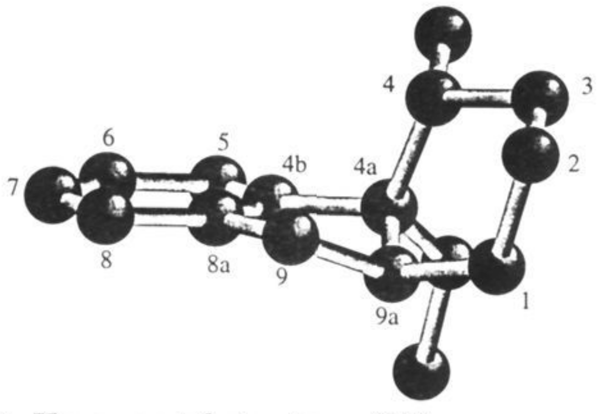


Figure 3. X-ray crystal structure of **13c**.

on noncompetitive NMDA antagonists (Figure 4; see the Experimental Section for details).[38-41] The modeled version of **13c** was designated $\mathbf{13c}_{\text{model}}$. For comparison with the modeled version, the crystal structure of **13c** was input, minimized, and designated $\mathbf{13c}_{\text{xray}}$.

In Figure 5 is shown a comparison between $\mathbf{13c}_{\text{model}}$ (red) and $\mathbf{13c}_{\text{xray}}$ (green). Similarities include the cis ring fusion, chair conformation of the cyclohexyl ring, cis arrangement of the methyl and methylamino substituents, and *RS* stereochemical configuration of the ring fusion atoms. The major difference is the pucker of the 5-membered "B" ring, with $\mathbf{13c}_{\text{model}}$ puckered up and $\mathbf{13c}_{\text{xray}}$ down. As a result of this down pucker in the crystal structure, the cyclohexyl C ring is now projected down toward the receptor interaction atom [N] location, where it would be expected to project steric hindrance (Figure 5).

Both MAXIMIN[42] and AM-1[43] were employed to investigate the energy differences between the two B ring puckered versions of **13c**. Using MAXIMIN (no electrostatic term used), a significant energy difference of 7.6 kcal/mol was found between $\mathbf{13c}_{\text{model}}$ and $\mathbf{13c}_{\text{xray}}$, with $\mathbf{13c}_{\text{xray}}$ being more stable. Roughly the same difference (6.8 kcal) in calculated heats of formation resulted when AM-1 was used with full geometry optimization. Such differences are likely to hamper interconversion of the two forms; gas-phase calculations predict that the "down-puckered" X-ray version is more stable.

To investigate whether the energy difference was due primarily to steric interactions of the C-ring methyl substituent, this group was removed from both versions and the minimizations were repeated on **1a**. The results show much smaller energy differences between the two forms, 1.7 kcal/mol and 3.8 kcal using MAXIMIN and AM-1 (full geometry optimization), respectively, with the "down puckered", X-ray version continuing to be more stable. These reduced energy differences relative to the methyl-substituted **13c** would not be expected to prevent interconversion of the two forms. Thus, a hydrogen bond interaction between a receptor atom and the basic amine within the unsubstituted analog **1a**, which would contribute roughly 4 kcal/mol in energy stabilization, could be expected to drive the structure into the "up puckered" B ring conformation, overcoming the 2–4 kcal/mol difference in energy of the two forms. The 7–8 kcal/mol difference in energy calculated for **13c** would be expected to hinder this conformational change. Indeed, the NMR analysis (vide infra) of the closely related unsubstituted compound **3** showed that the "up puckered" version predominates in solution.

Therefore, the reduced potency observed for **13c** relative to **1a** may not be due to volume intolerance of the methyl group itself, but rather a conformational change (downward pucker) induced in the ring system. This would result in a steric impact on the receptor site, as shown in Figure 5, when **13c** is fit to the pharmacophore model.

