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

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The (*R*)- and (*S*)-2-butylamides of *d*-lysergic acid were prepared and evaluated in behavioral and biochemical assays of 5-HT_2 agonist activity. In rats trained to discriminate 0.08 mg/kg LSD tartrate from saline, both isomers completely substituted for the training stimulus. Similarly, both isomers were found to possess very high affinity in displacing [^{125}I]-(*R*)-DOI ([^{125}I]-(*R*)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane) from rat cortical homogenate 5-HT_2 receptors and in displacing [^{3}H]-8-OH-DPAT ([^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetralin) from rat hippocampal 5-HT_{1A} receptors. The difference in activity between the two isomeric amides was significant in both the behavioral and binding assays, with the R isomer possessing greater potency. Molecular mechanics were used to predict the active geometries of the subject compounds. It was found that the (*R*)-2-butylamide has a conformation quite similar to LSD, while the (*S*)-2-butylamide does not. These results suggest that stereochemical properties of the amide substituent of hallucinogenic lysergamides may exert a critical influence on activity. It is concluded that the conformation of the amide function may directly affect binding through stereoselective interactions with a hydrophobic region on the receptor, indirectly by inducing conformational changes elsewhere in the molecule, or by a combination of these two mechanisms.

From studies relating structure and activity, it was noted more than 30 years ago that minor molecular modifications in ergot alkaloids produce surprisingly great changes in pharmacological effect.[1] Included among these compounds are various amides of lysergic acid. In the years following Hofmann's synthesis and discovery of the hallucinogenic properties of the *N*,*N*-diethylamide of *d*-lysergic acid (LSD, 1),[2] a large number of chemical modifications were investigated in structure–activity relationship (SAR) studies. As discussed previously,[3] the results of these early studies generally confirmed that hallucinogenic potency in the lysergamide series seemed to be optimized in 1. Although very few new compounds have been prepared in SAR studies of hallucinogenic lysergamides in recent years, evidence that 1 may *not* possess uniquely high potency has been reported. Derivatives in which various alkyl groups replaced the *N*(6)-methyl of 1 were identified that retained or even surpassed the potency of 1.[4-7] Of these compounds, the *N*(6)-*n*-propyl derivative was found to be equipotent to 1, while the *N*(6)-ethyl and -allyl derivatives were more potent than 1 in a rat behavioral model.[5]

In view of a renewed interest in hallucinogenic agents, related to the recent emphasis in the neurosciences on the role of serotonin in behavior,[8] further studies of 1 SAR using more up-to-date techniques were thought to be quite valuable. Previous work regarding stereochemical aspects of lysergamide pharmacology has demonstrated that inversion of the configurations of either of the two chiral centers (5*R*,8*R*) of 1 results in loss of activity. That is, the 5*S*,8*S*, 5*S*,8*R*, and 5*R*,8*S* isomers were inactive in man as

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hallucinogens.[9,10] This demonstrates the high degree of stereoselectivity in the ergoline ring. Very little is known, however, about possible stereochemical influences on the interaction between the amide moiety of hallucinogenic lysergamides and the biological target sites of these drug molecules.

The focus of this study was directed toward evaluating potential stereoselective effects of 1-like lysergamides containing a chiral *N*-alkyl substituent. The simplest of these are the *N*-2-butylamides of *d*-lysergic acid (2 and 3). These stereoisomers are novel compounds with a molecular weight identical to, and size very similar to the diethylamide (1). Although the mixture of 2 and 3 has previously been reported[11] for analytical purposes, the isomers had not been separated, nor had any biological data been reported for them. The pyrrolidyl amide (4) was also included in the behavioral pharmacology part of this study, since it represents an analogue of 1 with decreased potency in which the terminal methyl groups of the diethyl substituent are "connected" by an additional bond. Further, 4 has been studied clinically for hallucinogenic activity[12] and provided a point of comparison between the results from the bioassay employed here and those from human studies conducted previously.

Hallucinogenic activity in humans can be modeled in the drug discrimination (DD) paradigm by studying 1-like discriminative stimulus (DS) properties in animals.[13,14] In the typical procedure, rats are trained to press levers for positive reinforcement and to recognize the interoceptive state associated with the actions of a particular dose of 1 at a particular time after administration. The DS properties of 1 have been studied extensively and seem to be mediated critically by agonist activity at 5-HT_2 receptors.[13,15-18] Therefore, the DD paradigm was employed as a means of evaluating these chiral analogues in rats. In addition, receptor binding studies were employed as an in vitro measure of the 5-HT_2 and the 5-HT_{1A} receptor interactions for the two chiral lysergamides synthesized in this experiment, relative to 1. As in previous studies,[19,20] this was measured as affinity for [^{125}I]-(*R*)-DOI ([^{125}I]-(*R*)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane)-labeled 5-HT_2 receptors in rat cortical homogenates and as affinity for [^{3}H]-8-OH-DPAT ([^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetralin)-labeled 5-HT_{1A} receptors in rat hippocampal homogenates. Since [^{125}I]-(*R*)-DOI also binds with high affinity to 5-HT_{1C} receptors (see e.g. ref 8) it is possible that this receptor subtype is involved in hallucinogenesis. However, our focus has been on the more prevalent view that the 5-HT_2 subtype is more critical.

