Synthesis and Binding Properties of MK-801 Isothiocyanates; (+)-3-Isothiocyanato-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine Hydrochloride: A New, Potent and Selective Electrophilic Affinity Ligand for the NMDA Receptor-Coupled Phencyclidine Binding Site

Joannes T. M. Linders,† James A. Monn,‡ Mariena V. Mattson, Clifford George,§ Arthur E. Jacobson, and Kenner C. Rice*

Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, and Naval Research Laboratory, Washington, District of Columbia 20375

Received August 28, 1992

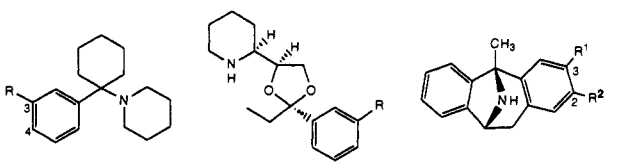
Three new site-directed irreversible (wash-resistant) ligands for the high-affinity phencyclidine (PCP) binding site associated with the *N*-methyl-D-aspartate (NMDA) receptor were synthesized and their binding characteristics were studied. (+)-3- And (+)-2-isothiocyanato-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine hydrochloride ((+)-**8a**,**b**·HCl) were prepared in four steps from the corresponding nitro derivatives (+)-**4a**,**b**, which were obtained by nitration of (+)-**3** (MK-801). In the same way the optical antipode (−)-**8a**·HCl was synthesized from (−)-**3**. At a concentration of 100 nM, the 3-isothiocyanate derivative (+)-**8a** irreversibly labeled approximately 50% of the (+)-[^{3}H]-**3** binding sites, compared to 20 μM needed for its optical antipode (−)-**8a** and the 2-isothiocyanate (+)-**8b**. The apparent K_i values for reversible inhibition of (+)-[^{3}H]-**3** binding by (+)- and (−)-**8a** and (+)-**8b** were 37, 838, and 843 nM, respectively. In contrast, metaphit (**1b**) and etoxadrol *m*-isothiocyanate (**2b**), two previously reported irreversible ligands for the PCP binding site, label about 50% of the (+)-[^{3}H]-**3** binding sites at 100 μM and 250 nM, respectively, with apparent K_i values for reversible inhibition of 535 and 94 nM. Compound (+)-**8a** is also a selective affinity ligand, displaying little or no irreversible in vitro affinity at 100 μM for opioid, benzodiazepine, muscarinic, and dopamine receptors. At a 25 μM concentration, (+)-**8a** caused an irreversible 52% reduction of binding to σ_1-receptors. Compound (+)-**8a** is the most potent known electrophilic affinity label for the PCP binding site. Its potency and selectivity should enable it to be a valuable tool for the elucidation of the structure and function of the NMDA receptor-associated PCP binding site in the mammalian central nervous system.

Introduction

The pharmacological role of the *N*-methyl-D-aspartate (NMDA) receptor in the mammalian central nervous system (CNS) has become the focus of an increasing amount of attention.[1-5] Activation of this receptor by endogenous excitatory amino acids such as L-glutamate and L-aspartate opens an ion channel allowing the influx of Ca^{2+} into the cell. During states of ischemia and hypoglycemia, the levels of aspartate and glutamate are elevated, causing overactivation of the NMDA receptor, resulting in neuronal degeneration and cell death.[6] The NMDA receptor may be involved as well in the pathogenesis of schizophrenia[7] and epilepsy,[8] and in neurodegenerative diseases such as Parkinson's,[9] Alzheimer's,[1,2] and Huntington's chorea.[1,2] In addition, the NMDA receptor has been shown to be essential for neuronal and behavioral plasticity, and hence has effects on learning and memory processes in the developing and adult brain.[10] The reported ability of dizocilpine ((+)-**3**, MK-801, Chart I) to inhibit morphine tolerance and dependence may be related to learning processes and neuronal plasticity.[11]

In recent years, a number of noncompetitive antagonists for the NMDA receptor have been found,[3,4] including the dissociative anesthetics PCP [*N*-(1-(phenylcyclohexyl)-piperidine, phencyclidine, **1a**] and ketamine [(±)-2-(2-chlorophenyl)-2-(methylamino)cyclohexanone], TCP [*N*-[1-(2-thienyl)cyclohexyl]piperidine], some of the 6,7-benzomorphans, substituted dioxolanes such as (4*S*,6*S*)-2,2-diphenyl-4-(2-piperidyl)-1,3-dioxolane (dexoxadrol) and (2*S*,4*S*,6*S*)-2-ethyl-2-phenyl-4-(2-piperidyl)-1,3-dioxolane (etoxadrol, **2a**) and the dibenzocycloheptenimine (+)-**3** (Chart I). These compounds show high affinity for a binding site inside the ion channel labeled by [^{3}H]PCP, [^{3}H]TCP, or (+)-[^{3}H]-**3** and, presumably, exhibit anticonvulsant and neuroprotective properties through blockade of the ion channel.[6] This binding site is now commonly referred to as the PCP site.[12] Recently, the cloning and sequencing of the NMDA receptor has been reported.[13]

Chart I



Electrophilic affinity ligands bearing the isothiocyanate moiety have proven to be extremely versatile tools in the study of a wide range of receptor systems.[14-16] The isothiocyanate group can be easily prepared from primary amines and is highly reactive toward amino-containing bionucleophiles and to a lesser extent sulfhydryl groups, while showing only low reactivity toward water and other

† Present address: Organon International bv, Postbus 20, 5340 BH Oss, The Netherlands.
‡ Present address: CNS Research, The Lilly Research Laboratories, Indianapolis, IN 46285.
§ Naval Research Laboratory.

hydroxyl functions.[14-16] In the past, our interest in delineating the structure and function of the PCP site has led to the development of two site-directed irreversible (wash-resistant) agents: **1b** [metaphit; 1-[1-(3-isothiocyanatophenyl)cyclohexyl]piperidine][17] and **2b** [etoxadrol *m*-isothiocyanate; (2*S*,4*S*,6*S*)-2-ethyl-2-(3-isothiocyanatophenyl)-4-(2-piperidyl)-1,3-dioxolane].[18] Although both **1b** and **2b** irreversibly interact with the high-affinity PCP binding site, they do so only at relatively high concentration [IC_{50} = 10 μM for **1b** (against [^{3}H]PCP) and 1 μM for **2b** (against [^{3}H]TCP)]. Furthermore, metaphit shows cross-affinity toward the dopamine reuptake site[19,20] and σ receptors.[21] Interestingly, a structural isomer of metaphit, fourphit [4-isothiocyanato-1-(phenylcyclohexyl)piperidine], irreversibly labels the dopamine reuptake site but binds reversibly to the PCP site.[17,22] Thiophit (1-[1-(4-isothiocyanatothienyl)cyclohexyl]piperidine), the thiophene analog of metaphit, and ethylphit [2-[1-(ethylamino)cyclohexyl]-3-isothiocyanatobenzene] were only marginally better than **1b**.[23] Recently, the preparation of an irreversible thienyl analog of the latter compound was reported,[24] having an apparent IC_{50} of 300 nM. It was shown to label up to 80% of the PCP binding sites in bovine brain at a 10 μM concentration.[24,25]

(+)-5-Methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine ((+)-**3**) is among the most potent and selective ligands yet described for this PCP binding site.[26-29] Its selectivity for the PCP site associated with the NMDA receptor over the dopamine reuptake site is especially noteworthy.[30,31] A site-directed electrophilic irreversible ligand based upon (+)-**3** might thus be expected to overcome the efficacy and selectivity problems associated with **1b** and, possibly, **2b**. Sonders et al.[32] reported the synthesis of the 3-azido derivative of (+)-**3**, which had a reversible binding potency equivalent to that of the parent compound. The azide in its [^{3}H]-labeled form was used for the photoaffinity labeling of the PCP site.[32]

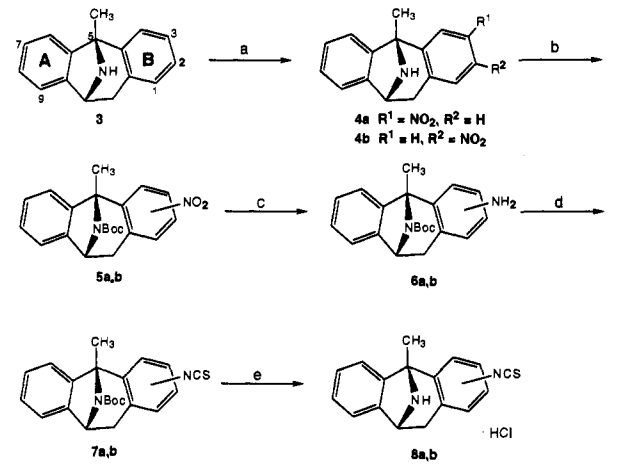
We now report the preparation of the 3- and 2-isothiocyanato analogs **8a,b**, derived from **3**, and their interaction with the NMDA receptor-associated PCP binding site. In order to determine its bioselectivity, the interaction of (+)-**8a** with other pharmacologically relevant CNS receptors was also studied.

Chemistry

The synthesis of the 3- and 2-isothiocyanates **8a,b**, respectively, is shown in Scheme I. Preparation of racemic **3** was accomplished according to the literature method of Lamanec et al.[33] Resolution was performed by recrystallization of the diastereomeric salts formed with di-*p*-toluoyl-L- or -D-tartaric acid.[34] To determine their enantiomeric purity, (+)- and (−)-**3** were derivatized with *N*-*tert*-butyloxycarbonyl-L-alanine in dimethylformamide in the presence of equivalent amounts of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole.[35] GC analysis of the resulting amides showed them to be >99% enantiomerically pure.