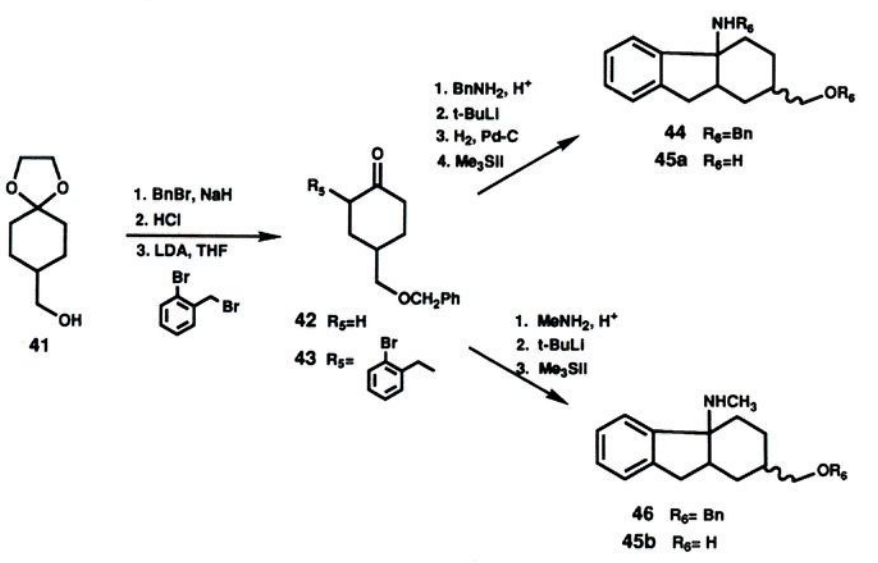
NMR Studies

To further investigate and compare the preferred solution conformations of **1a** and **13c**, several of the targeted compounds were investigated by NMR spectroscopy. In comparing the ^{1}H NMR spectra of **3** and **13c**, it was readily apparent that there were large differences in the appearance of the proton NMR spectra. In particular, compound **3**, which lacks a methyl group at the 4-position on the C ring, exhibited spectra with sharp lines, while **13c**, which contains a 4-methyl group, displayed spectra with broad lines. These results are consistent with a single low-energy conformation of the hexahydrofluorenamine ring system existing when there is no methyl group present on the aliphatic six-membered ring. Addition of the 4-methyl group cis to the amine disrupts the single low-energy state and is manifested in the proton NMR spectra as exchange broadening.

To further understand the conformational preferences of the hexahydrofluorenamines, the solution conformation of **3** was determined by a detailed analysis of the vicinal proton coupling constants. The crucial region of the molecule that determines the 5-member ring pucker, and thereby how well the conformation fits that of **1a** from the molecular modeling study, is centered around carbon 9a and includes the protons H9, H9′, H9a, H1, and H1′. Because H9 and H9′ are only coupled to each other and to H9a, one-dimensional decoupling experiments allowed close initial estimates of $^2J_{\text{H9,H9}'}$, $^3J_{\text{H9,H9a}}$, and $^3J_{\text{H9}'\text{H9a}}$. These data were input into the LAOCOON[44] spectral simulation program along with the experimental multiplet frequencies for proton 1. The program was allowed to iterate until the simulated spectrum had an acceptable fit. A comparison of a region of the simulated and experimental spectrum is shown in Figure 6. A general Karplus relationship was applied to the four vicinal coupling constants to extract the corresponding dihedral angles. The calculated vicinal coupling constants and the corresponding dihedral angles from the LAOCOON calculations are shown in Table II. Because of the constraints of the fused ring system and the availability of two vicinal coupling constants for each bond, a single solution was obtained in which the B ring was puckered "up" at C9a and the amine substituent was directed away (Figure 7). The solution structure of **3** is thus very unlike the crystal structure of **13c** and is consistent with the previously described conformation for **1a** used in the modeling studies.[23]

Consistent with the modeling study, the NMR spectra further suggest that the C ring of **3** adopts a chair conformation in which the amine and the C9 are close to equatorial. In this conformation, a bulky group attached to C4 cis to the amine (such as the methyl group in **13c**) may be forced to be axial. However, from the modeling analysis, this would result in a steric impact on the putative receptor interaction atom. Thus, two or more higher energy conformations with different B or C ring puckers may exist in equilibrium, thereby causing the broadening observed in the proton NMR spectrum of **13c**. It is probable that the bioactive conformation suggested from the modeling studies exists transiently in solution and that this conformation may be adopted by **13c** upon binding to the receptor, at some cost in internal energy.

