Preferred Antagonist Binding State of the NMDA Receptor: Synthesis, Pharmacology, and Computer Modeling of (Phosphonomethyl)phenylalanine Derivatives[1,2]

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A series of substituted [phosphono-, sulfo-, carboxy-, and (*N*-hydroxycarbamoyl)methyl]phenylalanines were synthesized as probes for the investigation of the preferred antagonist state of the NMDA receptor antagonists. The potency of these compounds was evaluated by measuring electrophysiological responses induced by NMDA in cultured mouse cortical neurons. 3-(Phosphonomethyl)phenylalanine [1(m)] a formal AP7 analogue, has been shown to be the most potent antagonist in this study with an IC_{50} of around 5 μM. The isomeric 2-(phosphonomethyl)phenylalanine [1(o)] was about half as active as 1(m) and as active as compound 5(3), a derivative which is cis-hydrogenated on the phenyl ring of 1(m). Replacement of a phosphono by a sulfo group led to a large reduction in the ability of these compounds to antagonize NMDA responses, although the ortho and meta isomers retained some activity in their reduced forms. In both series the para isomers were almost completely inactive at 100 μM. Introduction of a carboxyl or a bidentate HONHCO group in place of the phosphono moiety in the 3-position results in compounds devoid of activity. The active and inactive compounds of this study were used in conjunction with the most potent linear and cyclic phosphono-containing NMDA antagonists reported to date to determine, via computer modeling techniques, a three-dimensional model corresponding to an antagonist preferring state of the NMDA binding site. This structure defines a pharmacophore which is characterized by (i) well-defined distances between the central atoms of the polar groups PO_3H^-, NH_n^+, (n = 2, 3), and COO^- (P–N = 5.89 ± 0.12 Å, P–C = 6.66 ± 0.08 Å, and N–C = 2.28 ± 0.01 Å), (ii) a sterically allowed region between the C_5 methylene and the PO_3H^- group, and (iii) a molecular electrostatic field in which the positive, neutral, and negative potential zones are self-contained—with the negative potential zone connecting the PO_3H^- and COO^- groups as the largest. We have compared our results to a preliminary model of the NMDA antagonist site by Hutchison et al.[1] and to a topological model of the NMDA–glycine receptor site by Cordi et al.[2] Our proposed steric–electrostatic pharmacophore which refines, simplifies, and improves these models has now to be validated by the design of new NMDA antagonists.

The excitatory amino acids, L-glutamate and L-aspartate in particular, play an important role in central synaptic transmission.[3-6] Electrophysiological[6] and binding studies[7,8] have demonstrated the existence of a variety of receptors, among which the best characterized are those activated selectively by *N*-methyl-D-aspartate (NMDA). NMDA receptor activation appears to require the binding of both an excitatory amino acid at the NMDA site and glycine at a separate site of the receptor molecule.[9,10] There is growing evidence that excitatory amino acids could be implicated in central nervous system disorders such as epilepsy,[11] ischemia,[12-14] and Alzheimer's disease,[15,16] emphasizing the possible clinical interest of potent and selective antagonists. Several selective competitive antagonists for NMDA receptors have been described. These blocking agents have three common structural features: (i) an amino acid of D configuration, (ii) the presence of two acidic functions, one of which is preferentially a phosphonic acid, and (iii) a separation of four to six atoms between the two acidic functions.[17,18]

The first described potent NMDA antagonists were acyclic compounds (Figure 1) such as D-2-amino-5-phosphonopentanoic acid (**22**, D-AP5)[19,20] and D-2-amino-7-phosphonoheptanoic acid (**23**, D-AP7).[21] The increase in biological activity found for the derived cyclic compound 4-(3-phosphonoprop-1-yl)piperazine-2-carboxylic acid (**24**, CPP)[22-24,26] initiated pursuit of the classical medicinal chemistry strategy of conformational restriction and has very recently led to the synthesis of other potent cyclic and unsaturated compounds such as *cis*-4-(phosphonomethyl)piperidine-2-carboxylic acid (**25**, CGS 19755),[25,27] *cis*-4-(3-phosphonoprop-1-yl)piperidine-2-carboxylic acid (**26**, CPPP),[28] D-4-[(1*E*)-(3-phosphonoprop-2-enyl)]piperazine-2-carboxylic acid (**27**, D-CPP-

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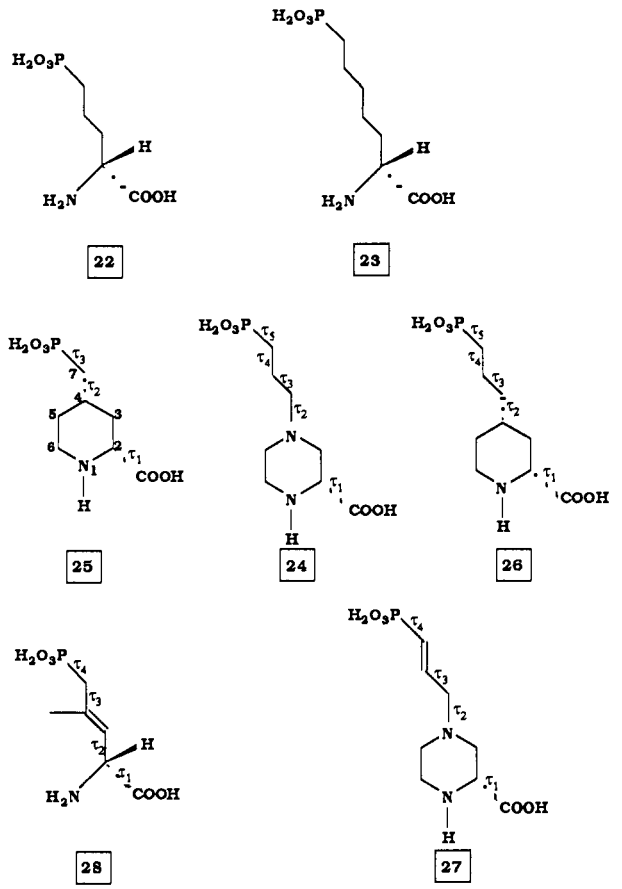
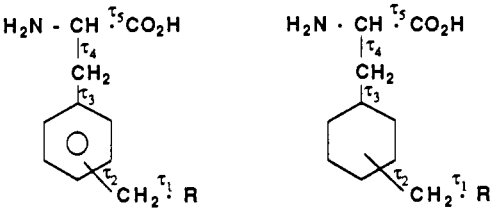


Figure 1. Structures of flexible and conformationally restricted competitive antagonists of the NMDA receptor. Those with defined rotatable bonds were used for the definition of the pharmacophore (see text). τ_1, τ_2, τ_3, τ_4, τ_5: rotatable bonds.

ene),[26] and D-(*E*)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (28, CGP 40116).[29] In all these compounds,

Table I. Percent Inhibition by Substituted Phenylalanine Derivatives of Electrophysiological Responses Induced by NMDA (10 μM) in Culture Mouse Cortical Neurons



R = PO_3H_2 1 (o, m, p) R = PO_3H_2 5 (2, 3, 4)
R = SO_3Na 2 (o, m, p) R = SO_3Na 6 (2, 3, 4)
R = CO_2H 3 (m)
R = CONHOH 4 (m)

antagonist[a]	% inhibn 100 μM antagonist	% inhibn 10 μM antagonist
22	100	68
1(o)	87	40
1(m)	100	80
1(p)	0	
2(o)	22	
2(m)	0	
2(p)	0	
3(m)	31	
4(m)	12	
5(2)	75	
5(3)	90	34
5(4)	18	
6(2)	50	
6(3)	50	
6(4)	20	

[a] All compounds except **22** were obtained as a mixture of stereoisomers.

incorporation of the α-amino acid functional groups into a piperidine or a piperazine ring led to a drastic reduction

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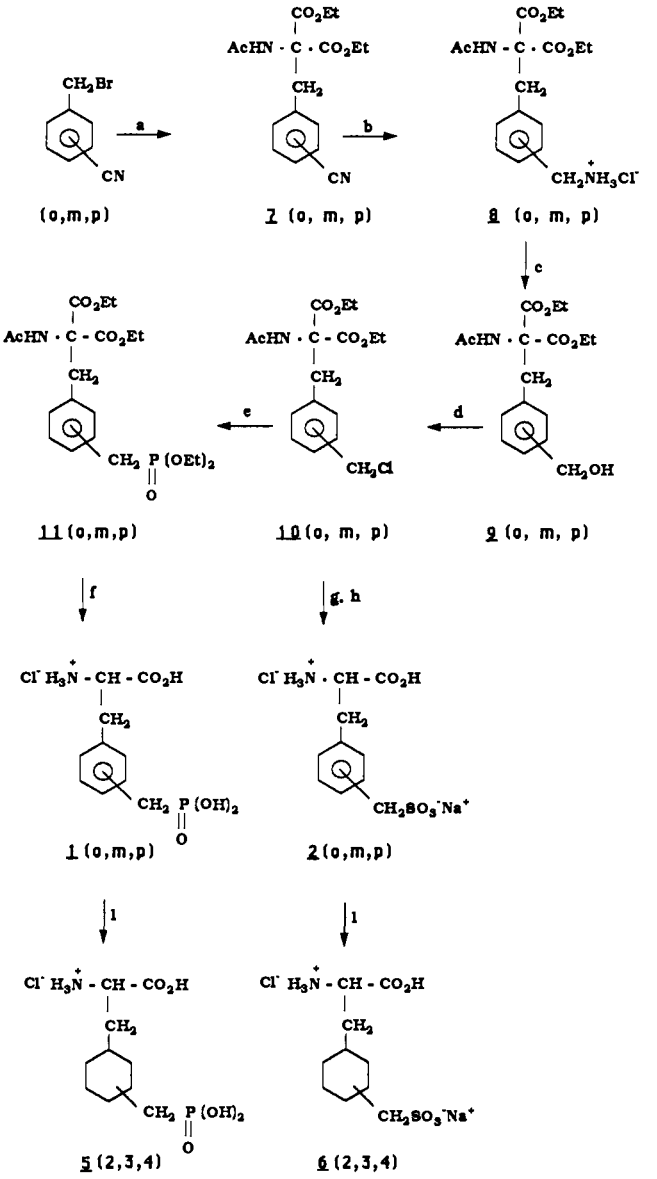
in conformational flexibility. Incorporation of the phosphonomethyl groups at various positions into the ring of phenylalanine or cyclohexylalanine residues was expected to favor the antagonist state receptor-recognition process, occurring probably through a zipper mechanism,[30,31] by increasing the conformational freedom at the level of the carboxyl and amino groups.