Nitration of (+)-**3** with fuming nitric acid in AcOH–H_2SO_4 (1:1) afforded a complex mixture. GC–EIMS analysis of this material demonstrated the presence of at least six mononitration and four dinitration products (integrated ratio of mononitro to dinitro products = 82:18). One of the mononitro compounds, the 3-nitro derivative (+)-**4a**, represented 50% of the total product mixture, and was conveniently isolated in 40% yield by crystallization as its oxalate salt. The structure of (+)-**4a**·oxalate was established unambiguously by X-ray crystallographic analysis (Figure 1; 5*S*,10*R*). One of the minor mononitro compounds was isolated from the mother liquor through crystallization of the fumarate salt. Single crystal X-ray analysis of the free base showed this compound to be the 2-nitro isomer (+)-**4b** (Figure 2; 5*S*,10*R*). Recently, Sonders et al.[32] reported the nitration of the *N*-acetyl derivative of (+)-**3** with ammonium nitrate and trifluoracetic anhydride. They obtained the 3-nitro compound only after repetitive preparative TLC followed by crystallization (no yield given). Their procedure also required a hydrazinolysis step to regenerate the secondary amine. Our procedure for the direct nitration of **3** with nitric acid in a mixture of acetic and sulfuric acid provides substantial

Scheme I. Synthesis of Isothiocyanates (+)- and (−)-**8a** and (+)-**8b**[a]



[a] Reagents: (a) HNO_3, H_2SO_4/AcOH; (b) $(Boc)_2O$, $NaHCO_3$, CH_2Cl_2 or $CHCl_3/H_2O$; (c) H_2, Pd/C, EtOH; (d) $CSCl_2$, $NaHCO_3$, $CHCl_3/H_2O$; (e) HCl (g), Et_2O.

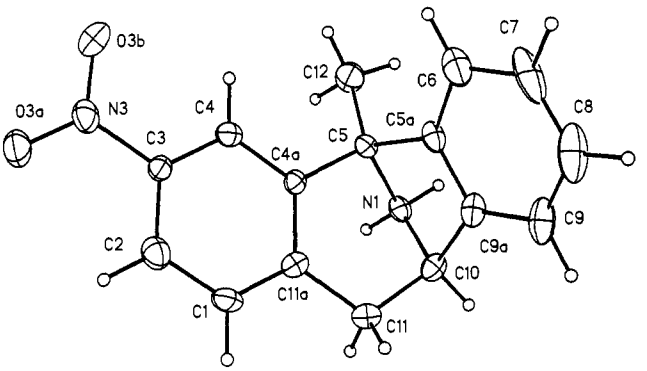


Figure 1. Thermal ellipsoid plot of (+)-**4a** cation. Oxalate anions and water omitted.

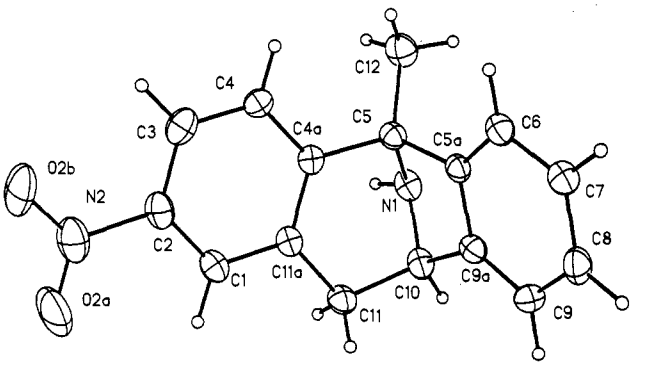


Figure 2. Thermal ellipsoid plot of (+)-**4b**. In the second molecule in the asymmetric unit (not shown) the imine hydrogen is trans to the nitro group.

advantages. Protection of the secondary amine is not necessary during the nitration. Possible side reactions such as *N*-nitration and aromatic sulfonation do not occur to any significant extent. Furthermore, our method is synthetically flexible, as it is easy to scale up and provides the opportunity for the facile introduction of different substituents at the nitrogen atom.

Conversion of nitro compounds (+)-**4a,b** into the desired isothiocyanates (+)-**8a,b** (Scheme I) followed the method employed in the preparation of **2b**.[18] The secondary amine function was expected to interfere with the isothiocyanate formation, making temporary protection as an amide necessary. Thus, the nitro compounds (+)-**4a,b** were treated with di-*tert*-butyl carbonate to give the carbamates (+)-**5a,b**, which were hydrogenated in the presence of Pd–C. Reaction of (+)-**6a,b** with thiophosgene in a two-phase system $CHCl_3$/2 M $NaHCO_3$ gave (+)-**7a,b** in high yield. The *N*-protected secondary amines (+)-**7a,b** were treated with gaseous HCl in dry diethyl ether to give the isothiocyanates (+)-**8a,b** as their HCl salts. In the same manner, (−)-**8a** was obtained from (−)-**3**. Attempted recrystallization of the isothiocyanates resulted in extensive decomposition. All spectral data (^1H and ^{13}C NMR, IR, and MS) were in accordance with the structures **8a** and **8b**. All compounds were homogenous on TLC. However, in the case of (+)- and (−)-**8a**, the combustion analyses showed serious deviations from the expected values. In particular, the value found for chlorine was consistently 4–8% higher than expected, suggesting that more than 1 mol of HCl was present. The excess HCl may be bound to the isothiocyanate moiety, forming a thiocarbamoyl chloride, which may have some stability in crystalline form, but will dissociate in solution. Some literature precedent for addition of HCl to isothiocyanates is known.[36–38] The only phenyl thiocarbamoyl chloride reported in the literature was obtained by "titrating" thiophosgene with aniline in dry ether. The compound was reported to be of limited thermal stability and to dissociate to phenyl isothiocyanate and HCl upon contact with solvents.[37] An effort to prepare the 3-thiocarbamoyl chloride of (+)-**3** unambiguously by adding a solution of (+)-**6a** in ether to 1 equiv of thiophosgene in ether, followed by deprotection with gaseous HCl, did not result in the anticipated thiocarbamoyl chloride but instead gave an isothiocyanate with spectral and biological properties identical to those of (+)-**8a**.

X-ray Crystallography

In both (+)-**4a** (oxalate salt) and (+)-**4b** (free base) the crystallographic asymmetric unit consists of two formula units. In (+)-**4a** the two $C_{16}H_{15}N_2^+O_2{\cdot}C_2HO_4^-{\cdot}H_2O$ units differ primarily with respect to nonbonded interactions while in (+)-**4b**, in addition to packing differences, the two dibenzocycloheptenimine molecules differ with respect to the orientation of the imine hydrogen, which is alternately cis and trans to the nitro group. The conformations of the (+)-2-nitro and (+)-3-nitro compounds are nearly identical, excluding the nitro substituents. Bond distances and angles in both are near expected values, and the dihedral angle formed by the least squares plane (average root mean square deviation from the plane ranges from 0.006 to 0.02 Å) through each aromatic ring and the two adjacent atoms in the central ring is 105.7 and 106.9° in (+)-**4a**, and 103.6 and 104.2° in (+)-**4b**. In the unsubstituted (+)-**3**[39] this dihedral angle is at 102.0°.

In (+)-**4b** there are no unusual intermolecular interactions and intermolecular separations are near van der Waals separations. In (+)-**4a** there are two intermolecular hydrogen bonds to each of the two cations in which the quaternary nitrogen acts as a donor to each of the oxalate anions. There are also intermolecular hydrogen bonds between the oxalates and the water molecules. The absolute configurations of (+)-**4a**, (+)-**4b**, and (+)-**3**[39] were determined to be 5*S*,10*R*.

Table I. Comparison of Isothiocyanates to Reversibly and Irreversibly Inhibit the Binding of (+)-[^{3}H]-**3** in Vitro

ligand	K_i (nM)[a]	% inactivation (concentration in μM)[a]
(+)-**8a**	37 (±5.3)	56 (±1.6) (0.10)[b]
(−)-**8a**	838 (±119)	61 (±2) (20)
(+)-**8b**	843 (±115)	41 (±3) (20)
1b	535 (±62)	50.5 (±7.5) (100)
2b	94 (±7.5)	55 (±1) (0.25)[b]

[a] Experiments were performed in triplicate and the values (±SEM) are from the mean of at least two separate experiments. [b] 100% inactivation at 10 μM.

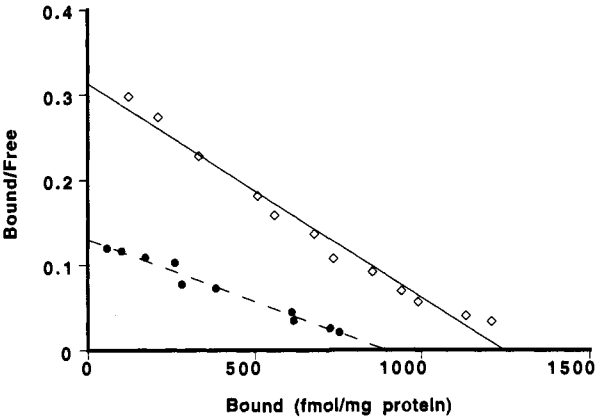


Figure 3. Scatchard plots of (+)-[^{3}H]-**3** binding in the presence (●) or absence (◇) of 0.1 μM (+)-**8a**·HCl.

Results

Isothiocyanates (+)- and (−)-**8a** and (+)-**8b** were tested for their ability to inhibit the in vitro binding of (+)-[^{3}H]-**3** from rat brain homogenates. To determine the effect of the isothiocyanate group on the affinity of **8a,b** for the PCP binding site, the isothiocyanates **8a,b** were tested under conditions of reversible inhibition. Isothiocyanate (+)-**8a** displayed an apparent K_i value of 37 nM. Its antipode (−)-**8a** and the 2-substituted isothiocyanate (+)-**8b** showed greatly reduced (about 330 times) affinity compared to the parent compound (+)-**3** (Table I). For the irreversible binding, displacement of (+)-[^{3}H]-**3** was studied using untreated control tissue and tissue pretreated with the isothiocyanates **8a,b** for 30 min and then well washed. At a concentration of 100 nM, (+)-**8a** revealed a loss of approximately 50% of the total binding capacity in the treated tissue (Table I). Compounds (−)-**8a** and (+)-**8b** were much less effective in the irreversible binding assay (Table I) and were not studied further.

