Identification, Synthesis, and Characterization of a Unique Class of *N*-Methyl-D-aspartate Antagonists. The 6,11-Ethanobenzo[*b*]quinolizinium Cation

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Received August 26, 1994®

A series of novel *N*-methyl-D-aspartate antagonists acting at the phencyclidine site has been identified. Compound **2** has a $K_i = 8 \pm 1$ nM (vs [^{3}H]thienylcyclidine, [^{3}H]TCP) as a mixture of enantiomers. Resolution and further testing indicate that (−)-**2**, $K_i = 4 \pm 0.7$ nM, is a potent and selective TCP site ligand with neuroprotective activity in cultured neurons in the presence of excitotoxic concentrations of NMDA ($IC_{50} = 26$ nM). Compound (−)-**2** is >1000-fold selective for the TCP site vs a panel of receptor types including opiate, adrenergic, serotonergic, dopamine, adenosine, dihydropyridine, and benzodiazepine and displays increased selectivity for the activated (open) NMDA receptor–ion channel complex vs PCP and MK801 as measured by patch recordings in cultured, voltage-clamped neurons. Highly enhanced "open-channel" selectivity leads to tentative classification of these ligands as *uncompetitive* vs NMDA. Ligands with these characteristics may enable deconvolution of the pharmacologic effects associated with typical noncompetitive NMDA antagonists. We report here on the identification, synthesis, and activity of compounds of this structural class.

Introduction

Several classes of excitatory amino acid (EAA) receptors have been identified with unique ligand specificities, cellular distributions, and (patho)physiological roles.[1] The delineation of EAA receptor subtypes and characterization of the several allosteric ligand sites associated with the *N*-methyl-D-aspartate (NMDA)-specific receptor–ionophore complex demonstrates the potential complexity of activity mediated by this system but also reveals many potential sites for molecular intervention.[2,3] In particular, NMDA sensitive receptors are involved in neuronal plasticity processes and, pathologically, in excitotoxicity. A site located within the ion channel specifically activated by NMDA is labeled by [^{3}H]TCP ("thienylcyclidine", the thiophene analog of PCP) and is the binding site for phencyclidine, ketamine, dextrorphan, and MK801. These compounds inhibit Ca^{2+} influx through the NMDA-sensitive receptor–ionophore complex and are neuroprotective in several laboratory models of neurodegenerative (excitotoxic) conditions.[4] NMDA receptor/ionophore inhibitors potentially have utility in several neurodegenerative conditions including ischemic stroke, head trauma, epilepsy, AIDS dementia, and Huntington's and Alzheimer's diseases.[5] Clinical investigations with several agents operating at the NMDA receptor–ionophore complex are currently underway; however, attenuation of the neurological loss and sequelae in these diseases with NMDA antagonists has yet to be conclusively demonstrated in man.

A common consequence of intervention at the TCP site is the development of behavioral and autonomic responses, observable in preclinical models, which when manifested clinically may confound medical care. Psychotomimetic activity observed in clinical trials with PCP halted its further development as an anesthetic,[6] and MK801 is reported to produce a PCP-like stereotype in preclinical behavioral and discrimination paradigms.[7] In addition to the psychotomimetic effects, a set of autonomic responses manifested principally as a reduction in blood pressure,[8] and reversible vacuolization[9] produced by the prototypical noncompetitive channel blocker MK801, are further impediments to clinical development. Clearly, dissociation of neuroprotective efficacy from the behavioral, autonomic, and neurotoxic effects of TCP site ligands would result in improved clinical potential for the therapeutic class.

Interaction with the TCP site, located in the ion channel,[10] is not a well-understood event. Possible *access* paths include (a) directly through the activated ion channel from the extracellular environment (open-channel state), (b) through the ion channel from the intracellular environment, and (c) via a membrane-mediated path where a lipophilic (neutral form) ligand gains access to the channel binding site via diffusion within the bilayer (open- or closed-channel state).[11] To this end, experimental conditions devised to measure "closed- vs open-" state antagonism, binding, kinetics, and the temperature effects on state-dependent binding have been reported.[12] It is likely that, for most ligands, combinations of these paths are involved, a result of factors specific to the test conditions and the nature of the ligand (see Figure 1). Conceptually, a sufficiently

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® Abstract published in *Advance ACS Abstracts*, December 1, 1994.

0022-2623/94/1837-4438$04.50/0

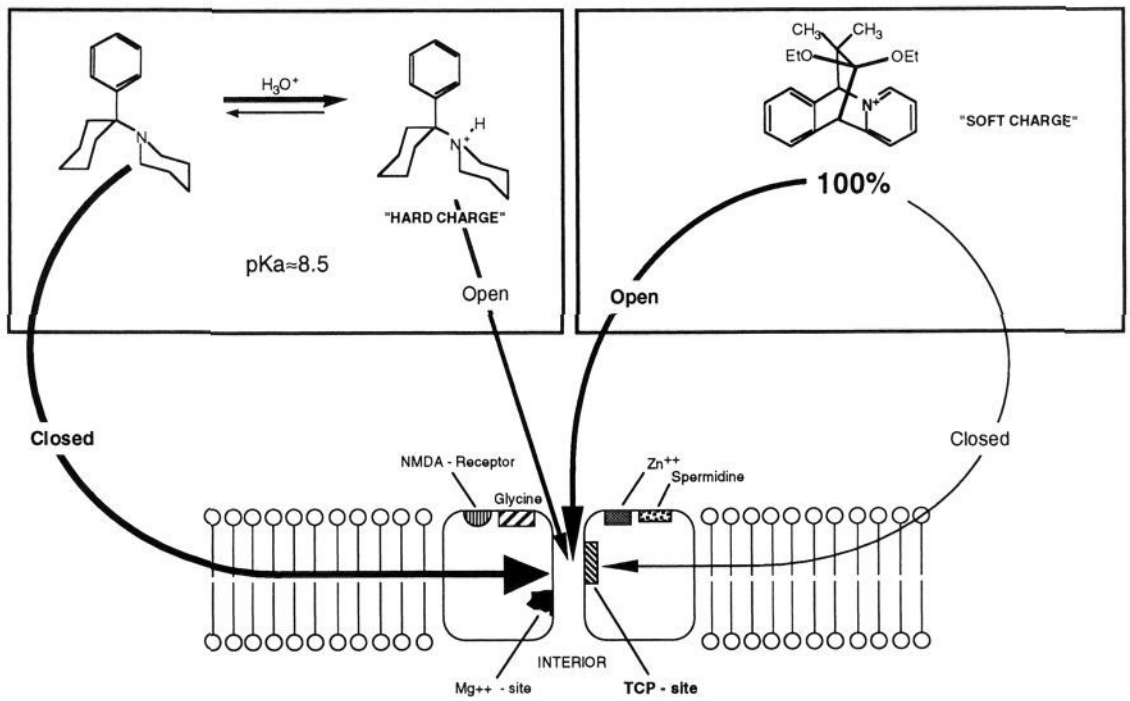


Figure 1. Possible access paths to the TCP binding site.

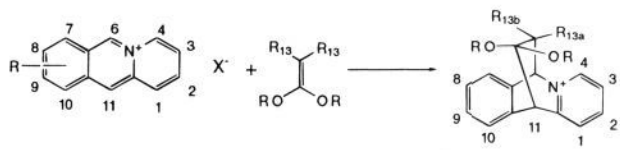
hydrophilic, potent ligand which exists in only one (polar) ionization state may provide a tool which could deconvolute these effects.

The mixture of geometric isomers **1a/b** was identified during high-volume screening for PCP ligands and was found to be highly selective for the TCP binding site. We report here that the derivative 6,11-ethanobenzo[*b*]quinolizinium cation **2** ($\log D = -1.3$) is a potent and selective ligand for the TCP site on the NMDA receptor–ionophore complex and is neuroprotective in primary cultures of cortical neurons in the presence of excitotoxic concentrations of NMDA.[13] Moreover, single-cell voltage-clamp studies in cortical neurons show that (−)-**2** is highly selective for the "open" NMDA ion channel, compared to PCP or MK801. This characteristic qualifies (−)-**2** as an uncompetitive NMDA antagonist.

Chemistry

Methodologies reported in the literature for the preparation of compounds of this type have proved satisfactory for the synthesis of most analogs reported here.[14] Inverse electron demand Diels–Alder reaction of an electron rich olefin with a substituted benzo[*b*]-quinolizinium perchlorate, hexafluorophosphate, or chloride, etc., provides the 6,11-ethano adducts in excellent yield. SAR development required regiospecific synthesis of several 10-(mono)substituted benzo[*b*]quinolizinium salts ("azoniaanthracenes" in many early reports). This was highly impractical using the known methodologies. Therefore, we developed a regiocontrolled method for their preparation during the course of this investigation.[15] In this report, we focus on those cycloadducts derived from oxygen-bearing olefins, specifically ketene acetals and enol ethers (Schemes 1–3). This efficient process is completely regiospecific as shown in Scheme 1. Analogs prepared in this general manner are shown in Table 1. Unsymmetric ketene acetals give a mixture of diastereomers at C13 although not in a 1:1 ratio. Addition of a methyl group to position 7 resulted in

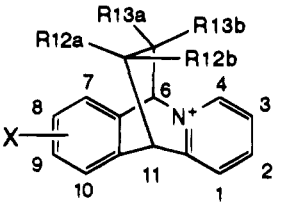
Scheme 1



nearly a 5-fold shift in the diastereomer ratio of **1a** to **1b**, Scheme 2. Assignment of the relative stereochemistry for these isomers was unequivocally established by the presence of a definitive NOE between H4 and the CH_3 at position 13 in **1a** and clear NOE's between H13 and H4, and CH_3 and H7 in **1b**. Enol ethers, symmetrical at carbon 2, undergo cycloaddition to give an unequal diastereomeric mixture at position 12, Scheme 3. The isomer ratio is near 4:1, preferring alkoxy syn to the benzo ring in the final product. Addition of a methyl group to position 10 reverses selectivity to 1:4, favoring alkoxy trans to the benzo ring. The product diastereomer ratio is insensitive to the nature of the counterion associated with the starting benzoquinolizinium cation. Here, the stereochemistry was assigned on the basis of the presence of a definitive NOE between H12 and H1 in **7a**. In both cases, the isomers can be separated by chromatography on silica gel or by repeated fractional crystallization.

