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**Supplementary Material Available:** Atomic numbering schemes for 4 and 5 and tables of atomic coordinates, thermal parameters, bond distances, and bond angles (15 pages). Ordering information is given on any current masthead page.

# 4-(Phosphonoalkyl)- and 4-(Phosphonoalkenyl)-2-piperidinecarboxylic Acids: Synthesis, Activity at *N*-Methyl-D-aspartic Acid Receptors, and Anticonvulsant Activity

Alan J. Hutchison,* Michael Williams, Christoff Angst, Reynalda de Jesus, Louis Blanchard, Robert H. Jackson, E. Jay Wilusz, Deborah E. Murphy, Patrick S. Bernard, Josef Schneider, Thomas Campbell, Wayne Guida, and Matthew A. Sills

*Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation, 556 Morris Avenue, Summit, New Jersey 07901.*
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A series of 4-(phosphonoalkyl)- and 4-(phosphonoalkenyl)-2-piperidinecarboxylic acids were synthesized, and their biological activity was assessed as competitive ligands for the NMDA receptor, both in vitro by using a receptor binding assay ([$^3$H]CGS 19755 binding) and in vivo by using an NMDA seizure model in mice. The analogues were also evaluated in [$^3$H]AMPA and [$^3$H]kainate binding to assess their affinity for non-NMDA excitatory amino acid receptor subtypes. A number of these analogues show potent and selective NMDA antagonistic activity both in vitro and in vivo. Most notable are 4-(phosphonomethyl)-2-piperidinecarboxylic acid (**1a**) (CGS 19755) and the phosphonopropenyl analogue **1i**, both of which show anticonvulsant activity in the 1–2 mg/kg ip range. With the aid of computer-assisted modeling, a putative bioactive conformation for AP-5 is hypothesized from the SAR data presented and a preliminary model for the antagonist-preferring state of the NMDA receptor is presented.

Amino acids have an intimate role in neutrotransmission processes in the mammalian CNS.[1] $\gamma$-Aminobutyric acid (GABA) and its analogues have inhibitory actions mediated via two distinct receptor subtypes termed GABA-A and GABA-B. The excitatory amino acids, aspartate and glutamate, mediate their actions via at least three classes of receptors which are generally represented by the prototypical agonists *N*-methyl-D-aspartatic acid (NMDA), quisqualic acid (QUIS), and kainic acid (KA).[2] Of these the NMDA receptor has been the most studied. Excess activity at this receptor has deleterious effects on CNS function. Antagonists of the NMDA receptor could thus have potential utility in a number of CNS disorders, most notably in the treatment of epilepsy and the neuronal damage resulting from cerebral ischemia.[3] The present paper describes the development of potent and selective ligands for the NMDA receptor subtype.

At the initiation of these studies the most potent competitive NMDA antagonists known were 2-amino-5-phosphonopentanoic acid (AP-5) and 2-amino-7-phosphonoheptanoic acid (AP-7), discovered by Watkins[4] (see Figure 1). We sought to enhance the biological activity of these templates by the classical medicinal chemistry strategy of conformational restriction. Although both AP-5 and AP-7 are extremely flexible molecules possessing many energetically accessible conformers, we made the initial assumption that the fully extended (all-anti) conformer was the bioactive one for AP-5 as shown in Figure 1. Furthermore, it was known that *cis*-piperidine-2,3-dicarboxylic acid (*cis*-PDA) was a reasonably potent NMDA partial agonist,[5] suggesting that the piperidine-2-carboxylic acid moiety could fit within the exclusion volume of the NMDA receptor site. The superimposition of the all-anti AP-5 conformer and *cis*-PDA led to the synthesis of **1a** (CGS 19755), which was initially identified in a functional assay involving acetylcholine release and subsequently characterized with a binding assay using [$^3$H]CPP [[3-(±)-(2-carboxypiperazin-4-yl)prop-1-yl]phosphonic acid] as the ligand.[6] As a result **1a** was found out to be a potent and selective competitive NMDA antagonist[7] that is an effective anticonvulsant[8] and antiischemic agent[9] which is presently undergoing extensive biological and toxicological evaluation.

This paper is concerned with the synthesis and SAR of a number of 4-(phosphonoalkyl)-2-piperidinecarboxylic acid analogues of **1a** as both receptor ligands and anticonvulsants. The present structure–activity data were derived by using [$^3$H]CGS 19755 (**1a**) as ligand because its higher affinity permits the use of a filtration methodology to isolate bound radioactivity, in contrast to CPP which requires the use of the more time-consuming centrifugation methodology.[10] Affinity of the analogues at quisqualate

* Present address: Nurogen Corp., 35 NE Industrial Rd, Branford, CT 06405.

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Table I. Physical and Analytical Data for the Analogues 1a–h

| compd no. | substituents[a] ($n$, $R_1$–$R_5$) | overal yield,[b] % | mp, °C | formula | anal.[c] |
|---|---|---|---|---|---|
| 1a | $n$ = 0, $R_1$–$R_5$ = H | 42 | 290–292 | $C_7H_{14}NO_5P$ | C, H, N |
| 1b | $n$ = 2, $R_1$–$R_5$ = H | 48 | 285–286 | $C_9H_{18}NO_5P$ | C, H, N |
| 1c | $n$ = 0, $R_2$–$R_5$ = H, $R_1$ = Me | 26 | 260–265 | $C_8H_{16}NO_5P$ | C, H, N |
| 1d | $n$ = 0, $R_1$, $R_2$, $R_4$, $R_5$ = H, $R_3$ = Me | 18 | 220–224 | $C_8H_{16}NO_5P$ | C, H, N |
| 1e | $n$ = 0, $R_1$, $R_2$, $R_5$ = H, $R_3$ = $R_4$ = Me | 17 | 250–252[d] | $C_9H_{19}ClNO_5P$ | C, H, N[e] |
| 1f | $n$ = 0, $R_1$–$R_4$ = H, $R_5$ = Me | 22 | 325–327 | $C_8H_{16}NO_5P$ | C, H, N |
| 1g | $n$ = 0, $R_1$, $R_3$–$R_5$ = H, $R_2$ = Me | 4 | 250–252 | $C_8H_{16}NO_5P$ | C, H, N |
| 1h | $n$ = 0, $R_1$–$R_5$ = H, $N$-Me of 1a | 31[f] | 256–260 | $C_8H_{16}NO_5P$ | C, H, N |

[a] As shown in Scheme I. [b] Overall yield from 2a–f unless otherwise noted. [c] Combustion analyses were within ± 0.04% of the theoretical value. [d] As the HCl salt. [e] Calculated with 1 mol of $NH_4^+Cl^-$. [f] Overall yield from 5a.

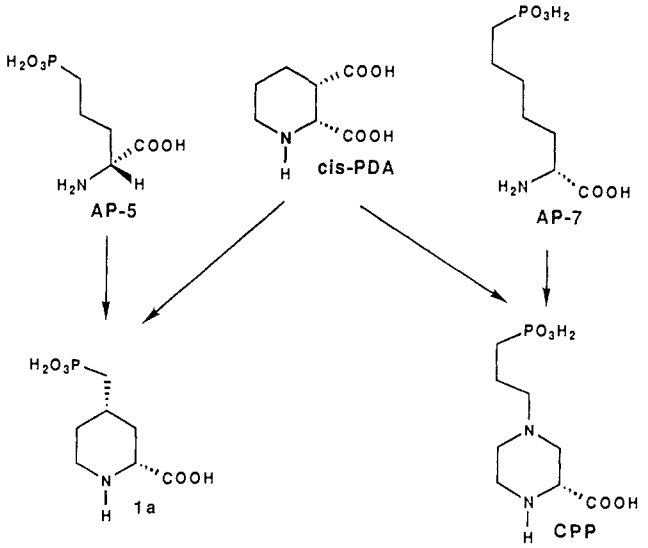



Figure 1. Rationale for synthesis of 1a.

