pounds which are unstable upon storage (8, 25, 29, 95, and 104) were tested for binding affinity immediately after their preparation.

Acknowledgment. We are grateful to The Hong Kong Jockey Club (Charities) Ltd. for financial support and to Yu-Xin Cui for his assistance in recording several 2D NMR spectra.

Supplementary Material Available: 2D ^1H-^1H COSY and NOESY of compounds 72 and 75 (4 pages). Ordering information is given on any current masthead page.

Synthesis of the 2-Amino-4-phosphonobutanoic Acid Analogues (*E*)- and (*Z*)-2-Amino-2,3-methano-4-phosphonobutanoic Acid and Their Evaluation as Inhibitors of Hippocampal Excitatory Neurotransmission

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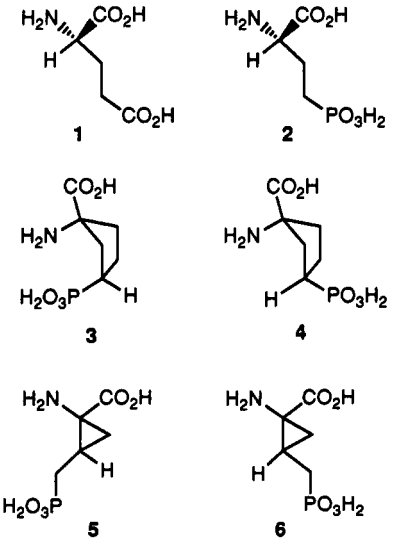
The cyclopropyl compounds (*Z*)- and (*E*)-2-amino-2,3-methano-4-phosphonobutanoic acid, **5** and **6**, respectively, were prepared as constrained analogues of 2-amino-4-phosphonobutanoic acid (AP4), a selective glutamate receptor ligand. A Horner–Emmons reaction of trimethyl *N*-(benzyloxycarbonyl)phosphonoglycinate with 2-(diethoxyphosphinyl)acetaldehyde gave the protected dehydroamino acids **9** and **10**, which were individually subjected to the following sequence of reactions: cycloaddition of diazomethane, photoelimination of N_2, and acid hydrolysis, to give **5** and **6**, respectively. Extracellular recording techniques were used to evaluate the abilities of **5** and **6** to block evoked synaptic transmission in specific neuronal pathways of the rat hippocampal slice. In the lateral perforant path (LPP) **5** and **6** were equipotent and possessed IC_{50} values of 18 and 17 μM, respectively. In the medial perforant path (MPP), **6** (IC_{50} = 81 μM) was much more potent than **5** (IC_{50} = 1580 μM). In paired pulse experiments which differentiate presynaptic and postsynaptic inhibition, **5** and **6** enhanced the second response to the same extent as L-AP4, suggesting a presynaptic site of action for these compounds. In contrast, the cyclopentyl AP4 analogues **3** and **4** enhanced the second response to a lesser extent. It was concluded that the biologically active conformation of AP4 in the LPP is different than in the MPP. In order to explain the same potency of **5** and **6** in the LPP, it was postulated that the two analogues assume a conformation that allows their functional groups to occupy the same relative place in space. Molecular modeling showed that the best overlap was achieved when the αC–βC–γC–P dihedral angle for **5** was in the range of 130° to 180° and that of **6** was in the range of −130° to −180°. The results suggest that the bioactive conformation of AP4 in the LPP is an extended one.

The importance of excitatory amino acids (EAA's), in particular L-glutamic acid (**1**), in central nervous system development, cognition, and disease is becoming increasingly apparent as more specific and selective EAA agonists and antagonists are developed.[1-3] Currently, there are postulated to be five defined EAA receptor subtypes.[1] One of these, the L-2-amino-4-phosphonobutanoic acid (L-AP4, **2**) EAA receptor subtype, is delineated by a unique responsiveness to L-AP4. To date, five systems have been studied which are particularly sensitive to L-AP4: the retina;[4] the spinal cord;[5,6] the lateral olfactory tract (LOT);[7,8] and in the hippocampus, the rat lateral perforant path (LPP),[9] and the guinea pig mossy fiber-CA3 pathway.[10,11] In the retina, L-AP4 shows postsynaptic agonist activity on ON-bipolar cells.[4,12] In contrast, L-AP4 is postulated to act at presynaptic inhibitory autoreceptors in the rat LPP,[13] in the guinea pig mossy fiber-CA3 pathway,[14] the spinal cord,[15] and the LOT.[16,17] In those systems where the receptor is proposed to be presynaptic, L-AP4 has been shown to stereoselectively antagonize evoked excitation without inhibiting responses due to application of the prototypical agonists NMDA, kainic



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acid, quisiqualic acid, L-aspartic acid, or L-glutamic acid.[10,18-21] In addition to these five systems, L-AP4 has been studied in its relation to the phenomenon known as the "Quis Effect" in which exposure of the hippocampal CA1-Schaffer collateral pathway to quisqualic acid results in a marked increase in the potency of L-AP4 to inhibit evoked synaptic field potentials.[22] The AP4 receptors responsible for the Quis Effect are pharmacologically distinct from the presynaptic AP4 receptor found in the rat perforant path.[23] However, the two classes of receptors are similar in that only phosphonates, phosphinates, and phosphates are presently known to exhibit specificity at these receptors.[24-26]

In order to investigate the structural requirements for antagonism of evoked electrophysiological activity, several L-AP4 analogues have been synthesized in the past and tested in rat hippocampal electrophysiological preparations.[24,25,27-30] In the perforant path of the rat hippocampal slice preparation, L-AP4 shows pathway selectivity for the LPP over the medial perforant path (MPP).[9] L-AP4 inhibits evoked field potentials in the LPP with an apparent K_d of 2.5 μM. The pattern of inhibition by L-AP4 in the MPP has two components with apparent K_d's of 45 μM and 10 mM.[24]

In one of our earlier studies,[27] conformationally constrained analogues of L-AP4 were prepared by linking the α- and γ-carbon atoms of the L-AP4 backbone with either two or three methylene units. These cyclopentyl and cyclohexyl analogues were designed to mimic possible folded and extended conformations of L-AP4. In this series of compounds, only the cyclopentyl AP4 analogues **3** and **4** showed significant activity. Analogue **3**, in which the phosphonate and amino groups are cis to one another, appeared to most resemble L-AP4 in that it showed appreciable activity in inhibiting synaptic activity in the LPP (IC_{50} = 130 μM) and also showed pathway selectivity for the LPP over the MPP. In contrast, **4**, wherein the phosphonate and carboxyl groups are cis to one another, showed little electrophysiological activity but was very potent and selective in inhibiting Ca^{2+}/Cl^--dependent [^{3}H]AP4 uptake.[27]

On the basis of the biological profile of **3**, we previously postulated that the active conformation of L-AP4 at the LPP AP4 receptor might be a folded one to allow for an ionic interaction between the amino and phosphonate moieties.[27] The relatively lower activity of **3** in comparison to L-AP4, however, suggested the possibility of limited steric tolerance at the receptor site. In order to test these hypotheses and to better define the available extended versus folded L-AP4 conformations, the 2,3-methano-AP4 analogues **5** and **6** have been synthesized. The methylene bridging unit was chosen because of its smaller size in comparison to the ethylene bridge used in **3** and **4**. Furthermore, by incorporating the bridge between the α- and β-carbon atoms of AP4 instead of between the α- and γ-carbon atoms as in **3** and **4** the steric bulk about the phosphonate moiety would be reduced and a different set of conformations attained. In this paper, we report that in terms of their pharmacological profiles **5** and **6** more closely mimic L-AP4 than do the cyclopentyl AP4 analogues **3** and **4** and that these results suggest that the bioactive conformation of L-AP4 at its receptor in the rat hippocampal LPP is an extended one.

