Expedited Articles

Discovery of 6,11-Ethano-12,12-diaryl-6,11-dihydrobenzo[b]quinolizinium Cations, a Novel Class of N-Methyl-D-aspartate Antagonists

Chakrapani Subramanyam,*,†,‡ John P. Mallamo,†,‡ John A. Dority, Jr.,† William G. Earley,† Virendra Kumar,† Lisa D. Aimone,§ Brian Ault,§ Matthew S. Miller,§,‡ Daniel A. Luttinger,§ and Diane L. DeHaven-Hudkins||

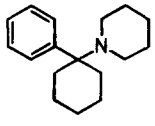
Departments of Medicinal Chemistry, Pharmacology, and Biochemistry, Sterling Winthrop Pharmaceuticals Research Division, 1250 South Collegeville Road, Collegeville, Pennsylvania 19426-0900

Received October 19, 1994®

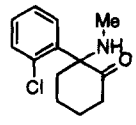
6,11-Ethano-12,12-diaryl-6,11-dihydrobenzo[b]quinolizinium cations **8**, a novel class of *N*-methyl-D-aspartate (NMDA) antagonists acting at the phencyclidine site, have been identified. Structure–activity relationship studies around the lead compound **8a** led to the identification of **12g** (WIN 67870-2), one of the most potent compounds in this series. Compound **12g** has a $K_i = 1.8 \pm 0.2$ nM vs [^{3}H]TCP binding, has 700-fold selectivity for binding to the open state of the NMDA receptor–ionophore, and was devoid of MK-801- and PCP-like behavioral effects in rats. Compound **12g** was neuroprotective in cultured mouse cortical neurons and exhibited antiischemic activity in a rat middle cerebral artery occlusion/reperfusion model of focal ischemia.

Introduction

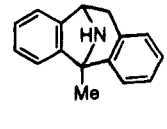
Excess glutamate, an excitatory amino acid which acts, in part, at the *N*-methyl-D-aspartate (NMDA) receptor subtype, has been implicated as the major causative factor for a number of neurodegenerative disorders such as ischemia, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis (ALS).[1] Many modulatory sites on the NMDA receptor–ion channel complex have been discovered, and these sites offer abundant opportunities for targeted drug discovery in the treatment of these disease states.[2] A site which is located within the receptor–ion channel complex is labeled by [^{3}H]TCP and is the binding site for the dissociative anesthetics PCP (**1**), ketamine (**2**), and the noncompetitive NMDA antagonist MK-801 (**3**).[3] Both PCP and MK-801 have been reported to be neuroprotective in various animal models of cerebral ischemia.[4] In spite of these beneficial effects, PCP and MK-801 have not been developed into clinically useful agents because of the undesirable behavioral and autonomic side effects associated with effective doses.[5] New agents that are devoid of such side effects would have improved therapeutic potential.



1

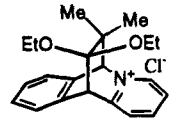


2

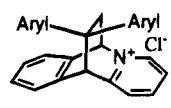


3

We recently reported that 6,11-ethanobenzo[b]quinolizinium cation **4** (WIN 63480-2) is a selective and potent antagonist of the NMDA receptor–ion channel complex.[6] Compound **4** interacts with the TCP site on the NMDA receptor–ionophore complex with a $K_i = 5.3 \pm 0.9$ nM and was efficacious in a middle cerebral artery occlusion (MCAO) model of cerebral ischemia in rats.[7] More importantly, at antiischemic and higher (up to the MTD) doses, **4** was found to be devoid of any of the psychotomimetic effects usually associated with classical PCP agonists such as MK-801.[6a] We postulated that selective binding of (>400-fold) of this highly hydrophilic ($\log D = -4.08$) TCP site ligand to the open state of the NMDA ion channel (2 orders of magnitude greater than PCP or MK-801) is responsible for the lack of behavioral effects.[8] In order to better understand the structural requirements (for activity) and to improve the potency, we undertook an extensive structure–activity relationship (SAR) study around **4**. We report herein that 6,-11-ethano-12,12-diaryl-6,11-dihydrobenzo[b]quinolizinium cations **8** are a novel class of potent and selective *uncompetitive* NMDA antagonists.



4



8

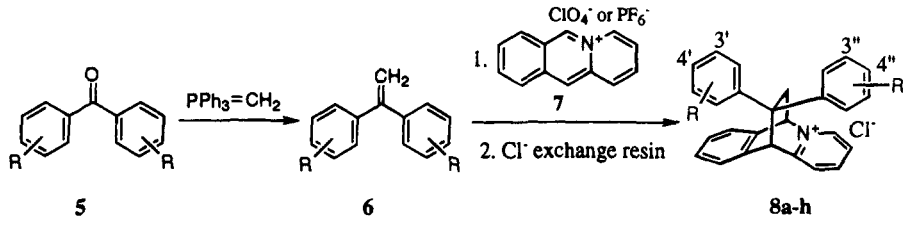
Chemistry

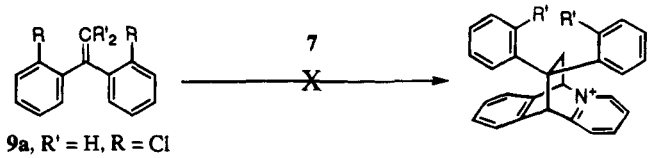
Syntheses of the target compounds **8a–h** chosen for this study were accomplished via an inverse-electron demand Diels–Alder reaction between the benzo[b]-quinolizinium cation (**7**) and the corresponding **1,1**-diarylethylenes (**6**)[9] (Scheme **1**). The requisite olefins **6** were prepared via Wittig methylenation of the corresponding ketones **5**.[10] All attempts to react the 2,2′-disubstituted analogs **9a,b**[11] with **7** under a variety of reaction conditions proved futile. It is conceivable that

* Address correspondence to this author at his present address.
† Department of Medicinal Chemistry.
‡ Present address: Cephalon Inc., 145 Brandywine Parkway, West Chester, PA 19380-4245.
§ Department of Pharmacology.
|| Department of Biochemistry.
® Abstract published in *Advance ACS Abstracts*, January 1, 1995.