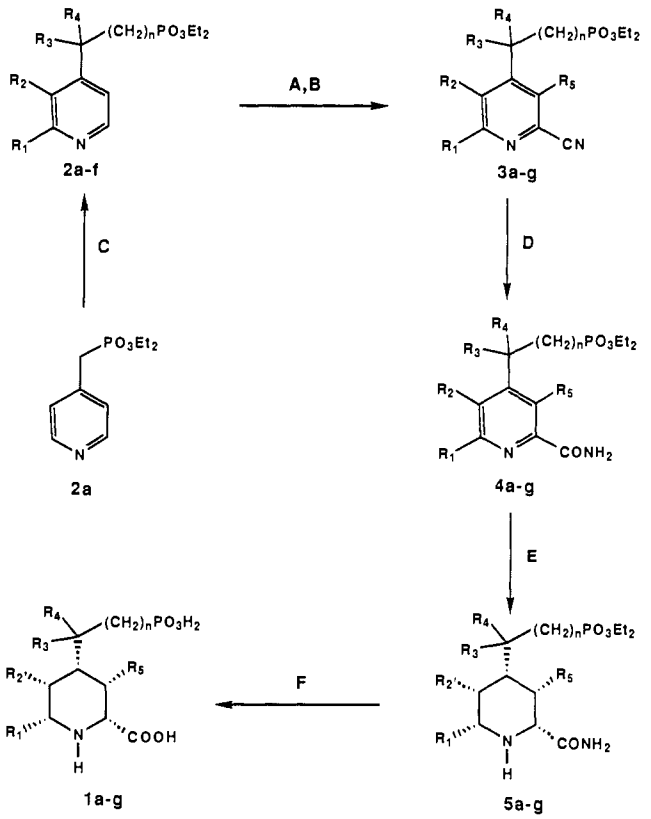
and kainate receptors was assessed by using [$^3$H]AMPA and [$^3$H]kainate binding.[11,12] All compounds were evaluated for their ability to antagonize NMDA convulsions in CF-1 mice (Crl: CF1BR) as described under Experimental Section.[8]

Finally, on the basis of the analysis of published data on competitive NMDA antagonists including those presented in this paper we have constructed a preliminary model for the antagonist preferring state of the NMDA receptor.

## Chemistry

The 4-(phosphonoalkyl)-2-piperidinecarboxylic acids **1a–g** were synthesized as outlined in Scheme I. The appropriately substituted 4-picolyl chlorides or 4-(3-chloropropyl)pyridines were reacted with the sodium salt of diethyl phosphite in toluene[13] to afford the corresponding 4-[(diethylphosphono)alkyl]pyridines **2a–c,f** in yields of 79–91%. The 4-[(diethylphosphono)alkyl]-pyridines **2d,e** were prepared by alkylation of **2a** with methyl iodide in either LDA/THF/HMPA (for **2d**) or NaH in a mixture of DMF and THF (for **2e**). These derivatives were converted to their corresponding $N$-oxides with MCPBA in $CH_2Cl_2$ in excellent yields. Conversion of these $N$-oxides to the 2-cyanopyridine derivatives **3a–g** was achieved in moderate to excellent yields by heating the $N$-oxides derived from **2a–f** with a mixture of trimethylsilyl cyanide and $Et_3N$ at 90 °C for 1–3 h.[14] Selective hydrolysis of the nitriles **3a–g** was accomplished with concentrated $H_2SO_4$ at 80 °C to afford the amides **4a–g** in yields of 60–85%. In the case of **2f** both regioisomers **3f** and **3g** were formed in a ratio of about 6:1. These regioisomers were separated by flash chromatography on silica gel with ethyl acetate/hexane/MeOH/$NH_4OH$ (100:10:5:1) as the eluent after they had been converted to the corresponding amides **4f** and **4g**. Hydrogenation of the amides **4a–g** with $PtO_2$ in acetic acid followed by neutralization with $Na_2CO_3$ in $CH_2Cl_2$ afforded the *cis*-piperidinecarboxamides **5a–g**, which were generally not purified but rather directly hydrolyzed in refluxing 6 N HCl to afford the desired (phosphonoalkyl)piperidinecarboxylic acids **1a–g**. The $N$-methyl derivative **1h** was prepared by reductive alkylation of **5a** in the presence of aqueous formaldehyde with 10% Pd/C as catalyst to afford the $N$-methyl derivative **5h**, which was hydrolyzed to **1h**. The substitution patterns, overall yields from the corresponding chlorides, and melting points for the ana-

Scheme I[a]




[a] Reagents: (A) MCPBA, $CH_2Cl_2$; (B) TMSCN, TEA; (C) NaH, DMF, MeI; (D) concentrated $H_2SO_4$; (E) $H_2$, $PtO_2$, HOAc; (F) 6 N HCl.

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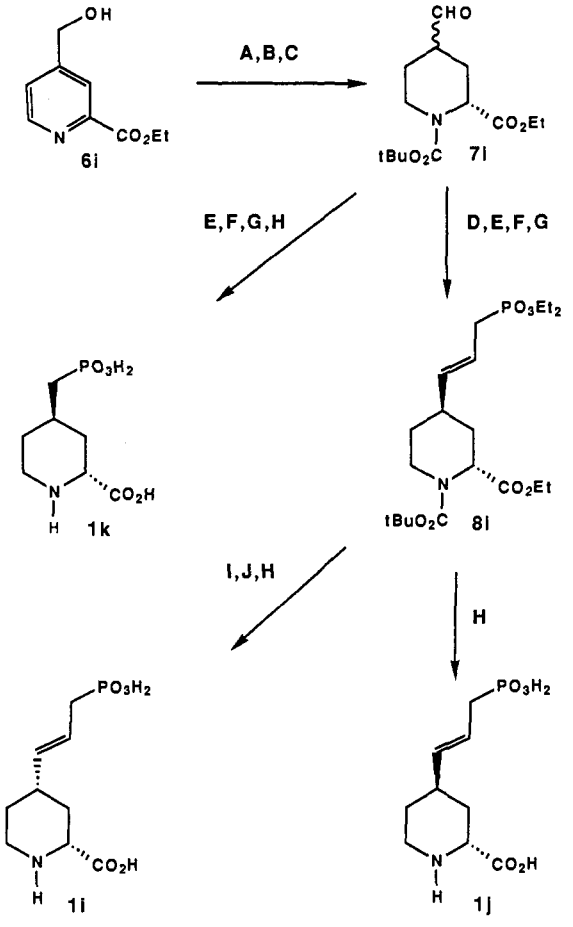
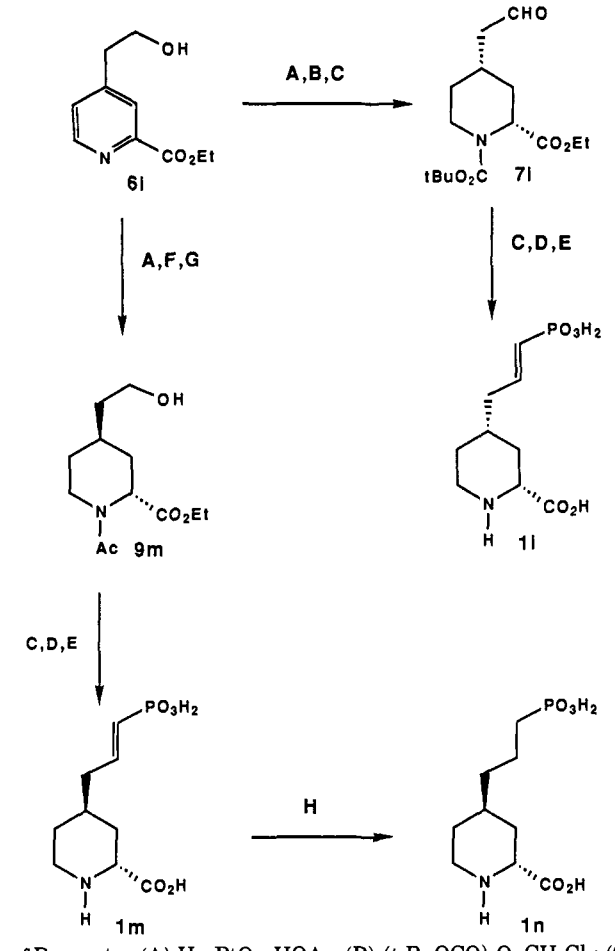
**Scheme II**[a]




[a] Reagents: (A) $H_2$, $PtO_2$; (B) $(t\text{-BuOCO})_2O$, TEA; (C) PCC, $CH_2Cl_2$; (D) $Ph_3P{=}CHCHO$; (E) $NaBH_4$; (F) NBS, $Ph_3P$; (G) $(EtO)_3P$; (H) 6 N HCl; (I) TFA; (J) LiOEt.