Table I. Horner-Wadsworth-Emmons Reaction Conditions for the Formation of the *Z* and *E* Dehydroamino Acids **9** and **10**[a]

base	temp, °C	solvent	time, h	yield, %	9:10[b]
nBuLi	−78 to 20	THF	2	70	13:1
nBuLi	−78 to 20	THF	20	75	12:1
nBuLi	−78	THF	2	NR[c]	
NaH	0 to 20	THF	2	36	14:1
tBuOK	−78 to 20	CH_2Cl_2	2	78	33:1

[a] Reaction conditions are described in the Experimental Section. [b] The ratio is based on the isolated yield of these two dehydroamino acids. [c] No reaction.

Results

Syntheses. The 2,3-methano-AP4 analogues **5** and **6** were synthesized as shown in Scheme I. The reaction between 2-(diethoxyphosphinyl)acetaldehyde (**8**)[31] and commercially available trimethyl *N*-(benzyloxycarbonyl)-phosphonoglycinate (**7**) was performed under Horner-Wadsworth-Emmons conditions[32] utilizing a variety of bases (Table I). Under the conditions examined, formation of the *Z*-olefin (**9**) was favored over the *E*-olefin (**10**). Ratios of *Z*:*E* varied from 12:1 with *n*-butyllithium to 50:1 with potassium *tert*-butoxide. The *Z*- and *E*-olefins were separated successfully by silica gel medium-pressure chromatography. This was a difficult separation, however, due to the small R_f difference between the two isomers and the tendency of **10** to convert to **9**, as well as to decompose when exposed to warm temperatures and light.

In order to obtain sufficient quantities of both isomers for further reactions, various techniques were investigated to invert the *Z*-olefin **9** to the *E*-olefin **10**. Several attempts were made to apply the method of Mazur and Pilipauskas[33] of conjugate addition of either benzeneselenol or benzenemethaneselenol to a *Z*-dehydro amino acid, followed by oxidation of the selenide to the selenoxide and then syn elimination of this intermediate to reform a mixture of *E*- and *Z*-dehydro amino acids. All attempts with this method proved unsatisfactory in our hands. Only minor yields of **10** were obtained, and partial deprotection of the methyl ester was observed.

Ultimately, it was found that **9** could be photoisomerized to **10**. Several reaction conditions were investigated for this photoisomerization, and these are summarized in Table II. On the basis of these results acetone was selected as the solvent to carry out the isomerization on a preparative scale. In the preparative reaction, longer time

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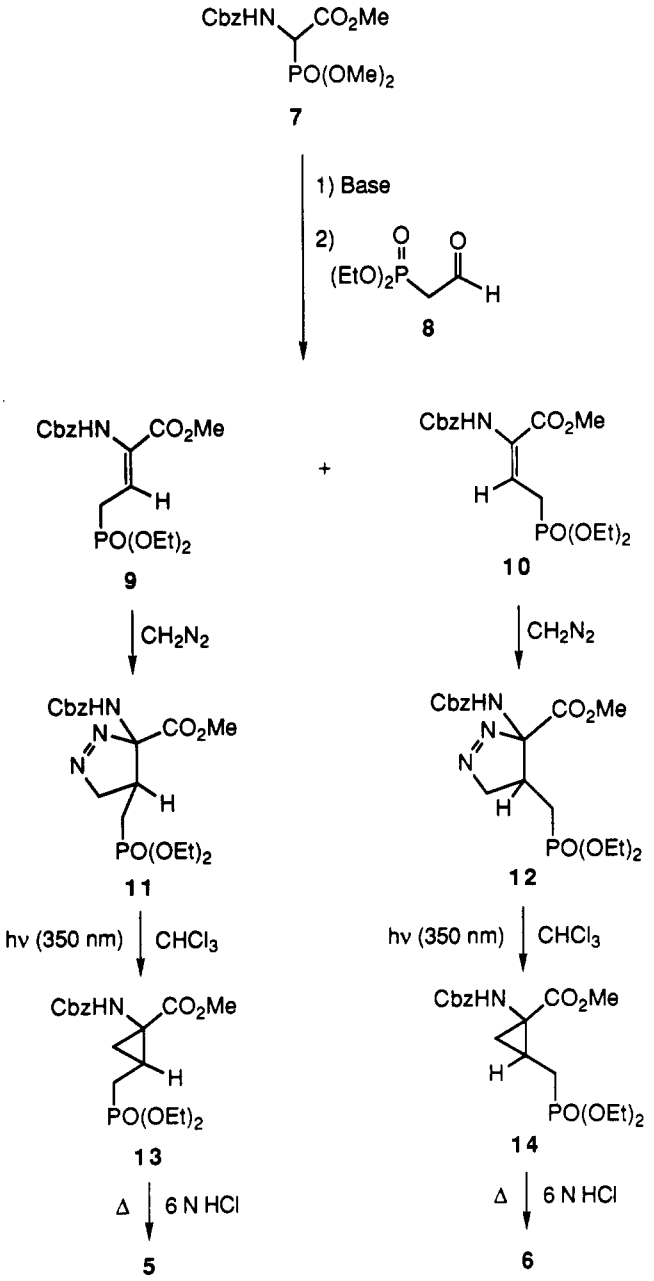
Scheme I



periods were required for the equivalent conversion of **9** to **10**. ^{1}H NMR of the reaction mixture indicated that the maximum ratio of **9** to **10** obtained was 5:3. However, because of the difficulty of separating **10** from **9** the ratio of isolated **9** to **10** was typically in the vicinity of 4:1. Several other reaction products also were formed in minor quantities during the preparative photoisomerization reaction. The products appeared to result from the isomerization of the double bond and subsequent 2 + 2 cycloaddition of the resultant β,γ-olefins. Also some removal of the benzyloxycarbonyl amino protecting group was observed.

The *Z*- and *E*-olefins **9** and **10** were distinguished from each other by comparison of the vinylic and allylic proton chemical shifts to those of other known dehydro amino acids and by ^{1}H NMR 1-D NOE difference experiments. The ^{1}H NMR chemical shifts in $CDCl_3$ of the vinylic (6.43 ppm) and allylic (2.76 ppm) protons of **9** were upfield from the vinylic (6.84 ppm) and allylic (3.26 ppm) protons of **10**. On the basis of the relative chemical shifts observed for analogous protons in other *E* and *Z* *N*-benzyloxycarbonyl-protected aliphatic β-substituted dehydro amino acid esters,[32] our results indicated that the dehydro amino acids **9** and **10** had the *Z* and *E* configurations, respectively. NOE experiments performed on **10** in the manner of Shimohigashi et al.[34] failed to show enhancement of the vinylic proton of **10** at 6.8 ppm upon irradiation of the amide proton at 7.0 ppm. However, NOE difference experiments carried out on **9** showed that, when the amide proton of this isomer was irradiated, there was a significant negative enhancement of the allylic protons at 2.7 ppm. This indicated close, through-space proximity of the amide and allylic protons in **9** and supported the assignment of the *Z* and *E* configuration to **9** and **10**, respectively.

