Synthesis and Biological Evaluation of Cyclopropyl Analogues of 2-Amino-5-phosphonopentanoic Acid

Michael S. Dappen,*,† Roberto Pellicciari,‡ Benedetto Natalini,‡ Joseph B. Monahan,§ Claudio Chiorri,‡ and Alex A. Cordi§

Searle Research and Development, 4901 Searle Parkway, Skokie, Illinois 60077, Universita di Perugia, Istituto di Chimica Farmaceutica e Tecnica Farmaceutica, Via del Liceo, 06100 Perugia, Italy, and Searle, c/o Monsanto Life Sciences Research Center, Mail Zone AA5C, 700 Chesterfield Village Parkway, St Louis, Missouri 63198. *Received April 30, 1990*

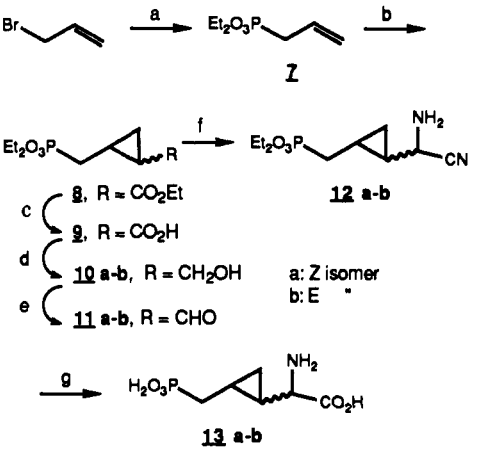
A series of cyclopropyl analogues related to 2-amino-5-phosphonopentanoic acid (AP5) were synthesized and their biological activity was assessed as competitive antagonists for the *N*-methyl-D-aspartate (NMDA) receptor. In vitro receptor binding using [^{3}H]-L-glutamate as the radioligand provided affinity data, while modulation of [^{3}H]MK-801 binding was used as a functional assay. The analogues were also evaluated in [^{3}H]kainate binding to assess selectivity over non-NMDA glutamate receptors. Of the compounds tested, 4,5-methano-AP5 analogue **26** was the most potent selective NMDA antagonist; however, potency was lower than that for [[(±)-2-carboxypiperidin-4-yl]methyl]phosphonic acid (CGS 19755, **5**).

During recent years it has become increasingly clear that excitatory amino acids (EAA) play a critical role as neurotransmitters[1] in the brain. Electrophysiological, biochemical, and pharmacological investigations of different agonists and antagonists have indicated that at least four different receptors mediate the action of the excitatory amino acids.[2,3] These receptors are named according to the most selective ligand used to characterize them: the *N*-methyl-D-aspartate (NMDA) receptor, the quisqualate receptor, the kainate receptor, and the AP4 (L-2-amino-4-phosphonobutanoic acid) receptor. Of these the NMDA receptor has been the most studied, not only for its interesting pharmacological characteristics[4] but also because of the general availability of specific antagonists and because it is hoped that major therapeutic progress can be obtained via its manipulation.[5]

In addition to the putative endogenous agonists L-glutamic acid,[6] L-aspartic acid,[7] and L-homocysteic acid,[8] synthetic agonists such as NMDA (**1**), *cis*-1(*R*)-amino-1,3(*R*)-dicarboxycyclopentane,[9] and (2*R*,3*S*,4*R*)-α-(carboxycyclopropyl)glycine[10] (**2**) have been identified (see Figure 1). These agonists have provided clues about the conformation of agonists at the receptor site. Fully rigid antagonists have not been identified so far, but substantial gains in receptor affinity have been reported in the series starting from D-2-amino-5-phosphonopentanoic acid[11] (AP5, **3a**), D-2-amino-7-phosphonoheptanoic acid[12] (AP7, **3b**), and [3-[(±)-carboxypiperazin-4-yl]prop-1-yl]-phosphonic acid[13] (CPP, **4**) and ending with [[(±)-2-carboxypiperidin-4-yl]methyl]phosphonic acid[14] (CGS 19755, **5**) and (*E*)-2-amino-4-methyl-5-phosphonopent-3-enoic acid[15] (CGP 37849, **6**) (see Figure 2).

We previously reported that a cyclopropyl ring was used successfully to conformationally restrict the glutamic acid skeleton. Some of the analogues reported have affinity and potency at the NMDA receptor similar to those of glutamic acid itself with decreased affinity at other EAA receptors. This led us to consider the synthesis of hybrid molecules wherein the AP5 skeleton has been rigidified by a cyclopropyl ring. We report herein the synthesis and biological evaluation of a series of AP5, AP6, and AP7 analogues wherein the carbon skeleton has been conformationally restricted by placement of a cyclopropyl ring at various positions along the carbon blackbone. The AP5 analogues (2,3-methano, 3,4-methano, and 4,5-methano) are structural isomers of CGP 37849 and were expected to provide NMDA antagonists with the affinity and the selectivity of (2*R*,3*S*,4*R*)-α-(carboxycyclopropyl)glycine.[10,16]

Scheme I[a]

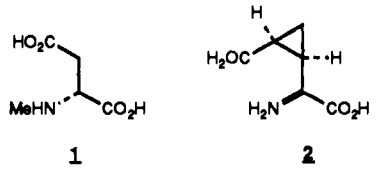


[a] (a) $P(OEt)_3$, 100 °C; (b) N_2CHCO_2Et, $CuSO_4$, C_6H_{10}, reflux; (c) KOH, THF/H_2O; (d) BH_3–THF, THF; (e) PCC, CH_2Cl_2; (f) KCN, NH_4Cl, Al_2O_3, CH_3CN, ultrasound; (g) 6 N HCl, reflux.

† Searle Research and Development.
‡ Universita di Perugia.
§ Searle.

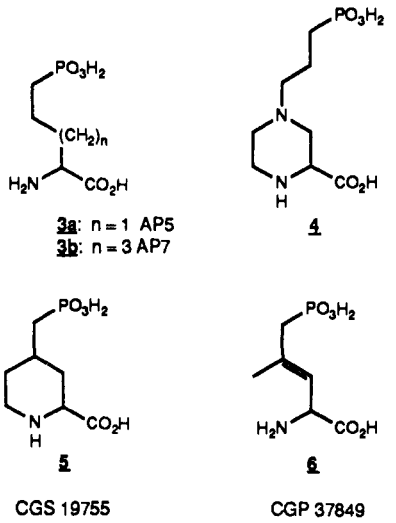
(1) Johnson, R. L.; Koerner, J. F. *J. Med. Chem.* **1988**, *31*, 2057.
(2) Watkins, J. C.; Olverman, H. J. *Trends Neurosci.* **1987**, *10*, 265.
(3) Lehmann, J.; Schneider, J. A.; Williams, M. *Annu. Rep. Med. Chem.* **1987**, *22*, 31.
(4) Wood, P. L.; Rao, T. S., Iyengar, S.; Lanthorn, T. L.; Monahan, J. B.; Cordi, A. A.; Sun, E. T.; Vazquez, M. L.; Gray, N. M.; Contreras, P. *Neurochem. Res.* **1989**, *14*, 217.
(5) Meldrum, B. *Clin. Sci.* **1985**, *68*, 113.
(6) Curtis, D. R.; Phillis, J. W.; Watkins, J. C. *J. Physiol. (London)* **1960**, *150*, 656.
(7) Evans, R. H.; Francis, A. A.; Hunt, K.; Oakes, D. J.; Watkins, J. C. *Br. J. Pharmacol.* **1979**, *67*, 591.
(8) Pullan, L. M.; Olney, J. W.; Price, M. T.; Compton, R. P.; Hood, W. F.; Monahan, J. B. *J. Neurochem.* **1987**, *49*, 1301.
(9) Curry, K.; Peet, M. J.; Magnuson, D. S. K.; McLennan, H. *J. Med. Chem.* **1988**, *31*, 864.
(10) (a) Pellicciari, R.; Natalini, B.; Marinozzi, M.; Selvi, L.; Chiorri, C.; Monahan, J. B.; Lanthorn, T. H.; Snyder, J. P. *Frontiers in Excitatory Amino Acid Res.*; E. A. Cavalheiro, J. Lehman, and L. Turski, Eds.; A. R. Liss, Inc.: New York, 1988, pp 67. (b) Pelliciari, R.; Natalini, B.; Marinozzi, M.; Monahan, J. B.; Snyder, J. P. *Tetrahedron Lett.* **1990**, *31*, 139.
(11) Perkins, M. N.; Stones, T. W.; Collins, J. F.; Curry, K. *Neurosci. Lett.* **1981**, *23*, 333.
(12) Davies, J.; Francis, A. A.; Jones, A. W.; Watkins, J. C. *Neurosci. Lett.* **1981**, *21*, 77.
(13) Davies, J.; Evans, R. H.; Francis, A. A.; Jones, A. W.; Olverman, H. J.; Pook, P.; Watkins, J. C. *Brain Res.* **1986**, *382*, 169.

0022-2623/91/1834-0161$02.50/0



1 2

Figure 1. Selective receptor agonists.



3a: n = 1 AP5
3b: n = 3 AP7
4
5
6
CGS 19755
CGP 37849

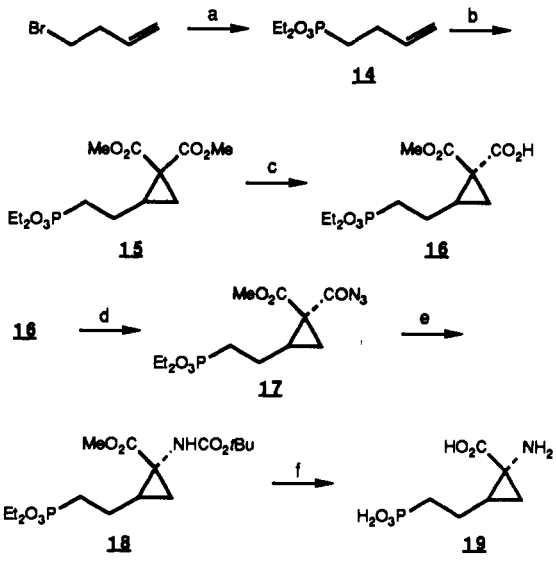
Figure 2. Selective NMDA antagonists.

