, ethanol-free $CHCl_3$ were placed in a 50 mL, three-neck, round-bottom flask fitted with a condenser and septa inlets. The dark slurry was stirred magnetically and warmed to reflux over a 90 °C oil bath. Racemic 2-aminopentane (Aldrich, Milwaukee, WI) (1.24 mL, 10.5 mmol) and phosphorus oxychloride (0.195 mL, 2.10 mmol), diluted to 1.25 mL with $CHCl_3$, were then added simultaneously to the refluxing mixture, via syringe, over a 3 min period. The mixture was heated at reflux for an additional 5 min, after which the oil bath was removed, and the clear, amber-colored solution was allowed to cool to room temperature over 15 min. The $CHCl_3$ solution was poured into 1 M NH_4OH, and the layers were separated. The aqueous phase was extracted twice with $CHCl_3$, and the organic fractions were combined and washed with H_2O and brine. After drying over $MgSO_4$ and filtration through Celite, the chloroform was removed by rotary evaporation to yield a dark brown oil. This oil was shown by TLC analysis to consist of the two diastereomeric *N*-2-butyllysergamides and an unidentified, high-running component.

The diastereomers were separated and purified by radial centrifugal chromatography ("Chromatotron", Harrison Research, Palo Alto, CA) using a silica gel rotor and elution with ethyl acetate. The Chromatotron chamber was continuously purged with N_2 and protected from light. After fractionation and solvent removal, the diastereomers were isolated as yellow oils. The faster moving diastereomer weighed 171 mg (48%) and the slower moving component weighed 153 mg (43%). The amines were precipitated as their maleate salts and isolated as white, crystalline solids. Crystals suitable for X-ray structure determination were grown by dissolving the appropriate maleate salt in a minimum amount of hot, aqueous 2% NaCl or KBr and allowing the solution to cool slowly to room temperature. X-ray analysis confirmed the identity of the higher running spot to be **3a**: $R_f = 0.36$; mp (maleate salt) 210−212 °C dec; ^{1}H NMR δ 0.91 (t, J = 7.3 Hz, 3 H), 1.14 (d, J = 6.5 Hz, 3 H), 1.35 (m, 2 H), 1.42 (p, J = 7.0 Hz, 2 H), 2.60 (s, 3 H), 2.7−2.8 (m, 2 H), 3.09 (dd, J = 11.5, 4.8 Hz, 1 H), 3.32 (m, 1 H), 3.40 (dd, J = 14.3, 5.2 Hz, 1 H), 3.50 (m, 1 H), 4.0 (p, J = 6.6 Hz, 1 H), 6.44 (dd, J = 4.0, 2.0 Hz, 1 H), 6.54 (bs, 1 H, amide N*H*), 6.92 (t, J = 1.7 Hz, 1 H), 7.14−7.24 (m,

3 H), 8.0 (bs, 1 H); MS (*m*/*z*) 338 (M + 1); $[\alpha]_D$ = +17 (*c* = 0.1, EtOH). Anal. ($C_{25}H_{31}N_3O_5$) C, H, N.

The lower running spot was **3b**: R_f = 0.29; mp (maleate salt) 230–232 °C dec; ^{1}H NMR δ 0.92 (t, *J* = 7.2 Hz, 3 H), 1.14 (d, *J* = 6.6 Hz, 3 H), 1.37 (m, 2 H), 1.42 (p, *J* = 6.7 Hz, 2 H), 2.60 (s, 3 H), 2.70–2.78 (m, 2 H), 3.08 (dd, *J* = 11.5, 4.6 Hz, 1 H), 3.35 (m, 1 H), 3.41 (dd *J* = 14.3, 5.2 Hz, 1 H), 3.51 (m, 1 H), 4.01 (p, *J* = 6.6 Hz, 1 H), 6.43 (dd, *J* = 4.0, 2.0 Hz, 1 H), 6.48 (bs, 1 H, amide N*H*), 6.92 (t, *J* = 1.6 Hz, 1 H), 7.14–7.24 (m, 3 H), 8.0 (bs, 1 H); MS (*m*/*z*) 338 (M + 1); $[\alpha]_D$ = +26 (*c* = 0.1, EtOH). Anal. ($C_{25}H_{31}N_3O_5$) C, H, N.