**Scheme III**[a]




[a] Reagents: (A) $H_2$, $PtO_2$, HOAc; (B) $(t\text{-BuOCO})_2O$, $CH_2Cl_2$; (C) PCC, $CH_2Cl_2$; (D) $CH_2(PO_3Et_2)_2$, *n*-BuLi, THF; (E) 6 N HCl; (F) $Ac_2O$/Py; (G) $K_2CO_3$, EtOH; (H) $H_2$, Pd/C, $H_2O$.

logues **1a–h** are collected in Table I.

The syntheses of the 4-(phosphonoalkenyl) derivative **1i**, its trans isomer **1j**, and the trans isomer of **1a** (**1k**) are outlined in Scheme II. The synthetic route employed utilizes the fact that when the piperidine nitrogen is acylated with acetyl or *t*-Boc protecting groups, the trans stereoisomers predominate under equilibrating conditions, whereas in the unprotected form the cis stereoisomers predominate. Conversion of 4-(hydroxymethyl)pyridine to the corresponding 2-carbethoxy derivative **6i** was accomplished by using a four-step sequence with no isolation of intermediates: protection of the alcohol with *tert*-butyldimethylsilyl chloride, *N*-oxide formation with MCPBA, nitrile formation using TMSCN in $Et_3N$, and nitrile/protecting group hydrolysis with NaOEt in EtOH followed by 6 N HCl treatment. The overall yield for this process was 59%. Hydrogenation of **6i** using a $Pt_2O$ in acetic acid system afforded the corresponding piperidine derivative, which was protected at nitrogen with di-*tert*-butyl carbonate followed by oxidation with pyridinium chlorochromate in $CH_2Cl_2$ to give the aldehyde **7i** in an overall yield of 36%. A $^1H$ NMR analysis of this material indicated that it consisted of a mixture of cis and trans stereoisomers in approximately equal amounts. Apparently the proton $\alpha$ to the aldehyde moiety is extremely labile since all subsequent transformations of this material led to only the thermodynamically more stable trans isomers. The aldehyde **7i** was reacted with (formylmethylene)triphenylphosphorane to give the corresponding $\alpha,\beta$-unsaturated aldehyde, which subsquently was reduced to the corresponding allylic alcohol with $NaBH_4$. This allylic alcohol was converted to the corresponding allylic bromide with NBS and $Ph_3P$ followed by treatment with $(EtO)_3P$ to afford the protected trans diethyl phosphonate ester **8i** in an overall yield of 17%. Direct hydrolysis of **8i** with 6 N HCl affords the trans phosphonic acid **1j** in a yield of 72%. Removal of the *t*-Boc group with trifluoroacetic acid followed by equilibration with LiOEt in EtOH afforded predominantly the cis isomer, which was hydrolized with 6 N HCl to afford the cis phosphonate derivative **1i** in an overall yield of 48%. The stereochemical assignments for **1i** and **1j** were made by analysis of their $^1H$ NMR spectra. The assignment of double bond geometry was made by examination of the coupling constants for the vinyl protons in the $\alpha,\beta$-unsaturated aldehyde intermediate derived from **7i** ($J_{AB}$ = 16 Hz). The conversion of **7i** to **1k** was accomplished by using a similar procedure to that used for the conversion of **7i** to **1j** except that the bromide was converted to its corresponding iodide prior to reaction with $(EtO)_3P$.

The synthesis of the $\alpha,\beta$-unsaturated phosphonic acid **1l**, its trans isomer **1m**, and the trans isomer of **1b** (**1n**) is outlined in Scheme III. The 4-(2-hydroxyethyl)picolinic ester **6l** was prepared in an analogous manner to **6i** starting from 4-(2-hydroxyethyl)pyridine[15] in an overall yield of 63%. This material was hydrogenated as before to the corresponding cis piperidine derivative, which was protected at nitrogen with di-*tert*-butyl carbonate and oxidized with PCC to afford the cis aldehyde **7l** in an overall

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Table II. Biological Activity of the Analogues 1a–n

| compd | [$^3$H]CGS 19755[a,b] binding $IC_{50}$, nM, ± SEM[c] | NMDA convulsions $ED_{50}$, mg/kg ip (95% CL)[d] Siezures[e] | traction[f] |
|---|---|---|---|
| AP-5 | 350 ± 15 | >154 | >154 |
| AP-7 | 912 ± 200 | 34 (23–47) | 118 (86–164) |
| CPP | 220 ± 7 | 1.9 (0.41–4.81) | 6.1 (3.7–10) |
| 1a | 32 ± 2 | 2.1 (1.0–3.4) | 5.4 (4.0–7.4) |
| 1b | 98 ± 9 | 2.0[g] | 5.4 (3.8–7.9) |
| 1c | 1600 ± 95 | 34 (20–44) | 84 (65–121) |
| 1d | 800 ± 42 | 21 (7.0–32) | 64 (38–143) |
| 1e | 1300 ± 67 | NT | NT |
| 1f | 1300 ± 48 | 26 (15–34) | 33 (25–46) |
| 1g | 78 ± 2 | 7.1[g] | 29 (23–40) |
| 1h | 28000 ± 2000 | NT | NT |
| 1i | 14 ± 1 | 1.2 (0.1–2.3) | 1.9 (1.2–3.0) |
| 1j | 39 ± 3 | 8.3[g] | 14 (8.5–22) |
| 1k | 452 ± 28 | NT | NT |
| 1l | 72 ± 2 | 2.5 (0.9–4.9) | 14 (8.2–25) |
| 1m | 80 ± 0.5 | 10 (2.8–28) | 20 (12–44) |
| 1n | 105 ± 6 | NT | NT |

[a] Compounds were run at 5–10 concentrations in triplicate. [b] [$^3$H]CGS 19755 binding.[14] [c] Concentration necessary to achieve half-maximal inhibition of specific bending ($IC_{50}$'s) ± the standard deviation (nM). [d] Assay described under Experimental Section. Numbers in parentheses are the 95% confidence limits. [e] Dose which gave 50% protection against tonic seizures. [f] Dose that caused 50% loss of traction reflex. [g] 95% confidence limit not determined.

yield of 73%. This material was reacted with tetraethyl methylenediphosphonate/*n*-butyllithium and hydrolyzed with 6 N HCl to afford the cis phosphonate **1l** in an overall yield of 65%. If the piperidine derivative obtained from **6l** after hydrogenation was treated with acetic anhydride in pyridine followed by $K_2CO_3$ in EtOH, the trans alcohol **9m** could be isolated in 34% yield. This material when processed as described above for **1l** afforded the trans phosphonate **1m** in an overall yield of 47%. Finally, hydrogenation of **1l** with 10% Pd/C as catalyst afforded the trans isomer of **1b** (**1n**) in quantitative yield.

## Results and Discussion

The biological test results for the analogues **1a–n** are listed in Table II along with relevant data on the standards AP-5, AP-7, and CPP. The compound **1a** itself was active, with an $IC_{50}$ of 32 nM. This was about 3-fold higher than previously reported,[10] a finding that can be attributed to differences in the specific activity of the ligand used. In the [$^3$H]CGS 19755 receptor binding assay a number of the analogues have potent NMDA receptor affinity with the most potent analogue **1i** ($IC_{50}$ = 14 nM) being over 10 times more potent in vitro than CPP ($IC_{50}$ = 220 nM) and about twice as potent in vivo. All compounds synthesized, including the standards, have negligible interaction with "non-NMDA" excitatory amino acid sites labeled by [$^3$H]AMPA and [$^3$H]kainate at concentrations of up to 10 μM. With respect to the in vivo activity of the analogues above it is clear that, in general, activity in the NMDA convulsion model ip parallels the in vitro activity in the [$^3$H]CGS 19755 binding assay with four of the most active compounds, **1a**, **1b**, **1i**, and **1l** being active in the 1–2 mg/kg range in the in vivo assay. Compounds active in the low micromolar range in the binding assay such as AP-7, **1c**, **1d**, and **1f** are considerably less active in the in vivo assay ($ED_{50}$ = 21–36 mg/kg). However, the correlation between the two assays is by no means perfect. For example, CPP is only moderately active in the binding assay and is quite potent in vivo, and **1j** is potent in binding ($IC_{50}$ = 39 nM) but only moderately active in vivo. These discrepancies between binding activity and in vivo anticonvulsant activity may be explained by several factors.