Accordingly, a series of 2-, 3-, and 4-(phosphonomethyl)phenylalanines **1(o,m,p)** (Table I) were preliminary electrophysiological evaluation as NMDA antagonists and molecular modeling of these compounds are reported here. 2-(Phosphonomethyl)phenylalanine has been previously synthesized using α,α'-dibromo-*o*-xylene as starting material.[32] 3- and 4-(Phosphonomethyl)phenylalanines have been reported[33] in independent studies performed during the present work using our previously published synthetic method.[34,35] In order to obtain additional information about the structural components of the NMDA binding site, the phosphonic group was replaced by either a sulfonic, carboxylic, or hydroxamic acid group in compounds **2–4**. The chelating hydroxamic acid group was selected to test the hypothesis of the presence within the NMDA binding site of a metal atom able to strongly interact with the acidic function present in most NMDA antagonists. This hypothesis was based upon the observed antagonism by Zn^{2+} of NMDA agonists.[36,37] The aromatic ring has been reduced in compounds **5** and **6** (Table I) to increase further the flexibility of the molecules.

Scheme I[a]



[a] (a) $AcNHCH(CO_2Et)_2$, Na, EtOH; (b) H_2 Pd/C HCl; (c) $NaNO_2$, H_2O, Δ; (d) $SOCl_2$, Δ; (e) $P(OEt)_3$, Δ; (f) 9 M HCl, Δ; (g) Na_2SO_3, Δ; (h) HCl, Δ; (i) PtO_2, AcOH.

On the basis of the abilities to antagonize NMDA-activated currents recorded from cultured mouse cortical neurons of the various compounds described here, an NMDA antagonist pharmacophore was derived taking into account the recently reported activity of semirigid molecules and the potencies of the compounds reported in this paper. The proposed model was compared to the recent ones by Hutchison et al.[1] and Cordi et al.[2]

Results and Discussion

Chemistry. 2- and 3-(phosphonomethyl- and sulfomethyl)phenylalanines were prepared as 4-(phosphonomethyl- and sulfomethyl)phenylalanines[35] (Scheme I): base-catalyzed alkylation of diethyl acetamidomalonate with 2-, 3-, or 4-substituted cyanobenzyl bromides (using various temperatures and time reactions as a function of the reactivity of these latter), followed by a three-step transformation of the nitrile function into the reactive chloromethyl group, led to the intermediates **10(o,m,p)**. Conversion to the racemic phosphonic acids **1(o,m,p)** was obtained by heating the corresponding **10(o,m,p)** with triethyl phosphite followed by an acid-catalyzed hydrolytic

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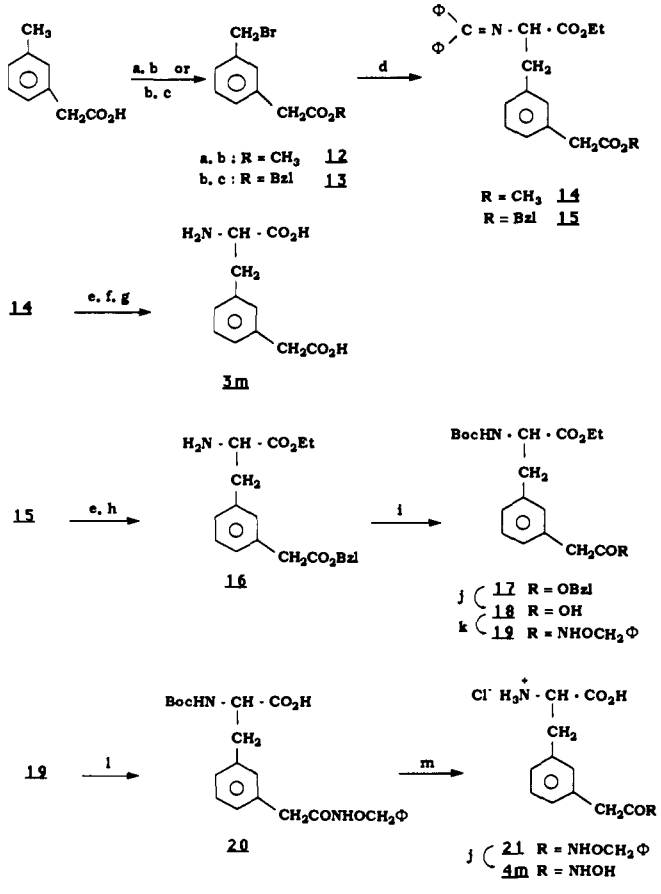
Scheme II[a]



[a] (a) MeOH, HCl, Δ; (b) NBS, peroxide, Δ; (c) $PhCH_2OH$, DCC, DMAP; (d) $Ph_2C{=}N\text{-}CH_2CO_2Et$, $PhCH_2(CH_3)_3N^+OH^-$, KI; (e) 1 M HCl; (f) 6 M HCl; (g) propylene oxide, EtOH; (h) $NaHCO_3$; (i) Boc_2O; (j) H_2 Pd/C; (k) DCC, HOBt, $PhCH_2ONH_2$; (l) NaOH, EtOH; (m) 3 M HCl in EtOAc.

dealkylation. The sulfonic acid derivatives **2(o,m,p)** were obtained directly from **10(o,m,p)** by reaction with sodium sulfite followed by acid hydrolysis.

Reduction of the aromatic rings of compounds **1(o,m,p)** and **2(o,m,p)** was performed with PtO_2 in AcOH yielding the hydrogenated phosphonic and sulfonic acid derivatives **5(2,3,4)** and **6(2,3,4)**. The evaluation of the cis/trans stereoisomers by ^1H NMR was difficult due to overlapping of the aliphatic resonances. However, in the case of the sulfonic acid-containing compounds, the evaluation of the relative stereochemistry was achieved for the 4-substituted derivative **6(4)**, on the basis of two distinct doublets corresponding to the $CH_2SO_3^-Na^+$ protons. The measured ratio of 7:3 was assumed to be in favor of the cis stereoisomer by analogy with the predominance of the cis stereoisomer of 4-[(diethylphosphono)methyl]piperidine-2-carboxamide (obtained by hydrogenation with PtO_2 in acetic acid of the 4-[(diethylphosphono)methyl]pyridine-2-carboxamide),[1] the structure of which is closely related to that of **5(3)** (pyridyl in place of phenyl ring).

The 3-[carboxy- and (*N'*-hydroxycarbamoyl)methyl]-phenylalanines were synthesized according to Scheme II as described for the corresponding 4-substituted compounds.[38] The synthesis of the amino acid derivatives involved one common step: the base-catalyzed alkylation of Schiff base derivatives of glycine with methyl and benzyl 3-(bromomethyl)phenylacetate.[39]

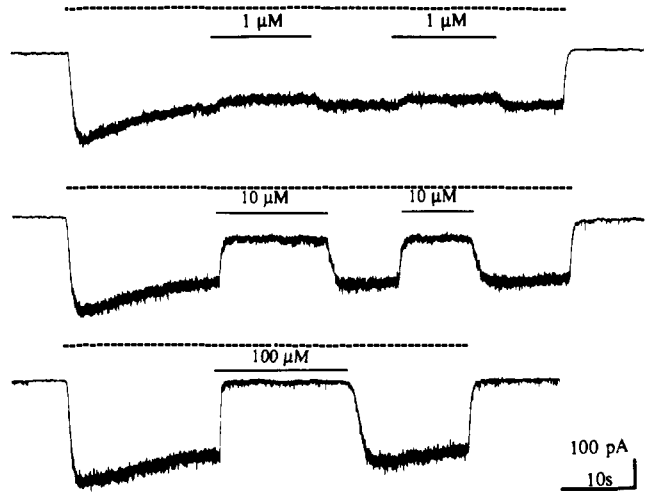


Figure 2. Voltage-clamp measurements of NMDA steady-concentration currents. The dotted line corresponds to the period of application of NMDA (10 μM) and glycine (1 μM). The antagonist **1m** was applied during the periods indicated by the full lines at concentration of 1, 10, and 100 μM.

A two-step acid hydrolysis of **14** allowed the amine to be deprotected[40] before the carboxylic acid groups, leading to compound **3(m)**.

For the synthesis of **4(m)**, the two ester functions present in the precursor **15** were chosen to be compatible with protection of the α-amino group and selective deprotection of the 3-carboxylic group by hydrogenation followed by coupling with *O*-benzylhydroxylamine. Indeed, attempts in our laboratory to directly prepare hydroxamic acids by condensation of hydroxylamine with ester groups failed. Likewise, in the para series, the introduction of the hydroxamate function by reaction of *O*-benzylhydroxylamine on the carboxylate of 4-(bromomethyl)phenylacetic acid before alkylation of ethyl *N*-(diphenylmethylene)glycinate gave, after hydrolysis, the corresponding amine in a yield of 4%. Thus, after hydrolysis of the imine **15**, the α-amino group was protected by a tert-butyloxycarbonyl group. Deprotection of the carboxylic acid by hydrogenolysis allowed coupling with *O*-benzylhydroxylamine by the classical DCC/HOBt method with a yield of 81%. Complete deprotection of compound **19** led to amino acid **4(m)**.

All modified amino acids described above were obtained and tested as racemic forms.