Chemistry

The syntheses involved the amide-forming condensation of the appropriate amines with *d*-lysergic acid. The classical methods for preparing amides by acylation of amines with esters or acid chlorides were known to fail when applied to lysergic acid.[21] The condensation was accomplished using the practical and efficient method of Johnson et al.[22] *d*-Lysergic acid and a 9-fold excess of amine were stirred under nitrogen in refluxing chloroform. Then $POCl_3$ was added dropwise over 5 min. This procedure was employed without difficulty with pyrrolidine in the synthesis of 4. The alternative procedure of Johnson et al.,[22] in which phosphorus oxychloride and the amine were added simultaneously to a chloroform suspension of lysergic acid at reflux, was employed for the more bulky 2-butylamines. After workup, the normal (8β) isomers were purified by centrifugal chromatography (chromatotron) as described previously.[5] The diastereomeric 2 and 3 could also be separated using the chromatotron. Data from analyses utilizing ^{1}H NMR, IR, and high-resolution mass spectrometry were all consistent with this structural assignment and agreed with relevant data previously reported for the unseparated mixture of 2 and 3.[11]

Pharmacology

Using methods described previously,[23] compounds 1, 2, 3, and 4 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, ip). Potencies were measured using ED_{50} values with 95% confidence intervals for those compounds that completely substituted for 1. The methods used in binding experiments have also been described in earlier reports.[7,20] Briefly, the ability of 1, 2, and 3 to displace 0.25 nM [^{125}I]-*R*-DOI from rat frontal cortex homogenate and 1.0 nM [^{3}H]-8-OH-DPAT from rat hippocampus homogenate was measured.

Molecular Modeling

Knowledge of the biologically active conformations of lysergic acid derivatives would clarify understanding of their hallucinogenic potencies and of the nature of the

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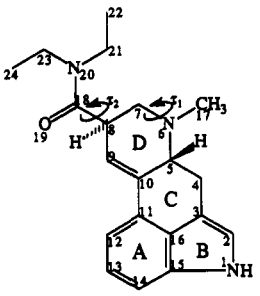
5-HT_2 receptor. Although lysergamides are relatively rigid, they do have conformational flexibility in two places, the amide substituent and the D ring. In particular, the D ring of 1, and of most related ergolenes, has been found to exist in the C(7) "flap-up" conformation in $CDCl_3$ solution[24-26] as well as in the crystal structure of 1 *p*-iodobenzoate monohydrate.[27,28] However, Pierri et al.[29] have reported that ergotamine exists in the C(7) "flap-down" conformation. The position of the amide substituent is variable and considered fluxional within the bounds of hydrogen bonding and steric influences.



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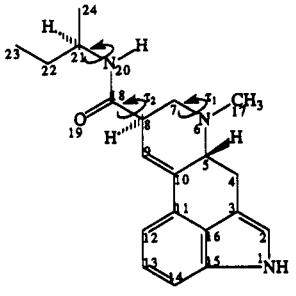

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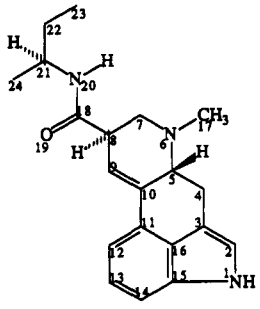

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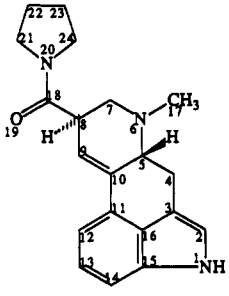

4

Classical molecular mechanics were performed on 1, 2, and 3. The molecular flexibility of the lysergamides is principally described by two dihedral angles, identified in structure 1. In addition, the 2-butyllysergamides, 2 and 3, exhibit a third important torsion angle, identified in structure 2.

$$\tau_1 = \text{C(5)–N(6)–C(7)–C(8)}$$

$$\tau_2 = \text{C(7)–C(8)–C(18)–O(19)}$$

$$\tau_3 = \text{C(18)–N(20)–C(21)–C(22)}$$

As such, τ_1 describes the envelope conformation of the D ring, with positive values of τ_1 corresponding to flap-down conformations and negative values of τ_1 corresponding to flap-up conformations. The angle τ_2 describes the position of the amide carbonyl group relative to the D ring, with values of τ_2 in the approximate range -90° to 90° corresponding to the amide carbonyl pointing above the D ring (β) and values of τ_2 in the approximate ranges -90° to -180° and 90° to 180° corresponding to the carbonyl pointing below the D ring (α). The angle τ_3 measures the position of the amide alkyl group relative to the carbonyl. All three dihedral angles were investigated by performing geometry searches, or grid scans, with minimization at each step. The structures as drawn illustrate the compounds with $\tau_1 \approx 65°$, $\tau_2 \approx -90°$, and $\tau_3 \approx 0°$.

Results and Discussion

The results of the drug discrimination testing are given in Table I. The two 2-butylamides, 2 and 3, and the pyrrolidide, 4, completely substituted for 1. All eight rats tested with saline selected the saline lever. Based on the 95% confidence intervals, 1 and 2 were of comparable potency, which was significantly greater than the potencies of 3 and 4. The latter two compounds did not have significantly different potencies in substituting for 1. In terms of disruptive effects, none of the test compounds produced large numbers of disruptions at stimulus-generalization doses. The compound which produced the highest degree of disruptive effects was 4.

The results suggest that stereochemical differences in the amide substituent of lysergamides may have a significant influence on hallucinogenic activity, as reflected in the DD model. The relative potency of 4 (16% of 1) is in agreement with clinical evaluations (5–10% of 1),[12,30] thereby lending support to the validity of this model. It is particularly noteworthy that the (*R*)-2-butyl group of 2 represents the first amide substituent to give a lysergamide with potency comparable to that of 1. This is the first indication that the diethylamide may not in fact be "unique". In addition, the sensitivity of activity to the amide substituent is not merely related to the size or physicochemical properties of this portion of the molecule. Since the two 2-butylamide isomers differ only in the spatial arrangement of identical atoms, amide conformational properties are likely of significance in optimizing interactive forces at the receptor(s) involved in 1-like actions.