Scheme VI



Structure–Activity Relationships

Amine Configuration, Substitution, and Position. In agreement with previous studies[23] in which the enantiomers of the primary amine **2** were examined, the (+)-**1a** isomer (absolute configuration 4a*R*) has greater affinity in [^{3}H]TCP binding than the (−)-**1b** isomer (absolute configuration 4a*S*). In general, optimal potency was observed with *N*-methyl substitution (Table I, compounds **1a,b** and **13a–c**). *N*-Benzyl substitution (**12a,b,d,g**) markedly reduced affinity while NH_2 and NHEt analogs (**15c** and **16b**) had comparable affinity. This suggests a steric intolerance in this region of the receptor for large N-substituents and supports an optimal substitution pattern such that methylamine > primary amine ≈ ethylamine ≫ benzylamine. Placement of the amino functionality at the alternate bridgehead position (**20, 24a,** and **24b**) resulted in a complete loss of receptor affinity. Although such compounds can be fit to previously described pharmacophore models[12,45–47] of the PCP site within the NMDA receptor complex, the angle at which a hydrogen bond is made between the amine and the putative receptor atom is altered, falling in a region described[47] as being detrimental to receptor affinity. Addition of a methylene spacer between the amine and

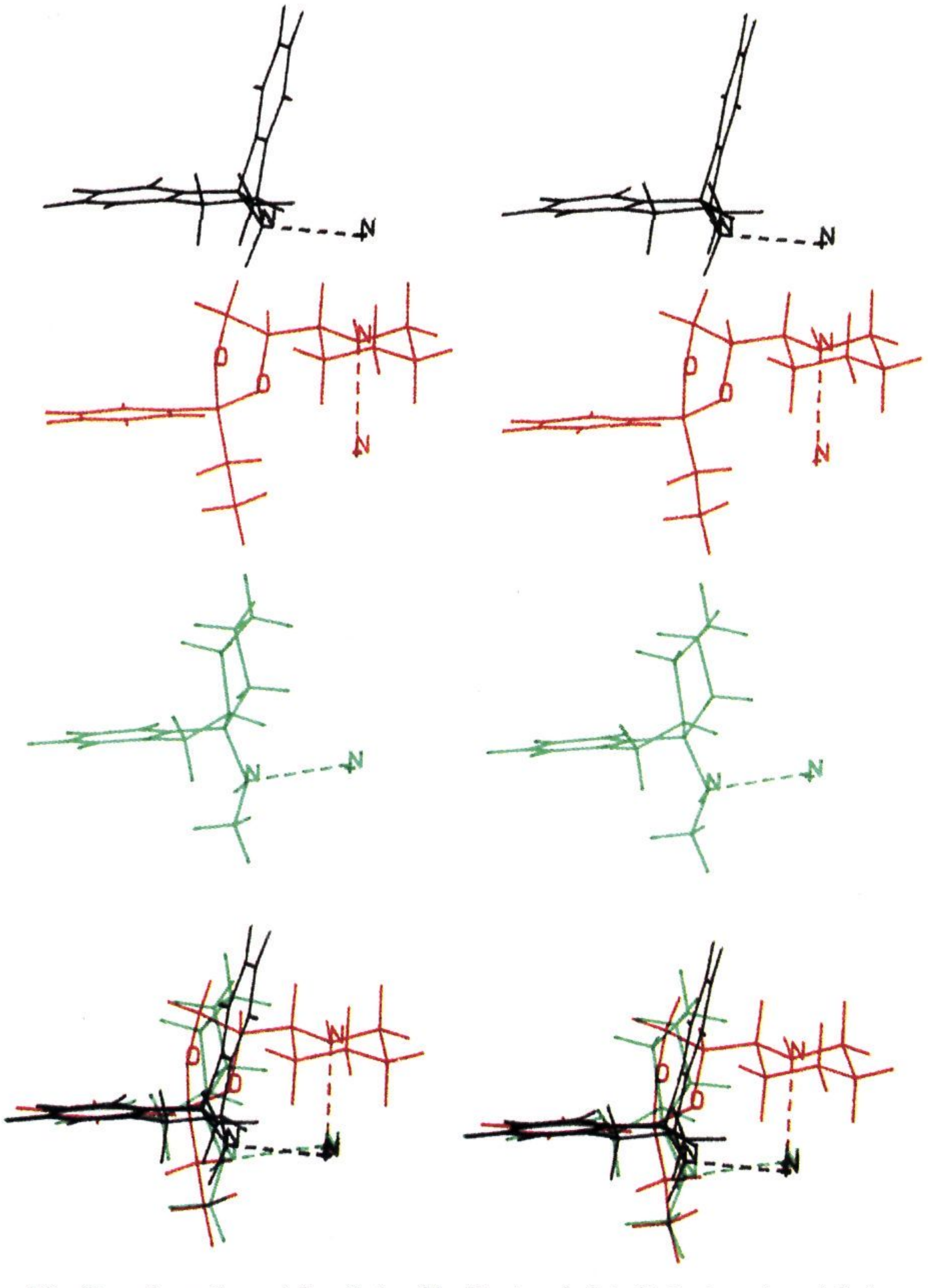


Figure 4. Stereoviews of the fit conformations of dizocilpine (black), etoxadrol (red), **1a** (green), and their superposition after fitting (bottom). A putative receptor site (N) atom used in the fitting process has been included.