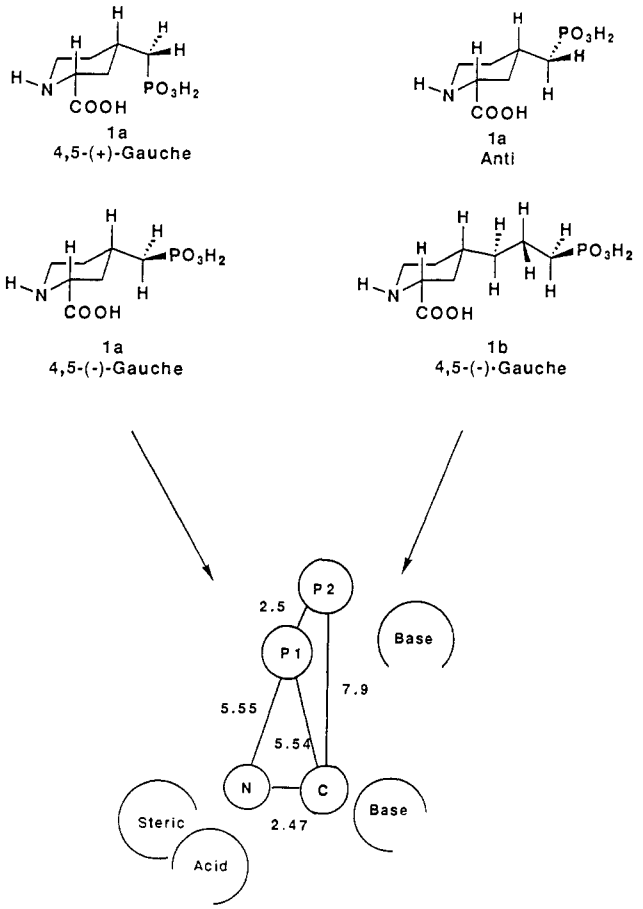


**Figure 2.** Proposed bioactive coformations and NMDA receptor model.

It may be possible that these compounds have differing abilities to cross the blood–brain barrier. All of the compounds studied are extremely polar and may enter the brain via an active transport process which has different recognition properties than that for binding to the NMDA receptor. These compounds may also have different absolute bioavailabilities and durations due to differences in metabolism or distribution. In most cases ataxia (traction deficit) was seen at about 2–4 times the anticonvulsant $ED_{50}$ (Table II). However, **1l** had an approximately 6-fold separation between the two effects whereas for **1f**, **1c**, and **1m** there was essentially no separation. In general, these compounds have poor oral bioavailability in both an absolute and relative sense.

Molecular mechanics based calculations were performed on a series of AP-5 analogues using the MULTIC(onformer) submode of the MacroModel program.[16] This approach relies on the torsional grid search method[17] to generate representative trial conformations, which are then subjected to energy minimization. A modified version[18] of the

(16) Available from Professor W. Clark Still, Columbia University, New York, NY 10027.
(17) Lipton, M.; Still, W. C. *J. Comput. Chem.* **1988**, *9*, 343.
(18) The AMBER force field was reparametrized to handle amino acid phosphonates. The modified parameter set was considered to be accurate enough for the present study when the crystal structure of AP-4 (Sawka-Dobrowolska, W.; Glowiak, T.; Siatecki, Z.; Soroka, M. *Acta Crystallogr.* **1985**, *C41*, 453) could be located as one of the low-energy conformations produced by MacroModel/MULTIC. Recent results from our laboratories (to be published elsewhere) have shown that this modified AMBER parameter set also reproduces the crystal structure of 1a (as the global minimum).

AMBER force field[19] was used to compute the molecular mechanics energies. For the nonrigid analogue AP-5, energy calculations indicate that, as anticipated, many possible bioactive conformations exist that lie within 5 kcal/mol above the ground state. However, for the rigid cyclic analogue **1a** there are only three conformations possible as shown in Figure 2. In all these conformations the bonds around C(2)–C(3) and C(3)–C(4) are restricted in the thermodynamically most stable anti orientation and the C(4)–C(5) bond (see Figure 2) is restricted to any one of three possible low-energy conformations: anti, (−)-gauche, and (+)-gauche. The (−)-gauche and (+)-gauche conformers were labeled as such on the basis of their derivation from the all-anti conformer via a counterclockwise (−) or a clockwise (+) rotation of 120°. Energy calculations indicate that the 4,5-(−)-gauche conformation of **1a** corresponds to the ground state, with the 4,5-anti conformation being approximately equal in energy. The (+)-gauche conformer is about 1 kcal/mol higher in energy but certainly cannot be excluded as a possible bioactive conformation for AP-5 on the basis of energic considerations. In order to further define the bioactive conformation of AP-5, we prepared the methyl-substituted analogues **1f** and **1g**. Addition of an axial methyl group in position 3 of the piperidine ring ($R^5$ = Me) to give **1f** renders the (+)-gauche conformation energetically inaccessible and stabilizes the anti versus the (−)-gauche conformation by 0.5–1.0 kcal/mol. Addition of an axial methyl group in position 5 of the piperidine ring ($R_2$ = Me) to give **1g** again renders the (+)-gauche conformation inaccessible, but in this case the (−)-gauche conformation is favored relative to the anti conformation, again by about 0.5–1.0 kcal/mol. In binding studies, **1g** has quite potent affinity for NMDA receptors ($IC_{50}$ = 78 nM) whereas **1f** is much less active ($IC_{50}$ = 1300 nM). One possible interpretation of these results is that the bioactive conformer of **1a** is 4,5-(−)-gauche. However, another perhaps more plausible explanation is that the methyl group in **1f** violates the NMDA receptor's exclusion volume. Thus the bioactive conformation for **1a** may be either anti or 4,5-(−)-gauche, but we tend to favor the 4,5-(−)-gauche conformation as the bioactive one for the above reasons. In addition, independent studies on acyclic AP-5 analogues[20] tend to support this hypothesis.

Determination of the bioactive conformation of AP-7 analogues such as CPP or **1b** is considerably more complex given the additional degrees of freedom these molecules possess relative to analogue **1a**. Whereas no definitive conclusions about the bioactive conformation for CPP or **1b** around C(5)–C(7) can be made on the basis of the data presented, the following results are supportive of the conformation for **1b** presented in Figure 2 as being one plausible candidate. The potent binding activity of **1l** ($IC_{50}$ = 72 nM) is consistent with the bioactive rotamer for C(6)–C(7) being anti. In addition, the potent activity of **1i** ($IC_{50}$ = 14 nM) supports the hypothesis that C(5)–C(6) is also anti in the bioactive conformation of **1b**. Moreover, it is impossible to overlap the phosphono group in **1l** and **1i** with the phosphono group of the AP-5 analogue **1a** in any of their energetically accessible conformations.

No direct information on the bioactive conformation around C(4)–C(5) of **1b** could be determined from this study, but we tentatively propose it to be (−)-gauche for the following reasons. First, this conformation allows for the possibility that the same basic group on the receptor could bind to the phosphonate group of both AP-5 and AP-7 type molecules. Second, independent studies on acyclic AP7 analogues[20] in which (*Z*)-4,5-dehydro-AP-7 as as active as AP-7 at NMDA receptors while (*E*)-4,5-dehydro-AP-7 was completely inactive also tend to support this hypothesis. Third, conformational energy calculations on CPP, **1i**, and **1l** indicate that the (−)-gauche conformation around C(4)–C(5) is energetically highly favorable for each of these species. In fact, it is the global minimum for CPP although in all cases there are other energetically accessible conformers.

In addition to analogues designed to probe the bioactive conformations of AP-5 and AP-7, a number of specifically methylated analogues of **1a** were prepared to probe the receptor exclusion volume of the NMDA receptor. Addition of either one or two methyl groups to the carbon $\alpha$ to the phosphono group (**1d** and **1e**) resulted in 20–50-fold loss in activity, suggesting unfavorable interactions with the receptor protein in this region. *N*-Methylation of **1a** (**1h**: $IC_{50}$ = 28000 nM) results in about a 1000-fold loss in activity, suggesting an intolerance to steric bulk in this region which is consistent with an acidic binding group being present in this region of the receptor. Most likely there is also an essential hydrogen bond necessary for binding to the receptor. Substitution at the nearby 6-position with an equatorial methyl substituent (**1c**: $IC_{50}$ = 1600 nM) also results in a significant loss in activity. In fact, the only methyl-substituted analogue of **1a** that retains potent binding is the 5 axial methyl substituted analogue **1g** ($IC_{50}$ = 78 nM).