Chemistry

The syntheses of (*Z*)- and (*E*)-3,4-methano-AP5 analogues are outlined in Scheme I. An Arbuzov reaction using allyl bromide and triethyl phosphite provided phosphonate **7** in 90‰ yield. The copper-catalyzed decomposition of ethyl diazoacetate[17] afforded cyclopropyl ester **8** (yield 30%) as an approximately 1:3 mixture of *Z* and *E* isomers. In contrast to the preparation of the 3,4-methano-AP6 and -AP7 analogues (vide infra), an attempted addition of the carbenoid to allyl bromide did not give cyclopropanes but rather carbene insertion into the carbon–bromine bond. In order to adjust the oxidation state at C-1, ester **8** was hydrolyzed to acid **9** (KOH, THF/H_2O, 72%), which was then treated with borane to give alcohols **10a,b** (77%). The alcohols were separated with silica gel chromatography and the isomers were characterized by ^{13}C NMR, with the signals for C(1) and C(3) the of *Z* isomer **10a** appearing at higher field than for the *E* isomer **10b**. Each alcohol was independently oxidized to aldehydes **11a,b** (pyridinium chlorochromate,[18] PCC; yields 83 and 86%, respectively). The aldehydes were transformed into amino nitriles **12a,b** via an alumina-catalyzed ultrasound Strecker reaction [19] in 45–75‰ yield. Finally hydrolysis of the nitrile and phosphonate using refluxing 6 N HCl provided the 3,4-methano-AP5 analogues **13a,b**. No evidence of cyclopropane ring opening was observed during the hydrolysis of any amino nitrile intermediate. The amino acids were generally purified by ion-exchange chromatography using strongly acidic resin (Dowex 50 8×100), eluting with 1 N aqueous pyridine. Note that the cyclopropyl aldehydes **11** were racemic and that the amino nitriles were generally a 50:50 to 60:40 mixture of diastereomers, as judged by ^1H NMR analysis. Thus the final amino acids **13** were a mixture of four stereoisomers (two pairs of diasteomers) in approximately equivalent ratios.

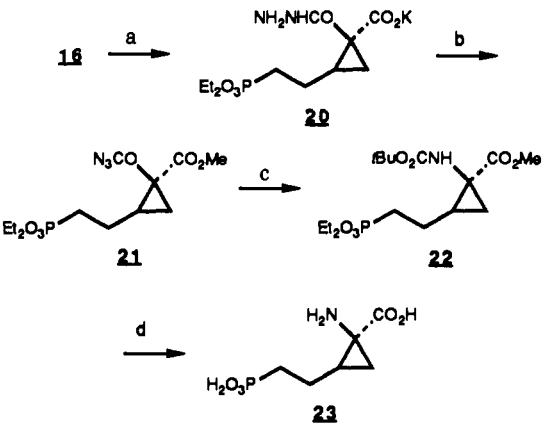
The syntheses of the (*E*)- and (*Z*)-2,3 methano-AP5 analogues were similar to methods reported by Stammer[20] and are depicted in Schemes II and III, respectively. An Arbuzov reaction afforded phosphonate **14** (32%), which was converted to a cyclopropane with dimethyl diazomalonate[21] to give 1,1-dicarboxylate **15** (17%). The methyl

Scheme II[a]



[a] (a) $P(OEt)_3$, 100 °C; (b) $N_2C(CO_2Me)_2$, C_6H_{10}, reflux; (c) NaOH $MeOH/H_2O$; (d) (i) K_2CO_3, EtOCOCl, 1,6-dicyclohexano-18-crown-6, THF, 0 °C, (ii) NaN_3, THF/H_2O; (e) *t*-BuOH, reflux; (f) 6 N HCl, reflux.

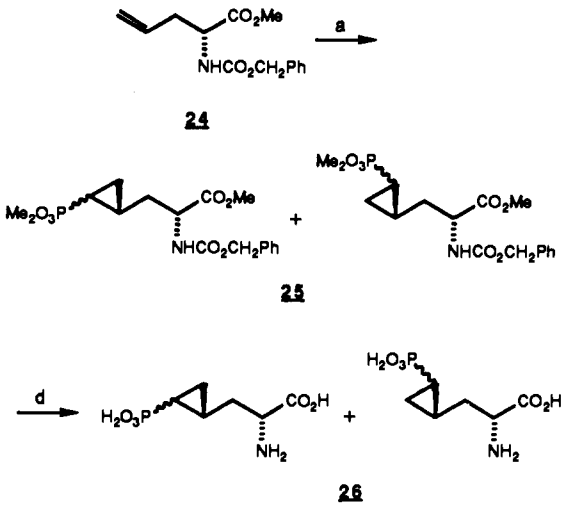
Scheme III[a]



[a] (a) (i) K_2CO_3, $MeOH/H_2O$, (ii) NH_2NH_2–H_2O, MeOH, reflux; (b) (i) $NaNO_2$, H_2SO_4, Et_2O/H_2O, 0 °C, (ii) CH_2N_2, Et_2O; (c) *t*-BuOH, reflux; (d) 6 N HCl, reflux.

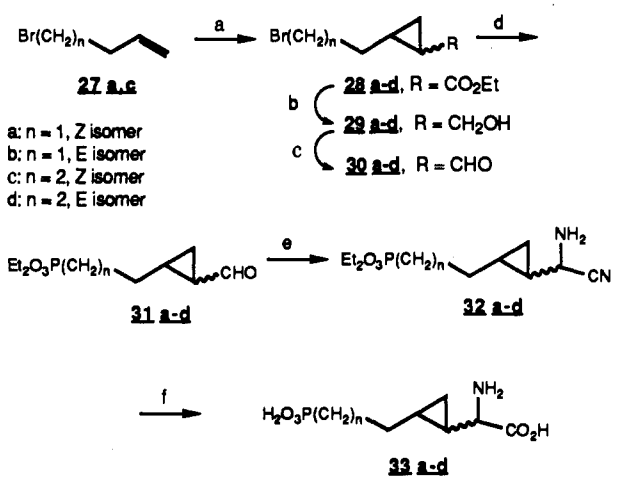
(14) Hutchinson, A. J.; Williams, W.; Angst, c.; de Jesus, R.; Blanchard, L.; Jackson, R. H.; Wilusz, E. J.; Murphy, D. E.; Bernard, P. S.; Schneider, J.; Campbell, T.; Guida, W.; Sills, M. A. *J. Med. Chem.* **1989**, *32*, 2171.
(15) Fagg, G. E.; Olpe, H.-R.; Pozza, M. F.; Bittiger, H.; Schmutz, M.; Angst, C.; Brundish, D.; Allgeier, H.; Heckendorn, R.; Dingwall, J. G. *Abstr. Soc. Neurosci.* **1988**, *381*, 941.
(16) Monahan, J. B.; Hood, W. F.; Compton, R. P.; Cordi, A. A.; Snyder, J. P.; Pellicciari, R.; Natalini, B. *Neurosci. Lett.*, in press.
(17) Tsuji, T.; Nishida, S. In *The Chemistry of the Cyclopropyl Group*; Rappoport, Z., Ed.; John Wiley and Sons Ltd.: Chichester, 1987; Chapter 7, pp 330–333.
(18) Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, 2647–2650.
(19) Hanafusa, T.; Ichiharqa, J.; Ashida, T. *Chem. Lett.* **1987**, 687–690.
(20) Mapelli, C.; Turocy, G.; Switzer, F. L.; Stammer, C. H. *J. Org. Chem.* **1971**, *36*, 1379–1386.

Scheme IV[a]



[a] (a) $N_2CHPO_3Me_2$, $Rh_2(OAc)_4$, CH_2Cl_2; (b) 6 N HCl, reflux.

Scheme V[a]



[a] (a) N_2CHCO_2Et, $CuSO_4$, C_6H_{10}, reflux; (b) $LiAlH_4$, Et_2O; (c) PCC, CH_2Cl_2; (d) $P(OEt)_3$, 100 °C; (e) KCN, NH_4Cl, Al_2O_3, CH_3CN, ultrasound; (f) 6 N HCL, reflux.

ester trans to the phosphonoethyl chain was selectively hydrolyzed with 1.0 equiv of sodium hydroxide in methanol to yield monoacid **16** (84%). From here the preparation of the two isomers diverge. For the *E* isomer **19**, mono acid **16** was first converted to acyl azide **17** (ethyl chloroformate, potassium carbonate; then aqueous sodium azide, 97%), which was decomposed in refluxing *tert*-butyl alcohol to form *N*-BOC amino ester **18** via a Curtius rearrangement (49%). Hydrolysis afforded racemic (*E*)-2,3-methano-AP5 analogue **19**.

Scheme III outlines the synthesis of *Z* analogue **23**. Monoacid **16** was converted to its potassium salt (potassium carbonate, methanol/water) and the ester functionality was transformed with hydrazine hydrate in refluxing methanol to give a quantitative yield of intermediate hydrazide **20**. Diazotization of the hydrazide using sodium nitrite and sulfuric acid, followed by esterification of the acid functionality with diazomethane, provided acyl azide/ester **21**. Again, treatment of **21** with refluxing *tert*-butyl alcohol provided ***N*-BOC** amino ester **22** (23% from **16**), which was hydrolyzed to (*Z*)-2,3-methano-AP5 analogue **23** (69%).

(21) Peace, B. W.; Carman, F.; Wulfman, D. S. *Synthesis* **1971**, 658–661.