A critical aspect of our chemical effort in this class of molecules was to develop methodology providing the enantiomers of interesting analogs. We initially undertook three approaches: (1) fractional crystallization of diastereomeric ion pairs, (2) separation of covalent diastereomeric derivatives, and (3) asymmetric synthesis. Our approach to asymmetric synthesis was predicated on a presumed close interaction between the substituent on C10 of the benzo[*b*]quinolizinium cation and the optically active, C_2 symmetric ketene acetal derived from 2,3-butanediol (as described above), Scheme 4. Initial experiments without incorporation of a substituent at position 10 gave no diastereomeric excess with the chiral ketene acetal derived from 2,3-butane-

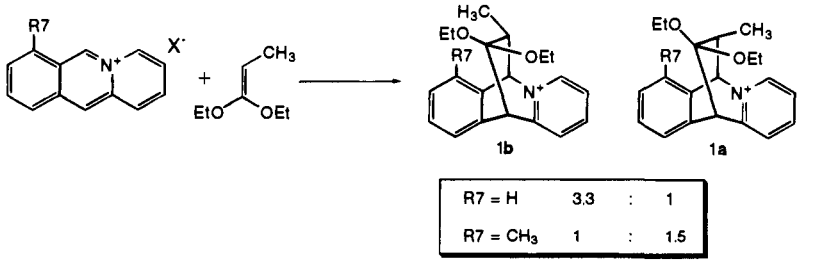
Table 1



no.	X	R12 a	R12 b	R13 a	R13 b	counterion	TCP K_i (nM)[a]	method[b]	mp (°C)
1a	H	OCH_2CH_3	OCH_2CH_3	H	CH_3	ClO_4^-	115 ± 23	B/EPAW	159–162
1b	H	OCH_2CH_3	OCH_2CH_3	CH_3	H	ClO_4^-	34 ± 7	B/EPAW	177–180
(±)-**2**	H	OCH_2CH_3	OCH_2CH_3	CH_3	CH_3	Cl^-	8 ± 1		124–126
(+)-**2**	H	OCH_2CH_3	OCH_2CH_3	CH_3	CH_3	Cl^-	11 ± 2		181 (dec)
(−)-**2**	H	OCH_2CH_3	OCH_2CH_3	CH_3	CH_3	Cl^-	4 ± 0.7		110–112
3	H	OCH_3	OCH_3	CH_3	CH_3	ClO_4^-	103 ± 8.6	ref 14d	
4	H	OCH_2CH_3	OCH_2CH_3	H	H	Cl^-	3805 ± 488	ref 14b	124–126
5	H	=O		CH_3	CH_3	Cl^-	>10 000	ref 14d	143–145
6	H	$OCH_2CH_2CH_3$	$OCH_2CH_2CH_3$	CH_3	CH_3	ClO_4^-	12 ± 2	A/EtOAc	150–155
7a	H	OCH_2CH_3	H	CH_3	CH_3	ClO_4^-	465 ± 67	B, 1/iPrOH	106–108
7b	H	H	OCH_2CH_3	CH_3	CH_3	ClO_4^-	1878 ± 276	B, 1/iPrOH	158–164
8	H	$-OCH_2CH_2CH_2O-$		CH_3	CH_3	Cl^-	2899 ± 442	A/iPrOH	123–125
9	H	$-OCH_2CH_2O-$		CH_3	CH_3	ClO_4^-	3456 ± 576	A/iPrOH	240–242
10	H	OCH_2CH_3	OCH_2CH_3	CH_2CH_3	CH_2CH_3	ClO_4^-	11 ± 1.5	B, 1/H_2O	121 (dec)
11	H	OCH_2CH_3	OCH_2CH_3	OCH_2CH_3	OCH_2CH_3	ClO_4^-	4987 ± 422	B	202–203
12	9–NO_2	OCH_2CH_2	OCH_2CH_3	CH_3	CH_3	ClO_4^-	2764 ± 163	A/EtOAc	193–195
13	9–Br	OCH_2CH_3	OCH_2CH_3	CH_3	CH_3	ClO_4^-	52 ± 13	B, 2/H_2O–MeOH	163–165
14	9–OCH_3	OCH_2CH_3	OCH_2CH_3	CH_3	CH_3	PF_6^-	4 ± 1	A/H_2O	76–81
15	9-Cl	OCH_2CH_3	OCH_2CH_3	CH_3	CH_3	ClO_4^-	12 ± 0.5	A/EtOAc–MeOH	96–101
16	9–CF_3	OCH_2CH_3	OCH_2CH_3	CH_3	CH_3	Cl^-	401 ± 14	A/H_2O	foam
17	10–OCH_3	OCH_2CH_3	OCH_2CH_3	CH_3	CH_3	Cl^-	13 ± 1.7	A/H_2O	foam
18	10–CH_3	OCH_2CH_3	OCH_2CH_3	CH_3	CH_3	ClO_4^-	46 ± 0.6	B, 1/H_2O	86–91
19	10–Br	OCH_2CH_3	OCH_2CH_3	CH_3	CH_3	ClO_4^-	132 ± 18	B, 1/H_2O–MeOH	188–194
20	10–OAc	OCH_2CH_3	OCH_2CH_3	CH_3	CH_3	ClO_4^-	26 ± 5	A/iPrOH	135–137
21	10–OH	OCH_2CH_3	OCH_2CH_3	CH_3	CH_3	ClO_4^-	8 ± 0.7		100 (dec)
22	10–OiPr	OCH_2CH_3	OCH_2CH_3	CH_3	CH_3	Cl^-	48 ± 4	A/H_2O	74–80
23	8–OCH_3	OCH_2CH_3	OCH_2CH_3	CH_3	CH_3	Cl^-	123 ± 24	A/H_2O	foam
24	7–OCH_3	OCH_2CH_3	OCH_2CH_3	CH_3	CH_3	PF_6^-	1843 ± 53	A/iPrOH	124 (dec)
25	7–CH_3	OCH_2CH_3	OCH_2CH_3	CH_3	CH_3	Cl^-	455 ± 20	A/H_2O	49–56
26	11–CH_3	OCH_2CH_3	OCH_2CH_3	CH_3	CH_3	ClO_4^-	11 ± 1	B, 1/Et_2O–EtOAc	144–146
27	4–CH_3	OCH_2CH_3	OCH_2CH_3	CH_3	CH_3	Cl^-	314 ± 6	B, 1/none	foam
31	7,10 $(OCH_3)_2$	OCH_2CH_3	OCH_2CH_3	CH_3	CH_3	ClO_4^-	>10 000	B, 1/H_2O–MeOH	foam
32	1–OCH_3	OCH_2CH_3	OCH_2CH_3	CH_3	CH_3	ClO_4^-	26 ± 2	B, 1/H_2O–MeOH	172–174
(+)-MK801							2.4 ± 0.3		
PCP							38 ± 2		

[a]Mean ± SEM for at least three separate determinations in triplicate unless otherwise indicated. [b]Given as purification method, chromatography solvent/crystallization solvent(s) for those compounds not individually detailed in the Experimental Section.

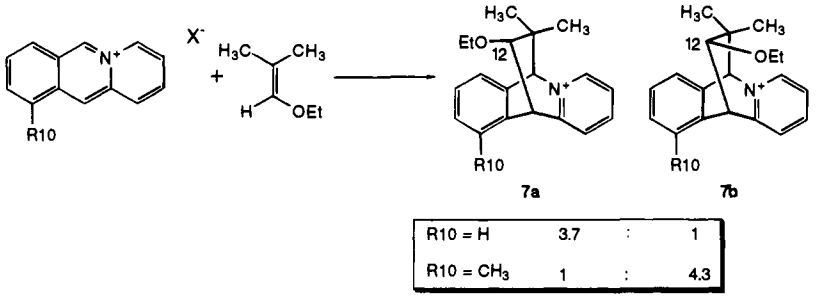
Scheme 2



diol, suggesting a need to further differentiate the *si* and *re* faces of the tricycle. Various substituents at position 10 were tried with *tert*-butyl providing a disappointing maximum diastereomeric excess of 3.2:1. Initial samples of (+)-**2** and (−)-**2** were obtained using the process depicted in Scheme 5. Preparative scale chromatographic separation of the optically active diastereomeric cyclic ketals **28a/b** (derived from optically active (2*R*,4*R*)-2,4-pentanediol) on C-18 reverse phase column packing provided resolution of this isoquinuclidinium system. The resulting optically pure diastereomers were converted to ketones (−)-**5** and (+)-**5** in hot 12 N HCl in >90% yield.