This is partially supported by the binding affinities for the test compounds using [^{125}I]-(*R*)-DOI and [^{3}H]-8-OH-DPAT as the radioligands (Tables II and III). Both 2 and 3 have high affinity for the 5-HT_2 and 5-HT_{1A} sites. It is noteworthy that the difference observed in the potency of the 1-like DS properties of these compounds is mirrored by their significantly different affinities for [^{125}I]-(*R*)-DOI-labeled 5-HT_2 binding sites and their affinities for [^{3}H]-8-OH-DPAT-labeled sites. In both cases the (*R*)-2-butyllysergamide (2) had higher affinity than the (*S*)-2-butyllysergamide (3). However, the higher affinity of 2 compared to 1 at both sites was not reflected in the behavioral potency, where 2 and 1 were approximately equipotent, while 3 was significantly less potent than either drug. The reasons for this are not clear, although this may indicate that factors other than 5-HT_2 and 5-HT_{1A} interactions are involved. For instance, it is possible that, compared to the hallucinogenic phenethylamines, lysergamides may produce DS effects that involve important contributions from activity at receptors other than the 5-HT_{1A} and/or 5-HT_2 subtypes. Alternatively, if 1-like drugs are acting as partial agonists at 5-HT receptors (e.g.,

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Table I. Potency of Lysergic Acid Amides in 1-Trained Rats

compd	dose (nmol/kg)	n^a	D^b	%SDL[c]	ED_{50} (nmol/kg)	(95% CI)	potency (relative to LSD)
LSD, 1	6	10	0	0	27.5	(20–39)	1.00
	23	11	0	36			
	47	9	0	78			
	93	9	0	100			
2	12	8	0	13	33	(17–66)	0.82
	23	8	0	50			
	47	8	0	75			
	93	8	0	75			
	186	8	0	88			
3	47	9	1	13	124	(74–209)	0.22
	93	8	0	50			
	186	11	3	75			
	290	10	1	67			
	372	8	0	75			
	465	10	1	100			
4	47	9	0	22	168	(109–258)	0.16
	93	10	1	22			
	139	12	1	27			
	163	11	1	40			
	186	16	1	60			
	279	13	4	67			
	301	16	5	82			
saline	-	8	0	0			

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

Table II. Results of 5-HT_2 Binding Studies with Lysergic Acid Amides

drug	K_i (nM)	Hill coeff	$\Delta G°$ (kcal/mol)
LSD, 1	6.31 ± 0.13	1.18 ± 0.08	-11.76
2	2.63 ± 0.38	1.18 ± 0.04	-12.19
3	7.76 ± 0.24	1.02 ± 0.03	-11.53

Table III. Results of 5-HT_{1A} Binding Studies with Lysergic Acid Amides

drug	K_i (nM)	Hill coeff	$\Delta G°$ (kcal/mol)
LSD, 1	5.05 ± 0.38	1.05 ± 0.04	-11.79
2	2.01 ± 0.16	0.83 ± 0.05	-12.35
3	4.61 ± 0.33	1.03 ± 0.03	-11.84

see ref 31), it is possible that 1, 2, and 3 have different efficacies at the 5-HT_2 and 5-HT_{1A} receptors. The contribution of pharmacokinetic factors to the difference in in vivo potencies between 1 and 2 would also need to be assessed. Clearly, more work is needed to clarify the role of 5-HT receptors in the DD cue of 1 and its analogues.

With current knowledge, it is unclear whether 1 and its analogues bind to the receptor as the free base or as the N(6)-protonated species. From its pK_b,[27] it is certain that approximately 75% of 1 exists as the N(6)-protonated cation in biological fluids. Further, the N(6) proton can be seen as possibly important in the docking process. However, it is not known whether the proton is retained after docking. Therefore, we have modeled both the free bases and the N(6)-protonated cations of 1, 2, and 3 to verify that protonation has little effect on the conformations of the species.

The outcome of the geometry search of τ_1 for the free bases of 1, 2, and 3 is given in Figure 1. As anticipated, two minima were identified, corresponding to the C(7) flap-up (-65°) and the C(7) flap-down (+65°) conformations. Conformational energy increases toward 0° due to the strain of flattening of the D ring. The slight discontinuity observed at 0–10° is an artifact of N(6) inversion, moving the methyl from β-pseudoequatorial to α-pseudoequatorial.

(31) Glennon, R. A. Serotonin receptors: clinical implications. *Neurosci. Biobehav. Rev.* **1990**, *14*, 35–47.

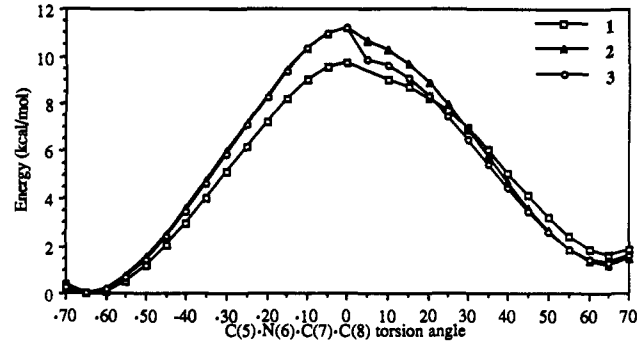

Figure 1. Energy of D-ring flap inversion (τ_1) for free base LSD (1), (*R*)-2-butyllysergamide (2), and (*S*)-2-butyllysergamide (3).