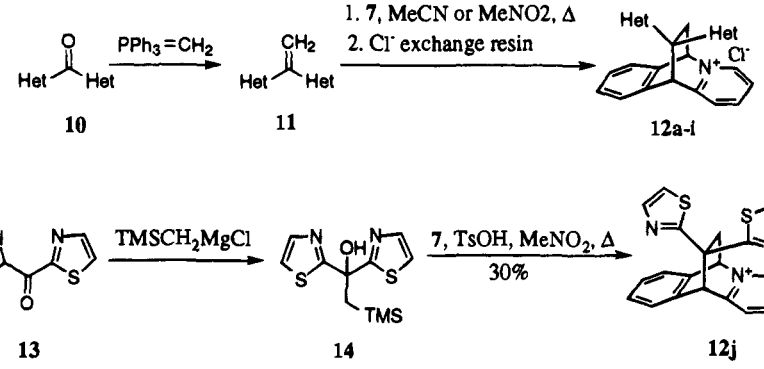
0022-2623/95/1838-0021$09.00/0

Scheme 1

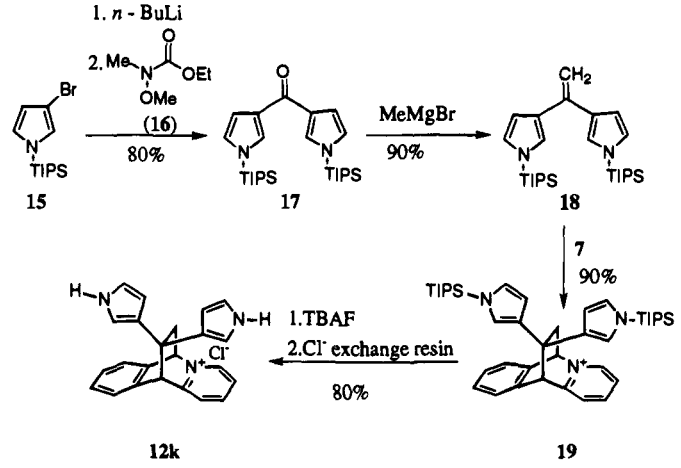




Scheme 2



Scheme 3

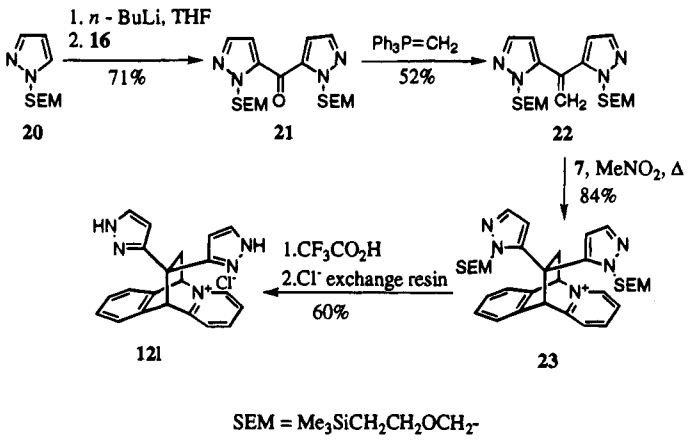


steric crowding around the olefinic double bond in **9a,b** may be responsible for this lack of reactivity. The tetrasubstituted ethylene **9c**[12] also failed to react with **7**.

The 12,12-bis-heteroaryl derivatives **12a–j** were synthesized as shown in Scheme 2. Thus Wittig methylenation of ketones **10**[13] followed by reaction of the intermediate olefin **12** with **7** in refluxing CH_3CN or CH_3NO_2 led to the desired targets **12** in very good yields. Interestingly, attempts to olefinate thiazole ketone **13**[14c] under standard Wittig conditions led to extensive decomposition of the starting material. This problem was overcome by the use of silylmethyl carbinol **14** in the Diels–Alder reaction with **7**.

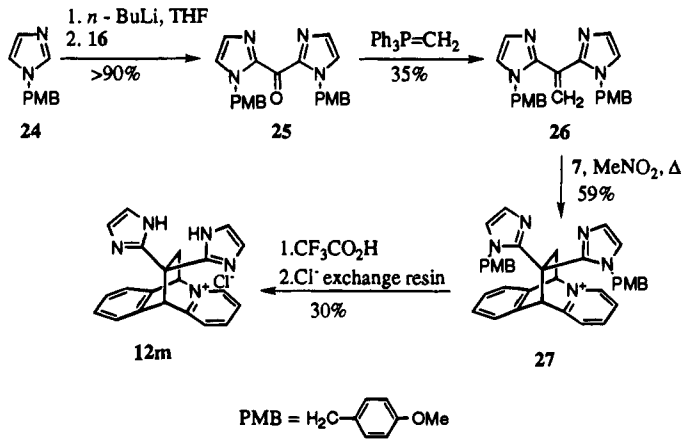
Compounds **12k–m** with heteroaryl groups containing an acidic hydrogen (hence participate as hydrogen bond donors in receptor–ligand interaction) were synthesized according to Schemes 3–5. The anion generated from 3-bromo-1-(triisopropylsilyl)pyrrole (**15**)[14] was treated with the urethane **16**,[15] which gave ketone **17**. Treatment of **17** with excess MeMgBr gave olefin **18**, which was reacted with azoniaanthracene **7** to provide adduct **19**. Finally, removal of the triisopropylsilyl protecting group ($nBu_4N^+F^-$) gave the target **12k**. Compound **12l** was prepared from the SEM pyrazole **20**[16] via a four-step sequence. Thus conversion of **20** to the ketone **21** (nBuLi and then **16**) followed by Wittig methylenation gave olefin **22**. Diels–Alder reaction of

Scheme 4



SEM = $Me_3SiCH_2CH_2OCH_2$-

Scheme 5



PMB = H_2C-C6H4-OMe

22 with **7** provided **23**, which upon removal of the SEM groups (refluxing TFA) provided the bis-pyrazole derivative **12l**. Surprisingly, preparation of the imidazole analog **12m** proved to be very difficult. After considerable effort, a methodology based on a new nitrogen protecting group, namely the 4-methoxybenzyl (PMB)[17] group, was developed to achieve the synthesis of **12m**. Olefin **26**, prepared from **24** via the ketone **25**, underwent smooth inverse demand cycloaddition with **7** to give the adduct **27**. Removal of the PMB groups from **27** was accomplished with trifluoroacetic acid to give **12m**. Since most of the cycloaddition reactions between **7** and the corresponding olefins were carried out with the perchlorate or hexafluorophosphate of salt **7**, the final adducts **8** and **12** were converted to the appropriate chloride salt via an ion exchange column.

Structure–Activity Relationship

The nature of the 12,12-aryl substituent demonstrated exceptional sensitivity toward binding to the TCP site of the NMDA receptor.[18] Substitution on the 12,12-diphenyl rings of analogs **8** had a detrimental effect on the binding affinity (Table 1). The 3,3′-disubstituted derivatives **8b–d** were 5–20-fold less potent than **8a**, while the 4,4′-substituted analogs **8e–h** were completely devoid of activity. We believe that both the electronic and steric effects exerted by the 3,3′ or 4,4′ substituents in **8b–h** are responsible for the observed results.