In addition to the analogues described above we have prepared the trans stereoisomers of **1a**, **1b**, **1i**, and **1l**. The trans isomer of the AP-5 analogue **1a** (**1k**: $IC_{50}$ = 452 nM) surprisingly retains about 10% of the binding affinity of **1a**. The trans isomer of the AP-7 analogue **1i** (**1j**: $IC_{50}$ = 39 nM) retains approximately 30% of the affinity of **1i** while the other trans AP-7 analogues **1m** and **1n** are almost equipotent to their cis counterparts in the receptor binding assay. This affinity cannot be explained by contamination by the cis isomer which in all cases above is less than 5% on the basis of high-field $^1H$ NMR analysis. One possible explanation for these unexpected findings is that the trans diastereomers, unlike their corresponding cis analogues which are effectively locked in a chair orientation, also can adopt a relatively low-energy twist-boat conformation within 3–5 kcal/mol of the chair ground state. Molecular modeling studies show that there can be an excellent overlap of these energetically accessible twist-boat conformers of **1k**, **1i**, **1m**, and **1n** with their corresponding cis isomers in the proposed bioactive conformations suggested above.

Using the information from the present study as well as other available information,[20] we have constructed a preliminary model of the antagonist-preferring state of the NMDA receptor, shown in Figure 2 along with our proposed bioactive conformation for **1a** and one possible bioactive conformer for **1b**. This model suggests that there is a basic group on the receptor protein which is located in a position that allows for the phosphonate groups of **1a** and **1b** to bind to this site from two different directions. The preferred distances between the ammonium nitrogen, carboxyl carbon, and phosphorus atoms for AP-5 ($P_1$) and AP-7 ($P_2$) analogues are also shown as well as the approximate positions of the complementary charged groups on the receptor protein and proposed steric barriers. All known potent competitive NMDA antagonists have en-

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ergetically accessible confomers that can closely fit this model.

In conclusion, we have demonstrated that the 4-(phsphonoalkyl)-2-piperidinecarboxylic acid **1a** and the 4-(phosphonoalkenyl)-2-piperidinecarboxylic acid **1i** are potent and selective ligands for NMDA receptors with good systemic anticonvulsant activity. Compound **1a** currently represents the most potent competitive NMDA antagonist yet described. Compound **1i** appears to be more potent than **1a** on the basis of its receptor binding.

## Experimental Section

**Chemistry.** All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. All 60-MHZ 1 H NMR spectra were recorded on a Perkin-Elmer R-12 spectrometer, all 90-MHz $^1$H NMR spectra were recorded on a Varian EM-390 spectrometer, and all 300-MHz $^1$H NMR spectra were recorded on a Varian XL-300 spectrometer. All chemical shifts are expressed in ppm relative to a TMS internal standard, and all reported coupling constants ($J$) are in hertz (Hz). All reactions were carried out under a nitrogen atmosphere. All silica gel flash chromatographies were carried out on Kieselgel 60 (0.040–0.063 mm) from EM Science. Reference compounds were obtained as generous gifts from their respective manufacturers or from Research Biochemicals, Natick, MA. 4-(Chloromethyl)-2-methylpyridine,[21] 4-(3-chloropropyl)pyridine,[22] and 4-(2-hydroxyethyl)pyridine[15] were prepared by the reported literature procedures. 4-(Chloromethyl)-3-methylpyridine was prepared from the corresponding alcohol[23] by the procedure described for (3-chloropropyl)pyridine.[22]

**4-[(Diethylphosphono)methyl]pyridine (2a).**[10] To a suspension of 24 g (500 mmol) of 50% NaH washed free of oil by hexane wash in 200 mL of toluene was added 139.2 g (1.01 mol) of diethyl phosphite in a dropwise fashion, after which the temperature was kept at 80 °C for 30 min. To this was added a solution of 4-picolyl chloride [freshly prepared from 82 g (500 mmol) of the hydrochloride] in 700 mL of toluene also in a dropwise manner. After 30 min at 80 °C the reaction was poured onto water, saturated with NaCl, and extracted three times with ethyl acetate. After drying over $MgSO_4$, the solvent was removed in vacuo to afford 104.4 g (91%) of **2a** as a colorless oil: $^1$H NMR ($CDCl_3$, 60 MHz) $\delta$ 1.3 (6 H, t, $J$ = 7 Hz), 3.15 (2 H, d, $J$ = 23 Hz), 4.06 (4 H, q, $J$ = 7 Hz), 7.25 (2 H, m), 8.55 (2 H, d, $J$ = 5 Hz).

**4-[1-(Diethylphosphono)ethyl]pyridine (2d).** To a solution of LDA at −78 °C prepared from 1.21 g (10 mmol) of diisopropylamine and 4.5 mL of 2.5 M $n$-butyllithium in hexane in 20 mL of THF is added 2.3 g (10 mmol) of **2a**. After 15 min at −78 °C, a mixture of 1.42 mL (22.8 mmol) of methyl iodide and 2 mL of HMPA was added, and the reaction was allowed to warm to room temperature over 1 h. The reaction mixture was poured onto brine, and the products were extracted with ethyl acetate. After drying over $MgSO_4$, the solvent was removed in vacuo and the residue was purified by flash chromatography on silica gel with acetone/hexane (1:1.5) as the eluent to afford 1.0 g (41%) of **2d** as a colorless oil: $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ 1.20 (6 H, dt, $J$ = 6, 7 Hz), 1.55 (3 H, dd, $J$ = 7, 18 Hz), 3.00 (1 H, m), 4.00 (4 H, st, $J$ = 7 Hz), 7.30 (2 H, m), 8.52 (2 H, d, $J$ = 5 Hz).

**4-[1-Methyl-1-(diethylphosphono)ethyl]pyridine (2e).** A mixture of 1.0 g (4.4 mmol) of **2a** and 480 mg (10 mmol) of 50% NaH in 16 mL of dry THF and 4 mL of dry DMF was refluxed for 10 min. After cooling to room temperature, 0.52 mL (8.35 mmol) of methyl iodide was added and the reaction was heated at reflux for 30 min. The solvents were removed in vacuo, and the residue was subjected to flash chromatography on silica gel with 10% methanol in $CH_2Cl_2$ as the eluent to afford 300 mg (27%) of **2e** as a colorless oil: $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ 1.21 (6 H, t, $J$ = 7 Hz), 1.55 (6 H, d, $J$ = 17 Hz), 3.95 (4 H, q, $J$ = 7 Hz), 7.45 (2 H, m), 8.60 (2 H, d, $J$ = 5 Hz).

**2-Cyano-4-[(diethylphosphono)methyl]pyridine (3a).** A mixtue of 103.4 g (444 mmol) of **2a** and 102.4 g (464 mmol) of MCPBA in 1100 mL of $CH_2Cl_2$ was kept at room temperature for 2 h. The solvent was removed in vacuo, and the product was partitioned between water and ether. The aqueous phase which contained the product was washed two additional times with ether, and the water was removed in vacuo. The residue was dissolved in $CH_2Cl_2$ and dried over $MgSO_4$ to afford 106 g of the $N$-oxide of **2a** as a colorless oil after removal of the solvent. To this material were added 171 g (1.72 mol) of TMSCN and 87 g (862 mmol) of $Et_3N$, and the reaction mixture was refluxed for 1 h. The solvents were removed in vacuo, and the residue was taken up in ethyl acetate and washed with saturated $NaHCO_3$ solution. After drying over $MgSO_4$, the solvent was removed in vacuo to afford 101.5 g (88%) of **3a** as a colorless oil: $^1$H NMR ($CDCl_3$, 60 MHz) $\delta$ 1.15 (6 H, t, $J$ = 7 Hz), 3.05 (2 H, d, $J$ = 23 Hz), 3.95 (4 H, q, $J$ = 7 Hz), 7.40 (1 H, m), 7.58 (1 H, bs), 8.60 (1 H, d, $J$ = 6 Hz).