Complete loss of (+)-[^{3}H]-**3** binding could be effected using a 10 μM concentration of (+)-**8a**. Scatchard analysis of the data (Figure 3) showed that the loss of binding sites (28% at 100 nM of (+)-**8a**) occurred with a small change in the affinity of (+)-**3** for the remaining sites [K_d for control, 4.8 nM (4.0–5.5 nM, 95% confidence interval); K_d for treated, 7.0 nM (5.9–8.1 nM, 95% confidence interval); B_{max} for control, 1310 pmol/mg protein (1244–1377 pmol/mg protein, 95% confidence interval); B_{max} for treated, 897 pmol/mg protein (850–945 pmol/mg protein, 95% confidence interval]. An examination of the time course

Table II. Ability of (+)-8a to Reversibly and Irreversibly Inhibit the Displacement of Various CNS Receptor Radioligands in Vitro[a]

ligand	K_i (nM) (+)-3	K_i (nM) (+)-8a	% inactivation by 100 nM (+)-8a
(+)-[^{3}H]-3[b]	2.5 (±0.57)	37 (±5.3)	53
[^{3}H]TCP[c]	6.3 (±2.1)	132 (±20)	50
[^{3}H]QNB[d]	>100 000[i]	>100 000[i]	0
[^{3}H]FOXY[e]	>100 000[i]	>100 000[i]	0
[^{3}H]flunitrazepam[f]	>100 000[i]	>100 000[i]	0
(+)-[^{3}H]pentazocine[g]	59 167 (±1 167)	4 815 (±585)	0 (52% at 25 μM)
[^{3}H]mazindol[h]	40 867 (±4 449)	6 950 (±980)	0

[a] Experiments were performed in triplicate and the K_i values (±SEM) are from the mean of at least two separate experiments, except where indicated. The ligand concentrations used were: 2 nM (+)-[^{3}H]-3, 2 nM [^{3}H]TCP, 0.5 nM [^{3}H]QNB, 2 nM [^{3}H]FOXY, 1 nM [^{3}H]flunitrazepam, 3 nM (+)-[^{3}H]pentazocine, and 5 nM [^{3}H]mazindol. Inactivation by (+)-8a refers to the percent inactivation in washed, treated tissue compared with similarly washed controls. Inhibition constants (K_i) for (+)-8a were calculated from the Cheng–Prusoff equation (ref 57). [b] (+)-[^{3}H]-5-Methyl-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-5,10-imine. [c] [^{3}H]-1-[1-(2-Thienyl)cyclohexyl]piperidine. [d] [^{3}H]-Quinuclidinyl benzilate. [e] (-)-[^{3}H]-3,14-Dihydroxy-4,5-epoxy-6β-fluoro-17-methylmorphinan. [f] [^{3}H]-5-(2-Fluorophenyl)-1,3-dihydro-1-methyl-7-nitro-2*H*-1,4-benzodiazepin-2-one. [g] (+)-[^{3}H]-2′-Hydroxy-5,9-α-dimethyl-*N*-(3-methyl-2-butenyl)-6,7-benzomorphan. [h] [^{3}H]5-(4-Chlorophenyl)-2,5-dihydro-3*H*-imidazo[2,1-a]isoindol-5-ol. [i] Results from a single experiment run in triplicate.

for irreversible loss in (+)-[^{3}H]-3 binding revealed that, at a concentration of 100 nM, less than 5 min was required to cause wash-resistant inhibition (data not shown). In concentrations up to 100 μM, isothiocyanate (+)-8a displayed no detectable irreversible inhibition of the in vitro binding of radioligands for the μ-opioid[40] or muscarinic,[41] benzodiazepine,[42] or dopamine receptors[20] (Table II). At 100 nM, (+)-8a did not irreversibly inhibit binding to σ_1-receptors.[43] However, at a 25 μM concentration of (+)-8a, a 52% wash-resistant reduction in binding of (+)-[^{3}H]pentazocine was observed.

Discussion

Optimally, site-directed irreversible agents should possess high affinity for the target biomacromolecule and display a significant degree of receptor selectivity at concentrations required for irreversible binding. Our data (Tables I and II, Figure 3) demonstrate that isothiocyanate (+)-8a meets these standards. Compound (+)-8a is potent (K_i = 37 nM) in binding to the (+)-[^{3}H]-3 site and compares favorably in this regard with both **1b** (K_i = 535 nM) and **2b** (K_i = 94 nM). In terms of irreversible inactivation of this site, incubation of rat brain nomogenates with 100 nM (+)-8a results in a 50% wash-resistant decrease in subsequent (+)-[^{3}H]-3 binding, compared to the 100 μM and 250 nM concentrations required for **1b** and **2b**, respectively. Furthermore, compound (+)-8a is exceptionally receptor selective (Table II). Concentrations of (+)-8a which are 1000 times greater than that required for 50% irreversible inactivation of the (+)-[^{3}H]-3 site have no effect on the reversible binding of radioligands which label the μ-opioid, muscarinic, or benzodiazepine receptors. However, (+)-8a does reversibly displace (+)-[^{3}H]pentazocine and [^{3}H]mazindol, markers for σ_1-receptors and the dopamine reuptake complex, respectively, albeit weakly. (+)-[^{3}H]Pentazocine is displaced with an apparent K_i of 4.8 μM, or 130 times the apparent K_i for (+)-8a. At high concentrations of (+)-8a (25 μM), 52% of the σ_1-receptors are irreversibly labeled. (+)-8a also shows low affinity for the dopamine reuptake complex (apparent K_i of 6.95 μM), but does not irreversibly label this site in contrast to **1b**.[19,20] Previous studies have revealed the reversible interaction of **1b** at muscarinic,[19] μ-opioid,[19] dopaminergic,[19,20] and σ_1-sites[21] and of **2b** at the muscarinic[20] and σ_1-receptors.[43]

The observed reduction in B_{max} of (+)-8a (Figure 3) is similar to earlier reported values for metaphit,[17] etoxadrol *m*-isothiocyanate,[18] thiophit,[23] ethylphit,[23] and similar irreversible ligands.[24,25] The decrease in K_d for (+)-3 from 4.8 nM (4.0–5.5 nM, 95% confidence interval) to 7.0 nM (5.9–8.1 nM, 95% confidence interval) is comparable to the values found for the PCP analogs,[24,25] when either [^{3}H]TCP or (+)-[^{3}H]-3 is used. Only in the case of etoxadrol *m*-isothiocyanate[18] (vs [^{3}H]TCP) and metaphit[17] (vs [^{3}H]PCP) is the K_d found to remain constant after irreversible labeling of the PCP binding site. The change in K_d has been rationalized by the suggestion of a possible competitive labeling of low affinity sites within the ion channel which would prevent the tritiated ligand from reaching the PCP binding site.[25]

In contrast to (+)-8a, both the (-)-3-isothiocyanate (-)-8a and the 2-isothiocyanate (+)-8b show only low affinity for the PCP site. The fact that the optical antipode of (+)-8a is not very effective is in accordance with the assumed asymmetric nature of this receptor site. Although (+)-3 and its optical antipode only differ in activity by a factor of 7,[45] increasing the asymmetry by adding a substituent increases the in vitro differences between the enantiomers. The inactivity of the (+)-2-isothiocyanate derivative may at first appear to be puzzling. However, it is presumed that the nucleophilic moiety on the receptor, with which the isothiocyanate must react, has to be in close proximity to make covalent binding to the receptor possible.[14] There is no *a priori* certainty that (+)-8b will covalently bind to the PCP site because (+)-8a did in spite of their close structural similarity. More importantly, other (±)-2-substituted derivatives of (+)-3 (Me, OH, OMe) were shown to be significantly less active than their 3-substituted counterparts.[46] The 3-substituted derivatives were at least equipotent with the parent compound, or showed even higher affinity as in the case of the 3-chloro and 3-bromo derivatives.[46] This may indicate that steric bulk at the 2-position cannot be tolerated because it interferes with the binding to the receptor.