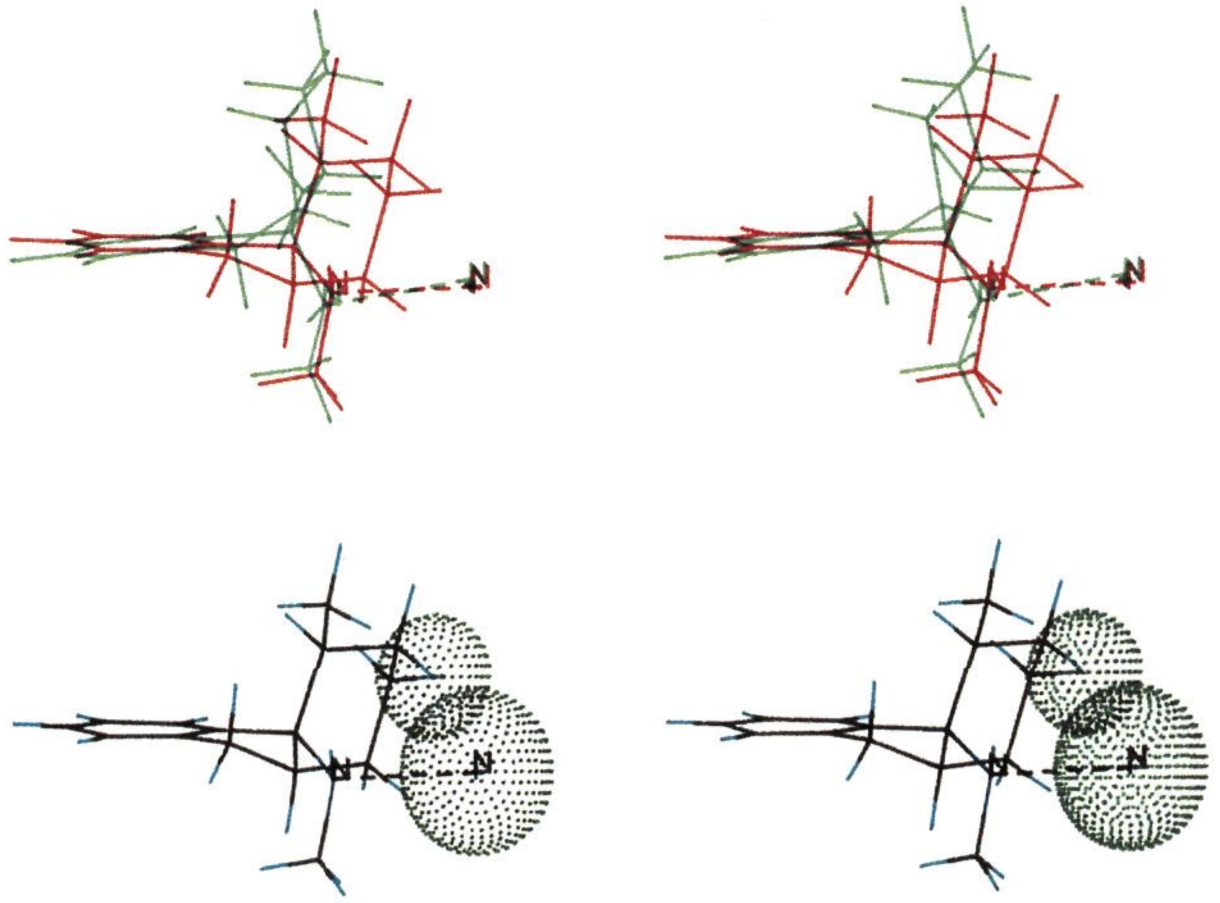


Figure 5. (Top) stereoview of the modeled (green) and X-ray (red) versions of **13c** (**13c**$_{\text{model}}$ and **13c**$_{\text{xray}}$, respectively). See the Experimental Section for details. (Bottom) **13c**$_{\text{xray}}$, color coded by atom type, with the putitive receptor site (N) atom used in the fitting process included. A steric contact between one of the cyclohexyl ring hydrogens and the receptor site atom is illustrated by the interpenetration of van der Waals dot surfaces.