***N*-((*R*)-2-Hexyl)-9,10-didehydro-6-methylergoline-8β-carboxamide (5a):** 40% as the free base from the condensation of *d*-lysergic acid monohydrate with 2-aminohexane (Aldrich, Milwaukee, WI); R_f = 0.38; mp (maleate) 214 °C dec; ^{1}H NMR δ 0.89 (t, *J* = 6.9 Hz, 3 H), 1.14 (d, *J* = 6.6 Hz, 3 H), 1.31 (m, 2 H), 1.44 (p, *J* = 6.5 Hz, 2 H), 2.60 (s, 3 H), 2.7–2.8 (m, 2 H), 3.09 (dd, *J* = 11.4, 4.8 Hz, 1 H), 3.34 (m, 1 H), 3.41 (dd, *J* = 14.2, 5.1 Hz, 1 H), 3.51 (m, 1 H), 3.99 (p, *J* = 6.6 Hz, 1 H), 6.44 (m, 1 H), 6.58 (bs, 1 H, amide N*H*), 6.93 (s, 1 H), 7.14–7.24 (m, 3 H), 7.98 (bs, 1 H); MS (*m*/*z*) 352 (M + 1); $[\alpha]_D$ = +19 (*c* = 0.1, EtOH); IR (free base, neat) 1644 cm^{-1} (C=O). Anal. ($C_{26}H_{33}N_3O_5$) C, H, N.

***N*-((*S*)-2-Hexyl)-9,10-didehydro-6-methylergoline-8β-carboxamide (5b):** 52% as the free base from the condensation of *d*-lysergic acid monohydrate with 2-aminohexane (Aldrich, Milwaukee, WI); R_f = 0.29; mp (maleate) 220 °C dec; ^{1}H NMR δ 0.91 (t, *J* = 7.2 Hz, 3 H), 1.14 (d, *J* = 6.6 Hz, 3 H), 1.32 (m, 4 H), 1.44 (p, *J* = 6.6 Hz, 2 H), 2.60 (s, 3 H), 2.70–2.78 (m, 2 H), 3.08 (dd *J* = 11.5, 4.6 Hz, 1 H), 3.34 (m, 1 H), 3.40 (dd, *J* = 14.3, 5.2 Hz, 1 H), 3.52 (m, 1 H), 4.0 (p, *J* = 6.6 Hz, 1 H), 6.44 (m, 1 H), 6.50 (bs, 1 H, amide N*H*), 6.93 (s, 1 H), 7.14–7.24 (m, 3 H), 8.0 (bs, 1 H); MS (*m*/*z*) 352 (M + 1); $[\alpha]_D$ = +27 (*c* = 0.1, EtOH); IR (free base, neat) 1646 cm^{-1} (C=O). Anal. ($C_{26}H_{33}N_3O_5$) C, H, N.

***N*-((*R*)-2-Heptyl)-9,10-didehydro-6-methylergoline-8β-carboxamide (6a):** 27% as the maleate salt from the condensation of *d*-lysergic acid monohydrate with 2-aminoheptane (Pfaltz & Bauer Inc., Waterbury, CT); R_f = 0.50; mp (maleate) 218 °C dec; ^{1}H NMR δ 0.87 (t, *J* = 6.9 Hz, 3 H), 1.14 (d, *J* = 6.5 Hz, 3 H), 1.29 (m, 6 H), 1.42 (m, 2 H), 2.60 (s, 3 H), 2.7–2.8 (m, 2 H), 3.09 (dd, *J* = 11.5, 4.7 Hz, 1 H), 3.34 (m, 1 H), 3.41 (dd, *J* = 14.2, 5.4 Hz, 1 H), 3.51 (m, 1 H), 3.99 (p, *J* = 6.8 Hz, 1 H), 6.44 (q, *J* = 2.0 Hz, 1 H), 6.56 (bs, 1 H, amide N*H*), 6.73 (t, 1.7 Hz, 1 H), 7.13–7.24 (m, 3 H), 7.96 (bs, 1 H); MS (*m*/*z*) 366 (M + 1); $[\alpha]_D$ = +22 (*c* = 0.1, EtOH). Anal. ($C_{27}H_{35}N_3O_5$) C, H, N.