Pharmacology. Compounds **1–6** were initially tested for their ability to antagonize responses induced by NMDA (10 μM in the presence of 1 μM glycine) in cultured mouse cortical neurons. The antagonist properties of these compounds were analyzed in voltage clamp experiments using the whole-cell configuration of the patch-clamp method. The current produced by applying a steady concentration of NMDA (10 μM) often showed a slow decay in the first minutes of the experiment. After a few minutes, however, the response usually became non-desensitizing, although eventually after a longer period (>30 min), a new, faster desensitization process could be observed. Most of the analysis of the effect of **1(m)** and of the **1(m)** analogues was done in the period during which desensitization was

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minimal (Figure 2). An increasing blockade was observed with increasing concentrations of the antagonist and eventually complete blockade of the NMDA response could be observed. Electrophysiological results are listed in Table I. Under the experimental conditions used, the unsaturated sulfonic acid derivatives **2(p)** and **2(m)** show no antagonism, or a weak effect (20%) in the case of the 2-substituted amino acid **2(o)**. Reduction of the aromatic ring led to cyclohexyl derivatives **6** with restoration of some effect (20–50%). The carboxylic acid- and hydroxamic acid-substituted compounds **3(m)** and **4(m)** weakly inhibited (31% and 12%, respectively) the NMDA response. In contrast, at a concentration of 100 μM, the 2- and 3-(phosphonomethyl)phenylalanines **1(o)** and **1(m)** which can be considered as cyclic analogues of AP6 and AP7, were shown to be able to block the response produced by NMDA by 87% and 100%, respectively. At 10 times lower concentration (10 μM), **1(o)** produced 40% inhibition versus 80% for **1(m)**. Hydrogenation of the phenyl ring of the phosphono derivatives **1(o,m,p)** led to reduction in the pharmacological activity of **5(2)** and **5(3)**. Nevertheless, it is interesting to observe that **5(3)** remains almost as potent as inhibitor as its precursor **1(m)** in the aromatic series. The IC_{50} of **1(m)** was evaluated as 5.6 μM (three experiments). The blockade produced by 10 μM **1(m)** on the response to 10 μM NMDA was on average 56%. In four cells, the same concentration of **1(m)** was found to produce a larger reduction (about 76%) of the response to 1 μM NMDA, in qualitative agreement with the predictions of competitive antagonism. Neglecting the cooperativity of the NMDA response, an approximate value of the inhibition constant K_i of **1(m)** can be calculated from $K_i = (IC_{50} \times EC_{50})/(EC_{50} + [NDMA])$.

Patneau and Mayer[41] have evaluated the EC_{50} of NMDA as 35 μM, which would lead to a value of $K_i = 4.4$ μM. These values, however, do not predict the marked difference between the inhibition of the responses to 10 μM and to 1 μM NMDA, and it is probable that, to explain this difference, one would require a model involving two binding sites for NMDA.

The IC_{50} of **1(m)** is in agreement with its potency in displacing [^{3}H]CPP from the NMDA binding site of rat cortical membranes.[33,42] The agreement is somewhat lower for compound **1(o)**, which was reported to inhibit less than 20% of the binding of [^{3}H]AP7 to rat brain membranes at 100 μM concentration,[32] whereas in this study an IC_{50} of around 20 μM can be estimated for its potency in blocking NMDA-induced currents. One of the reasons for this slight discrepancy could be the use of different binding conditions. The experiments of Benveniste et al.,[43] which were done under conditions comparable to those used in the present paper, indicated that **22** inhibited the response to 100 μM NMDA with an IC_{50} of about 5 μM; this suggests that **1(m)** and **22** have comparable antagonist potencies.

These results show that the conformational restriction induced by a phenyl or a cyclohexyl ring can generally be

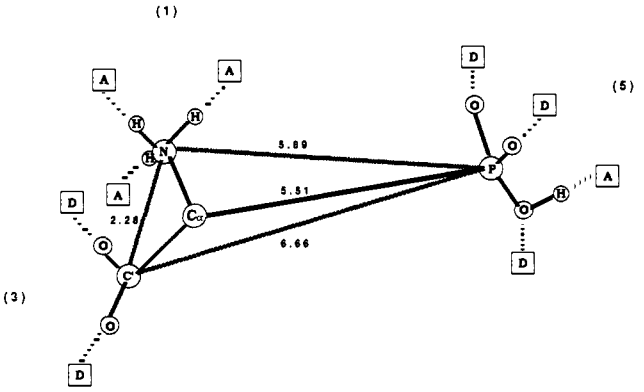

Figure 3. Proposed configuration of the NMDA receptor/ligand complex in the antagonist preferring state including chemical virtual, and hydrogen-bonds. Each of the interacting atoms may establish multiple interactions with the receptor atoms. In this model, permutation of the terminal groups bound to the phosphorus atom is allowed: D, proton, positive charge, proton donors, or water molecule; A, oxygen, negative charge, proton acceptors, or water molecule. (Charges are meant to be partial. The numbers in parentheses correspond to the model of Cordi et al.[2])

accepted in the NMDA binding site, but its position between the two acidic functions is important for biological activity (meta > ortho > para). In agreement with a structure–activity relationship concerning the antagonist recognition site of the NMDA receptor,[17,18] the order of the nonaminated acidic function for the meta series is $PO_3H_2 > CO_2H > SO_3H$. This preference cannot be explained just on the basis of conformational arguments (see below), but perhaps by the ability of PO_3H^- to form charge–charge Coulombic and/or hydrogen-bond interactions with hydrogen acceptor and donor groups in the site of the receptor at physiological pH.[2] The low affinity of compound **4(m)** suggests that there is no zinc atom associated with the hydroxamic function in the NMDA antagonist recognition site; nevertheless, this does not exclude the hypothesis of the presence of the metal elsewhere in the NMDA binding site.

Computational Chemistry. Computer-assisted molecular modeling was used to try to delineate a pharmacophore for the NMDA antagonist recognition site. For this purpose, five molecules, namely **25**, **24**, **27**, **26**, and **28** (Figure 1), were selected to determine a possible bioactive structure for the NMDA antagonist recognition site using the molecular modeling software SYBYL 5.3,[44] as described in the Experimental Section.

These compounds are among those exhibiting the highest affinities[28,45] and selectivities[25] in the series of phosphonates for the NMDA recognition site. All of them contain the three functional groups, $-PO_3H_2$, –COOH, and >NH, which have been shown to be essential for activity.[3,19,26,46] A systematic conformational search on the zwitterionic molecules whose ionized functional groups now become PO_3H^-,[25,47] COO^-, and NH_n^+ (n = 2, 3) was per-

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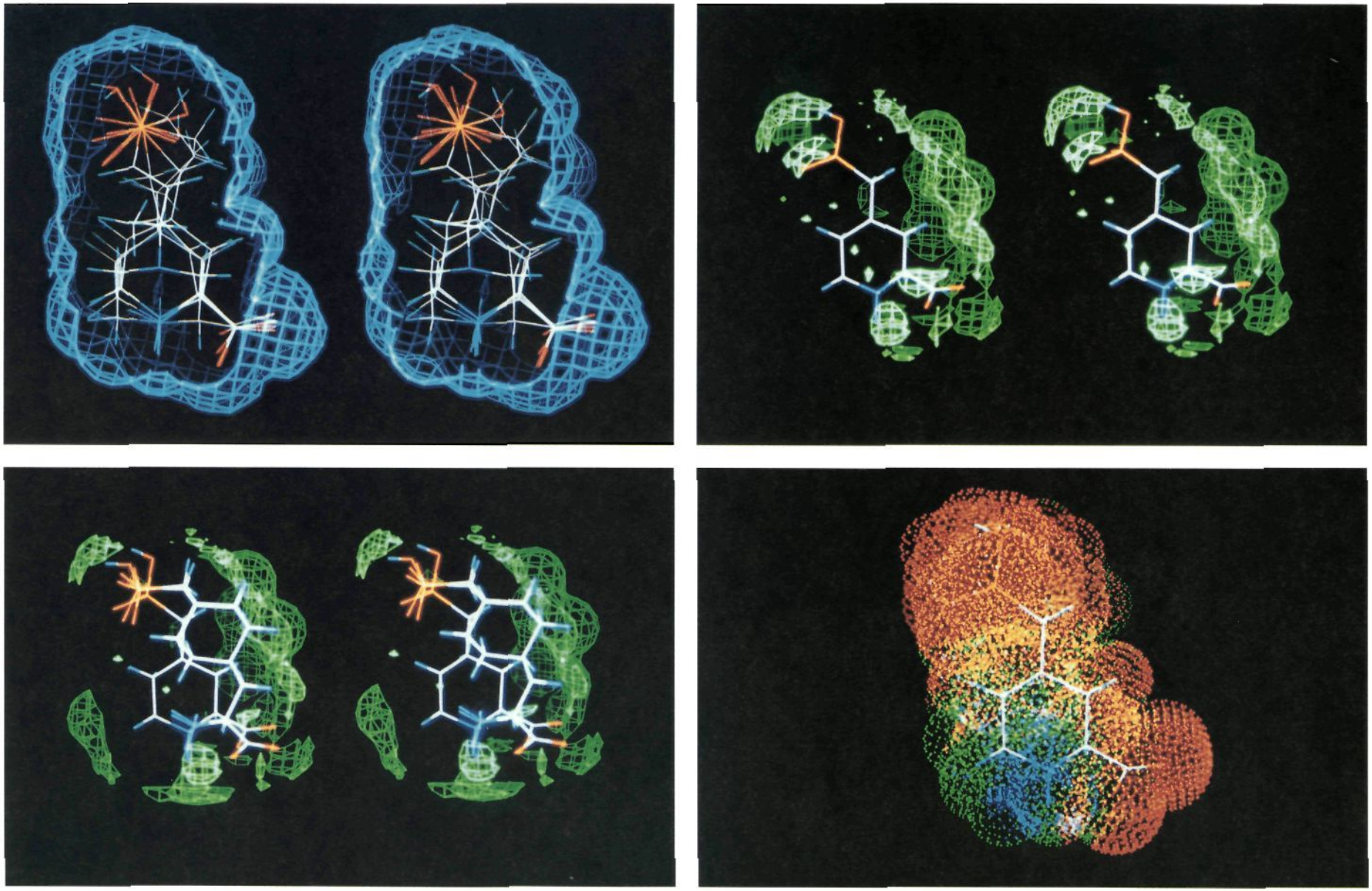

Figure 4. (a, upper left) "Relaxed" stereoscopic view of the ligand-accessible volume V_1 (blue) in the NMDA antagonist recognition site generated by the five conformationally-restricted molecules of Figure 1 in their bioactive conformations and least rms orientations with respect to **25**. Molecules are shown inserted in the volume. Phosphorus is in yellow, oxygen in red, and nitrogen in blue (see the text for additional details). (b, upper right) "Relaxed" spectroscopic view of the inactive ligand-occupied volume V_2 (green) for the NMDA receptor antagonist recognition site, in the same orientation shown in part a. Compound **25** inserted (see the text). (c, lower left) "Relaxed" stereoscopic view of the excluded volume V_5 (green) for the NMDA receptor antagonist recognition site (see the text). (d, lower right) Superimposition of the molecular electrostatic potential surfaces for the molecules of part a, with compound **25** inserted. Contour levels: red, $V < -10$ kcal/mol; green, -10 kcal/mol $< V < +10$ kcal/mol; blue, $V > +10$ kcal/mol.