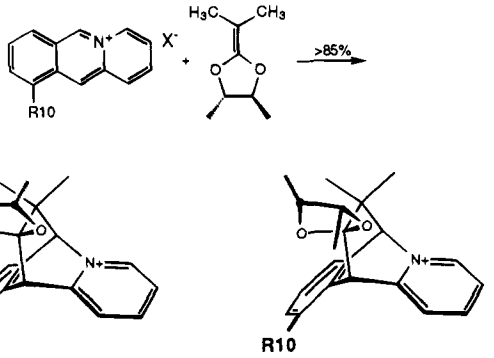
Conversion of ketones (−)-**5** and (+)-**5** to the corresponding diethyl ketals (−)-**2** and (+)-**2** proved exceedingly problematic. Lack of reactivity at the 12-oxo position is possibly due to the cationic nature of the material which retards a second protonation during standard ketalization procedures. In addition, a hypothesized orbital interaction between the carbonyl π system and the π systems of the two flanking aromatic rings might affect the carbonyl HOMO and LUMO, slowing reaction with electrophiles (on oxygen) and reaction with nucleophiles at carbon.[16] Quantum mechanical examination[17] and comparison of the parent 6,11-ethano-6,11-dihydrobenzo[*b*]quinolizinium cation **A**

Scheme 3



R10 = H	3.7	:	1
R10 = CH_3	1	:	4.3

Scheme 4



R10 = H	1	:	1
CH_3	1	:	1.1
OCH_3	1	:	2.2
$C(CH_3)_3$	1	:	3.2

with ketone **5** and 9,10-ethano-11-oxo-9,10-dihydroanthracene **B**, depicted in Figure 2, illustrate this effect. Lack of symmetry in 6,11-ethanodihydrobenzo[*b*]quinolizinium cation **5** and subsequent transfer of π-electron density have dramatic effects on the HOMO and LUMO of the carbonyl at position 12 when compared to the anthracene system **B**. The effect visualized in Figure 2 is consistent with our experimentally observed reactivity of **5**.[18] After an extensive investigation of methods, ketal formation was accomplished in a high-pressure reactor: 10 k Bar, $(EtO)_4Si$, TFA, CH_2Cl_2. The scope of this robust process is under further exploration. Scale quantities (10–50 g) of the enantiomers of **2** were subsequently obtained by crystallization of the ion pair formed with K^+DBT^- after removal of residual KCl. Optical purity is achieved after a final crystallization from dichloroethane. The extent of resolution during recrystallizations and the optical purity of the final material were verified by HPLC on a Cyclobond I-RN column[19] which provided base line separation of (+)-**2** and (−)-**2**.

Results and Discussion

Receptor affinity ([^{3}H]TCP) demonstrated exceptional sensitivity to initial substitution at position 13 (R13a and R13b, Table 1). Addition of a methyl group to unsubstituted compound **4** results in at least a 30-fold increase in affinity (1**a**) with a 3-fold preference for diastereomer 1**b**. Gem-dimethyl substitution at this position (compound **2**) gave nearly 500-fold improvement over **4**. The gem-diethyl analog **10** had slightly reduced affinity compared to **2**. Further extension of this alkyl substituent did not improve potency. Variation of the substituents at position 12 indicated a preference for the diethoxy ketal. Cyclic ketals **8** and **9** had dramatically reduced affinity. Although binding data for only two cyclic ketals are reported here, all others we have prepared show poor receptor affinity.[20] Substitution at positions 1–10, where synthetically accessible, led to reductions in affinity with only the 9-methoxy analog displaying a 2-fold increase in affinity. Little effect on affinity was observed with bridgehead substitution at position 11.[21]

Receptor selectivity for the enantiomers (+)-**2** and (−)-**2** is presented in Table 2. Although neither enantiomer demonstrated appreciable displacement of the indicated tritiated ligand at 10 μM, (−)-**2** had, in general, a slightly cleaner profile. Minor cross-affinity is observed in two cases: 50% displacement of [^{3}H]QNB and [^{3}H]naloxone. If these values are taken to approximate the IC_{50} or K_i, then (−)-**2** has at least 2500-fold higher affinity for the [^{3}H]TCP site vs this panel of receptor types.

In vitro neuroprotective efficacy for representative compounds is presented in Table 3. The assay is a measure of a test compound's ability to protect cultured mouse cortical neurons from an excitotoxic exposure to NMDA as assessed by lactate dehydrogenase activity released into the growth media. Compound (−)-**2** demonstrated potent neuroprotective activity under these conditions. (±)-**17** was the most efficacious compound tested with an IC_{50} = 22 nM. The 9-methoxy derivative, **14**, was 5–7-fold less effective than anticipated by its binding affinity. However, binding affinity correlated fairly well with efficacy in this assay (see Figure 4).

Electrophysiology. As described in the Introduction, we hypothesized that a sufficiently polar (permanently charged) TCP site ligand would demonstrate improved selectivity for blockade of the open or activated NMDA-sensitive receptor–ionophore complex. Conditions which could reveal this effect were devised. Primary cultures of mouse cortical neurons were voltage-clamped at −60 mV to enable single-cell recordings of NMDA-induced current. Two paradigms were utilized in order to measure the ability of a compound to block NMDA channels: open-channel block was assessed by application of NMDA concurrently with the test compound; closed-channel block was assessed by inhibition of NMDA responses following the incubation of cells for 30 min with test compound and 10 μM 7-chlorokynurenic acid, in the absence of NMDA stimulation. In the first paradigm, NMDA opens channels that are subsequently blocked following entry of inhibitor which binds to the TCP site. This leads to a decay in NMDA-induced current with time as compared to application of NMDA alone. In the closed-channel protocol, NMDA responses are assessed following re-

Scheme 5

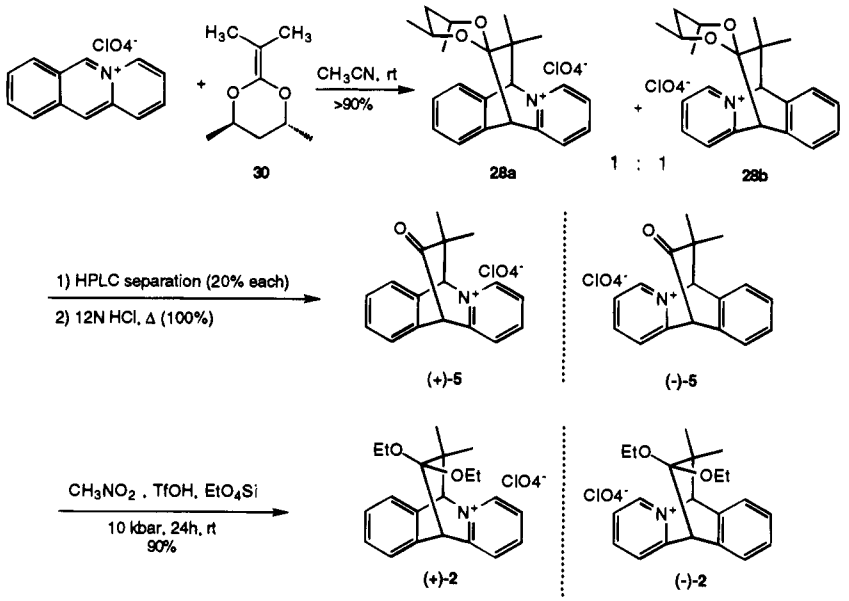


Table 2. Receptor Selectivity of (+)- and (−)-**2**

		% inhibition at 10 μM	
binding site	(ligand)	(+)-**2**	(−)-**2**
opiate	([^{3}H]naloxone)	73	51
α-1	([^{3}H]prazocine)	51	23
α-2	([^{3}H]rauw.)	52	32
BDZ	([^{3}H]flunarazine)	14	−3[a]
β	([^{3}H]DHAP)	4	3
5-HT	([^{3}H]LSD)	12	11
Ca^{2+}	([^{3}H]nitrendipine)	−23	−6
musc	([^{3}H]QNB)	53	50
H1	([^{3}H]pyrilamine)	55	0
D1	([^{3}H]SCH23390)	7	1
D2	([^{3}H]raclopride)	20	9
A1, A2	([^{3}H]NECA)	1	3
σ	([^{3}H]DTG)	31	21

[a]Indicates stimulation of binding of the ^{3}H-ligand used.

Table 3. Neuroprotection in Cell Culture

no.	TCP K_i (nM)	IC_{50} (nM)[a]
1a	115 ± 23	810
1b	34 ± 7	1160
4	3805 ± 488	95 000
6	12 ± 2	85
12	2764 ± 163	15 270
13	52 ± 13	173
14	4 ± 1	157
17	13 ± 1.7	22
19	132 ± 18	1040
26	11 ± 1	32
(+)-**2**	11 ± 2	50
(−)-**2**	4 ± 0.7	26

[a]Neuroprotection in cultured mouse cortical neurons.

moval of inhibitor and glycine site antagonist; therefore, inhibition principally depends on binding of inhibitor to the closed channel, presumably via a lipid membrane route. Under these conditions, with inhibitor bound prior to application of NMDA, compound (±)-**2** is *greater than* 400-fold selective favoring the conditions of open-channel block . In contrast, (+)-MK801 is effective under both experimental conditions, favoring open-channel conditions by 60-fold, as shown in Table 4. Open-channel block depends on the degree of agonist stimulation and would appear uncompetitive. Closed-channel block allows inhibitor to establish equilibrium in the absence of agonist and thus should produce an apparent noncompetitive inhibition profile at equilibrium. At low agonist concentrations, a highly selective open-channel blocker is predicted to produce much less inhibition (less disruption of normal glutamate function) compared to a noncompetitive inhibitor and therefore may differentially affect neural systems in the central nervous system.

Conclusions

We have identified a novel class of ligand for the PCP binding site in the NMDA-sensitive receptor−ionophore complex. Synthesis of the analogs described here has provided compounds which competitively displace TCP from this ion channel,[22] are greater than 1000-fold selective for the TCP site, and appear to interact with this site in a selective manner. The prototypical member of this class of ligand, (−)-**2**, demonstrates highly selective blockade of current flow in voltage-clamped neurons under experimental conditions providing the open-channel state and has little efficacy in blocking current flow under experimentally induced closed-channel conditions. These results suggest that (−)-**2** is a functionally uncompetitive antagonist of glutamate—a highly "use-dependent" channel blocker. Compound (−)-**2** is an effective neuroprotective agent in cultured mouse cortical neurons at nanomolar concentrations and has been examined in experimental models of cerebral ischemia and in in vivo behavioral paradigms. (−)-**2** is neuroprotective in the rat middle cerebral artery occlusion model of focal ischemia (MCAO, 40%−70% reduction of infarct volume at 1.8−6.0 mg/kg/h iv vs vehicle-treated controls, n = 18). Using equivalent administration methods, at these doses and higher (up to the MTD) (−)-**2** does not produce PCP-like stereotypes or ataxia in conscious rats.[23] Detailed results of these studies will be reported elsewhere.[24]

Experimental Section[25]