Table II. Photoisomerization of **9** to **10**[a]

solvent	time, h	λ, nm	% **10**[b]
$CDCl_3$	72	254	<1
$CDCl_3$[c]	24	350	0[d]
d_6-benzene	72	350	33
d_6-acetone	7	350	20
d_6-acetone	25	350	24
d_6-acetone	52	350	37

[a] Reactions were performed in 5-mm NMR tubes with 10–20 mg of **9** dissolved in 1 mL of solvent. The tubes were placed in a Rayonet Photoreactor containing an array of 16 light tubes at the λ indicated. The reactions were performed in a cold room to provide an ambient temperature in the photoreactor of approximately 22 °C. [b] The percent of isomerization was determined by comparing the ^{1}H NMR integration values of the vinylic and allylic protons in the resulting mixture of **9** and **10**. [c] Benzophenone was added as a photosensitizer. [d] Only decomposition of **9** occurred.

Olefins **9** and **10** were converted to their corresponding pyrazolines **11** and **12** by cycloaddition of diazomethane in Et_2O at room temperature for several days.[35] Progress of the reaction was monitored by TLC and by measuring the UV absorbance at a λ_{max} at 330 nm. Since such [3 + 2] cycloadditions result in the stereospecific syn addition of diazomethane to either face of the olefin,[36] **11** and **12** were each obtained as a racemic mixture. They were purified by silica gel chromatography and were found to be stable under these conditions. Similar to other α-amino acid pyrazolines,[36] both pyrazolines were stable to thermal decomposition. Photochemical conversion of pyrazolines **11** and **12** to the corresponding racemic cyclopropanes **13** and **14** occurred in $CHCl_3$ with the release of N_2 upon irradiation with light (λ = 350 nm) for several days. The products were purified by MPLC with EtOAc elution from silica gel. These protected cyclopropyl intermediates slowly decomposed to form multiple olefinic products during long-term storage in the dark, but they remained stable for several months if kept below 0 °C.

Deprotection of **13** and **14** gave racemic **5** and **6**, respectively, and was accomplished by heating at reflux the protected compounds in 6 N HCl for several hours. The final 2,3-methano amino acids were purified by elution from a strong cation exchange resin with H_2O. Although the rather harsh method of deprotection gave undesirably low yields of **5** and **6**, milder attempts such as HBr/HOAc at 25 °C for 20 h and 6 N HCl at reflux for <5 h or TMSI in $CHCl_3$ at 55 °C for 2 h resulted in the retention of at least one phosphonate ethyl ester or the carboxyl methyl ester, respectively. These partially deprotected intermediates could not be cleanly separated from the completely

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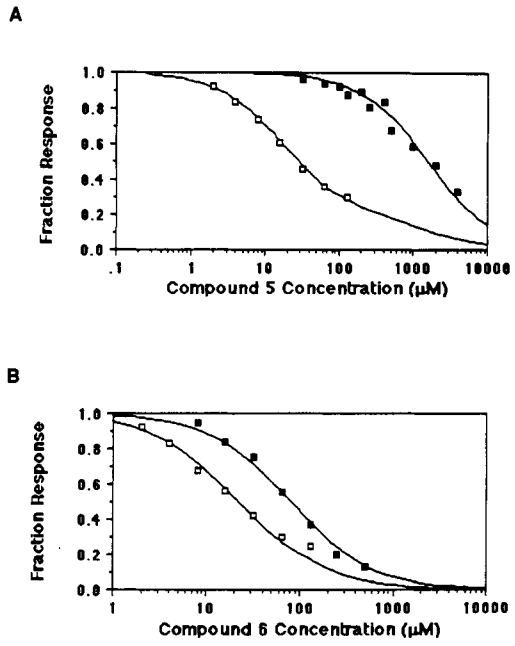

Figure 1. Concentration–response curves for the inhibition of perforant path responses by the (*Z*)- and (*E*)-2,3-methano-AP4 analogues **5** (A) and **6** (B), respectively. Amplitudes of the synaptic field potential were recorded in the absence and the presence of increasing drug concentrations and plotted as a fraction of the original uninhibited amplitude. The mean data for medial perforant path responses (■, n = 5) are presented and were contaminated with no more than 15% lateral responses. The mean data for lateral perforant path responses (□, n = 5–8) are presented and were contaminated with no more than 25% medial responses.

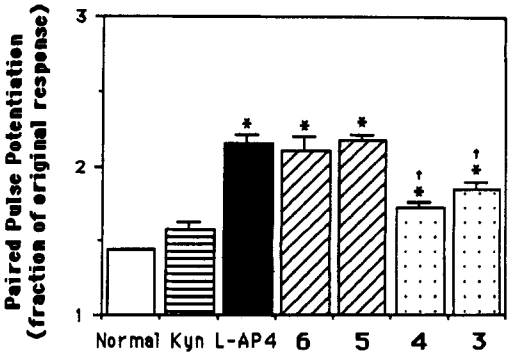

Figure 2. Paired pulse potentiation (PPP). Data are plotted as the mean (±SEM) potentiation during exposure to medium, kynurenic acid (Kyn, 200–500 μM), L-AP4 (5–10 μM), 2,3-methano AP4 analogues **5** (20–80 μM) and **6** (20–80 μM), and cyclopentyl AP4 analogues **3** (200–500 μM) and **4** (1–2 mM). Drugs were added at concentrations which reduced the LPP responses to 30–60% of the original response. Potentiation was calculated by dividing the amplitude of the second response by the amplitude of the first response (see Experimental Section). (*) Significantly different ($p < 0.001$, paired difference test) from the normal. (†) Significantly different ($p < 0.001$, Student's t test) from L-AP4. In the presence of normal medium, the PPP was 1.43 fold (±0.01 SEM, n = 62). Kynurenic acid enhanced the second response 1.57 fold (±0.05 SEM, n = 8) whereas L-AP4 enhanced the second response 2.15-fold (±0.07 SEM, n = 20). These results are similar to those published previously by Harris and Cotman.[13] Exposure of the silice to **5** and **6** enhanced the second response 2.18-fold (±0.04 SEM, n = 6) and 2.10-fold (±0.10 SEM, n = 6), respectively. Compounds **3** and **4** enhanced the second response 1.85-fold (±0.05 SEM, n = 13) and 1.72-fold (±0.04 SEM, n = 13), respectively.

deprotected final compounds by ion-exchange chromatography.

The ^{1}H NMR spectra of **5** and **6** in 10% NaOD/D_2O were quite distinct. Assignments were made with the aid of ^{31}P-decoupled experiments. The most notable difference between the two was the different chemical shifts of the cyclopropyl-CH_2 protons. In the case of compound **6**, the resonances were observed at 0.86 and 0.78 ppm while for **5** they were observed at 0.73 and −0.01 ppm. The large upfield chemical shift observed for one of the cyclopropyl-CH_2 protons of **5** is probably the result of the syn cyclopropyl-CH_2 proton being shielded by the phosphonate group. The *Z* configuration of **5** would allow the phosphonate oxygen atoms to participate in hydrogen bonding with the amino hydrogens. In such a folded conformation the phosphonate moiety would come into close proximity to the syn cyclopropyl-CH_2 proton. This type of folded conformation would not be possible for **6** with the *E* configuration because of the repulsion between the negatively charged carboxylate and phosphonate moieties. Intramolecular hydrogen bonding in **5** might also explain the fact that the ^{13}C resonance for the C-5 methylene carbon adjacent to phosphorus in **5** is observed at 30.8 ppm ($^1J_{C-P}$ = 129 Hz) while for **6** it is observed at 23.42 ppm ($^1J_{C-P}$ = 82 Hz). The presence of P–O···H–N hydrogen bonding would result in a more electropositive phosphorus atom which in turn would shift the adjacent carbon resonance farther downfield than normal.