It is of interest to note that in (+)-8a the isothiocyanate group is positioned on aromatic ring B of the parent compound (+)-3. This ring was not previously considered to be as important for binding as the A-ring in (+)-3.[45] The aromatic ring of metaphit or etoxadrol *m*-isothiocyanate, which carries the isothiocyanate group, is essential for binding. If we assume that metaphit, etoxadrol *m*-isothiocyanate, and (+)-8a interact similarly with the PCP binding site, then it is likely that the aromatic rings of the three compounds are similarly located at the binding site and their isothiocyanate moieties interact with either the same amino acid at the binding site or closely situated amino acids in three-dimensional space. Also, our comparison of structure–activity data for 3 and for PCP show a resemblance which has not been taken into account in a previous study.[45] Structure–activity studies on PCP show an almost complete loss of binding when the phenyl ring is substituted in the 4-position, while substitution with OH or NH_2 in the 3-position leads to increased affinity for the PCP site.[47,48] A similar pattern of affinities is found for the 2- and 3-position of the B-ring in 3.[46] In contrast, substitution with OH or NH_2 in both the C7- and 8-positions of the other aromatic ring of 3 increases the

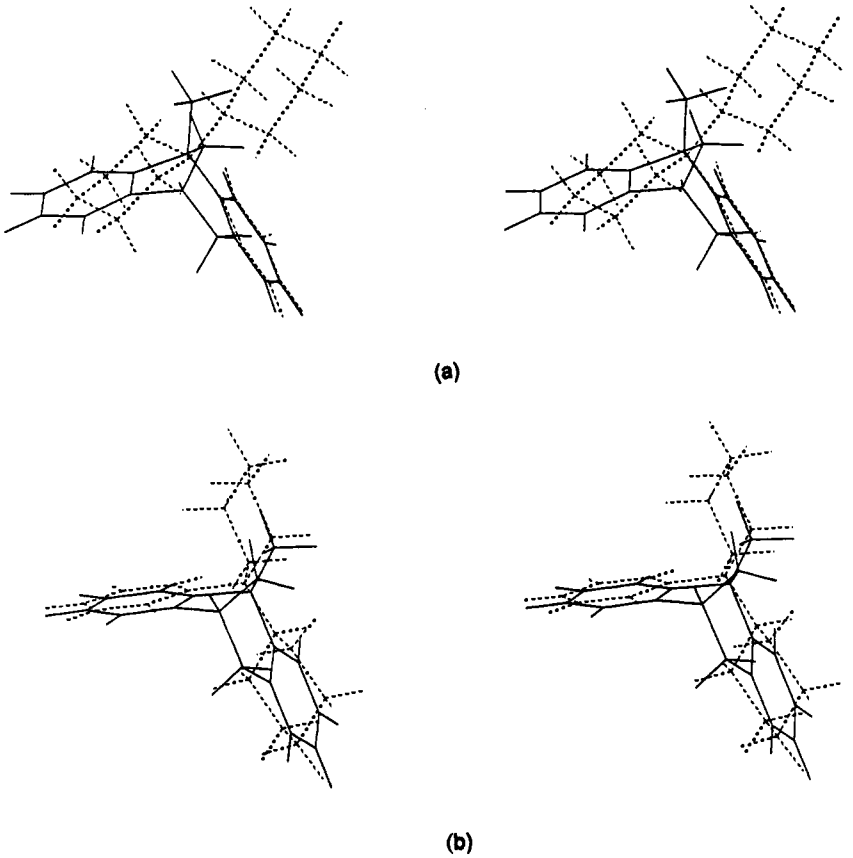


Figure 4. (a) Stereoview of the overlap of PCP (dotted line) and (+)-3 (solid line) using the B-ring of (+)-3. $\Sigma d^2 = 0.033$ Å^2, RMS $d = 0.104$ Å, $d_{average} = 0.102$ Å, $d_{max} = 0.126$ Å. (b) Stereoview of the overlap of PCP (dotted line) and (+)-3 (solid line) using the A-ring of (+)-3. $\Sigma d^2 = 0.359$ Å^2, RMS $d = 0.346$ Å, $d_{average} = 0.333$ Å, $d_{max} = 0.404$ Å.

in vitro binding by about 2-fold.[46] When the aromatic ring of PCP is overlapped with the B-ring of (+)-3, the 3- and 4-positions in PCP are in almost perfect overlap with the 3- and 2-positions in (+)-3 (Figure 4a; for computational procedures used see Experimental Section), which is in agreement with the structure–activity data for these compounds. The alternate orientation (Figure 4b) shows limited overlap between the 7- and 8-positions of ring A and the 4- and 3-positions, respectively, in PCP. Additionally, we have noted previously that the fit of (+)-3 to the PCP pharmacophore is better when the B-ring instead of the A-ring is used as part of the pharmacophore.[49] Leeson et al.[45] used a water molecule, positioned *syn* to the piperidine ring, as a model for the hydrogen-bond-acceptor group to which the protonated nitrogen atom binds. However, our alternative orientation for PCP and derivatives will require a more extended group such as a carboxylate as a hydrogen-bond-acceptor group to accommodate both PCP and (+)-3. Indeed, the PCP site has been shown to have acidic groups.[50] We will address this problem in more detail in a subsequent paper.[51]

Recently, binding data on the *N*-(2-isothiocyanatoethyl) derivative of (±)-3 were published.[44] This compound showed wash-resistant inhibition of both the PCP site and the haloperidol-sensitive σ-receptor site in guinea pig brain, albeit at much higher concentrations (46% and 40% inhibition, respectively, at 100 μM) than (+)-8a. From structure–activity correlations and molecular modeling studies by Leeson et al.,[45] it is clear that potent compounds based on (+)-3 may only have small substituents on the nitrogen atom. This observation might explain why the *N*-(2-isothiocyanatoethyl) derivative of (±)-3 is relatively inactive.

In summary, the extremely high affinity and selectivity displayed by isothiocyanate (+)-8a clearly indicate that the ligand will be a valuable tool in the further elucidation of the structure and function of the NMDA receptor.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and were uncorrected. Gas chromatographic (GC) analyses were performed using a Hewlett-Packard Model 5880A instrument employing a 10-m SE-30 capillary column (0.32 mm i.d.). Thin-layer chromatographic (TLC) analyses were performed using 250-μm silica gel GHLF plates (Analtech). Eluent systems were as follows: A, ethyl acetate/hexanes 1:6; B, $CHCl_3$/CH_3OH/concentrated ammonia 9:1:0.1. Preparative centrifugal thin-layer chromatography (PCTLC) was performed on a Harrison Model 7924 Chromatotron using silica gel PF254 containing $CaSO_4 \cdot 0.5H_2O$ binder (Merck). Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance spectra were obtained on a Varian XL-300 spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (Me_4Si; 0.0 ppm) for ^{1}H NMR and $CDCl_3$ (77.0 ppm) for ^{13}C NMR. Coupling constants (J) are reported in hertz (Hz) and s, bs, d, t, q, and m refer to singlet, broadened singlet, doublet, triplet, quartet, and multiplet, respectively. Infrared spectra (IR) were obtained using a Beckman IR 4230 spectrophotometer, and absorbances are reported in reciprocal centimeters (cm^{-1}). High-resolution mass spectra (HRMS) and electron impact mass spectra (EIMS) were obtained on a VG 7070F. Chemical ionization mass spectra (CIMS) were obtained using a Finigan 1015D mass spectrometer. Optical rotations were measured with a Perkin-Elmer Model 241 MC polarimeter. Combustion analyses were performed by Atlantic Microlabs (Atlanta, GA) and are within 0.4% of the calculated values, except when indicated otherwise. The purity of the compounds for which no satisfactory combustion analysis could be obtained was verified by HRMS combined with TLC (two different eluent systems). A Nicolet Model R3m/V automatic X-ray diffractometer in $\theta/2\theta$ collection mode was used for X-ray crystallography.

Nitration of (+)-3. Preparation of (+)-3- and (+)-2-Nitro-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (4a and 4b). Imine (+)-3[33,34] (19.02 g, 86 mmol) was dissolved in 250 mL of a 1:1 mixture of sulfuric and glacial acetic acid over a period of 24 h. Fuming nitric acid (90% HNO_3, d 1.5, 6.00 g, 90%, 86 mmol) in 10 mL of sulfuric acid was added and the reaction was stirred at room temperature. After 16 and 40 h, respectively, two 0.60-g (8.6 mmol) portions of nitric acid were added. After 48 h, the reaction mixture was poured on 1 kg of ice, concentrated ammonia was added until the pH was 9, and the resulting emulsion was extracted with ethyl acetate (500 mL, 2 × 100 mL). The combined organic extracts were washed with 1 N ammonia (100 mL) and water (100 mL), dried (Na_2SO_4), and evaporated to give 23.0 g of yellow oil. The oil was dissolved in 200 mL of methanol and mixed with a solution of oxalic acid (9.1 g, 100 mmol) in 200 mL of methanol. The foam obtained after evaporation of the methanol was refluxed in 500 mL of ethyl acetate for 1 h and left overnight at room temperature, yielding 18.0 g of crystalline material, of which 80% (GC) was the 3-nitro compound (+)-4a. This material was refluxed two times with 100 mL of methanol for 1 h to give 12.4 g of (+)-4a·oxalate (35 mmol, 40% yield, 98% pure by GC). An analytical sample was obtained by repetitive PCTLC of the free base (1% methanol in dichloromethane), followed by recrystallization of the oxalate salt from methanol/2-propanol 1:1. (+)-4a·Oxalate decomposes slowly above 240 °C, terminating at 250 °C in a black melt: $[\alpha]^{22}_D$ +251° (*c* 0.39, CH_3OH); IR (film of free base) 3220 (NH), 1545 and 1350 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$, free base) δ 1.99 (s, 3H, CH_3), 2.32 (bs, 1H, NH), 2.84 (d, 1H, *J* = 17 Hz, H_{11}endo), 3.53 (dd, 1H, *J* = 17 and 5 Hz, H_{11}exo), 4.75 (d, 1H, *J* = 5 Hz, H_{10}), 7.09–7.34 (m, 5H, ArH), 7.95 (dd, 1H, *J* = 8 and 2 Hz, ArH), 8.12 (d, 1H, *J* = 2 Hz, ArH); ^{13}C NMR ($CDCl_3$, free base) δ 19.91, 34.90, 57.85, 64.18, 116.62, 118.85, 121.70, 122.03, 127.27, 130.98, 114.04, 144.45, 145.89, 151.26; CIMS (NH_3, oxalate salt) *m/e* 267 (M^+ + 1), 251, 137. Anal. ($C_{18}H_{16}N_2O_6$) C, H, N.