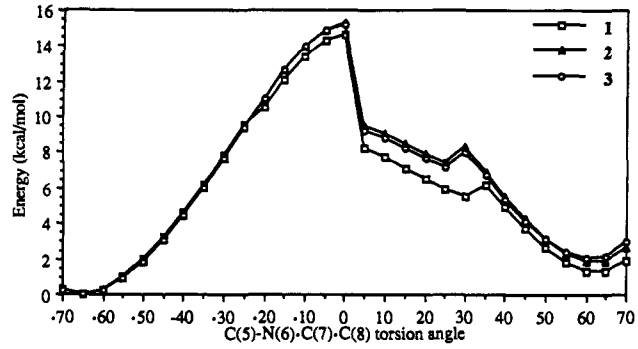

Figure 2. Energy of D-ring flap inversion (τ_1) for N(6)-protonated LSD (1), (*R*)-2-butyllysergamide (2), and (*S*)-2-butyllysergamide (3).

From the Boltzman distribution, $N_u/N_d = \exp(-(E_u - E_d)/RT)$, where N_u = the molar fraction of molecules in the flap-up conformation, N_d = the molar fraction of molecules in the flap-down conformation, and E_u and E_d are the energies of the conformations, respectively, 6.72% of free base LSD (1) molecules, 13.02% of free base (*R*)-2-butyllysergamide (2) molecules, and 10.61% of free base (*S*)-2-butyllysergamide (3) molecules at 37 °C are predicted to be in the flap-down conformation.

The outcome of the geometry search of τ_1 for the N-(6)-protonated (unsolvated) form of 1, 2, and 3 is given in

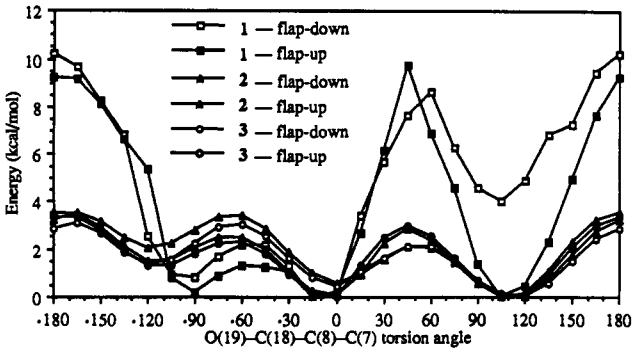

Figure 3. Energy of carbonyl torsion (τ_2) for LSD (1) and 2-butyllysergamide free bases 2 and 3.

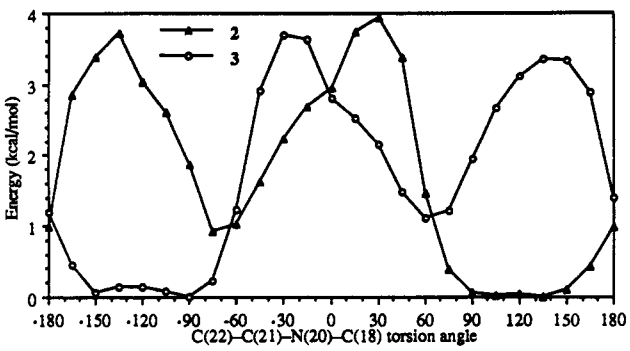

Figure 4. Energy of amide torsion (τ_3) for 2-butyllysergamide free bases 2 and 3 in the flap-up conformation.

Figure 2. Two minima occur, corresponding to the C(7) flap-up (−65°) and the C(7) flap-down (+60°) conformations. Conformational energy increases toward 0° due to the strain of flattening of the D ring. The discontinuity observed at 0–15° is an artifact of both N(6) and C(7) adopting "down" positions.

From $N_u/N_d = \exp(-(E_u - E_d)/RT)$, 10.72% of protonated LSD (1) molecules, 4.66% of protonated (*R*)-2-butyllysergamide (2) molecules, and 3.66% of protonated (*S*)-2-butyllysergamide (3) molecules at 37 °C are predicted to be in the flap-down conformation.

Geometry searches were also performed on the position of the amide functionality for 1 and the two 2-butyllysergamide isomers (2 and 3) in the flap-up and the flap-down conformations. The searches of τ_2, summarized in Figure 3, show two important features. First, 1 is consistently higher in energy than either of the 2-butyllysergamides. This is reasonable because 1 is a dialkylamide and, as such, one ethyl group is always in close proximity to the D ring, providing a nonbonding interaction. By contrast, the 2-butyllysergamides have an amide hydrogen which does not interact with the D ring as strongly as does the 1 ethyl group. Second, for all of the compounds, the preferred orientation of the amide group occurs at $\tau_2 \approx 0°$, although minima of comparable energy occur at $\tau_2 \approx 105°$. In the former case, the amide group extends away from the D ring and the carbonyl points toward the β-face. In the latter case, the amide group lies above the D ring and the carbonyl points toward the α-face.

Figure 4 shows the energy of rotation of the 2-butyl group in 2 and 3, τ_3 = C(18)–N(20)–C(21)–C(22). The flap-up free base conformation was used and the carbonyl position, τ_2, was initially set to its energy minimum, 0°. The (*R*)-2-butyllysergamide 2 prefers a conformation where the C(21) hydrogen is eclipsed with the carbonyl oxygen, while in 3 this hydrogen is anti to the carbonyl.

It is clear from the data that protonated forms of 1, 2, and 3 are lower in energy than the corresponding free base by 3–4 kcal/mol, with few excepting conformations. Further, the flap-up conformers are lower in energy than the corresponding flap-down conformer by 1–3 kcal/mol, also with few exceptions.