Melting points were determined on a Mel-Temp apparatus and are uncorrected. Infrared spectra were recorded on a Nicolet 20SX FTIR spectrometer. NMR spectra were acquired in the indicated solvent on a JEOL-FX270, General Electric QE-300, or Bruker-AC200 FTNMR spectrometer. HETCOR (^{1}H−^{13}C correlation), DEPT, COSY, and NOESY experiments were utilized to assist in peak assignments and the assignment of stereochemistry. Mass spectra were recorded on a Nermag

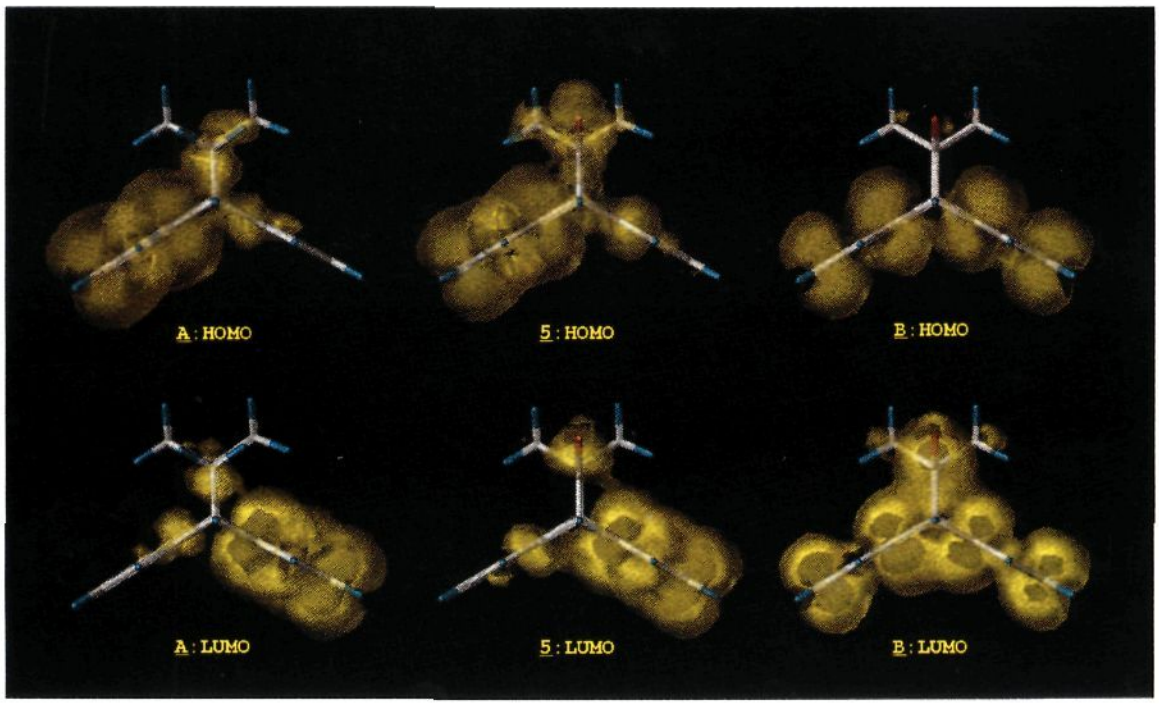


Figure 2. Comparison of frontier molecular orbitals of ketone **5** with 12-dihydro-6,11-ethanobenzo[*b*]quinolizidine cation **A** and 9,10-ethano-11-oxoanthracene **B**. Orbital density plots were calculated at the RHF/PM3 level.

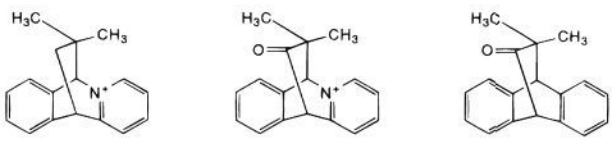


Figure 3. Representations of model compounds shown with MO (psi²) plots in Figure 2.

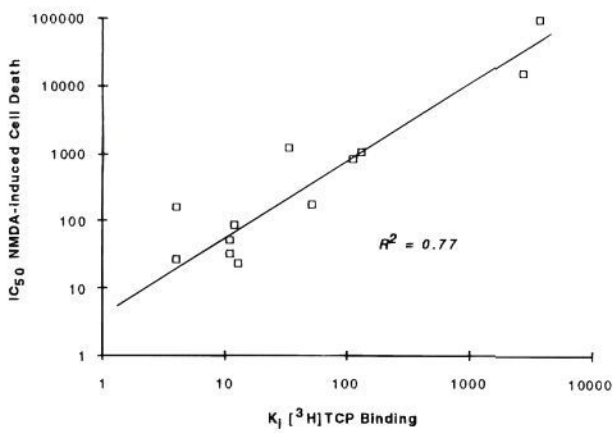


Figure 4. Correlation of TCP site binding affinity with neuroprotection in cell culture.

Table 4. Comparison of Open- vs Closed-Channel Inhibition

	MK801	(±)-**2**
open-channel IC_{50} (μM)	0.1	0.26
closed-channel IC_{50} (μM)	5.8	>100
ratio (CC/OC)	*58*	*>384*

R10/10 spectrometer coupled to a Varian 3400 gas chromatograph or on a JEOL JMS-01SC spectrometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Where analyses are indicated only by symbols of the elements, analytical results are within ±0.45% of the theoretical values, except where indicated by individual analyses. Thin layer chromatography (TLC) was performed on E. Merck (5 × 20 cm) Kieselgel 60 F-254 plates. Preparative chromatography was performed using a Buchi B680 MPLC system coupled to an ISCO UV detector and fraction collector or by the flash method as described by Stille.[26] Columns were packed with Kieselgel 60, 230–400 mesh. High-boiling solvents (DMF) were stage-dried over molecular sieves.[27] Anhydrous THF was distilled from sodium–benzophenone ketyl. Alkyllithium reagents were titrated with diphenylacetic acid.[28] Other materials and reagents were purified by standard procedures where needed. Known benzo[*b*]quinolizinium bromides, perchlorates, or hexafluorophosphates were prepared according to the published procedures.[29] Tetraethoxyethylene was purchased from Fluka Chemicals and used without further purification.

General Synthesis Method. A mixture of the benzo[*b*]-quinolizinium perchlorate or hexafluorophosphate (5 mmol) and di(mono)alkylketene dialkylacetal (10 mmol) (or enol ether) in acetonitrile or nitromethane (40 mL) was heated to reflux in an inert atmosphere (N_2 or Ar) for 8 h, or until the reaction was complete by TLC, and then stirred at room temperature for 16 h. The volatiles are removed under reduced pressure, and the residue was treated with ether (75 mL) for trituration in a sonication bath. The resulting solid was collected by filtration, washed successively with water, ether, and then hexanes, and dried under reduced pressure at ambient temperature to afford the crude product.

Purification Methods. A. The crude Diels–Alder product perchlorate or hexafluorophosphate (5 mmol) was dissolved in warm solvent (100–200 mL), filtered through a pad of activated charcoal on a filter aid, and concentrated with warming to one-half of the original volume. After cooling, the solid was collected by filtration, washed with cold, distilled water, and dried under reduced pressure keeping the temperature below 50 °C.

B. The crude Diels–Alder product perchlorate or hexafluorophosphate was dissolved in a minimal amount of solvent (methanol, acetonitrile) and loaded onto a column packed with silica gel. Elution of the material with either 1:1 E/PAW, solvent 1 (ethyl acetate/PAW, PAW = pyridine/acetic acid/water, 55:35:10), or 9:1 methylene chloride/acetonitrile, solvent 2, and removal of the volatiles from pooled fractions provided the pure product which was crystallized as described in method A.

Ion-Exchange Procedure. A column of Dowex 1 × 2-200 ion-exchange resin (300 g) was eluted with 0.5 N HCl until eluant was clear and then washed with distilled water until a pH of about 6.5-7.5 was obtained. A solution of the appropriate 6,11-ethano-6,11-dihydrobenzo[*b*]quinolizinium perchlorate, hexafluorophosphate, or dibenzoyltartarate (ca. 30-200 mmol) in a minimum amount of acetonitrile or methanol was loaded and the column rinsed with distilled water (ca. 1000 mL) until the eluant no longer contained the product as detected by TLC (these materials produce a robust staining with Dragendorf indicator). The water was removed under reduced pressure (lyophilization or rotary evaporation) to provide the pure chloride salt in near quantitative yield.

1,1-[(4′*R*,6′*R*-Dimethyl-1′,3′-dioxan-2′-yl]-2,2-dimethylethylene (30). Part A: To a solution of 15.6 g (0.105 mol) of 2-methyl-1,1,1-triethoxypropane in toluene (150 mL) were added 10.0 g (9.6 mmol) of (2*R*,4*R*)-pentanediol and 0.251 g (1.0 mmol) of pyridinium *p*-toluenesulfonate. The resulting mixture was refluxed with azeotropic removal of water under N_2 for 1.5 h and cooled to room temperature, and potassium *tert*-butoxide (K^+tBuO^-, 1.2 g) was added. After removal of toluene under atmospheric pressure, the reaction mixture was diluted with ether (500 mL) and filtered through a pad of filter aid, and the ether was removed under reduced pressure. The residue was distilled [bp 90-93 °C (18 Torr)] to give (4*R*,6*R*)-dimethyl-2-methoxy-2-(1-methylethyl)-1,3-dioxane (14.0 g, 77%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.8 (6H, m), 1.2 (3H, d, *J* = 6 Hz), 1.4 (3H, d, *J* = 6 Hz), 1.6 (2H, m), 2.1 (1H, m), 3.3 (3H, s), 4.0 (1H, m), 4.2 (1H, m).

Part B: A mixture of the above ortho ester (10.0 g, 5.3 mmol) and aluminum tri-*tert*-butoxide (13.1 g, 5.3 mmol) was heated in an oil bath, and the temperature was slowly raised to 180 °C over a 30 min period and maintained at this temperature for an additional hour, during which time 2-butanol distilled off. The resulting mixture was cooled to room temperature and the residue distilled under reduced pressure [bp 75-78 °C (18 Torr)] to give ketene acetal **30** (5.3 g, 63%) as a colorless liquid: ^{1}H NMR ($CDCl_3$) δ 1.2 (6H, d, *J* = 6 Hz), 1.5 (6H, s), 1.7 (2H, t, *J* = 6 Hz), 4.1 (2H, m).