Table II. Fitting Index, Torsional Angle, and Relative Energies of the Active Conformers

no.	rms[a] (Å)	torsional angle values for best conformers (deg) τ_1	τ_2	τ_3	τ_4	τ_5	total energy (kcal/mol),[b] E	ΔE (kcal/mol),[c] $E - E_m$
25	0.000	252	185	179			−72.36	0.53
24	0.023	249	277	270	63	177	−69.68	3.89
27	0.104	238	60	88	45		−49.52	1.11
26	0.099	253	295	310	313	299	−66.40	0.62
28	0.123	236	188	217	66		−83.74	1.50

[a] rms of the three atoms N_α, C′, and P, with respect to **25**. [b] Total energy is the sum of bond length, bond angle, torsional angle, van der Waals, and electrostatic (with $\epsilon = r$) energy terms. [c] Difference between the minimized intramolecular total energy and its corresponding value after MULTIFIT (E_M).

formed by making use of the technique of distance maps[48] in order to reduce the number of conformations generated by restraining the conformational search of related molecules. Subsequently, an energy minimization[49] for each conformation generated was undertaken. Superimposition of the central atoms of the three functional groups, i.e., the P, N_α, and C′ (of the COOH group) was achieved with the MULTIFIT option of SYBYL (see Molecular Modeling in the Experimental Section).

Upon interaction, more than one of the oxygen, nitrogen, and hydrogen atoms of the ionized phosphonate, carboxyl, and amino functional groups will be among the actual points of contact with the NMDA receptor, locking in a given configuration the corresponding functional groups through H-bonding interactions. These multiple interactions have been clearly established from crystallographic data on complexes between, for example, the bacterial metallopeptidase thermolysin and inhibitors bearing phosphonate or carboxyl functions.[50] Hughes and Andrews[51] use a "receptor fit point" method which consists of adding receptor guide points located at hydrogen-bond distances from the acceptor or donor groups. In such a case, one common or two separate receptor guide points can be used for the carboxylate oxygens. In the present study, we have adopted the more "rigid" fit procedure, supposing therefore that there are two receptor guide points for the carboxylate oxygens. In this way, only the P, N_α, and C′ atoms were used in the molecular modeling studies for delineating the pharmacophore. The quality of the superimposition of the three central atoms was evaluated by the root mean square (rms) deviation between the atoms constituting the pharmacophore in the reference molecule (**25**) and the corresponding atoms in the other molecules (Table II). From the various solutions resulting from the MULTIFIT approach, one set presented good interatomic overlap and best minimized intramolecular energy (Table II). This set was chosen as that containing the possible bioactive conformations for **25**, **24**, **27**, **26**, and **28**. In this set the active structure of **25** has a conformation 4,7-trans for torsion τ_2 (see Figure 1). The resulting pharmacophore is characterized by the mean internuclear distances among N, C′, and P shown in Figure 3. A van der Waals surface corresponding to the envelope of the superimposed antagonists in their allowed bioactive conformations and representing an accessible volume (V_1) for ligands of the NMDA receptor antagonist recognition site is shown in Figure 4a.

All the compounds synthesized in this work were subsequently studied by the same conformational techniques in order to select which compounds, if any, presented structural properties matching those of the proposed pharmacophore (the reduced forms **5(2)**, **5(3)**, **5(4)**, **6(2)**, **6(3)** and **6(4)** were in the cis configuration). A conformational search for these molecules was performed allowing the bonds to rotate with a chosen stepwise increment of 10° or 120° [30° for **4(m)**] of the dihedral angles. We selected the conformations which had the Y–N and Y–C (where Y = P, C, S) distances within ±0.3 Å to those obtained in the previously defined pharmacophore (P–N, 5.89 ± 0.12 Å; P–C, 6.66 ± 0.08 Å). We then obtained a volume corresponding to the Boolean difference between the total volume of the superimposed inactive compounds and the total volume of the best antagonists **25**, **24**, **27**, **26**, **28**, **1(m)**, **1(o)**, and **5(3)**, all in their supposed bioactive conformations. This volume represents an excluded van der Waals volume (V_2) for the NMDA receptor antagonist recognition site (Figure 4b), in which the region between the C_6 methylene and the PO_3H^- group results as the only one occupied by a potential ligand.

Using the previously described techniques, a new allowed volume (V_3) containing the active phosphono derivatives **1(m)**, **1(o)**, and **5(3)** was obtained. The union of V_1 and V_3 led to an allowed volume V_4. V_4 is characterized essentially by two supplementary zones with respect to V_1, adding up 188 Å^3. One is present above and below the mean plane of the ring in the bioactive conformation of **25** on the side of the C_5–C_6 bond and is due to the aromatic parts of both enantiomers of **1(o)**; the other one forms a protuberance on the side of the C_3–C_4 bond, but only on the C_4 axial direction (Figure 4a) and is a result of the aromatic ring of **1(m)**-(*R*) and of the cyclohexyl rings of the *R* and *S* enantiomers of **5(3)** (*R* and *S* referred to the absolute configuration of the C_α of the amino acid). However, when we calculated the van der Waals surface of **5(2)**-(*R*), its volume intersected by 4.0 Å^3 the excluded volume V_2 whereas that of **5(2)**-(*S*) intersected V_2 by 14.8 Å^3. We hypothesize therefore that the relatively good activity of the racemic mixture of compound **5(2)** is due to the *R* enantiomer, a result which agrees with previous structure–activity relationship studies in which the *R* enantiomers have been proposed as the active isomers.[17,18]

As compared to their corresponding active phosphono derivatives, the sulfo-containing molecules **2(m)**, **6(3)**, **2(o)**, and **6(2)** present poor or no biological activity (Table I), even though the differences in the corresponding electronic properties of the sulfonate and the phosphonate groups are rather small (our unpublished calculations). In addition, when we calculated the corresponding molecular electrostatic potentials from the charge distribution,[52,53]

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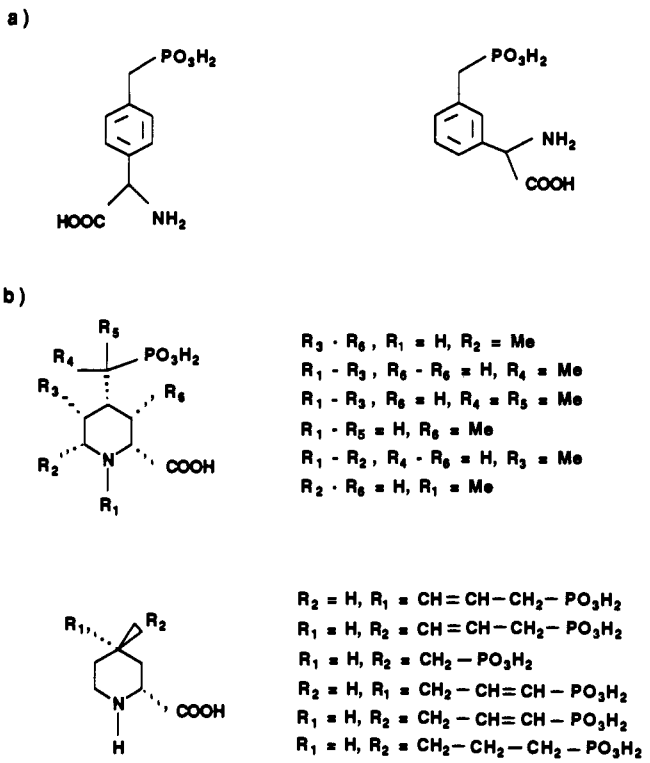

Figure 5. Structures of (a) 3- and 4-(phosphonomethyl)-phenylglycine[31] and (b) 4-(phosphonoalkyl)- and 4-(phosphonoalkenyl)-2-piperidinecarboxylic acids[1] used for refinement of the final excluded volume V_5 (see the text).

we found no differences between the sulfo and carboxyl analogues and the phosphonates **1(m)**, **25**, **24**, **27**, **26**, and **28**. However, it will be noted that, in contrast to the phosphonate, the sulfonate and carboxylate groups bear no protons in the ionized state. In the case of **4(m)** (a very poor antagonist), its hydroxamic acid group possesses a potential proton donor hydroxyl group, but at physiological pH it carries no formal charge.[54] Thus, in this series of antagonists, a negatively charged moiety with proton donor abilities seems to be necessary for receptor blockade.

Finally, in order to improve the excluded volume at the NMDA antagonist receptor site, we selected another set of active and inactive molecules from the works of Bigge et al.[33] and of Hutchison et al.[1] This set includes 4- and 3-(phosphonomethyl)phenylglycine[33] (Figure 5a) and 4-(phosphonoalkyl)- and 4-(phosphonoalkenyl)-2-piperidinecarboxylic acids[1] (Figure 5b). We then applied once more the methodology used for the first ensemble of compounds. The excluded volume obtained previously (V_2) was slightly improved, resulting in a final volume V_5 (Figure 4c). V_5, representing a forbidden volume, is characterized by a small reduction near the phosphonate group, and a significant increase near the N_α, C_5, and C_6 atoms of **25** in its bioactive conformation. It shall be noted that the internal faces of the volume fragments making up V_5 that are facing the antagonist molecule represent the receptor/ligand interface of the active site cavity (Figure 4c).

The electrostatic field generated by the polarity of the molecules is "a powerful tool that can provide insights into the molecular properties of small molecules";[52] it can be obtained by calculation of the net atomic charge distribution. At neutral pH, one expects the amino acids to be in the zwitterionic forms and the phosphono group to carry a single negative charge.[47] The electrostatic molecular potential was then calculated for **25**, **24**, **27**, **26**, and **28**. After superimposition of all the electrostatic potential molecular surfaces, we observed essentially three self-contained zones (Figure 4d), corresponding to each of three defined intervals of the calculated electrostatic potential (see the Experimental Section). In general terms, the region of positive potential (>10 kcal/mol) is small and is localized on the N_1 atom; the negative potential (<−10 kcal/mol) is widely distributed on the side of PO_3H^- and COO^- and "connects" these two groups. The potential corresponding to nonpolarity (−10 to +10 kcal/mol) is located around the cyclic moieties and aliphatic chains of **24**, **26**, and **27**, and would bring into play interactions of the hydrophobic type. A complementarity of the electrostatic potential between the ligand and the NMDA receptor site is expected to encourage a mutually favorable interaction.