Table I. Binding Data[a]

compd	[^{3}H]-L-glutamate; K_i, μM	[^{3}H]MK-801; IC_{50}, μM	[^{3}H]kainate; K_i, μM
3a, D-AP5	0.42 ± 0.02	0.29 ± 0.03	>100
5	0.16 ± 0.01	0.14 ± 0.03	>100
13a	18.9 ± 1.6	22.6 ± 5.1	>100
13b	9.5 ± 1.9	13.3 ± 2.7	>100
19	18.7 ± 1.0	15.2 ± 2.3	>100
23	26.7 ± 1.8	18.1 ± 3.8	>100
26	1.6 ± 0.4	2.7 ± 0.6	>100
33a	>50	ND[b]	>100
33b	>50	ND	>100
33c	12.5 ± 1.7	agonistlike[c]	>100
33d	>50	ND	>100

[a] Various concentrations of compounds were incubated with either 10 nM [^{3}H]glutamate, 5 nM [^{3}H]MK-801, or 0.5 nM [^{3}H]kainate as described in the Experimental Section. Logit-log analysis was used for K_i determination and IC_{50} values. The results are expressed as mean ± SEM from at least three separate experiments, each performed in triplicate. [b] ND = not determined. [c] Stimulated [^{3}H]MK-801 binding which is indicative of agonistlike activity.

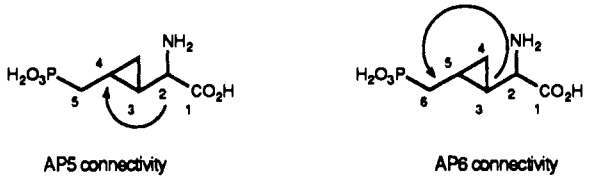


Figure 3. AP5 and AP6 connectivity of molecule **13**.

Scheme IV describes the preparation of D-4,5-methano-AP5 analogue **26**. (*R*)-*N*-Boc-allylglycine (**24**),[22] was treated with dimethyl diazomethylphosphonate[23] and rhodium acetate dimer to give an inseparable mixture of all four possible diastereomers of cyclopropane **25**. Hydrolysis using refluxing 6 N HCl and purification via ion-exchange chromatography provided amino acids **26**.

The preparations of the AP6 and AP7 analogues are shown in Scheme V. 4-Bromo-1-butene (**27a**) or 5-bromo-1-pentene (**27c**) was converted to cyclopropanes with ethyl diazoacetate to give esters **28a-d**. The (*Z*)- and (*E*)-cyclopropyl esters were cleanly separated at this stage by silica gel chromatography; however, the *E* isomers were mixed with side products diethyl fumarate and maleate (carbene dimerization). This posed no problem in that these side products were removed during workup of the subsequent ester reduction. Esters **28a–d** were treated separately with lithium aluminum hydride to provide alcohols **29a–d**. Again, (*Z*)- and (*E*)-cyclopropane geometry was assigned with ^{13}C NMR. Oxidation using PCC afforded bromo aldehydes **30a–d** (yields 88–95%). The Arbuzov reaction provided the alkyl phosphonates **31a–d**; however the yields were much lower for the (*Z*)-cyclopropyl aldehydes compared to those of the *E* isomers (12–27% versus 58–62%). Presumably, this can be attributed to unwanted condensations with the **cis**-disposed aldehyde functionality. Phosphono aldehydes **31a–d** were converted into amino nitriles **32a–d** via the alumina/ultrasound Strecker reaction (yields 41–47%). Hydrolysis provided the amino acids **33a–d**, again as a mixture of four stereoisomers.

Results and Discussion

The results of the biological evaluation are presented in Table I. It is clear from this table that none of the compounds met the goal of a potent NMDA antagonist.

(22) Hanessian, S.; Sahoo, S. P. *Tetrahedron Lett.* **1984**, *25*, 1425.
(23) Seyferth, D.; Marmor, R. S.; Hilbert, P. *J. Org. Chem.* **1971**, *36*, 1379–1386.

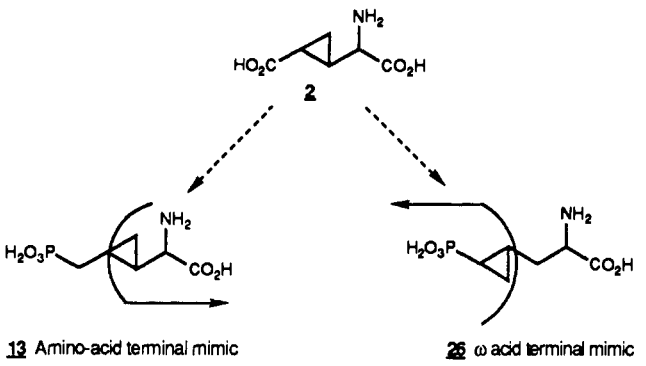


Figure 4. Design of two AP5 analogues from 2.

However, these results deserve some comment.

First, by introducing a cyclopropyl ring in the AP5 skeleton, we created some ambiguity about the connectivity linking the amino acid residue to the phosphonic acid moiety. Indeed, the molecules **23**, **19**, **13a,b**, and **26** could be considered as AP5 analogues as well as AP6 analogues, depending on how the cyclopropyl ring was considered (Figure 3). The results of Table I show clearly that what we considered as AP5 analogues (**23**, **19**, **13a,b**, and **26**) have higher affinity for the NMDA receptor than the so called AP7 analogues (**33c** and **33d**) and that the AP6 analogues (**33a** and **33b**) are totally inactive. This structure–activity relationship (SAR) parallels completely that reported for AP5, AP6, and AP7,[24] indicating that among the cyclopropyl derivatives, the shorter connectivity must be considered.

Further support for this idea is brought by the analysis of the efficacy of **2** at the level of the NMDA receptor.[16] Indeed, **2** could be considered as having either a glutamic acid skeleton or a 2-aminoadipic acid frame. Agonist or antagonist efficacy would have been anticipated,[25] respectively. The agonist efficacy of **2** reinforces the perception that the cyclopropyl must be considered as a double-bond equivalent rather than a one-carbon homologue.

Second, among the AP5 analogues, the position of the methano bridge appears to become less and less favorable as it moves from the 4,5-position to the 2,3-position. In the parallel glutamic acid series, the cyclopropyl in the 2,3-position is deleterious for the affinity[26] and therefore the inactivity of the 2,3-methano-AP5 was not completely unexpected. On the other hand, as we tried to extrapolate from the affinity of 3,4-methanoglutamic acid for developing potent AP5 derivatives, we discovered that the 3,4-methano- as well as 4,5-methano-AP5 could be designed, depending on how the 3,4-methanoglutamic acid molecule is viewed. In one case the amino acid terminal present in **2** remains (**13**) while in the other case the ω-acidic terminal of **2** is conserved (**26**, Figure 4). Our results show that the 4,5-methano-AP5 compound has a higher affinity for the receptor than its 3,4-isomer. In addition, the most favored isomer for the 3,4-methano-AP5 molecule is trans (*E*) while among the isomers of **2** cis (*Z*) is preferred.

CGP 37849 (**6**) also possesses an *E* configuration around its double bond, indicating that even though the structure–activity relationships generated from the agonists are not always helpful for the design of antagonist molecules, SAR among the antagonists appear consistent. The analogy between CGP 37849 and **26** is not perfect because even though the two molecules are isomers, the position of the cyclopropyl ring cannot be correlated to the position of the double bond. The closest analogue to CGP 37849, **13b**, displays even poorer affinity for the receptor.

A more surprising result is the agonist-like efficacy manifested by **33c**, an AP7 analogue. To our knowledge, it is the only reported example of a ω-phosphono amino acid with an inverted efficacy at the level of the NMDA receptor. This effect could be related to the significant affinity of **33c** for the glycine B receptor (88 ± 10% inhibition of [^{3}H]glycine binding at 100 μM concentration) where it can act as an agonist.

Finally, the compound which manifested the highest affinity for the NMDA receptor (**26**) is still a mixture of four isomers (in approximately equal amounts). The limit of a potential K_i for the best of the isomers can be calculated as 0.40 μM if we make the assumption that all the activity resides in the least abundant isomer. As can be seen, we are still far away from the high affinities displayed by CGS 19755 and CGP 37849.

Experimental Section

^{1}H and ^{13}C NMR spectra were recorded on a Varian XL-300 spectrometer at 300 and 75 MHz, respectively. Deuteriochloroform with tetramethylsilane internal standard was used as the solvent for NMR spectra, except for amino acids, where D_2O was used without internal standard. Reported coupling constants (*J*) are in hertz; splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; p, pentuplet; h, hextuplet; m, multiplet, comp m, complex multiplet; br, broad. Low-resolution FAB mass spectra were recorded on a VG4250 triple-quadrapole mass spectrometer. All solvents and reagents were used as obtained. All experiments were run under a positive pressure of nitrogen. Silica gel chromatography of amounts less than 1 g was carried out with Kieselgel 60 (40–63 mM, EM Science). Chromatography of amounts greater than 1 g was carried out with a Waters Prep 500A LC instrument. GC analyses were done on a Varian 3500 capillary gas chromatograph fitted with a J and W DB5 megabore column (15 M × 0.534 mm (i.d.)). Retention times (t_R) are reported in min (temperature program: initial temperature 50 °C, hold time 2 min, temperature rate 20 °C/min, final temperature 250 °C).

Diethyl Allylphosphonate (7). A mixture of allyl bromide (18.0 g, 149 mmol) and triethyl phosphite (20.6 g, 124 mmol) was heated at reflux for 21 h. The residue was distilled (bp 58.5–60 at 0.65 Torr; GC t_R = 4.88 min) to give phosphonate **7** (19.8 g, 90%) which GC analysis indicated contained less than 5% diethyl ethylphosphonate (t_R = 4.20 min): ^{1}H NMR ($CDCl_3$) δ 5.89–5.73 (m, 1 H), 5.27–5.16 (m, 2 H), 4.19–4.02 (m, 4 H), 2.61 (dd, *J* = 24, 7.5 Hz, 2 H), 1.32 (t, *J* = 7 Hz, 6 H).