12,2′-Spiro[(4′*R*,6′*R*)-dimethyl-1′,3′-dioxane]-13,13-dimethyl-6,11-ethano-6,11-dihydrobenzo[*b*]qulnolizinium Perchlorate (28a/b). A mixture of ketene acetal **30** (5.0 g, 0.032 mol) and benzo[*b*]quinolizinium perchlorate (7.2 g, 0.025 mol) in CH_3CN (100 mL) was stirred at room temperature for 20 h and concentrated in vacuo. The residue was triturated with ether, and the solids were collected by filtration and air-dried to provide the 10.3 g (92%) of **28a/b** as a colorless solid. This material was found to be a 1:1 mixture of the two diastereomers as evidenced by NMR: ^{1}H NMR ($CDCl_3$) δ 0.8 (3H, d, *J* = 7 Hz), 0.9 (3H, d, *J* = 7 Hz), 1.05 (6H, m), 1.6 (1H, m), 1.8 (1H, m), 3.8 (1H, m), 4.3 (1H, m), 5.25 (0.5 H, s), 5.35 (0.5 H, s), 6.0 (0.5 H, s), 6.05 (0.5 H, s), 7.25 (1H, m), 7.4-7.7 (4 H, m), 8.0-8.2 (2 H, m), 9.2 (1H, m).

The above diastereomers were separated on a Waters Prep-500 instrument using two DeltaPak C-18 reverse phase silica cartridges in series eluting with 1:1 MeOH/0.05 N NH_4OAc. Fractions were analyzed by analytical HPLC using a C-18 reverse phase column (Dynamax 60A) eluting with the same solvent. The fractions that contained pure diastereomers were combined, and the solvent was removed in vacuo. The residue was taken up in water and treated with 10% $NaClO_4$ solution. The precipitated product was collected by filtration, washed with ether, and air-dried. From 13.0 g of **28a/b** (30 mmol), 1.9 g (4.4 mmol) of pure diastereomer **28a** and 0.7 g (1.6 mmol) of pure diastereomer **28b** were obtained. The remaining fractions contained mixtures. **28a**: colorless solid; mp 191-192 °C; $[\alpha]^{25}_D$ = +3.5° (c = 0.25, $CHCl_3$); IR (KBr) 2924, 1793, 1461, 1101, 624.1 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.82 (3H, s), 0.94 (3H, s), 1.0 (6H, overlapping doublets), 1.6 (1H, m), 1.8 (1H, m), 3.8 (1H, m), 4.3 (1H, m), 5.29 (1H, s), 6.0 (1H, s), 7.25 (2H, m), 7.51 (1H, dd, *J* = 2.4, 6.0 Hz), 7.58 (1H, t, *J* = 6.6 Hz), 7.66 (1H, dd, *J* = 2.1, 6.1 Hz), 8.08 (1H, d, *J* = 7.7 Hz), 8.18 (1H, t, *J* = 7.8 Hz), 9.17 (1H, d, *J* = 5.9 Hz). Anal. ($C_{22}H_{26}ClNO_6$) C,H,N. **28b**: colorless solid; mp 192-93 °C; $[\alpha]^{25}_D$ = -73.0° (c = 0.25, $CHCl_3$); IR (KBr) 2975, 1504, 1379, 1099, 622 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.85 (3H, s), 0.92 (3H, s), 1.03 (3H, d, *J* = 6.3 Hz), 1.08 (3H, d, *J* = 6.3 Hz), 1.6 (1H, m), 1.8 (1H, m), 3.75 (1H, m), 4.24 (1H, m), 5.14 (1H, s), 6.05 (1H, s), 7.33 (2H, m), 7.40 (1H, m), 7.64 (1H, m), 7.70 (1H, dd, *J* = 3.4, 6.2 Hz), 7.99 (1H, d, *J* = 7.8 Hz), 8.19 (1H, t, *J* = 8.4 Hz), 9.28 (1H, *J* = 6.0 Hz). Anal. ($C_{22}H_{26}ClNO_6$) C,H,N.

(+)-12-Oxo-13,13-dimethyl-6,11-ethano-6,11-dihydrobenzo[*b*]quinolizinium Perchlorate [(+)-5]. A mixture of 0.805 g (1.8 mmol) of (+)-12,12-[(4*R*,6*R*)-dimethyldioxanyl]-13,13-dimethyl-6,11-ethano-6,11-dihydrobenzo[*b*]quinolizinium perchlorate (**28a**) in concentrated HCl (15 mL) was heated at 80 °C under N_2 for 1.5 h and cooled to room temperature. The reaction mixture was concentrated in vacuo, the residue diluted with H_2O (10 mL), and 10% sodium perchlorate in water (2 mL) added. The solids that precipitated were collected by filtration and crystallized from ethanol:acetonitrile to give 0.6 g (93%) of (+)-**5** (perchlorate salt) as a white crystalline solid: mp 281-283 °C, $[\alpha]^{25}_{435}$ = +49.5° (*c* = 1, CH_3CN); IR (KBr) 3059, 1733, 1627, 1098, 819, 624, 525 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.83 (3H, s), 0.90 (3H, s), 5.87 (1H, s), 6.66 (1H, s), 7.50 (2H, m), 7.6-7.7 (2H, m), 8.15 (1H, t, *J* = 7.2 Hz), 8.40 (1H, d, *J* = 7.9 Hz), 8.65 (1H, t, *J* = 7.8 Hz), 9.36 (1H, d, *J* = 5.8 Hz). Anal. ($C_{17}H_{16}ClNO_5$) C,H,N.

Similarly, starting from **28b**, (-)-**5** was obtained in 95% yield (perchlorate salt) as a white crystalline solid: mp 282-284 °C, $[\alpha]^{25}_{435}$ = -45.8° (*c* = 1, CH_3CN); IR (KBr) 3020, 2975, 1733, 1627, 1576, 697, 525 cm^{-1}; ^{1}H NMR ($CDCl_3$/DMSO-d_6) δ 0.82 (3H, s), 0.93 (3H, s), 5.70 (1H, s), 6.62 (1H, s), 7.41 (2H, m), 7.53-7.71 (2H, m), 8.05 (1H, t, *J* = 7.0 Hz), 8.34 (1H, d, *J* = 7.8 Hz), 8.53 (1H, dd, *J* = 1.0, 8.0 Hz), 9.40 (1H, d, *J* = 6.0 Hz). Anal. ($C_{17}H_{16}ClNO_5$) C,H,N.

Ketalization of (+)- and (-)-5. To a solution of (+)-**5** (0.56 g, 1.6 mmol) in nitromethane (8 mL), tetraethoxysilane (3.07 g, 16 mmol), and ethanol (0.74 g, 10 mmol) was added chloroform (200 mL). Then most of the chloroform was removed using a Dean-Stark water separator. The mixture was cooled to room temperature and treated with triflic acid (0.24 g, 1.6 mmol). The solution was then taken up in a disposable plastic syringe (20 mL), and after capping, the syringe was submersed into the ultra-high-pressure reactor (Leco Tem-Pres, model PG200HPC) using kerosene as the transfer media and the reactor pressurized to 10 Kbar (approximately 150 000 psi) for 24 h. The reaction mixture was then poured into a cold 10% sodium bicarbonate solution, and the product was extracted with methylene chloride (3 × 50 mL). The combined extract was dried over anhydrous sodium sulfate and evaporated to dryness to give the crude product. The ^{1}H NMR of the crude product indicated >80% conversion to the corresponding ketal, (+)-**2**. The perchlorate counterion was exchanged with chloride anion using Dowex 1 × 2-200 (Cl^-) ion-exchange resin in the usual manner to give (+)-**2** chloride (0.46 g) along with a minor amount of ketone (+)-**5**. The product was purified by silica gel chromatography eluting with methylene chloride/methanol (9:1) and then recrystallized from methylene chloride/ether (1:1) to give (+)-**2** chloride, 0.29 g (50%), as an amorphous powder (>97.8% pure by HPLC; Aztec Cyclobond I-RN column, CH_3CN/0.1 M NH_4Cl, 22:78, flow rate = 0.3 mL/min, retention time = 66.87 min, detection at 269 nm): $[\alpha]^{25}_D$ = +43.8° (*c* = 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 M Hz) δ 1.0 (12H, m), 3.45 (2H, q, *J* = 4.0 Hz), 3.62 (2H, q, *J* = 3.8 Hz), 5.08 (1H, s), 7.08 (1H, s), 7.40 (3H, m), 7.78 (1H, t, *J* = 4.5 Hz), 7.85 (1H, m), 7.95 (1H, d, *J* = 5.5 Hz), 8.30 (1H, t, *J* = 4.8 Hz), 10.62 (1H, d, *J* = 5.6 Hz). Anal. ($C_{21}H_{26}ClNO_2$·0.75H_2O) C,H,N.

In a similar manner, (-)-**2** was prepared from (-)-**5** (0.3 g, 0.86 mmol) following the above procedure. The crude product was purified on a silica gel column as the chloride anion and recrystallized from methylene chloride/ether (1:1) to give (-)-**2**, 0.17 g (55%), as an amorphous solid (>96.4% pure by HPLC; Aztec Cyclobond I-RN column, CH_3CN/0.1 M NH_4Cl, 22:78, flow rate = 0.3 mL/min, retention time = 70.71 min, detection at 269 nm); $[\alpha]^{25}_D$ = -42.3° (c = 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 M Hz) δ 1.05 (12H, m), 3.50 (2H, q, *J* = 3.6 Hz), 3.68 (2H, q, *J* = 3.8 Hz), 5.10 (1H, s), 6.96 (1H, s), 7.38 (2H, m), 7.56 (1H, d, *J* = 5.4 Hz), 7.80 (2H, m), 8.20 (1H, d, *J* = 5.5 Hz), 8.48 (1H, t, *J* = 5.6 Hz), 10.56 (1H, d, *J* = 5.6 Hz). Anal. ($C_{21}H_{26}ClNO_2$·0.5H_2O) C,H,N.

