lectivity and the binding affinity by employing computer-assisted modeling as well as by expanding the series of compounds.

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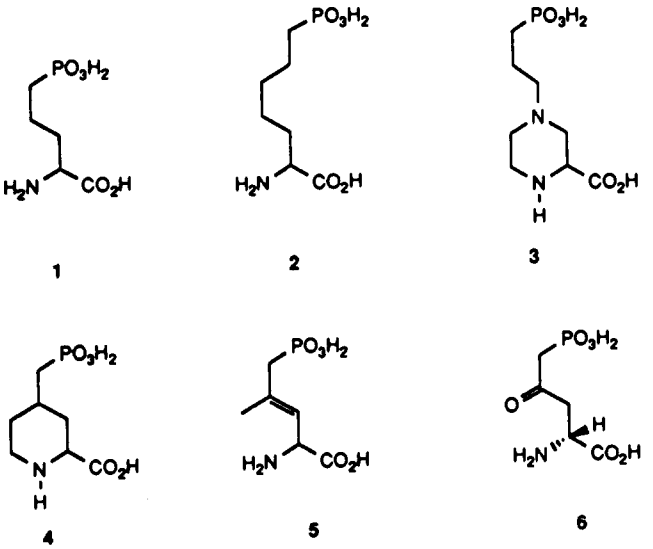
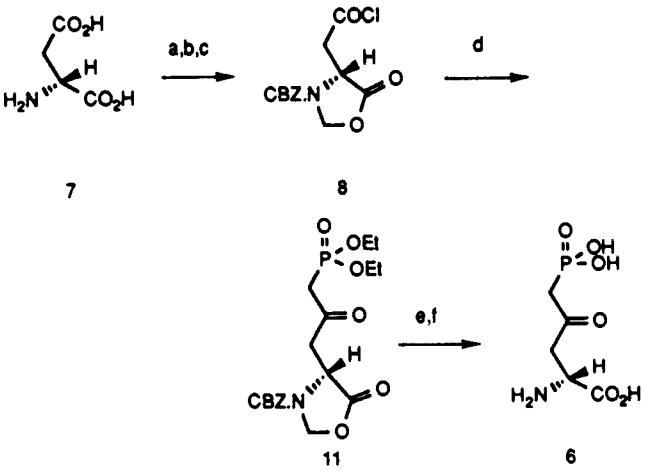
(*R*)-4-Oxo-5-phosphononorvaline: A New Competitive Glutamate Antagonist at the NMDA Receptor Complex

The neurochemical functions of the excitatory amino acids glutamic acid and aspartic acid have been extensively studied in recent years. These neurotransmitters can activate at least three receptor complexes, which are designated according to their sensitivity to the ligands *N*-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and kainic acid.[1] Abnormal physiological conditions, such as epilepsy, Huntington's chorea, memory disorders, and neuronal damage, which occur following an ischemic episode, have been associated with hyperactivity of one or more of these receptor complexes.[2] The NMDA receptor complex, which is the best understood to date, contains individual binding sites for glutamic acid,[3] glycine,[3] Mg^{2+},[4] Zn^{2+},[5] and polyamines,[6] all of which play a key role in modulating the flow of Ca^{2+} through the receptor-associated ion channel. In this paper we wish to communicate the discovery of a new, systematically active antagonist of the glutamate binding site of the NMDA receptor complex.

A number of antagonists have been reported in the literature. The first of these, 2-amino-5-phosphonopentanoic acid (AP5, **1**) and 2-amino-7-phosphonoheptanoic acid (AP7, **2**), while reasonably potent in binding assays, have difficulty in penetrating the blood–brain barrier, resulting in weak systemic activity in various animal models.[7] More recently, second-generation phosphonic acid derivatives, such as 4-(3-phosphonomethyl)-2-piperazinecarboxylic acid (CPP, **3**),[8] *cis*-4-(phosphonomethyl)-2-piperidinecarboxylic acid (CGS 19,755, **4**),[9] and (*E*)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 37,849, **5**)[10] have been shown to combine good binding affinities with the ability to cross the blood–brain barrier. In this context, on the basis of extensive molecular modeling studies, we designed and synthesized (*R*)-4-oxo-5-phosphononorvaline (**6**), a structural analogue of AP5 (**1**).[20]



Scheme I[a]



[a] Reagents: (a) CBZCl, NaOH; 93%; (b) CH_2O, PTSA refluxing benzene; 96%; (c) $SOCl_2$; 100%; (d) $((EtO)_2POCH_2)_2CuI$, Et_2O/THF, −30 °C; 37%; (f) TMSI; (g) Dowex 50W-X8; 68%.