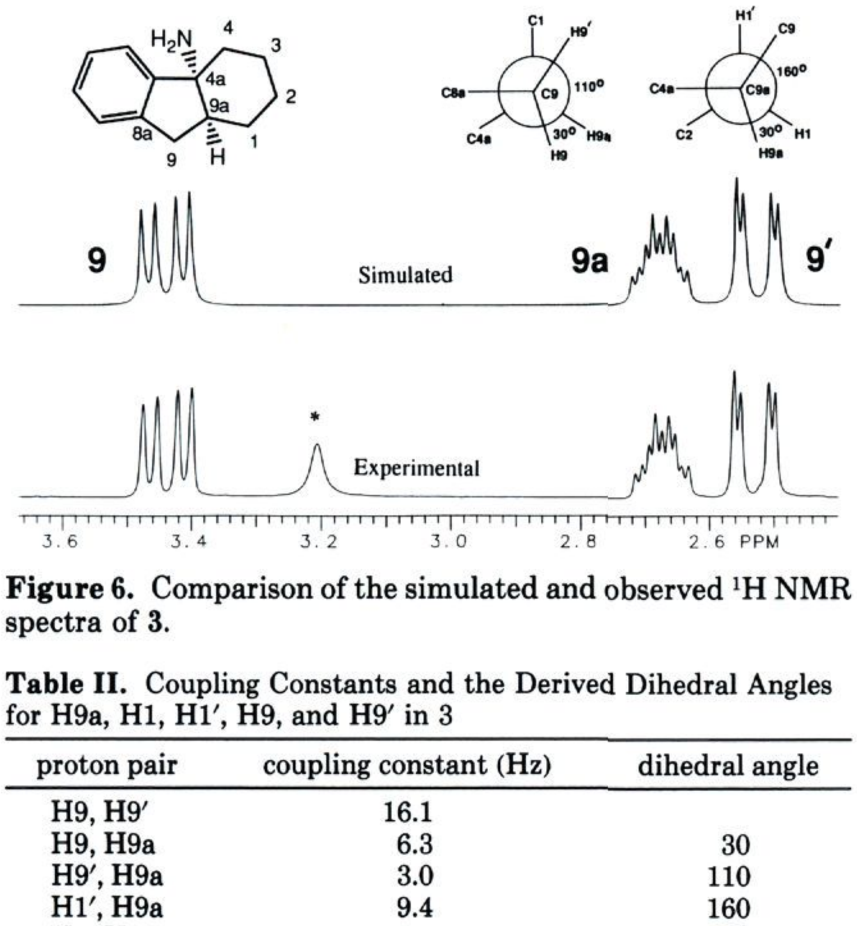


Figure 6. Comparison of the simulated and observed ^{1}H NMR spectra of **3**.

Table II. Coupling Constants and the Derived Dihedral Angles for H9a, H1, H1′, H9, and H9′ in **3**

proton pair	coupling constant (Hz)	dihedral angle
H9, H9′	16.1	
H9, H9a	6.3	30
H9′, H9a	3.0	110
H1′, H9a	9.4	160
H1, H9a	6.3	30
H1, H1′	16.0	

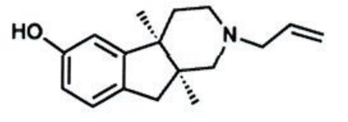
the ring system (**25a** and **25b**) did not restore binding affinity to these analogs.

Aromatic Substitution. 6-Methoxy and hydroxy substitution (**13g** and **13h**) provided a slight improvement in receptor binding affinity. This is in contrast to the SAR exhibited by phencyclidine analogs, where *m*-methoxy and hydroxyphenyl substitution improved binding affinity more than 5-fold over the unsubstituted parent.[48] Substitution of a methoxy group at the 7-position (**13f**) drastically reduced affinity at the NMDA receptor. Replacement of the benzo ring by thiophene also reduced potency somewhat (Table I, compare **13d** with **1**).

B and C Ring Modifications. Methylene substitution at the benzylic position of the B ring (**32a** and **32b**) resulted in a large reduction in binding affinity. Replacement of the benzylic methylene in the B-ring of **2** with heteroatoms resulted in compounds with equivalent (O-containing analog **16a**) or slightly improved (S-containing analog **16b**) affinity when compared to the racemic parent **1**.