(±)-12,12-Diethoxy-13,13-dimethyl-6,11-ethano-6,11-dihydrobenzo[*b*]quinolizinium Hexafluorophosphate [(±)-

2 Hexafluorophosphate]. A mixture of benzo[*b*]quinolizinium hexafluorophosphate (460 g, 1.4 mol) in acetonitrile (1.84 L) was heated on a steam bath until a solution resulted, and dimethylketene diethylacetal[30] (46.2 g, 2.12 mol) was added. The mixture was heated to reflux for 4 h and then stirred at room temperature for 16 h. The solvent was removed in vacuo; the residue was dissolved in ethyl acetate (14 L) and stirred with water (5 L). The solution was decolorized with charcoal, dried over $MgSO_4$, and concentrated in vacuo to afford an off-white solid. The solid was slurried with 2-propanol (5.5 L), heated to reflux, and then stirred at room temperature for 16 h. The solution was cooled in an ice bath, and the product was collected by filtration and dried in high vacuum to afford 627 g (94.5%) of (±)-**2**·PF_6^- as a white solid: mp 165–167 °C. Anal. ($C_{21}H_{26}F_6NO_2P$) C,H,N. This material was converted to its chloride salt in portions following the general procedure described above.

Potassium (+)-O,O′-Dibenzoyl-D-tartaric Monobasic Acid. (+)-O,O′-Dibenzoyl-D-tartaric acid [143 g, 0.399 mol; (+)-DBT] was dissolved in methanol (500 mL), and an aqueous solution of $KHCO_3$ (40.04 g, 0.40 mol) in water (150 mL) was slowly added. When the addition was completed, methanol (275 mL) and water (300 mL) were added and the mixture was stirred for 4 h. A white precipitate formed, which was collected by filtration and washed with methanol (3 × 50 mL) to afford 130 g (82%) of the monopotassium salt of (+)-dibenzoyl-D-tartaric acid: mp 164–166 °C; $[\alpha]^{24}_D$ = +125° (c = 1, methanol).

(−)-12,12-Diethoxy-13,13-dimethyl-6,11-ethano-6,11-dihydrobenzo[*b*]quinolizinium Chloride [(−)-2] by Fractional Crystallization. Part A: A mixture of the monopotassium salt of (+)-dibenzoyl-D-tartaric acid (33.63 g, 0.086 mol) and methanol (300 mL) was heated to reflux, and water (300 mL) was slowly added. The hot solution was then added to a solution of (±)-**2** chloride (30.03 g, 0.0835 mol) in methanol (500 mL). The reaction mixture was stirred at room temperature for 16 h, and any solids which formed were removed by filtration. The filtrate was extracted with CH_2Cl_2 (1 × 1000 mL, 2 × 500 mL), and the organic layers were combined and concentrated in vacuo. The residue was azeotroped (2×) with methanol to dryness to remove any residual water, and the residue was dissolved in CH_2Cl_2 (200 mL) and filtered. The filtrate was concentrated in vacuo to afford 51.1 g (90%) of (±)-**2** [(+)-dibenzoyltartarate salt], a 1:1 mixture of diastereomers, as an off-white solid: $[\alpha]^{26.8}_D$ = +114.6° (*c* = 1, methanol). This material (49.24 g, 0.072 mol) was dissolved in acetonitrile (500 mL) with heating and then cooled slightly. *tert*-Butyl methyl ether (ca. 725 mL) was then added, and the mixture was warmed for 4 min and then cooled to room temperature. The mixture was stirred for 6.5 h, and the product which precipitated was collected by filtration, washed with *tert*-butyl methyl ether (2 × 500 mL), and dried at 45 °C under vacuum to afford 31.2 g (63%) of (±)-**2** [(+)-dibenzoyltartarate salt], as approximately a 2:1 mixture of diastereomers: $[\alpha]^{25}_D$ = +53.6° (c = 1, methanol). An additional 52.6 g of product was obtained from three other experimental runs, for a total of 83.8 g.

Part B: To the 2:1 mixture of diastereomers above (83.8 g, 0.123 mol) was added 1,2-dichloroethane (500 mL), and the mixture was heated to reflux until all of the material had dissolved. The solution was filtered through solca floc; the filtrate was warmed on a steam bath for 5 min and then gradually cooled to room temperature. The solution was seeded, and crystals were allowed to form for 6 h. The crystals were then collected by filtration, washed with cold dichloroethane (2 × 40 mL), and dried at 45 °C under reduced pressure to afford 37.4 g (45%) of (−)-**2** (+)-dibenzoyltartarate salt as a single diastereomer (>99.8% pure by HPLC, Cyclobond I-RN column, CH_3CN/0.1 M NH_4Cl, 22:78, flow rate = 1 mL/min): $[\alpha]^{25}_D$ = +32.9° (*c* = 1, methanol), mp 124–126 °C. A solution of the single diastereomer from above (10.02 g, 0.0147 mol) in warm methanol (50 mL) was added rapidly to a stirred aqueous solution of sodium perchlorate (11.94 g, 0.0976 mol), $NaHCO_3$ (3.55 g, 0.0423 mol), and water (1 L). The reaction mixture was stirred at room temperature for 16 h, and the white solid which precipitated was isolated by filtration and washed with water (20 mL). The solid was dried at 45 °C under reduced pressure to afford 5.21 g (84%) of (−)-**2** as the perchlorate salt: mp 143–144 °C; $[\alpha]^{25}_D$ = −45.5° (*c* = 1, methanol). This material was converted to its chloride salt using the ion-exchange procedure described in the general methods section to give (−)-**2** chloride: mp 110–112 °C; $[\alpha]^{25}_D$ = −54.4° (c =1, methanol); ^{13}C NMR (DMSO-d_6) δ 14.76, 15.04, 23.14, 23.94, 44.74, 51.62, 58.01, 59.04, 76.54, 103.58, 125.97, 126.20, 126.55, 127.91, 129.44, 134.23, 134.89, 143.50, 146.89, 153.13, 174.9; IR (KBr) 3414 (br, s) 3031 (m), 2977 (s), 2933 (m), 2900 (m), 1632 (s), 1585 (m), 1504 (m), 1478 (s), 1462 (m), 1392 (m), 1161 (w), 1125 (s), 1112 (m), 1088 (s), 1060 (s); MS-FAB (*m/z*) 324 (M^+, 84), 250 (29) 180 (100). Anal. ($C_{21}H_{26}ClNO_2$·0.5H_2O) C,H,N.

(+)-12,12-Diethoxy-13,13-dimethyl-6,11-ethano-6,11-dihydrobenzo[*b*]quinolizinium Chloride [(+)-2] by Fractional Crystallization. The monopotassium salt of (−)-dibenzoyl-L-tartaric acid [99.1 g, 0.25 mol; prepared as described above for (+)-DBT] was dissolved in 2-propanol (4.8 L) and water (2.4 L) with heating on a steam bath. A solution of (±)-**2** (60.0 g, 0.167 mol) in 2-propanol (600 mL) was then added, and the reaction mixture was cooled to room temperature and stirred for 16 h. The mixture was warmed on a steam bath, and the solvent was removed in vacuo. Dichloromethane (6 L) was added to the residue, and 16 h later the solution was filtered to remove any inorganic salts. The solution was washed with water (3 L), dried over $MgSO_4$, and concentrated in vacuo to afford 105.9 g (93.2%) of (±)-**2** (dibenzoyltartarate salt) as a 1:1 mixture of diastereomers. This material (105.9 g) was dissolved in 1,2-dichloroethane (1060 mL) with gentle heating on a steam bath. The resulting solution was cooled to room temperature and allowed to stand for 40 h. A white solid precipitated, was collected by filtration, and was washed with cold 1,2-dichloroethane (2 × 125 mL) to afford 29.62 g (28%) of (−)-**2** (dibenzoyltartarate salt) as a single diastereomer: $[\alpha]^{25}_D$ = +32.5° (*c* =1, methanol); mp 126–128 °C. A 18.2 g portion of this material was directly converted to the chloride salt according to the standard procedure to give 8.84 g (92%) of (+)-**2** (chloride salt) after drying under high vacuum for 5 days at 40 °C: $[\alpha]^{25}_D$ = +51.5°, (c =1, methanol); mp 108–110 °C. Anal. ($C_{21}H_{26}ClNO_2$·0.75H_2O) C,H,N.

10-Hydroxybenzo[*b*]quinolizinium Perchlorate (29). A solution of 10-methoxybenzo[*b*]quinolizinium perchlorate[15] (4.0 g, 0.013 mol) in 48% HBr (50 mL) was heated at 100 °C for 20 h and cooled to room temperature. The solids that precipitated were collected by filtration, redissolved in hot water, and treated with 20% aqueous $NaClO_4$ (50 mL). The precipitated yellow solid was collected by filtration and crystallized from iPrOH to give 2.9 g (76%) of **29** as a pale yellow solid: mp 218–220 °C dec; ^1H NMR (DMSO-d_6) δ 7.34 (1H, d, *J* = 6.9 Hz), 7.79–8.03 (4H, m), 8.63 (1H, d, *J* = 8.8 Hz), 9.21 (1H, d, *J* = 7.0 Hz), 9.28 (1H, s), 10.31 (1H, s), 11.57 (1H, br s). Anal. ($C_{13}H_{10}ClNO_6$) C,H,N.