Table I. Relative in Vitro Potency of Phosphono Amino Acid Antagonists[a]

compound	K_i, nM, vs [^{3}H]CPP	IC_{50}, μM, vs cGMP
1 (AP5)	176 ± 16	5.5
2 (AP7)	730 ± 64	14.1
3 (CPP)	131 ± 18	0.8
4 (CGS 19,755)	99 ± 32	1.1 ± 0.4
6 (MDL 100,453)	109 ± 12	5.9 ± 1.4

[a] Methods are described by Baron *et al.*[14] Results are means of at least three determinations except the functional assays with no range, which are the results of a single determination.

A convergent synthesis of **6** utilizing a key copper-catalyzed coupling step,[11] with D-aspartic acid as the chiral educt, is outlined in Scheme I. D-Aspartic acid (**7**), protected as the *N*-(carbobenzyloxy)-4-oxazolidone[12] by

(1) Watkins, J. C.; Krogsgaard-Larsen, P.; Honorē, T. *Trends Pharmacol. Sci.* **1990**, *11*, 25.
(2) Olney, J. W. *Biol. Psychiatry* **1989**, *26*, 505.
(3) Wood, P. L.; Rao, T. S.; Iyengar, S.; Lanthorn, T.; Monahan, J.; Cordi, A.; Sun, E.; Vazquez, M.; Gray, N.; Contreras, P. *Neurochem. Res.* **1990**, *15*, 217.
(4) Mayer, M. L.; Westbrook, G. L.; Guthrie, P. B. *Nature* **1984**, *309*, 261. Nowak, L.; Bregastovski, P.; Ascher, P.; Herbet, A.; Prochiantz, A. *Nature* **1984**, *307*, 462.
(5) Peters, S.; Koh, J.; Choi, D. W. *Science* **1987**, *236*, 589. Westbrook, G. L.; Mayer, M. L. *Nature* **1987**, *328*, 640.
(6) Ransom, R. W.; Stec, N. L. *J. Neurochem.* **1988**, *51*, 830.
(7) Evans, R. H.; Watkins, J. C. *Life Sci.* **1981**, *28*, 1303. Lehmann, J.; Schneider, J.; McPherson, S.; Murphy, D. E.; Bernard, P.; Tsai, C.; Bennett, D. A.; Pastor, G.; Steel, D. J.; Boehm, C.; Cheney, D. L.; Liebman, J. M.; Williams, M.; Wood, P. L. *J. Pharmacol. Exp. Ther.* **1987**, *240*, 737.
(8) Davies, J.; Evans, R. H.; Herrling, P. L.; Jones, A. W.; Olverman, H. J.; Pook, P.; Watkins, J. C. *Brain Res.* **1986**, *382*, 169.
(9) Lehmann, J.; Hutchinson, A. J.; McPherson, S. E.; Mondadori, C.; Schmutz, M.; Sinton, C. M.; Tsai, C.; Murphy, D. E.; Street, D. J.; Williams, M.; Cheney, D. L.; Wood, P. L. *J. Pharmacol. Exp. Ther.* **1988**, *246*, 65.
(10) Fagg, G. E.; Olpe, H.-R.; Pozza, M. F.; Baud, J.; Steinmann, M.; Schmutz, M.; Portet, C.; Baumann, P.; Thedinga, K.; Bittiger, H.; Allgeier; Heckendorn, R.; Angst, C.; Brundish, D.; Dingwall, J. G. *Br. J. Pharmacol.* **1990**, *99*, 791.
(11) Vaslet, J. M.; Collignon, N.; Savignac, P. *Can. J. Chem.* **1979**, *57*, 3216.