The heteroaryl-substituted compounds **12a–m** displayed an interesting range of activity (Table 2). Among the three pyridyl derivatives, only the 2-pyridyl analog **12a** was active and equipotent to **8a**, whereas the 3- and 4-pyridyl compounds **12b,c** were inactive. Analogs **12d–g** with electron rich thiophene and furyl rings at the 12 position were 3–4-fold more potent than the lead compound **8a**. The oxazole and thiazole derivatives **12h–j** were synthesized with the hope that combining the beneficial effect of the 2-pyridyl nitrogen of **12a** and the 3-furyl oxygen (or the 2-thienyl sulfur) of **12g** would lead to improvement in binding affinity. To our surprise, both **12h,i** were >50-fold less active than either **12a** or **12g**. Interestingly, the 2-thiazole derivative **12j** was only 10-fold less potent than **12g**.

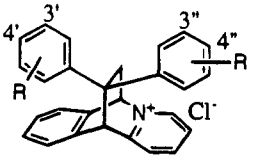
The azole analogs **12k–m** were synthesized to probe the effect of hydrogen donor groups at the 12 position. As the data reveals, such exercise did not have a significant adverse effect on the binding affinity. The imidazole derivative **12m** was however totally inactive in displacing ^{3}[H]TCP from the receptor site.

A selected group of the more potent analogs was further tested for their ability to inhibit NMDA-induced cell death in cultured mouse cortical neurons.[19] As shown in Table 3 and Figure 1, there is a very good correlation ($r = 0.89, p < 0.01$) between ^{3}[H]TCP binding affinity and the efficacy in the NMDA-induced cell death assay.

Electrophysiology and In Vivo Antiischemic Activity

Compound **12g**, the most potent inhibitor in this new class of NMDA antagonists, was evaluated in patch

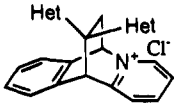
Table 1. Potencies of Compounds **8a–h** in Displacing [^{3}H]TCP Bound to Rat Brain Homogenates



compd	R	formula[a]	cryst solvent	yield[b] (%)	mp (°C)	K_i (nM)[c]
MK-801						2
4						5.3 ± 0.9
8a	H	$C_{27}H_{22}N{\cdot}ClO_4{\cdot}0.5H_2O$	amorph powder[d]	80	150–152	8.2 ± 1.6
8b	3',3"-di-Cl	$C_{27}H_{20}Cl_2N{\cdot}Cl{\cdot}0.75H_2O$	amorph powder	80	166–181	104 ± 7
8c	3',3"-di-Br	$C_{27}H_{20}Br_2N{\cdot}Cl{\cdot}1.0H_2O$	amorph powder	60	194–198	185 ± 38
8d	3',3"-di-OMe	$C_{29}H_{26}NO_2{\cdot}Cl{\cdot}1.5H_2O$	amorph powder	80	164–168	41 ± 5
8e	4',4"-di-F	$C_{27}H_{20}F_2N{\cdot}Cl{\cdot}2.25H_2O$	amorph powder	75	155–161	2207 ± 331
8f	4',4"-di-Cl	$C_{27}H_{20}Cl_2N{\cdot}PF_6$	EtOAc/ether	60	212–215	45% ± 6%[e]
8g	4',4"-di-Br	$C_{27}H_{20}Br_2N{\cdot}Cl{\cdot}2.0H_2O$	amorph powder	85	194–197	22% ± 6%[e]
8h	4',4"-di-OMe	$C_{29}H_{26}NO_2{\cdot}PF_6$	EtOAc/ether	66	238–240	28122 ± 2526

[a] Satisfactory analyses were obtained. [b] Combined yield for the Diels–Alder and ion exchange steps. [c] The binding potencies (K_i) were determined as described in ref 19, and the values reported are mean ± SEM for at least three separate determinations in triplicate. [d] These compounds were isolated as amorphous powders after removal of solvent (usually water) from a Cl^- ion exchange column. [e] Value reported is percent inhibition at 10 μM.

Table 2. [^{3}H]TCP-Displacing Potency of Heteroaryl Analogs **12**



compd	R	formula[a]	cryst solvent	yield[b] (%)	mp (°C)	K_i (nM)[c]
MK-801						2
8a	phenyl					8.2 ± 1.6
12a	2-pyridyl	$C_{25}H_{20}N_3{\cdot}Cl{\cdot}2.0H_2O$	amorph powder	25		8.4 ± 1.7
12b	3-pyridyl	$C_{25}H_{20}N_3{\cdot}Cl{\cdot}2.5H_2O$	amorph powder	30	>225 dec	2267 ± 126
12c	4-pyridyl	$C_{25}H_{20}N_3{\cdot}Cl$[d]	amorph powder	8		54% ± 1%[e]
12d	2-furyl	$C_{23}H_{18}NO_2{\cdot}Cl{\cdot}1.0H_2O$	amorph powder	50	136–141	1.9 ± 0.2
12e	2-thienyl	$C_{23}H_{18}NS_2{\cdot}Cl$	CH_3CN	30	>250 dec	2.1 ± 0.5
12f	3-thieny	$C_{23}H_{18}NS_2{\cdot}Cl{\cdot}2.0H_2O$	CH_3CN	55	>230 dec	1.8 ± 0.4
12g	3-furyl	$C_{23}H_{18}NO_2{\cdot}Cl$	MeOH/CH_3CN	85	260–265	1.8 ± 0.2
12h	5-thiazolyl	$C_{21}H_{16}NS_2{\cdot}ClO_4$[d]	iPrOH	28		120 ± 3
12i	4-oxazolyl	$C_{21}H_{16}N_3O_2{\cdot}ClO_4$	MeOH	76	236–238	93 ± 3
12j	2-thiazolyl	$C_{21}H_{18}NS_2{\cdot}ClO_4$	iPrOH	10	235–237	17 ± 2
12k	3-pyrrolyl	$C_{23}H_{20}N_3{\cdot}Cl{\cdot}0.25H_2O{\cdot}0.25iPrOH$	iPrOH	40	>200 dec	3.1 ± 0.2
12l	3(5)-pyrazolyl	$C_{21}H_{18}N_5{\cdot}Cl{\cdot}0.25H_2O$	water	60	>340	13 ± 0.5
12m	2-imidazolyl	$C_{21}H_{18}N_5{\cdot}Cl$[f]	amorph powder	30		1670 ± 29

[a] Satisfactory analyses were obtained. [b] Combined yield for the Diels–Alder and ion exchange steps. [c] The binding potencies (K_i) were determined as described in ref 19, and the values reported are mean ± SEM for at least three separate determinations in triplicate. [d] Satisfactory elemental analysis could not be obtained for this compound. Characterized by ^{1}H NMR and ^{13}C NMR. [e] Value reported is percent inhibition at 10 μM. [f] HRMS calcd for (M + Cl^-) 340.1562, found 340.1552.