Electrophysiological Studies. The 2,3-methano AP4 analogues **5** and **6** were tested for their ability to inhibit evoked synaptic field potentials in the perforant path of the rat hippocampal slice as measured by extracellular recording. Neither of these compounds induced population spiking when stimulus intensity was just subthreshold for evoking such a response. Additionally, the data for inhibition of the evoked field potential showed parallel theoretical binding curves with a Hill coefficient of one as shown in Figure 1. The LPP theoretical curves for both **5** and **6** were empirically corrected for 20% contamination of medial responses. By maintaining a high Mg^{2+} concentration in the bathing medium, a stimulation rate of 0.1 Hz, and by recording the responses 4–8 ms after stimulation, responses due to any possible interaction of the AP4 analogues with NMDA receptors was excluded.[1-3]

The LPP and MPP apparent K_d values for **5** were 18 and 1580 μM, respectively, while the LPP and MPP apparent K_d values for **6** were 17 and 81 μM, respectively. As the data show, both **5** and **6** possessed similar potencies in the lateral perforant path. In contrast, analogue **6** was approximately 20 times more potent than **5** in the MPP. Compound **6** did not exhibit the same degree of pathway specificity as L-AP4 as shown by the difference in their IC_{50} (MPP) to IC_{50} (LPP) ratio of 4.8 and 18, respectively. Compound **5**, on the other hand, with an IC_{50} (MPP) to IC_{50} (LPP) ratio of 88, showed high pathway specificity.

The mechanisms of inhibition by the constrained AP4 analogues **3–6** were investigated with use of paired pulse potentiation (PPP) on LPP responses. Previous paired pulse potentiation experiments suggested that L-AP4 may act presynaptically at inhibitory autoreceptors in the rat LPP.[13] It was found that if the LPP was stimulated twice in quick succession (40-ms interval) then the second synaptic response was potentiated when compared to the first response. Presynaptic inhibitors such as L-AP4 or low extracellular calcium caused a reduction in LPP responses but increased the percent potentiation of that response. In contrast, the postsynaptic inhibitor kynurenic acid decreased the LPP responses with little increase in the percent potentiation. As shown in Figure 2, the 2,3-methano AP4 analogues **5** and **6** enhanced PPP in a manner similar to L-AP4, whereas the cyclopentyl AP4

Table III. Inhibition of Hippocampal Excitatory Neurotransmission[a]

compd	IC_{50}(LPP[b]) μM	IC_{50}(MPP[c]) μM	MPP IC_{50}/ LPP IC_{50}
2 (L-AP4)	2.5	45[d]	18
3[e]	130	1850	31
4[e]	960	2400	2.5
5[e]	18	1580	88
6[e]	17	81	4.8

[a] Data for 2–4 are from ref 27. [b] LPP, lateral perforant path. [c] MPP, medial perforant path. [d] A second component is observed with an IC_{50} = 10 mM. [e] Tested as racemic mixtures.

analogues 3 and 4 potentiated the response to a lesser extent.

Discussion

In a previous study,[27] we synthesized the cyclopentyl-AP4 analogues 3 and 4 in an attempt to define the biologically active conformation of L-AP4. Since compound 3, where the amino and phosphonate groups are cis to one another, resembled L-AP4 in its electrophysiological profile, we postulated that the biologically active conformation of L-AP4 might be a folded one whereby the amino and phosphonate groups could participate in an ionic interaction. However, we also recognized at the time that other conformations of 3 were possible because of pseudorotation of the cyclopentane ring system.[37] In addition, the much lower potency of 3 in comparison to L-AP4 suggested that the cyclopentyl constraint might be the source of unfavorable steric interactions with the receptor.

In order to test the hypothesis put forward in our previous study, as well as to further elucidate the bioactive conformation of L-AP4, we initiated the synthesis of the (*Z*)- and (*E*)-2,3-methano-AP4 analogues 5 and 6. The cyclopropyl ring system was chosen as the conformational constraint so as to minimize any potential adverse steric interactions with the AP4 receptor as well as to provide AP4 analogues that would be constrained in a different manner than the previously synthesized cyclopentyl AP4 analogues. On the basis of our previous hypothesis of a folded conformation, we expected the *Z*-isomer 5 to possess greater activity than the *E*-isomer 6 in the hippocampal perforant path. The results in Table III clearly show this is not the case. For comparison, data for the inhibition of the perforant path by L-AP4 and the cyclopentyl AP4 analogues 3 and 4 are also presented.[27] L-AP4 has previously been shown to inhibit synaptic transmission in a concentration-dependent and stereospecific manner. Studies describing these properties of L-AP4 have been reported elsewhere.[9]

In the lateral perforant path, racemic 5 and 6 exhibited the same potency. With IC_{50} values of 18 and 17 μM, respectively, these two compounds are the most potent L-AP4 analogues synthesized to date. They are some 7 times more potent than the cyclopentyl-AP4 analogue 3. A comparison of 5 and 6 with 3 (Figure 3) suggests that part of the difference in potency between the cyclopropyl and cyclopentyl conformationally constrained AP4 analogues may be due to steric effects. There is in 3 more steric bulk about the carbon atom to which the phosphonate group is attached than in either 5 or 6. Also, in 3 as compared to either 5 or 6, one of the ring methylene groups projects into a space opposite that which is occupied by the 2,3-methano bridge. In contrast to the LPP, 6 was 20 times more potent than 5 in the medial perforant path (MPP). It is not known if the two analogues interact with different receptor sites in this pathway or whether they interact with a single receptor type which prefers a ligand conformation more easily assumed by 6. Previously, the MPP was found to have two L-AP4 sensitive components, one with an apparent K_d of 45 μM and another with an apparent K_d of 10 mM.[24] Unlike L-AP4 itself, neither 5 nor 6 separated the medial perforant path into two components.

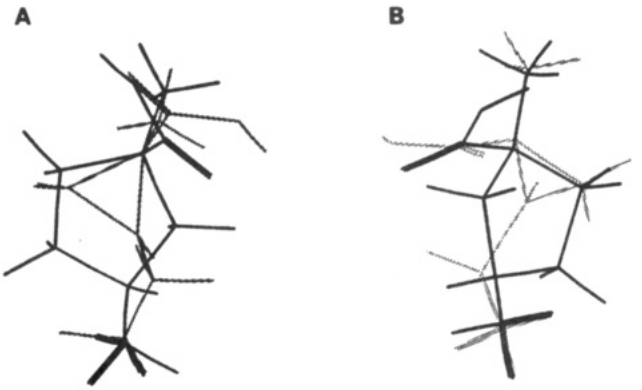

Figure 3. Superimposed ALCHEMY II energy-minimized structures of *trans*-1(*S*)-amino-3(*R*)-phosphonocyclopentanecarboxylic acid (3, black) with (2*S*,3*R*)-5 (A, gray) and (2*S*,3*S*)-6 (B, gray).