Cordi et al.[2] have presented a topological model for the NMDA–glycine receptor complex consisting of two negative charges and two positive charges arranged arbitrarily in a tetrahedral arrangement, plus a negative charge above one of the faces of the tetrahedron. In this "five point" model only Coulombic interactions are taken into account. Given the molecular volumes computed in this paper (V_1–V_5) and Figure 3, our proposed pharmacophore model gives the steric interactions not considered in ref 2 and provides the interatomic distances involved on the NMDA side of the receptor complex in its antagonist state;[2] however, it must be considered that the arrangement of the ionized basic and acidic side chains of the amino acid residues in the receptor protein does not in general correspond precisely to the arrangement of the corresponding opposite charges on the antagonist. To Cordi et al.'s schematic model for the receptor should be added, as observed crystallographically for other systems,[50] the knowledge of the existence of numerous contact points on the receptor, some of them carrying partial electric charges, others being acceptors or donors of protons (see Figure 3). In our view, the five points in the model of Cordi et al. correspond rather to the central atoms of the bound ligands, and *not* to atoms or entities of the doubly occupied glycine–NMDA receptor. The receptor itself, due to the complex network of polyfurcated interactions (electrostatic, H-bonds) involving, in addition, water molecules, becomes actually a highly distorted polyhedron.

Hutchison and co-workers[1] assessed a series of substituted piperidine carboxylic acids as competitive ligands of the NMDA receptor. With computer-assisted modeling techniques using a torsional grid method, these authors propose bioactive conformations for two molecules (**25** and **26**) of the series, and subsequently present a NMDA receptor model in the antagonist state. Their favored conformations are characterized by the gauche (−) conformation for torsion τ_2 of **25** and torsion τ_4 of **26** (Figure 1). As a result of the extensive conformational space search, multifitting, and energy minimization techniques described in this paper we favor, however, the trans (or anti) conformation for torsion τ_2 of **25** as that of the bioactive conformation. Our studies include, but are not limited to, the analogues of AP5 and AP7 studied by Hutchison et al.[1] Moreover, since it is possible to find excellent su-

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perimpositions of the three central atoms N, C′, and P of **26** with those of **25**, only one location is found for the phosphorus atom, and not two as in ref 1. This allows the formulation of a generalized model for the antagonist state of the NMDA receptor as shown in Figure 3. As can be seen in that figure, the interatomic distances between the pair of central atoms N–C′ (2.28 Å) and the pair P–C′ (6.66 Å) compare rather well to those of Hutchison et al. (2.47 and 6.70 Å, respectively; for P–C′ we take an average of their P1 and P2). In addition, it is not necessary to try to interpret some affinity results from binding studies of NMDA receptors by making recourse of twist-boat conformations for the trans stereoisomers of the analogue compounds studied, since the formulation of our model incorporates these in their chair conformations. The computation of the molecular surfaces, and their visualization by molecular graphics (Figure 4), gives a quantitative picture concerning the steric hindrances present in the receptor protein, such as the one in front of the amino group (and already described by Hutchison et al.[1]) and the new one extending from the carboxylate to the phosphonate groups along the P–C_α molecular surface profile of the pharmacophore (Figure 4c).

Conclusion

In this study, compounds endowed with interesting antagonist properties such as **1(m)** have been obtained by the insertion of a phenyl ring between the two acidic functions present in phosphono containing NMDA antagonists. Using previously described and newly synthesized compounds, a model for the NMDA receptor antagonist recognition site has been proposed. In this model, which is subject to the validity of the used molecular mechanics force field, the active molecules **25** and **28** are in an extended conformation, whereas **24**, **27**, and **26** are in a folded conformation. They present together excluded volumes (Figure 4c in green) (i) around the amino group and (ii) extending from the carboxylic to the phosphonic acid moieties. This latter corresponds to a zone of negative electrostatic potential (Figure 4d in red color). Conversely, there are also several potential sites where hydrophobic substituents can be introduced onto the ring for instance to increase the bioavailability of the antagonists.

The proposed pharmacophore model gives the electrostatic, geometric, and stereochemical features of the ligand/receptor cavity, illustrating its topographic and electrostatic complementarity. Using these features for predicting binding properties of NMDA antagonists, more efficient and selective, competitive NMDA receptor blockers could be designed.

Experimental Section

Chemistry. (2-, 3-, and 4-cyanobenzyl bromide, ethyl *N*-(diphenylmethylene)glycinate, and benzyltrimethylammonium hydroxide (40%) were obtained from Aldrich. Diethyl acetamidomalonate, 3-methylphenylacetic acid, and *O*-benzylhydroxylamine were obtained from Janssen-Chimica. D-2-amino-5-phosphonopentanoic acid (**22**) was obtained from Sigma-Chimie. All the other reagents and solvents (Normapur label) were obtained from Prolabo. Methyl 3-methylphenylacetate was synthesized from 3-methylphenylacetic acid by a classical esterification method. Melting points of the crystallized compounds were taken on an electrothermal melting point apparatus and are uncorrected. Chromatography was carried out with Merck silica gel (230–400 mesh). TLC was performed on precoated silica gel plates (60F-254, 0.2 mm thick, Merck) with the following solvent systems (v/v): A, CH_2Cl_2–MeOH (9:1); B, cyclohexane–EtOAc–MeOH (6:4:0.4); C, 2-propanol–NH_4OH (28%) (6:4); D, 2-propanol–NH_4OH (28%) (7:3); E, hexane–EtOAc (9:1); F, CH_2Cl_2–Et_2O (9:1); G, $CHCl_3$–MeOH–H_2O–AcOH–EtOAc (7:3:0.6:0.3:10.9); H, CH_2Cl_2–MeOH (95:5): I, CH_2Cl_2; J, cyclohexane–EtOAc (1:1); K, $CHCl_3$–MeOH (8:2).

Plates were developed with UV light, iodine vapor, or ninhydrin. The structure of the compounds was confirmed by ^1H NMR spectroscopy (Bruker WH, 270 MHz). Mass spectra were recorded using a double-focusing VG 70-250 SEQ instrument. Elementary analysis (C, H, N) were in agreement with theoretical values.

The following abbreviations are used: THF, tetrahydrofuran; Boc_2O, di-*tert*-butyl dicarbonate. Other abbreviations used are those recommended by the IUPAC–IUB commission (*Biochem. J.* **1984**, *219*, 345).

Diethyl (3-Cyanobenzyl)acetamidomalonate [7(m)]. Procedure A. To a solution of Na (2.60 g, 113 mmol), in EtOH (150 mmol), were added successively diethyl acetamidomalonate (22.20 g, 102 mmol) and 3-cyanobenzyl bromide (20 g, 102 mmol). The solution was stirred for 30 min at room temperature and the resulting precipitate was collected by filtration and washed twice with water. **7(m)** was obtained as a white solid (30.85 g, 91% yield): R_f (A) 0.70; ^1H NMR (DMSO-d_6) δ 1.12 (t, 6 H, 2 OCH_2CH_3), 1.90 (s, 3 H, CH_3CO), 3.43 (s, 2 H, $PhCH_2C$), 4.11 (q, 4 H, 2 OCH_2CH_3), 7.26–7.73 (m, 4 H, aromatic protons), 8.17 (s, 1 H, NH). Anal. ($C_{17}H_{20}N_2O_5$) C, H, N.

Diethyl (2-Cyanobenzyl)acetamidomalonate [7(o)] and Diethyl (4-Cyanobenzyl)acetamidomalonate [7(p)]. Compound **7(o)** was synthesized via procedure A (stirred overnight) from 2-cyanobenzyl bromide in 95% yield. **7(o)**: R_f (A) 0.70; ^1H NMR (DMSO-d_6) δ 1.12 (t, 6 H, 2 OCH_2CH_3), 1.91 (s, 3 H, CH_3CO), 3.57 (s, 2 H, $PhCH_2C$), 4.10 (q, 4 H, 2 OCH_2CH_3), 7.11–7.78 (m, 4 H, aromatic protons), 8.20 (s, 1 H, NH). Anal. ($C_{17}H_{20}N_2O_5$) C, H, N. Compound **7(p)** was obtained as previously described,[35] R_f (A) 0.70.

Diethyl [3-(Aminomethyl)benzyl]acetamidomalonate [8(m)]. Procedure B. To a suspension of 10% Pd on charcoal (3.60 g) in EtOH (40 mL), saturated with hydrogen, was added a solution of **7(m)** (16.4 g, 49.4 mmol) in $CHCl_3$ (200 mL), EtOH (100 mL), and concentrated HCl (15 mL). The resulting mixture was stirred at room temperature for 24 h. After filtration and evaporation of the solvent, the residue was taken up in water (200 mL) and the unreacted material was filtered. Evaporation of the filtrate gave **8(m)** (11.22 g, 68% yield) as a white solid: R_f (A) 0.15; ^1H NMR (DMSO-d_6) δ 1.13 (t, 6 H, 2 OCH_2CH_3), 1.94 (s, 3 H, CH_3CO), 3.40 (s, 2 H, $PhCH_2C$), 3.93 (s, 2 H, $PhCH_2N$), 4.12 (q, 4 H, 2 OCH_2CH_3), 6.92–7.37 (m, 4 H, aromatic protons), 7.90 (s, 1 H, NH), 8.87 (brs, 3 H, NH_3^+). Anal. ($C_{17}H_{24}N_2O_5$·HCl) C, H, N.

Diethyl [2-(Aminomethyl)benzyl]acetamidomalonate [8(o)] and Diethyl [4-(Aminomethyl)benzyl]acetamidomalonate [8(p)]. These compounds were synthesized via procedure B from **7(o)** and **7(p)**, respectively, in 61% and 75% yields. **8(o)**: R_f (A) 0.20; ^1H NMR (DMSO-d_6) δ 1.13 (t, 6 H, 2 OCH_2CH_3), 1.89 (s, 3 H, CH_3CO), 3.50 (s, 2 H, $PhCH_2C$), 3.82 (d, 2 H, $PhCH_2N$), 4.11 (q, 4 H, 2 OCH_2CH_3), 6.94–7.55 (m, 4 H, aromatic protons), 8.18 (s, 1 H, NH), 8.26 (brs, 3 H, NH_3^+). Anal. ($C_{17}H_{24}N_2O_5$·HCl) C, H, N. **8(p)**: R_f (A) 0.10. Physical data are in accordance with the literature.[35]

Diethyl [3-(Hydroxymethyl)benzyl]acetamidomalonate [9(m)]. Procedure C. A solution of compound **8(m)** (9.0 g, 24.16 mmol) in water (150 mL) was adjusted to pH 6 with 10% NaOH and then $NaNO_2$ (2.33 g, 33.76 mmol) was added and the reaction mixture was refluxed for 5 h, cooled, and extracted with EtOAc (4 × 100 mL). The organic extracts were washed with 1 M HCl (20 mL), water (20 mL), 5% $NaHCO_3$, (20 mL), water (20 mL), and brine (20 mL) and dried over Na_2SO_4. Evaporation of the solvent gave **9(m)** as a white solid (7.90 g, 97% yield): R_f (A) 0.47; ^1H NMR (DMSO-d_6) δ 1.12 (t, 6 H, 2 OCH_2CH_3), 1.90 (s, 3 H, CH_3CO), 3.37 (s, 2 H, $PhCH_2C$), 4.12 (q, 4 H, 2 OCH_2CH_3), 4.39 (d, 2 H, $PhCH_2O$), 5.07 (t, 1 H, OH), 6.72–7.22 (m, 4 H, aromatic protons), 7.92 (s, 1 H, NH). Anal. ($C_{17}H_{23}NO_6$) C, H, N.