The mother liquor from the first crystallization was treated with 100 mL of a 1 N KOH solution. The organic layer was washed with 25 mL of water and dried (Na_2SO_4), and the solvent was evaporated in vacuo to give 9.0 g of a yellow oil, which was dissolved in 100 mL of 2-propanol and treated with fumaric acid (4.7 g, 41 mmol, 1.2 equiv) in 100 mL of 2-propanol. The crystalline precipitate was filtered and refluxed first with 50 mL of 2-propanol and then with 50 mL of ethyl acetate, giving 1.74 g of 98% pure 2-nitro compound (+)-4b as its fumarate salt. The base was liberated with 2 N KOH and crystallized from ethyl acetate, giving 0.67 g (3%) of crystalline (+)-4b: mp 200–201 °C; $[\alpha]^{25}_D$ +114° (*c* 0.81, $CH_3OH/CHCl_3$ 4:1); IR (KBr) 3280, 3240 (NH), 1510, 1340 ($ArNO_2$) cm^{-1}; ^1H NMR ($CDCl_3$, free base) δ 1.76 (bs, 1H, NH), 1.99 (s, 3H, CH_3), 2.84 (d, 1H, *J* = 17.1 Hz, H_{11}endo); 3.57 (dd, 1H, *J* = 17.1 and 5.6 Hz, H_{11}exo), 4.78 (d, *J* = 5.5 Hz, H_{10}); 7.06–7.44 (m, 5H, ArH), 7.83 (d, 1H, *J* = 2.2 Hz, ArH), 7.95 (dd, 1H, *J* = 8.5 and 2.3 Hz, ArH); CIMS (NH_3) *m/e* 267 (M^+ + 1). Anal. ($C_{18}H_{14}N_2O_2$) C, H, N.

(+)-*N*-[(*tert*-Butyloxy)carbonyl]-3-nitro-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine ((+)-5a). A solution of the free base (+)-4a (0.36 g, 1.35 mmol) in CH_2Cl_2 (20 mL) was treated successively with a solution of $NaHCO_3$ (2.30 g, 27.4 mmol) in 20 mL of H_2O, and di-*tert*-butyl dicarbonate (0.60 g, 2.74 mmol). The reaction mixture was vigorously stirred for 39 h at room temperature, at which time TLC analysis (hexanes/ethyl acetate 2:1) demonstrated complete consumption of the starting material. The mixture was separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were dried (K_2CO_3), and the solvent was removed in vacuo, affording a colorless oil (1.11 g), which was purified by PCTLC (hexanes/ethyl acetate 2:1). Homogeneous fractions were combined and concentrated to dryness, affording pure (+)-5a as a white foam (0.49 g, 1.34 mmol, 98%): mp 74–75 °C; $[\alpha]^{25}_D$ +194° (*c* 0.46, $CH_3OH/CHCl_3$ 4:1); IR (KBr) 1705 (NCO_2t-Bu), 1515 and 1345 ($ArNO_2$) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (s, 9H, *t*-Bu), 2.32 (s, 3H, CH_3), 2.73 (d, 1H, *J* = 17.8 Hz, H_{11}endo), 3.72 (dd, 1H, *J* = 17.8 and 5 Hz, H_{11}exo), 5.40 (d, 1H, *J* = 5.5 Hz, H_{10}), 7.08–7.35 (m, 5H, ArH), 7.97 (dd, *J* = 2.3 and 8.4 Hz, 1H, ArH), 8.17 (d, *J* = 2.2 Hz, 1H, ArH); EIMS *m/e* 367 (M^+), 336, 310, 293, 280, 266, 265, 249, 235, 219, 204, 57. Anal. ($C_{21}H_{22}N_2O_4$) C, H, N.

(+)-*N*-[(*tert*-Butyloxy)carbonyl]-3-amino-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine ((+)-6a). A solution of (+)-5a (0.48 g, 1.31 mmol) in EtOH (50 mL) containing 50 mg of 10% Pd on carbon was hydrogenated (30 psi) for 16 h at room temperature. Filtration of the catalyst and removal of the solvent in vacuo afforded a crude product (0.45 g) which was purified by PCTLC (hexanes/ethyl acetate 2:1), yielding (+)-6a as a white foam (0.40 g, 1.19 mmol, 91%), which was homogenous on TLC (system A). Compound (+)-6a undergoes slow decomposition above 70 °C, terminating in a yellow melt at 150 °C: $[\alpha]^{22}_D$ +193° (*c* 0.4, CH_3OH); IR (KBr) 3460 and 3370 (NH_2), 1695 (NCO_2t-Bu); ^1H NMR ($CDCl_3$) δ 1.40 (s, 9H, *t*-Bu), 2.20 (s, 3H, CH_3), 2.49 (d, 1H, *J* = 16 Hz, H_{11}endo), 3.53 (m, 3H, $ArNH_2$ and H_{11}exo), 5.31 (d, 1H, *J* = 5 Hz, H_{10}), 6.40–6.44 (m, 1H, ArH), 6.65–6.69 (m, 2H, ArH), 7.01–7.04 (m, 1H, ArH), 7.13–7.18 (m, 2H, ArH), 7.27–7.30 (m, 1H, ArH); EIMS *m/e* 336 (M^+), 280, 279, 336, 335, 220, 208. Anal. ($C_{21}H_{24}N_2O_2 \cdot 0.1H_2O$) C, H, N.

(+)-3-Isothiocyanato-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine Hydrochloride ((+)-8a·HCl). An ice-water chilled solution of (+)-6a (0.25 g, 0.74 mmol) in pentene-stabilized chloroform (10 mL) was first treated with 10 mL of a 2 *N* solution of $NaHCO_3$ and then with a solution of freshly distilled thiophosgene (87 μL, 1.15 mmol) in chloroform (5 mL). After 10 min, the phases were separated, and the aqueous layer was extracted with Et_2O (3 × 20 mL). The combined organic extracts were dried (Na_2SO_4), and the solvent was removed in vacuo, affording a viscous yellow oil which was purified by chromatography (SiO_2, hexanes/ethyl acetate 4:1) to give 0.25 g (0.66 mmol, 90%) of (+)-7a as a colorless oil, pure by TLC (systems A and B): EIMS *m/e* 378 (M^+), 322, 277, 57, ^1H NMR ($CDCl_3$) δ 1.41 (s, 9H, *t*-Bu), 2.24 (s, 3H, CH_3), 2.61 (d, 1H, *J* = 17.3 Hz, H_{11}endo), 3.63 (dd, 1H, *J* = 17.3 and 5.4 Hz, H_{11}exo), 5.36 (d, 1H, *J* = 5.4 Hz, H_{10}), 6.88–7.34 (m, 7H, ArH); IR (neat) 2140–2040 (NCS), 1710 (NCO_2t-Bu) cm^{-1}. HRMS calcd for $C_{22}H_{22}N_2O_2S$ 378.1402, found 378.1415.

The isothiocyanate (+)-7a (0.12 g, 0.32 mmol) was dissolved in 5 mL of dry ether and cooled at 0 °C, and HCl was bubbled through the solution for 15 min. The flask was capped and the solution was stirred for 45 min at room temperature, after which TLC showed complete conversion of the starting material. The mixture was evaporated under reduced pressure to dryness and the white residue was dissolved in 3 mL of pentene-stabilized chloroform. Slow addition of 6 mL of hexanes while stirring resulted in precipitation of (+)-8a·HCl. After cooling to 0 °C for 2 h, the solids were filtered, washed with 15 mL of cold hexanes, and dried in vacuo to yield 0.09 g of (+)-8a·HCl (0.29 mmol, 89%), which was homogenous on TLC (systems A and B). Compound (+)-8a·HCl decomposes slowly above 170 °C: $[\alpha]^{22}_D$ +375° (*c* 0.4, MeOH); IR (KBr) 3500–3400 (NH), 2900–2500 (NH_2^+), 2120–2040 (NCS) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.33 (s, 3H, CH_3), 2.89 (d, 1H, *J* = 17.6 Hz, H_{11}endo), 3.96 (dd, 1H, *J* = 17.6 and 2.7 Hz, H_{11}exo), 5.32 (d, 1H, *J* = 1.7 Hz, H_{10}), 6.97–7.39 (m, 7H, ArH), 10.92 (bs, 1H, NH), 10.93 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 17.39, 32.09, 57.86, 66.94, 119.37, 119.45, 122.91, 126.20, 128.97, 129.39, 129.51, 130.28, 131.61, 136.06, 139.60, 144.73; CIMS (NH_3) *m/e* 279 (M^+ + 1), 263, 247; HRMS calcd for $C_{17}H_{14}N_2S$ 278.0878, found 278.0881.

(−)-3-Isothiocyanato-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine Hydrochloride ((−)-8a·HCl). The optical antipode of (+)-8a was obtained starting from (−)-3 via the synthesis described above and was homogenous on TLC (systems A and B): mp 170 °C (dec); $[\alpha]^{22}_D$ −378° (*c* 0.4, MeOH). HRMS calcd for $C_{17}H_{14}N_2S$ 278.0878, found 278.0876.

(+)-*N*-[(*tert*-Butyloxy)carbonyl]-2-nitro-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine ((+)-5b). To a solution of (+)-4b (0.50 g, 1.88 mmol) in pentene-stabilized $CHCl_3$ (25 mL) was added a solution of $NaHCO_3$ (3.20 g, 38.0 mmol) in 20 mL of H_2O and di-*tert*-butyl dicarbonate (0.60 g, 3.74 mmol). The reaction mixture was vigorously stirred for 36 h at room temperature, at which time TLC analysis (hexanes/ethyl acetate 9:1) showed complete consumption of the starting material. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was removed in vacuo to give a colorless oil which was purified by chromatography (SiO_2, hexanes/ethyl acetate 9:1). Pure (TLC,

systems A and B) (+)-**5b** was obtained as a white solid (0.62 g, 1.69 mmol, 90%): mp 120–122 °C (hexanes); $[\alpha]^{25}_D$ +116° (*c* 0.39, CH_3OH); IR (KBr) 1700 (NCO_2t-Bu), 1520, 1350 ($ArNO_2$) cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.40 (s, 9H, *t*-Bu), 2.29 (s, 3H, CH_3), 2.73 (d, 1H, *J* = 17.2 Hz, H_{11}endo), 3.73 (dd, 1H, *J* = 17.2 and 5.4 Hz, H_{11}exo), 5.40 (d, 1H, *J* = 5.4 Hz, H_{10}), 7.05–7.49 (m, 5H, ArH), 7.81 (d, 1H, *J* = 2.2 Hz, ArH), 7.97 (dd, 1H, *J* = 2.2 and 8.6 Hz, ArH); CIMS (NH_3) *m/e* 367 (M^+ + 1), 311, 267 (–CO_2t-Bu). HRMS calcd for $C_{21}H_{22}N_2O_4$ 366.1580, found 366.1591.