Earlier studies[24] showed L-AP4 to be pathway specific, being 18 times more potent for the LPP than the more sensitive component in the MPP and 4000 times more potent than the less sensitive medial component. Both 5 and 6 showed pathway selectivity for the LPP over the MPP as shown in Table III. There were, however, differences in the degree of selectivity shown by these two analogues. Compound 6 was only 4.8 times more selective toward the LPP than the MPP whereas 5 was 88 times more selective. This makes 5 the most selective agent described to date. Because neither compound induced population spiking when stimulus intensity was just subthreshold for such a response, and since both sets of data for inhibition of the evoked field potentials parallel theoretical binding curves with Hill coefficients of 1 as shown in Figure 1, the cyclopropyl analogues appear to be acting either presynaptically as agonists of the L-AP4 receptor or postsynaptically as antagonists of excitatory transmission. Postsynaptic agonists evoke population spiking and do not give concentration–response curves conforming to single ligand–receptor interactions.[38]

Earlier, studies measuring paired pulse potentiation showed the LPP receptor to be presynaptic.[13] In this study, PPP experiments showed analogues 5 and 6 to both inhibit the LPP response and increase the percent potentiation (Figure 2). This suggests that the cyclopropyl analogues are acting presynaptically to inhibit the LPP responses by a mechanism similar to that of L-AP4. In contrast, PPP experiments with the cyclopentyl AP4 analogues 3 and 4 resulted in potentiation which was dissimilar to both L-AP4 and kynurenic acid; the potentiation by 3 and 4 was greater than that of kynurenic acid but less than the potentiation of L-AP4. This suggests that the cyclopentyl analogues may be acting both pre- and postsynaptically to give a mixed response. Previously, it was hypothesized that 4 was acting postsynaptically as an agonist.[27]

These results have important implications regarding the conformation of L-AP4 when it interacts with its receptors in the LPP and MPP to inhibit evoked field potentials.

(37) Altona, C. *Conformational Analysis*, Chiurdoglu, G., Ed.; Academic Press: New York, 1971; p 1.

(38) Koerner, J. F.; Cotman, C. W. *Brain Res.* **1982**, *251*, 105.

The results indicate that there is a difference in the preferred conformations of L-AP4 at the LPP and the MPP sites. Although the 2,3-methano bridge in **5** and **6** provides a very rigid constraint for this portion of the L-AP4 molecule, rotation about the βC–γC bond is still possible and thus allows for some flexibility in these conformationally constrained molecules. The fact that **5** and **6** are equipotent in the LPP and that they act similar to AP4 in PPP experiments strongly suggests that these two analogues bind to the same receptor site in the LPP. If this is true, then both *Z*- and *E*-2,3-methano analogues **5** and **6** must be able to assume a conformation that allows their charged moieties to occupy the same relative place in space, since it has been shown that the amino, carboxyl, and phosphonate moieties are all required for the activity of L-AP4 in the LPP.[24,39] Evidently, the LPP receptor site is able to accommodate the differences in the positions of the structural constraints of **5** or **6** that are necessary to allow for this functional group overlap.

In an effort to determine which conformations of **5** and **6** might meet these requirements, the molecular modeling software program ALCHEMY II[40] was used to compare the structures of **5** and **6**. Since it is known that the AP4 receptor in the LPP and MPP is enantioselective for the L-isomer of AP4,[9] we expect that **5** and **6** will also exhibit enantioselectivity. A comparison of the enantiomers of **5** and **6** with L-AP4 indicates that the enantiomers of **5** and **6** that possess the same relative configuration at the C-2 carbon atom as does L-AP4, (2*S*,3*R*)-**5** and (2*S*,3*S*)-**6**, project their methylene bridge in the same relative area of space as does the α-H of L-AP4. The (2*R*,3*S*)-**5** and (2*R*,3*R*)-**6** enantiomers, on the other hand, resemble the relatively inactive D-AP4 molecule in this regard. Thus, (2*S*,3*R*)-**5** and (2*S*,3*S*)-**6** are used to illustrate the results of our molecular modeling analysis. It should be pointed out, however, that analogous results would be obtained if the other possible combination of enantiomers were used. The only difference would be that the cyclopropyl ring would be projected in the opposite direction.

The dihedral angle of **5** as defined by the four atoms αC–βC–γC–P was initially set at 10° while the respective dihedral angle of **6** was set at −10°. A root mean square (rms) fit between these two conformations of **5** and **6** was derived from the formula: rms = $(\sum^n \Delta_i^2/n)^{1/2}$, where Δ_i is the distance between two paired atoms and n is the number of pairs. The paired atoms N, C-1, and P (n = 3) were chosen because they are the central atoms of the functional groups shown necessary for the receptor interaction. It should be emphasized, however, that it is likely that one or more of the ionized oxygen atoms of the phosphonate and carboxyl moieties are the actual points of contact with the AP4 receptor. The central C-1 and P atoms were used in the modeling studies to simplify the analysis. This process was repeated at 20° intervals over the full dihedral angle range (0 to ±180°). The results are shown in Figure 4. The best fit occurs when **5** and **6** are in a relatively extended conformation with αC–βC–γC–P dihedral angles of 150° and −150°, respectively. The superimposition of these two particular conformations of **5** and **6** is illustrated in Figure 5. The results in Figure 4 also show that there is a range of dihedral angles in which the overlap of the amino, carboxyl, and phosphonate groups of **5** and **6** is quite good. This range of the αC–βC–γC–P dihedral angle is from 130 to 180° for **5** and from −130 to −180° for **6**. These data suggest that the bioactive conformation of L-AP4 when it interacts with its receptor in the LPP is an extended one.

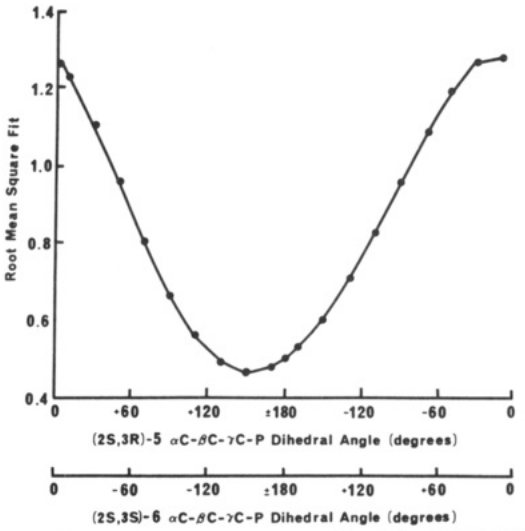

Figure 4. Plot of the root mean square (rms) fit of (2*S*,3*R*)-**5** and (2*S*,3*S*)-**6** with varying αC–βC–γC–P dihedral angles. The rms values were derived from the ALCHEMY II molecular modeling program[40] by the formula rms = $(\sum^n \Delta_i^2/n)^{1/2}$, where Δ_i is the distance between two paired atoms and n is the number of pairs. In this case, n = 3 and the paired atoms were N, C-1, and P.

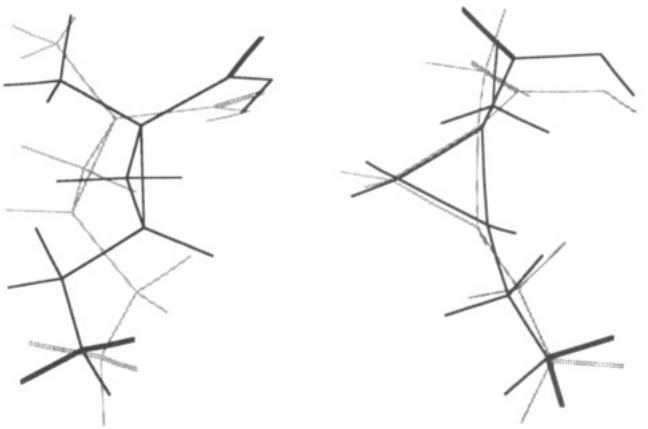

Figure 5. Two views of the superimposed conformations of (2*S*,3*R*)-**5** (black) and (2*S*,3*S*)-**6** (gray) that give the best root mean square fit. The αC–βC–γC–P dihedral angle of **5** = 150° and that of **6** = −150°. The structures were built and superimposed using ALCHEMY II molecular modeling software.[40]