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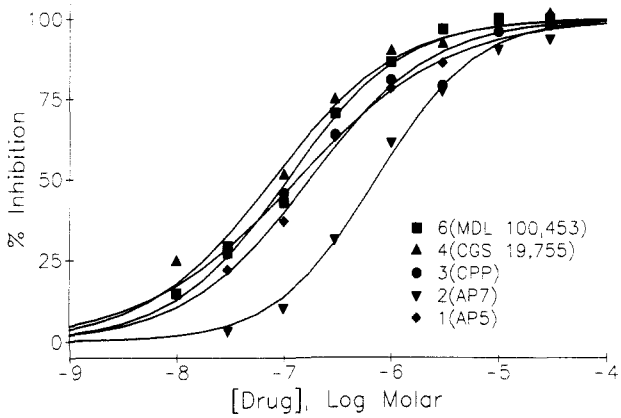


Figure 1. Effect of phosphono amino acid antagonists (1–4, 6) on [^{3}H]CPP binding. Rat brain membranes were incubated with 10 nM [^{3}H]CPP in the presence of various concentrations of the unlabeled antagonists. Specific binding was measured by using a centrifugation assay. Percent inhibition of specific binding is plotted versus drug concentration. Results are means of three independent experiments.

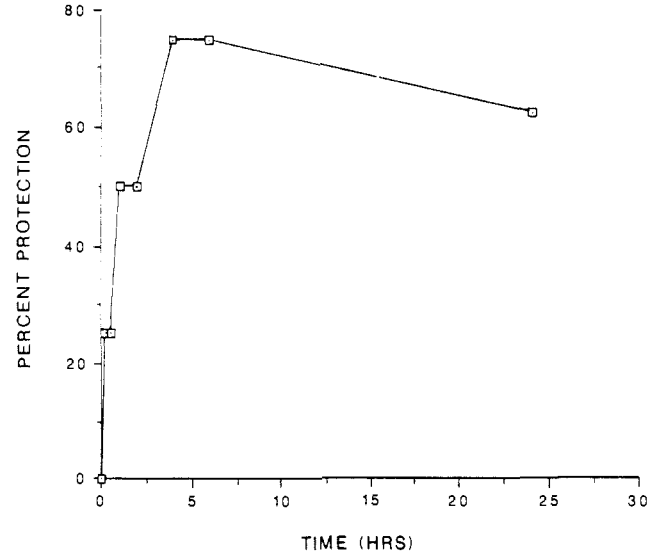


Figure 2. Effect of 6 on the expression of kindled seizures in the rat. Rats were exposed daily to a low-level electric shock (8 ma, 4 s) delivered through corneal electrodes until the stimulus reliably evoked stage 5 seizures. These "kindled" animals were then given a single dose of 6 (50 mg/kg) orally and groups of eight animals were tested at various time intervals for their responses to the corneal shock.

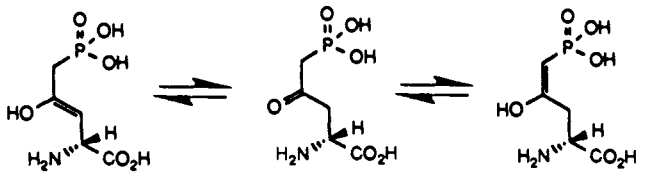
standard procedures, was converted to acid chloride 8 by treatment with thionyl chloride. Reaction of 8 with cuprate 10, derived from diethyl methylphosphonate (9) and cuprous iodide, afforded 11 in 61% yield. All five protecting groups of 11 were removed simultaneously by the use of trimethylsilyl iodide in CH_2Cl_2/CH_3CN, followed by ion exchange on Dowex 50W-X8. Conventional methods for deprotection such as refluxing acid were unsatisfactory. (*R*)-4-Oxo-5-phosphononorvaline (6) was isolated as an amorphous, colorless powder in 47% yield.[13]

The relative affinities of 1–6 for the NMDA binding site of the glutamate receptor was determined by measuring the ability of the unlabeled compounds to inhibit the binding of [^{3}H]CPP to rat cerebral cortical membranes (Figure 1, Table I).[14] K_i values were 176 nM and 109 nM for 1 and 6, respectively. These compounds were essentially inactive (K_i values >100 μM) in competition experiments with [^{3}H]kainic acid and [^{3}H]AMPA.[16] Functional antagonism was measured as the ability of the compound to inhibit NMDA-stimulated cGMP accumulation in rat cerebellar slices.[14] It can be seen from the values for compounds 1–6 (Table I) that 6 acts as an antagonist and is equipotent to AP5 (1) in this functional test, although somewhat less potent than CPP (3) and CGS 19,755 (4) (approximately $^1/_7$ and $^1/_5$ as active, respectively). The reason for the apparent lower activity of 6 relative to 3 and 4 is unclear at present, but may be related to the existence of subtypes of the NMDA receptor which are present in differing amounts in the cortical membranes used for the binding assays versus the cerebellar slices used in the cyclic GMP determinations.