(*Z*)- and (*E*)-Ethyl 2,3-Methano-4-(diethylphosphono)-butanoate (8). $CuSO_4$ (0.54 g, 3.4 mmol) was added to a solution of **7** (6.01 g, 33.7 mmol) in cyclohexane (10 mL) and the mixture was heated to reflux. A mixture of ethyl diazoacetate (19.2 g, 169 mmol) and cyclohexane (7 mL) was added via syringe drive over 18 h. After an additional 1 h at reflux, the mixture was cooled and passed through a plug of SiO_2 (50 mm × 10 cm), eluting with hexane/EtOAc (1:1) to remove insoluble inorganic material. After rotary evaporation of the solvent, the resulting residue was purified by preparative SiO_2 chromatography (eluted 1:1 hexane/EtOAc) to give a mixture of cyclopropanes **8** (2.67 g, 30%): ^{1}H NMR ($CDCl_3$) δ 4.20–4.06 (comp m, 6 H), 2.09 (dd, *J* = 18, 7 Hz, 2 H, *E* diastereomer), 1.93–1.49 comp m), 1.32 (t, *J* = 7 Hz, 6 H), 1.31 (t, *J* = 7 Hz, 3 H, *Z* diastereomer), 1.26 (t, *J* = 7 Hz, 3 H, *E* diastereomer), 1.2–1.10 (m, 0.5 H), 1.03–0.97 (m, 0.5 H), 0.88–0.81 (m, 1 H).

(*Z*)- and (*E*)-Ethyl 2,3-Methano-5-bromopentanoate (28a,b). $CuSO_4$ (0.44 g, 2.7 mmol) was added to a solution of 4-bromo-1-butene (10.0 g, 67.1 mmol) in cyclohexane (10 mL) and the mixture was heated to reflux. A mixture of ethyl diazoacetate (31.1 g, 273 mmol) and cyclohexane (21 mL) was added via syringe drive over 20 h. After an additional 17 h at reflux, the mixture

(24) Evans, R. H.; Francis, A. A.; Jones, A. W.; Smith, D. A. S.; Watkins, J. C. *Br. J. Pharmacol.* **1982**, *75*, 65.
(25) Olverman, H. J.; Jones, A. W.; Mewett, K. N.; Watkins, J. C. *Neuroscience* **1988**, *26*, 17.
(26) Monahan, J. B., unpublished observation.

was cooled and passed through a plug of SiO_2 (50 mm × 10 cm), eluting with hexane/EtOAc (20:1) to remove insoluble inorganic material. After rotary evaporation of the solvent, the resulting residue was purified by preparative SiO_2 chromatography (eluted with 40:1 hexane/EtOAc, one recycle) to give two fractions: A contains **(*Z*)-ethyl 2,3-methano-5-bromopentanoate (28a**; 3.98 g, 33%); B contains **(*E*)-ethyl 2,3-methano-5-bromopentanoate (28b)** and diethyl fumarate (11.89 g total in an approximately 1:2 ratio was judged by ^1H NMR analysis. For A: ^1H NMR ($CDCl_3$) δ 4.13 (q, J = 7 Hz, 2 H), 3.40 (comp dt, J = 6.8, 1.5 Hz, 2 H), 2.12 (nontet, J = 7 Hz, 2 H), 1.74 (dt, J = 6, 8.5 Hz, 1 H), 1.44 (dh, J = 6.5, 1 Hz, 1 H), 1.27 (t, J = 7 Hz, 3 H), 1.09 (dt, J = 8, 4 Hz, 1 H), 0.98 (m, 1 H). For **B**: ^1H NMR ($CDCl_3$) δ 6.83 (s, fumarate 2 H), 4.26 (q, fumarate 4 H), 4.12 (q, J = 7 Hz, 2 H), 3.43 (t, J = 7 Hz, 2 H), 1.87 (structured m, 2 H), 1.57–1.42 (m, 2 H), 1.31 t, J = 7 Hz, fumarate), 1.27 (t, J = 7 Hz, 3 H), 1.25–1.18 (m, 1 H), 0.80–0.73 (m, 1 H).

(*Z*)- and (*E*)-Ethyl 2,3-Methano-6-bromohexanoate (28c,d). $CuSO_4$ (1.07 g, 6.70 mmol) was added to a solution of 5-bromo-1-pentene (10.0 g, 67.1 mmol) in cyclohexane (30 mL) and the mixture was heated to reflux. A mixture of ethyl diazoacetate (38.3 g, 335 mmol) and cyclohexane (15 mL) was added via syringe drive over 18 h. After an additional 1 h at reflux, the mixture was cooled and passed through a plug of SiO_2 (50 mm × 10 cm), eluting with hexane/EtOAc (20:1) to remove insoluble inorganic material. After rotary evaporation of the solvent, the resulting residue was purified by preparative SiO_2 chromatography (eluted 40:1 hexane/EtOAC, one recycle) to give two fractions: A contains **(*Z*)-ethyl 2,3-methano-6-bromohexanoate (28c**; 5.62 g, 35.6%); B contains **(*E*)-ethyl 2,3-methano-6-bromohexanoate (28d)** and diethyl fumarate (11.34 g total) in an approximately 2:1 ratio as judged by ^1H NMR analysis. For A: ^1H NMR ($CDCl_3$) δ 4.14 (q, J = 7 Hz, 2 H), 3.42 (t, J = 7 Hz, 2 H), 2.02–1.58 (m, 5 H), 1.27 (t, J = 7 Hz, 3 H), 1.32–1.22 (m, 1 H), 1.07–0.83 (m, 2 H). For B: ^1H NMR ($CDCl_3$) δ 6.84 (s, fumarate 2 H), 4.26 (q, fumarate 4 H), 4.12 (q, J = 7 Hz, 2 H), 3.44 (t, J = 7 Hz, 2 H), 1.97 (m, 2 H), 1.54–1.14 (m, 5 H), 1.32 (t, fumarate), 1.27 (t, J = Hz, 3 H), 0.77–0.70 (m, 1 H).

(*Z*)- and (*E*)-2,3-Methano-4-(diethylphosphono)butanoic Acid (9). To a solution of ester **8** (880 mg, 3.33 mmol) in 2:1 THF/H_2O (9 mL) was added potassium hydroxide (192 mg, 3.4 mmol) at room temperature and the resulting red mixture was stirred for 20 h. The mixture was poured into H_2O (25 mL) and extracted with ether (25 mL). The aqueous layer was acidified to pH <1 with 6 N HCl and then continuously extracted with ether for 20 h. The ether layer was dried ($MgSO_4$) and evaporated in vacuo to give acid **9** (569 mg, 72%): ^1H NMR ($CDCl_3$) δ 9.35 (br s, 1 H), 4.20–4.08 (m, 4 H), 2.14 (dd, J = 18, 7.5 Hz, 0.5 H), 1.90–1.75 (m, 1 H), 1.81 (dd, J = 18, 3 Hz, 0.5 H), 1.79 (dd, J = 18, 2 Hz, 0.5 H), 1.69–2.58 (m, 1 H), 1.53 (p, J = 4 Hz, 1 H), 1.36–1.28 (comp m, 6 H), 1.25–1.00 (m, 1 H), 0.91–0.84 (m, 1 H).

(*Z*)- and (*E*)-2,3-Methano-4-(diethylphosphono)butan-1-ol (10a,b). Borane–THF complex (1 M in THF, 2.9 mmol) was added dropwise to a solution of acid **9** (652 mg, 2.76 mmol) in THF (9 mL) at 0 °C. The mixture was warmed to room temperature and stirred for 2.5 h. The mixture was cooled to 0 °C and methanol (1.5 mL) was added dropwise. The mixture was allowed to warm to room temperature before the solvent was removed by rotary evaporation. The residue was purified by chromatography on SiO_2 (eluted with CH_2Cl_2/MeOH 96:4) to separate the two diastereomeric alcohols. Fraction A contains **(*Z*)-2,3-methano-4-(diethylphosphono)butan-1-ol (10a**; 107 mg, 17%). Fraction B contains **(*E*)-2,3-methano-4-(diethylphosphono)butan-1-ol (10b**; 367 mg, 60%). For A: ^1H NMR ($CDCl_3$) δ 4.83 (br s, 1 H), 4.21–2.04 (m, 4 H), 3.96 (dd, J = 12, 4 Hz, 1 H), 3.20 (dd, J = 12, 9 Hz, 1 H), 2.24 (ddd, J = 18, 15, 4 Hz, 1 H), 1.50 (ddd, J = 18, 15, 12 Hz, 1 H), 1.36 (t, J = 7 Hz, 3 H), 1.32 (t, J = 7 Hz, 3 H), 1.36–1.26 (m, 1 H), 1.16–1.02 (m, 1 H), 0.84–0.75 (m, 1 H), –0.03 (br q, J = 6 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 65.8, 62.4, 61.9, 25.9, 24.0, 18.8, 16.5, 9.4, 8.6. For B: ^1H NMR ($CDCl_3$) δ 4.20–2.05 (m, 4 H), 3.73 (dd, J = 11, 6 Hz, 1 H), 3.18 (dd, J = 11, 8.5 Hz, 1 H), 2.89 (br s, 1 H), 21.98 (dt, J = 16, 5.5 Hz, 1 H), 1.48 (ddd, J = 17, 16, 9 Hz, 1 H), 1.33 (dt, J = 7, 1.5 Hz, 3 H), 1.07–0.96 (m, 1 H), 0.92–0.78 (m, 1 H) 0.60–0.54 (m, 1 H), 0.49 (br dt, J = 8, 6 Hz, 1 H); ^{13}C NMR ($CDCl_3$ δ 66.4, 61.8, 61.7, 30.7, 28.4, 21.7, 16.4, 11.0, 10.8.

General Procedure for Reduction of Bromo Esters to Alcohols. The ester (1.0 equiv) as a 0.5 M solution in ether was added dropwise to a slurry of $LiAlH_4$ (1.0 equiv) in ether at 0–5 °C. The mixture was stirred for 40 min before dropwise addition of EtOAc (3 equiv.). Water (1 mL/g of $LiAlH_4$), 15% aqueous NaOH (1 mL/g of $LiAlH_4$), and water (3 mL/g of $LiAlH_4$) were added in succession, and the resulting white solid was removed by filtration and washed with ether. The combined ether phases were dried ($MgSO_4$) and evaporated in vacuo to yield a residue which was passed through a plug of SiO_2 and eluted with hexane/EtOAc. Evaporation of the solvent in vacuo provided the alcohol.