Table 3. Protection against NMDA-Induced Cell Death in Cortical Neurons[a]

compd	K_i (nM)	NMDA-induced cell death: IC_{50} (nM)
MK-801	2	67
8a	8.2 ± 1.6	197
12d	1.9 ± 0.2	36
12e	2.1 ± 0.5	23
12f	1.8 ± 0.2	45
12g	1.8 ± 04	39
12k	3.1 ± 0.2	30
12l	13 ± 0.5	271

[a] Determined as described in ref 6a.

clamp recordings designed to measure closed vs open state binding to the NMDA receptor–ionophore complex.[20] The results are collated in Table 4. As evident from this data, **12g** was >700-fold selective (2 orders of magnitude better than MK-801 and PCP) for binding to the "open state" of the NMDA receptor–channel complex. This is consistent with our hypothesis that a sufficiently hydrophilic (negative log *D*) and potent ligand would be selective for binding to the activated (open) state of the NMDA-sensitive receptor–ionophore complex.[6a,21]

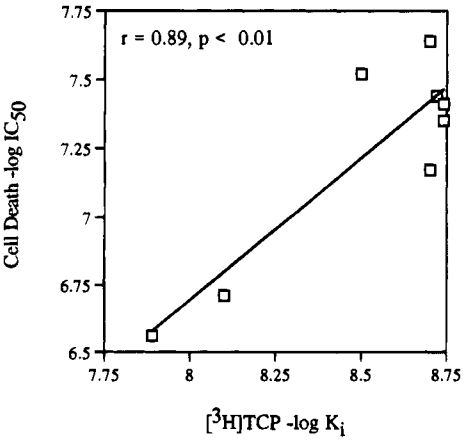


Figure 1. Correlation between ^{3}[H]TCP binding affinity and neuroprotection in cultured mouse cortical neurons.

Table 4. Open vs Closed Channel Binding in Mouse Cortical Neuronal Cultures[a]

compd	log D[b]	IC_{50} (μM) open channel	IC_{50} (μM) closed channel	ratio (CC/OC)
PCP	1.76	0.27	13.9	51
MK-801	1.79	0.1	5.8	58
4	−4.08	0.26	>100 (−8.5%)[c]	>384
12g	−3.41	0.14	>100 (12%)[c]	>700

[a] The open and closed channel binding were determined as described in ref 6a. [b] The log D was measured as described in ref 24. [c] The values in parentheses are the actual percent inhibition of NMDA-induced current at 100 μM.

We postulated that the lack of behavioral effect observed with the TCP site ligand **4** is probably due to its selectivity for the open state of the NMDA receptor. If this contention is correct, compound **12g** that is highly selective for binding to the open state of the NMDA–ion channel complex should also be devoid of the psychomimetic effects usually associated with ligands acting at the PCP site. In conscious rats, when administered via continuous intravenous infusion, **12g** showed no PCP- nor MK-801-like stereotypical behavioral effects up to a dose of 18 mg/kg/h for 6 h.[22,23]

The in vivo antiischemic activity of **12g** was evaluated in a rat middle cerebral artery occlusion/reperfusion model of focal ischemia.[24] At a dose of 6 mg/kg/h for 6.5 h (iv, continuous infusion), **12g** produced ≈50% (n = 15) reduction in infarct volume.

Conclusions

In summary, we have identified a novel series of uncompetitive NMDA antagonists based on the ethanobenzo[*b*]quinolizinium cation template. These compounds competitively displace TCP from the ion channel and were >1000-fold selective for the TCP site.[25] Compound **12g** (WIN 67870-2), with a K_i = 1.8 ± 0.2 nM, is one of the most potent NMDA ion channel blockers known and was efficacious in in vitro and in vivo models of neuroprotection. It was devoid of PCP- and MK-801-like behavioral effects in rats. Studies are underway to probe the effects of substituents on the pyridinium and benzo rings on the potency of this series of compounds. Results of these efforts and the relationship between the physicochemical charactor (e.g., log D) and the open vs closed channel selectivity of this class of NMDA antagonists will be the subject of future publications.

Experimental Section

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 FT NMR spectrometer, and the chemical shifts are expressed in δ units from tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a Nermag 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, or by QTI Technologies. Where analyses are indicated only by symbols of the elements, analytical results are within ±0.4% of the theoretical values except where indicated by individual analyses. Thin layer chromatography (TLC) was performed on E. Merck 5 × 20, 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 Still. Columns were packed with Kieselgel 60, 230-400 mesh. All solvents and reagents were reagent grade unless otherwise noted.

1,1-Di(3′-furyl)ethylene (11g). To a stirred suspension of $Ph_3P^+Br^-$ (6.0 g, 0.017 mol) in anhydrous THF (100 mL) at 0 °C was added 2.5 M nBuLi in hexanes (6.8 mL, 0.017 mol), and the resulting suspension was stirred at room temperature for 1 h. A solution of ketone **10g** (2.5 g 0.0154 mol) in THF (50 mL) was added and the mixture stirred for 1 h. Hexanes (100 mL) were added, and the resulting mixture was filtered through a pad of florisil. The filtrate was concentrated in vacuo and the residue purified by flash chromatography (100% hexanes) to give 2.2 g (88%) of olefin **11g** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.30 (s, 2H), 6.57 (s, 2H), 7.42 (d, J = 1.6 Hz, 2H), 7.51 (d, J = 1.6 Hz, 2H).

Olefins **6b–h** and **11a–i** were prepared in an analogous manner from the corresponding ketones **5** and **10**, respectively.

6,11-Ethano-12,12-di(3′-furanyl)-6,11-dihydrobenzo[*b*]quinolizinium Chloride (12g). A reaction mixture containing benzo[*b*]quinolizinium perchlorate (**7**) (1.4 g, 0.005 mol) and 1,1-di(3′-furyl)ethylene (**11g**) (0.96 g, 0.006 mol) in CH_3CN (50 mL) was heated to reflux for 3 h and cooled to room temperature. The solvents were removed, and the residue was triturated with MeOH. The precipitated solids were collected by filtration and air dried to give 1.9 g (86%) of **12g** (perchlorate salt) as a colorless solid: ^{1}H NMR (DMSO-d_6) δ 2.62 (d, J = 13.2 Hz, 1H), 2.93 (dd, J = 14.3 and 3.2 Hz, 1H), 5.8 (s, 1H), 6.23 (d, J = 1.6 Hz, 1H), 6.48 (s, 1H), 6.68 (s, 1H), 7.28–7.32 (m, 3H), 7.40–7.44 (m, 3H), 7.51 (s, 1H), 7.61 (m, 1H), 7.93 (t, J = 6.9 Hz, 1H), 8.03 (d, J = 7.8 Hz, 1H), 8.40 (t, J = 7.8 Hz, 1H), 9.24 (d, J = 6.0 Hz, 1H).