Fluorenamine derivatives with a methyl group in the 2- or 4-position of the C ring retained significant binding when the amino group was methyl substituted (**13c** and **13e**). It was not clear why the analogous primary amine **14c** did not show similar binding affinity. Contraction of the cyclohexyl C ring to cyclopentyl (**13a**) or expansion to cycloheptyl (**13b**) reduced affinity, supporting an ideal ring size of six carbons for high affinity. The cyclopentyl ring may be inadequately filling the "upper lipophilic cleft" in the PCP pharmacophore,[45] while the cycloheptyl analog may be consuming receptor-excluded volume. The marked reduction in affinity of the C ring cyclopentyl, NH_2 analog **14a** may also be due to its low log P (Table I).

Comparison of the noncompetitive antagonist etoxadrol to other active compounds by overlapping the aromatic rings suggests that the nitrogen atom of etoxadrol approaches the NMDA noncompetitive receptor from the top face of the receptor binding site (Figures 4 and 8).[46] Additional evidence for receptor interaction from this direction comes from the relatively rigid hexahydroindeno[1,2-*c*]pyridine **47**,[49] a compound reported to have an IC_{50} of 62 nM versus [^{3}H]PCP (Figure 8). These unusual fits in the binding site model suggested that compounds might be designed to take advantage of this receptor's apparent ability to bind the heteroatom of ligands from more than