(*Z*)-2,3-Methano-5-bromopentan-1-ol (29a): yield 75%; ^1H NMR ($CDCl_3$) δ 3.75 (dd, J = 11, 6 Hz, 1 H), 3.51 (t, J = 7 Hz, 2 H), 3.48 (dd, J = 11, 8 Hz, 1 H), 2.51 (br s, 1 H), 2.02 (h, J = 7 Hz, 1 H), 1.88 (h, J = 7 Hz, 1 H), 1.18 (m, 1 H), 1.03 (br h, 1 H), 0.77 (dt, J = 8, 5 Hz, 1 H), 0.06 (q, J = 6 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 62.6, 34.5, 32.6, 17.8, 15.0, 8.9.

(*E*)-2,3-Methano-5-bromopentan-1-ol (29b): yield 47% (This yield is reduced due to the presence of ca. 50% diethyl fumarate in ester **28b**. The diethyl fumarate does not interfere with the reduction): ^1H NMR ($CDCl_3$) δ 3.50 (dd, J = 12, 6.5 Hz, 1 H), 3.47 (t, J = 7 Hz, 2 H), 3.42 (dd, J = 12, 7 Hz, 1 H), 2.34 (br s, 1 H), 1.82 (dectet, J = 7 Hz, 2 H), 1.00–0.91 (m, 1 H), 0.83–0.73 (m, 1 H), 0.51–0.37 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 66.5, 36.6, 33.4, 20.8, 16.0, 9.5.

(*Z*)-2,3-Methano-6-bromohexan-1-ol (29c): yield 95%; ^1H NMR ($CDCl_3$) δ 3.69 (dd, J = 6.3, 11.5 Hz, 1 H), 3.55 (dd, J = 8.4, 11.5 Hz, 1 H), 3.47 (t, J = 7 Hz, 2 H), 2.13 (br s, 1 H), 2.06–1.95 (m, 2 H), 1.68–1.57 (m, 1 H), 1.47–1.35 (m, 1 H), 1.19–1.06 (m, 1 H), 0.93–0.81 (m, 1 H), 0.78–0.70 (m, 1 H), 0.08–0.00 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 62.7, 33.8, 33.1, 26.9, 17.9, 15.1, 9.3.

(*E*)-2,3-Methano-6-bromohexan-1-ol (29d): yield 69% (This yield is reduced due to the presence of ca. 30% diethyl fumarate in ester **28d**. The diethyl fumarate does not interfere with the reduction); ^1H NMR ($CDCl_3$) δ 3.41 (t, J = 7 Hz, 2 H), 3.35 (br d, J = 6.9 Hz, 2 H), 2.71 (br s, 1 H), 1.92–1.83 (m, 2 H), 1.52–1.24 (m, 2 H), 0.83–0.72 (m, 1 H), 0.57–0.47 (m, 1 H), 0.35–0.20 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 66.4, 33.7, 32.7, 31.8, 20.9, 16.1, 9.8.

General Procedure for Oxidation of Cyclopropyl Alcohols. A solution of the alcohol (1 equiv) in CH_2Cl_2 (5 mL/equiv) was rapidly added to a slurry of pyridinium chlorochromate (1.5 equiv) in CH_2CL_2 (5 mL/equiv) and this mixture was stirred for 1.5 h. Ether (30 mL/equiv) was added and the supernate was decanted from the resulting black residue, which was washed with ether (2×). The combined ether phases were passed through a plug of florisil (30 mm × 15 cm), eluting with ether (300 mL). The combined solutions were carefully evaporated in vacuo to yield the volatile aldehydes.

(*E*)-2,3-Methano-4-(diethylphosphono)butanal (11b): yield 83%; ^1H NMR ($CDCl_3$) δ 9.13 (d, J = 4 Hz, 1 H), 4.18–4.06 (m, 4 H), 1.95–1.65 (comp m, 4 H), 1.43–1.35 (m, 1 H), 1.33 (t, J = 7 Hz, 6 H), 1.10–1.03 (m, 1 H).

(*Z*)-2,3-Methano-4-(diethylphosphono)butanal (11a): yield 86%; ^1H NMR ($CDCl_3$) δ 9.69 (d, J = 1.5 Hz, 1 H), 4.16–4.04 (m, 4 H), 2.20–1.70 (comp m, 4 H), 1.33 (t, J = 7 Hz, 6 H), 1.30–1.20 (m, 2 H).

(*Z*)-2,3-Methano-5-bromopentanal (30a): yield 92%; GC t_R = 4.95 min; ^1H NMR ($CDCl_3$) δ 9.60 (dd, J = 4, 0.2 Hz, 1 H), 3.47–3.35 (m, 2 H), 2.21–2.00 (comp m, 3 H), 1.69 (h, J = 7 Hz, 1 H), 1.31–1.20 (comp m, 2 H); ^{13}C NMR ($CDCl_3$) δ 200.9, 32.9, 30.4, 26.8, 23.7, 14.3.

(*E*)-2,3-Methano-5-bromopentanal (30b): yield 88%; GC t_R = 5.02 min; ^1H NMR ($CDCl_3$) δ 9.08 (d, J = 5 Hz, 1 H), 3.46 (t, J = 7 Hz, 2 H), 1.93 (q, J = 7 Hz, 2 H), 1.78–1.70 (m, 1 H), 1.69–1.60 (m, 1 H), 1.40–1.33 (m, 1 H), 1.04–0.97 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 200.2, 35.6, 31.8, 29.6, 21.1, 14.2.

(*Z*)-2,3-Methano-6-bromohexanal (30c): yield 95%; GC t_R = 5.77 min; ^1H NMR ($CDCl_3$) δ 9.45 (d, J = 5 Hz, 1 H), 3.42 (t, J = 7 Hz, 2 H), 2.0–1.18 (m, 8 H).

(*E*)-2,3-Methano-6-bromohexanal (30d): yield 89%; GC t_R = 6.57 min; ^1H NMR ($CDCl_3$) δ 9.08 (d, J = 5 Hz, 1 H), 3.46 (t, J = 7 Hz, 2 H), 2.04–1.95 (m, 2 H), 1.73–1.66 (m, 1 H), 1.58–1.42 (m, 3 H), 1.36–1.30 (m, 1 H), 1.02–0.95 (m, 1 H).

General Procedure for Formation of Diethyl Phosphonates from Alkyl Bromides (Arbuzov Reaction). A mixture

of the alkyl bromide (1 equiv) and triethyl phosphite (1.2 equiv) was heated to 100–110 °C under an N_2 stream until the alkyl bromide was consumed (generally 24–48 h), as judged by GC analysis. To some reactions was added an additional 0.25–0.50 equiv of triethyl phosphite if GC analysis indicated unconsumed alkyl bromide after 24 h. Upon consumption of the alkyl bromide, the reaction mixture was cooled to room temperature and the alkyl phosphonate was purified by chromatography on SiO2 (CH_2Cl_2/MeOH 19:1).

(*Z*)-2,3-Methano-5-(diethylphosphono)pentanal (31a): yield 27%; GC t_R = 8.20 min; ^{1}H NMR ($CDCl_3$) δ 9.49 (d, *J* = 5 Hz, 1 H), 4.15–4.04 (m, 4 H), 2.01–1.56 (m, 7 H), 1.33 (t, *H* = 7 Hz, 6 H), 1.28–1.20 (m, 1 H).

(*E*)-2,3-Methano-5-(diethylphosphono)pentanal (31b): yield 62%; GC t_R = 8.33 min: ^{1}H NMR ($CDCl_3$) δ 9.08 (d, *J* = 5 Hz, 1 H), 4.16–4.03 (m, 4 H), 1.75–1.53 (comp m, 7 H), 1.32 (t, *J* = 7 Hz, 6 H), 0.97 (dt, *J* = 8, 5 Hz, 1 H).

(*Z*)-2,3-Methano-6-(diethylphosphono)hexanal (31c): yield 12%; GC t_R = 8.73 min; ^{1}H NMR ($CDCl_3$) δ 9.60 (d, *J* = 5 Hz, 1 H), 4.14–4.03 (m, 4 H), 1.95–1.58 (m, 8 H), 1.32 (t, *J* = 7 Hz, 6 H), 1.28–1.19 (m, 1 H), 0.97–0.88 (m, 1 H).

(*E*)-2,3-Methano-6-(diethylphosphono)hexanal (31d): yield 58%; GC t_R = 9.08 min; ^{1}H NMR ($CDCl_3$) δ 9.06 (d, *J* = 5.7 Hz, 1 H), 4.16–4.04 (m, 4 H), 1.81–1.62 (m, 6 H), 1.49–1.43 (m, 2 H), 1.33 (t, *J* = 7 Hz, 6 H), 0.98–0.91 (m, 1 H).

General Procedure for Amino Nitrile Formation (Al_2O_3-Catalyzed Ultrasound Strecker Reaction). A mixture of KCN (2 equiv), NH_4Cl (2.25 equiv), and Al_2O_3 (1.5 mg/mg of aldehyde) in CH_3CN (1 mL/mmol of aldehyde) was sonicated for 10 min before addition of the aldehyde (1 equiv in CH_3CN (2 mL/mmol of aldehyde). The mixture was sonicated an additional 16–24 h, during which time the reaction temperature rose to ca. 50–55 °C. The mixture was then filtered to remove solid materials, which were further washed with CH_3CN. The combined CH_3CN fractions were combined and evaporated in vacuo and then taken up in ether. The ether layer was extracted with 1 N HCl (2×) which was separated and made basic with 2.5 N NaOH (pH 11). The resulting aqueous layer was extracted with ether (2×), which was dried (Na_2SO_4), and evaporated in vacuo to give a crude residue, which was purified by chromatography on SiO_2 (generally CH_2Cl_2/MeOH/NH_4OH 90:10:1) to give the amino nitrile.

(*Z*)-2-Amino-3,4-methano-5-(diethylphosphono)pentanenitrile (12a): yield 46% (75% based on consumed aldehyde); ^{1}H NMR ($CDCl_3$) δ 4.21–4.07 (m, 4 H), 3.67 (d, *J* = 7 Hz, 0.33 H, one diastereomer), 3.38 (d, *J* = 9 Hz, 0.67 H, one diastereomer), 2.34–2.25 (comp m, 2.5 H), 2.00–1.67 (comp m, 2.5 H), 1.38–1.25 (m, 7 H), 1.08–0.98 (m, 1 H), 0.49 (q, *J* = 6 Hz, 0.33 H), 0.33 (q, *J* = 6 Hz, 0.67 H).