Diethyl [2-(Hydroxymethyl)benzyl]acetamidomalonate [9(o)] and Diethyl [4-(Hydroxymethyl)benzyl]acetamidomalonate [9(p)]. These compounds were synthesized via procedure C from **8(o)** and **8(p)**, respectively, in 89% and 78% yields. **9(o)**: R_f (A) 0.47; ^1H NMR (DMSO-d_6) δ 1.09 (t, 6 H, 2 OCH_2CH_3), 1.87 (s, 3 H, CH_3CO), 3.43 (s, 2 H, $PhCH_2C$), 4.08 (q, 4 H, 2 OCH_2CH_3), 4.32 (d, 2 H, $PhCH_2O$), 4.98 (t, 1 H, OH), 6.83–7.41 (m, 4 H, aromatic protons), 8.09 (s, 1 H, NH). Anal. ($C_{17}H_{23}NO_6$)

C, H, N. **9(p)**: R_f (A) 0.47. Physical data are in accordance with the literature.[35]

Diethyl [3-(Chloromethyl)benzyl]acetamidomalonate [10(m)]. Procedure D. A solution of compound **9(m)** (7.85 g, 23.3 mmol) and thionyl chloride (34 mL, 46.6 mmol) in CH_2Cl_2 (200 mL) was refluxed for 20 h and concentrated to dryness. The residue was washed twice with Et_2O to give a white solid **10(m)** (8.05 g, 97% yield): R_f (B) 0.36; ^{1}H NMR (DMSO-d_6) δ 1.13 (t, 6 H, 2 OCH_2CH_3), 1.91 (s, 3 H, CH_3CO), 3.40 (s, 2 H, $PhCH_2C$), 4.12 (q, 4 H, 2 OCH_2CH_3), 4.67 (s, 2 H, $PhCH_2Cl$), 6.85–7.33 (m, 4 H, aromatic protons), 7.99 (s, 1 H, NH). Anal. ($C_{17}H_{22}NO_5Cl$) C, H, N.

Diethyl [2-(Chloromethyl)benzyl]acetamidomalonate [10(o)] and Diethyl [4-(Chloromethyl)benzyl]acetamidomalonate [10(p)]. These compounds were synthesized via procedure D from **9(o)** and **9(p)**, respectively, in 90% and 95% yields. **10(o)**: R_f (B) 0.37; ^{1}H NMR (DMSO-d_6) δ 1.12 (t, 6 H, 2 OCH_2CH_3), 1.88 (s, 3 H, CH_3CO), 3.55 (s, 2 H, $PhCH_2C$), 4.12 (q, 4 H, 2 OCH_2CH_3), 4.54 (s, 2 H, $PhCH_2Cl$), 6.93–7.44 (m, 4 H, aromatic protons), 8.21 (s, 1 H, NH). Anal. ($C_{17}H_{22}NO_5Cl$) C, H, N. **10(p)**: R_f (B) 0.35. Physical data are in accordance with the literature.[35]

Diethyl [3-[(Diethoxyphosphinyl)methyl]benzyl]acetamidomalonate [11(m)]. Procedure E. Compound **10(m)** (2.5 g, 7.03 mmol) was dissolved in triethyl phosphite (20 mL), refluxed overnight, and concentrated to dryness. Flash chromatography of the residue on silica gel with cyclohexane–EtOAc–MeOH (6:4:0.4) gave **11(m)** as a white solid (2.51 g, 78% yield): mp 93–95 °C (lit.[33] mp 92–93 °C); R_f (B) 0.13; ^{1}H NMR (DMSO-d_6) δ 1.12 (m, 12 H, 4 OCH_2CH_3), 1.92 (s, 3 H, CH_3CO), 3.06 and 3.16 (s, 2 H, $PhCH_2P$), 3.35 (s, 2 H, $PhCH_2C$), 3.88 (m, 4 H, $P(OCH_2CH_3)_2$), 4.12 (q, 4 H, 2 OCH_2CH_3), 6.74–7.21 (m, 4 H, aromatic protons), 7.87 (s, 1 H, NH). Anal. ($C_{21}H_{32}NO_8P$) C, H, N.

Diethyl [2-[(Diethoxyphosphinyl)methyl]benzyl]acetamidomalonate [11(o)] and Diethyl [4-[(Diethoxyphosphinyl)methyl]benzyl]acetamidomalonate [11(p)]. These compounds were synthesized via procedure E from **10(o)** and **10(p)**, respectively, in 65% and 82% yields. **11(o)**: R_f (B) 0.16; ^{1}H NMR (DMSO-d_6) δ 1.11 (m, 12 H, 4 OCH_2CH_3), 1.87 (s, 3 H, CH_3CO), 3.02 and 3.10 (s, 2 H, $PhCH_2P$), 3.52 (s, 2 H, $PhCH_2C$), 3.83 (m, 4 H, $P(OCH_2CH_3)_2$), 4.10 (q, 4 H, 2 OCH_2CH_3), 6.85–7.25 (m, 4 H, aromatic protons), 8.09 (s, 1 H, NH). Anal. ($C_{21}H_{32}NO_8P$) C, H, N. **11(p)**: mp 115 °C (lit.[33] mp 95–96 °C); R_f (B) 0.10. Physical data are in accordance with the literature.[35]

3-(Phosphonomethyl)-DL-phenylalanine [1(m)]. Procedure F. Compound **11(m)** (563 mg, 1.23 mmol) in 9 M HCl (20 mL) was refluxed overnight and concentrated to dryness. Flash chromatography of the residue on silica gel with 2-propanol–NH_4OH (28%) (6:4) as eluent gave **1(m)** as a white solid (309.4 mg, 85% yield): R_f (C) 0.10; ^{1}H NMR (D_2O) δ 3.09 and 3.17 (s, 2 H, $PhCH_2P$), 3.09 and 3.17 (s, 2 H, $PhCH_2C$), 4.12 (dd, 1 H, CH), 7.27–7.52 (m, 4 H, aromatic protons); FB^+ (MH^+) calcd, 296; found, 296. Anal. ($C_{10}H_{14}NO_5P{\cdot}HCl$) C, H, N.

2-(Phosphonomethyl)-DL-phenylalanine [1(o)] and 4-(Phosphonomethyl)-DL-phenylalanine [1(p)]. These compounds were synthesized via procedure F from **11(o)** and **11(p)**, respectively, in 91% and 80% yields. **1(o)**: R_f (C) 0.12; ^{1}H NMR (D_2O) δ 3.03 and 3.10 (s, 2 H, $PhCH_2P$), 3.24 and 3.42 (dd, 2 H, $PhCH_2CH$), 4.03 (dd, 1 H, CH), 7.21–7.42 (m, 4 H, aromatic protons). Anal. ($C_{10}H_{14}NO_5P{\cdot}HCl$) C, H, N. **1(p)**: R_f (C) 0.08. Physical data are in accordance with the literature.[35]

3-(Sulfomethyl)-DL-phenylalanine [2(m)]. Procedure G. A solution of compound **10(m)** (500 g, 1.41 mmol) and Na_2SO_3 (1.0 g, 7.9 mmol) in water (50 mL) was refluxed for 2 h and acidified with 9 M HCl (30 mL). The reaction mixture was still-heated overnight and concentrated to dryness. Flash chromatography of the residue on silica gel with 2-propanol–NH_4OH (28%) (7:3) as eluent gave **2(m)** as a white solid (273 mg, 61% yield): R_f (D) 0.24; ^{1}H NMR (D_2O) δ 3.20 and 3.47 (dd, 2 H, $PhCH_2CH$), 4.12 (dd, 1 H, CH), 4.31 (s, 2 H, $PhCH_2S$), 7.41–7.60 (m, 4 H, aromatic protons); FB^+ (MH^+ – Cl – Na) calcd, 260; found, 260.

2-(Sulfomethyl)-DL-phenylalanine [2(o)] and 4-(Sulfomethyl)-DL-phenylalanine [2(p)]. These compounds were synthesized via procedure G from **10(o)** and **10(p)**, respectively, in 61% yields. **2(o)**: R_f (D) 0.24; ^{1}H NMR (D_2O) δ 3.37 and 3.57 (dd, 2 H, $PhCH_2CH$), 4.15 (dd, 1 H, CH), 4.42 (s, 2 H, $PhCH_2S$), 7.43–7.62 (m, 4 H, aromatic protons). **2(p)**: R_f (D) 0.24; ^{1}H NMR (D_2O) δ 3.22 and 3.48 (dd, 2 H, $PhCH_2CH$), 4.13 (dd, 1 H, CH), 4.32 (s, 2 H, $PhCH_2S$), 7.45–7.57 (2 d, 4 H, aromatic protons).

β-[3-(Phosphonomethyl)cyclohexyl]-DL-alanine [5(3)]. Procedure H. To a suspension of PtO_2 (30 mg) in 80% AcOH (10 mL), saturated with hydrogen, was added a solution of **1(m)** (50 mg, 0.169 mmol) in 80% AcOH (10 mL). The resulting mixture was stirred at room temperature for 2 days. After filtration and evaporation of the solvent and flash chromatography of the residue on silica gel with 2-propanol–NH_4OH (28%) (6:4) as eluent, **5(3)** was obtained quantitatively (51 mg) as a white solid: R_f (C) 0.12; ^{1}H NMR (D_2O) δ 0.65–2.30 (m, 14 H, $CHCH_2$-*c*H*x*-CH_2P), 3.70 (m, 1 H, CH); FB^+ (MH^+ – Cl) calcd, 266; found, 266. Anal. ($C_{10}H_{20}NO_5P{\cdot}HCl$) C, H, N.