(+)-*N*-[(*tert*-Butyloxy)carbonyl]-2-amino-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine ((+)-6b). A solution of (+)-**5b** (0.57 g, 1.56 mmol) in ethyl acetate (50 mL) containing 50 mg of 10% Pd on carbon was hydrogenated (45 psi) at room temperature. After 16 h, another portion of catalyst (50 mg) was added and hydrogenation was continued for 24 h. Filtration of the catalyst and removal of the solvent in vacuo afforded a crude product. This was crystallized from a small volume of ethyl acetate, yielding 0.30 g (0.89 mmol, 57%) of (+)-**6b**: mp 179–180 °C; $[\alpha]^{22}_D$ +99° (*c* 0.6, $CH_3OH/CHCl_3$ 9:1); CIMS (NH_3) *m/e* 337 (M^+ + 1), 293, 281 (–*t*-Bu), 237 (–CO_2t-Bu); IR (KBr) 3430, 3360 ($ArNH_2$), 1680 (NCO_2t-Bu) cm^{-1}. ^1H NMR δ 1.39 (s, 9H, *t*-Bu), 2.19 (s, 3H, CH_3), 2.52 (d, 1H, *J* = 16.9 Hz, H_{11}endo), 3.57 (dd, 1H, *J* = 16.9 and 5.6 Hz, H_{11}exo), 3.75 (bs, 2H, $ArNH_2$), 5.30 (d, 1H, *J* = 5.5 Hz, H_{10}), 6.28 (d, 1H, *J* = 2.2 Hz, ArH), 6.42 (dd, 1H, *J* = 8.2 and 2.3 Hz), 7.00–7.29 (m, 5H, ArH). Anal. ($C_{21}H_{24}N_2O_2$) C, H, N.

(+)-2-Isothiocyanato-5-methyl-10,11-dihydro-5*H*-dibenzo-[*a*,*d*]cyclohepten-5,10-imine Hydrochloride ((+)-8b·HCl). An ice-water chilled solution of (+)-**6b** (0.15 g, 0.44 mmol) in pentene-stabilized chloroform (10 mL) was first treated with a solution of $NaHCO_3$ (0.35 g, 42 mmol) in 20 mL of water and then with a solution of freshly distilled thiophosgene (50 μL, 0.65 mmol) in chloroform (2 mL). After 20 min, the mixture was separated and the aqueous layer was extracted with Et_2O (3 × 10 mL). The combined organic layers were dried (Na_2SO_4), and the solvents were removed in vacuo to afford a viscous yellow oil, which was purified to homogeneity by column chromatography (SiO_2, hexanes/ethyl acetate 3:1), yielding 0.16 g (0.42 mmol, 95%) of (+)-**7b** as a colorless oil, pure by TLC (systems A and B): EIMS *m/e* 378 (M^+); IR (neat) 2140–2040 (NCS), 1710 (NCO_2t-Bu) cm^{-1}; HRMS calcd for $C_{22}H_{22}N_2O_2S$ 378.1402, found 378.1401. The isothiocyanate (+)-**7b** (0.16 g, 0.42 mmol) was dissolved in 10 mL of dry ether and cooled to 0 °C, and HCl was bubbled through for 15 min. The flask was capped and the solution was stirred for 1 h at room temperature, after which TLC and GC showed complete conversion of the starting material. The solvent was evaporated in vacuo and the resulting white residue was dissolved in 5 mL of pentene-stabilized chloroform. Slow addition of 10 mL of hexanes while being stirred resulted in precipitation of (+)-**8b**·HCl. After cooling at 0 °C for 2 h, the solids were filtered, washed with 15 mL of cold hexanes, and dried in vacuo to yield 0.10 g of (+)-**8b**·HCl (0.32 mmol, 75%), which was homogenous on TLC (systems A and B). (+)-**8b**·HCl decomposes slowly above 200 °C, terminating in a brown melt at 240 °C: $[\alpha]^{22}_D$ +57° (*c* 0.4, MeOH); IR (KBr) 3400 (NH), 2900–2500 (NH_2^+), 2160–2040 (NCS) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.34 (s, 3H, CH_3), 2.88 (d, 1H, *J* = 16.4 Hz, H_{11}endo), 3.98 (dd, 1H, *J* = 16.4 and 1.4 Hz, H_{11}exo), 5.30 (bs, 1H, H_{10}), 6.88–7.39 (m, 7H, ArH), 10.85 (bs, 1H, NH), 11.11 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 17.4, 32.11, 57.63, 67.03, 110.55, 119.23, 122.86, 123.21, 124.30, 127.33, 129.31, 129.50, 131.57, 135.87, 137.01, 144.90; EIMS *m/e* 278 (M^+), 263, 251, 236; HRMS calcd for $C_{17}H_{14}N_2S$ 278.0878, found 278.0881. Anal. ($C_{17}H_{15}ClN_2S·H_2O$) C, H, N; Cl: calcd, 10.65; found, 12.44; S: calcd, 9.63; found, 9.11.

Single Crystal X-ray Analysis of (+)-4a·Oxalate·Hydrate and 4b. Clear crystals of (+)-**4a**·oxalate·hydrate and (+)-**4b** (crystallized from methanol/2-propanol 1:1 and ethyl acetate, respectively) were selected for data collection in the $\theta/2\theta$ mode on a computer-controlled automated diffractometer (Nicolet R3m/V). The space group determinations were based on observed extinctions, *E* value statistics, and structure solutions. The data were corrected for Lorentz and polarization effects but not for absorption. Both structures were solved by direct methods with the aid of the program SHELXTL[52] and refined by a full-matrix least-squares.[52] The parameters refined include the coordinates and anisotropic thermal parameters for all non-hydrogen atoms. Carbon hydrogens used a riding model in which the coordinate shifts of the carbons were applied to the attached hydrogens with C–H = 0.96 Å, H angles idealized, and U_{iso}(H) set at fixed values. Experimental and structural analysis details are given in Table III and tables of crystal coordinates, bond distances, and bond angles are available as supplementary material.

Table III. Crystal and Refinement Data for the Two Nitro Compounds **4a** and **4b** Studied by X-Ray Diffraction

	(+)-4a.oxalate.H_2O	(+)-4b
formula	$C_{16}H_{15}N_2^+O_2 \cdot C_2HO_4^- \cdot H_2O$	$C_{16}H_{14}N_2O_2$
crystal system	orthorhombic	monoclinic
space group	$P22_12_1$	$C2$
a, Å	8.987(2)	22.348(3)
b, Å	12.865(2)	8.952(1)
c, Å	30.855(5)	14.836(2)
β, deg		116.13(9)
V, Å^3	3567.4(11)	2664.9(6)
Z	8	8
formula weight	373.8	266.3
F(000)	1568	1120
ρ(calc), g cm^{-3}	1.394	1.327
temp, °C	22	22
crystal dim., mm	0.07 × 0.48 × 0.52	0.06 × 0.11 × 0.28
λ, wavelength, Å	1.541 84	1.541 84
μ, absorption coeff, cm^{-1}	8.77	6.83
2θ max, deg	115	120
2θ scan speed, deg/min	variable 10–30	variable 8.0–30.0
2θ scan range, deg	2.0 + $\Delta_{\alpha1\alpha2}$	2.0 + $\Delta_{\alpha1\alpha2}$
data collected, *hkl*	0–9, 0–14, 0–33	–24–22, 0–10, 0–16
unique data	2785	2114
R_{int}	0.040	0.017
unique data, $F_o > 3\sigma(F_o)$	2519	1636
parameters refined	522	369
weighting function, g^a	0.00023	0.00023
R,^{b} wR,^{c} S^d	0.051, 0.058, 2.06	0.047, 0.044, 1.38
Fourier excursions, e Å^{-3}	0.22, –0.20	0.19, –0.19

^{a} $w^{-1} = \sigma^2(F_o) + gF_o^2$. ^{b} $\sum|\Delta|/\sum|F_o|$. ^{c} $\sum[(w\Delta^2)/\sum(wF_o^2)]^{1/2}$. ^{d} $[\sum w(\Delta^2)/(N_o - N_p)]^{1/2}$.

Binding Assays. Displacement curves for (+)-[^{3}H]-**3** were conducted with adult Sprague–Dawley rats (Taconic Farms, Germantown, NY), employing the following procedure. Following rapid decapitation, whole brain minus cerebellum was diluted in 45 volumes of ice cold 5 mM Tris·HCl, pH 7.4, and homogenized with a Brinkman Polytron, setting 6 for 20 s. The membranes were washed by centrifugation three times for 20 min with Tris buffer at 20000*g* with the last reconstitution quick frozen on dry ice and stored at –20 °C for later use. On the day of the experiment, the thawed membranes were spun an additional three times at 20000*g* for 20 min. A final tissue resuspension in 45 volumes of 5 mM Tris·HCl was added to a 1-mL total incubation mixture containing a final concentration of 2 nM (+)-[^{3}H]-**3**, 10 μM glutamate, 10 μM glycine, and test compounds. Nonspecific binding was determined by an excess of (+)-**3** (10 μM). Following a 90-min incubation at 5 °C, the assay was filtered through presoaked filters [Schleicher and Schuell #32 in 0.03% poly(L-lysine)] and placed in vials containing Hydrofluor scintillation cocktail (National Diagnostics). After an overnight elution of the filters, the vials were counted using a Packard Tri-Carb 2200CA with an approximate efficiency of 50%.