(*E*)-2-Amino-3,4-methano-5-(diethylphosphono)pentanenitrile (12b): yield 19% (45% based on consumed aldehyde): ^{1}H NMR ($CDCl_3$) δ 4.21–4.05 (m, 4 H), 3.81 (d, *J* = 5 Hz, 0.5 H, one diastereomer), 3.48 (d, *J* = 7 Hz, 0.5 H, one diastereomer), 2.00–1.84 (comp m, 3 H), 1.61 (ddd, *J* = 18, 15, 7.5 Hz, 1 H), 1.38–1.30 (m, 6 H), 1.20–1.04 (comp m, 2 H), 0.85–0.77 (m, 1 H), 0.67–0.58 (m, 1 H).

(*Z*)-2-Amino-3,4-methano-6-(diethylphosphono)hexanenitrile (32a): yield 47%; ^{1}H NMR ($CDCl_3$) δ 4.17–4.07 (m, 4 H), 3.33 (d, *J* = 9.5 Hz, 0.4 H, one diastereomer), 3.28 (d, *J* = 9.5 Hz, 0.6 H, one diastereomer), 2.11–1.50 (comp m, 6 H), 1.32 (t, *J* = 7 Hz, 6 H), 1.30–1.20 (m, 1 H), 1.15–1.05 (m, 1 H), 0.97–0.89 (m, 1 H), 0.27–0.18 (h, *J* = 6 Hz, 1 H).

(*E*)-2-Amino-3,4-methano-6-(diethylphosphono)hexanenitrile (32b): yield 44%; ^{1}H NMR ($CDCl_3$) δ 4.16–4.02 (m, 4 H), 3.74 (d, *J* = 6 Hz, 0.4 H, one diastereomer), 3.51 (d, *J* = 6.5 Hz, 0.6 H, one diastereomer), 2.52 (br s, 2 H), 1.94–1.62 (comp m, 3 H), 1.55–1.38 (m, 1 H), 1.33 (t, *J* = 7 Hz, 6 H), 1.11–0.96 (m, 2 H), 0.77–0.68 (m, 1 H), 0.57–0.50 (m, 1 H).

(*E*)-2-Amino-3,4-methano-7-(diethylphosphono)heptanenitrile (32d): yield 41%; ^{1}H NMR ($CDCl_3$) δ 4.17–4.03 (m, 4 H), 3.56–3.53 (m, 2 × 1 H, two diastereomers), 1.82–1.64 (m, 6 H), 1.42–1.35 (m, 2 H), 1.33 (t, *J* = 7 Hz, 6 H), 1.00–0.92 (m, 1 H), 0.90–0.81 (m, 1 H), 0.70–0.61 (m, 1 H), 0.53–0.46 (m, 1 H).

General Procedure for Hydrolysis of Amino Nitriles/Phosphonates to Triacids. Amino nitrile/diethyl phosphonate 12 or 32 (1 equiv) was dissolved in 6 N HCl (10 equiv) and heated to reflux for 3 days. At this time TLC analysis (reverse phase, H_2O/*i*-PrOH/HOAc/TFA 70:20:10:5) indicated a single ninhydrin-active product (R_f = 0.95). The mixture was cooled and the solvent was evaporated in vacuo to give a residue of the amino acid HCl salt. This material was purified by ion-exchange chromatography using strongly acidic resin (Dowex 1 8×100), eluting with 1 *N* pyridine in H_2O. Evaporation of the solvent, followed by lyophilization from H_2O, yielded the pure amino acids 13 or 33.

(*Z*)-2-Amino-3,4-methano-5-phosphonopentanoic acid (13a): yield 61%; ^{1}H NMR (D_2O) δ 3.46 (d, *J* = 10 Hz, 0.33 H, one diastereomer), 3.33 (d, *J* = 10 Hz, 0.67 H, one diastereomer), 2.11–1.98 (m, 1 H), 1.54–1.10 (comp m, 3 H), 1.00–0.93 (m, 1 H), 0.42 (q, *J* = 6 Hz, 0.67 H), 0.32 (q, *J* = 6 Hz, 0.33 H); MS (FAB$^+$) *m/z* 210 (M + H, 100), 175, 164, 153, 149. Anal. ($C_6H_{12}NO_5P$ + 0.1C_5H_5N) C, H, N.

(*E*)-2-Amino-3,4-methano-5-phosphonopentanoic acid (13b): yield 98%; ^{1}H NMR (D_2O) δ 3.28 (d, *J* = 9 Hz, 0.5 H, one diastereomer), 3.13 (d, *J* = 10 Hz, 0.5 H, one diastereomer), 1.72–1.38 (comp m, 2.5 H), 1.20–0.80 (comp m, 2.5 H), 0.70–0.55 (m, 1 H); MS (FAB$^+$) *m/z* 210 (M + H, 100), 175, 164, 153. Anal. ($C_6H_{12}NO_5P$ + 0.1C_5H_5N) C, H, N.

(*Z*)-2-Amino-3,4-methano-6-phosphonohexanoic acid (33a): yield 95%; ^{1}H NMR (D_2O) δ 3.44 (d, *J* = 9 Hz, 0.4 H, one diastereomer), 3.32 (d, *J* = 10.5 Hz, 0.6 H, one diastereomer), 1.88–1.62 (comp m, 3 H), 1.35–1.10 (comp m, 3 H), 0.90–0.80 (m, 1 H), 0.35 (q, *J* = 6 Hz, 0.6 H), 0.18 (q, *J* = 6 Hz, 0.4 H); MS (FAB$^+$) *m/z* 224 (M + H, 100), 208, 197, 178, 153. Anal. ($C_7H_{14}NO_5P$ + 0.1C_5H_5N) C, H, N.

(*E*)-2-Amino-3,4-methano-6-phosphonohexanoic acid (33b): yield 86%; ^{1}H NMR (D_2O) δ 3.36 (d, *J* = 9 Hz, 0.45 H, one diastereomer), 3.26 (d, *J* = 9 Hz, 0.55 H, one diastereomer), 1.83–1.65 (comp m, 4 H), 1.50–1.30 (m, 1 H), 1.24–1.17 (m, 0.5 H), 1.10–0.95 (m, 1 H), 0.95–0.85 (m, 0.5 H), 0.77–0.63 (m, 1 H); MS (FAB$^+$) *m/z* 224 (M + H, 100), 197, 178, 153. Anal. ($C_7H_{14}NO_5P$ + 0.1C_5H_5N) C, H, N.

(*Z*)-2-Amino-3,4-methano-7-phosphonoheptanoic acid (33c): yield 17%; ^{1}H NMR (D_2O) δ 3.56 (d, *J* = 10 Hz, 0.4 H), one diastereomer), 3.47 d, *J* = 9 Hz, 0.6 H, one diastereomer), 2.1–0.9 (comp m, 9 H), 0.5 (m, 0.5 H), 0.3 (m, 0.5 H); MS (FAB$^+$) *m/z* 238 (M + H, 100), 221, 217, 215, 210, 207.

(*E*)-2-Amino-3,4-methano-7-phosphonoheptanoic acid (33d): yield 91%; ^{1}H NMR (D_2O) δ 3.48 (d, *J* = 9 Hz, 0.4 H, one diastereomer), 3.41 (d, *J* = 10 Hz, 0.6 H, one diastereomer), 1.9–0.6 (comp m, 10 H); MS (FAB$^+$) *m/z* 238 (M + H, 100), 192; MS (FAB$^-$) *m/z* 236 (M - H, 100), 218, 192, 175. Anal. ($C_8H_{16}NO_5P$) C, H, N.

4-(Diethylphosphono)-1-butene (14). A mixture of 4-bromo-1-butene (47.4 g, 351 mmol) and triethyl phosphite (117 g, 703 mmol) was heated at reflux for 55 h under an N_2 stream. The residue was distilled (bp 58.5–60 at 0.65 Torr) to give phosphonate 14 (21.5 g, 32%; GC t_R = 5.78 min) which GC analysis indicated contained less than 5% diethyl ethylphosphonate (GC t_R = 4.72 min): ^{1}H NMR ($CDCl_3$) δ 5.92–5.78 (m, 1 H), 5.10–4.97 (m, 2 H), 4.20–4.05 (m, 4 H), 2.41–2.29 (m, 2 H), 1.89–1.77 (m, 2 H), 1.32 (t, *J* = 7 Hz, 6 H).

Dimethyl 2-[2-(Diethylphosphono)ethyl]cyclopropane-1,1-dicarboxylate (15). Cupric acetylacetonate (0.50 g, 1.9 mmol) was added to a solution of 14 (21.32 g, 111 mmol) in cyclohexane (25 mL) and the mixture was heated to reflux. A mixture of dimethyl diazomalonate (35.1 g, 222 mmol) and cyclohexane (15 mL) was added via syringe drive over 25 h. After an additional 5 h at reflux, the mixture was cooled and passed through a plug of SiO_2 (50 mm × 10 cm), eluting with CH_2Cl_2/MeOH (95:5) to remove insoluble inorganic material. After rotary evaporation of the solvent, the resulting residue (40 g) was purified by preparative SiO_2 chromatography (Waters Prep 500, 100% EtOAc) to give cyclopropane 15 (6.06 g, 17%): ^{1}H NMR ($CDCl_3$) δ 4.15–4.02 (m, 4 H), 3.77 (s, 3 H), 3.72 (s, 3 H), 1.96 (h, *J* = 8 Hz, 1 H), 1.92–1.80 (comp m, 2 H), 1.72–1.58 (m, 1 H), 1.47–1.38 (comp m, 2 H), 1.35–1.30 (m, 1 H), 1.32 (t, *J* = 7 Hz, 6 H).