***N*-((*S*)-2-Heptyl)-9,10-didehydro-6-methylergoline-8β-carboxamide (6b):** 22% as the maleate salt from the condensation of *d*-lysergic acid monohydrate with 2-aminoheptane (Pfaltz & Bauer Inc., Waterbury, CT); R_f = 0.40; mp (maleate) 220 °C dec; ^{1}H NMR δ 0.89 (t, *J* = 6.8 Hz, 3 H), 1.14 (d, *J* = 6.4 Hz, 3 H), 1.30 (m, 6 H), 1.42 (m, 2 H), 2.60 (s, 3 H), 2.71–2.78 (m, 2 H), 3.08 (dd, *J* = 11.5, 4.6, 1 H), 3.34 (m, 1 H), 3.40 (dd, *J* = 14.3, 5.2 Hz, 1 H), 3.52 (m, 1 H), 3.99 (p, *J* = 6.6, 1 H), 6.44 (q, *J* = 2.1 Hz, 1 H), 6.50 (bs, 1 H, amide N*H*), 6.92 (t, *J* = 1.6 Hz, 1 H), 7.14–7.24 (m, 3 H), 8.0 (bs, 1 H); MS (*m*/*z*) 366 (M + 1); $[\alpha]_D$ = +29 (*c* = 0.1, EtOH). Anal. ($C_{27}H_{35}N_3O_5$) C, H, N.

***N*-(3-Pentyl)-9,10-didehydro-6-methylergoline-8β-carboxamide (4):** 95% as the free base from the condensation of *d*-lysergic acid monohydrate with 1-ethylpropylamine (Aldrich, Milwaukee, WI); R_f = 0.33; mp (maleate) 212–214 °C dec; ^{1}H NMR δ 0.88–0.96 (m, 6 H), 1.34–1.42 (m, 2 H), 1.54–1.60 (m, 2 H), 2.60 (s, 3 H), 2.74–2.80 (m, 2 H), 3.09 (dd, *J* = 11.5, 4.6, 1 H), 3.36 (m, 1 H), 3.40 (dd, *J* = 14.3, 5.2 Hz, 1 H), 3.56 (m, 1 H), 3.82 (m, 1 H), 6.46 (q, *J* = 2.3 Hz, 1 H), 6.63 (bs, 1 H, amide N*H*), 6.92 (s, 1 H), 7.12–7.24 (m, 3 H, Ar*H*), 8.5 (bs, 1H); MS (*m*/*z*) 338 (M + 1); $[\alpha]_D$ = +23 (*c* = 0.1, EtOH). Anal. ($C_{25}H_{31}N_3O_5$) C, H, N.

Pharmacology. Drug Discrimination Studies. Twelve male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 200–220 g at the beginning of the drug discrimination study were employed. None of these rats had previously received drugs or behavioral training. Water was freely available in their individual home cages, and a rationed amount of rat feed (Purina Lab Blox) was made available after experimental sessions to maintain approximately 80% of the free-feeding weight. The temperature of the animal facility and of the laboratory remained within the range of 22–24 °C. The humidity was maintained at 40–50%, and the lights were on between 7 a.m. and 7 p.m.

Apparatus. Six standard operant chambers (Model F10-10RF, 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 test cage, which was also equipped with two response levers, separated by a food hopper, all positioned 2.5 cm above the floor. Solid-state logic in an adjacent room was linked through a Med Associates interface to a 486-based PC that controlled reinforcement and data acquisition with locally written software.

Drug Administration. The training drug, **1**-tartrate (*d*-LSD) (NIDA, 186 nmol/kg, 0.08 mg/kg), or saline was administered ip 30 min prior to sessions. All drugs were administered dissolved in saline such that a volume of 1 mL/kg was used. Solutions were sterilized prior to use by filtration through a sterile 0.2 μm filter (Millipore) into an autoclaved vial.

Discrimination Training and Testing. The DD training and testing procedures described previously by Oberlender and Nichols[15] were followed with some modifications. A fixed ratio (FR) 50 schedule of food reinforcement (Bioserv 45 mg dustless pellets) in a two-lever paradigm was used. Initially, rats were taught to lever press on an FR 1 schedule so that one food pellet was dispensed for each press. Half the rats were trained on drug-left, saline-right, and the other half on drug-right, saline-left, to avoid positional preference. Training sessions lasted 15 min and were conducted at the same time each day, Monday through Friday. Levers were cleaned with a 10% ethanol solution in order to avoid olfactory cues.[44] Only the appropriate lever was present during the first 10 sessions of training. Afterward, both levers were present during all phases of training, but reinforcements were delivered only after responses on the stimulus-appropriate lever. Presses on the incorrect lever were recorded but had no programmed consequence. After initially learning to lever-press for food, saline and drug sessions were randomly ordered, with neither treatment given more than three consecutive sessions. As responding rates stabilized, the schedule of reinforcement was gradually increased from FR 1 to FR 50. Once at the FR 50, training continued until an accuracy of at least 85% (number of correct presses × 100/total number of presses) was attained for 8 of 10 consecutive 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 traning sessions following a test session.[23] Test sessions were run under conditions of extinction, with rats removed from the operant box when 50 presses were made on either lever. If 50 presses on one lever were not completed within 5 min, the session was ended and scored as a "disruption" (D). Treatments were randomized at the beginning of the study.