**4-[(Diethylphosphono)methyl]pyridine-2-carboxamide (4a).** Concentrated $H_2SO_4$ (239 g, 2.44 mol) which had been cooled to 0 °C was added to 51 g (199 mmol) of **3a** which had also been cooled to 0 °c. An exothermic reaction ensued, and the temperature rose to 70 °C. After the addition was complete the reaction mixture was heated at 80 °C for 10 min after which it was cooled to room temperature and cautiously poured onto a mixture of 1000 mL of $CH_2Cl_2$, 1000 mL of water, and 302.5 g (2.44 mol) of $Na_2CO_3$. The organic layer was separated, and the aqueous layer was extracted with $CH_2Cl_2$. The combined extracts were dried over $MgSO_4$, and the solvent was removed in vacuo. The resulting solid was triturated with ether to afford 38.7 g (71%) of **4a**: mp 135–140 °C; $^1$H NMR ($CDCl_3$, 90 MHz) $\delta$ 1.25 (6 H, t, $J$ = 7 Hz), 3.16 (2 H, d, $J$ = 24 Hz), 4.05 (4 H, q, $J$ = 7 Hz), 5.95 (1 H, bs), 7.40 (1 H, m), 7.75 (1 H, bs), 8.05 (1 H, s), 8.45 (1 H, d, $J$ = 6 Hz). Anal. ($C_{11}H_{17}N_2O_4P$) C, H, N.

***cis*-4-[(Diethylphosphono)methyl]piperidine-2-carboxamide (5a).** A mixture of 38 g of **4a** (139 mmol), 500 mL of HOAc, and 5.0 g (22 mmol) of $Pt_2O$ was hydrogenated at room temperature at 40 psi for 6 h. The reaction mixture was filtered, and the solvent was removed in vacuo. The residue was dissolved in $CH_2Cl_2$ and stirred with anhydrous $Na_2CO_3$ for 1 h. After filtration the solvent was again removed in vacuo to afford 38.8 g (100%) of **5a** as a colorless oil.

***cis*-4-[(Diethylphosphono)methyl]-1-methylpiperidine-2-carboxamide (5h).** A mixture of 500 mg (1.8 mmol) of **5a**, 3.0 mL (40 mmol) of 37% aqueous HCHO, 50 mL of MeOH, and 500 mg of 10% Pd/C catalyst was hydrogenated at room temperature and 40 psi for 6 h. The solvent was removed in vacuo, and the residue was subjected to flash chromatography on silica gel with $CH_2Cl_2$/MeOH (20:1) as the eluent to afford 290 mg (55%) of **5h** as an amorphous solid: $^1$H NMR ($CDCl_3$, 60 MHz) $\delta$ 1.30 (6 H, t, $J$ = 7 Hz), 1.15–3.15 (9 H, m), 2.30 (3 H, s), 4.05 (1 H, m), 4.10 (4 H, q, $J$ = 7 Hz), 6.60 (2 H, bs).

***cis*-4-(Phosphonomethyl)piperidine-2-carboxylic Acid (1a).** A mixture of 38.8 g (139 mmol) of **5a** and 640 mL of 6 N HCl was refluxed with stirring for 16 h. The solvent was removed in vacuo, and the residue was dissolved in 200 mL of 70% EtOH and treated with 48.4 mL (690 mmol) of propylene oxide. The resulting precipitate was collected and washed with 70% EtOH to afford 22.1 g (74%) of **1a**: mp 290–292 °C; $^1$H NMR ($D_2O$, 300 MHz) $\delta$ 1.33 (2 H, m), 1.58 (2 H, m), 1.93 (1 H, m), 2.03 (1 H, dm, $J$ = 14 Hz), 2.92 (1 H, dt, $J$ = 13, 2 Hz), 3.36 (1 H, dm, $J$ = 13 Hz), 3.72 (1 H, dd, $J$ = 3, 13 Hz). Anal. ($C_7H_{14}NO_5P$) C, H, N.

**Ethyl 4-(Hydroxymethyl)pyridine-2-carboxylate (6i).** A mixture of 85 g (780 mmol) of 4-pyridylcarbinol, 130 g (860 mmol) of TBDMSCI, 64 g (960 mmol) of imidazole in 850 mL of dry DMF, and 85 mL of $CH_2Cl_2$ is kept at room temperature for 3 h. The reaction mixture was poured into water, and the product was extracted with ethyl acetate/hexane (1:1). After drying over $Na_2CO_3$, the solvent was removed in vacuo. The residue was dissolved in 2 L of $CH_2Cl_2$ and treated with 155 g (900 mmol) of MCPBA. After 20 h at room temperature the reaction mixture was washed with 1 N NaOH and dried over $Na_2SO_4$, and the solvent was removed in vacuo. The residue was treated with 209 mL (1.5 mol) of $Et_3N$ and 400 mL (3 mol) of TMSCN and heated at 90 °C for 3 h. The reaction mixture was poured onto water,

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and the product was extracted with ethyl acetate. After drying over $MgSO_4$, the solvent was removed in vacuo and the residue was subjected to flash chromatography with ethyl acetate/hexane (15:100) as the eluent to afford 2-cyano-4-[[(*tert*-butyldimethylsilyl)oxy]methyl]pyridine as a colorless oil. To this material in 1 L of anhydrous EtOH was added a solution of 1.2 g (519 mmol) of Na dissolved in 300 mL of EtOH. After 20 h at room temperature 130 mL of 6 N HCl was added dropwise while cooling on an ice bath. After 20 h at room temperature the pH was adjusted to 6.5 and 6 N NaOH, the reaction mixture was diluted with $NaHCO_3$ solution and water, and the product was extracted with $CH_2Cl_2$. After drying over $Na_2SO_4$, the solvent was removed in vacuo and the residue was triturated with ether/hexane to afford 83.6 g (59% overall) of **6i**: mp 78–79 °C; $^1H$ NMR ($CDCl_3$, 90 MHz) $\delta$ 1.40 (3 H, t, $J$ = 7 Hz), 3.00 (1 H, b), 4.48 (2 H, q, $J$ = 7 Hz), 4.78 (2 H, s), 7.48 (1 H, d, $J$ = 6 Hz), 8.10 (1 H, s), 8.70 (1 H, d, $J$ = 6 Hz). Anal. ($C_9H_{11}NO_3$) C, H, N.

***cis*- and *trans*-Ethyl 1-(*tert*-Butoxycarbonyl)-4-formylpiperidine-2-carboxylate (7i).** A mixture of 43.5 g (240 mmol) of **6i**, 4.5 g (19.8 mmol) of $Pt_2O$, and 900 mL of HOAc was hydrogenated for 6 h at 60 psi at room temperature. After filtration and removal of solvent the residue was dissolved in $CH_2Cl_2$ and stirred with excess $K_2CO_3$. After filtration and removal of solvent the residue was subjected to flash chromatography on silica gel with $CH_2Cl_2$/MeOH($NH_3$) (20:1) as the eluent to afford the corresponding cis piperidine derivative. To a solution of this material (21.5 g, 115 mmol) in 300 mL of $CH_2Cl_2$ was added 25.1 g (115 mmol) of di-*tert*-butyl carbonate. After 24 h at room temperature all volatiles were removed in vacuo and the residue was dissolved in 400 mL of $CH_2Cl_2$ and treated with 43 g (200 mmol) of PCC. After being stirred at room temperature for 3 h, the reaction mixture was filtered through silica gel with ethyl acetate/hexane (1:3) as the eluent to afford 24.6 g (36%) of **7i** as a colorless oil.