(*Z*)-1-(Methoxycarbonyl)-2-[2-(diethylphosphono)ethyl]cyclopropanecarboxylic Acid (16). A solution of diester 15 (6.06 g, 18.8 mmol) in MeOH (40 mL) was treated at 0 °C with a solution of NaOH (752 mg, 18.8 mmol) in H_2O (4 mL). After stirring at 0 °C for 2 h, the mixture was warmed to room temperature and stirred for 48 h. The methanol was removed by

rotary evaporation and the residue was poured into H_2O (70 mL). The aqueous mixture was extracted with ether (2 × 50 mL), separated, and cooled in an ice bath before acidifying with 2 N HCl (11 mL). The resulting mixture was extracted with EtOAc (4 × 60 mL). The combined EtOAc extracts were dried (Na_2SO_4) and evaporated in vacuo to give monoacid **16** (4.85 g, 84%): ^{1}H NMR ($CDCl_3$) δ 4.16–4.03 (m, 4 H), 4.33 (s, 1 H), 2.17–1.73 (comp m, 6 H), 1.68 (dd, J = 8, 5 Hz, 1 H), 1.32 (t, J = 7 Hz, 6 H).

(*E*)-Methyl 1-[*N*-*tert*-(Butoxycarbonyl)amino]-2-[2-(diethylphosphono)ethyl]cyclopropanecarboxylate (18). A solution of monoacid **16** (1.52 g, 4.93 mmol) in THF (15 mL) was treated with potassium carbonate (1.36 g, 9.81 mmol) and *cis*-1,6-dicyclohexano-18-crown-6[27] (0.2 g) at 0 °C. A solution of ethyl chloroformate (589 mg, 5.42 mmol) in THF (3 mL) was added dropwise and the mixture was then stirred for 30 min at 0 °C and 90 min at room temperature. The solution was quickly filtered to remove solids (washed with 10 mL of THF). The solution was recooled to 0 °C whereupon a solution of sodium azide (353 mg, 5.42 mmol) in H_2O (5 mL) was rapidly added. After stirring for 30 min, the mixture was poured into H_2O (60 mL) and extracted with ether (3 × 60 mL). The recombined ether extracts were washed with brine (70 mL), dried ($MgSO_4$), and evaporated in vacuo to give the intermediate acyl azide **17** (1.59 g, 97%).

The acyl azide was taken up in *tert*-butyl alcohol (30 mL) and heated to reflux for 17 h. An initial gas evolution was noted. The mixture was cooled and the solvent was removed by rotary evaporation. The resulting residue was purified by chromatography on SiO_2 (eluted with CH_2Cl_2/MeOH 95:5) to give product **18** (911 mg, 49%) as a thick oil. ^{1}H NMR ($CDCl_3$) δ 4.16–4.02 (m, 4 H), 3.73 (s, 3 H), 2.02–1.37 (comp m, 7 H), 1.44 (s, 9 H), 1.32 (t, J = 7 Hz, 6 H).

(*E*)-1-Amino-2-(2-phosphonoethyl)cyclopropanecarboxylic Acid (19). The procedure was identical with that described above for hydrolysis of amino nitriles/diethyl phosphonates: yield 95% (NMR analysis indicated the presence of ca. 5% of the *Z* diastereomer **13**); ^{1}H NMR (D_2O) δ 1.88–1.45 (comp m, 5 H), 1.38 (d, J = 8.5 Hz, 2 H); MS (FAB$^+$) m/z 210 (M + H, 100), 181, 153. Anal. ($C_6H_{12}NO_5P$ + 0.05C_5H_5N) C, H, N.

(*Z*)-Methyl-[*N*-(*tert*-butoxycarbonyl)amino]-2-[2-(diethylphosphono)ethyl]cyclopropanecarboxylate (22). A solution of monoacid **16** (1.02 g, 3.32 mmol) in 1:1 MeOH/H_2O (10 mL) was treated with a 0.5 M aqueous solution of potassium carbonate (3.31 mL, 1.66 mmol) and the mixture was stirred until CO_2 evolution ceased. The solvent was removed by rotary evaporation and the intermediate potassium salt was lyophilized from H_2O (20 mL). The resulting solid was taken up in MeOH (3 mL), hydrazine hydrate (10 mL) was added, and the mixture was heated to reflux for 20 h. The mixture was cooled to room temperature, the solvents were removed by rotary evaporation, and the residue was lyophilized from H_2O (20 mL) to give an intermediate acyl hydrazide/potassium carboxylate, **20** (1.18 g, 102%).

A two-phase solution of the potassium salt in 2 H_2O/ether (30 mL) at 0 °C was treated with sodium nitrite (286 mg, 4.15 mmol), and then H_2SO_4 (1 M, 7.47 mL) was added dropwise over 10 min with rapid stirring. The mixture was stirred for an additional 60 min before separation of the ether and aqueous phases. The aqueous phase was extracted with EtOAc (3 × 20 mL), and the combined organic phases were dried (Na_2SO_4) and then evaporated in vacuo to give a crude residue (525 mg). The residue was taken up in ether (20 mL), cooled to 0 °C, and treated dropwise with a freshly prepared ethereal diazomethane solution until the yellow color persisted. Low-temperature rotary evaporation yielded an acyl azide/methyl ester, **21** (527 mg).

The acyl azide was taken up in *tert*-butyl alcohol (25 mL) and heated to reflux for 18 h. The mixture was cooled and the solvent was removed by rotary evaporation. The resulting residue was purified by chromatography on SiO_2 (eluted CH_2Cl_3/MeOH 95:5) to give product **22** (290 mg, 23%) as a thick oil. Proton NMR analysis indicated a mixture of products as judged by multiple methyl ester signals. The desired product predominated and the material was used for the hydrolysis step. ^{1}H NMR ($CDCl_3$) δ 4.16–4.05 (m, 4 H), 3.74 (s), 3.72 (s), 3.71 (s), 3.70 (s, 3 H), 3.98 (s), 1.98–1.65 (comp m, 6 H), 1.46 (s, 9 H), 1.44 (s), 1.33 (dt, J = 7, 3 Hz, 6 H), 0.93–0.90 (m).

(*Z*)-1-Amino-2-(2-phosphonoethyl)cyclopropanecarboxylic Acid (23). The procedure was identical with that described above for hydrolysis of amino nitriles/diethyl phosphonates: yield 69% (NMR analyis indicated the presence of ca. 10% of the *E* diastereomer **19**); ^{1}H NMR (D_2O) δ 1.78–1.48 (comp m, 5 H), 1.45 dd, J = 6, 9 Hz, 1 H), 0.95 (t, J = 6.5 Hz, 1 H). Anal. ($C_6H_{12}NO_5P$ + 0.1C_5H_5N) C, H, N.

(*Z*)- and (*E*)-Methyl 2-[*N*-[(Benyloxy)carbonyl]-amino]-4,5-methano-5-(dimethylphosphono)pentanoate (25). A solution of dimethyl (diazomethyl)phosphonate (0.068 g, 0.45 mmol) in anhydrous dichloromethane (11 mL) was added dropwise over 4 h to a solution of (*R*)-(*N*-benzyloxycarbonyl)allylglycine (**24**) in dichloromethane containing rhodium acetate dimer (0.050 g, 0.11 mmol) under an argon atmosphere. After evaporation of the solvent, the residue (0.170 g) was purified by SiO_2 chromatography (chloroform elution) to afford cyclopropane **25** (0.030 g, 34%) as an inseparable mixture of four diastereomers: ^{1}H NMR ($CDCl_3$) δ 7.26 (s, 5 H), 5.62 (m, 1 H), 5.06 (s, 2 H), 4.39 (m, 1 H), 3.69 (s, 9 H), 2.05 (m, 2 H), 2.20–0.30 (comp m, 4 H). Anal. ($C_{16}H_{24}NO_7P$) C, H, N.

(*Z*)- and (*E*)-2-Amino-4,5-methano-5-phosphonopentanoic Acid (26). A suspension of ester **25** (0.34 g, 0.88 mmol) in 6 N HCl (15 mL) was heated to reflux for 3 days. The mixture was cooled and the solvent was evaporated in vacuo to give a residue which was purified by ion-exchange chromatography (Dowex 1 8×100, eluted with 1 N acetic acid) to provide **26** (0.054 g, 30%) as an inseparable mixture of four diastereomers: ^{1}H NMR (D_2O) δ 3.95 (m, 1 H), 2.30–1.40 (comp m, 3 H), 1.25–0.40 (comp m, 3 H). Anal. ($C_6H_{12}NO_5P$) C, H, N.

NMDA and Kainate Binding Assay. Synaptic plasma membranes (SPM) were prepared from rat forebrain (30–45 day old, male, Sprague–Dawley) and stored as previously described.[28] In preparation for the binding studies, the SPM were thawed at room temperature, treated with 0.04% Triton X-100, and incubated for 30 min at 37 °C. The Triton-treated SPM were then washed four times in 50 mM Tris/acetate, pH 7.4 at 4 °C by centrifugation and resuspension. The SPM are finally resuspended with homogenization to a concentration of 2.5–3.5 mg/mL in 50 mM Tris/acetate buffer just prior to the initiation of the assay. The NMDA receptor-selective [^{3}H]-L-glutamate binding and [^{3}H]kainate binding assays are carried out in a similar manner as previously described.[1,8] The general method involved adding the radioligand [10 mM [^{3}H]-L-glutamate (54.7 Ci/mmol), 0.5 nM [^{3}H]kainate (60.0 Ci/mmol), both obtained from New England Nuclear] to the appropriate concentration of the test compound and initiating the assay via addition of ice-cold SPM (final concentration of 0.25–0.35 mg/mL). All additions were made in 50 mM Tris/acetate, pH 7.4. Both assays were allowed to proceed to equilibrium (10 min, 4 °C and 60 min, 4 °C for the glutamate and kainate assays, respectively). Incubations were terminated and bound ligand separated from free using either centrifugation ([^{3}H]kainate) or vacuum filtration through Whatman GF/B filters pretreated with 0.05% polyethylenimine using a Brandel MB-18 Harvester ([^{3}H]glutamate). The radioactivity was quantitated by liquid-scintillation spectrometry. Nonspecific binding was defined as the residual binding in the presence of either excess L-glutamate (0.1 mM) or kainate (0.1 mM). Displacement of radioligand binding was analyzed with a logit–log transformation to calculate the IC_{50}, while the K_i values were determined with the Cheng–Prusoff equation.[29]

[^{3}H]MK-801 Binding Assay. Modulation of [^{3}H]MK-801 binding was performed with Triton X-100 (0.04% v/v) treated rat SPM that had been extensively washed. Assay incubations were at 25 °C for 30 min and contained 5.0 nM [^{3}H]MK-801, L-glutamate (10.0 nM), and various concentrations of the tested compounds in 50 mM Tris/acetate, pH 7.4. The assay was stopped by rapid filtration, using Brandel MB-48 Harvester, through Whatman GF/B filters treated with 0.05% polyethylenimine, and the samples were washed four times with 2.0

(27) Wheeler, T. N.; Ray, J. *J. Org. Chem.* **1987**, *52*, 4875–4877.