Data Analysis. The data were scored in 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. If that drug was one which completely substituted for the training drug (at least one dose resulted in the % SDL = 80% or higher), the method of Litchfield and Wilcoxon[45] was used to determine the ED_{50} and 95% confidence interval (95% CI). This method also allowed for tests of parallelism between the dose–response curves of the test drugs and that of **1**.

Pharmacology. Radioligand Competition Studies. [^{3}H]Ketanserin and [^{3}H]-8-OH-DPAT were purchased from New England Nuclear (Boston, MA) at specific activities of 63.7

Table 4. Summary of X-ray Crystallographic Data for Compounds **3a**, **3b**, and **5a**

unit cell formula	**3a**·HCl·2H$_2$0	**3b**·HCl·H$_2$O	**5a**·HBr
empirical formula	$C_{21}H_{32}N_3O_3Cl$	$C_{21}H_{30}N_3O_2Cl$	$C_{22}H_{30}N_3OBr$
formula weight	409.96	391.94	432.41
space group	$P2_1$ (No. 4)	$P2_1$ (No. 4)	$P2_12_12_1$ (No. 19)
a, Å	11.554	11.518	5.700
b, Å	7.945	7.752	15.228
c, Å	13.501	12.394	25.081
β, deg	112.935	112.265	2177.2
volume, Å^3	1141.4	1024.0	2177.2
Z	2	2	4
density calcd, g/cm^3	1.190	1.268	1.319
crystal dimensions, mm	0.25 × 0.22 × 0.04	0.25 × 0.24 × 0.08	0.28 × 0.08 × 0.05
temperature, K	199	200	293
radiation	Cu Kα	Cu Kα	Cu Kα
wavelength, Å	1.541 84	1.541 84	1.541 84
absorption coefficient, cm^{-1}	16.81	18.16	26.90
scan method	$\omega-2\theta$	$\omega-2\theta$	$\omega-2\theta$
h, k, l, index ranges	0 to 13, 0 to 9, −15 to 14	−13 to 12, 0 to 9, 0 to 14	0 to 6, 0 to 17, 0 to 28
2θ range, deg	4.00 to 130.00	4.00 to 130.00	4.00 to 120.00
scan width, deg	0.68 + 0.15 tan (θ)	0.68 + 0.15 tan (θ)	1.52 + 0.15 tan (θ)
take-off angle, deg	6.00	6.00	6.00
programs used	Enraf-Nonius MolEN	Enraf-Nonius MolEN	Enraf-Nonius MolEN
F_{000}	438.0	418.0	904.0
p factor used in weighting	0.040	0.040	0.040
reflections collected	2073	1898	1929
unique data	2073	1898	1929
data with $I > 3.0\sigma(I)$	1741	1769	1271
number of variables	276	259	176
R	0.055	0.032	0.047
R_w	0.067	0.042	0.053
goodness of fit	1.813	1.476	1.297

and 169.9 Ci/mmol, respectively. The procedure of Johnson *et al.*[16] was employed for competition and saturation experiments conducted with [^{3}H]ketanserin. Briefly, the frontal cortex and hippocampal brain regions from 10 to 20 male Sprague–Dawley rats (175–190 g, Harlan Laboratories, Indianapolis, IN) were pooled and homogenized (Brinkman Polytron, setting 6 for 2 × 20 s) in 4 or 8 volumes of 0.32 M sucrose for frontal cortex or hippocampus, respectively. The homogenate was centrifuged at 36500g for 10 min, and the resulting pellets were resuspended in the same volume of sucrose. Separate aliquots of tissue were then frozen at −70 °C until assay.

For each experiment, a tissue aliquot was thawed slowly and diluted 1 to 20 with 50 mM Tris HCl buffer (pH 7.4). The homogenate was then incubated at 37 °C for 10 min and centrifuged twice at 36500g for 10 min, with an intermittent wash. The resulting pellet was resuspended in 50 mM Tris HCl with 0.5 mM Na_2EDTA, 0.1% sodium ascorbate, and 10 μM pargyline hydrochloride (pH 7.4). In experiments with [^{3}H]-ketanserin, 5.7 mM $CaCl_2$ was also included. A second preincubation for 10 min at 37 °C was conducted, and the tissues were then cooled in an ice bath.