A column of Dowex 1X 2–200 ion exchange resin (100 g) was eluted with 0.5 N HCl until the eluent was clear. The resulting resin was washed with distilled water until the pH of the washings was about 6.5–7.0. A solution of the above perchlorate (1.9 g) in a minimum amount of acetonitrile was loaded onto the resin column and eluted with water until no product was detected by TLC in the eluents. The water was removed under reduced pressure to provide 1.3 g (80%) of chloride **12g** as a colorless solid: mp 260–265 °C; ^{1}H NMR (DMSO-d_6) δ 2.61 (d, J = 14.9 Hz, 1H), 2.95 (dd, J = 14.7 and 3.0 Hz, 1H), 5.96 (s, 1H), 6.26 (s, 1H), 6.52 (s, 1H), 6.85 (s, 1H), 7.28–7.31 (m, 3H), 7.41 (m, 2H), 7.51 (s, 2H), 7.62 (m, 1H), 7.94 (t, J = 7.8 Hz, 1H), 8.09 (d, J = 7.8 Hz, 1H), 8.41 (t, J = 7.8 Hz, 1H), 9.40 (d, J = 5.9 Hz, 1H). Anal. ($C_{13}H_{18}NO_2 \cdot Cl$) C, H, N.

Compounds **8b–i** and **12a–i** were prepared in an analogous manner from **7** and the appropriate olefin.

^{1}H NMR data for 6,11-Ethano-12,12-di(5′-thiazolyl)-6,11-dihydrobenzo[*b*]quinolizinium perchlorate (12h): ^{1}H NMR (DMSO-d_6) δ 3.10 (d, J = 14.6 Hz, 1H), 3.34 (dd, J = 2.8 and 14.3 Hz, 1H), 6.18 (s, 1H), 6.76 (s, 1H), 7.33 (m, 3H), 7.48 (s, 1H), 7.65 (d, J = 7.3 Hz, 1H), 7.90 (s, 1H), 7.98 (t, J = 7.0 Hz, 1H), 8.20 (d, J = 7.7 Hz, 1H), 8.45 (t, J = 7.7 Hz, 1H), 8.9 (s, 1H), 8.98 (s, 1H), 9.26 (d, J = 5.9 Hz, 1H).

6,11-Ethano-12,12-di(2′-thiazolyl)-6,11-dihydrobenzo[*b*]quinolizinium Perchlorate (12j). To a solution of 1,1-di(2′-thiazolyl) ketone (**13**) (1.96 g, 0.001 mol) in THF (75 mL) cooled to 0 °C under nitrogen was added 1 M $TMSCH_2MgCl$ in ether (15 mL, 0.015 mol), and the resulting mixture was stirred at room temperature, for 1 h. Saturated NH_4Cl (3 mL) was added and the mixture diluted with CH_2Cl_2 (100 mL) and filtered. The filtrate was washed with saturated NH_4Cl and the organic layer dried and concentrated in vacuo. The residue was purified by passing through a pad of silica gel, eluting with hexane/ether/CH_2Cl_2 (4:2:1), to afford 1.1 g (39%) of carbinol **14**: ^{1}H NMR ($CDCl_3$) δ −0.21 (s, 9H), 1.91 (s, 2H), 5.43 (br s, 1H), 7.32 (d, J = 1.8 Hz, 1H), 7.75 (d, J = 1.7 Hz, 1H).

A mixture of **7** (0.18 g, 0.0007 mol), carbinol **14** (0.24 g, 0.0009 mol), and *p*-toluenesulfonic acid (0.21 g, 0.0007 mol) in CH_3NO_2 (10 mL) was refluxed for 3 h under nitrogen and cooled to room temperature. The resulting solution was concentrated in vacuo and the residue purified by chromatography (EtOAc and then EtOAc/CH_2Cl_2/MeOH, 2:6:1) to yield a foamy solid. Crystallization from 2-PrOH gave 0.03 g (10%)

of **12j** as a colorless solid: mp 235–237 °C; ^{1}H NMR (DMSO-d_6) δ 3.32 (d, J = 14.5 Hz, 1H), 3.62 (dd, J = 14.4 and 2.9 Hz, 1H), 6.09 (s, 1H), 6.79 (s, 1H), 7.25 (m, 4H), 7.75 (m, 4H), 8.01 (m, 2H), 8.39 (t, J = 7.7 Hz, 1H), 9.38 (d, J = 6.0 Hz, 1H). Anal. ($C_{21}H_{16}N_3S_2 \cdot ClO_4$) C, H, N.

6,11-Ethano-12,12-di(3′-pyrrolyl)-6,11-dihydrobenzo[*b*]quinolizinium Chloride (12k). To a cooled (−78 °C) solution of 3-bromo-1-(triisopropylsilyl)pyrrole (**15**) (10.4 g, 0.029 mol) in anhydrous THF (500 mL) was added 2.5 M nBuLi in hexanes (12.3 mL, 0.031 mol) over a 30 min period, and the mixture was stirred at −78 °C for 0.5 h. *N*-Methyl-*N*-methoxyurethane (**16**) (1.9 g, 0.014 mol) in THF (30 mL) was added and the mixture slowly warmed to room temperature and stirred for 20 h. After the reaction was quenched with saturated $NaHCO_3$ (100 mL), the organic layer was separated, dried, and concentrated in vacuo. Purification of the residue by chromatography (2:1 hexanes/ether) gave 4.25 g (64%) of ketone **17** as a viscous liquid: ^{1}H NMR ($CDCl_3$) δ 1.11 (d, J = 7.5 Hz, 36H), 1.45 (m, 1H), 6.73 (m, 2H), 6.82 (m, 2H), 7.39 (m, 2H).