Irreversible (wash-resistant) binding experiments were performed using rat brain homogenates as prepared above. After thawing, the homogenates were divided into equal portions and incubated with the isothiocyanates for 30 min at 5 °C and washed three times with buffer containing glutamate (100 μM), glycine (30 μM), and $MgCl_2$ (300 μM). This wash procedure was found to be sufficient to remove a 100 nM concentration of (+)-**3** without loss of (+)-[^{3}H]-**3** binding. There was no significant difference between three and six washes. Data from these experiments were analyzed using GraphPAD software.[53] Binding methodologies for benzodiazepine, muscarinic, μ-opioid, dopamine, and σ-receptors were performed as described in published accounts[40-43] and modified to include additional washes sufficient to remove unreacted compound. Where irreversible binding was detected, in the σ-[(+)-[^{3}H]-pentazocine] and (+)-[^{3}H]-MK-801 binding assays, the control compounds, (+)-pentazocine and (+)-

MK-801, were included to demonstrate wash-resistant binding. The washing procedure was sufficient to remove these control compounds.

Molecular Modeling Studies. Molecular models were constructed on a Silicon Graphics 4D70GT workstation starting from the X-ray structural data[39,54] using the 2D and 3D graphic options of Quanta 3.0 (Molecular Simulations Inc., Burlington, MA).[55] The structures were energy-minimized using the Newton–Raphson routine (dielectric constant (ϵ) of 80) in the CHARMm software.[56] Geometries were considered minimized when either the energy change between two subsequent structures was less than 0.001 kcal/mol or the root mean square (RMS) of the deviation in the geometry of two subsequent structures was less than 0.01. For the overlap studies, the following procedure was used. Dummy atoms were built onto the aromatic rings (3.5 Å under and above) and on a vector aligned with the protonated lone pair of the nitrogen atom (2.8 Å from the nitrogen). The COMPARISON option in CHARMm was then applied, which uses a least squares fitting algorithm to minimize the displacement (d) between matching atoms in the structures that are superimposed. The "quality" of the fit was expressed by the value of Σd^2, RMS d, $d_{average}$, and d_{max}.

Acknowledgment. J.A.M. was supported by the National Institute of Arthritis, Musculoskeletal and Skin Diseases through the National Research Service Award 5 F32 AR07980. J.A.M. also gratefully acknowledges partial financial support from G. D. Searle and Co. J.T.M.L. was supported by the Fogarty Foundation. The authors with to thank Dr. A. Buonanno, LDN, NICHD, NIH, for his suggestions, Dr. N. A. Grayson, LMC, NIDDK, NIH, for helpful discussions, and N. F. Whittaker, W. White, and H. Yeh, LAC, NIDDK, NIH, for the mass spectral and nuclear magnetic resonance data.

Supplementary Material Available: Tables of atomic coordinates, bond lengths, bond angles, anisotropic displacement coefficients, and H-atom coordinates for compounds (+)-**4a** and (+)-**4b** and hydrogen bond parameters for compound (+)-**4a** (17 pages). Ordering information is given on any current masthead page.

References

(1) Stone, T. W.; Burton, N. R. NMDA Receptors and Ligands in the Vertebrate CNS. *Progr. Neurobiol.* **1988**, *30*, 333–368.
(2) Johnson, R. L.; Koerner, J. F. Excitatory Amino Acid Neurotransmission. *J. Med. Chem.* **1988**, *31*, 2057–2066.
(3) Honoré, T. Excitatory Amino Acid Receptor Subtypes and Specific Antagonists. *Med. Res. Rev.* **1989**, *9*, 1–23.
(4) Hansen, J. J.; Krogsgaard-Larsen, P. Structural, Conformational and Stereochemical Requirements of Central Excitatory Amino Acid Receptors. *Med. Res. Rev.* **1990**, *10*, 55–94.
(5) *The NMDA Receptor*, Watkins, J. C.; Collingridge, G. L., Eds., IRL Press at Oxford University Press, Oxford, 1989.
(6) Rottman, S. M.; Olney, J. W. Excitotoxicity and the NMDA Receptor. *Trends Neurosci.* **1987**, *19*, 299–302.
(7) Wachtel, H.; Turski, L. Glutamate: a New Target in Schizophrenia. *Trends Pharmacol. Sci.* **1990**, *11*, 219–220. See also: Kornhuber, J. Glutamate and Schizophrenia. *Trends Pharmacol. Sci.* **1990**, *11*, 357.
(8) Dingledine, R.; McBain, C. J.; McNamara, J. O. Excitatory Amino Acids Receptors in Epilepsy. *Trends Pharmacol. Sci.* **1990**, *11*, 334–338.
(9) Turski, L.; Bressler, K.; Rettig, K.-J.; Löschmann, P.-A.; Wachtel, H. Protection of Substantia Nigra from MPP+ Neurotoxicity by *N*-Methyl-D-aspartate Antagonists. *Nature* **1991**, *349*, 414–418.
(10) Collingridge, G. L.; Singer, W. Excitatory Amino Acids Receptors and Synaptic Plasticity. *Trends Pharmacol. Sci.* **1990**, *11*, 290–296.
(11) Trujillo, K. A.; Akil, H. Inhibition of Morphine Tolerance and Dependence by the NMDA Receptor Antagonist MK-801. *Science* **1991**, *251*, 85–87.
(12) Quirion, R.; Chicheportiche, R.; Contreras, P. C.; Johnson, K. M.; Lodge, D.; Tam, S. W.; Woods, J. H.; Zukin, S. R. Classification and Nomenclature of Phencyclidine and Sigma Receptor Sites. *Trends Neurosci.* **1987**, *10*, 444–446.
(13) Moriyoshi, K.; Masu, M.; Ishii, T.; Shigemoto, R.; Mizuno, N.; Nakanishi, S. Molecular Cloning and Characterization of the Rat NMDA Receptor. *Nature* **1991**, *354*, 31–37.
(14) Takemori, A. E.; Portoghese, P. S. Affinity Labels for Opioid Receptors. *Annu. Rev. Pharmacol. Toxicol.* **1985**, *25*, 193–223.
(15) Williams, E. F.; Rice, K. C.; Mattson, M.; Paul, S. M.; Skolnick, P. *In Vivo* Effects of Two Novel Alkylating Benzodiazepines, Irazepine and Kenazepine. *Pharmacol. Biochem. Behav.* **1981**, *14*, 487–491.
(16) Newman, A. H. Irreversible Ligands for Drug Receptor Characterization. *Annu. Rep. Med. Chem.* **1990**, *25*, 271–280.
(17) Rafferty, M. F.; Mattson, M.; Jacobson, A. E.; Rice, K. C. A Specific Acylating Agent for the [^{3}H]Phencyclidine Receptors in Rat Brain. *FEBS Lett.* **1985**, *181*, 318–322.
(18) Thurkauf, A.; Mattson, M. V.; Huguenin, P. N.; Rice, K. C.; Jacobson, A. E. Etoxadrol-*meta*-isothiocyanate: A Potent, Enantioselective, Electrophilic Affinity Ligand for the Phencyclidine-Binding Site. *FEBS Lett.* **1988**, *238*, 369–374.
(19) Sershen, H.; Berger, P.; Jacobson, A. E.; Rice, K. C.; Reith, M. E. A. Metaphit Prevents Locomotor Activation Induced by Various Psychostimulants and Interferes with the Dopaminergic System in Mice. *Neuropharmacol.* **1988**, *27*, 23–30.
(20) Zimanyi, I.; Jacobson, A. E.; Rice, K. C.; Lajtha, A.; Reith, M. E. A. Long-Term Blockade of the Dopamine Uptake Complex by Metaphit, an Isothiocyanate Derivative of Phencyclidine. *Synapse* **1989**, *3*, 239–245.
(21) Bluth, L. S.; Rice, K. C.; Jacobson, A. E.; Bowen, W. D. Acylation of σ Receptors by Metaphit, an Isothiocyanate Derivative of Phencyclidine. *Eur. J. Pharmacol.* **1989**, *161*, 273–277.
(22) Schweri, M. M.; Thurkauf, A.; Mattson, M. V.; Rice, K. C. Fourphit: A Selective Probe for the Methylphenidate Binding Site on the Dopamine Transporter. *J. Pharmacol. Exp. Ther.* **1992**, *261*, 936–942.
(23) Lessor, R. A.; Mattson, M. V.; Rice, K. C.; Jacobson, A. E. Electrophilic Affinity Ligands for the Phencyclidine (PCP) Receptor. In *Problems of Drug Dependence 1985: Proceedings of the 47th Annual Scientific Meeting, NIDA Research Monograph* 67, Harris, L. R., Ed.; Supt. of Docs., U.S. Govt. Print. Off.: Washington, D.C., 1986; pp 138–144.
(24) Kozikowski, A. P.; Fauq, A. H.; Tückmantel, W.; Casalotti, S. O.; Krueger, K. E. Synthesis of *N*-[1-(2-Thienyl)cyclohexyl]piperidine (TCP) Based Irreversible Alkylators of the Phencyclidine (PCP) Recognition Site. *Synlet.* **1991**, 654–656.
(25) Casalotti, S. O.; Kozikowski, A. P.; Fauq, A.; Tückmantel, W.; Krueger, K. E. Design of an Irreversible Affinity Ligand for the Phencyclidine Recognition Site on *N*-Methyl-D-aspartate-Type Glutamate Receptors. *J. Pharmacol. Exp. Ther.* **1992**, *260*, 21–28.
(26) Wong, E. H. F.; Kemp, J. A.; Priestley, T.; Knight, A. R.; Woodruff, G. N.; Iversen, L. L. The Anticonvulsant MK-801 Is a Potent *N*-Methyl-D-aspartate Antagonist. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 7104–7108.
(27) Loo, P. A.; Braunwalder, A. F.; Williams, M.; Sills, M. A. The Novel Anticonvulsant MK-801 Interacts with Central Phencyclidine Recognition Sites in Rat Brain. *Eur. J. Pharmacol.* **1987**, *135*, 261–263.
(28) Sircar, R.; Rappaport, M.; Nichtenhauser, R.; Zukin, S. R. The Novel Anticonvulsant MK-801: a Potent and Specific Ligand of the Brain Phencyclidine/σ-Receptor. *Brain Res.* **1987**, *435*, 235–240.
(29) Wong, E. H. F.; Knight, A. R.; Woodruff, G. N. [^{3}H]MK-801 Labels a Site on the *N*-Methyl-D-aspartate Receptor Channel Complex in Rat Brain Membranes. *J. Neurochem.* **1988**, *50*, 274–281.
(30) Snell, L. D.; Yi, S.-J.; Johnson, K. M. Comparison of the Effects of MK-801 and Phencyclidine on Catecholamine Uptake and NMDA-Induced Norepinephrine Release. *Eur. J. Pharmacol.* **1988**, *145*, 223–226.
(31) Rothman, R. B.; Reid, A. A.; Monn, J. A.; Jacobson, A. E.; Rice, K. C. The Psychotomimetic Drug Phencyclidine Labels Two High Affinity Binding Sites in Guinea Pig Brain: Evidence for *N*-Methyl-D-aspartate-Coupled and Dopamine Reuptake Carrier-Associated Phencyclidine Binding Sites. *Mol. Pharmacol.* **1989**, *36*, 887–896.
(32) Sonders, M. S.; Barmettler, P.; Lee, J. A.; Kitahara, Y.; Keana, J. F. W.; Weber, E. A. Novel Photoaffinity Ligand for the Phencyclidine Site of the *N*-Methyl-D-aspartate Receptors Labels a M_r 120,000 Polypeptide. *J. Biol. Chem.* **1990**, *265*, 6776–6781.
(33) Lamanec, T. R.; Bender, D. R.; DeMarco, A. M.; Karady, S.; Reamer, R. A.; Weinstock, L. M. α-Effect Nucleophiles: A Novel and Convenient Method for the Synthesis of Dibenzo[*a*,*d*]cycloheptenimines. *J. Org. Chem.* **1988**, *53*, 1768–1774.
(34) Anderson, P. S.; Christy, M. E.; Evans, B. E. 5-Substituted 10,-11-Dihydro-5H-dibenzocyclo[*a*,*d*]hepten-5,10-imines. U. K. Patent GB 2,004,872, 1982; *Chem. Abstr.* **1979**, *91*, 5126j. See also: Anderson, P. S.; Christy, M. E.; Evans, B. E. 5-Alkyl or Hydroxyalkyl Substituted 10,11-Dihydro-5H-dibenzo[*a*,*d*]cycloheptene-5,10-imines and Their Anticonvulsant Use. U. S. Patent US 4,399,141, 1983; *Chem. Abstr.* **1984**, *100*, 68191b.
(35) Rittle, K. E.; Evans, B. E.; Bock, M. G.; DiPardo, R. M.; Whitter, W. L.; Veber, D. F.; Freidinger, R. M. A New Amine Resolution Method and its Application to 3-Aminobenzodiazepines. *Tetrahedron Lett.* **1987**, *28*, 521–522.
(36) Houben-Weyl *Methoden der Organischen Chemie*; Thieme: Stuttgart, 1983; Band E4, pp 415–420.
(37) Dyson, G. M.; George, H. J. The Reactions of Thiocarbonyl Chloride. Part I. Reactions with Aromatic Primary Amino Compounds. *J. Chem. Soc.* **1924**, *124*, 1702–1708.
(38) Allenstein, E.; Quis, R. *Chem. Ber.* **1964**, *97*, 3162–3172.