Assuming similar pharmacokinetics, the differences observed in the behavioral assay can probably be attributed to effects related to the drug–receptor interaction. Stereochemical changes in portions of a drug molecule which are involved in binding interactions are known to produce the greatest effect on potency.[32] The magnitude of the difference in potency between 2 and 3 is consistent with the idea that the amide substituent interacts with a sterically sensitive region of the receptor. The existence of such a hydrophobic region which can accommodate, in a predictable way, the substituents of the amide nitrogen has been suggested previously by Dunn and Bederka.[33] These workers also speculated that the conformational rigidity of cyclic amides may impair receptor contact. The results of the present study suggest that if a hydrophobic bonding site is involved, stereochemical aspects of the amide alkyl groups that interact with it exert an important influence on the drug–receptor interaction.

Alternatively, the alkyl group(s) of the amide substituent may indirectly affect binding by influencing the conformation of another portion of the molecule. For example, the orientation of the carbonyl oxygen of 1 is probably influenced by factors including the relative stability of the possible amide *N*-alkyl conformations. The various optimally stable positions adopted by the different alkyl groups in the lysergamide series may force the carbonyl oxygen to reside in slightly different orientations in each derivative. It is conceivable, therefore, that the diethylamide of 1 may help to orient the carbonyl oxygen in such a way as to optimize hydrogen-bond formation between its unshared electron pairs and a proton of one of the amino acids which comprise the receptor. This possibility has been discussed previously by Kumbar[34] in an empirically derived conformational analysis of 1 and several other lysergamides. It was found in that study that the directionality of the carbonyl oxygen, observed in 1, changed when the diethyl groups were substituted with other alkyl substituents. The angle of deviation from the preferred carbonyl conformation of 1 correlated with hallucinogenic potency, thus providing support for the importance of a hydrogen-bonding interaction involving the oxygen.[34] It is clear from our study, for example (Figure 3), that the carbonyl will resist orientations where τ_2 is near 45° or 180°.

However, from our modeling data it appears that the differences in potency and binding shown by these compounds are not directly due to conformational energy considerations. Stereoviews of the fully minimized structures of the free bases of 1, 2, and 3 (shown in Figure 5) indicate that all three possess virtually identical con-

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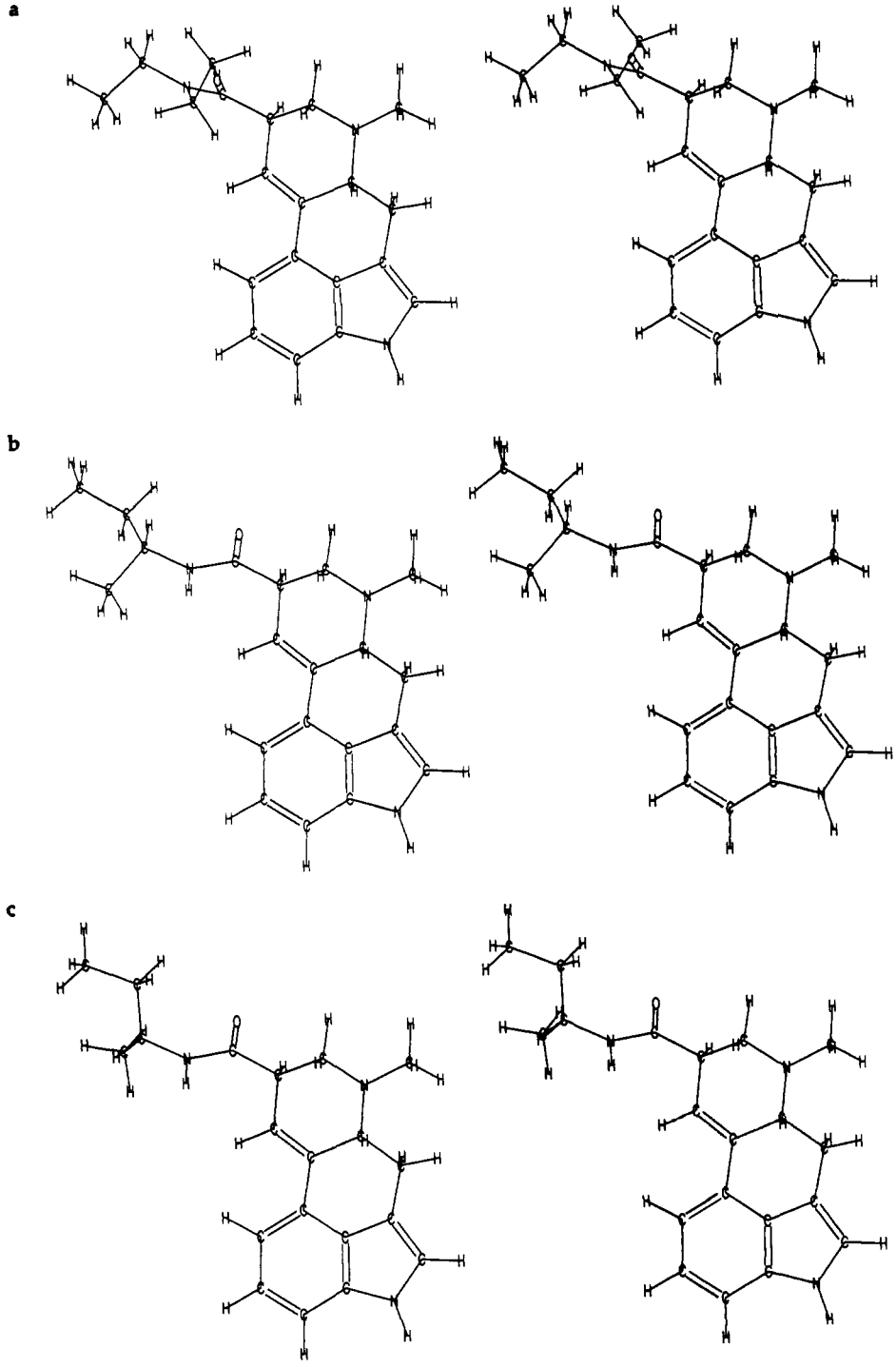

Figure 5. Stereoviews of fully minimized structures: (a) LSD (1), (b) (*R*)-2-butyllysergamide (2), and (c) (*S*)-2-butyllysergamide (3) as the free bases in the flap-up conformation.

formations, differing most in the positions of the amide alkyl groups. Figure 6 illustrates the most detailed comparison of the D ring and amide functionalities.