To a solution of **17** (1.0 g, 0.002 mol) in THF (30 mL) at room temperature was added 3.0 M MeMgBr in ether (3 mL, 0.009 mol). After the mixture had stirred for 2 h at room temperature, the reaction was carefully quenched with saturated $NaHCO_3$ solution. The resulting mixture was diluted with CH_2Cl_2 and filtered through a pad of florisil. The filtrate was concentrated in vacuo to provide 1.1 g (100%) of olefin **18** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.11 (d, J = 7.5 Hz, 36H), 1.45 (m, 6H), 5.28 (s, 2H), 6.52 (m, 2H), 6.78 (m, 2H), 6.92 (m, 2H).

Reaction of **18** (1.1 g, 0.023 mol) with **7** (0.59 g, 0.021 mol) in refluxing acetonitrile (1 h) provided, after workup as described for **12g**, 1.25 g (79%) of adduct **19** as a bright yellow solid: ^{1}H NMR ($CDCl_3$) δ 0.93 (m, 36H), 1.22 (m, 6H), 2.61 (d, J = 14.5 Hz, 1H), 3.10 (dd, J = 3.2 and 14.7 Hz, 1H), 5.13 (s, 1H), 5.53 (m, 1H), 5.99 (m, 2H), 6.26 (s, 1H), 6.43 (m, 1H), 6.56 (m, 1H), 6.71 (s, 1H), 7.25 (m, 3H), 7.42 (m, 1H), 7.61 (d, J = 7.8 Hz, 1H), 7.72 (d, J = 7.6 Hz, 1H), 7.90 (t, J = 8.1 Hz, 1H), 9.25 (d, J = 6.0 Hz, 1H).

To a solution of **19** (1.22 g, 1.6 mmol) in CH_2Cl_2 (50 mL) was added 1 M tetrabutylammonium fluoride in THF (2.8 mL, 3.1 mmol). After the mixture had stirred for 15 min at room temperature, the solids that precipitated were collected by filtration to provide crude **12k** as its perchlorate salt. Conversion of the perchlorate salt to the corresponding chloride using the ion exchange procedure provided 0.35 g (39% from **19**) of crude **12k**. Crystallization from 2-PrOH provided analytically pure **12k**: mp >200 °C dec; ^{1}H NMR (D_2O) δ 2.67 (d, J = 14.6 Hz, 1H), 3.03 (dd, J = 3.1 and 14.4 Hz, 1H), 5.51 (s, 1H), 5.79 (m, 1H), 5.99 (m, 1H), 6.43 (m, 3H), 6.56 (s, 1H), 6.63 (s, 1H), 7.25 (m, 2H), 7.41 (m, 1H), 7.57 (m, 1H), 7.62 (t, J = 7.1 Hz, 1H), 7.72 (d, J = 7.9 Hz, 1H), 8.08 (t, J = 7.8 Hz, 1H), 8.86 (d, J = 6.0 Hz, 1H). Anal. ($C_{23}H_{20}N_3 \cdot Cl \cdot 0.25H_2O \cdot 0.25$ 2-PrOH) C, H, N.

6,11-Ethano-12,12-di(3′-pyrazolyl)-6,11-dihydrobenzo[*b*]quinolizinium Chloride (12l). Starting from 1-[[1-(trimethylsilyl)ethoxy]methyl]pyrazole (**20**) (19.8 g, 0.1 mol), ketone **21** was prepared in 71% yield by a procedure similar to the one described above for **17**: ^{1}H NMR ($CDCl_3$) δ −0.01 (s, 9H), 0.84 (t, J = 8.4 Hz, 4H), 3.57 (t, J = 8.0 Hz, 4H), 5.95 (s, 4H), 6.95 (d, J = 1.8 Hz, 2H), 7.62 (d, J = 1.7 Hz, 2H).

Ketone **21** was converted to the olefin **22** in 52% yield by a procedure similar to that described above for **12g**: ^{1}H NMR ($CDCl_3$) δ −0.01 (s, 9H), 0.84 (t, J = 8.4 Hz, 4H), 3.57 (t, J = 8.0 Hz, 4H), 5.30 (s, 4H), 5.93 (s, 2H), 6.23 (d, J = 1.8 Hz, 2H), 7.50 (d, J = 1.7 Hz, 2H).

A reaction mixture containing **7** (1.9 g, 0.007 mol) and olefin **22** (3.6 g, 0.009 mol) in nitromethane (70 mL) was heated at reflux for 9 h and cooled to room temperature. The solvent was removed in vacuo. The residue was triturated with 2-propanol, and the solids were collected to give 4.0 g (84%) of 6,11-ethano-12,12-bis[5-[[1-(trimethylsilyl)ethoxy]methyl]-pyrazolyl]-6,11-dihydrobenzo[*b*]quinolizinium perchlorate (**23**) as a colorless solid: mp 123–125 °C. Anal. ($C_{33}H_{46}N_5O_2Si_2 \cdot ClO_4$) C, H, N.

A solution of **23** (0.53 g, 0.0007 mol) in dichloroethane (10 mL) containing CF_3CO_2H (5 mL) was stirred at room temperature for 2 h. After removal of the volatiles in vacuo, the residue was purified by chromatography on silica gel using EtOAc/PAW (1:1; PAW = pyridine/acetic acid/water, 55:25:20) to give 0.17 g (60%) of **12l** as a mixture of perchlorate and acetate salts. This mixture was converted to the corresponding Cl^- salt using the ion exchange method described above for **12g**. Crystallization from water provided analytically pure **12l**: mp >340 °C; ^{1}H NMR (D_2O) δ 3.06 (dd, J = 1.6 and 14.1 Hz, 1H), 3.34 (dd, J = 3.2 and 14.2 Hz, 1H), 5.78 (s, 1H), 6.22 (m, 2H), 6.53 (s, 1H), 7.26 (m, 2H), 7.44 (m, 3H), 7.58 (m, 1H), 7.70 (t, J = 7.3 Hz, 1H), 7.87 (d, J = 7.8 Hz, 1H), 8.16 (t, J = 8.0 Hz, 1H), 8.99 (d, J = 6.0 Hz, 1H). Anal. ($C_{21}H_{18}N_5 \cdot Cl \cdot 0.25H_2O$) C, H, N.

6,11-Ethano-12,12-di(2′-imidazolyl)-6,11-dihydrobenzo[*b*]quinolizinium Chloride (12m). To a solution of imidazole (10.88 g, 0.16 mol) in DMF (150 mL) was added 60% suspension of NaH (6.4 g, 0.16 mol). After stirring for 1 h at room temperature, 4-methoxybenzyl chloride (25.0 g, 0.16 mol) was added. The resulting mixture was stirred at room temperature for 24 h and evaporated to dryness in vacuo. The residue was partitioned between CH_2Cl_2 and water, and the layers were separated. The organic phase was dried and concentrated in vacuo. Purification by chromatography (CH_2Cl_2/MeOH, 9:1) gave 25.3 g of 1-(4-methoxybenzyl)imidazole (**24**) as an oil.