In vivo, 6 displayed anticonvulsant activity in both mice and rats. In the genetically seizure prone DBA/2J mouse,[17] compound 6 had an ED_{50} of 3.5 mg/kg (ip) whereas AP5 has a reported ED_{50} value of 78.8 mg/kg (ip).[17] Compound 6 is also a potent antagonist of maximal electroshock-induced convulsions in the rat[15] with an ED_{50} (ip) of 2.3 mg/kg and displays a very fast onset of action (peak effect following iv administration was measured at the first time point, i.e., 5 min). Oral administration of 6 to rats at a dose of 50 mg/kg markedly suppressed the expression of kindled seizures for more than 24 h (Figure 2).

Although AP5 (1) represented a significant advance in the development of NMDA antagonists and has been an important tool for evaluating the role of this receptor in central nervous system pharmacology, the lack of blood–brain barrier penetration initially proved to be a major obstacle to the in vivo evaluation of this class of compounds in various animal models.[3,21] Compound 6 is approximately twice as active as AP5 at the receptor binding site, but in contrast to AP5 has good blood–brain barrier penetration with rapid onset of action. Thus, introduction of a carbonyl functionality in the β-position relative to the phosphonate moiety not only increases binding but also enables better penetration into the brain. The reason for the increased antagonist activity is not clear, but it could possibly be due to the ability of the ketone in 6 to enolize.[22]

(12) Itoh, M. *Chem. Pharm. Bull.* **1969**, *17*, 1679.
(13) Compound 6 gave spectral data and C, H, and N combustion analyses consistent with the structure.
(14) Baron, B. M.; Dudley, M. W.; McCarthy, J. R.; Miller, F. P.; Reynolds, I. J.; Schmidt, C. J. *J. Pharmacol. Exp. Ther.* **1989**, *250*, 162.
(15) [^{3}H]Kainic acid binding was conducted according to the method of London and Coyle: London, E. D.; Coyle, J. T. *Mol. Pharmacol.* **1979**, *15*, 492.
(16) [^{3}H]AMPA binding was performed as in the following: Honoré, T.; Drejer, J. *J. Neurochem.* **1988**, *51*, 457.
(17) Meldrum, B. In *Excitatory Amino Acid Transmission*; Hicks, T. P., Lodge, D., McLennan, H., Eds.; Alan R. Liss, Inc.: New York, 1987; pp 189–196.
(18) Swinyard, E. A.; Woodhead, J. H.; White, H. S.; Franklin, M. R. In *Antiepileptic Drugs*, 3rd ed.; Levy, R., Mattson, R., Meldrum, B., Penry, J. K., Dreifuss, F. E., Eds.; Raven: New York, 1989; pp 85–102.
(19) Skeen, G.; Woodhead, J.; Wolf, H.; Swingard, E.; Tietz, E.; White, H. S. *Soc. Neurosci. Abstr.*, in press. White, H. S.; Sheen, G.; Woodhead, J.; Wolf, H.; Swingard, E.; Tietz, E. *Epilepsy Res.*, in press.
(20) Whitten, J. P.; Harrison, B. L.; McDonald, I. A. in preparation.
(21) Several recently reported NMDA antagonists are also brain permeable; for example, see: Chapman, A. G.; Graham, J.; Meldrum, B. S. *Eur. J. Pharmacol.* **1990**, *178*, 97. Hutchison, A. J.; Williams, M.; Angst, C.; Reynalda de J.; Blanchard, L.; Jackson, R. H.; Wilusz, E. J.; Murphy, D. E.; Bernard, P. S.; Schneider, J.; Campbell, T.; Guida, W.; Sills, M. A. *J. Med. Chem.* **1989**, *32*, 2171.
(22) This has been demonstrated indirectly by NMR deuterium-exchange experiments. For instance, the α phosphonate protons (δ 3.13) completely exchange with D_2O within 4 h.



6

In conclusion, this β-ketophosphonate represents an important new example of an NMDA receptor glutamate antagonist, combining good binding affinities with effective in vivo activity.