(±)-10-Acetoxy-12,12-diethoxy-13,13-dimethyl-6,11-ethano-6,11-dihydrobenzo[*b*]quinolizinium Perchlorate (20). Part A, 10-acetoxybenzo[*b*]quinolizinium perchlorate: To a solution of 6.5 g (0.024 mol) of **29** in 1:1 CH_2Cl_2:pyridine (400 mL) were added acetic anhydride (50 mL) and 4-(dimethylamino)pyridine (0.1 g), and the mixture was stirred at room temperature under argon for 2 h. The reaction mixture was concentrated in vacuo and the residue partitioned between CH_2Cl_2 (200 mL) and 10% $NaClO_4$ (100 mL) and sonicated. The solids that precipitated were collected by filtration, dissolved in boiling water, and filtered. The filtrate was treated with 30% $NaClO_4$; the solids were collected by filtration and air-dried to give 1.9 g (24%) of **20** as a pale yellow solid: ^1H NMR (DMSO-d_6) δ 2.56 (3H, s), 7.92–8.04 (3H, m), 8.12 (1H, t, *J* = 8.5 Hz), 8.40 (1H, d, *J* = 8.4 Hz), 8.61 (1H, d, *J* = 8.8 Hz), 9.27 (2H, m), 10.49 (1H, s).

Part B: A solution of 1.9 g (0.0056 mol) of 10-acetoxybenzo[*b*]quinolizinium perchlorate and 1.71 g (0.011 mol) of dimethylketene diethylacetal in acetonitrile (35 mL) was heated at 60 °C under argon for 8 h and cooled to room temperature. The reaction mixture was evaporated to dryness and the residue triturated with ether, and the solids were collected by filtration. The crude product was recrystallized from 2-propanol after treatment with activated charcoal to provide 1.9 g (70%) of (±)-**20** as a colorless solid: mp 135–137 °C; IR (KBr)

2986, 1766, 1632, 1477, 1199, 1089, 625 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.91–1.06 (12H, m), 2.40 (3H, s), 3.4–3.60 (4H, m), 5.01 (1H, s), 6.11 (1H, s), 7.13 (1H, d, J = 8.2 Hz), 7.34 (1H, t, J = 7.7 Hz), 7.53 (1H, t, J = 6.9 Hz), 7.65 (1H, d, J = 7.4 Hz), 7.98 (1H, d, J = 7.7 Hz), 8.18 (1H, d, J = 8.1 Hz), 9.19 (1H, J = 5.9 Hz). Anal. ($C_{23}H_{28}ClNO_8 \cdot 1H_2O$) C,H,N.

(±)-10-Hydroxy-12,12-diethoxy-13,13-dimethyl-6,11-ethano-6,11-dihydrobenzo[*b*]quinolizinium Perchlorate (21). To a solution of acetate **20** (0.5 g, 1.0 mmol) in methanol (25 mL) was added powdered NaOH (0.12 g, 3.0 mmol). The resulting yellow solution was stirred at room temperature for 15 min, the reaction quenched with 1.5 M acetic acid in methanol (2.25 mL, 3.3 mmol), and the solution evaporated to dryness in vacuo. The residue was diluted with 10% aqueous $NaClO_4$ (1 mL) and extracted with CH_2Cl_2 (2 × 50 mL). The organic phase was dried and concentrated in vacuo and the resulting foamy solid crystallized from water to give 0.24 g (52%) of (±)-**22** as a fluffy solid: IR (KBr) 3549, 3503, 2983, 1628, 1575, 1091, 627 cm^{-1}; ^{1}H NMR (DMSO-d_6/10% $CDCl_3$) δ 0.8–1.05 (12H, m), 3.4–3.8 (4H, m), 5.4 (1H, s), 5.85 (1H, s), 6.9 (1H, d, J = 7.1 Hz), 7.05 (1H, d, J = 6.0 Hz), 7.20 (1H, t, J = 6.0 Hz), 7.85 (1H, t, J = 5.5 Hz), 8.1 (1H, d, J = 7.0 Hz), 8.45 (1H, t, J = 6.0 Hz), 9.25 (1H, d, J = 5.5 Hz). Anal. ($C_{21}H_{26}ClNO_7 \cdot 1H_2O$) C,H,N.

12,12,13,13-Tetraethoxy-6,11-ethano-6,11-dihydrobenzo[*b*]quinolizinium Perchlorate (11). A mixture of benzo[*b*]quinolizinium bromide (1.8 g, 6.9 mmol) and tetraethoxyethylene (14.13 g, 69 mmol) in 25 mL of acetonitrile was stirred at room temperature overnight (14 h). The resulting mixture was concentrated under reduced pressure and partitioned between 100 mL of water and 50 mL of ether. The aqueous layer was separated, washed again with 50 mL of ether, and then treated with 10% aqueous $NaClO_4$ until precipitation was complete. The resulting white solid was collected by filtration, washed with water, and air-dried. The white perchlorate salt was then dried under reduced pressure at 60 °C to give 2.6 g (98%) of 11 as a white powder: mp 202–203 °C; DCI-MS (m/z) 384 (MH^+), 204, 180 (100); ^{1}H NMR (DMSO-d_6) δ 1.14 (12H, 4 × t), 3.5–4.0 (8H, 4 × q), 5.6 (1H, s), 6.71 (1H, s), 7.40 (m, 2H), 7.59 (m, 2H), 8.1 (1H, d), 8.31 (1H, d), 8.6 (1H, m), 9.3 (1H, d). Anal. ($C_{23}H_{30}ClNO_8$) C,H,N.

12,2′-Spiro(1′,3′-dioxane)-13,13-dimethyl-6,11-ethano-6,11-dihydrobenzo[*b*]quinolizinium Chloride (8). This compound was prepared in 40% overall yield by a procedure similar to that of **28a/b**, starting from 1,3-propanediol: IR (KBr) 3422, 2976, 1631, 1480, 1249, 1107, 1003, 786 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.72 (3H, s), 0.73 (3H, s), 1.49–1.53 (1H, m), 1.82–1.88 (1H, m), 3.68–3.77 (2H, m), 4.27–4.35 (1H, m), 4.46–4.54 (1H, m), 6.37 (1H, s), 6.77 (1H, s), 7.33–7.44 (2H, m), 7.56 (1H, d, J = 7.1 Hz), 7.63 (1H, d, J = 7.3 Hz), 8.03 (1H, t, J = 7.0 Hz), 8.40 (1H, d, J = 7.8 Hz), 8.60 (1H, t, J = 7.8 Hz), 9.39 (1H, d, J 6.0 Hz). Anal. ($C_{20}H_{22}NO_2Cl \cdot 1.5H_2O$) C,H,N.

12,12-Diethoxy-13,13-diethyl-6,11-ethano-6,11-dihydrobenzo[*b*]quinolizinium Perchlorate (10). Part A: A mixture of benzo[*b*]quinolizinium perchlorate (3.8 g, 0.014 mol) and 2,2-diethyl-1-methoxy-1-(trimethylsiloxy)ethylene[31] (6.3 g, 0.03 mol) in acetonitrile (100 mL) was stirred at room temperature for 18 h and evaporated to dryness in vacuo. The residue was triturated with ether, and the solids were collected by filtration and air-dried to give 6.3 g (99%) of the initial Diels–Alder adduct as a colorless solid (a 1.6:1 mixture of diastereomers at position 12). A solution of 4.8 g (0.01 mol) of this material in concentrated HCl (80 mL) was heated at 80 °C for 2 h and then cooled to room temperature. The resulting solution was diluted with water (100 mL), 10% aqueous $NaClO_4$ (200 mL) was added, and the solution was cooled in an ice bath. The solids were collected by filtration, washed with water and ether, and dried to give 3.72 g (99%) of the desired ketone as a colorless solid: ^{1}H NMR ($CDCl_3$/DMSO-d_6) δ 0.75–1.10 (6H, m), 1.20–1.60 (2H, m), 5.58 (1H, s), 6.71 (1H, s), 7.35–7.38 (2H, m), 7.51–7.56 (1H, m), 7.66–7.69 (1H, m), 8.0 (1H, t, J = 6.9 Hz), 8.30 (1H, d, J = 7.9 Hz), 8.49 (1H, t, J = 7.8 Hz), 9.42 (1H, d, J = 6.0 Hz). This material was used in the following step without further purification.

Part B: Ketalization of 1.0 g (0.0026 mol) of the above ketone with tetraethoxysilane by the method similar to that of (±)-**5** gave, after chromatography on silica gel with 3:2 E/PAW, 0.21 g (18%) of **10** as a gummy solid. This compound was further purified by conversion to the corresponding chloride by ion exchange followed by treatment with 10% $NaClO_4$ in water to give ketal **10** as a colorless amorphous solid: IR (KBr) 2981, 1633, 1087, 623 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.73–0.99 (14H, m), 1.09–1.13 (1H, m), 1.23–1.27 (1H, m), 3.47–3.62 (2H, m), 5.55 (1H, s), 6.30 (1H, s), 7.37–7.42 (2H, m), 7.59–7.61 (1H, m), 8.01 (1H, t, J = 6.8 Hz), 8.31 (1H, d, J = 7.8 Hz), 8.57 (1H, t, J = 7.8 Hz), 9.25 (1H, d, J = 5.9 Hz). Anal. Calcd for $C_{23}H_{30}ClNO_6 \cdot 2.0H_20$: C, 56.61; H, 7.02; N, 2.92. Found: C, 56.57; H, 6.14; N, 2.87.

(1′,3′-Dioxolan-2′-yl)-2,2-dimethylethylene.[32] Part A: A mixture of 12.0 g (0.067 mol) of 1,1-diethoxy-2-chloro-2-methylpropane, ethylene glycol (15.0 g, 0.24 mol), and pyridinium *p*-toluenesulfonate (0.5 g, 0.002 mol) in toluene (200 mL) was refluxed with azeotropic removal of water for 16 h and cooled to room temperature. The reaction mixture was washed with saturated $NaHCO_3$ (1 × 50 mL) and brine and dried. Removal of the solvent in vacuo and distillation under reduced pressure (bp 55–60 °C at 20 mmHg) gave 7.0 g (70%) of 2-(2-chloro-2-propyl)-1,3-dioxolane as a colorless liquid: ^{1}H NMR ($CDCl_3$) δ 1.5 (6H, s), 3.9–4.1 (4H, m), 4.85 (1H, s).