Conversion of **24** to the ketone **25** was achieved in 100% yield by a procedure similar to the one reported above for **17**: ^{1}H NMR ($CDCl_3$) δ 3.82 (s, 6H), 5.55 (s, 4H), 6.85 (d, J = 7.8 Hz, 4H), 7.15 (s, 1H), 7.22 (d, J = 7.9 Hz, 4H), 7.35 (s, 1H).

To a suspension of K^+tBuO^- (3.56 g, 0.032 mol) in THF (150 mL) was added methyltriphenylphosphonium bromide (11.4 g, 0.03 mol). The resulting mixture was stirred at room temperature for 2 h, and a solution of ketone **25** (8.5 g, 0.021 mol) in THF (100 mL) was added. After stirring at room temperature for 2 h, acetone was added and the mixture filtered through supercel, eluting with EtOAc. The combined eluents were concentrated in vacuo, and the residue was purified by chromatography (EtOAc) to give 3.0 g (35%) of 1,1-bis[2-[1-(4-methoxybenzyl)imidazolyl]]ethylene (**26**) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 3.8 (s, 6H), 4.6 (s, 4H), 5.8 (s, 2H), 6.8 (m, 10H), 7.1 (s, 2H).

Diels–Alder reaction of **26** with **5** in refluxing nitromethane (4 days) gave, after chromatographic purification, 2.1 g (59%) of adduct **27**. A solution of **27** in trifluoroacetic acid (15 mL) was heated at reflux for 24 h. After cooling to room temperature, the mixture was evaporated to dryness. The residue was taken up in boiling MeOH/H_2O (200 mL) and filtered. The filtrate was evaporated to dryness and the residue converted to the chloride **12m** by the ion exchange procedure: yield, 0.9 g (30% from **26**); ^{1}H NMR (DMSO-d_6) δ 3.4 (m, 2H), 6.6 (s, 1H), 7.05 (s, 1H), 7.35 (m, 6H), 7.42 (d, J = 7.7 Hz, 1H), 7.62 (d, J = 6.7 Hz, 1H), 8.01 (m, 1H), 8.43 (m, 2H), 9.45 (d, J = 5.9 Hz, 1H); HRMS (M^+ − Cl) calcd for $C_{21}H_{18}N_5$ 340.1562, found 340.1552.

Acknowledgment. We thank the expert technical assistance of Larry Wagner, Lee Hildebrand, Lorraine Lanyon, Felicia Ford-Rice, Connie Zobre, Fran Casiano, and Susan Chippari in acquiring biological data, Dr. Philip Carabateas and Mr. Gary Pilling for their assistance in the synthesis of compounds **5b** and **12f**, Dr. Ethel Krasney and Mr. Walt Wheeler for log *D* data, and Mr. Al Hlavac for mass spectroscopic data. We also wish to thank Drs. Susan Ward and R. Everett for many helpful discussions.

References

(1) Lehmann, J. The NMDA Receptor. *Drugs Future* **1989**, *14*, 1059–1070.
(2) Wong, E. H. F.; Kemp, J. A. Sites for Antagonism on the N-Methyl-D-Aspartate Receptor Channel Complex. *Annu. Rev. Pharmacol. Toxicol.* **1991**, *31*, 401–425.

(3) (a) Honey, C. R.; Miljkovic, Z.; MacDonald, J. F. Ketamine and Phencyclidine Cause a Voltage-Dependent Block of Responses to L-Aspartic Acid. *Neurosci. Lett.* **1985**, *61*, 135–139. (b) Wong, E. H. F.; Kemp, J. A.; Priestly, T.; Knight, A. R.; Woodruff, G. N.; Iversen, L. L. The Anti-Convulsant MK-801 is a potent N-Methyl-D-Aspartate Antagonist. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 7104–7108.

(4) Sauer, D.; Nuglisch, J.; Rossberg, C.; Mennel, H.; Beck, T.; Bielenberg, G. W.; Krieglstein. Phencyclidine Reduces Postischemic Neuronal Necrosis in Rat Hippocampus Without Changing Blood Flow. *J. Neurosci. Lett.* **1988**, *91*, 327. (b) Gill, R.; Brazell, G. N.; Wooddruff, G. N.; Kemp, J. A. The Neuroprotective Action of Dizocilpine (MK-801) in the Rat Middle Cerebral Artery Occusion Model of Focal Ischemia. *Br. J. Pharmacol.* **1991**, *103*, 2030–2036.

(5) Tricklebank, M. D.; Singh, L.; Oles, R. J.; Preston, C.; Iversen, S. D. The behavioural Effects of MK-801: A Comparison with Antagonists Acting Non-competitively and Competitively at the NMDA Receptor. *Eur. J. Pharmacol.* **1989**, *167*, 127.

(6) (a) Mallamo, J. P.; Earley, W. G.; Kumar, V.; Subramanyam, C.; Dority, J. A., Jr.; Miller, M. S.; DeHaven-Hudkins, D. L.; Ault, B.; Herrmann, J. L., Jr.; Dung, J. S.; McMullen, L. A.; Kullnig, R.; Magee, L. J. Identification, Synthesis and Characterization of a Unique Class of NMDA antagonists. The 6,11-Ethano-Benzo(b)quinolizinium Cation. *J. Med. Chem.* **1994**, *37*, 4438–4448. (b) DeHaven-Hudkins, D. L.; Ford-Rice, F. Y.; Daubert, J. D.; Earley, W. G.; Mallamo, J. P.; Lanyon, L. F. Binding of the Novel Ligand [^{3}H] WIN 63480 to the Phencyclidine (PCP) Receptor. Presented at the Society for Neuroscience Meeting, Miami Beach, FL, November 13–18, 1994.

(7) Miller, M. S.; Ault, B.; DeHaven-Hudkins, D. L.; Earley, W. G.; Luttinger, D.; Mallamo, J. P.; Ward, S. J. WIN 63480: A Novel, Open Channel-Selective NMDA-Antagonist with Antiischemic Activity. Presented at the Society for Neuroscience Meeting, Miami Beach, FL, November 13–18, 1994.

(8) The experimental protocol to determine the open vs closed channel block of the NMDA receptor by compound **4** and MK-801 is detailed in ref 6a. Also, see: Ault, B.; Luttinger, D.; Ward, S. J.; Mallamo, J. P.; Martin, D.; Salt, T. E.; Eaton, S. A.; Zheng, X.; Zukin, R. S.; Miller, M. S. WIN 63480, a High Affinity Uncompetitive NMDA Channel Blocker, has Reduced Closed Channel Access Compared to MK-801. Abstracts of Papers, Society for Neuroscience Meeting, Miami Beach, FL, November 13–18, 1994; Abstr. 121.5.