β-[2-(Phosphonomethyl)cyclohexyl]-DL-alanine [5(2)], β-[4-(Phosphonomethyl)cyclohexyl]-DL-alanine [5(4)], β-[3-(Sulfomethyl)cyclohexyl]-DL-alanine [6(3)], β-[2-(Sulfomethyl)cyclohexyl-DL-alanine [6(2)], and β-[4-(Sulfomethyl)cyclohexyl]-DL-alanine [6(4)]. These compounds were synthesized via procedure H from **1(o)**, **1(p)**, **2(m)**, **2(o)**, and **2(p)**, respectively, in 88%, 64%, 100%, and 50% yields. **5(2)**: R_f (C) 0.15; ^{1}H NMR (D_2O) δ 0.90–2.85 (m, 14 H, $CHCH_2$-*c*H*x*-CH_2P), 3.75 (m, 1 H, CH). Anal. ($C_{10}H_{20}NO_5P{\cdot}HCl$) C, H, N. **5(4)**: R_f (C) 0.10; ^{1}H NMR (D_2O) δ 1.0–2.12 (m, 14 H, $CHCH_2$-*c*H*x*-CH_2P), 3.87 (m, 1 H, CH). Anal. ($C_{10}H_{20}NO_5P{\cdot}HCl$) C, H, N. **6(3)**: R_f (D) 0.29; ^{1}H NMR (D_2O) δ 0.70–2.40 (m, 12 H, $CHCH_2$-*c*H*x*), 3.0 (m, 2 H, CH_2S), 3.70 (m, 1 H, CH); FB^+ (MH^+ – Cl – Na) calcd, 266; found, 266. **6(2)**: R_f (D) 0.29; ^{1}H NMR (D_2O) δ 1.0–2.30 (m, 12 H, $CHCH_2$-*c*H*x*), 3.0 (m, 2 H, CH_2S), 3.75 (m, 1 H, CH). **6(4)**: R_f (D) 0.29; ^{1}H NMR (D_2O) δ 1.08–2.33 (m, 12 H, $CHCH_2$-*c*H*x*), 2.97 and 3.08 (d, 2 H, CH_2S), 3.88 (m, 1 H, CH).

Methyl 3-(Bromomethyl)phenylacetate (12). Procedure I. To a solution of methyl 3-methylphenylacetate (3.20 g, 19.2 mmol) in CCl_4 (166 mL) was added *N*-bromosuccinimide (3.47 g, 19.2 mmol) and dibenzoyl peroxide (100 mg) and was refluxed overnight. Hot filtration of succinimide, evaporation of the filtrate, and flash chromatography of the residue on silica gel with hexane–Et_2O (9:1) as eluent gave **12** (2.37 g, 50% yield) as an oil: R_f (E) 0.14; ^{1}H NMR (DMSO-d_6) δ 3.57 and 3.67 (s, 5 H, $CH_2CO_2CH_3$), 4.65 (s, 2 H, $PhCH_2Br$), 7.08–7.35 (m, 4 H, aromatic protons). Anal. ($C_{10}H_{11}O_2Br$) C, H.

Benzyl 3-(Bromomethyl)phenylacetate (13). From 3-methylphenylacetic acid (5 g, 33.3 mmol) and *N*-bromosuccinimide (5.92 g, 33.3 mmol) was obtained 11.5 g of crude 3-(bromomethyl)phenylacetic acid via procedure I. Esterification of this compound by benzyl alcohol was performed by using the procedure of Ziegler and Berger.[55] Flash chromatography of the residue on silica gel with hexane–Et_2O (9:1) as eluent gave **13** (3.5 g, 50% yield) as an oil: R_f (E) 0.16; ^{1}H NMR (DMSO-d_6) δ 3.70 (s, 2 H, $PhCH_2C$), 4.64 (s, 2 H, $PhCH_2Br$), 5.07 (s, 2 H, OCH_2Ph), 7.12–7.42 (m, 9 H, aromatic protons). Anal. ($C_{16}H_{15}O_2Br$) C, H.

Ethyl *N*-(Diphenylmethylene)-3-[(carbomethoxy)methyl]-DL-phenylalaninate (14). Procedure J. To a cold (10 °C) stirring solution of methyl 3-(bromomethyl)phenylacetate **12** (972.4 mg, 4 mmol), ethyl *N*-(diphenylmethylene)glycinate (1.17 g, 4.4 mmol), and potassium iodide (73 mg, 0.44 mmol) in dioxane (35 mL) was added dropwise over 45 min benzyltrimethylammonium hydroxide (1.57 mL, 40% aqueous). The reaction mixture was then brought to room temperature and stirred for an additional 3 h. After cooling at 0 °C, water (30 mL) was added and the mixture was extracted with toluene (6 × 50 mL). The organic extracts were washed with water (20 mL) and dried over $MgSO_4$. Evaporation of the solvent gave **14** quantitatively (1.72 g) as a yellow oil, which was not further purified: R_f (F) 0.77; ^{1}H NMR (DMSO-d_6) δ 1.12 (t, 3 H, OCH_2CH_3), 2.97 and 3.09 (dd, 2 H, $PhCH_2CH$), 3.44 and 3.49 (s, 5 H, $CH_2CO_2CH_3$), 4.02 (m, 3 H, $CHCO_2CH_2$), 6.42–7.87 (m, 14 H, aromatic protons).

Ethyl *N*-(Diphenylmethylene)-3-[(carbobenzoxy)methyl]-DL-phenylalaninate (15). This compound was synthesized via procedure J. From compound **13** (3.5 g, 10.96 mmol)

(55) Ziegler, F. E.; Berger, G. D. A mild method for the esterification of fatty acids. *Synth. Commun.* **1979**, *9*, 539–543.

and ethyl *N*-(diphenylmethylene)glycinate (3.22 g, 12.06 mmol) was obtained a yellow oil **15** quantitatively (5.54 g) which was not further purified: R_f (F) 0.80; ^{1}H NMR (DMSO-d_6) δ 1.10 (t, 3 H, OCH_2CH_3), 2.88–3.20 (m, 2 H, $PhCH_2CH$), 3.56 (s, 2 H, $PhCH_2C$), 3.95–4.17 (m, 3 H, $CHCO_2CH_2$), 5.07 (s, 2 H, OCH_2Ph), 6.44–7.79 (m, 19 H, aromatic protons).

3-(Carboxymethyl)-DL-phenylalanine [3(m)]. Compound **14** (1.72 g, 4 mmol) was dissolved in ether (12 mL) and 1 M HCl (12 mL) and stirred for 2 h at room temperature. The ethereal phase was then separated and the aqueous layer was concentrated to dryness in vacuo. HCl (6 M, 12 mL) was then added, refluxed for 5 h, and evaporated. The residue was concentrated twice from water to give a foam, which was dissolved in EtOH (6 mL) and treated with propylene oxide (2 mL). A fine white precipitate **3**(m) was collected by filtration, washed successively with EtOH (2 × 5 mL) and Et_2O (5 mL), and dried in vacuo (223 mg, 25% yield): mp 236–238 °C dec; R_f (G) 0.14; ^{1}H NMR (D_2O) δ 2.80 and 3.02 (dd, 2 H, $PhCH_2CH$), 3.45 (s, 2 H, $PhCH_2CO_2H$), 3.73 (m, 1 H, CH), 6.85–7.27 (m, 4 H, aromatic protons); FB^+ (MH^+) calcd, 224; found, 224. Anal. ($C_{11}H_{13}NO_4$) C, H, N.

Ethyl 3-[(Carbobenzoxy)methyl]-DL-phenylalaninate (16). Compound **15** (5.54 g, 10.96 mmol) was dissolved in Et_2O (35 mL) and 1 M HCl (35 mL) and stirred for 2 h at room temperature. The ethereal phase was then separated; the aqueous layer was made alkaline with 10% $NaHCO_3$ and extracted with CH_2Cl_2 (7 × 50 mL). The organic extracts were dried over $MgSO_4$ and evaporated in vacuo to yield a yellow oil, **16** (1.5 g, 40% yield), which was not further purified: R_f (H) 0.29; ^{1}H NMR (DMSO-d_6) δ 1.04 (t, 3 H, OCH_2CH_3), 2.0 (brs, 2 H, NH_2), 2.73 (m, 2 H, $PhCH_2CH$), 3.46 (m, 1 H, CH), 3.64 (s, 2 H, $PhCH_2C$), 3.96 (q, 2 H, OCH_2CH_3), 5.04 (s, 2 H, OCH_2Ph), 6.88–7.51 (m, 9 H, aromatic protons).

Ethyl *N*-(*tert*-Butyloxycarbonyl)-3-[(carbobenzoxy)methyl]-DL-phenylalaninate (17). To a cold (0 °C) stirring solution of the preceding compound **16** (1.5 g, 4.39 mmol) in THF (75 mL) was added Boc_2O (1.72 g, 7.87 mmol). After 1 h at 0 °C and 2 h at room temperature, the THF was added and the mixture was extracted with CH_2Cl_2 (3 × 50 mL). The organic extracts were dried over $MgSO_4$ and evaporated. Flash chromatography of the residue on silica gel with CH_2Cl_2 as eluent gave **17** (900 mg, 46% yield) as a colorless oil: R_f (I) 0.14; ^{1}H NMR (DMSO-d_6) δ 1.05 (t, 3 H, OCH_2CH_3), 1.27 (s, 9 H, $C(CH_3)_3$), 2.68–2.95 (m, 2 H, $PhCH_2CH$), 3.65 (s, $PhCH_2C$), 4.0 (m, 3 H, $CHCO_2CH_2$), 5.06 (s, 2 H, OCH_2Ph), 6.93–7.50 (m, 10 H, aromatic protons, NH). Anal. ($C_{25}H_{31}NO_6$) C, H, N.

Ethyl *N*-(*tert*-Butyloxycarbonyl)-3-(carboxymethyl)-DL-phenylalaninate (18). Procedure K. To a suspension of 10% Pd on charcoal (100 mg) in MeOH (4 mL), saturated with hydrogen, was added **17** (720 mg, 1.63 mmol) in MeOH (4 mL). The mixture was stirred for 1 h at room temperature. After filtration and evaporation of the solvent, **18** was obtained quantitatively (573 mg) as a colorless oil: R_f (H) 0.24; ^{1}H NMR (DMSO-d_6) δ 1.05 (t, 3 H, OCH_2CH_3), 1.28 (s, 9 H, $C(CH_3)_3$), 2.79 and 2.87 (dd, 2 H, $PHCH_2CH$), 3.46 (s, 2 H, $PhCH_2C$), 3.91–4.10 (m, 3 H, $CHCO_2CH_2$), 6.96–7.29 (m, 10 H, aromatic protons, NH). Anal. ($C_{18}H_{25}NO_6$) C, H, N.