Part B: To a solution of K^+tBuO^- (7.15 g, 0.063 mol) in THF (100 mL) was added 2-(2-chloro-2-propyl)-1,3-dioxolane (6.4 g, 0.042 mol) in THF (10 mL) over 15 min. After stirring under nitrogen for 15 min, the THF was removed at atmospheric pressure. The residue was evacuated (aspirator pressure) for 15 min, and all the volatiles were collected in a flask cooled in a dry ice–acetone bath. The distillate was dissolved in ether (50 mL), NaH (0.5 g) was added, and the mixture was refluxed for 30 min and filtered through a pad of supercel. The solvent was removed at atmospheric pressure and the residue distilled under reduced pressure. The fraction boiling at 95–105 °C at 110 mmHg was collected to give 1.5 g of a colorless liquid: ^{1}H NMR ($CDCl_3$) indicated it to be a mixture of the desired olefin (1′,3′-dioxolan-2′-yl)-2,2-dimethylethylene (30%), the regioisomeric olefin (1′,3′-dioxolan-2′-yl)-1-methylethylene (30%), and tBuOH (40%). This mixture was used as such in the preparation of **9**.

12,2′-Spiro(1′,3′-dioxolane)-13,13-dimethyl-6,11-ethano-6,11-dihydrobenzo[*b*]quinolizinium Perchlorate (9). To a solution of (1′,3′-dioxolan-2′-yl)-2,2-dimethylethylene (1.5 g of 30% pure material) in acetonitrile (50 mL) was added benzo[*b*]quinolizinium perchlorate (1.84 g, 0.0065 mol), and the resulting mixture was refluxed under nitrogen for 8 h. Removal of the solvent in vacuo and purification of the crude product by flash chromatography (6:1 CH_2Cl_2/iPrOH) followed by crystallization from iPrOH gave 0.42 g (25%) of **9** as a colorless solid: mp 240–242 °C; IR (KBr) 3086, 2886, 1632, 1502, 1090, 789, 624 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.72 (3H, s), 0.74 (3H, s), 3.83–3.91 (2H, m), 4.03–4.13 (2H, m), 5.27 (1H, s), 6.22 (1H, s), 7.36–7.45 (2H, m), 7.52–7.59 (2H, m), 8.02–8.07 (1H, m), 8.25 (1H, d, J =7.8 Hz), 8.59 (1H, dd, J = 1.0, 8.0 Hz), 9.23 (1H, d, J = 5.8 Hz). Anal. ($C_{19}H_{20}ClNO_6 \cdot 0.25H_2O$) C,H,N.

Biological Methods. [^{3}H]TCP Radioreceptor Assay. [^{3}H]TCP binding to PCP recognition sites was performed as described by Vignon.[33] Male Sprague–Dawley rats were sacrificed by decapitation, and whole brains were homogenized in 10 volumes (w/v) of cold Tris-HCl buffer (50 mM, pH 7.7) using a Brinkmann Polytron instrument (setting 6, 30 s). The homogenate was centrifuged at 40 000g for 10 min at 4 °C. The supernatant was decanted, and the homogenization and centrifugation steps were repeated twice as described above. Following this, the pellet was resuspended in Tris-HCl (5 mM, pH 7.7) at a tissue concentration of 0.5–0.75 g/mL, and 1 mL aliquots were frozen at −70 °C until use. The binding characteristics for PCP recognition sites were not altered by the freezing of membrane suspensions.

On the day of the assay, membrane aliquots were thawed, resuspended in fresh 5 mM Tris-HCl buffer at a tissue concentration of 1 mg/mL, and stored on ice until use. Each assay tube contained 100 μL of [^{3}H]TCP at a final concentration of approximately 1 nM, 100 μL of various concentrations of the compounds of interest, 500 μL of the tissue suspension, and 300 μL of buffer to a final assay volume of 1 mL and a

final protein concentration of 0.5 mg/tube. Nonspecific binding was defined by addition of a final concentration of 100 μM PCP to blank tubes. All tubes were incubated at room temperature for 25 min before termination of the reaction by rapid filtration over Whatman GF/B glass fiber filters that had been presoaked in a solution of 0.5% poly(ethylenimine) for at least 1 h prior to use. Filters were washed with three 4 mL volumes of cold Tris buffer. Following addition of scintillation cocktail, the amount of bound radioactivity was determined by liquid scintillation spectrometry using a Beckman LS 5000TA liquid scintillation counter with an efficiency for tritium of approximately 55%. Inhibition constants (K_i values) were calculated using the EBDA/LIGAND program,[34] purchased from Elsevier/Biosoft, Inc.

Neuroprotection in Cultured Mouse Cortical Neurons. Pregnant, Swiss–Webster mice were obtained from Taconic Farms and sacrificed 16 days postconception. Fetuses were removed and placed in a sterile dish containing Hank's balanced salt solution (HBSS), pH 7.4. Brain cortices were dissected, meninges were removed, and the tissue was minced and placed into a solution of HBSS containing 0.25% (w/v) trypsin at 37 °C for 15 min. Tissue was then triturated with a sterile Pasteur pipette and diluted with minimal essential media (Gibco 330-1430), pH 7.4, supplemented with 10% horse serum, 10% fetal calf serum, 2 mM *l*-glutamine, 21 mM *d*-glucose, 2.2 g/L sodium bicarbonate, 1000 U/mL penicillin, and 1000 μg/mL streptomycin. Cells were plated onto Falcon Primaria 96-well plates at a final density of 50 000 cells/well and incubated at 37 °C in the presence of 5% (v/v) carbon dioxide. After 5 days, plating media was replaced with maintenance media containing minimal essential media (Gibco 330-1430), pH 7.4, supplemented with 10% horse serum, 10% *l*-glutamine, 21 mM *d*-glucose, 2.2 g/L sodium bicarbonate, 1000 U/mL penicillin, 1000 μg/mL streptomycin, and 10 μM cytosine arabinoside. On days 7 and 10, media were replaced with maintenance media as above lacking the cytosine arabinoside. Experiments were conducted on day 13.

Day 13-cultured cortical neurons were washed twice with minimal essential media, pH 7.4, and then exposed for 30 min to 500 μM *N*-methyl-D-aspartic acid (NMDA) with or without varying concentrations of test agents. MK801 at a final concentration of 10 μM was routinely included as a positive control. MK801 and test agents were prepared in minimal essential media supplemented with 21 mM *d*-glucose and 2.2 g/L sodium bicarbonate (MEM). After 30 min, media were replaced with MEM alone. Exposure of neurons to test agents was limited to the NMDA treatment period. Twenty-four hours after removal of NMDA, an aliquot of media from each well was removed for assessment of cell injury by determining lactate dehydrogenase (LDH) activity by the method of Wroblewski and LaDue (1955).

Electrophysiology (State-dependent channel blockade). Electrophysiologic responses to NMDA were recorded in 13-day old mouse cortical neuronal cultures (described above). Cells grown on 35 mm dishes were superfused from a tube of approximately 800 μm i.d. and placed approximately 2 mm from the cell body. Perfusion solution approximated extracellular fluid and consisted of 150 mM NaCl, 4.5 mM KCl, 1 mM $CaCl_2$, 10 mM HEPES, 12.5 mM dextrose, 3 μM glycine, and 0.1 μM tetrodotoxin, pH 7.4. Cultures were perfused at a rate of 2 mL/min. Patch electrodes (1–4 MΩ) were filled with solution containing 120 mM KCl, 1 mM $CaCl_2$, 11 mM EGTA, 10 mM HEPES, 10 mM NaCl, and 2 mM MgATP, at pH 7.3. On-cell patches with GΩ resistances were established, and the patch was ruptured to establish whole cell recording. The preparation was allowed to equilibrate without voltage clamp for 15 min, at which time the resting potential was routinely at least −40 mV. For all protocols, the cells were voltage-clamped at −60 mV using an Axoclamp-2A instrument (Axon Instruments). Experiments were performed at room temperature (ca. 23 °C). To evaluate NMDA antagonism, a submaximal concentration of NMDA (100 μM) was chosen from dose–response studies.

Open-channel block was assessed by application of NMDA alone or in combination with a test compound. Prolonged application (300 s) was used to allow inhibitor equilibrium. Only a single drug application was performed in individual cells due to tachyphylaxis. Current at 300 s was normalized to the initial peak current, the values were averaged for the control group, and the inhibition was calculated as [(control − test)/control] × 100%. To assess closed-channel blockade, base line responses were established by applying two boluses of NMDA (100 μM), of approximately 3 s contact time, at 15 min intervals. Cells were maintained for 30 min with 10 μM 7-chlorokynurenate to minimize spontaneous channel opening and then washed for 1–2 min, and the response to an additional application of 100 μM NMDA was quantified. Inhibition was calculated for the final NMDA application relative to the mean of the two initial applications. In control experiments, incubation with 10 μM 7-chlorokynurenic acid followed by a 1 min wash period did not affect subsequent NMDA responses (−1.0 ± 5.8% inhibition, $n = 8$), indicating that this glycine receptor antagonist was adequately removed during the wash period and that NMDA-induced responses were relatively stable using this protocol. Test agents were applied to cells during the 30 min incubation period concurrently with 7-chlorokynurenate. IC_{50} values were calculated by fitting a nonlinear curve according to the equation: % inhibition = $100/\{1 + (IC_{50}/[\text{drug}])^n\}$ to all data points using a fitting program (Graphpad[35]).

Acknowledgment. The authors acknowledge the expert assistance of Dr. Eugene Baizman, Tim Allen, Harry Bentley, Felica Ford-Rice, Lee Hildebrand, Lorraine Lanyon, and Connie Zobre in acquiring biological data and Dr. S. Clemens and Mr. A. Halvac for expert mass spectrometry. We also wish to thank Drs. A. M. Treasurywala, S. J. Ward, J. Reczek, and R. Everett for many helpful discussions and insights during this investigation.

Supplementary Material Available: Full X-ray crystallographic data, atomic coordinates, and experimental details for (±)-**2** chloride salt (WIN 63480-2) (26 pages). Ordering information is given on any current masthead page.

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(25) All research involving animals described in this publication was performed in accord with the Sterling Winthrop Pharmaceuticals Research Division (SWPRD) Policy on Animal Use and all national and federal legislation. All SWPRD animal facilities and programs are accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

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