***trans*-Ethyl 1-(*tert*-Butyoxycarbonyl)-4-[(1*E*)-[3-(diethylphosphono)prop-1-enyl]]piperidine-2-carboxylate (8i).** A solution of 25.5 g (89 mmol) of **7i** and 43.5 g (143 mmol) of (formylmethylene)triphenylphosphorane in 350 mL of toluene was heated at 105 °C for 4 h. After removal of solvent in vacuo the residue was subjected to flash chromatography on silica gel with ethyl acetate/hexane (1:3) as the eluent to afford 20.4 g of the corresponding $\alpha,\beta$-unsaturated aldehyde. This material was dissolved in 200 mL of EtOH and treated with 1.24 g (66 mmol) of $NaBH_4$. After 1 h the solvent was removed in vacuo and the residue was again subjected to flash chromatography as above to afford 9.4 g of the corresponding allylic alcohol. This material was treated with 5.9 g (33 mmol) of NBS and 10.2 g (39 mmol) of $Ph_3P$ in 180 mL of $CH_2Cl_2$ at 0 °C. After 30 min at 0 °C the solvent was removed in vacuo and the residue chromatographed as above to afford 6.3 g of the bromide. This material was refluxed in 65 mL of $(EtO)_3P$ for 2 h. After removal of the solvent and chromatography as before 6.4 g (17% overall) of **8i** was obtained as a colorless oil: $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 1.35 (9 H, m), 1.35 (4.5 H, s), 1.45 (4.5 H, s), 1.70 (2 H, m), 2.04 (1 H, m), 2.25 (1 H, tm, $J$ = 14 Hz), 2.54 (2 H, dd, $J$ = 9, 24 Hz), 2.92 (0.5 H, tm, $J$ = 15 Hz), 3.02 (0.5 H, tm, $J$ = 15 Hz), 4.14 (8 H, m), 4.77 (0.5 H, d, $J$ = 4 Hz), 4.92 (0.5 H, d, $J$ = 4 Hz), 5.48 (2 H, m).

***trans*-4-[(1*E*)-(3-Phosphonoprop-1-enyl)]piperidine-2-carboxylic Acid (1j).** Following the same procedure as for **1a**, 240 mg (0.55 mmol) of **8i** affords 99 mg (72%) of **1j**: mp 130–135 °C; $^1H$ NMR ($D_2O$, 300 MHz) $\delta$ 1.78 (1 H, m), 2.03 (2 H, m), 2.21 (1 H, m), 2.55 (2 H, dd, $J$ = 7, 24 Hz), 3.33 (2 H, m), 4.14 (1 H, dd, $J$ = 6, 8 Hz), 5.64 (2 H, m). Anal. ($C_9H_{18}NO_5P$) C, H; N: calcd, 5.24; found, 4.82.

***cis*-4-[(1*E*)-(3-Phosphonoprop-1-enyl)]piperidine-2-carboxylic Acid (1i).** To a solution of 6.33 g (14.6 mmol) of **8i** in 15 mL of $CH_2Cl_2$ is added 8 mL of TFA. After 2 h at room temperature the solvents were removed in vacuo and the residue was taken up in $CH_2Cl_2$, washed with saturated $NaHCO_3$ solution, and dried over $Na_2SO_4$, and the solvent was removed in vacuo. This material was dissolved in 80 mL of anhydrous EtOH and treated with 1.34 mL of 1.6 M *n*-butyllithium in hexane. After 72 h at 80 °C the solvent was removed in vacuo and the residue was subjected to flash chromatography on silica gel with $CH_2Cl_2$/MeOH (20:1) as the eluent to afford 2.4 g of the pure cis isomer. This material was refluxed for 16 h with 50 mL of 6 N HCl. After removal of solvent and ethanolic propylene oxide treatment 1.74 g (48%) of **1i** was obtained: mp 170–175 °C; $^1H$ NMR ($D_2O$, 300 MHz) $\delta$ 1.55 (2 H, m), 2.01 (1 H, dm, $J$ = 16 Hz), 2.41 (1 H, dm, $J$ = 16 Hz), 2.49 (2 H, dd, $J$ = 7, 23 Hz), 3.08 (1 H, dt, $J$ = 4, 15 Hz), 3.53 (1 H, dm, $J$ = 15 Hz), 3.90 (1 H, dd, $J$ = 3, 15 Hz), 5.62 (2 H, m). Anal. ($C_9H_{18}NO_5P$) C, H, N.

***trans*-4-(Phosphonomethyl)-2-piperidinecarboxylic Acid (1k).** To a solution of 1.39 g (4.87 mmol) of **7i** in 3 mL of EtOH is added 0.46 mL of 1.6 M *n*-butyllithium in hexane. After 2 h at room temperature 75 mg of $NaBH_4$ (2 mmol) is added and the reaction mixture is stirred for an additional 10 min. After dilution with water the product is extracted with ethyl acetate, the organic layer is dried over $MgSO_4$, and the solvent is removed in vacuo to afford 1.4 g of trans alcohol. This material is processed in an analogous manner to the conversion of the allylic alcohol derived from **7i** to **1j** except that the bromide was converted to its corresponding iodide via treatment with 2 equiv of NaI in 8 mL of refluxing acetone for 1 h prior to reaction with $(EtO)_3P$ to afford 470 mg (43%) of **1k**: mp at 132–135 °C; $^1H$ NMR ($D_2O$, 300 MHz) $\delta$ 1.57 (1 H, m), 1.80 (2 H, dd, $J$ = 8, 23 Hz), 1.98 (3 H, m), 2.28 (1 H, dm, $J$ = 16 Hz), 3.22 (2 H, m), 4.23 (2 H, t, $J$ = 6 Hz).

***cis*-Ethyl 1-(*tert*-Butoxycarbonyl)-4-(formylmethyl)-piperidine-2-carboxylate (7l).** Starting with 8.37 g (43 mmol) of **6l** and according to the procedure described for the preparation of **7i** from **6i**, 9.28 g (73%) of **7l** was obtained as a colorless oil.

***cis*-4-[(1*E*)-(3-Phosphonoprop-2-enyl)]piperidine-2-carboxylic Acid (1l).** To a stirred solution of 8.5 mL (34 mmol) of tetraethyl methylenediphosphonate in 150 mL of dry THF was added 20.3 mL (32.5 mmol) of 1.6 M *n*-butyllithium in hexane in a dropwise manner at −78 °C, and the mixture was stirred for 5 min at −78 °C. This solution was added to a solution of 9.26 g (31 mmol) of **7l** in 100 mL of dry THF also at −78 °C. After cooling to room temperature over 30 min, the reaction mixture was refluxed for 18 h. The reaction was concentrated in vacuo and diluted with water, and the products were extracted with $CH_2Cl_2$. After drying over $Na_2SO_4$, the solvent was removed and the residue was subjected to flash chromatography on silica gel with ethyl acetate/hexane (1:3) as the eluent to afford 9.5 g of the corresponding $\alpha,\beta$-unsaturated phosphonic ester. This material was refluxed for 16 with 150 mL of 6 N HCl. The solvent was removed and the residue treated with ethanolic propylene oxide to afford 4.9 g (65%) of **1l**: mp 170–175 °C; $^1H$ NMR ($D_2O$, 300 MHz) $\delta$ 1.42 (2 H, m), 1.95 (2 H, m), 2.28 (2 H, t, $J$ = 7 Hz), 2.39 (1 H, d, $J$ = 16 Hz), 3.08 (1 H, dt, $J$ = 4, 15 Hz), 3.53 (1 H, dm, $J$ = 15 Hz), 3.89 (1 H, dd, $J$ = 3, 15 Hz), 5.88 (1 H, dd, $J$ = 16, 23 Hz), 6.50 (1 H, m). Anal. ($C_9H_{18}NO_5P$) C, H, N.

***trans*-Ethyl 1-Acetyl-4-(2-hydroxyethyl)piperidine-2-carboxylate (9m).** A mixture of 13.1 g (67 mmol) of **6l**, 7 g (30.8 mmol) of $Pt_2O$, and 200 mL of HOAc is hydrogenated at 40 psi at room temperature for 6 h. After filtration the solvent was removed in vacuo and the residue treated with 9.5 mL (101 mmol) of $Ac_2O$ and 75 mL of pyridine. After 30 min the solvents were removed in vacuo and the residue was dissolved in 200 mL of EtOH, and 8 g (58 mmol) of $K_2CO_3$ was added. This mixture was vigorously stirred for 4 h. After filtration the solvent was removed and the residue subjected to flash chromatography on silica gel with $CH_2Cl_2$/MeOH (20:1) as the eluent to afford 5.3 g (34%) of **9m** as a colorless oil.