(9) (a) Fields, D. L.; Regan, T. H.; Dignan, J. C. Diels-Alder Reactions Involving Azonia Polycyclic Aromatic Compounds and Nucleophilic Dienophiles. *J. Org. Chem.* **1968**, *33*, 390–395. (b) The lead compound in this series, **8a**, is known in the literature and was prepared as described in: Bradsher, C. K.; Westerman, I. J. Stereochemistry of Polar Cycloadditions. *J. Org. Chem.* **1979**, *44*, 727.

(10) (a) Ketones **5b**,c were prepared according to the method of: Gomberg, M.; Bailar, J. C., Jr. Halogen Substituted Aromatic Pinacols and the Formation of Ketyl Radicals, $R_2(IMgO)C$-. *J. Am. Chem. Soc.* **1955**, *51*, 2229–2238. (b) Ketone **5d** was prepared as described in: Dharanipragada, R.; Ferguson, S. B.; Diederich, F. A Novel Optically Active Host: Design, Computer Graphics, Synthesis and Diastereomeric Complex Formation in Aqueous Solution. *J. Am. Chem. Soc.* **1988**, *110*, 1679. (c) All the 4,4′-substituted benzophenones (compounds **5e**–**h**) used in this study were available from Aldrich Chemical Co.

(11) Olefin **9a** was prepared from the corresponding ketone (commercially available from Lancaster Synthesis) via Wittig methylenation. Olefin **9b** is commercially available from Frinton Laboratories.

(12) McMurry, J. E.; Krepski, L. R. Synthesis of Unsymmetrical Olefins by Titanium (0) Induced Mixed Carbonyl Coupling. Some Comments on the Mechanism of the Pinacol Reaction. *J. Org. Chem.* **1976**, *24*, 3929.

(13) The ketones **10a**–**h** were prepared as per the following reported methods. (a) **10a**: Commercially available from Aldrich Chemical Co. (b) **10b**,**c**: Henze, H. R.; Knowles, M. B. Synthesis of 5-(Pyridyl substituted)hydantoins. *J. Org. Chem.* **1954**, *19*, 1127–1135. (c) **10d**–**f**, **13**: Strekowski, L.; Wydra, R. L.; Cegla, M. T.; Czarny, A.; Patterson, S. Efficient Preparation of Ketones from N-(Ethoxymethylene)aniline and Organometallic Reagents. *J. Org. Chem.* **1989**, *54*, 6120–6123. (d) **10g**: Benassi, R.; Folli, U.; Iarossi, D.; Schenetti, L.; Taddesi, F.; Musatti, A.; Nardelli, M. Conformational Analysis of Organic Carbonyl Compounds. Part 11. *J. Chem. Soc., Perkin Trans. II* **1989**, 1741–1751. (e) Ketone **10h** was prepared by a modification of the procedure used by Dondoni for the preparation of bis-thiazolyl ketone: Dondoni, A.; Mastellari, A. R.; Medici, A.; Negrini, E.; Pedrini, P. Synthesis of Stannylthiazoles and Mixed Stannyl-Silylthiazoles and their Use for a Convenient Preparation of Mono- and Bis-halothiazoles. *Synthesis* **1986**, 757–780. (f) The bis-oxazole ketone **10i** was prepared by Collins oxidation of the corresponding carbinol which in turn was synthesized according to the method of: Hodges, J. C.; Patt, W. C.; Connolly, C. J. Reactions of Lithiooxazole. *J. Org. Chem.* **1991**, *56*, 449.

(14) Bray, B. L.; Mathies, P. H.; Naef, R.; Solas, D. R.; Tidwell, T. T.; Artis, D. R.; Muchowski, J. M. N-(Triisopropylsilyl)pyrrole. A Progenitor "Par Excellence" of 3-Substituted Pyrroles. *J. Org. Chem.* **1990**, *55*, 6317–6328.

(15) Available from Aldrich Chemical Co., Milwaukee, WI.

(16) Fugina, N.; Holzer, W.; Wasicky, M. The [2-(Trimethylsilyl)-ethoxy]methyl Function as a Suitable N-1 Protecting Group in Lithiation Reactions with Pyrazoles and 1,2,4-Triazoles. *Heterocycles* **1992**, *34*, 303–314.

(17) Subramanyam, C. 4-Methoxybenzyl (PMB), a Versatile Protecting Group for The Regiospecific Lithiation and Functionalisation of Pyrazoles. *Synth. Commun.* in press.

(18) The [^{3}H]TCP binding affinity of the compounds reported were determined according to the method of: Vignon, J.; Chicheportiche, R.; Chicheportiche, M.; Kamenka, J. M.; Geneste, P.; Lazdunski, M. [^{3}H] TCP: A New Tool with High Affinity for the PCP Receptor in Rat Brain. *Brain Res.* **1983**, *140*, 194–197.

(19) The activity in this assay was determined as described in ref 6a.

(20) The open vs closed channel blockage of the NMDA receptor of **12g** and the other compounds reported in Table 4 was determined as described in ref 6a.

(21) A similar observation of binding of permanently charged local anesthetic QX314 to the open state of Na^+ channels was recently reported; see: Ragsdale, D. S.; McPhee, J. C.; Scheuer, T.; Catterall, W. A. Molecular Determinants of State-Dependent Block of Na^+ Channels by Local Anesthetics. *Science* **1994**, *265*, 1724–1728.

(22) This was the maximum tolerated sublethal dose (MTD) and is 3 times the dose (ED_{50}) that produced 50% reduction in infarct volume in a rat model of focal ischemia.

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

(24) He, Y. Y.; Hsu, C. Y.; Ezrin, A. M.; Miller, M. S. Polyethylene glycol-conjugated Superoxide Dismutase in Focal Cerebral Ischemia-Reperfusion. *Am. J. Physiol.* **1993**, *265*, H252–256.

(25) The selectivity was measured against the following receptors: opiate, α-1, α-2, BDZ, β, 5-Ht, Ca^{2+}, muscarinic, H1, D1, D2, A1, A2, and σ.

(26) Log *D*s (partition coefficient between pH 7.4 buffer and octanol) were measured on a Sirius PCA 101 instrument by Water Technology Associates of Reading, PA, using the methodology described in: Avdeef, A. Difference Plots for Determining Ion-Pair Octanol-Water Partition Coefficients of Multiprotic Substances. *Quant. Struct.-Act. Relat.* **1992**, 510–517.

JM940695C