In view of the conformational similarities identified for 1, 2, and 3, it seems most probable that the differences in measured affinity are rooted in subtle steric effects. In terms of the portions of the 2-butyl group directed toward the β-face of the molecule (up in Figure 6), 2 has an ethyl extended up, similar to 1. By contrast, 3 has a propyl extended up. Therefore, the β-face of 2 seems similar to that of 1, while the β-face of 3 is considerably different. This may suggest that neither the 5-HT_2 nor the 5-HT_{1A} receptor can optimally accommodate a group larger than ethyl on the β-side of the molecule.

In addition, the role of the lone pair electrons of the tertiary nitrogen atom in the ergoline D ring has been discussed extensively with respect to binding forces of the drug–receptor interaction.[24,35-38] Variations of the amide

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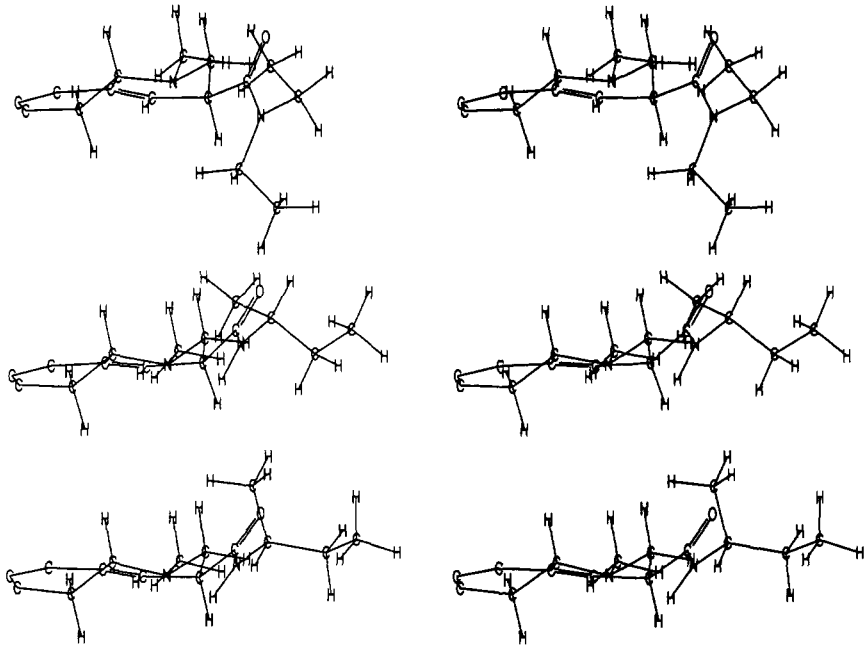

Figure 6. Stereoviews of the fully minimized D ring and amide functionality of LSD (1, top), (*R*)-2-butyllysergamide (2, center), and (*S*)-2-butyllysergamide (3, bottom) as the free bases in the flap-up conformation.

alkyl substituent may also affect the conformation of the D ring and consequently the directionality of the nitrogen lone pair electrons. In fact, the rigidity of 1 tends to increase the importance of conformational properties of the amide as influential in the determination of the shape of the molecule as a whole.

In conclusion, the present study has demonstrated that stereochemical properties of the amide substituent of lysergamides exert a significant influence on potency in producing 1-like discriminative stimulus effects in rats. Since this behavioral paradigm seems to provide a reasonably good model for human hallucinogenic activity, a similar influence on the latter may also exist. Receptor affinities are also sensitive to stereochemical effects of the amide substituent. The conformation of the amide function may directly affect binding through stereoselective interactions with a hydrophobic region on the receptor, or indirectly by inducing conformational changes elsewhere in the molecule.

Experimental Section

Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. ^{1}H NMR (200 MHz) were obtained in $CDCl_3$ with a Chemagnetics A-200 spectrometer. Chemical shifts are reported in δ values (parts per million) relative to an internal reference of tetramethylsilane (δ 0). Abbreviations used in NMR analysis are as follows: br s = broad singlet, s = singlet, d = doublet, t = triplet, m = multiplet. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN, and were within ±0.4% of the calculated values.

***N*-((*R*)-1-Methylpropyl)-9,10-didehydro-6-methylergoline-8β-carboxamide (2).** (+)-Lysergic acid monohydrate (150 mg, 0.52 mmol) and 25 mL of dry, ethanol-free $CHCl_3$ were placed in a flame-dried 50 mL, three-necked, round-bottom flask equipped with a condenser, N_2 line, and septa inlets. The stirred slurry was brought to reflux in a preheated 90 °C oil bath after which 384 mg (5.2 mmol) of (*R*)-(−)-2-butylamine (Aldrich) in 1.0 mL of $CHCl_3$ and 160 mg (1.04 mmol) of $POCl_3$ were added simultaneously, via syringe, over 3 min. The mixture was allowed to stir at reflux for an additional 5 min and was then cooled to room temperature. The clear amber $CHCl_3$ solution was then washed with 1 M NH_4OH (3 × 30 mL) and brine (1 × 10 mL) and dried (Na_2SO_4). The drying agent was removed by filtration, and the solution was concentrated in the dark by rotary evaporation at 30 °C.