***trans*-4-[(1*E*)-(3-Phosphonoprop-2-enyl)]piperidine-2-carboxylic Acid (1m).** Starting from 3.7 g (15.2 mmol) of **9m** and according to the procedure outlined above for the preparation of **1l**, 1.8 g (47%) of **1m** was obtained: 132–140 °C; $^1H$ NMR ($D_2O$, 300 MHz) $\delta$ 1.57 (1 H, m), 1.85 (1 H, m), 1.85 (2 H, m), 2.31 (3 H, m), 3.36 (1 H, t, $J$ = 4 Hz), 4.27 (1 H, dt, $J$ = 2, 8 Hz), 5.90 (1 H, dd, $J$ = 16, 23 Hz), 6.53 (1 H, m). Anal. ($C_9H_{18}NO_5P$) C, H, N.

***trans*-4-(3-Phosphonopropyl)piperidine-2-carboxylic Acid (1n).** A mixture of 300 mg (0.83 mmol) of **1m**, 300 mg of 10% Pd/C catalyst, and 15 mL of water was hydrogenated at 50 psi at room temperature for 3 h. The solvent was removed in vacuo to afford 302 mg (100%) of **1n**: mp 150–160 °C; $^1H$ NMR ($D_2O$, 300 MHz) $\delta$ 1.60 (9 H, m), 1.92 (1 H, dm, $J$ = 16 Hz), 2.22 (1 H, dd, $J$ = 6, 15 Hz), 3.30 (2 H, t, $J$ = 7 Hz), 4.18 (1 H, t, $J$ = 6 Hz). Anal. ($C_9H_{18}NO_5P$) C, H, N.

**Pharmacology. Binding Studies: CGS 19755 Binding.** Binding to NMDA receptors labeled by [$^3$H]CGS 19755 (specific

activity 30–40 Ci/mmol; Du Pont NEN, Boston, MA) was measured as previously described.[10] Crude synaptic membranes were prepared from male Sprague-Dawley rats (Crl: CDBR) and pretreated with 0.04% Triton X-100 to remove endogenous excitatory amino acids. An aliquot (200–400 μg/mL) of membrane protein was incubated in 50 mL Tris-HCl buffer, pH 8.0, together with a final concentration of 10 nM [$^3$H]CGS 19755. Nonspecific binding was determined in the presence of 1 mM L-glutamate. Incubation was continued for 15 min at 4 °C and bound radioactivity isolated by vacuum filtration over Whatman GF/B glass fiber filters. Under these conditions, [$^3$H]CGS 19755 showed 80–85% specific binding to a single site with a $K_d$ value of 24 nM.[10] Compounds were run at 5–10 concentrations in triplicate for $IC_{50}$ determinations.

**Other Excitatory Amino Acid Receptor Binding.** Binding of CGS 19755 and its analogues to quisqualate and kainate receptors was measured by using [$^3$H]AMPA and [$^3$H]kainate as previously described.[11,12]

**Behavioral Studies: NMDA Convulsion Model.** Male mice (Crl: CF1BR; 18–22 g; Charles River, Wilmington, MA) were administered test compounds 30 min ip before testing for impairment of traction reflex as assessed by the ability of the mice to grasp a thin wire with their forepaws. Mice that did not bring their hindpaws up to the wire within 10 s were considered to have "lost traction", an indication of muscle relaxation.[8] Immediately following the traction test, mice were administered NMDA (154 mg/kg ip) and observed for a 30-min period for the appearance of tonic convulsions. $ED_{50}$ values for seizure protection and traction deficit were determined by probit analysis.[24]

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**Registry No.** AP-5, 76726-92-6; AP-7, 85797-13-3; CPP, 108549-42-4; TMSCN, 7677-24-9; TBDMSCl, 18162-48-6; **1a**, 110347-85-8; **1b**, 113190-92-4; **1c**, 121570-54-5; **1d**, 121524-85-4; **1e**, 113229-88-2; **1f**, 121524-86-5; **1g**, 121524-87-6; **1h**, 113229-64-4; **1i**, 121570-55-6; **1j**, 121570-56-7; **1k**, 113229-89-3; **1l**, 121570-57-8; **1m**, 121570-58-9; **1n**, 121524-88-7; **2a**, 77047-42-8; **2a** (*N*-oxide), 35469-52-4; **2d**, 121524-89-8; **2e**, 121524-90-1; **3a**, 118892-60-7; **4a**, 113190-80-0; **5a**, 113190-81-1; **5b**, 121541-51-3; **6i**, 59663-96-6; **6l**, 117423-32-2; **7i**, 117423-46-8; **7l**, 121524-92-3; **8i**, 121524-91-2; **9m**, 117423-38-8; AP-5, 76726-92-6; AP-7, 85797-13-3; CPP, 108549-42-4; NMDA, 6384-92-5; TMSCN, 7677-24-9; TBDMSCl, 18162-48-6; $Ph_3P{=}CHCHO$, 2136-75-6; $CH_2(PO_3Et_2)$, 1660-94-2; diethyl phosphite, 762-04-9; 4-picolyl chloride, 10445-91-7; imidazole, 288-32-4; 4-pyridylcarbinol, 586-95-8; 2-cyano-4-[[(*tert*-butyldimethylsilyl)oxy]methyl]pyridine, 117423-43-5; *cis*-ethyl 1-(*tert*-butoxycarbonyl)-4-[1-(*E*)-3-(diethylphosphonoprop-2-enyl)]piperidine-2-carboxylate, 121524-93-4.

**Supplementary Material Available:** A table listing analysis and melting point data for **4a,b,f,g**, **6i**, and **1b–n** (2 pages). Ordering information is given on any current masthead page.

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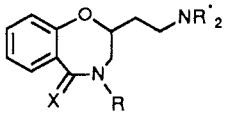
# Benzo- and Pyrido-1,4-oxazepin-5-ones and -thiones: Synthesis and Structure–Activity Relationships of a New Series of $H_1$ Antihistamines

Albert D. Cale, Jr.,*,† Thomas W. Gero,† Kathleen R. Walker,† Young S. Lo,† William J. Welstead, Jr.,† Larry W. Jaques,† Ashby F. Johnson,† Charles A. Leonard,‡ Joseph C. Nolan,‡ and David N. Johnson‡

*Research Laboratories, A. H. Robins Company, Inc., Richmond, Virginia 23261-6609.* *Received October 31, 1988*

A series of novel benzo- and pyrido-1,4-oxazepinones and -thiones which represents a new structural class of compounds possessing $H_1$ antihistaminic activity was synthesized, and the SARs were evaluated. The antihistaminic activity was determined by blockade of histamine-induced lethality in guinea pigs. The sedative potential was determined by comparison of the EEG profiles of the compounds with those of known sedating and nonsedating antihistamines. Several of the compounds were shown to possess potent $H_1$ antihistaminic activity and to be free of the cortical slowing with synchronized waves and spindling activity found in the EEG of sedative antihistamines. One compound, 2-[2-(dimethylamino)ethyl]-3,4-dihydro-4-methylpyrido[3,2-*f*]-1,4-oxazepine-5(2*H*)-thione (rocastine) is currently undergoing clinical evaluation as a nonsedating $H_1$ antihistamine.

In recent years, the search for an $H_1$ antihistamine which would be free from sedative side effects has been in progress. General screening of some novel compounds synthesized in these laboratories detected several compounds of the general structure I which possessed weak $H_1$ antagonism when tested in vitro against histamine-induced contractions of the guinea pig ileum.

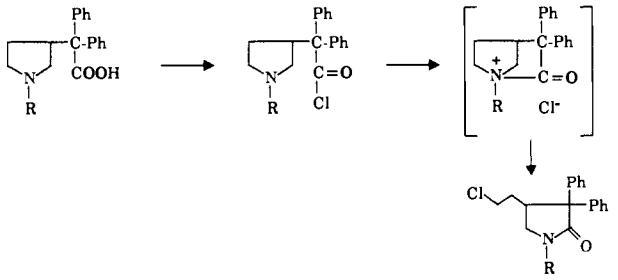


I

X = O, S
R = alkyl
R' = alkyl

The most potent of these compounds (I: R = $CH_3$;R' = $CH_3$; X = S) was shown to offer moderate protection against histamine-induced lethality in the guinea pig and did not produce EEG patterns in the cat which are believed to be indicative of sedation.[1] The following investigation was initiated on the basis of these findings with the goal of preparing a novel, potent nonsedative antihistamine.

**Scheme I**



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‡ Department of Pharmacology.

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