(28) Monahan, J. B.; Michel, J. *J. Neurochem.* **1987**, *48*, 1699.

(29) Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.

mL of cold buffer. The radioactivity associated with the filter was determined by liquid-scintillation spectrometry. Nonspecific binding was defined with 60 μM MK-801. IC_{50} were determined using a logit-log transformation of the binding data.

[^{3}H]Glycine Binding Assay. [^{3}H]Glycine binding to the NMDA receptor associated strychnine-insensitive recognition site was performed as previously described.[30]

Registry No. **7**, 1067-87-4; (*Z*)-**8**, 129920-47-4; (*E*)-**8**, 129920-48-5; (*Z*)-**9**, 129920-49-6; (*E*)-**9**, 129920-50-9; **10a**, 129920-51-0; **10b**, 129920-52-1; **11a**, 129920-53-2; **11b**, 129920-54-3; **12a** (diastereomer 1), 129920-55-4; **12a** (diastereomer 2), 130008-24-1; **12b** (diastereomer 1), 130008-31-0; **12b** (diastereomer 2), 130008-32-1; **13a** (diastereomer 1), 129920-81-6; **13a** (diastereomer 2), 130008-33-2; **13b** (diastereomer 1), 130008-34-3; **13b** (diastereomer 2), 130008-35-4; **14**, 15916-48-0; **15**, 129920-56-5; **16**, 129920-57-6; **17**, 129920-58-7; **18**, 129920-59-8; **19**, 129920-84-9; **20**, 129920-60-1; **21**, 129920-61-2; **22**, 129920-62-3; **23**, 129920-85-0; **24**, 127515-28-0; (*Z*)-**25** (diastereomer 1), 129920-87-2; (*Z*)-**25** (diastereomer 2), 130008-45-6; (*E*)-**25** (diastereomer 1), 130008-46-7; (*E*)-**25**, 130008-47-8; (*Z*)-**26** (diastereomer 1), 129920-86-1; (*Z*)-**26** (diastereomer 2), 130008-42-3; (*E*)-**26** (diastereomer 1), 130008-43-4; (*E*)-**26** (diastereomer 2), 130008-44-5; **27a**, 5162-44-7; **27c**, 1119-51-3; **28a**, 129920-63-4; **28b**, 129920-64-5; **28c**, 129920-65-6; **28d**, 129920-66-7; **29a**, 129920-67-8; **29b**, 129920-68-9; **29c**, 129920-69-0; **29d**, 129920-70-3; **30a**, 129920-71-4; **30b**, 129920-72-5; **30c**, 129920-73-6; **30d**, 129920-74-7; **31a**, 129920-75-8; **31b**, 129920-76-9; **31c**, 129920-77-0; **31d**, 129920-78-1; **32a** (diastereomer 1), 129920-79-2; **32a** (diastereomer 2), 130008-25-2; **32b** (diastereomer 1), 130008-26-3; **32b** (diastereomer 2), 130008-27-4; **32c** (diastereomer 1), 129920-80-5; **32c** (diastereomer 2), 130008-28-5; **32d** (diastereomer 1), 130008-29-6; **32d** (diastereomer 2), 130008-30-9; **33a** (diastereomer 1), 129920-82-7; **33a** (diastereomer 2), 130008-36-5; **33b** (diastereomer 1), 130008-37-6; **33b** (diastereomer 2), 130008-38-7; **33c** (diastereomer 1), 129920-83-8; **33c** (diastereomer 2), 130008-39-8; **33d** (diastereomer 1), 130008-40-1; **33d** (diastereomer 2), 130008-41-2; NMDA, 6384-92-5; $BrCH_2C{-}H{=}CH_2$, 106-95-6; $N_2{=}CHCOOEt$, 623-73-4; $N_2{=}C(COOMe)_2$, 6773-29-1; $N_2{=}CHPO_3Me_2$, 27491-70-9.

(30) Monahan, J. B.; Corpus, V. M.; Hood, W. F.; Thomas, J. W.; Compton, R. P. *J. Neurochem.* **1987**, *53*, 370-375.

Synthesis, Antibacterial Activities, and Pharmacological Properties of Enantiomers of Temafloxacin Hydrochloride[1]

Daniel T. W. Chu,* Carl W. Nordeen, Dwight J. Hardy, Robert N. Swanson, William J. Giardina, Andre G. Pernet, and Jacob J. Plattner

Anti-infective Research Division, Abbott Laboratories, Abbott Park, Illinois 60064-3500. *Received June 6, 1990*

Temafloxacin hydrochloride [(±)-7-(3-methylpiperazin-1-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid hydrochloride] is a potent member of the 4-pyridone-3-carboxylic acid class of antibacterial agents and is currently under clinical development as a broad-spectrum antimicrobial agent. It is a racemate having a chiral center at the C_3 of the 7-piperazin-1-yl group. The two enantiomers were synthesized and tested for their antibacterial activities. Although no difference in in vitro antibacterial activities was observed, a minor difference in in vivo antibacterial activities was observed. However, they both exhibited similar pharmacological profiles.

In recent years, many clinically important antibacterial agents (such as ciprofloxacin (**1**)[2] and norfloxacin (**2**)[3]) having the 1-substituted-1,4-dihydro-4-oxopyridine-3-carboxylic acid moiety and collectively known as quinolones have been discovered.[4] These agents have been shown to inhibit the topoisomerase enzyme DNA gyrase.[5,6] Hence, it may be expected that chirality in the quinolone molecule can have a great impact on the biological activity. Nearly all clinically useful quinolones developed to date, however, are either achiral or racemic mixtures. The *S* enantiomers of ofloxacin (**3**)[7,8] and S-25930 (**4**)[9] have recently been reported to possess greater biological activities (10–100-fold) than their antipodes. The *R* enantiomer of 7-(2-substituted-pyrrolidin-1-yl)quinolone derivative **5a** possesses 10–60-fold greater potency than the *S* enantiomer.[10] Minor differences in activity are observed with the two enantiomers of the 7-(3-aminopyrrolin-1-yl)-naphthyridine derivative **5b**.[11] The enantiomers of the 3-[(ethylamino)methyl]pyrrolidin-1-yl derivative **6**, however, were reported to have similar biological activity.[12]

Temafloxacin hydrochloride (**7**) [(±)-7-(3-methylpiperazin-1-yl)-6-fluoro-1-(2,4-difluorophenyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid hydrochloride] is a potent quinolone antibacterial agent. It is currently under clinical development and a NDA has been filed in the U.S. It possesses excellent activity against both Gram-positive and Gram-negative bacteria.[12] Temafloxacin is a racemate having a chiral center at C-3 of the 7-piperazin-1-yl group. Because of the excellent biological activity of **7** and the presence of a chiral center, both enantiomers were synthesized to evaluate their potential differences in biological, pharmacological, and toxicological properties. In this paper, we now report the synthesis and properties of the enantiomers of temafloxacin hydrochloride.

(1) A preliminary account of this work has been presented at the 29th ICAAC, Sept 17-20, Houston (Chu, D. T. W.; Nordeen, C. W.; Maleczka, R. E.; Hardy, D. J.; Pernet, A.; Clement, J. J.; Plattner, J. J.), 1989; Abstract No. 1245.
(2) Wise, R.; Andrew, J.; Edward, E. *Antimicrob. Agents Chemother.* **1983**, *23*, 559.
(3) Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. *J. Med. Chem.* **1980**, *23*, 1358.
(4) For a recent review, see: Chu, D. T. W.; Fernandes, P. B. *Antimicrob. Agents Chemother.* **1989**, *33*, 131.
(5) Cornett, J. B.; Wentland, M. P. *Annu. Rep. Med. Chem.* **1986**, *21*, 139 and references therein.
(6) (a) Fernandes, P. B.; Chu, D. T. W. *Annu. Rep. Med. Chem.* **1987**, *22*, 117 and references therein. (b) Fernandes, P. B.; Chu, D. T. W. *Ibid.* **1988**, *23*, 133 and references therein.
(7) Imamura, M.; Shibamura, S.; Hayakawa, I.; Osada, Y. *Antimicrob. Agents Chemother.* **1986**, *29*, 163.
(8) Mitscher, L. A.; Sharma, P. N.; Shen, L. L.; Chu, D. T. W. *J. Med. Chem.* **1987**, *30*, 2283.
(9) Gerster, J. F.; Rohlfing, S. R.; Pecore, S. E.; Winandy, R. M.; Stern, R. M.; Landmesser, J. E.; Olsen, R. A.; Gleason, W. B. *J. Med. Chem.* **1987**, *30*, 839.
(10) Cooper, C. S.; Chu, D. T. W.; Fernandes, P. B.; Pihuleac, E.; Pernet, A. 25th ICAAC, Sept 29–Oct 2, Minneapolis, 1985; Abstract No. 130.
(11) Rosen, T.; Chu, D. T. W.; Lico, I. M.; Fernandes, P. B.; Shen, L.; Borodkin, S.; Pernet, A. G. *J. Med. Chem.* **1988**, *31*, 1586.
(12) Culberton, T. P.; Domagala, J. M.; Nichols, J. B.; Priebe, S.; Skeean, R. W. *J. Med. Chem.* **1988**, *31*, 503.

0022-2623/91/1834-0168$02.50/0