The residue was purified and fractionated by radial centrifugal chromatography (Chromatotron, Harrison Research) using a silica gel rotor and eluting with ethyl acetate in an N_2-ammonia atmosphere. TLC (silica gel, EtOAc–NH_3) showed a large blue fluorescent product spot at R_f 0.21 corresponding to the (*R*)-2-butyllysergamide and a much smaller spot at R_f 0.15 corresponding to the S isomer. The faster moving component was collected and concentrated by rotary evaporation. The residue was taken up into CH_2Cl_2, washed with H_2O, and dried ($MgSO_4$), and the CH_2Cl_2 was removed by rotary evaporation followed by pumping under high vacuum. The free base (158 mg, 94% yield) was taken up into 2 mL of methanol, and 57 mg of maleic acid in 0.75 mL of methanol was added. The maleate salt (1:1 stoichiometry) spontaneously crystallized as a white crystalline solid: mp 210 °C dec; ^{1}H NMR (free base, $CDCl_3$) δ 0.91 (t, J = 7.5 Hz, 3 H), 1.13 (d, J = 6.7 Hz, 3 H), 1.36–1.56 (m, 2 H), 2.60 (s, 3 H), 2.68–2.82 (m, 2 H), 3.05–3.13 (m, 1 H), 3.31–3.56 (m, 3 H), 3.89–3.98 (m, 1 H), 6.45 (s, 1 H), 6.60 (d, J = 8.0 Hz, 1 H), 6.91 (s, 1 H), 7.12–7.22 (m, 3 H), 8.15 (br s, 1 H); $[\alpha]_D$ = +48° (c = 0.1, H_2O); IR (free base, neat) 1650 cm^{-1} (C=O). Anal. ($C_{24}H_{29}N_3O_5$) C, H, N.

***N*-((*S*)-1-Methylpropyl)-9,10-didehydro-6-methylergoline-8β-carboxamide (3).** An exact replication of the above procedure using 150 mg of (+)-lysergic acid monohydrate and 384 mg of (*S*)-(+)-2-butylamine (Aldrich) gave 154 mg (91.7% yield) of the free base. The free base in methanol was combined with 55 mg of maleic acid in methanol to again yield a white crystalline solid with 1:1 stoichiometry: mp 213 °C dec; ^{1}H NMR (free base, $CDCl_3$) δ 0.93 (t, J = 7.5 Hz, 3 H), 1.13 (d, J = 6.7 Hz, 3 H), 1.37–1.58 (m, 2 H), 2.60 (s, 3 H), 2.66–2.82 (m, 2 H), 3.05–3.13 (m, 1 H), 3.31–3.56 (m, 3 H), 3.89–3.98 (m, 1 H), 6.45 (s, 1 H), 6.60 (d, J = 8.0 Hz, 1 H), 6.92 (s, 1 H), 7.12–7.23 (m, 3 H), 8.02 (br s, 1 H); $[\alpha]_D$ = +59° (c = 0.1, H_2O); IR (free base, neat) 1650 cm^{-1} (C=O). Anal. ($C_{24}H_{29}N_3O_5$) C, H, N.

Pharmacology Methods. Drug Discrimination Studies. Twenty male Sprague–Dawley rats (harlan Laboratories, Indianapolis, IN) weighing 200–240 g at the beginning of their use as experimental subjects were employed for this study. These rats had previously received drugs and behavioral training as part of our ongoing investigations.[39] Water was freely available in

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their individual home cages, and a rationed amount of supplemental feed (Purina Lab Blox) was made available after experimental sessions so as to maintain approximately 85% of the free-feeding weight. The temperature of the animal facility remained within the range of 22–24 °C. The humidity was maintained at 40–50% and the lights were on between 6 am and 8 pm.

Apparatus. Six standard operant chambers (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, interfaced through a Coulbourn Instruments Dynaport to an IBM PC, controlled reinforcement and data acquisition with locally written software.

Drug Administration. The training drug, 1 (*d*-LSD) tartrate (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 of body weight was used. Solutions were sterilized prior to use by filtration through a sterile 0.2 μm filter (Millipore) into an autoclaved vial.

Discrimination Training. 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 FT 1 schedule so that one food pellet was dispensed for each press. Half the rats were trained on drug-L, saline-R, and the other half drug-R, saline-L, 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.[40] 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 FR 50, training continued until an accuracy of at least 85% (number of correct presses × 100/number of total 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 training sessions following a test session.[15] Test sessions were run under conditions of extinction, with rats removed from the operant box when 50 presses were emitted on one 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[41] 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 Methods. Radioligand Binding Studies. [^{125}I]-(*R*)-DOI was synthesized by the procedure of Mathis et al.[42] at a specific activity of 2000 Ci/mmol. [^{3}H]-8-OH-DPAT was purchased from New England Nuclear (Boston, MA) at a specific activity of 169.9 Ci/mmol. The procedure of Johnson et al.[7] was employed with minor modifications. 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 36500*g* 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 separate experiment, a tissue aliquot was thawed slowly and diluted 1 to 25 with 50 mM Tris HCl (pH 7.4). The homogenate was then incubated at 37 °C for 10 min and centrifuged twice at 36500*g* 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% Na ascorbate, and 10 μM pargyline HCl (pH 7.4). In experiments with [^{125}I]-(*R*)-DOI, 10 mM $MgCl_2$ 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. Displacement and saturation experiments were conducted with [^{125}I]-(*R*)-DOI as described in Nichols et al.[39] Tubes were allowed to equilibrate for 15 min at 37 °C before filtering through GF/C filters. Specific binding was defined as that displaceable with 1 μM cinanserin. Under these conditions [^{125}I]-(*R*)-DOI was found to bind to a single site (Hill coefficient of 0.99 ± 0.03) with a B_{max} of 46 ± 3 fmol/mg protein and a K_D of 1.34 ± 0.12 nM. The ability of the test drugs to displace 0.25 nM [^{125}I]-(*R*)-DOI was determined. Filters were allowed to air dry before counting with a gamma counter.