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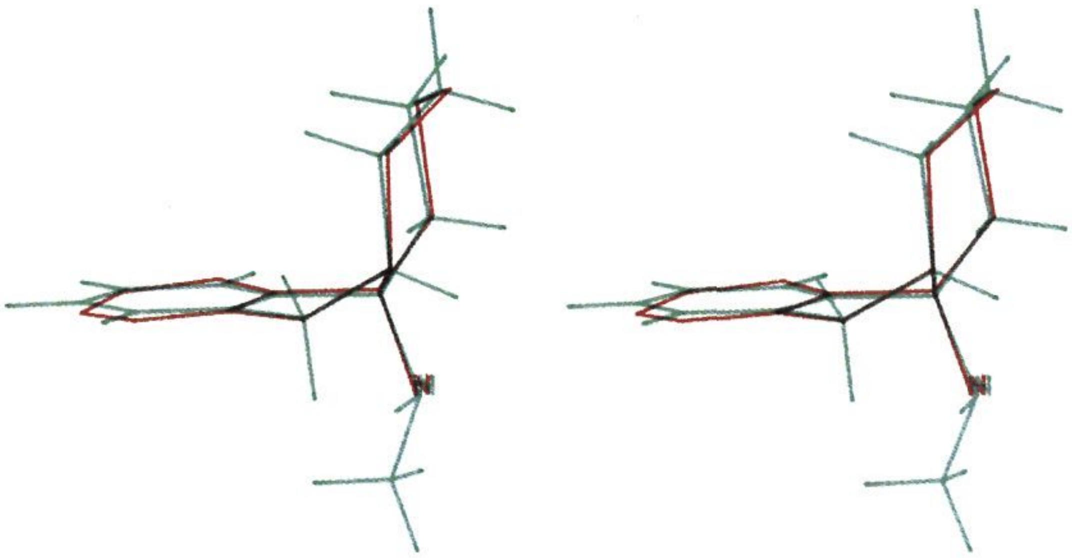

Figure 7. Comparison of the NMR-derived conformation of **3** (red, without hydrogens) and the modeled version of **1c** (green, with hydrogens added).

one direction. It was envisioned that an additional amino or hydroxyl group could hydrogen bond to the putative receptor atom from an analogous direction to that of the basic amine on etoxadrol while maintaining the interaction present in PCP. Molecular modeling studies suggested that 2-substitution of the C ring by $-CH_2X$ groups in the S configuration, where X was a hydrogen bonding group such as OH or NH_2, would present a group in the proper orientation for hydrogen bonding to the putative receptor atom, similar to what has been reported for etoxadrol[12] (Figure 8). In addition, the high receptor affinity retained *by the 2-Me analog* **13e** *in the present hexahydrofluorenamine* series suggested that there was steric tolerance in this region of the receptor.

The resulting 2-substituted analogs (**40a,b** and **45a,b**) all displayed reduced affinity relative to the parent **1** (or **2**). This may be due to a number of factors. Aside from increased entropy due to the introduction of a flexible 2-substituent, the reduced log *P* exhibited by **40a** and **40b** at pH 7.4 (Table I), due to protonation of the amine side chain, may be adversely affecting binding. All 2-substituted analogs were tested as a mixture of four stereoisomers (racemic at the 2 and 4a positions); resolution would certainly result in an increase in affinity. The debenzylation of the *O*-benzyl ester for **45b** involved the use of trimethylsilyl iodide, and the resulting HI salt was quite tightly complexed (see the Experimental Section). This HI complex may be adversely affecting the binding of this analog.