Ethyl *N*-(*tert*-Butyloxycarbonyl)-3-[[*N'*-(benzyloxy)carbamoyl]methyl]-DL-phenylalaninate (19). To a cold (0 °C) stirring solution of the preceding compound **18** (573 mg, 1.63 mmol) in THF (2 mL) were added 1-hydroxybenzotriazole (250 mg, 1.63 mmol) in THF (3 mL) and dicyclohexylcarbodiimide (367 mg, 1.79 mmol) in THF (2 mL). After being stirred for 3 h at room temperature, the reaction mixture was recooled to 0 °C and a solution of *O*-benzylhydroxylamine hydrochloride (260 mg, 1.63 mmol) and Et_3N (1.63 mmol, 0.23 mL) in $CHCl_3$ (2 mL) was added. The resulting mixture was stirred for 1 h at 0 °C and then at room temperature overnight. After filtration of dicyclohexylurea (DCU) and evaporation of the solvents, the residue was dissolved in EtOAc and washed successively with water (6 mL), 10% citric acid (2 × 6 mL), water (6 mL), 10% $NaHCO_3$ (2 × 6 mL), water (6 mL), and finally, saturated NaCl (6 mL). The organic layer was dried over Na_2SO_4 and evaporated in vacuo. Flash chromatography of the residue on silica gel with cyclohexane–EtOAc (1:1) as eluent gave **19** (605 mg, 81% yield) as a colorless oil: R_f (J) 0.23; ^{1}H NMR (DMSO-d_6) δ 1.05 (t, 3 H, OCH_2CH_3), 1.29 (s, 9 H, $C(CH_3)_3$), 2.81 and 2.90 (dd, 2 H, $PhCH_2CH$), 3.22 (s, 2 H, $PhCH_2C$), 4.03 (m, 3 H, $CHCO_2CH_2$), 4.76 (s, 2 H, OCH_2Ph), 6.90–7.42 (m, 10 H, aromatic protons, *NH*CH), 11.19 (s, 1 H, NHO). Anal. ($C_{25}H_{32}N_2O_6$) C, H, N.

***N*-(*tert*-Butyloxycarbonyl)-3-[[*N'*-(benzyloxy)carbamoyl]methyl]-DL-phenylalanine (20).** To a solution of the preceding compound **19** (600 mg, 1.31 mmol) in EtOH (5 mL) and water (1.25 mL), cooled to 0 °C, was slowly added 1 N aqueous NaOH (1.44 mL, 1.1 equiv). After 15 min at 0 °C and room temperature overnight, EtOH was evaporated. The residue was dissolved in water (10 mL), washed with EtOAc (5 mL), and then acidified carefully to pH 2 with 1 M HCl and extracted with EtOAc (3 × 7 mL). The organic layer was dried over Na_2SO_4 and evaporated in vacuo. Flash chromatography of the residue on silica gel with $CHCl_3$–MeOH (8:2) as eluent gave a white solid (562 mg, 100% yield): mp 184 °C; R_f (K) 0.23; ^{1}H NMR (DMSO-d_6) δ 1.27 (s, 9 H, $C(CH_3)_3$), 2.83 and 3.03 (dd, 2 H, $PhCH_2CH$), 3.18 (s, 2 H, $PhCH_2C$), 3.89 (m, 1 H, CH), 4.73 (s, 2 H, OCH_2Ph), 6.05 (s, 1 H, Boc*NH*), 6.84–7.50 (m, 9 H, aromatic protons), 11.26 (s, 1 H, NHO). Anal. ($C_{23}H_{28}N_2O_6$) C, H, N.

3-[[*N'*-(Benzyloxy)carbamoyl]methyl]-DL-phenylalanine Hydrochloride (21). The preceding compound **20** (280 mg, 0.65 mmol) was dissolved in 3 M HCl in EtOAc (2 mL) at room temperature and stirred for 30 min.[56] A white solid was collected by filtration and washed with EtOAc (216 mg, 91% yield): mp 222–224 °C dec; R_f (L) 0.17; ^{1}H NMR (DMSO-d_6) δ 3.08 (m, 2 H, $PhCH_2CH$), 3.25 (s, 2 H, $PhCH_2C$), 4.06 (m, 1 H, CH), 4.74 (s, 2 H, OCH_2Ph), 6.97–7.48 (m, 9 H, aromatic protons), 8.48 (s, 3 H, NH_3^+), 11.35 (s, 1 H, NHO).

3-[(*N'*-Hydroxycarbamoyl)methyl]-DL-phenylalanine Hydrochloride [4(m)]. This compound was obtained via procedure K. From the preceding compound **21** (211 mg, 0.58 mmol) was obtained a pale pink solid quantitatively (158 mg): ^{1}H NMR (DMSO-d_6) δ 3.05 (d, 2 H, $PhCH_2CH$), 3.24 (s, 2 H, $PhCH_2C$), 4.02 (t, 1 H, CH), 7.03–7.27 (m, 4 H, aromatic protons), 8.40 (brs, 3 H, NH_3^+), 8.79 (s, 1 H, OH), 10.65 (s, 1 H, NHO); FB^+ (MH^+ – Cl) calcd, 239; found, 239.

Electrophysiology. The methods were those described by Henderson et al.[57] All experiments were performed on primary cultures of cortical neurons taken from 15- or 16-day-old mouse embryos. The responses to *N*-methyl-D-aspartic acid (NMDA) were studied using the whole cell configuration of the patch-clamp method. The recording pipet was filled with (in mM): CsF, 140; EGTA, 10; $CaCl_2$, 1; HEPES, 10 (pH 7.2).

The extracellular solution contained (in mM) NaCl, 140; KCl, 2.8; $CaCl_2$, 1; HEPES, 10, to which were added tetrodotoxin (0.2 mM) and glycine (1 μM). NMDA (obtained from Cambridge Research Biochemicals) was applied in most experiments at a concentration of 10 μM, using a three-barrel fast-perfusion system. The membrane potential was held at −50 mV.

Molecular Modeling. The topographical and electrostatic characterization of the NMDA pharmacophore was performed using the following steps: (i) Models of compounds **25**, **24**, **27**, and **26** in the chair conformation, and of **28**, were built from the Tripos database using the SYBYL[44] software on an Evans & Sutherland PS390 picture system driven by a Microvax 2000. (ii) Torsion angles were defined around the indicated single bonds for each compound (see Figure 1), and a systematic conformational search was performed, allowing the bonds to rotate with a chosen stepwise increment. Selected increments for torsions τ_1, τ_2, and τ_3 in compound **25** were 120°, 10°, and 120°, respectively; in **24** and **26** they were τ_1, 120°; τ_2, 10°; τ_3, 10°; τ_4, 10°; and τ_5, 120°; in **27** they were τ_1, 120°; τ_2, 10°; τ_3, 10°; and τ_4, 120°; and in **28** they were τ_1, 120°, τ_2, 10°; τ_3, 10°; τ_4, 120°. (iii) Evaluation of the internal energy and geometry optimization for all generated conformers were achieved using the molecular mechanics empirical Tripos force field[49,58–61] including the electrostatic term. However,

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the bonds P(sp^3)–C(sp^2) and S(sp^3)–O(sp^3) are not defined in this force field. We assigned the values for the corresponding stretching, bending, and torsional force constants by using an iterative minimization procedure in which the values for the force constants were adjusted so as to reproduce the crystallographic structures of related molecules (values available upon request). (iv) A multimolecule structural fitting was done by using the MAXIMIN MULTIFIT option of SYBYL. The MULTIFIT program performs a flexible fit between two or more molecules by selecting the atoms to be fitted. The fit is done by minimization of an energy expression that contains additional spring constants (20 kcal/mol Å^2) between the fitted points, thus forcing atoms of different molecules to occupy the same place, while the geometry of each molecule is simultaneously adjusted in order to relieve any strain. (v) The van der Waals surface and corresponding molecular volumes of the molecules were computed. Logical operations between volumes such as union, subtraction, and intersection were then performed in order to obtain certain regions of interest at the level of the receptor. (vi) The net atomic charges generating the electrostatic charge distribution of the molecules was obtained with the PM3 method[53] of the AMPAC package, which resulted in atomic charge values more in line with chemical intuition, as compared to other methods such as MNDO. The molecular electrostatic potential surfaces in the monopole–monopole approximation were then calculated as implemented in SYBYL according to the approach of Weiner et al.[52] and partitioned into three intervals as follows: −10 kcal/mol and down, from +10 to −10 kcal/mol, and +10 kcal/mol and up.

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Supplementary Material Available: A listing of the values for the corresponding force constants for the P–C and S–O bonds, the Cartesian coordinates, and the electrostatic PM3 charges of the final structures of compounds **25**, **24**, **27**, **26**, and **28** (6 pages). Ordering information is given on any current masthead page.

Structure–Activity Relationships of a Series of 2-Amino-4-thiazole-Containing Renin Inhibitors

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A series of renin inhibitors was synthesized that contained a 2-amino-4-thiazolyl moiety at the P_2 position. These derivatives are potent inhibitors of monkey renin in vitro and are selective in that they only weakly inhibit the closely related aspartic proteinase, bovine cathepsin D. Four compounds exhibited oral blood pressure lowering activity in high-renin normotensive monkeys. One of these compounds, **22** (PD 134672), was selected for further evaluation in renal hypertensive monkeys, on the basis of its superior efficacy and duration of action in the in vitro assays and the normotensive primate model.

Introduction

The renin–angiotensin system (RAS) has long been a target of the medicinal chemist for the treatment of hypertension in man.[1] The first event in this cascade is the cleavage of angiotensinogen by the aspartic proteinase renin to yield the decapeptide angiotensin I (AI). AI is then transformed by angiotensin converting enzyme (ACE) to produce the extremely potent vasoconstrictor angiotensin II (AII). Inhibitors of ACE are effective agents for treatment of hypertension and congestive heart failure.[2-4] More recently, selective AII receptor antagonists have been reported that may potentially be useful as antihypertensive agents.[5]

Each point of attack for inhibition of the renin–angiotensin system has advantages and disadvantages. For example, angiotensinogen is the only known natural substrate for renin. Thus, renin inhibitors may produce a highly selective inhibition of the RAS, leading to an improved side-effect profile for therapeutic agents.[6,7] Nu-

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