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Articles

Antiarthritic and Suppressor Cell Inducing Activity of Azaspiranes: Structure–Function Relationships of a Novel Class of Immunomodulatory Agents

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Spirogermanium (1; 8,8-diethyl-*N*,*N*-dimethyl-2-aza-8-germaspiro[4.5]decane-2-propanamine dihydrochloride) is a potent cytotoxic agent in vitro which has demonstrated limited activity in experimental animal tumor models. Subsequently, it has been reported that spirogermanium has antiarthritic and suppressor cell-inducing activity. We have synthesized a series of substituted 8-hetero-2-azaspiro[4.5]decane and 9-hetero-3-azaspiro[5.5]undecane analogues of spirogermanium to identify the heteroatom requirements for in vivo antiarthritic and suppressor cell-inducing activity. This structure–activity relationship study has identified that appropriately substituted silicon and carbon analogues of spirogermanium retain both antiarthritic and immunosuppressive activity, with the 8,8-dipropyl (carbon) analogue being among the most active. Following the identification of *N*,*N*-dimethyl-8,8-dipropyl-2-azaspiro[4.5]decane-2-propanamine dihydrochloride (**9**) as a more active analogue than spirogermanium, a series of 8,8-dipropyl analogues with various amine substituents were synthesized. A number of these analogues had activity similar to that of **9**. A correlation between activity in the adjuvant arthritic rat and the ability to induce suppressor cells ($r = 0.894$, $p < 0.001$) suggests an association between the two pharmacologic effects. While the precise biochemical mechanism(s) for the pharmacological activity is unclear, these data suggest that compounds within this series, e.g., *N*,*N*-dimethyl-8,8-dipropyl-2-azaspiro[4.5]decane-2-propanamine dihydrochloride, may provide effective therapy in diseases of autoimmune origin and/or the prevention of rejection in tissue transplantation.

Introduction

The demonstration of disorders of suppressor cells in animal models of autoimmune disease[1-4] and in patients with autoimmunity[5-9] suggests that their regulation may be a rational approach for immunotherapy. If the loss of suppressor cells in patients with autoimmunity is an important link in the causation of the disease(s), therapy directed toward augmenting suppressor mechanisms may be effective in treating the manifestations of autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, autoimmune diabetes) and rejection following tissue/organ transplantation. Suppressor cells are introduced by most, if not all, forms of immunogenic challenge and can be antigen-specific T cells, as first described by Gershon and Kondo,[10] or they can be nonspecific in nature and of various phenotypes.[11-14]

Suppressor cells can also be induced by a wide variety of compounds and treatments, one of the most notable of which is cyclosporin A (for review see[15]), a fungal metab-

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(1) Barthold, D. R.; Kysela, S.; Steinberg, A. D. *J. Immunol.* **1974**, *112*, 9.
(2) Bernard, C. C. A. *Clin. Exp. Immunol.* **1977**, *29*, 100.
(3) Steinberg, A. D.; Huston, D. P.; Taurog, J. D.; Cowdery, J. S.; Raveche, S. *Immunol. Rev.* **1981**, *55*, 121.
(4) Binderup, L. *Ann. Rheum. Dis.* **1983**, *42*, 693.
(5) Fauci, A. S.; Steinberg, A. D.; Haynes, B. F.; Whalen, G. *J. Immunol.* **1978**, *121*, 1473.
(6) De Galocsy, C.; Jenkins, P. J.; Mielivergani, G.; Eddleston, A. L. W. F.; Williams, R. *Clin. Exp. Immunol.* **1981**, *43*, 486.
(7) Antel, J. P.; Bania, M. B.; Reder, A.; Cashman, N. *J. Immunol.* **1986**, *137*, 137.
(8) Goto, M.; Miyamoto, T.; Nishioka, K.; Uchida, S. *Arthritis Rheum.* **1987**, *30*, 737.
(9) Bach, J. F. *Clin. Exp. Immunol.* **1988**, *72*, 1.
(10) Gershon, R. K.; Kondo, K. *Immunology* **1970**, *18*, 723.
(11) Katz, D. H.; Benacerraf, B. *Adv. Immunol.* **1972**, *15*, 1.
(12) Gilbert, K. M.; Hoffmann, M. K. *Immunol. Today* **1983**, *4*, 253.
(13) Allison, A. C. *Immunol. Rev.* **1978**, *40*, 3.
(14) Strober, S. *Annu. Rev. Immunol.* **1984**, *2*, 219.

0022-2623/90/1833-2963$02.50/0