All experiments were performed with triplicate determinations using the appropriate buffer, to which 200–400 μg of protein was added, giving a final volume of 1 mL. The tubes were allowed to equilibrate for 15 min at 37 °C before rapid filtration through GF/C filters using a Brandel cell harvester (Gaithersburg, MD). Specific binding was defined as that displaceable with 10 μm cinanserin. Under these conditions, [^{3}H]ketanserin was found to bind to a single site (Hill coefficient of 1.08 ± 0.06) with a B_{max} of 297 ± 40 fmol/mg protein and a K_D of 0.71 ± 0.03 nM. The ability of test drugs to displace 0.75 nM of [^{3}H]ketanserin was determined. Filters were air-dried, placed in scintillation vials containing 10 mL of Ecolite scintillation cocktail, and allowed to sit overnight before counting.

Displacement and saturation experiments were conducted with [^{3}H]-8-OH-DPAT according to the methods of Gozlan *et al.*[17] Tubes were allowed to equilibrate for 10 min at 37 °C before filtering through GF/C filters. Specific binding was defined as that displaceable with 10 μM serotonin. Under these conditions, [^{3}H]-8-OH-DPAT was found to bind to a single site (Hill coefficient of 1.00 ± 0.01) with a B_{max} of 119 ± 8 fmol/mg protein and a K_D of 2.5 ± 0.2 nM. The ability of the test drugs to displace 0.75 nM [^{3}H]-8-OH-DPAT was determined. Filters were air-dried and placed in vials, 10 mL of scintillation cocktail was added, and the vials were allowed to sit overnight before counting.

After counting at an efficiency of 34% for tritium, the data were analyzed using the computer programs EBDA and LIGAND.[46] The values from three to four separate experiments were combined. Protein determinations were made using the procedure of Bradford.[47] The values for free energy of binding at 37 °C (310 K) were estimated from $\Delta G° = -RT \ln(1/K_I)$, where R = 0.001 99 kcal/K·mol.[48]

Pharmacology. Phosphoinositide Hydrolysis. NIH 3T3 fibroblasts transfected with 5-HT$_{2A}$ receptor cDNA[19] plated in 11 mm diameter wells were grown in Dulbelco's modified Eagle's medium with 10% calf serum in a humidified atmosphere of 5% CO_2 at 37 °C. Cells were used in phosphoinositide hydrolysis assays upon reaching confluency. To radiolabel phosphoinositides, the medium was changed to CMRL 1066 (Gibco Laboratories, Long Island, NY) containing 1 μCi/mL [^{3}H]myoinositol. Twenty hours later, the cells were treated with test drug (agonist) in the presence of 10 mM lithium chloride for 45 min. Accumulated [^{3}H]inositol monophosphate was extracted and isolated by anion exchange chromatography.[18]

X-ray Crystallography. The specific crystallographic data for compounds **3a**, **3b**, and **5a** are summarized in Table 4. In general, the X-ray structures were solved by mounting the colorless crystal on a glass fiber in a random orientation. Preliminary examination and data collection were performed with Cu Kα radiation (λ = 1.541 84 Å) on an Enraf-Nonius CAD computer-controlled κ axis diffractometer equipped with a graphite crystal, incident beam monochromator. Cell constants and an orientation matrix for data collection were obtained from least-squares refinement according to the details listed in the table. The space groups were determined from the systematic absences followed by least-squares refinement of the data. The scan range, in degrees, was determined as a function of θ to correct for the separation of the Kα doublet.[49] Lorentz and polarization corrections were applied to the data in addition to an empirical absorption correction based on the method of Walker and Stuart.[50]

The structures of **3a** and **3b** (Figure 1) were solved using the structure solution program SHELX-86.[51] The structure of **5a** (Figure 2) was solved using the Patterson heavy-atom

method which revealed the position of the Br atom, and the remaining atoms of **5a** were located using DIRDIF[52] and in succeeding difference Fourier syntheses. For all structures, hydrogen atoms were located and added to the structure factor calculations but not refined. The structures were refined in full-matrix least-squares where the function minimized was $\Sigma w(|F_o| - |F_c|)^2$ and the weight w is defined by the Killean and Lawrence method with terms of 0.020 and 1.0.[53] Scattering factors were taken from Cromer and Waber.[54] Anomalous dispersion effects were included in F_c,[55] and the values of f' and f'' were those of Cromer.[56] All calculations were performed on a VAX computer, and refinement was done using MolEN.[57]

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Supplementary Material Available: A listing of positional parameters, anisotropic temperature factor coefficients, bond distances and angles, torsion angles, and atomic multiplicities for compounds **3a**, **3b**, and **5a** (41 pages). Ordering information is given on any current masthead page.

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