Experimental Section

All reagents were purchased from the Aldrich Chemical Co. unless otherwise stated. Thin-layer chromatography (TLC) was done on either Analtech 250-μm silica gel GHLF, 250-μm C18 RPS-F reverse phase, or 250-μm DEAE-cellulose plates. Visualization was achieved by either UV light, I_2, ninhydrin, or 2,6-dichlorophenol–indophenol spray. Chromatographic separation via medium-pressure liquid chromatography (MPLC) was done with silica gel 60 (40–63 mm) from EM Reagents. Ion-exchange chromatography was performed with AG-50W-X8 cation exchange resin (200–400 mesh) from Bio-Rad Laboratories. IR spectra were performed on a Nicolet 5DXC FT-IR spectrometer. UV spectra were obtained from a Nicolet 5DXC FT-UV spectrometer. ^{1}H NMR spectroscopy was done on either a JEOL FX90Q 90 MHz FT-NMR, a Nicolet 300 MHz FT-NMR, or a GE Omega 500 MHz FT-NMR spectrometer. ^{13}C NMR spectra were obtained on either a JEOL spectrometer at 22.5 MHz or a Nicolet spectrometer at 75 MHz. ^{31}P NMR spectroscopy was conducted on either an IBM spectrometer at 81 MHz or a Nicolet spectrometer at 121 MHz.

(39) In the lateral perforant path, 3-aminopropylphosphonic acid inhibits the response 10% at 8 mM while 4-phosphonobutanoic acid inhibits the response 10% at 8 mM.

(40) ALCHEMY II, version 1.0 was obtained from Tripos Associates, St. Louis, MO, and run on a Macintosh IIcx microcomputer.

Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Unless otherwise indicated, all analytical results were within ±0.4% of the theoretical values.

(*Z*)- and (*E*)-Methyl 2-[*N*-(Benzyloxycarbonyl)amino]-4-(diethoxyphosphinyl)-2-butenoate (9 and 10). Potassium *tert*-butoxide (1.68 g, 15 mmol) was placed in a flame-dried 200-mL round-bottom flask and diluted with 5 mL of CH_2Cl_2 which had been distilled from P_2O_5 and stored over molecular sieves. The flask was sealed with a septum, flushed with N_2, and chilled to −78 °C. Trimethyl *N*-(benzyloxycarbonyl)-phosphonoglycinate (7; 5.0 g, 15 mmol; Fluka Chemical Co.) was dissolved in 25 mL of CH_2Cl_2 and added dropwise to the reaction flask while the mixture was stirred. The mixture was allowed to stir for 20 min at −78 °C and then 2-(diethylphosphinyl)-acetaldehyde[31] (8; 2.7 g, 15 mmol) dissolved in 5 mL of CH_2Cl_2 was added dropwise. The reaction mixture was stirred constantly while it was allowed to come to room temperature over a period of 2 h. The solvent was removed in vacuo and the residue was diluted with H_2O and then extracted with EtOAc (3×). The combined organic layers were washed with saturated NH_4Cl, dried over Na_2SO_4, filtered, and evaporated to give a pale yellow oil. Purification and separation by MPLC (silica gel, EtOAc) gave a combined yield of **9** and **10** of 4.48 g (77.5%) with the ratio of isolated *Z*:*E* = 33:1.

***Z*-Isomer 9.** TLC R_f (silica gel, EtOAc, UV visualization) = 0.41; UV λ_{max} (MeOH) 205, 231 nm; ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.35 (s, 5 H, Ph Hs), 7.20 (s, 1 H, NH), 6.43 (dt, *J* = 7.9 and 8.3 Hz, 1 H, C=CH), 5.14 (s, 2 H, CH_2Ph), 4.11 (dq, *J* = 7.0 and 8.4 Hz, 4 H, $POCH_2CH_3$), 3.74 (s, 3 H, OCH_3), 2.76 (dd, *J* = 8.4 and 22.4 Hz, 2 H, PCH_2), 1.31 (t, *J* = 7.0 Hz, 6 H, $POCH_2CH_3$); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 164.4 (CO_2CH_3), 154.0 (NCO_2), 135.1 (Ph quaternary C), 130.3 (d, $^3J_{C-P}$ = 13.4 Hz, α-C), 128.4, 128.1 (Ph Cs), 123.0 (d, $^2J_{C-P}$ = 12.2 Hz, β-C), 67.3 ($PhCH_2$), 62.4 (d, $^2J_{C-P}$ = 7.3 Hz, $POCH_2CH_3$), 52.3 (OCH_3), 26.4 (d, $^1J_{C-P}$ = 139.2 Hz, PCH_2), 16.2 (d, $^3J_{C-P}$ = 6.1 Hz, $POCH_2CH_3$); FAB-MS *m*/*z* 386 $(MH)^+$. Anal. ($C_{17}H_{24}NO_7P$) C, H, N, P.

***E*-Isomer 10.** TLC R_f (silica gel, EtOAc, UV visualization) = 0.35; ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.36 (s, 5 H, Ph Hs), 6.94 (s, 1 H, NH), 6.84 (dt, *J* = 8.4 and 8.8 Hz, 1 H, C=CH), 5.13 (s, 2 H, CH_2Ph), 4.12 (dq, *J* = 7.0 and 7.5 Hz, 4 H, $POCH_2CH_3$), 3.82 (s, 3 H, OCH_3), 3.26 (dd, *J* = 8.8 and 23.3 Hz, 2 H, PCH_2), 1.31 (t, *J* = 7.0 Hz, 6 H, $POCH_2CH_3$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 163.28 (CO_2Me), 153.01 (NCO_2), 135.43, 128.04, 127.80, 127.73 (Ph Cs), 127.05 (d, $^3J_{C-P}$ = 15.1 Hz, α-C), 117.16 (br s, β-C), 66.50 (CH_2Ph), 61.63 (d, $^2J_{C-P}$ = 6.6 Hz, $POCH_2CH_3$), 51.96 (OCH_3), 25.96 (d, $^1J_{C-P}$ = 139.2 Hz, PCH_2), 15.87 (d, $^3J_{C-P}$ = 6.1 Hz, $POCH_2CH_3$). Due to its instability this compound was not characterized further, but taken directly onto the next reaction.

The above procedure was carried out with a number of different bases besides potassium *tert*-butoxide in an effort to increase the amount of the *E*-isomer **10**. The results obtained are summarized in Table I.

Photochemical Inversion of 9 to 10. A photochemical inversion of the *Z*-olefin **9** was carried out whereby **9** (800 mg, 2.3 mmol) was dissolved in 10 mL of acetone which had been dried with 4-Å molecular sieves, filtered, and degassed with a fine stream of N_2. The solution was divided between two 10-mm NMR tubes, covered, and irradiated at 350 nm for 5 days in Rayonet photochemical reactor in a cold room. The average reactor temperature was 20 °C. The progress of the reaction was monitored by NMR and TLC. After removal of the solvent in vacuo, purification and separation of the olefins was performed by MPLC (silica gel, EtOAc). *Z*- and *E*-olefins **9** and **10** were recovered in a 90% yield as colorless oils with *Z*:*E* = 4:1.