Displacement and saturation experiments were conducted with [^{3}H]-8-OH-DPAT according to the methods of Gozlan et al.[20] 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 2.49 ± 0.23 nM. The ability of the test drugs to displace 1.0 nM [^{3}H]-8-OH-DPAT was determined. Filters were air-dried and placed in vials, 10 mL of scintillation fluid was added (Budget Solv, Research Products International, Mount Prospect, IL), and the vials were allowed to set overnight before counting.

After counting at an efficiency of 79% for [^{125}I] and 34% for [^{3}H], binding parameters were determined using the computer programs EBDA and LIGAND as described elsewhere.[43] The values from three to four separate experiments were combined. Protein determinations were made using the procedure of Bradford.[44] Free energy of binding at 37 °C (310 K) was estimated from $\Delta G^\circ = -RT \ln (1/K_I)$.

Molecular Modeling. Preliminary computations were performed on a Silicon Graphics Personal Iris workstation running Polygen Quanta and CHARMM molecular mechanics software.[45,46] Further calculations were done using the programs CNINDO (Quantum Chemistry Program Exchange program 141) and AMPAC

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v1.21 (Quantum Chemistry Program Exchange program 506). Final computations were performed on a Tektronix CAChe worksystem running Tektronix proprietary software[47] including their implementations of ZINDO v2.2 and MOPAC v5.10.[48] CAChe molecular mechanics uses Allinger's MM2 force field[49] as augmented by Tektronix. With both AMPAC and MOPAC, the AM1 Hamiltonian[50] was used.

The molecular mechanics of 1, 2, and 3 were investigated on the CAChe system[51] by minimizing the total molecular energy according to the molecular mechanics expression

$$E_{total} = E_{bonding} + E_{\theta} + E_{\phi} + E_{improp} + E_{elec} + E_{vdW} + E_{hb}$$

where $E_{bonding}$ describes bond lengths, E_{θ} describes bond angles, E_{ϕ} describes dihedral angles, E_{improp} describes improper torsions, E_{elec} describes electrostatic potential, E_{vdW} describes the van der Waals interactions, and E_{hb} describes hydrogen bonding. Further, molecular mechanics calculates energies relative to a hypothetical "perfect" geometry, rather than an absolute energy, and uses a temperature of 0 K. The structures cited here are those obtained from molecular mechanics, and the structure of LSD is in good agreement with the reported X-ray results.[27]

Calculations were performed on the free bases as well as on the N(6)-protonated cations, in vacuo, and no attempt was made to consider the effect of solvation. In addition, conformations where the N(6)-methyl was equatorial were of lower energy than those in which the methyl was axial. Therefore, only the equatorial conformations were used in the analyses.

As stated above, the molecular flexibility of 1, 2, and 3, is described primarily by the torsion angles τ_1, τ_2, and τ_3. All three dihedral angles were investigated on the CAChe system by performing geometry searches, or grid scans, with minimization at each step. In these searches, the molecular mechanics settings were as stated above. The angle τ_1 was searched over the ranges -70° to 0° and +70° to 0°, in 5-deg increments. This angle was scanned from both directions in order to better control the inversion of N(6) and to maintain the equatorial position of the N(6)-methyl. Similarly, τ_2 and τ_3 were searched over the range -180° to +180°, in 15-deg increments.

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Registry No. 2, 137765-82-3; 2·maleate, 137765-83-4; 3, 137765-84-5; (+)-lysergic acid, 82-58-6; (*R*)-(−)-2-butylamine, 13250-12-9; (*S*)-(+)-2-butylamine, 513-49-5.

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(48) MOPAC v5.10 settings: optimization by the AM1 Hamiltonian; singlet multiplicity; CI level = default; maximum SCF cycles = 200; BFGS converger, XYZ coordinates. ZINDO v2.2 settings: energy only; singlet multiplicity; maximum SCF cycles = 200; INDO/1; SCF type = RHF.
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The Three Binding Domain Model of Adenosine Receptors: Molecular Modeling Aspects

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Using molecular modeling, adenosine receptor ligands were fitted together to maximize correlations between the three most important factors controlling binding to the receptor, namely steric, hydrophobic, and electrostatic complimentarity. Structure–activity relationships can be explained by three binding domains on the receptors. These are hydrophobic, aromatic, and ribose binding domains. We propose that the N^6, C2, and C8 hydrophobic binding domains are not discreet but occupy the same region of the receptor.

Adenosine (1) is a naturally occurring endogenous nucleoside which has generated much interest due to its biological activity. Much of this activity is mediated via membrane-bound extracellular receptors which bind adenosine, its analogues, alkylxanthines, and various miscellaneous heterocycles.[1-3] Pharmacologically distinct receptors which inhibit (A_1) or stimulate (A_2) adenylate cyclase activity have been identified on the basis of differing structure–activity profiles. Interpretations of structure–activity relationships, based on the structure of (*R*)-(phenylisopropyl)adenosine and other N^6-substituted analogues, have resulted in details of the N^6 binding domain. The four atoms of the alkylamine moiety of (*R*)-(phenylisopropyl)adenosine lie in a Y-shaped groove, there is a site to fit the phenyl ring and in close proximity, capacious binding domains called S1 and S1-A based on evidence for little stereoselectivity and the ability to bind three carbons in this area.[4] There is a potential hydrogen-bond acceptor next to this N^6-subregion.[4] Novel N^6-bicyclo[2.2.1]alkyladenosines with unusually high potency allowed the receptor-excluded volume to be probed in considerable detail.[5] The sophistication of the model was increased using computer molecular modeling.[6] A

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