(39) Calculated from the X-ray structure of (+)-3. George, C., unpublished results; Springer, J. P., personal communication.
(40) Rothman, R. B.; Danks, J. A.; Jacobson, A. E.; Burke, T. R., Jr.; Rice, K. C.; Pert, C. B. Tritiated-6-beta-fluoro-6-deoxy-oxymorphone: A Highly Selective Ligand for the Opiate Mu Receptor Whose Binding is Characterized by Low Nonspecific Binding. *Neuropeptides* **1984**, *4*, 311–317.
(41) Yamamura, H. I.; Snyder, S. H. Muscarinic Cholinergic Binding in Rat Brain. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 1725–1729.
(42) Bolger, G. T.; Mezey, E.; Cott, J.; Weissman, B. A.; Paul, S. M.; Skolnick, P. Differential Regulation of Central and Peripheral Benzodiazepine Binding Sites in the Rat Olfactory Bulb. *Eur. J. Pharmacol.* **1984**, *105*, 143–148.
(43) de Costa, B. R.; Bowen, W. D.; Hellewell, S. B.; Walker, M. J.; Thurkauf, A.; Jacobson, A. E.; Rice, K. C. Synthesis and Evaluation of Optically Pure [^{3}H]-(+)-Pentazocine, a Highly Potent and Selective Radioligand for σ Receptors. *FEBS Lett.* **1989**, *251*, 53–58.
(44) Reid, A. A.; Kim, C.-H.; Thurkauf, A.; Monn, J. A.; de Costa, B. R.; Jacobson, A. E.; Rice, K. C.; Bowen, W. D.; Rothman, R. B. Wash-Resistant Inhibition of Phencyclidine- and Haloperidol-Sensitive Sigma Receptor Sites in Guinea Pig Brain by Putative Affinity Ligands: Determination of Selectivity. *Neuropharmacology* **1990**, *29*, 1047–1053.
(45) Leeson, P. D.; Carling, W. W.; James, K.; Smith, J. D.; Moore, K. W.; Wong, E. H. F.; Baker, R. Role of Hydrogen Bonding in Ligand Interaction with the *N*-Methyl-D-aspartate Receptor Channel. *J. Med. Chem.* **1990**, *33*, 1296–1305.
(46) Thompson, W. J.; Anderson, P. S.; Britcher, S. F.; Lyle, T. A.; Thies, J. E.; Magill, C. A.; Varga, S. L.; Schwering, J. E.; Lyle, P. A.; Christy, M. E.; Evans, B. E.; Colton, C. D.; Holloway, M. K.; Springer, J. P.; Hirshfield, J. M.; Ball, R. G.; Amato, J. S.; Larsen, R. D.; Wong, E. H. F.; Kemp, J. A.; Tricklebank, M. D.; Singh, L.; Oles, R.; Priestly, T.; Marshall, G. R.; Knight, A. R.; Middlemiss, B. N.; Woodruff, G. N.; Iversen, L. L. Synthesis and Pharmacological Evaluation of a Series of Dibenzo[*a*,*d*]cycloalkenimines as *N*-Methyl-D-aspartate Antagonists. *J. Med. Chem.* **1990**, *33*, 789–808.
(47) Kamenka, J. M.; Chiche, B.; Goudal, R.; Geneste, P.; Vignon, J.; Vincent, J. P.; Lazdunski, M. Chemical Synthesis and Molecular Pharmacology of Hydroxylated 1-(1-Phenylcyclohexyl)piperidine Derivatives. *J. Med. Chem.* **1982**, *25*, 431–435.
(48) Geneste, P.; Kamenka, J. M.; Ung, S. N.; Herrmann, P.; Goudal, R.; Trouiller, G. *Eur. J. Med. Chem.* **1979**, *14*, 301–308.
(49) Jacobson, A. E.; Linders, J. T. M.; Rice, K. C.; Monn, J. A.; Mattson, M. V.; George, C. Three Generations of Electrophilic Affinity Ligands for Phencyclidine Binding Sites. In Harris, L. R. (Ed.), *Problems of Drug Dependence 1991: Proceedings of the 53rd Annual Scientific Meeting, NIDA Research Monograph 119*, Supt. of Docs., U.S. Govt. Print. Off.: Washington, D.C., 1992, 81–85.
(50) Vignon, J.; Vincent, J. P.; Bidard, J. N.; Kamenka, J. M.; Geneste, P.; Monier, S.; Lazdunski, M. Biochemical Properties of the Brain Phencyclidine Receptor. *Eur. J. Pharm.* **1982**, *81*, 531–542.
(51) Linders, J. T. M.; Mattson, M. V.; Furlano, D. C.; Jacobson, A. E., unpublished results.
(52) Sheldrick, G. M. *SHELXTL80. An Integrated System for Solving, Refining, and Displaying Crystal Structures from Diffraction Data.* University of Göttingen, Federal Republic of Germany, 1980.
(53) H. J. Motulsky, *GraphPAD*; ISI Software, Institute for Scientific Information, Philadelphia, PA 19104.
(54) Argos, P.; Barr, R. E.; Weber, A. H. The Crystal and Molecular Structure of 1-(1-Phenylcyclohexyl)piperidine Hydrochloride. *Acta Crystallogr.* **1970**, *B26*, 53–61.
(55) Molecular Simulations Inc., 16 New England Executive Park, Burlington, MA 01803–5297.
(56) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. CHARMM: a Program for Macromolecular Energy, Minimizations and Dynamics Calculations. *J. Comput. Chem.* **1983**, *4*, 187–217.
(57) Cheng, Y. C.; Prusoff, W. H. Relationship Between the Inhibition Constant (K_i) and the Concentration of Inhibitor which Causes Fifty Percent Inhibition (IC_{50}) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3103.