(*Z*)-(3*RS*,4*RS*)-Methyl 3-[*N*-(Benzyloxycarbonyl)-amino]-4-[(diethoxyphosphinyl)methyl]pyrazoline-3-carboxylate (11). *Z*-Olefin **9** (440 mg, 1.2 mmol) was placed in a dry 25-mL round-bottom flask and diluted with 10 mL of Et_2O. After chilling the flask to 0 °C, excess CH_2N_2 in Et_2O was added dropwise to the solution which was then stoppered and stirred for 72 h at room temperature. Evaporation of the solvent gave a milky, viscous oil. The major impurity proved to be the starting olefin **9** which could be recycled to **11** after recovery. The crude material was purified via MPLC (silica gel, EtOAc) to give a 67% yield of racemic **11** as a transparent glass: TLC R_f (silica gel, EtOAc, UV visualization) = 0.23; UV λ_{max} (EtOH) 219, 251, 257, 327 nm; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (s, 1 H, Ph H), 7.34 (s, 2 H, Ph Hs), 7.30 (s, 2 H, Ph Hs), 6.67 (s, 1 H, NH), 5.05 (s, 2 H, CH_2Ph), 5.0–5.13 (m, 1 H, NNCH), 4.80 (dd, *J* = 7.0 and 12.2 Hz, 1 H, NNCH), 4.03–4.10 (m, 4 H, $POCH_2CH_3$), 3.83 (s, 3 H, OCH_3), 2.75–2.82 (m, 1 H, 4-CH), 1.65–1.95 (br m, 2 H, PCH_2), 1.30 (t, *J* = 7.0 Hz, 6 H, $POCH_2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.79 (CO_2Me), 153.87 (NCO_2), 135.51, 128.41, 128.19, 127.92 (Ph Cs), 101.43 (d, $^3J_{C-P}$ = 12.2 Hz, 3-C), 84.86 (d, $^3J_{C-P}$ = 4.9 Hz, $NNCH_2$), 67.35 (CH_2Ph), 61.78 (d, $^2J_{C-P}$ = 7.3 Hz, $POCH_2CH_3$), 53.81 (OCH_3), 32.31 (4-C), 23.99 (d, $^1J_{C-P}$ = 142.8 Hz, PCH_2), 16.19 (d, $^3J_{C-P}$ = 5.5 Hz $POCH_2CH_3$). Anal. ($C_{18}H_{26}N_3O_7P$) C, H, N.

(*E*)-(3*RS*,4*SR*)-Methyl 3-[*N*-(Benzyloxycarbonyl)-amino]-4-[(diethoxyphosphinyl)methyl]pyrazoline-3-carboxylate (12). The *E*-pyrazoline **12** was synthesized in an identical manner to the *Z*-pyrazoline **11** described above. From 220 mg (0.57 mmol) of *E*-olefin **10**, 215 mg (88%) of racemic **12** was produced: TLC R_f (silica gel, EtOAc, UV visualization) = 0.29; ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.34 (s, 5 H, Ph Hs), 6.72 (s, 1 H, NH), 5.05–5.09 (m, 1 H, NNCH), 5.08 (s, 2 H, CH_2Ph), 4.05–4.11 (m, 4 H, $POCH_2CH_3$), 3.88–3.91 (m, 1 H, NNCH), 3.78 (s, 3 H, OCH_3), 2.90–2.94 (m, 1 H, 4-CH), 1.84–1.90 (br m, 2 H, PCH_2), 1.32 (t, *J* = 7.0 Hz, 6 H, $POCH_2CH_3$); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 167.7 (CO_2Me), 153.8 (NCO_2), 135.4, 128.4, 128.2 (Ph Cs), 101.3 (d, $^3J_{C-P}$ = 14.6 Hz, 3-C), 84.70 (d, $^3J_{C-P}$ = 4.8 Hz, $NNCH_2$), 67.14 (CH_2Ph), 61.65 (d, $^2J_{C-P}$ = 6.1 Hz, $POCH_2CH_3$), 53.80 (OCH_3), 32.04 (4-C), 23.77 (d, $^1J_{C-P}$ = 142.8 Hz, PCH_2), 16.16 (d, $^3J_{C-P}$ = 4.8 Hz, $POCHCH_3$). Anal. ($C_{18}H_{26}N_3O_7P$) C, H, N.

(*Z*)-(2*RS*,3*SR*)-Methyl 2-[*N*-(Benzyloxycarbonyl)-amino]-2,3-methano-4-(diethoxyphosphinyl)butanoate (13). Pyrazoline **11** (1.47 g, 3.4 mmol) was dissolved in 10 mL of $CHCl_3$ and placed in a 50-mL Pyrex, pear-shaped flask. The flask was stoppered with a septum containing a needle to allow for N_2 escape. The flask was placed in a Rayonet photochemical reactor in a cold room (the average reactor temperature was 20 °C) and irradiated with light at a wavelength of 350 nm for 96 h. The reaction was monitored both by TLC and by the disappearance of the UV λ_{max} at 330 nm. Removal of solvent in vacuo gave 1.51 g of a crude, pale yellow oil. Purification by MPLC (silica gel, EtOAc) gave 1.01 g (74.3%) of racemic **13** as a buff-colored, viscous oil: TLC R_f (silica gel, EtOAc) = 0.28; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.33 (s, 5 H, Ph Hs), 6.34 (s, 1 H, NH), 5.11 (m, 2 H, CH_2Ph), 4.08 (dq, 4 H, *J* = 7.0 and 7.1 Hz, $POCH_2CH_3$), 3.66 (s, 3 H, OCH_3), 2.11 (m, 1 H, PCH*H*), 1.86 (m, 2 H, CH, CH*H*), 1.69 (m, 1 H, PCH*H*), 1.31 (t, *J* = 7.0 Hz, 6 H, $POCH_2CH_3$), 1.08 (m, 1 H, CH*H*); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 172.73 (CO_2Me), 157.02 (NCO_2), 136.54, 128.36, 127.82 (Ph Cs), 66.76 (CH_2Ph), 61.92 (d, $^2J_{C-P}$ = 6.1 Hz, $POCH_2CH_3$), 52.35 (OCH_3), 37.94 (d, $^3J_{C-P}$ = 7.3 Hz, α-C), 25.59 (d, $^1J_{C-P}$ = 141.6 Hz, CH_2P), 23.80 (d, $^3J_{C-P}$ = 12.2 Hz, β-CH_2), 21.80 (d, $^2J_{C-P}$ = 4.9 Hz, β-CH), 16.30 (d, $^3J_{C-P}$ = 6.1 Hz, $POCH_2CH_3$); ^{31}P NMR (81 MHz, $CDCl_3$) δ 29.75; FAB-MS *m*/*z* 400 $(MH)^+$. Anal. ($C_{18}H_{26}NO_7P$) C, H, N, P.

(*E*)-(2*RS*,3*RS*)-Methyl 2-[*N*-(Benzyloxycarbonyl)-amino]-2,3-methano-4-(diethoxyphosphinyl)butanoate (14). Pyrazoline **12** (488 mg, 0.50 mmol) was dissolved in 8 mL of $CHCl_3$ and divided between two 10-mm NMR tubes which were irradiated at 350 nm in a Rayonet photoreactor for 70 h at 20 °C. The solvent was removed in vacuo to give a milky, pale yellow oil which was purified by MPLC (silica gel, EtOAc) to give a 54.1% yield of racemic **14**: TLC R_f (silica gel, EtOAc) = 0.20; ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.34 (s, 5 H, Ph Hs), 5.60 (s, 1 H, NH), 5.12 (s, 2 H, CH_2Ph), 4.08 (dq, *J* = 7.0 and 7.1 Hz, 4 H, $POCH_2CH_3$), 3.69 (s, 3 H, OCH_3), 2.22 (m, 1 H, PCH), 2.01 (m, 2 H, PCH and β-CH), 1.57 (m, 2 H, β-CH_2), 1.31 (t, *J* = 7.0 Hz, 6 H, $POCH_2CH_3$); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 171.43 (CO_2Me), 156.42 (NCO_2), 136.27, 128.30, 127.92, 127.82 (Ph Cs), 66.76 (CH_2Ph), 61.86 (d, $^2J_{C-P}$ = 7.2 Hz, $POCH_2CH_3$), 52.35 (OCH_3), 38.49 (d, $^3J_{C-P}$ = 7.4 Hz, α-C), 24.61 (br s, β-CH), 23.56 (d, $^3J_{C-P}$ = 6.1 Hz, β-CH_2), 23.21 (d, $^1J_{C-P}$ = 141.6 Hz, PCH_2), 16.24 (d, $^3J_{C-P}$ = 6.2 Hz, $POCH_2CH_3$); ^{31}P NMR (81 Mz, $CDCl_3$) δ 30.05. Anal. ($C_{18}H_{26}NO_7P$) C, H, N, P.