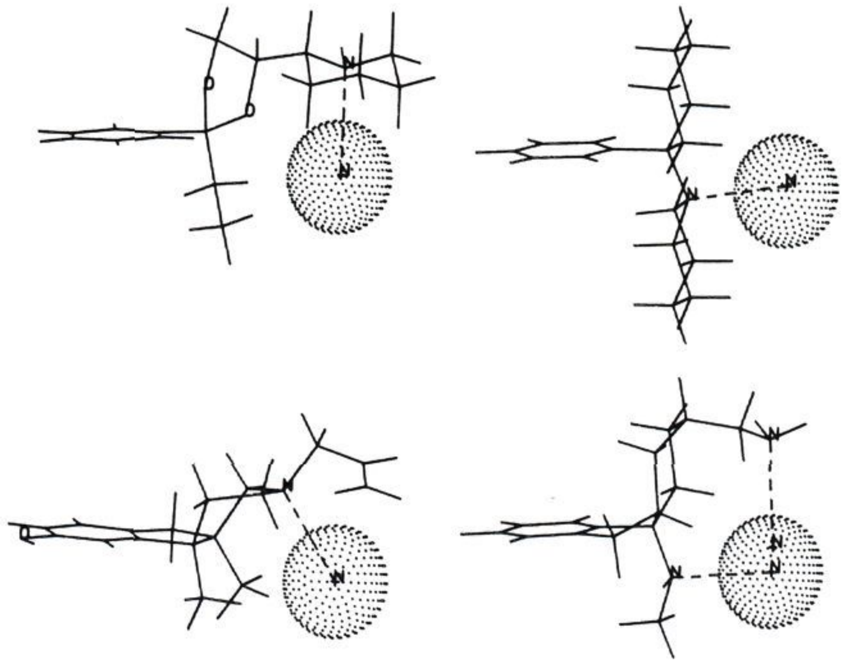

Figure 8. The noncompetitive antagonists etoxadrol (top left), PCP (top right), the hexahydroindeno[1,2-*c*]pyridine **47** (bottom left), and **40b** (bottom right), as viewed edge-on to the common phenyl rings. A hypothetical receptor atom has been added (a nitrogen surrounded by dots), 2.8 Å distant from the basic amines, along the lone pair direction (dotted lines) indicating a hydrogen bonding interaction in common to these antagonists.

Conclusions

Consistent with literature reports that describe a tight SAR for other classes of noncompetitive NMDA antagonists, the present series of hexahydrofluorenamines demonstrated a wide range of affinities with fairly subtle changes in substitution pattern. In line with previous studies,[23] the (+)-enantiomer **1a** was approximately 10-fold more potent than the (–)-isomer **1b**. There appears to be a size-limiting pocket near the basic amine that tolerates up to a benzyl group. Interestingly, heteroatom substitution in the central "B" ring was tolerated, with slightly decreased and increased affinities observed with oxygen and sulfur insertion, respectively (**16a** and **16b**). Similar to the PCP SAR, improved potency was seen with 6-hydroxy substitution (**13h**), while 7-substitution abolished activity (**13f**). These positions correspond to the meta and para positions, respectively, of PCP when the two structures are superimposed (Figures 4 and 8). However, the magnitude of the potency increase resulting from *m*-OH substitution was less than that reported for PCP. Reduced lipophilicity may be playing a role in reducing the affinity of **13h** (expected log *P* of 0–0.4 based on the measured values for the unsubstituted parent **1** and the methoxy-substituted **13g**; see Table I).

Consistent with the strict structural requirements reported to be present at this receptor, certain alterations in the directionality of hydrogen bonding between the basic amine and a putative receptor atom (see **20**, **24**, and **25**; Table I) markedly reduced affinity. Analogues containing C-ring substituents designed to interact with a receptor site from two directions were generally less potent. However, a number of other mitigating factors were also present in these analogs. The reduced affinity observed for **40a** and **b** was likely due to their low log *P* values. Very tight complexation of iodine by **45b** is likely preventing this compound from tight receptor binding. Compound **45a**, tested as a mixture of four isomers, nonetheless retains significant affinity for the receptor site (253 nM). It remains to test this theory by the preparation of additional analogs and/or the separation of isomers in the case of **45**.

As mentioned previously, log *P* also appears to play a role in determining affinity to the PCP site, a relationship