(*Z*)-(2*RS*,3*SR*)-2-Amino-2,3-methano-4-phosphonobutanoic Acid (5). Compound **13** (0.26 g, 0.65 mmol) was refluxed in 10 mL of 6 N HCl for 18 h. The solvent was evaporated and the remaining white residue was repeatedly rinsed with H_2O and dried in vacuo. The residue was dissolved in 3 mL of H_2O

and applied to an AG 50W-X8 strong cation-exchange column. The column was eluted with H_2O and the eluent was monitored at 210 nm. Lyophilization of the combined fractions containing the desired material gave a transparent glass which crystallized from H_2O to give a 57% yield of racemic **5** as a white solid: mp 219 °C (sinters), 256 °C (dec); TLC R_f (C18, MeOH–H_2O, 1:1, ninhydrin visualization) = 0.34, R_f (DEAE cellulose, 1-butanol–HOAc–H_2O, 12:3:5, 2,6-chlorophenol–indolphenol visualization) = 0.14; ^1H NMR (300 MHz, 10% NaOD–D_2O) δ 1.12 (ddd, J = 4.4, 14.1, and 16.7 Hz, 1 H, PCH), 0.85–0.95 (m, 1 H, β-CH), 0.75 (ddd, J = 14.2, 16.7, and 22.9 Hz, 1 H, PCH), 0.73 (dd, J = 4.2 and 9.2 Hz, 1 H, β-CH*H*), −0.01 (dd, J = 4.3 and 6.6 Hz, 1 H, β-CH*H*), ^{13}C NMR (75 MHz, 10% NaOD–D_2O) δ 186.89 (CO_2H), 42.02 (d, $^3J_{C-P}$ = 9.4 Hz, α-C), 30.81 (d, $^1J_{C-P}$ = 128.5 Hz, PCH_2), 25.25 (d, $^3J_{C-P}$ = 4.4 Hz, β-CH_2), 24.91 (d, $^2J_{C-P}$ = 5.0 Hz, β-CH); ^{31}P NMR (121 MHz, 10% NaOD–D_2O) δ 21.83; FAB-MS m/z 196 $(MH)^+$. Anal. ($C_5H_{11}NO_5P \cdot H_2O$) C, H, N, P.

(*E*)-(2*RS*,3*RS*)-2-Amino-2,3-methano-4-phosphonobutanoic Acid (6). Compound **14** (145 mg, 0.36 mmol) was dissolved in 15 mL of 6 N HCl and heated at reflux for 9 h. The solvent was removed in vacuo and the residue was repeatedly rinsed with H_2O and dried under vacuum. The residual orange glass was dissolved in 2 mL of H_2O and applied to an AG 50W-X8 cation-exchange column. The column eluted with H_2O and the eluent was monitored at 210 nm. The product was obtained as a transparent glass which recrystallized from H_2O to give a 36.3% yield of racemic **6** as a white solid: mp 263 °C (dec); TLC R_f (C18, MeOH–H_2O, 1:1, ninhydrin visualization) = 0.42, R_f (DEAE-cellulose, 1-butanol–HOAc–H_2O, 12:3:5, 2,6-chlorophenol–indophenol visualization) = 0.12; ^1H NMR (300 MHz, 10% NaOD–D_2O) δ 1.46 (dd, J = 11.6 and 17.4 Hz, 1 H, PCH*H*), 0.9–1.1 (m, 2 H, β-CH and PCH*H*), 0.86 (dd, J = 4.8 and 5.1 Hz, 1 H, β-CH*H*), 0.78 (dd, J = 5.1 and 12.4 Hz, 1 H, β-C*H*H); ^{13}C NMR (125.7 MHz, 10% NaOD–D_2O) δ 184.35 (CO_2H), 43.98 (d, $^3J_{C-P}$ = 12.8 Hz, α-C), 26.91 (d, $^3J_{C-P}$ = 8.5 Hz, β-CH_2), 26.16 (β-CH), 23.42 (d, $^1J_{C-P}$ = 82.2 Hz, PCH_2); ^{31}P NMR (121 MHz, 10% NaOD/D_2O) δ 20.99; FAB-MS m/z 196 $(MH)^+$.

Electrophysiological Experiments. Experiments were performed using transverse slices of hippocampus obtained from male Sprague–Dawley (Biolab Co.) rats (30–100 days old). The animals were anesthetized with uretane (1.5 g/kg, ip) followed by decapitation. The forebrain was quickly removed and placed in cold (0 °C) incubation medium comprised of 124 mM NaCl, 3.3 mM KCl, 2.4 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, and 26 mM $NaHCO_3$ equilibrated with 95% O_2–5% CO_2 to maintain the pH at 7.4. The hippocampus was isolated by blunt dissection. Transverse slices (450 μm thick) were prepared with a McIlwain tissue chopper and incubated in room temperature (20–25 °C) medium. After at least 1 h, individual slices were transferred to a microperfusion recording chamber.[41] The slice was placed on a nylon mesh so that its upper surface was exposed to a humid atmosphere (95% O_2–5% CO_2), while the lower surface was in contact with medium at 34 °C. Stimulation (0.1 ms, 10–40 V) was delivered by a pair of twisted, Teflon-insulated stainless steel wires (0.003 in diameter) at a frequency of 0.1 Hz. The recording electrode was a glass micropipet of 2–15 Mohm impedence when filled with 2 M NaCl. Extracellular synaptic field potentials were monitored by an oscilloscope and their amplitudes as a function of time were displayed on a chart recorder.

The entorhinal inputs to granule cells were recorded in the middle or outer one-third of the molecular layer of the dentate gyrus (the medial and lateral perforant paths, respectively). Because the responses recorded in the perforant path often represent mixed medial and lateral responses, electrode placement was confirmed by applying test concentrations of L-AP4, a selective lateral perforant path inhibitor. The level of inhibition was used to calculate the relative contributions of each pathway to the perforant path field potential.[24] By this criterion, lateral responses were contaminated 7–45% with medial responses and medial responses were contaminated with 0–11% lateral responses.

After electrode placement, the slice was submerged in oxygenated and rapidly stirred medium. When the response stabilized, the medium was equilibrated with drug at a threshold concentration for inhibition of field potentials. The drug concentration was doubled every 4 min, which is sufficient time for the response to restabilize. The fraction of the uninhibited response was plotted as a function of the log concentration of the drug. The IC_{50} values reported are weighted means of IC_{50} values for each experimental point.

Paired pulse experiments were performed in the LPP similar to the above except two stimuli were delivered at a 40-ms interval every 10 s. The peak amplitude of each response was measured as it was displayed on the oscilloscope after 4-min exposure to drug. The response to the second stimuli was potentiated, i.e., the amplitude of the second response was larger than that of the first response. The potentiation was calculated as the amplitude of the second response divided by the amplitude of the first response.

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